



Universiteit  
Leiden  
The Netherlands

## Exploring the relationships of gamma-hydroxybutyrate and sleep on metabolism, physiology, and behavior in humans

Pardi, D.J.

### Citation

Pardi, D. J. (2019, January 24). *Exploring the relationships of gamma-hydroxybutyrate and sleep on metabolism, physiology, and behavior in humans*. Retrieved from <https://hdl.handle.net/1887/68643>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/68643>

**Note:** To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/68643> holds various files of this Leiden University dissertation.

**Author:** Pardi, D.J.

**Title:** Exploring the relationships of gamma-hydroxybutyrate and sleep on metabolism, physiology, and behavior in humans

**Issue Date:** 2019-01-24

**Exploring the Relationships of  
*gamma*-Hydroxybutyrate and  
Sleep on Metabolism,  
Physiology, and Behavior in  
Humans**

**Daniel John Pardi**

ISBN: 978-0-578-44458-1

Layout: Daniel John Pardi

Cover Design: Daniel John Pardi

Printed By: Ram Print & Communications

Cover Painting: *"The Great Escape"*, Eric Zener, 2010.

Cover Icons: Vignesh Raja.

© 2019 Daniel John Pardi

Copyright of the individual chapters lies with the publisher of the journal listed at the beginning of each respective chapter. No part of this thesis may be reproduced in any form, by print, photocopy, digital file, internet, or any other means without written permission from the author.

Financial support for the publication of this thesis was generously provided by: Stanford Research Grant Fund.

# **Exploring the Relationships of *gamma*-Hydroxybutyrate and Sleep on Metabolism, Physiology, and Behavior in Humans**

Proefschrift

ter verkrijging van de graad van Doctor aan de Universiteit Leiden,  
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,  
volgens besluit van het College voor Promoties  
te verdedigen op donderdag 24 januari 2019  
klokke 16:15 uur

door

**Daniel John Pardi, MS**

Geboren te Marin Country, California, USA  
in 1974

**Promotor**

Prof. dr. H. Pijl

**Co-promoter**

Dr. G.J. Lammers

**Leden Promotiecommissie**

Prof. dr. N.R. Biermasz, Leiden University

Prof. dr. P.C.N. Rensen, Leiden University

Prof. dr. S. Overeem

Dr. Jamie Zeitzer, Stanford University

If you don't have time to do it right,  
when will you have time to do it over?

**John Wooden**

1910 – 2010

I have no special talents, I am only  
passionately curious.

**Albert Einstein**

1879 – 1955

The method of science is the method of  
bold conjectures and ingenious and severe attempts to refute  
them.

**Karl Popper**

1902 - 1994

For my family and my other advisors



# CONTENTS

|                                                                                                              |               |
|--------------------------------------------------------------------------------------------------------------|---------------|
| <b>CHAPTER 1</b>                                                                                             | <b>- 12 -</b> |
| <b>THESIS INTRODUCTION AND AIMS</b>                                                                          | <b>- 12 -</b> |
| 1.1 THESIS INTRODUCTION                                                                                      | - 13 -        |
| 1.2 ABSTRACT FOR PART I - NEUROBIOLOGICAL AND CLINICAL EFFECTS OF SODIUM OXYBATE                             | - 13 -        |
| 1.3 ABSTRACT FOR PART II - SLEEP, EATING, AND ENERGY REGULATION                                              | - 15 -        |
| 1.4 AIMS                                                                                                     | - 17 -        |
| <b>CHAPTER 2</b>                                                                                             | <b>- 23 -</b> |
| <b>INTRODUCTION TO GAMMA-HYDROXYBUTYRATE (GHB) / SODIUM OXYBATE (SXB): NEUROBIOLOGY AND CLINICAL EFFECTS</b> | <b>- 23 -</b> |
| <b>1. INTRODUCTION TO GHB / SXB</b>                                                                          | <b>- 26 -</b> |
| 1.1 BACKGROUND                                                                                               | - 26 -        |
| 1.2 ABUSE LIABILITY                                                                                          | - 27 -        |
| <b>2. BIOLOGY AND PHARMACOLOGY OF GHB / SXB</b>                                                              | <b>- 29 -</b> |
| 2.1 DISTRIBUTION                                                                                             | - 29 -        |
| 2.2 SYNTHESIS                                                                                                | - 30 -        |
| 2.3 METABOLISM                                                                                               | - 31 -        |
| 2.4 GHB AS A NEUROTRANSMITTER                                                                                | - 32 -        |
| 2.5 GHB BINDING SITE(S) IN THE BRAIN                                                                         | - 32 -        |
| <b>2.5.1 Stimulation of GHB Binding Site(s)</b>                                                              | <b>- 32 -</b> |
| <b>2.5.2 Stimulation of GABA<sub>B</sub> Receptors by GHB</b>                                                | <b>- 33 -</b> |
| 2.6 INTRACELLULAR RESPONSE TO GHB                                                                            | - 33 -        |
| 2.7 EFFECTS ON NEUROTRANSMITTER SYSTEMS                                                                      | - 34 -        |
| <b>2.7.1 Dopaminergic System</b>                                                                             | <b>- 34 -</b> |
| <b>2.7.2 Serotonergic System</b>                                                                             | <b>- 35 -</b> |
| <b>2.7.3 Opioidergic System</b>                                                                              | <b>- 35 -</b> |
| <b>2.7.4 Cholinergic System</b>                                                                              | <b>- 36 -</b> |
| <b>2.7.5 Noradrenergic System</b>                                                                            | <b>- 36 -</b> |
| <b>2.7.6 Glutamatergic System</b>                                                                            | <b>- 37 -</b> |
| <b>3. HORMONAL AND METABOLIC EFFECTS OF GHB / SXB</b>                                                        | <b>- 37 -</b> |
| 3.1 HORMONAL EFFECTS OF GHB / SXB                                                                            | - 37 -        |
| <b>3.1.1 Growth Hormone</b>                                                                                  | <b>- 37 -</b> |
| <b>3.1.2 Neurosteroids</b>                                                                                   | <b>- 39 -</b> |

|                                                                                                                                                    |                                               |        |
|----------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|--------|
| <b>3.1.3</b>                                                                                                                                       | <i>Prolactin</i>                              | - 39 - |
| <b>3.1.4</b>                                                                                                                                       | <i>Melatonin</i>                              | - 40 - |
| <b>3.1.5</b>                                                                                                                                       | <i>Ghrelin</i>                                | - 40 - |
| <b>3.2</b>                                                                                                                                         | <b>METABOLIC EFFECTS OF GHB / SXB</b>         | - 41 - |
| <b>3.2.1</b>                                                                                                                                       | <i>Insulin Sensitivity</i>                    | - 41 - |
| <b>3.2.2</b>                                                                                                                                       | <i>Thermoregulation</i>                       | - 42 - |
| <b>3.2.3</b>                                                                                                                                       | <i>Weight and Energy Balance</i>              | - 43 - |
| <b>4.</b>                                                                                                                                          | <b>CLINICAL EFFECTS OF GHB / SXB</b>          | - 47 - |
| <b>4.1</b>                                                                                                                                         | <b>HEALTHY SUBJECTS</b>                       | - 48 - |
| <b>4.1.1</b>                                                                                                                                       | <i>Sleep</i>                                  | - 48 - |
| <b>4.1.2</b>                                                                                                                                       | <i>Mood, Prosocial, and Prosexual Effects</i> | - 48 - |
| <b>4.2</b>                                                                                                                                         | <b>CLINICAL POPULATIONS</b>                   | - 49 - |
| <b>4.2.1</b>                                                                                                                                       | <i>Insomnia</i>                               | - 49 - |
| <b>4.2.2</b>                                                                                                                                       | <i>Fibromyalgia</i>                           | - 50 - |
| <b>4.2.3</b>                                                                                                                                       | <i>Parkinson's Disease</i>                    | - 52 - |
| <b>4.2.4</b>                                                                                                                                       | <i>Alzheimer's Disease</i>                    | - 52 - |
| <b>4.2.5</b>                                                                                                                                       | <i>Narcolepsy</i>                             | - 55 - |
| <b>5.</b>                                                                                                                                          | <b>CONCLUSIONS</b>                            | - 61 - |
| <b>5.1</b>                                                                                                                                         | <b>NEUROBIOLOGY</b>                           | - 61 - |
| <b>5.2</b>                                                                                                                                         | <b>CLINICAL EFFECTS</b>                       | - 62 - |
| <b>CHAPTER 3</b>                                                                                                                                   |                                               | - 63 - |
| <b>SODIUM OXYBATE FOR EXCESSIVE DAYTIME SLEEPINESS IN PARKINSON DISEASE - AN OPEN-LABEL POLYSOMNOGRAPHIC STUDY</b>                                 |                                               |        |
|                                                                                                                                                    |                                               | - 63 - |
| ABSTRACT                                                                                                                                           |                                               | - 64 - |
| INTRODUCTION                                                                                                                                       |                                               | - 65 - |
| METHODS                                                                                                                                            |                                               | - 65 - |
| RESULTS                                                                                                                                            |                                               | - 67 - |
| COMMENT                                                                                                                                            |                                               | - 69 - |
| <b>CHAPTER 4</b>                                                                                                                                   |                                               | - 73 - |
| <b>THE NIGHTLY ADMINISTRATION OF SODIUM OXYBATE RESULTS IN SIGNIFICANT REDUCTION IN THE NOCTURNAL SLEEP DISRUPTION OF PATIENTS WITH NARCOLEPSY</b> |                                               |        |
|                                                                                                                                                    |                                               | - 73 - |
| ABSTRACT                                                                                                                                           |                                               | - 74 - |
| INTRODUCTION                                                                                                                                       |                                               | - 75 - |

|                                                                                                                                                                             |                |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| METHODS                                                                                                                                                                     | - 75 -         |
| RESULTS                                                                                                                                                                     | - 79 -         |
| DISCUSSION                                                                                                                                                                  | - 84 -         |
| CONCLUSION                                                                                                                                                                  | - 85 -         |
| <b><u>CHAPTER 5</u></b>                                                                                                                                                     | <b>- 91 -</b>  |
| <b>ILLICIT <i>GAMMA</i>-HYDROXYBUTYRATE (GHB) AND PHARMACEUTICAL SODIUM OXYBATE (SXB; XYREM®): DIFFERENCES IN CHARACTERISTICS AND MISUSE.</b>                               | <b>- 91 -</b>  |
| ABSTRACT                                                                                                                                                                    | - 92 -         |
| INTRODUCTION                                                                                                                                                                | - 93 -         |
| 1. CHARACTERISTICS OF ILLICIT GHB COMPARED TO THOSE OF SXB                                                                                                                  | - 94 -         |
| 2. SUBSTANCE ABUSE, SUBSTANCE DEPENDENCE, AND MISUSE WITH ILLICIT GHB COMPARED TO SXB                                                                                       | - 100 -        |
| 3. DISCUSSION AND CONCLUSIONS                                                                                                                                               | - 108 -        |
| <b><u>CHAPTER 6</u></b>                                                                                                                                                     | <b>- 120 -</b> |
| <b>THE NIGHTLY USE OF SODIUM OXYBATE IS ASSOCIATED WITH A REDUCTION IN NOCTURNAL SLEEP DISRUPTION: A DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY IN PATIENTS WITH NARCOLEPSY</b> | <b>- 120 -</b> |
| INTRODUCTION                                                                                                                                                                | - 122 -        |
| RESULTS                                                                                                                                                                     | - 127 -        |
| DISCUSSION                                                                                                                                                                  | - 130 -        |
| CONCLUSION                                                                                                                                                                  | - 133 -        |
| <b><u>CHAPTER 7</u></b>                                                                                                                                                     | <b>- 137 -</b> |
| <b>PLASMA TOTAL GHRELIN AND LEPTIN LEVELS IN HUMAN NARCOLEPSY AND MATCHED HEALTHY CONTROLS: BASAL CONCENTRATIONS AND RESPONSE TO SODIUM OXYBATE</b>                         | <b>- 137 -</b> |
| MATERIALS AND METHODS                                                                                                                                                       | - 140 -        |
| RESULTS                                                                                                                                                                     | - 142 -        |
| DISCUSSION                                                                                                                                                                  | - 148 -        |
| REFERENCES                                                                                                                                                                  | - 150 -        |
| <b><u>CHAPTER 8</u></b>                                                                                                                                                     | <b>- 155 -</b> |
| <b>INTRODUCTION TO SLEEP, EATING, AND METABOLISM</b>                                                                                                                        | <b>- 155 -</b> |
| 1. SLEEP AND WEIGHT                                                                                                                                                         | - 157 -        |
| 1.1 OBESITY RATES HAVE RISEN DRASTICALLY                                                                                                                                    | - 157 -        |

|           |                                                                |                |
|-----------|----------------------------------------------------------------|----------------|
| 1.2       | OBSESITY IS A MAJOR PUBLIC HEALTH CONCERN                      | - 157 -        |
| 1.3       | OBSESITY IS MULTIFACTORIAL AND RELATED TO SLEEP                | - 157 -        |
| 1.4       | SLEEP TIMES ARE REDUCED                                        | - 158 -        |
| <b>2.</b> | <b>SLEEP TIMES IMPACT BODY WEIGHT</b>                          | <b>- 158 -</b> |
| 2.1       | REDUCED SLEEP, WEIGHT, AND ENERGY REGULATIONS                  | - 158 -        |
| 2.1.1     | <i>Epidemiological Evidence</i>                                | - 158 -        |
| 2.1.2     | <i>Actigraphy and PSG Evidence</i>                             | - 159 -        |
| 2.2       | SLEEP ELONGATION, WEIGHT, AND ENERGY REGULATION                | - 160 -        |
| <b>3.</b> | <b>POTENTIAL CAUSES OF WEIGHT GAIN WITH SLEEP DISTURBANCE</b>  | <b>- 161 -</b> |
| 3.1       | METABOLIC, ENDOCRINE, IMMUNE, AND AUTONOMIC RELATIONSHIPS      | - 161 -        |
| 3.1.1     | <i>Glucose Metabolism</i>                                      | - 161 -        |
| 3.1.2     | <i>Fatty Acid Metabolism</i>                                   | - 163 -        |
| 3.1.3     | <i>Hormones</i>                                                | - 163 -        |
| 3.1.4     | <i>Endocannabinoids</i>                                        | - 165 -        |
| 3.1.5     | <i>Immune system</i>                                           | - 166 -        |
| 3.1.6     | <i>Energy Expenditure and Temperature</i>                      | - 166 -        |
| 3.2       | CHRONOBIOLOGY                                                  | - 167 -        |
| 3.2.1     | <i>Circadian Introduction</i>                                  | - 167 -        |
| 3.2.2     | <i>Circadian Metabolism</i>                                    | - 167 -        |
| 3.3       | ALTERED ENERGY INTAKE AND EXPENDITURE                          | - 168 -        |
| 3.3.1     | <i>Energy Intake</i>                                           | - 168 -        |
| 3.3.2     | <i>Energy Expenditure</i>                                      | - 169 -        |
| 3.3.3     | <i>Altered Energy Regulation Under Calorie Restriction</i>     | - 170 -        |
| <b>4.</b> | <b>SLEEP, BRAIN PROCESSING, AND ENERGY REGULATION</b>          | <b>- 170 -</b> |
| 4.1       | AROUSAL, ATTENTION, COGNITION, AFFECTIVE PROCESSING, AND SLEEP | - 170 -        |
| 4.1.1     | <i>Vigilance Regulation</i>                                    | - 170 -        |
| 4.1.2     | <i>Compensatory Sleep</i>                                      | - 171 -        |
| 4.1.3     | <i>Executive Functioning</i>                                   | - 172 -        |
| 4.2       | SLEEP DISTURBANCE, BRAIN PROCESSING, AND ENERGY REGULATION     | - 175 -        |
| 4.2.1     | <i>Altered Inhibitory Control and Energy Regulation</i>        | - 175 -        |
| 4.2.2     | <i>Altered Memory and Energy Regulation</i>                    | - 176 -        |
| 4.2.3     | <i>Altered Mood and Energy Regulation</i>                      | - 176 -        |
| 4.2.4     | <i>Altered Reward Processing and Energy Regulation</i>         | - 176 -        |

|                                                                                                                                                 |                |
|-------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b><u>CHAPTER 9</u></b>                                                                                                                         | <b>- 178 -</b> |
| <b>EATING DECISIONS BASED ON ALERTNESS LEVELS AFTER A SINGLE NIGHT OF SLEEP MANIPULATION: A RANDOMIZED CLINICAL TRIAL</b>                       | <b>- 178 -</b> |
| ABSTRACT                                                                                                                                        | - 179 -        |
| INTRODUCTION                                                                                                                                    | - 180 -        |
| RESULTS                                                                                                                                         | - 186 -        |
| DISCUSSION                                                                                                                                      | - 189 -        |
| <b><u>CHAPTER 10</u></b>                                                                                                                        | <b>- 197 -</b> |
| <b>BACK TO THE FUTURE. METABOLIC EFFECTS OF A 4-DAY OUTDOOR TRIP UNDER SIMULATED PALEOLITHIC CONDITIONS – NEW INSIGHTS FROM THE EIFEL STUDY</b> | <b>- 197 -</b> |
| BACKGROUND                                                                                                                                      | - 199 -        |
| METHODS                                                                                                                                         | - 199 -        |
| RESULTS                                                                                                                                         | - 203 -        |
| DISCUSSION                                                                                                                                      | - 211 -        |
| CONCLUSION                                                                                                                                      | - 215 -        |
| <b><u>CHAPTER 11</u></b>                                                                                                                        | <b>- 222 -</b> |
| <b>SUMMARY, CONCLUSIONS &amp; FUTURE PERSPECTIVES</b>                                                                                           | <b>- 222 -</b> |
| SUMMARY AND CONCLUSIONS                                                                                                                         | - 223 -        |
| FUTURE PERSPECTIVES                                                                                                                             | - 233 -        |
| <b>REFERENCES</b>                                                                                                                               | <b>- 236 -</b> |
| <b>APPENDICES</b>                                                                                                                               | <b>- 274 -</b> |
| ACKNOWLEDGEMENTS                                                                                                                                | - 274 -        |
| CURRICULUM VITAE                                                                                                                                | - 276 -        |

# **CHAPTER 1**

## **Thesis Introduction and Aims**



## 1.1 Thesis Introduction

This thesis is divided into two discrete parts. The first part focuses on the impact of the drug sodium oxybate in various populations, especially in humans with narcolepsy, both with and without cataplexy. The second part of the thesis focuses on better understanding the relationship of sleep, eating, and metabolism in healthy humans.

## 1.2 Abstract for Part I - Neurobiological and Clinical Effects of Sodium Oxybate

### *Neurobiology of Gamma-hydroxybutyrate and Sodium Oxybate*

Gamma-hydroxybutyrate (GHB) is an endogenous short chain fatty acid and a, mostly oral, pharmacological compound that has been utilized in a variety of ways. Endogenously, GHB is synthesized locally within the CNS, mostly from its parent compound gamma-Aminobutyric acid (GABA). Evidence suggests a role for GHB as a neuromodulator/neurotransmitter. Under endogenous conditions and concentrations, and depending on the cell group affected, GHB may increase or decrease neuronal activity by inhibiting the release of neurotransmitters that are co-localized with GHB. Sodium oxybate (SXB) is the sodium salt of GHB and is used for the exogenous oral administration of GHB. Supraphysiological concentrations of GHB from exogenous administration are likely to produce qualitatively different neuronal actions than those produced by endogenous GHB concentrations. After exogenous administration, most of the observed behavioral effects appear to be mediated via the activity of GHB at GABA<sub>B</sub> receptors, as long as the concentration is sufficient to elicit binding, which does not happen at endogenous concentrations. Endogenous and exogenous GHB is rapidly and completely converted into CO<sub>2</sub> and H<sub>2</sub>O through the tricarboxylic acid cycle (Krebs cycle).

### *Clinical Effects of Sodium Oxybate*

Sodium oxybate has been observed to modulate sleep in nonclinical study participants, and sleep and wakefulness in clinical populations, including groups with insomnia, fibromyalgia, narcolepsy. Furthermore, multiple measures of daytime sleepiness and cataplexy demonstrated consistent short- and long-term improvement in response to nighttime SXB therapy. The most common reported adverse events include dose-related headache, nausea, dizziness and somnolence. In this thesis, with my collaborators, I characterized the sleep effects of SXB in individuals with Parkinson's disease (PD) and further characterized the sleep effects of SXB in individuals with narcolepsy.

In PD, many patients experience excessive daytime sleepiness and numerous nocturnal sleep abnormalities. To determine the safety and efficacy of SXB in this population, we enrolled 38 individuals in a multicenter, open-label, PSG study. The administration of nightly SXB correlated with significant improvements in

subjective sleepiness, subjective sleep quality, reduced fatigue severity, and a mean increase in time spent in slow wave sleep (SWS).

In narcolepsy, previous research indicates that nightly SXB administration reduces nocturnal sleep disruption. In this thesis, I present the findings from two clinical trials that provided an opportunity to further characterize these sleep-related effects in this population. The first study enrolled 278 individuals and evaluated treatment with SXB as monotherapy or in combination with modafinil and performed polysomnography (PSG) and the Maintenance of Wakefulness Test (MWT) at baseline and after 4 and 8 weeks. The second study enrolled 228 individuals across the United States, Canada, and Europe and randomized them to receive 6 and 9 g/night and measured nocturnal sleep after 8 weeks of treatment. In both studies, the individuals on SXB demonstrated several significant dose-related changes to their sleep compared their baseline measures. These include significant increases in time spent in total sleep time, SWS, and delta power. At the 6 and 9g/night doses, we observed a significant median decrease in nocturnal awakenings, and a significantly decreased in stage 1 sleep and the frequency of nocturnal awakenings in first and second study, respectively. In addition to its previously established efficacy for the treatment of cataplexy and EDS in narcolepsy, we further demonstrated that nightly SXB administration significantly impacts measures of SWS, wake after sleep onset, awakenings, total sleep time, and stage 1 sleep in a dose-related manner.

#### *Differences in Abuse Liability between 'Illicit GHB' and Sodium Oxybate*

There are distinct differences in the accessibility, purity, dosing, and misuse associated with illicit GHB compared to pharmaceutical SXB. Gamma-hydroxybutyrate sodium and SXB are the chemical and drug names, respectively, for the pharmaceutical product Xyrem® (sodium oxybate) oral solution. However, the acronym 'GHB' is also used to refer to illicit formulations that are used for non-medical purposes. In a review paper, we highlight important differences between illicit GHB and sodium oxybate with regard to their relative abuse liability, which includes the likelihood and consequences of abuse. Data are summarized from the scientific literature; from national surveillance systems in the U.S., Europe, and Australia (for illicit GHB); and from clinical trials and post-marketing surveillance with sodium oxybate (Xyrem). In the U.S., the prevalence of illicit GHB use, abuse, intoxication, and overdose has declined from 2000, the year that GHB was scheduled, to the present and is lower than that of most other licit and illicit drugs. Abuse and misuse of the pharmaceutical product, SXB, has been rare over the 5 years since its introduction to the market, which is likely due in part to the risk management program associated with this product. Differences in the accessibility, purity, dosing, and misuse of illicit GHB and sodium oxybate suggest that risks associated with illicit GHB are greater than those associated with the pharmaceutical product SXB.

### *Metabolic Effects of Sodium Oxybate*

The effects of SXB on various endocrinological parameters has been explored, including its impact on melatonin, ghrelin, growth hormone, insulin, cortisol and glucagon, endocannabinoids, and more. Because ghrelin and leptin interact with hypocretin neurons to influence energy homeostasis, we evaluated whether human hypocretin deficiency, or the narcolepsy therapeutic SXB, alter the levels of these hormones. To do this, we assessed 8 male, medication free, hypocretin deficient, narcolepsy with cataplexy patients, and 8 healthy controls matched for age, sex, body mass index (BMI), waist-to-hip ratio, and body fat percentage. Blood samples of total ghrelin and leptin were collected over 24 hours at 60 and 20-min intervals, respectively, during two study occasions: baseline, and during the last night of 5 consecutive nights of SXB administration (2 x 3.0g/night). At baseline, mean 24-h total ghrelin and leptin levels were not different between hypocretin deficient narcolepsy patients and controls. Furthermore, sodium oxybate did not significantly affect the plasma concentration of either one of these hormones. Therefore, the increased BMI of narcolepsy patients is unlikely to be mediated by hypocretin deficiency-mediated alterations in total ghrelin or leptin levels. Thus, the effects of these hormones on hypocretin neurons may be mainly unidirectional. Although SXB may influence body weight, the underlying mechanism is unlikely to involve changes in total ghrelin or leptin secretion.

### **1.3 Abstract for Part II - Sleep, Eating, and Energy Regulation**

#### *Sleep and Eating*

Substantial epidemiological evidence shows a significant association between reduced sleep and increased body weight, and a multitude of energy-regulation mechanisms have been explored to better understand this association. Cross-sectional and prospective data show that short-duration sleepers have modified eating behaviors, including altered within-day eating timing, increased snacking behavior, and increased calories from beverages. Controlled, prospective research demonstrates an increase in caloric intake on the day following one to several nights of partial sleep restriction in normal weight adults. While it is common for self-reported hunger to increase after sleep deprivation, some studies show no difference in hunger between the sleep-rested and sleep-deprived conditions, despite difference in food selection behavior. The increase in caloric intake may in part be explained by reduced satiety.

Some increase in caloric intake after sleep loss would be expected to accommodate for increased energy expenditure from additional wake time. Indeed, several studies show that homeostatic factors contribute to increased caloric intake after sleep loss; however, this increased caloric intake seems to exceed the level expected to accommodate for energy expenditure associated with the additional time. It has been argued that altered hedonic-valuation factors increase portion size and alter food selection after sleep loss.

A well-described and consistent response to sleep loss is impaired alertness, which can be observed in multiple ways, including decreases in both subjective and objective alertness measurements. Moreover, it has been hypothesized that sleep deprivation-induced cognitive impairments, such as reduced alertness and attention, result from instability in the wake state and that such an increase in moment-to-moment variability of attention impairs a wide variety of cognitive tasks, including goal-directed activities. Eating is one such goal-directed activity with food decisions being made 200 times or more each day. Eating is influenced by a wide variety of factors. Internal factors include metabolic state, health beliefs and objectives, emotional state, and the behavioral and metabolic consequences of dietary habits. External influences include food presentation and environmental conditions. External factors can shift awareness away from internal drivers of food intake, potentially causing diminished accordance with health goals and internal signals of satiety. Thus, the act of self-monitoring food intake volume and food type, as well as satiety requires awareness, which is influenced by alertness, which is influenced by sleep.

We hypothesized that lowered alertness would lead to less surveillance in both total caloric intake and decisions about the types of food one eats. In the current study, we examined whether experimentally induced changes in subjective or objective alertness were associated with changes in total calorie consumption, and calorie consumption based on several categorizations, including the healthfulness, the tempting nature, and the caloric density of the offered foods.

#### *Metabolic Effects of a 4-Day Outdoor Trip Under Simulated Paleolithic Conditions*

The observation that the emergence of common Western diseases (WD) – from obesity to coronary heart disease to cancers – takes place with much greater prevalence as societies migrate from natural-living cultures to those that increasingly assume the characteristics of wealthier, modernized societies, has been well documented. This is highlighted clearly by, for example, the drastically increased prevalence of obesity and diabetes in recently urbanized vs rural-living indigenous peoples. For instance, in Nauru, since the 1920s, royalties for the natural resource phosphate has allowed these people to become one of the world's richest *per capita*. This wealth, however, has also afforded a rapid change in lifestyle. In this population, the first case of type 2 diabetes was noted only in 1925. Now, however, the Nauruans are the world's most obese people (92.8%), have the highest blood pressure in the Western Pacific region, and two-thirds of their population over age 55 suffer from type 2 diabetes. For approximately 84,000 generations, humans lived under hunter and gatherer conditions. But recently, humans have endured dramatic change from their native lifestyle with the occurrence of the agricultural, industrial, and digital revolutions. Despite the massive technological innovation that has taken place during these revolutions, they have all occurred within a relatively recent time frame. These innovations have enabled humans to live in a manner that is discordant with expectancies of our genes, which were largely established during our pre-agricultural past.

The metabolic dysregulation that appears to accompany the rural-modern lifestyle transition is supported by an abundance of evidence indicting elements of the modern lifestyle as causative in WD. These include, but are not limited to: overnutrition, low dietary fiber intake, sugar-rich diet, physical inactivity, vitamin D deficiency, psychosocial stress, sleep deprivation and circadian rhythms disturbances, and more. Therefore, the shift from a natural to a modern lifestyle likely promotes a gene-environment mismatch, which causes metabolic dysregulation, which causes disease. In contrast to single-intervention studies, our study aimed to have participants emulate a modern-day, Paleolithic-like lifestyle pattern during a short nature trip – which included multiple alterations from the default lifestyle pattern of modern living – to assess signs of favorable metabolic changes. We hypothesize that adopting a more Paleolithic-like lifestyle pattern will yield favorable and observable effects on metabolism, even in the short term.

## **1.4 Aims**

### **Part I - Neurobiological and Clinical Effects of Sodium Oxybate**

GHB is an active metabolite of the inhibitory neurotransmitter GABA. SXB is the sodium salt of GHB and is used for the exogenous oral administration of GHB. GHB has a suggested role as a neuromodulator/neurotransmitter. After exogenous administration, most of the observed behavioral effects of SXB appear to be mediated via the activity of GHB at GABA<sub>B</sub> receptors. GHB has been shown to have modulatory activity on multiple neurotransmitter systems, as well as hormonal and metabolic effects.

In Chapter 2, I review the biology and pharmacology of GHB and SXB, as well as their modulatory effects on neurotransmitters, hormones and metabolism. I then review the evidence supporting a modulatory effect of GHB and SXB on sleep and wakefulness, both in healthy and in clinical populations. I examine the data showing that GHB can dose-dependently decrease sleep onset latency, promote delta activity and enhance sleep maintenance in healthy populations. Next, I provide an overview of the effects of GHB in enhancing mood and prosocial behavior in healthy populations and discuss its putative antidepressant effects.

In the following section of Chapter 2, I review the effects of GHB and SXB in clinical populations. I present data indicating that GHB may improve sleep in patients with insomnia. I also review the evidence indicating a beneficial effect of GHB/SXB in decreasing sleep disruption in patients with fibromyalgia and, importantly, in also decreasing pain, fatigue and overall multidimensional function. Next, I review the evidence of the effects of GHB/SXB in modulating sleep in patients with neurodegenerative diseases, specifically Parkinson's and Alzheimer's disease. I also discuss the association between sleep impairment and Alzheimer's disease pathogenesis and explore the potential therapeutic benefit of the neurobiological effects of GHB on those pathogenic processes. Finally, I analyze the effects of GHB/SXB in narcolepsy and discuss how SXB has consistently shown short- and

long-term improvement on various properties of sleep, including increases in SWS duration and delta power, and a reduced number of night-time awakenings.

In Chapter 3, I examine the safety and efficacy of SXB in subjects with Parkinson's disease (PD) and sleep disorders. In a multicenter, open-label, polysomnographic study<sup>1</sup>, we hypothesized that using SXB as a specific treatment for nocturnal sleep abnormalities could also decrease the excessive daytime sleepiness often observed in the PD population. In the 27 subjects with PD who completed the study, receiving a mean dose of 7.8 g SXB per night for 6 weeks, it was observed that SXB was well tolerated, increased SWS, improved subjective nighttime and daytime sleep problems, and decreased daytime fatigue. These results suggested that nocturnal administration of SXB can have beneficial effects on excessive daytime sleepiness and fatigue in PD and that the potential therapeutic effects of SXB for PD-associated sleep dysfunctions are worth pursuing in controlled trials using objective measures of daytime sleepiness.

In Chapter 4, I build upon previous studies indicating that nightly SXB administration reduces nocturnal sleep disruption in narcolepsy. Aiming at further characterizing the sleep-related effects of SXB in the context of narcolepsy, we conducted a double-blind, placebo-controlled study in patients with narcolepsy treated with SXB as monotherapy or in combination with modafinil<sup>2</sup>. The intent-to-treat population consisted of 222 patients randomized to receive treatment with placebo (n=55), SXB (n=50), modafinil (n=63), or SXB + modafinil (n=54). Treatment efficacy was assessed using overnight polysomnography (PSG), Maintenance of Wakefulness Test, Epworth Sleepiness Scale scores and daily diary recordings. After 8 weeks, significant changes in sleep architecture were observed among patients receiving SXB as monotherapy or in combination with modafinil, whereas no significant changes in PSG parameters were found after treatment with placebo or modafinil alone. Changes induced by SXB or SXB + modafinil included increased stage 3 and 4 sleep and delta power, and decreased nocturnal awakenings, showing that SXB can significantly reduce measures of sleep disruption and significantly increase SWS in patients with narcolepsy.

In Chapter 5, I review the differences in the accessibility, purity, dosing, and misuse between illicit GHB and pharmaceutical SXB (Xyrem®)<sup>3</sup>. Although gamma-hydroxybutyrate sodium is the chemical name for sodium oxybate, the acronym GHB also refers to the illicit formulations used for non-medical purposes. In this review, I emphasize critical differences between illicit GHB and pharmaceutical SXB, namely in what concerns their relative abuse liability and its consequences. Based on data from the scientific literature, from national surveillance systems in the United States, Europe, and Australia for illicit GHB, and from clinical trials and post-marketing surveillance for pharmaceutical SXB, I discuss how the prevalence of illicit GHB use, abuse, intoxication, and overdose has declined in the United States since it became an illegal drug in the year 2000, how and why the abuse and misuse of pharmaceutical SXB has been rare since its introduction to the market, and how and why the risks associated with illicit GHB are greater than those associated with pharmaceutical SXB.

In Chapter 6, I further analyze the effects of nightly SXB administration on nocturnal sleep in narcolepsy patients. I describe the first large randomized, double-blind, placebo-controlled, parallel group trial examining the impact of SXB on sleep architecture and narcolepsy symptoms, conducted with 228 adult patients with narcolepsy/cataplexy in the United States, Canada, and Europe<sup>4</sup>. In patients receiving either 4.5, 6, or 9 g SXB or placebo nightly for 8 weeks, changes in sleep architecture were measured using PSG and changes in narcolepsy symptoms and adverse events were recorded in daily diaries. After 8 weeks of nightly SXB administration, a significant dose-dependent impact on the measures of SWS was observed, including increases in the duration of stage 3 and 4 sleep, and increased delta power in all dose groups; the doses of 6 and 9 g/night also significantly decreased stage 1 sleep and the frequency of nocturnal awakenings. The effects of SXB on nocturnal sleep concurred with significant decreases in the severity and frequency of narcolepsy symptoms. The results I describe in this chapter indicate that SXB may improve the sleep fragmentation that is commonly associated with narcolepsy.

In Chapter 7, I examine the link between narcolepsy, hypocretin neurons, the hormones ghrelin and leptin, and SXB. Given that narcolepsy is caused by a selective loss of hypocretin neurons and is associated with obesity, and that ghrelin and leptin interact with hypocretin neurons to influence energy homeostasis, we aimed at evaluating whether human hypocretin deficiency or SXB can alter the levels of these hormones<sup>5</sup>. The rationale for this study was that ghrelin and leptin hormonal disturbances induced by hypocretin deficiency could provide an explanation for the weight and ingestive behavior phenotype of the narcoleptic population, and for the influence of SXB on body weight. To do so, we studied the blood levels of ghrelin and leptin in hypocretin deficient narcoleptic patients at baseline and after five consecutive nights of SXB administration ( $2 \times 3.0$  g/night). We found no differences in ghrelin or leptin levels nor any effects of SXB on the plasma levels of either hormone. These results therefore indicated that the increased body-mass index of narcolepsy patients is unlikely to be driven by hypocretin deficiency-mediated changes in total ghrelin or leptin levels, and that the influence of SXB on body weight is unlikely to involve changes in the secretion of the hormones. I discuss how these findings contribute to a better understanding of the hormonal phenotype of the narcoleptic population.

## **Part II: Sleep, Eating, and Metabolism**

Obesity is a major public health issue in today's world, and it appears likely that multiple factors of modern life contribute to its cause. Concurrently, various factors of modern life promote reduced and disturbed nighttime sleep. For example, in 2012, 70 million Americans reported sleeping less than six hours, which according to the National Sleep Foundation of the United States, is below recommended levels for human health and wellbeing.

There is strong epidemiologic evidence connecting reduced sleep duration and/or disturbed sleep with an increased risk for obesity. Subsequent to, and in parallel

with these associative observations, various investigations have revealed that altered sleep may disturb energy regulation and brain-processing circuits related to appetitive behavior, thus contributing to alterations in metabolism, body weight, and body composition.

In Chapter 8, I review the latest findings relevant to sleep and energy-regulating processes. First, I examine how obesity rates have risen in both adults and children worldwide, and how the prevalence of obesity may be related to both sleep duration and sleep quality. Next, I discuss epidemiological studies that seek to answer whether sleep times really have decreased over the last 60 years. In addition, I discuss how epidemiological evidence of sleep length and quality relates to weight and energy regulation, and how sleep disturbance affects metabolic, endocrine, immune, and circadian processes. Finally, I examine how brain-processing circuits related to alertness, memory, inhibition control, mood, and reward behavior are altered by sleep loss. This literature review provides the background for the human studies I contributed to in this field, which are presented in detail in chapter 9 and chapter 10.

In chapter 9, I explain how manipulation of a single night sleep influences participants' food preferences<sup>6</sup>. In that study, we hypothesized that lowered alertness by artificially restricting a person's natural sleep period would alter total calorie consumption or modify preference for the source of calories from a range of healthy and unhealthy foods. We show that ecologically-relevant impairments in both subjective and objective alertness is associated with altered eating behaviors and food choice. We also show that some of these effects differentiate between whether the alertness impairment was subjective (i.e., sleepiness) or objective (e.g., reaction time) in nature. Overall, impairments in alertness associated with increased caloric intake, preference for less healthy foods (as rated by the participants' subjective rating of the food), and consumption of more calorically-dense food options. These findings suggest that reduced alertness after modest sleep loss may alter a person's food choices towards less healthy, more calorically-dense foods.

In chapter 10, I examine how a short-term return to Paleolithic eating, living, and sleeping conditions via a several-day nature trip may have beneficial effects on human physiology<sup>7</sup>. The recent shift from natural to modern living environments may cause an unintended mismatch between genes shaped for a natural environmental niche and typical modern lifestyles. For example, blue light from hand-held electronic devices prolongs sleep onset and shifts circadian timing, reduced sleep promotes the consumption of calorically dense processed foods, and modern diet can disrupt sleep quality. Using a within-participant design, we examined whether a four-day and four-night intervention aimed to mimic Paleolithic-like living conditions could alter metabolic and physiological parameters in two groups of 14 volunteers. Participants lived outdoors without tents, hiked extensively during the day, and had reduced and delayed caloric intake (two meals per day after noon). Body weight, body mass index, body fat, visceral fat, and waist-hip ratio all significantly decreased. In addition, metabolic parameter

values, such as fasting glucose, basal insulin, and fatty liver index, decreased, which suggests that a brief nature trip may be an effective intervention to improve modern metabolic health.

# **PART I**

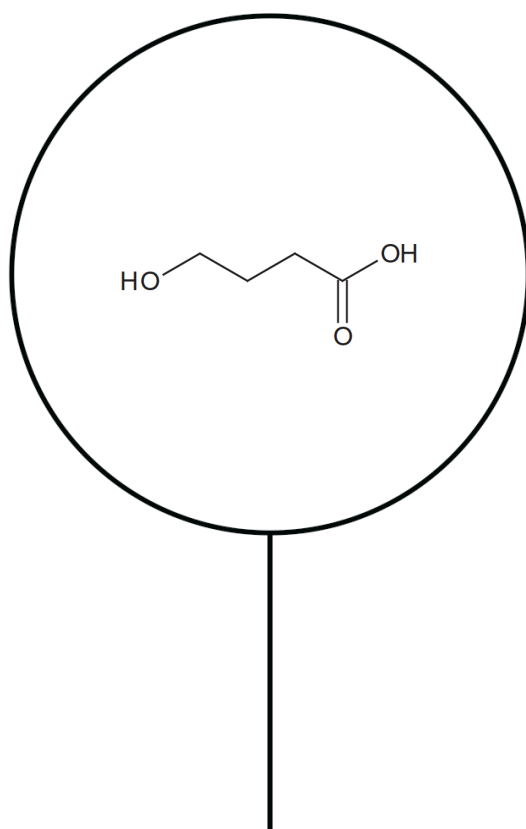
## **Neurobiological and Clinical Effects of *gamma*-Hydroxybutyrate / Sodium Oxybate**

## CHAPTER 2

### Introduction to *gamma*-Hydroxybutyrate / Sodium Oxybate: Neurobiology and Clinical Effects

*CNS Drugs (2006) 20(12): 993-1018. (Updated)*

**Authors:** Daniel Pardi and Jed Black.



## CHAPTER 2 CONTENTS

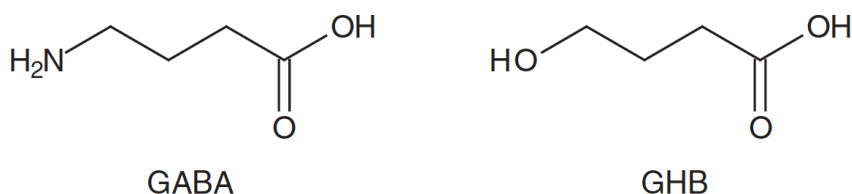
|       |                                             |        |
|-------|---------------------------------------------|--------|
| 1.    | Introduction To GHB / SXB                   | - 25 - |
| 1.1   | Background                                  | - 25 - |
| 1.2   | Abuse Liability                             | - 26 - |
| 2.    | Biology and Pharmacology of GHB / SXB       | - 28 - |
| 2.1   | Distribution                                | - 28 - |
| 2.2   | Synthesis                                   | - 29 - |
| 2.3   | Metabolism                                  | - 30 - |
| 2.4   | GHB as a Neurotransmitter                   | - 30 - |
| 2.5   | Ghb Binding Site(S) in the Brain            | - 31 - |
| 2.5.1 | Stimulation of GHB Binding Site(S)          | - 31 - |
| 2.5.2 | Stimulation Of GABAB Receptors by GHB       | - 32 - |
| 2.6   | Intracellular Response to GHB               | - 32 - |
| 2.7   | Effects on Neurotransmitter Systems         | - 33 - |
| 2.7.1 | Dopaminergic System                         | - 33 - |
| 2.7.2 | Serotonergic System                         | - 34 - |
| 2.7.3 | Opioidergic System                          | - 34 - |
| 2.7.4 | Cholinergic System                          | - 35 - |
| 2.7.5 | Noradrenergic System                        | - 35 - |
| 2.7.6 | Glutamatergic System                        | - 36 - |
| 3.    | Hormonal and Metabolic Effects of GHB / SXB | - 36 - |
| 3.1   | Hormonal Effects of GHB / SXB               | - 36 - |
| 3.1.1 | Growth Hormone                              | - 36 - |
| 3.1.2 | Neurosteroids                               | - 38 - |
| 3.1.3 | Prolactin                                   | - 38 - |
| 3.1.4 | Melatonin                                   | - 39 - |
| 3.1.5 | Ghrelin                                     | - 39 - |
| 3.2   | Metabolic Effects of GHB / SXB              | - 40 - |
| 3.2.1 | Insulin Sensitivity                         | - 40 - |
| 3.2.2 | Thermoregulation                            | - 41 - |
| 3.2.3 | Weight and Energy Balance                   | - 42 - |
| 4.    | Clinical Effects of GHB / SXB               | - 46 - |
| 4.1   | Healthy Subjects                            | - 46 - |
| 4.1.1 | Sleep                                       | - 46 - |
| 4.1.2 | Mood, Prosocial, and Prosexual Effects      | - 47 - |
| 4.2   | Clinical Populations                        | - 48 - |
| 4.2.1 | Insomnia                                    | - 48 - |
| 4.2.2 | Fibromyalgia                                | - 48 - |
| 4.2.3 | Parkinson's Disease                         | - 50 - |
| 4.2.4 | Alzheimer's Disease                         | - 51 - |
| 4.2.5 | Narcolepsy                                  | - 53 - |
| 5.    | Conclusions                                 | - 60 - |

|     |                  |        |
|-----|------------------|--------|
| 5.1 | Neurobiology     | - 60 - |
| 5.2 | Clinical Effects | - 61 - |

## 1. Introduction to GHB / SXB

### 1.1 Background

Gamma-Hydroxybutyrate (GHB) is an endogenous short-chain fatty acid that is synthesized within the central nervous system (CNS)<sup>8</sup>. It is an active metabolite of the inhibitory neurotransmitter gamma-Aminobutyric acid (GABA)<sup>9–11</sup> (Figure 1) and, to a much lesser extent, of 1,4-butanediol (1,4-BD)<sup>12</sup> and gamma-butyrolactone (GBL)<sup>8</sup>. The latter two substances are also commercially produced synthetically for a wide range of industrial purposes. Sodium oxybate (SXB) is the sodium salt of GHB and is the form of GHB that is used therapeutically in humans. In this review, the term 'illicit GHB' is used to refer to illegally manufactured GHB and/or congener substances, such as industrial 1,4-BD and GBL, substances manufactured as unfit for human consumption.



**FIGURE 1.** Chemical structure of gamma-hydroxybutyrate (GHB) and gamma-aminobutyric acid (GABA).

GHB was first synthesized in the early 1960s (by Henry Laborit) and was found to readily cross the blood-brain barrier into the CNS<sup>13</sup>, where it imparts distinct pharmacological effects. Soon thereafter, it was discovered that GHB is an endogenous substance<sup>8</sup>. Further evidence suggested a role for GHB as a neuromodulator/neurotransmitter, as GHB is synthesized in neurons heterogeneously distributed throughout the CNS, stored in vesicles, released via potassium-dependent depolarization into the synaptic cleft, and undergoes reuptake into the nerve terminal (see section 2). Additionally, specific GHB binding sites have been identified in the CNS (see section 2). The GHB binding sites appear to share functional similarity with GABA<sub>B</sub> receptors, with both being G protein-coupled receptors.

Henry Laborit synthesized GHB in an attempt to create a GABA analogue that replicated the CNS function of GABA, but that was more slowly metabolized and that would readily cross the blood brain barrier<sup>13</sup>. Early work in humans demonstrated a correlation between increased blood concentrations of exogenously administered GHB and decreased levels of consciousness<sup>14</sup>. In 1962, the first human study of GHB was reported, in which GHB was used as a surgical anesthetic in 26 patients<sup>15</sup>; indeed, anesthesia became its first clinical use<sup>16</sup>. A remarkable feature of GHB appears to be its unique ability to induce CNS

depression with minimal effects on the respiratory and circulatory system, and without significant adverse effects<sup>17,18</sup>. Early research with GHB also examined its ability to induce a sleep-like state<sup>19,20</sup>. In 1964, Helrich *et al.*<sup>14</sup> induced sleep in 16 healthy study participants with intravenous administration of GHB at doses ranging from 5.9 to 9 g.

While initial evaluation of GHB in animals and humans explored its sleep-promoting or anesthesia-inducing capacity, most of the more recent human therapeutic research has focused on its use in narcolepsy. Nevertheless, other potential therapeutic effects of GHB have been explored, including: reduction of intracranial pressure<sup>21,22</sup>; tissue-sparing and neuroprotective effects in ischemia-induced challenges<sup>23,24</sup>; relief of pain, anxiety and tension during childbirth<sup>25</sup>; impact on anxiety conditions<sup>26</sup>; treatment of the alcohol withdrawal syndrome<sup>27–34</sup>; treatment of heroin (diamorphine) dependence<sup>35,36</sup>; efficacy in improving the pain, fatigue and sleep fragmentation of fibromyalgia syndrome<sup>37,38</sup>; antidepressant effects<sup>39</sup>; reduction of the negative effects of cocaine self-administration<sup>40,41</sup>; treatment for hyperkinetic movement disorders<sup>42–44</sup>; treatment of infection<sup>45</sup>; and hypnotic potential in healthy individuals<sup>46</sup> and those with insomnia<sup>47</sup>.

As mentioned above, there has been extensive recent investigation into the usefulness of GHB (in the form of SXB) in the treatment of excessive daytime sleepiness, nocturnal sleep fragmentation and cataplexy in patients with narcolepsy. This research has established SXB as an important first-line agent in the treatment of narcolepsy. SXB (Xyrem®) was approved in July 2002 by the United States' Food and Drug Administration (FDA) as a drug for the treatment of cataplexy in patients with narcolepsy<sup>48</sup>. Furthermore, in November 2005, the FDA approved a label extension to include SXB as a treatment for excessive daytime sleepiness in patients with narcolepsy. These data, together with other preliminary data on the impact of SXB on sleep and wakefulness, are addressed in detail in this review, as is the neurobiology of GHB. (For a further review describing the effects of SXB in the treatment of cataplexy in narcolepsy, readers are referred to the Xyrem® International Study Group<sup>49</sup>.)

## **1.2 Abuse Liability**

Illicit GHB is a term used to describe multiple substances, including substances with structural similarity to GHB, which have been reported to have abuse potential when consumed in a certain fashion and, since March 2000, have been considered Schedule I controlled substances by the United States' Drug Enforcement Agency (DEA)<sup>50</sup>. Some of these substances have been manufactured for a variety of valid industrial and research purposes; their method of use makes them legal or illicit. It should be noted that most of these substances are not produced under good manufacturing practices necessary for human consumption, as they are intended for manufacturing purposes; thus, these substances can contain toxic impurities that may contribute to some of the negative effects imputed to illicit GHB. These substances include GHB analogues such as GBL and 1,4 BD, gamma-

hydroxyvalerate (GHV) and gamma-valerolactone (GVL). Furthermore, the various GHB analogues can have distinct toxicity and pharmacokinetic profiles from pure GHB; for example, GBL crosses the blood-brain barrier more easily and, therefore, smaller oral doses can cause much higher CNS exposure<sup>12</sup>. The main category of abuse of these agents relates mostly to intoxicant usage; however, their usage in sexual assault (the 'date rape drug') has received notoriety.

Physical dependence has been described with the usage of these substances. Most frequently, dependence is reported after long periods of abuse and repetitive high doses throughout the day and night<sup>51</sup>; some abusers report escalating their daily GHB intake to 150 g/day<sup>52</sup>. High frequency administration, across the day and night, appears to be a common feature to those who present with withdrawal symptoms<sup>29</sup>. When serious withdrawal has been induced, symptoms may be severe and take up to 12 days to resolve, usually without sequelae, but can result in death<sup>29,51</sup>.

To examine the abuse liability of GHB in rhesus monkeys, Woolverton *et al.*<sup>53</sup> evaluated GHB in procedures predictive of abuse and dependence. After being trained in drug discrimination paradigms to discriminate D-amphetamine, pentobarbital or triazolam from saline, monkeys given GHB (0.01–10 mg/kg per intravenous injection) maintained self-administration marginally above saline levels. These researchers concluded that GHB has low potential for abuse. McMahon *et al.*<sup>54</sup> also used rhesus monkeys to evaluate the effects of 1,4 BD and GBL on various behavioral procedures that measure positive reinforcing effects and discriminative stimulus effects versus GABA<sub>A</sub> receptor modulators. These GHB analogues did not substitute for pentobarbital, midazolam or flumazenil, indicating qualitatively different effects from the abuse-related, positive reinforcing effects of GABA<sub>A</sub> receptor modulators.

Most recently, the work of Carter *et al.*<sup>55</sup> evaluated the relative abuse liability of GHB in 14 volunteers with a history of drug abuse. Psychomotor, subjective and cognitive effects of a broad range of GHB doses (2–18g/70kg) were compared to placebo and two abused sedative/hypnotic drugs, triazolam (0.5mg/70kg and 1mg/70kg) and pentobarbital (200mg/70kg and 400mg/70kg). This study was conducted at a residential research facility under double-blind, double-dummy conditions. Generally, on most measures of likelihood of abuse (e.g., ratings of liking the reinforcing effects), the effects of pentobarbital were significantly greater than those of triazolam, and the effects of GHB were between two reference compounds. Furthermore, GHB produced relatively less memory impairment and more negative effects such as nausea and sedation, occasionally leading to accidental overdose (defined as greater sedation than intended). In total, the authors noted that the negative effects limited the desirability of GHB for the participants.

To monitor the trends in abuse of illicit GHB (including analogues), Anderson *et al.*<sup>56</sup> identified cases from the California Poison Control System computerized database between 1999 and 2003. Illicit GHB exposures decreased precipitously by

76% from baseline (1999; n=426) to the final study year (2003; n=101). These data were then compared to the American Association of Poison Control Centers and Drug Abuse Warning Network (DAWN) data and drug use prevalence from the National Institute for Drug Abuse survey data. These organizations reported a parallel decreased trend to those of the California Poison Control System. These authors concluded that, nationally, a clear decrease in illicit GHB abuse case incidence is likely<sup>56</sup>. For comparative purposes, the absolute and relative abuse incidence of illicit GHB is further discussed. All proceeding comparisons in this paragraph are based on the DAWN quarter 3 (Q3) and quarter 4 (Q4) 2003 report. For Q3-Q4 2003, DAWN estimates 305,731 drug-related emergency department (ED) visits that involved a major substance of abuse. For this time period, drug-related ED visits were alcohol associated in 141,343 visits (46% of the total), cocaine associated in 125,921 visits (41% of the total) and marijuana associated in 79,663 visits (26% of the total), while illicit GHB (including analogues) accounted for 990 drug-related ED visits (0.3% of the total)<sup>57</sup>.

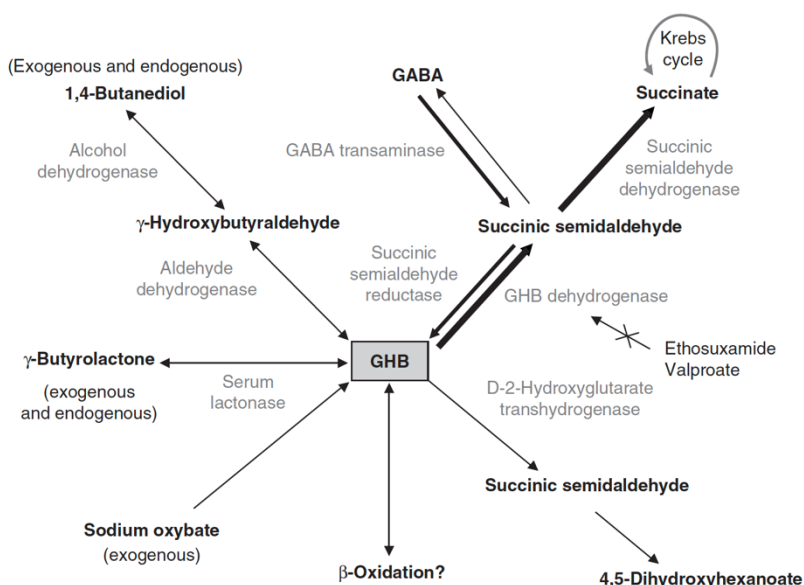
Like many CNS depressants, illicit GHB has been implicated in a number of sexual assault cases. Over 60 chemicals have been associated with sexual assault<sup>57</sup> and any drug that has CNS depressant properties can be used as such. In response to this social problem, the Violence Intervention Program, Women's Hospital, University of Southern California Medical Center, Los Angeles, CA, USA, performed a study to obtain information about the relationship of alcohol and drug usage in these victims<sup>58</sup>. In this study, a total of 2003 women who presented to rape treatment centers throughout the U.S. allowed for their urine specimens to be analyzed via gas chromatography combined with mass spectrometry. Nearly two-thirds of the samples contained alcohol and/or drugs; a substantial subset of the specimens contained more than one substance. The predominant substances found were alcohol and marijuana, present in 63% and 30% of samples, respectively. Other substances that appeared in the collected samples included cocaine, diazepam, amphetamines, methamphetamines and others. Illicit GHB was found less frequently than the above-mentioned drugs; <3% of the positive samples contained GHB. Additionally, over the 2-year study period, illicit GHB, as a drug of sexual assault, appeared to be declining in frequency of appearance in tested samples. The author of the study concluded that the attention currently being focused on 'date-rape drugs', such as illicit GHB, is misleading the public about the drugs most commonly related to sex crimes, namely alcohol and marijuana<sup>58</sup>.

## **2. Biology and Pharmacology of GHB / SXB**

### **2.1 Distribution**

In mammals, GHB is present in micromolar concentrations in all brain regions investigated, although the concentration differs by region<sup>59,60</sup>. The highest relative concentration is found in the striatum of humans and monkeys, reaching concentrations between ~11 and 25  $\mu\text{mol/L}$ <sup>11</sup>. Localization of GHB within cytosolic and synaptosomal fractions<sup>61</sup> suggests a mechanism for presynaptic accumulation. The concentration of GHB is higher in the developing brain than in the adult brain<sup>62</sup>.

GHB is also found in micromolar concentrations in peripheral tissues such as heart, kidney, liver and muscle, with the highest concentration found in brown adipose tissue (37  $\mu\text{mol/L}$ )<sup>63</sup>.



**FIGURE 2.** Synthesis and metabolism of gamma-hydroxybutyrate (GHB). The major pathways are shown by the thick arrows. GABA = gamma-aminobutyric acid.

## 2.2 Synthesis

Radiolabel studies have shown that GABA is the major precursor of GHB in the brain<sup>9,64</sup>. GHB formation occurs in GABAergic neurons<sup>65</sup> as approximately 0.05% (*in vitro*)<sup>66</sup> to 0.16% (*in vivo*)<sup>64</sup> of GABA is metabolized into GHB under normal conditions. Thus, the concentration of GHB is roughly 0.1% of the concentration of GABA<sup>67</sup> in the central nervous system.

The synthesis of GHB from GABA occurs mainly via the enzyme GABA transaminase, which converts GABA to succinic semialdehyde (SSA)<sup>12,68</sup> (see Figure 2). SSA is reduced to form GHB via the enzyme SSA reductase (SSR), an aldehyde reductase enzyme with high affinity and specificity for SSA. This enzyme is mostly present in the cytosol and, to a lesser extent, in the synaptosomal fraction<sup>69,70</sup>; a second catabolic pathway through a mitochondrial SSR has also been described<sup>71</sup>, although evidence for the existence of this pathway is limited<sup>72</sup>.

Another postulated precursor of endogenous GHB is 1,4-BD<sup>73</sup>. Snead *et al.*<sup>12</sup> showed that, in the brain, 1,4-BD is metabolized to gamma-hydroxybutyraldehyde by the enzyme alcohol dehydrogenase. Subsequently, gamma-

hydroxybutyraldehyde is metabolized via aldehyde dehydrogenase to produce GHB. It has also been observed that GBL is metabolized to GHB via serum lactonases<sup>74</sup>. Minor pathways for the production of GHB, including conversion from 1,4-BD and GBL, are shown in Figure 2.

### 2.3 Metabolism

The major route of GHB metabolism is via conversion to SSA and succinate, as shown in Figure 2. Radio-isotope studies show that intraventricularly administered GHB is metabolized rapidly into SSA<sup>75,76</sup>. Studies using [<sup>3</sup>H]-GABA showed total brain turnover time of 26 minutes for GHB<sup>64</sup>. GHB is catabolized by the SSR enzyme (aldehyde reductase 1) in the presence of nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>)<sup>77-79</sup>; this enzyme is now known as NADP-dependent GHB dehydrogenase (GHB-DH)<sup>80</sup>. SSA and the co-substrate NADPH<sup>65</sup> are the products formed by GHB-DH from GHB and NADP<sup>+</sup><sup>79</sup>. This process is also coupled to the reduction of D-glucuronate, which releases NADPH<sup>81,82</sup> and activates the pentose phosphate pathway (potentially more prevalent during metabolism of exogenously administration GHB than seen with endogenous levels)<sup>83</sup>. After GHB is converted into SSA, SSA is converted to succinate, which is eventually metabolized to CO<sub>2</sub> and H<sub>2</sub>O via the tricarboxylic acid cycle (Krebs cycle).

The enzyme GHB-DH is inhibited by various antiepileptic drugs (valproate, ethosuximide, barbiturates) and certain short-chain fatty acids<sup>84</sup>. These compounds induce increased brain GHB concentrations by inhibiting GHB metabolism<sup>66,84</sup>.

SSA dehydrogenase (SSADH) deficiency is a rare autosomal recessive disorder, linked to the ALDH5A1 gene on the short arm of chromosome 6. People with this condition have elevated CNS concentrations of GABA and GHB due to an inability to break down these substances. This condition has a serious impact and can result in delayed development, ataxia, hypotonia, hyporelexia, seizures, mental retardation, hyperkinesia, psychosis, myopathy, ocular abnormalities and other neurological manifestations<sup>85</sup>.

Direct transport of GHB to the mitochondria may also be possible. A mitochondrial enzyme, GHB-oxoacid-transhydrogenase (D-2-hydroxyglutarate transhydrogenase), is capable of reducing GHB to SSA, suggesting that the mitochondria is a location of GHB metabolism<sup>82,86,87</sup>.

*In vivo* experiments suggest that it is unlikely that a significant amount of GHB converts back to GABA<sup>75,88,89</sup>; however, *in vitro* research has shown that it is possible<sup>89-91</sup>. Other possible routes for GHB degradation include  $\beta$ -oxidation, and conversion to GBL and 1,4-BD<sup>92</sup> (Figure 2).

## 2.4 GHB as a Neurotransmitter

Several lines of evidence suggest that GHB acts as a neurotransmitter. First, studies using [ $^3\text{H}$ ]-GHB have shown that neuronal and synaptosomal GHB is released via potassium-induced neuronal depolarization; this release is calcium dependent<sup>93–95</sup>. Interestingly, there is suggestion of heterogeneous GHB release within the CNS, with greater extracellular release occurring within the cortex, hippocampus and striatum, and lesser exocytotic release in caudal regions<sup>67</sup>. Second, there appears to be an active transport mechanism, as the existence of a high-affinity sodium- and potassium-dependent GHB uptake system has been observed in the synaptosomal membrane<sup>96,97</sup>.

## 2.5 GHB Binding Site(s) in the Brain

Two GHB binding sites have been discovered, a high-affinity binding site (30–580 nmol/L, Kd1) and a low-affinity binding site (2.3–16  $\mu\text{mol/L}$ , Kd2)<sup>98,99</sup>. Both sites are G protein linked and are of the  $G_i$  or  $G_o$  family<sup>100</sup>. In the CNS, the highest density of high- and low-affinity GHB binding sites is found at the neuronal synapse<sup>98</sup>, and [ $^3\text{H}$ ]-GHB binding is displaced only by GHB and analogues — findings that support the existence of discrete GHB binding site(s) and the role of GHB as a neurotransmitter<sup>96,99</sup>. The GHB high-affinity binding site is absent from peripheral tissues such as kidney, liver, muscle or heart, although GHB is present in significant amounts in these organs<sup>99</sup>. GHB might function as a metabolic intermediate in the periphery<sup>101</sup>.

Studies analyzing the location of GHB binding sites show heterogeneous CNS distribution. Maximal high-affinity binding occurs in the hippocampus, dentate gyrus, olfactory system, nucleus accumbens, septum, caudate putamen, substantia nigra, ventral tegmental area, and in the cortex (prefrontal, frontal, parietal, temporal, cingulate and entorhinal)<sup>99,102,103</sup>. Intermediate concentrations of GHB high-affinity binding are found in the amygdala and the thalamus. Snead and Liu<sup>99</sup> also showed significant binding in the pons and hypothalamus.

The distribution of GHB high-affinity binding sites in rat brain appears specific, as it does not match the distribution of GABA<sub>A</sub> or GABA<sub>B</sub> receptors<sup>104</sup>. Some GHB binding sites, however, have been shown to be located on cholinergic interneurons and on a population of GABAergic interneurons containing enkephalin immunoreactivity<sup>105</sup>.

It has been demonstrated that some antipsychotics bind to GHB binding site(s). Among them is (–)-sulpiride, which, at therapeutic dosages, may have an influence on psychotic symptoms partly via an interaction with GHB binding sites<sup>106</sup>.

### 2.5.1 Stimulation of GHB Binding Site(s)

Discrete activity has been associated with GHB binding site(s), as the selective GHB binding site antagonist NCS-382 has been shown to induce inhibition of calcium

conductance<sup>107</sup> and a decrease in nitric oxide synthase (NOS) activity<sup>108</sup>. Stimulation of presynaptic GHB binding sites, via exogenously administered GHB, induces neuronal cell membrane hyperpolarization via potassium extrusion or entry of chloride ions<sup>109,110</sup>. However, much remains unclear regarding the role of the GHB binding site(s), including the effect of endogenous GHB at its binding site(s) and the contribution of this response to the behavioral effects associated with supraphysiological brain concentrations of GHB induced by exogenous GHB administration. Furthermore, these effects are not antagonized by GABA<sub>B</sub> receptor antagonists, suggesting these effects are mediated at the GHB binding site<sup>107</sup>.

### **2.5.2 Stimulation of GABA<sub>B</sub> Receptors by GHB**

GHB has been shown to have selective but weak affinity for GABA<sub>B</sub> receptors, with an IC<sub>50</sub> of 150 μmol/L<sup>111</sup>. This suggests that supraphysiological brain GHB concentrations, achieved by exogenous administration of GHB<sup>95,112</sup>, are necessary for GHB to bind to GABA<sub>B</sub> receptors<sup>99,113</sup>.

Binding of GHB to postsynaptic GABA<sub>B</sub> receptors augments potassium conductance via inwardly rectifying potassium [GIRK or Kir3] currents<sup>111</sup>; Kir3 channels are of particular importance in anti-nociception in animal models<sup>114</sup>. In *Xenopus* oocytes, GHB activated GABA<sub>B</sub> R1/R2 receptors, with a maximal stimulation of 69% compared with the GABA<sub>B</sub> receptor agonist (–)-baclofen. A combination of GHB and (–)-baclofen did not amplify the effect of each agent alone and did not stimulate the GABA<sub>B</sub> receptor in a linearly additive manner<sup>115</sup>. Cellular recordings of hippocampal or thalamocortical neurons after local application of GHB also demonstrated neuronal hyperpolarization. GABA<sub>B</sub> receptor antagonists block this GHB-induced response<sup>116–118</sup>. Furthermore, in the prefrontal cortex, the GHB binding site antagonist NCS-382 did not suppress these hyperpolarizations<sup>119</sup>. Kaupmann *et al.*<sup>104</sup> studied the effects of GHB in GABA<sub>B</sub> R1 –/– mice, which lack functional GABA<sub>B</sub> receptors. After GHB or GBL application, GABA<sub>B</sub> R1 –/– mice did not show the hypolocomotion, hypothermia, increase in striatal dopamine synthesis or the electroencephalogram (EEG) delta wave induction seen in wild-type mice. These authors concluded that all the effects of GHB studied were GABA<sub>B</sub> receptor dependent.

## **2.6 Intracellular Response to GHB**

Several cellular effects in various brain regions have been observed after GHB has been applied in experimental conditions, including alterations in intracellular calcium<sup>107</sup>, cyclic guanosine monophosphate (cGMP)<sup>120</sup>, cyclic adenosine monophosphate (cAMP)<sup>120</sup>, NOS<sup>108</sup> and increases of inositol phosphate concentrations<sup>121</sup>. Stimulation of GHB binding site(s) has been shown to cause an increase of up to 123% in the concentration of cGMP in the hippocampus 45 minutes after intraperitoneal administration of GHB 500 mg/kg<sup>121</sup>. The stimulation of GHB binding site(s) by GHB induces a progressive decrease in NOS activity (this reduced NOS activity presumably explains the increase in cGMP concentrations previously reported); other agonists of the GHB binding site(s) reproduce this

effect. The effect on these substances is blocked by GABA<sub>B</sub> and GHB antagonists, antiepileptic drugs and opioid receptor antagonists<sup>108,122,123</sup>.

GHB has also been shown to induce a G protein-mediated decrease in adenylyl cyclase activity via the presynaptic GHB binding site(s); this effect is blocked by a GHB antagonist but not by a GABA<sub>B</sub> receptor antagonist<sup>120</sup>. The effect of GHB on intracellular response depends on the type of receptor stimulated and manifests regional variability within the brain.

## **2.7 Effects on Neurotransmitter Systems**

### **2.7.1 Dopaminergic System**

Exogenous administration of GHB raises concentrations of GHB to many times higher than endogenous concentrations in dopaminergic regions of the brain<sup>124,125</sup>. It appears possible that the GHBergic system participates physiologically in the control of the dopaminergic system in the nigrostriatal and in the mesocorticolimbic pathways<sup>126,127</sup>. Research has shown that GHB has multiple effects on dopaminergic neurons. Initially, at certain concentrations, less dopamine is released under the influence of GHB, as GHB reduces impulse flow and inhibits firing of dopaminergic terminals<sup>128,129</sup>. Decreased dopamine release is also seen with administration of baclofen, a GABA<sub>B</sub> receptor agonist<sup>116,126,130–133</sup>. It is possible that the effects of GHB on dopaminergic neurons are mediated via the GHB binding site(s) and/or GABA<sub>B</sub> receptors. With GHB, the decrease in dopamine release is followed by tissue accumulation of dopamine in the neuron<sup>134–140</sup>. By inhibiting impulse flow, administration of GHB stimulates the kinetic properties of tyrosine hydroxylase, the rate-limiting enzyme controlling the synthesis of dopamine from tyrosine<sup>141–146</sup>. Histofluorescent analysis of different catecholamine systems has shown that dopamine fibers appear swollen and abnormal following administration of GHB, suggesting that GHB alters metabolism within the neurons<sup>147</sup>. In summary, it appears that pharmacologic GHB affects dopaminergic neurons by inhibiting dopamine release, increasing dopamine synthesis and inhibiting end-product inhibition of the enzyme tyrosine hydroxylase. Both latter mechanisms increase the amount of available dopamine in the neurons prior to GHB washout<sup>148</sup>.

Subsequent to the GHB-mediated decrease in dopamine release and increase in intercellular dopamine synthesis, a transient increase in dopamine release is observed during washout<sup>149,150</sup>. Animals pretreated with a GHB receptor antagonist showed no increase in extracellular dopamine induced by GHB. Therefore, in a similar way, GHB may be affecting dopaminergic neurons via GHB binding site(s) and GABA<sub>B</sub> receptors<sup>126,130,131,151</sup>. These neurons could be enkephalinergic<sup>105</sup> and partly controlled via GHB binding site(s), as naloxone (an opioid receptor antagonist) has been reported to block the GHB-induced increase in dopamine synthesis in the striatum<sup>152</sup>; it should be noted that naloxone can antagonize GABA receptors<sup>153</sup>.

Furthermore, 6 hours after GHB administration (500 mg/kg intraperitoneally), an increase in dopamine D1 and D2 receptor mRNA expression has been observed. Repeated exposure to GHB (500 mg/kg intraperitoneally twice daily for 10 days), followed by a 14-hour withdrawal period, induced increased mRNA expression for the D1 and D2 receptors. The author cautions that this study analyzed concentrations of dopamine receptor mRNA, not actual receptor numbers or activity<sup>154</sup>.

### **2.7.2 Serotonergic System**

GHB can induce an increase in serotonin turnover in the striatum and in the mesolimbic areas<sup>133,141,155,156</sup> without significantly changing absolute concentrations of serotonin. This effect can be seen as an accumulation of 5-hydroxy-indoleacetic acid (5-HIAA), the main metabolite of serotonin, together with no significant change in potassium-induced increase in extracellular serotonin concentrations after administration of GHB<sup>128,129</sup>. Furthermore, GHB may affect transport of tryptophan, the precursor of serotonin, through the blood-brain barrier and/or through the neuronal membrane, as evidenced by tryptophan accumulation after *in vivo* administration of GHB<sup>157</sup>.

Baclofen-induced activation of GABA<sub>B</sub> receptors mimics some aspects of the serotonergic activity of GHB<sup>133</sup>, suggesting the effect of GHB on serotonergic neurons may be in part mediated by GHB-induced activation of GABA<sub>B</sub> receptors; the role of GHB binding site(s) in the effects of GHB on serotonergic activity is unclear.

This action on serotonin may account for the ability of GHB to stimulate growth hormone (GH) release (see section 3.1.1), as co-administration with the serotonin receptor antagonist metergoline significantly reduces this increase<sup>158</sup>. Metergoline has also been shown to lower GH concentrations in patients with acromegaly<sup>159</sup>.

### **2.7.3 Opioidergic System**

GHB has been found to increase brain concentrations of the endogenous opioids dynorphin and enkephalin<sup>160,161</sup>. After a single intraperitoneal dose of GHB 500 mg/kg, an increase in proenkephalin mRNA concentrations in the whole dorsal striatum (+60%), but not in other areas such as the nucleus accumbens, was seen between 15 and 90 minutes after injection. An increase in prodynorphin mRNA expression was observed in the frontal cortex (+90%) and hippocampus (+55%) 6 hours after GHB administration<sup>123</sup>. Chronic exposure to GHB (500 mg/kg intraperitoneally twice daily for 10 days) induced significant increases in both proenkephalin and prodynorphin mRNA concentrations in various brain regions<sup>161,162</sup>.

GHB appears not to act as an agonist at the  $\mu$ -,  $\delta$ -, and  $\kappa$ -opioid receptors<sup>162</sup>. The opioid receptor antagonist naloxone (10 mg/kg) blocks the GHB-induced increase in cGMP and inositol phosphate turnover in response to GHB<sup>121</sup> and decrease in

glucose utilization<sup>163</sup>; however, these effects may be mediated via GABA receptors as naloxone has no effect on GHB-induced sleep or dopamine metabolism<sup>152,164,165</sup>. Thus, GHB could act on opioid interneurons via GABAergic and/or GHBergic agonism. Again, it should be noted that naloxone can antagonize GABA receptors<sup>153</sup>, and GHB binding site(s) antagonists block the GHB-induced accumulation of met-enkephalin and inhibition of release of met-enkephalin, which participates in the presynaptic regulation of dopamine release<sup>165</sup>. Thus, GHB could act on opioid interneurons via GABAergic and/or GHBergic agonism.

#### **2.7.4 Cholinergic System**

The effects of GHB administration on the cholinergic system are unclear, although GHB binding site(s) have been found on cholinergic interneurons<sup>105</sup>. A study by Sethy *et al.*<sup>166</sup>, using anesthetic doses of GBL (750 mg/kg intraperitoneally), produced a time-dependent increase in acetylcholine in the striatum and hippocampus, with maximal increase occurring 15 minutes after administration. However, a correlation between the increase in acetylcholine and the depth of anesthesia produced by GBL was not observed<sup>166</sup>. In contrast, a microdialysis study in freely moving rats showed a dose- dependent reduction in extracellular hippocampal acetylcholine concentrations induced by both GHB (200 and 500 mg/kg intraperitoneally) and baclofen (10 and 20 mg/kg intraperitoneally). Furthermore, a GABA<sub>B</sub> receptor antagonist prevented this effect, while a GHB receptor antagonist did not block this effect. These findings indicate that the GHB-induced reduction of hippocampal acetylcholine release is mediated by GABA<sub>B</sub> receptors<sup>167</sup>. Of note, the disparity in responses between the two studies may be mediated by the different dose levels utilized but may also highlight neuropharmacological differences between GHB and GBL.

#### **2.7.5 Noradrenergic System**

Anatomical, neurochemical and electrophysiological studies have provided evidence that GABA<sub>B</sub> receptors are involved in the regulation of noradrenergic neurons emanating from the locus coeruleus (LC). Activation of GABA<sub>B</sub> receptors by baclofen inhibits spontaneous firing of these neurons and causes membrane hyperpolarization due to an increase in potassium conductance<sup>168</sup>. In reports as early as 1980, GHB was shown to affect noradrenergic transmission in the CNS. Intraperitoneal administration of GHB increases brain noradrenaline (norepinephrine) synthesis and utilization, particularly in the neocortex<sup>169</sup>. Recently, Szabo *et al.*<sup>170</sup> used *in vivo* extracellular unitary recordings to monitor the effect of sustained administration of GHB (40 mg/kg/day) on the burst firing of LC noradrenergic neurons. Two days and 10 days of continuous 24-hour GHB administration decreased the firing activity of LC neurons by ~50% when compared with controls. In contrast, withdrawal of GHB administration after 10 days of continuous 24-hour treatment resulted in a 33% augmentation in LC activity for 36 hours compared with controls. Thus, chronic GHB treatment inhibits the burst firing of LC noradrenergic neurons while the drug is present in meaningful

concentrations and enhances LC noradrenergic firing during and beyond drug washout.

Speculatively, as noradrenergic activity is implicated in arousal<sup>171</sup>, inhibition of LC noradrenergic activity by GHB, as seen in animal models<sup>170</sup>, may contribute to the observed sleep enhancements noted in the literature<sup>47,172–174</sup>. Conversely, the augmentation of LC noradrenergic neurons on washout, as seen in animal models<sup>170</sup>, might help explain the observations of improved wakefulness and reduced cataplexy seen during the daytime after chronic nighttime administration of GHB in patients with narcolepsy<sup>49,175,176</sup>.

Lastly, withdrawal effects have not been seen in clinical trials<sup>177</sup>, but have been reported in the literature with regard to frequent, regular dosing of illicit GHB<sup>51,178</sup>. These data on noradrenergic neurons might partially or fully explain any GHB withdrawal phenomenon. Further research is necessary to assess these hypotheses.

#### **2.7.6 Glutamatergic System**

GHB has been shown to affect glutamate transmission via GHB site(s) and/or GABA<sub>B</sub> receptors<sup>179</sup>. The ability of GHB to depress the amplitude of the first and the second evoked alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate excitatory postsynaptic potentials suggests that GHB inhibits excitatory synaptic transmission by decreasing the probability of release of glutamate<sup>180</sup>. Further research has shown that this effect of GHB on glutamate levels is concentration dependent, whereby a direct GHB binding site-mediated facilitation is observed at nanomolar concentrations and an indirect GABA<sub>B</sub> receptor-mediated inhibition predominates at millimolar concentrations<sup>179,181</sup>. This effect is partially reversed by GHB binding site(s) antagonists. Of note, GABA<sub>B</sub> agonists also produce a concentration-dependent decrease in basal and potassium-evoked concentrations of glutamate<sup>182</sup>.

### **3. Hormonal and Metabolic Effects of GHB / SXB**

#### **3.1 Hormonal Effects of GHB / SXB**

##### **3.1.1 Growth Hormone**

The effects of GHB on GH had been described as early as 1970<sup>183</sup>. Van Cauter *et al.*<sup>184</sup> investigated the GH response to GHB in healthy young men. Eight such men each participated in four experiments involving bedtime oral administration of placebo, GHB 2.5 g, GHB 3.0 g or GHB 3.5 g. Polysomnography (PSG) sleep recordings were performed every night, and blood samples were obtained at 15-minute intervals from 20:00–08:00 hours. There was a doubling of GH secretion in all GHB-treated groups, resulting from an increase in the amplitude and the duration of the first GH pulse after sleep onset. This stimulation of GH secretion was significantly correlated with a simultaneous increase in the amount of stage IV non-rapid eye movement (NREM) sleep.

It is uncertain whether the ability of GHB to stimulate GH secretion is exclusively tied to its ability to stimulate slow-wave sleep (SWS) during NREM sleep, which is the phase of sleep during a human's 24-hour diurnal cycle when most GH is released, particularly in men<sup>184</sup>. There appears to be a linear relationship between SWS and GH secretion, so that during aging, SWS and GH secretion decrease with the same chronology<sup>185</sup>.

Interestingly, many of the studies investigating the ability of GHB to stimulate GH release were performed during the daytime without allowing the study participant to sleep. This suggests that the ability of GHB to stimulate GH secretion is not dependent on sleep induction. Some studies that evaluated the effect of GHB on GH in the absence of sleep are notable. Gerra *et al.*<sup>186</sup> investigated GH responses to GHB with or without the benzodiazepine receptor antagonist flumazenil. Nine male healthy volunteers (aged  $23.2 \pm 2.5$  years) were submitted to three tests in random order: (i) oral GHB administration; (ii) oral GHB and intravenous flumazenil administered 15 minutes prior to GHB; and (iii) oral placebo and intravenous saline administration. Blood samples for GH assays were collected during the three tests 15 minutes prior to drug administration, at the time of administration, and 15, 30, 45, 60 and 90 minutes afterward. GHB induced a significant increase in plasma GH concentrations; however, intravenous flumazenil 15 minutes prior to GHB administration antagonized the action of GHB on GH secretion. No changes in GH concentrations were obtained with placebo and saline. This study suggests that some of the effects of GHB on GH are mediated at GABA<sub>A</sub> receptors.

The observation that baclofen stimulates GH secretion in healthy men but not in patients with Parkinson's disease<sup>187</sup> prompted research to test the effect of GHB on GH secretion in these patients. GHB, like baclofen, is a GABA<sub>B</sub> receptor agonist. GABA<sub>B</sub> receptor activation has been shown to have a positive effect on GH releasing hormone (GHRH), which in turn has a positive effect on the release of GH<sup>188</sup>. Volpi *et al.*<sup>187</sup> studied ten healthy men and ten patients with *de novo* parkinsonism who were administered sodium valproate (800 mg orally), GHB (25 mg/kg/body weight orally) or baclofen (10 mg orally). All drugs induced a significant increase in serum GH concentrations in the healthy controls. Growth hormone secretion in parkinsonian patients did not change after baclofen or sodium valproate administration, whereas normal responsiveness to GHB was observed. This suggests a different mechanism underlying the GH response to GHB compared with the other drugs. Volpi *et al.*<sup>189</sup> administered GHB to healthy and parkinsonian patients to investigate whether muscarinic cholinergic receptors mediate the GH secretion induced by GHB; both study groups were tested in the absence and the presence of the anticholinergic agent pirenzepine. Both healthy controls and parkinsonian patients showed a significant rise in serum GH concentration in response to GHB (25 mg/kg/ bodyweight orally), although a slightly, but significantly, lower response was observed in parkinsonian patients. Pretreatment with pirenzepine (100 mg orally 2 hours before GHB) completely suppressed the GHB-induced GH release in both controls and parkinsonian patients. These data indicate that a cholinergic mechanism mediates the GH

response to GHB in healthy men and that this is preserved in the parkinsonian brain.

In narcolepsy, Overeem *et al.*<sup>190</sup> observed that GH secretion does still occurs during SWS. Since sleep in narcoleptics is distributed across a 24-hour period, however, so are the GH pulses leading to a more dispersed 24-hour GH profile and greater daytime secretion. To follow up on this work characterizing GH secretion patterns in narcoleptics, Donjacour *et al.*,<sup>191</sup> evaluated the influence of nighttime SXB administration (two times 3 g/night for 5 consecutive nights) on GH and sleep in eight male hypocretin-deficient patients with narcolepsy and cataplexy and eight controls matched for sex, age, body-mass index (BMI), waist-to-hip ratio, and fat percentage. On the fifth day of SXB administration, both groups underwent 24 hours of blood sampling at 10-minute intervals. Administration of SXB caused a significant increase in total 24-hour GH secretion in narcolepsy patients, but not in controls. However, SWS, and the cross-correlation between GH levels and SWS, more than doubled in both groups, strengthening the temporal relation between SWS and GH secretion.

### **3.1.2 Neurosteroids**

GHB has been shown to increase the concentrations of the neurosteroids allopregnanolone and allotetrahydrodeoxycorticosterone (allo-THDOC)<sup>192</sup>. These neurosteroids are positive allosteric modulators of GABA<sub>A</sub> receptors and could therefore contribute to the hypnotic properties of GHB.

In rats, the effect of GHB on the concentrations of allopregnanolone and allo-THDOC and on righting reflex was assessed. GHB induced a loss of righting reflex that lasted up to 90 minutes and declined 180 minutes after GHB administration. An increase in the concentrations of the neurosteroids was also seen after administration of GHB, with a time course that matched that of the loss of righting reflex. The authors of the study concluded that the GHB-induced increase in neurosteroid concentrations was implicated in the sedative/hypnotic properties of GHB<sup>192</sup>.

### **3.1.3 Prolactin**

Prolactin is a pituitary-derived protein that plays an important role in metabolism and the regulation of the immune system. Its secretion is regulated by inhibitory control of dopaminergic tuberoinfundibulum neurons on D2 receptors of the arcuate nucleus and stimulated by thyrotropin-releasing factor<sup>193,194</sup>. At sufficient concentrations, GHB can bind to GABA<sub>B</sub> receptors and influence the release of dopamine and serotonin.<sup>195</sup> Accordingly, Donjacour *et al.*, designed an open-label intervention study to examine the influence of five nights of SXB administration (3 g given twice per night.) on prolactin levels in eight hypocretin-deficient patients and eight controls matched for sex, age, BMI, waist-to-hip ratio and fat percentage. Administration of SXB markedly increased prolactin secretion and enhanced the association between prolactin release and SWS in both groups. The authors

speculated that changes in the tuberoinfundibular output of dopamine could be the cause of the effect of SXB on prolactin release, and that the hypocretin system is not involved in this mechanism since SXB treatment-stimulated prolactin release did not significantly differ between narcolepsy patients and controls<sup>196</sup>.

#### **3.1.4 Melatonin**

Melatonin is a pineal gland-derived hormone involved in endocrine timing, the timing of behaviors, and communication of night length in seasonal-breeding mammals<sup>197</sup>. It is also involved in regulation of the sleep-wake cycle, and as such, it can modulate sleep and even induce sleepiness at sufficient concentrations<sup>198</sup>.

The secretion of melatonin is regulated by the release of norepinephrine (NE) from local sympathetic nerve fibers. This NE release happens exclusively under darkness conditions. Once released, NE binds to  $\beta$ -adrenergic receptors on the pinealocyte to activate cAMP-dependent protein kinase A (PKA), which in turn regulates the rate limiting enzyme in melatonin synthesis: it phosphorylates arylalkylamine N-acetyltransferase (AANAT). Under conditions of light exposure to the eye, there is an absence of local NE stimulation, and AANAT is degraded by proteasomal proteolysis. Production of melatonin is started again, typically in the evening, under conditions of dim light.

As described earlier in the article, GHB has been shown to affect noradrenergic transmission in the CNS, likely via binding to GABA<sub>B</sub> receptors when at sufficient concentrations. Given that GHB can influence NE transmission, the key neurotransmitter responsible for regulating melatonin, and including the fact that therapeutic SXB is administered chronically in certain populations, the investigation of SXB on the melatonin level is warranted.

Interestingly, the pineal gland is innervated by hypocretinergic neurons, therefore making hypocretin activity a possible modulator of diurnal melatonin synthesis and secretion<sup>199</sup>. Therefore, Donjacour and colleagues<sup>200</sup> measured plasma melatonin after 5 days of SXB administration (3 g, 2x per night) in both hypocretin-deficient narcoleptic patients and matched controls. Mean melatonin concentrations were similar between patients and controls; however, the percentage of 24-hour melatonin secreted during the daytime was significantly higher in narcoleptic patients – possibly secondary to light exposure during wake periods during nighttime fragmented sleep – and melatonin secretion exhibited a weaker coupling to sleep. SXB did not affect melatonin secretion.

#### **3.1.5 Ghrelin**

Ghrelin is a peptide hormone mainly produced by endocrine cells in the stomach and gastrointestinal tract, and is an important endogenous regulator of energy balance and GH secretion<sup>201</sup>. Ghrelin has an excitatory influence on hypocretin neurons and these systems have been shown to interact in ingestive behavior<sup>202</sup>. Like ghrelin, SXB administration stimulates GH release<sup>184</sup>. Research detailed below

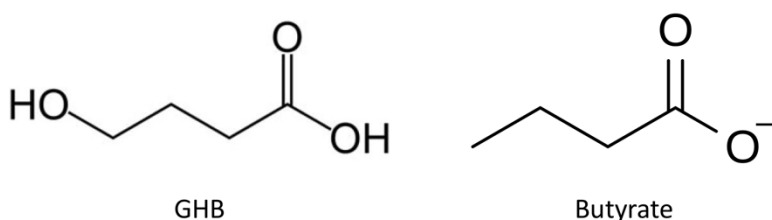
in this thesis describes the effects of SXB on the ghrelin level in narcoleptics and controls.

### 3.2 Metabolic Effects of GHB / SXB

#### 3.2.1 Insulin Sensitivity

##### *Butyrate*

The short chain fatty acid butyrate is structurally similar to (Figure 3) and can be produced from  $\beta$ -oxidation of GHB. Supplemental butyrate has been shown to improve insulin sensitivity in mice<sup>203</sup>. Likewise, a meta-analysis evaluating human butter consumption—which contains a significant amount of butyrate per serving—showed a modest inverse association with the risk for type 2 diabetes<sup>204</sup>. Furthermore, inducing an increase in butyrate-producing gut bacteria, such as *Roseburia* in the feces and *Eubacterium halii* in the small intestine, associates with increased peripheral insulin sensitivity in males with metabolic syndrome who underwent a fecal transplant<sup>205</sup>.



**FIGURE 3.** Chemical structure of gamma-hydroxybutyrate (GHB) and butyrate.

Butyrate may induce beneficial metabolic effects through enhancement of mitochondrial activity, prevention of metabolic endotoxemia by strengthening the gut lining, and activation of intestinal gluconeogenesis via different routes of gene expression and hormone regulation<sup>206</sup>. The anti-diabetic properties of butyrate may also in part come through its influence as a histone deacetylase (HDAC) inhibitor, which is a property it shares with GHB. Some HDAC inhibitors have been shown to promote  $\beta$ -cell reprogramming, differentiation, proliferation and functioning, and to improve insulin resistance<sup>207</sup>.

##### *HDAC Inhibition*

Multiple physiological and functional roles are attributed to the modification of histone acetylation and its consequences at the level of gene expression. Pharmacological concentrations of GHB significantly induce brain histone H3 acetylation and reduce *in vitro* HDAC activity<sup>208</sup>. It is therefore possible that GHB, at sufficient levels, could directly participate in the epigenetic modification of gene expression related to insulin-sensitizing mechanisms.

Also of interest is SXB's influence in increasing GH release<sup>184</sup>. While GH administration can promote reductions in body fat, which itself promotes an insulin-sensitizing effect, long-term elevations in its level have been associated with both increases and decrements in insulin sensitivity<sup>209,210</sup>.

Given that the use of this medication in narcoleptic patients is chronic, and that multiple insulin-sensitizing mechanisms are plausible, evaluating the long-term impact of SXB therapy on insulin sensitivity is important. As such, Donjacour and colleagues studied the effects of nightly SXB treatment on  $\beta$ -cell functioning and insulin sensitivity in seven hypocretin-deficient patients over three months. Compared to baseline, SXB treatment increased hepatic, but decreased whole-body insulin sensitivity<sup>211</sup>. It should be noted that the sample of narcoleptic patients studied demonstrated increased peripheral insulin sensitivity, but normal hepatic insulin sensitivity and  $\beta$ -cell function, compared to matched healthy controls at baseline. Therefore, future studies should further evaluate the effect of SXB on insulin sensitivity and  $\beta$ -cell functioning in this population, and in populations other than hypocretin-deficient narcoleptics.

### **3.2.2 Thermoregulation**

The hypothalamus integrates sensory information to help control basic life functions, including the regulation of temperature and sleep/wake states. To regulate body temperature, the median preoptic nucleus (MePOA) of the hypothalamus sends descending output to two key sites. The first is to the dorsomedial nucleus (DMH), in which activation of glutamatergic neurons elevates body temperature. The MePOA, as well as the DMH, also innervates the raphe pallidus nucleus, which in turn innervates sympathetic preganglionic neurons that cause an increase in body temperature by activating brown adipose tissue to cause thermogenesis, and by promoting vasoconstriction of superficial vascular beds, thus reducing heat loss through the skin<sup>212</sup>.

In relation to wake and sleep states, changes in skin temperature may moderate the efficacy by which the clock and homeostat manage to initiate or maintain sleep or wakefulness<sup>213</sup>. In healthy individuals, body temperature, both core and skin, closely associate with sleep and arousal<sup>214</sup>. Relatively speaking, during wakefulness, core temperature is high while skin temperature is low. When transitioning between wake and sleep, temperature changes can be observed reliably. Just prior to the onset of sleep, skin temperature increases, allowing convective heat loss and a drop in core temperature. During sleep, core temperature stays depressed while skin temperature remains elevated<sup>215</sup>.

Aligned with the above findings, recent work from Siegel and colleagues evaluated the association of sleep and wake to skin and environmental temperature in three natural-living, hunter-gatherer communities in their ecological niche. They found that sleep onset, in both the winter and summer, occurred during falling ambient temperature. Morning awakenings were accompanied by strong vasoconstriction in the hands and in the feet. The authors proposed that the physiological purpose

of this vasoconstriction could be to increase the temperature of the core and brain to evoke awakening<sup>216</sup>.

Interestingly, hypocretin-deficient narcoleptic patients are characterized by skin temperature dynamics that deviate from normal controls: during wake, their skin temperature resembles that of the healthy at sleep onset<sup>217</sup>. Daytime sleepiness is a cardinal symptom of narcolepsy, and it has been proposed that changes in skin and core body temperature modulate neuronal activity of thermosensitive neurons in brain areas that regulate vigilance and sleepiness<sup>218</sup>.

### *GHB / SXB and Body Temperature*

The effects of GHB on body temperature appear to vary by dose. In rats, an intraperitoneal dose of 10 mg/kg raises core body temperature slightly, while doses of 200 mg/kg or greater lower it<sup>219</sup>. A similar temperature-lowering effect with higher doses of GHB has also been observed in gerbils and rabbits<sup>220,221</sup>, and in humans under conditions of GHB abuse<sup>222,223</sup>.

The main temperature-lowering effect of GHB seems to be dependent on GABA<sub>B</sub> receptor binding<sup>104,224</sup>. However, GHB may also affect body temperature through decreased heat production, via reductions in metabolism and energy utilization and increases in cutaneous circulation<sup>101,225</sup>.

Given the impact of GHB on temperature regulation, the altered pattern of skin temperature dynamics in narcolepsy, and the positive effects of SXB on both sleep and sleepiness in narcolepsy patients, van der Heide *et al.* investigated whether the beneficial effect of SXB occurs, at least partially, by restoring physiological temperature regulation. The effects of five nights of SXB (3g, twice per night) treatment on temperature and sleep-wakefulness was tested in eight male hypocretin-deficient narcoleptic patients and matched controls<sup>226</sup>. At baseline, all participants underwent measurement of core, as well as proximal and distal skin temperatures, and 24-hour PSG to assess sleep/wake measures. For narcoleptic individuals, core and proximal skin temperatures were significantly lower than controls during daytime. Immediately after the fifth night of SXB treatment, the narcoleptic patients underwent a second round of temperature and PSG over a 24-hour period. The nightly administration of SXB increased the daytime proximal skin temperature in narcoleptic patients to a level similar to that of controls and normalized the predictive value of distal skin temperature and distal-proximal temperature gradient for the onset of daytime naps. This treatment protocol, however, did not affect core body temperature, distal temperature, or distal-proximal temperature gradient. Further studies should explore the role of SXB in temperature regulation and sleep in narcolepsy, as well as in other populations.

### **3.2.3**    *Weight and Energy Balance*

When GHB was legal to purchase as a dietary supplement in the U.S., it was advertised as an agent to help reduce body fat. The effects of SXB on weight loss have been reported on in some clinical settings and there are several plausible

mechanisms for how GHB might influence adiposity. However, SXB remains to be tested rigorously as a clinical intervention for weight loss.

## Clinical Reports

### *Weight Loss in Narcolepsy*

The first clinical report found on this subject was from Dr. Ruzica Ristanovic, published in abstract form at the 2003 Associated Professional Sleep Society (APSS) conference. She analyzed the change in BMI in narcolepsy patients on SXB from baseline (n=184; median BMI=28) to 2 (n=156), 6 (n=175), and 12 months (n=97) of treatment. At each time point, there was a statistically significant reduction in BMI from baseline (2 mo = -0.20; 6 mo = -0.425; and 12 mo = -1.103).

In 2005, Husain *et al.*, published an abstract (APSS) on a chart review of 17 narcolepsy patients (11 males, 6 females; mean age  $43 \pm 16.6$  years) in their clinic who had been on SXB ( $7.0 \pm 1.8$  g/n avg.) for a mean duration of 10.7 ( $\pm 7.2$ ) months. The average weight loss was 10.4 pounds (pre-SXB weight =  $182.1 \pm 33.3$ , BMI =  $27.6 \pm 4.0$ ; post-treatment weight =  $171.7 \pm 31.2$ , BMI =  $26.0 \pm 3.5$ ), with a maximum loss of 41 pounds.

Similarly, Turner *et al.*, published an abstract at the 2006 APSS meeting on a chart review of 86 patients (22 males, 20-76 years old; 64 females, 16-76 years old) being treated with SXB therapy (3-10.8 g/night). Chart audits were conducted at three time points: baseline, first, and second office visit. At the first office visit (mean 1.7 months), 56% of patients experienced weight loss ranging from 1 to 16 lbs., with 10 patients experiencing weight loss of 10 lbs. or more. This finding was not statistically significant. At the second visit, three months after the first, 60% of the patients experienced some degree of weight loss, yet still, across the group, the amount of weight loss was not significant. Comparatively, however, from baseline to the second visit, women lost significantly more weight than men did ( $14 \text{ lbs} \pm 10 \text{ lbs.}$ ,  $8.3 \text{ lbs} \pm 4 \text{ lbs.}$ , respectively).

More recently, Donjacour *et al.*, conducted a study looking at the effects of SXB on glucose and fat metabolism in narcoleptic patients (as reported above)<sup>211</sup>. Compared to baseline, three months of SXB treatment significantly reduced body weight ( $99.2 \pm 6.0$  vs.  $94.0 \pm 5.4$  kg) and increased lipolysis ( $4.9 \pm 0.4$  vs.  $6.5 \pm 0.6$   $\mu\text{mol/kg FM/min}$ ), possibly accounting for the observed weight loss from treatment. This change in body weight strongly associated with alterations in glucose disposal rate per unit serum insulin ( $r=-0.93$ ,  $p=0.003$ ), but not with changes in endogenous glucose production per unit serum insulin ( $r = 0.29$ ,  $p=0.535$ ). While SXB showed a tendency to decrease systemic insulin sensitivity, it increased hepatic insulin sensitivity, suggesting tissue-specific effects<sup>211</sup>.

### *Weight Change in Fibromyalgia*

Fibromyalgia is a complex musculoskeletal disorder clinically characterized by widespread pain, usually accompanied by fatigue, sleep disturbance, and dyscognition<sup>227</sup>. A 14-week, phase 3, double-blind, randomized, controlled trial evaluated SXB (4.5 g, n = 182; 6 g, n = 183) versus placebo (n = 183) in patients with fibromyalgia. At study endpoint, compared to placebo (1.2%), a greater proportion of patients treated with SXB (4.5 g = 7.6%; 6 g = 14.3%) had a decrease in weight of  $\geq 7\%$ . Furthermore, a decrease in weight was reported as an adverse event in five patients in each SXB group, while an increase in weight was reported in one patient in each SXB group. Additionally, a decreased appetite was reported as an adverse event in five patients in the SXB 4.5 g group, and in three in the SXB 6 g group<sup>228</sup>.

In a separate 14-week phase 3 study evaluating SXB in fibromyalgia, 573 patients that were enrolled at 108 centers in eight countries were randomly assigned to placebo, SXB 4.5 g/night or SXB 6 g/night. In this study, weight loss was observed in the SXB-treated groups, with a mean (standard error) weight change from baseline of  $-1.19$  ( $0.22$ ) kg in the SXB 6 g/night group and  $-0.43$  ( $0.20$ ) kg in the SXB 4.5 g/night group compared with a mean weight gain of  $0.43$  ( $0.16$ ) kg in the placebo group<sup>229</sup>.

The weight loss in both the narcoleptic and fibromyalgia patients is impressive as these reductions in weight took place in populations not enriched for weight loss.

### **Potential Mechanisms for SXB and Weight Loss**

#### *Sleep*

Both feeding and sleeping behaviors in mammals are thought to result from the integration of homeostatic and circadian influences. Drug-induced alterations of one behavior (sleepiness-sleep) may hypothetically lead to changes in the other behavior (hunger-food consumption), or both behaviors can be altered via drug effect on common mechanism(s) they share.

Sleep deprivation contributes to a number of molecular, immune, and neural changes with broad effects on body systems (endocrine, metabolic, immune, and cognitive)<sup>230</sup>, all of which can play a role in increasing adiposity. The connection between sleep and weight will be discussed in greater detail in the second half of this thesis.

The ability of SXB to reduce sleep disruption in some populations and increase SWS in all populations tested, is well-established. It is plausible that SXB-induced sleep enhancement can influence adiposity in susceptible individuals.

### *Arousal*

The effects of SXB on wakefulness in narcolepsy, Parkinson's disease, and healthy sleep deprived adults are well documented<sup>1,231</sup>. This increased wakefulness may lead to increased physical activity and energy expenditure, which are key elements in the energy balance equation.

The daytime wake-enhancing effects from nighttime SXB treatment may be a result of several interacting mechanisms, including increased SWS during nocturnal sleep and/or direct pharmacological effects on wake-associated systems. For instance, after acclimation to a week or more of nightly SXB use, and subsequent down regulation of GABA<sub>B</sub> receptors, SXB may promote daytime wakefulness by desensitizing adenosine receptors (A<sub>1A</sub>) on thalamic relay neurons to reduce the drowsiness-inducing tonic adenosine-mediated inhibition in the thalamus. Additionally, acclimation to SXB may promote arousal by disinhibiting excitatory glutamatergic transmission within thalamic relay neurons circuits, excitatory dopaminergic of the VTA, and excitatory noradrenergic circuits emanating from the locus coeruleus.

As per the second half of my thesis, impairments in arousal, and thus subjective and objective alertness, may lead to altered food decisions that could promote a defection from personal health standards<sup>7</sup>.

### *Anorexigenic Effects*

SXB may also promote an anorexigenic effect via an influence on neuromodulators like cocaine- and amphetamine-regulated transcript (CART) that are involved in feeding behavior and energy expenditure. Furthermore, the closely-related ketone  $\beta$ -hydroxybutyrate has been shown to induce proliferative remodeling of neurons in the energy-regulation arcuate nucleus of the hypothalamus, which may lower the body fat set point<sup>232</sup>. Given the clinical weight loss that has been observed, and the impressive weight loss effects from  $\beta$ -hydroxybutyrate, the effects of chronic exposure to SXB on body fat set point neurons should be evaluated directly. Interestingly, Fisler *et al.*<sup>233</sup> showed that obese rats have lower brain levels of GHB than their lean counterparts, despite an identical diet. This suggests endogenous GHB could play a role in the regulation of bodyweight.

### *Hormones*

As discussed previously, SXB administration promotes the release of GH, which promotes lipolysis by upregulating hormone sensitive lipase (HSL) to mobilize free fatty acids from triglycerides. Additionally, three months of SXB treatment in narcoleptic patients significantly increased lipolysis and lowered body weight, with tissue-specific effects on insulin sensitivity. This increased lipolysis may have accounted for the weight loss observed in this same study<sup>211</sup>.

### *Temperature*

A study in rats showed a remarkable increase in body temperature after administration of very low doses of the GHB precursor, GBL. Following intraperitoneal injection of 5 mg/kg, body temperature rose rapidly to 1.8°F above normal at 15 minutes, reached a peak of almost 2.2°F at the hour mark, and was still elevated by 2°F at the 75-minute mark, dropping gradually thereafter. This thermogenic effect could contribute to increased energy expenditure and reduced adiposity over time<sup>234</sup>.

In summary, a potent connection between sleep and energy regulation is known, as well as a unique positive effect of SXB on sleep in clinical and non-clinical populations. Given the connection between sleep and weight, and SXB's effects on sleep — in addition to its other pharmacological effects on neuronal and hormonal systems of the body — as well as the impressive clinical reports that have been published on weight loss after SXB therapy, a more thorough, direct investigation into SXB as a therapy for weight loss is warranted.

## **4. Clinical Effects of GHB/SXB**

The effects of GHB, in the form of SXB, on sleep in humans and animals have been examined since the early 1960s<sup>19,20,235,236</sup>. Much of the recent research has continued to focus on the somnopharmacological properties of this compound. Animal studies included the exploration of intravenous or intraperitoneal doses as low as 10 mg/kg to as high as 1200 mg/kg. The sample sizes, doses and other aspects of the methodologies varied greatly across studies, resulting in a variety of responses ranging from sedation to torpor<sup>235,237,238</sup>.

Much more meaningful analysis of GHB on sleep and wakefulness has been conducted in humans during the past 30 years. The clinical findings are fairly consistent and are discussed in the following sections. In humans, administration of GHB affects many aspects of sleep; additionally, a growing data pool demonstrates that it also promotes next-day wakefulness (i.e., ameliorates excessive daytime sleepiness), at least in certain clinical populations. The following sections review research on sleep and wakefulness in healthy humans and across various clinical populations. It should be noted that in the sections on fibromyalgia and narcolepsy (sections 4.2.2 and 4.2.5), all doses of GHB discussed represent a total nightly dose. In all the clinical research trials in these two indications, the total nightly dose has been split into two equal halves, with the first dose administered at bedtime and the second dose administered somewhere between 2.5 and 4 hours later. For example, a total nightly dose of 6 g would equate to a bedtime dose of 3 g and a night-time dose of 3 g, 2.5-4 hours later.

## 4.1 Healthy Subjects

### 4.1.1 Sleep

Early work with GHB in a healthy human population examined the ability of the drug to induce a sleep-like state<sup>19,20</sup>. In this population, GHB was shown to decrease sleep onset latency, promote delta activity and enhance sleep maintenance; these effects were dependent on dose and timing of administration<sup>239</sup>. In 1964, Helrich *et al.*<sup>14</sup> induced sleep in 16 healthy study participants with intravenous administration of GHB at doses ranging between 5.9 g and 9 g. Lapiere *et al.*<sup>46</sup> documented an effect of GHB on sleep organization in 12 healthy individuals (six male, six female; age range 23–63 years). All subjects were free from a history or current symptoms of psychopathology, as well as of any medical conditions known to influence sleep. Each participant received a single oral dose of GHB 2.25 g or placebo, in double-blind fashion, 15 minutes prior to undergoing nocturnal PSG recording. In this study, GHB had no significant effect on total sleep time (TST), and sleep latency decreased, although not significantly; stage 1 sleep decreased from a mean of 10.1% to 8.1% of TST; the percentage of TST spent in stage 2 remained unchanged; SWS increased significantly from a mean of 10.5% to 13.6% of TST and SWS latency shortened significantly. No difference was found in REM sleep latency or the percentage of time spent in REM sleep; however, REM sleep efficiency increased significantly. As expected, these effects, from single bedtime doses of GHB, were seen only during the first third of the night.

Research by Van Cauter *et al.*<sup>184</sup> has supported this earlier work, showing that, in addition to increasing GH secretion (see section 3.1.1), GHB increases the time spent in deep SWS in healthy human subjects. These researchers evaluated eight healthy young men who participated in each of four experiments involving bedtime oral administration of placebo, GHB 2.5 g, GHB 3.0 g and GHB 3.5 g; PSG sleep recordings were performed every night. Subjects showed reduced sleep latency at all doses of GHB compared with placebo ( $24 \pm 5$  minutes); these sleep latency scores were significantly reduced in the GHB 2.5 g ( $13 \pm 3$  minutes) and 3.5g ( $14 \pm 2$  minutes) dose groups. Subjects showed increases in deep SWS time with the 3.0 g ( $105 \pm 9$  minutes) and 3.5 g ( $99 \pm 18$  minutes) doses and decreases in deep SWS at the 2.5 g ( $88 \pm 8$  minutes) dose, which were not significant when compared with placebo ( $91 \pm 9$  minutes). Again, as expected with a single nightly dose, these effects occurred mainly during the first third of the night after sleep onset. None of these studies were designed to evaluate next-day wakefulness or cognition parameters.

### 4.1.2 Mood, Prosocial, and Prosexual Effects

In humans, GHB has a wide spectrum of properties ranging from stimulation and euphoria in lower doses, to sedation, deep sleep, and coma after ingestion of high doses. Reports from abuse cases suggest that the ingestion of certain doses of GHB elicit euphoric, prosocial, prosexual, libido-enhancing and empathogenic effects in users. Until recently, these commonly-reported effects had not been tested

directly. In 2015, Bosch et al., gave GHB (20 mg/kg) to 16 healthy males, using a randomized, placebo-controlled, cross-over design. They observed that GHB showed both stimulating and sedating effects, and elicited euphoria, disinhibition, and enhanced vitality<sup>240</sup>. In 2017, Bosch et al., reported on two experiments of the putative prosexual effects of GHB<sup>241</sup>. In the first experiment, the research group gave 20 and 35mg/kg GHB vs. placebo in 32 participants using a randomized, placebo-controlled, double-blind, balanced, and cross-over study designs to measure subject prosexual effects assessed using the Sexual Arousal and Desire Inventory (SADI). The prosexual effects of GHB were shown by increased SADI ratings regarding physiological, evaluative, and motivational aspects of sexual arousal. In the second experiment, brain reactivity towards erotic vs. neutral pictures was investigated in 15 participants using fMRI after 35mg/kg GHB vs. placebo. Under the placebo condition, erotic visual stimuli activated the bilateral insula, NAcc, fusiform gyrus, thalamus, and left occipital pole. Post administration of GHB, even sexually neutral pictures of persons induced subjective sexual arousal and increased activation of the bilateral NAcc and right anterior cingulate cortex, which significantly correlated (left NAcc by trend). GHB also increased connectivity between NAcc and ventromedial prefrontal cortex during processing of visual erotic cues, i.e., in the condition in which subjective sexual arousal was highest.

Together, these findings align with the subject reports by demonstrating that GHB between doses of 20-35mg/kg can increase physiological, evaluative, and motivational ratings of sexual arousal, likely by stimulating hedonic sexual functioning and lowering the threshold for erotic perception, which is related to increased susceptibility of mesolimbic reward pathways.

## **4.2 Clinical Populations**

### **4.2.1 Insomnia**

The early work of Laborit *et al.*<sup>13,16,242–244</sup>, Helrich *et al.*<sup>14</sup> and Jouany *et al.*<sup>245</sup> characterizing the sleep-promoting effects of GHB led to speculation by Mamelak that this substance may improve the sleep of patients with insomnia or fragmented sleep associated with a history of psychiatric illness. In their 1973 report, Mamelak *et al.*<sup>47</sup> describe the placebo-controlled, crossover administration of low-dose GHB to five patients (three males and two females; age range 35–60 years) with all-night PSG monitoring. In all subjects, an increase in delta sleep was observed after GHB compared with placebo, along with a subjective improvement in sleep quality and the restorative nature of sleep; no rebound insomnia or withdrawal effects were seen. In 1995, Reder *et al.*<sup>246</sup> explored the use of GHB to induce SWS in a patient with fatal familial insomnia. In this patient, who had been devoid of SWS, GHB induced 3 hours of SWS and had no effect on REM sleep. Furthermore, this patient showed enhanced daytime alertness, as evidenced by his greatly improved ability to answer simple questions.

#### 4.2.2 Fibromyalgia

Fibromyalgia is a perplexing disorder where disrupted sleep is believed to result from its primary pathophysiology or to perhaps even contribute to or exacerbate symptoms. Patients with fibromyalgia often exhibit an electroencephalogram (EEG) pattern of excess alpha activity during NREM sleep, especially during SWS, and also evidence of reduced SWS, which may correlate with musculoskeletal pain and mood disturbance<sup>247,248</sup>. Of interest, work by Lentz *et al.*<sup>249</sup> reproduced results previously reported by Moldofsky *et al.*<sup>248</sup> that disrupted SWS correlated with the rapid appearance of discomfort/pain in healthy controls. This research showed that disrupting deep SWS, without reducing total sleep or sleep efficiency, for three consecutive nights in 12 healthy, middle-aged, sedentary women without muscle discomfort was associated with the appearance of fibromyalgia symptoms, including a decreased pain threshold, increased discomfort, fatigue and the inflammatory flare response in skin.

Two trials conducted by Scharf *et al.*<sup>37,38</sup> evaluated the effects of GHB in the treatment of fibromyalgia. The first 11 patients underwent a 4-week open-label pilot trial to evaluate the effect of GHB on nighttime sleep and daytime fatigue, among other endpoints. PSG recordings were used to evaluate sleep stages, sleep efficiency and the presence of the alpha anomaly in NREM sleep. Daily diaries were kept to assess fatigue levels. After administration of GHB, there was a significant increase in SWS, a decrease in the severity of the alpha anomaly, and a significant improvement in subjective fatigue by more than 110% compared with baseline<sup>38</sup>. Moreover, EEG registrations of sleep showed that GHB induced an increase in delta sleep by ~60% and decreases in NREM sleep periods with alpha intrusion by ~70%.

This preliminary finding led Scharf *et al.*<sup>37</sup> to conduct a second, more rigorous study to evaluate the effect of GHB on fibromyalgia. This 24-patient (all female), double-blind, randomized, placebo-controlled, crossover trial evaluated the effects of GHB on subjective sleep quality; objective PSG sleep variables such as alpha intrusion, SWS (stage 3/4) and sleep efficiency; and subjective daytime fatigue and alertness, among other endpoints. During GHB treatment, subjective sleep quality significantly improved by 33%, compared with only 10% during placebo treatment. SWS (stage 3/4) was significantly increased from  $14.9 \pm 6.5$  minutes at baseline to  $21.5 \pm 7.1$  minutes at endpoint, with a change of  $6.6 \pm 8.6$  minutes. Additionally, alpha intrusion, measured as a percentage of NREM sleep, significantly decreased changed from a baseline of  $36.9 \pm 10.7$  minutes to  $25.8 \pm 11.8$  minutes, which represented a  $-11.1 \pm 7.1$ -minute change, and sleep latency and REM sleep were significantly decreased compared with placebo. Subjective fatigue scores were evaluated by assessing morning fatigue, end-of-day fatigue and overall fatigue. Morning alertness significantly improved by 18% for GHB compared with 2% for placebo. The authors concluded that GHB effectively addressed nighttime sleep and excessive daytime sleepiness in this study group.

A double-blind, placebo-controlled study by Moldofsky *et al.*<sup>250</sup> assessed the effects of SXB 4.5 g and 6 g/night administered to fibromyalgia patients for 8 weeks on sleep physiology and sleep/wake-related symptoms; 151 patients completed the study (54

placebo, 51 SXB 4.5 g, and 46 SXB 6 g). Data showed that SXB treatment improved EEG sleep physiology and sleep-related fibromyalgia symptoms. Compared with placebo, both doses of SXB achieved statistically significant improvements in daytime sleepiness, morning fatigue, sleep, daytime functioning, vitality, and in general and morning tiredness; both doses also demonstrated decreased REM sleep. SXB 6 g/night improved afternoon, evening and overall fatigue, reduced wakefulness after sleep onset, and increased stage 2, slow-wave, and total NREM sleep versus placebo. Moderate correlations were noted between changes in subjective sleep and pain measures.

A phase 3 double-blind, randomized, placebo-controlled trial conducted by Russel *et al.*<sup>228</sup> evaluated the effect of SXB administered at doses of 4.5 and 6 g per night during 14 weeks in patients with fibromyalgia (a previous prospective, randomized clinical trial to evaluate the potential role of SXB in the management of fibromyalgia had already been carried out by the same group<sup>251</sup>); 334 patients completed the study (111 placebo, 119 SXB 4.5 g, and 104 SXB 6 g). In general, significant effects of SXB were observed as early as 1 week after initiating therapy and maintained throughout the 14-week trial duration. Significant reductions in pain, fatigue and in the Fibromyalgia Impact Questionnaire total score, an indicator of improvement in fibromyalgia patient functioning, were observed with SXB relative to placebo. There was a statistically significant reduction in patient-reported sleep disturbance with SXB relative to placebo, observed in both SXB groups at the first evaluation (week 4) and maintained throughout the study; a significantly reduced frequency of waking up several times per night was also observed for both SXB doses. The proportion of patients who reported a global improvement of “much” or “very much” better was 48.3% in the SXB 4.5 g group and 45.4% in the SXB 6 g group, significantly greater than the 27.2% for placebo<sup>228</sup>.

Another phase 3 double-blind, randomized, placebo-controlled 14-week trial conducted by Spaeth *et al.*<sup>229</sup> (376 patients completed the study; 131 placebo, 129 SXB 4.5 g, and 116 SXB 6 g) also showed significant improvements in function, fatigue, tenderness, health-related quality of life, and subject's impression of change in overall wellbeing. A significant improvement in sleep quality was observed for both SXB 4.5 g/night and SXB 6 g/night versus placebo. Improvements in functionality related to sleep were also significantly greater for SXB 4.5 g/night and SXB 6 g/night versus placebo. There were strong correlations between a subjective measure of restorative sleep and pain ( $r=0.68$ ;  $p<0.001$ ), fatigue ( $r=0.78$ ;  $p<0.001$ ), global assessments ( $r=0.59$ ;  $p<0.001$ ) and sleep quality ( $r=0.56$ ;  $p<0.001$ ). Post-hoc analyses demonstrated statistically significant associations of moderate strength between the changes in the Jenkins Sleep Scale (JSS) and in the Functional Outcomes of Sleep Questionnaire (FOSQ), and the changes in other clinical outcomes (pain, multidimensional function, fatigue). Significantly greater proportions of subjects reported feeling ‘much better’ or ‘very much better’ at week 14 and had their condition rated as ‘very much improved’ or ‘much improved’ by investigators with SXB 4.5 g/night and SXB 6 g/night versus placebo.

Overall, multiple large, robust and well-designed clinical trials have shown that SXB has therapeutic effects not only on sleep, but also on other important clinical fibromyalgia symptoms, such as fatigue and pain. Since the induction of alpha activity during SWS (often observed in fibromyalgia patients) has been shown to produce fibromyalgia-like symptoms in healthy individuals<sup>248,249</sup>, the increased pattern of alpha-delta sleep may underlie the exacerbated pain experienced by fibromyalgia patients<sup>252</sup>. Given that SXB was shown to reduce the incidence of alpha-delta sleep in fibromyalgia patients<sup>37,250</sup>, and that SXB was able to restore normal delta sleep in an experimental model of alpha-delta sleep<sup>253</sup>, it is possible that the therapeutic effects of SXB on fibromyalgia-associated pain may be linked to its action on alpha activity during SWS. But further research is needed to fully understand SXB's effects on fibromyalgia pathophysiology.

#### **4.2.3**     *Parkinson's Disease*

Parkinson's Disease (PD) is strongly associated with excessive daytime sleepiness (EDS) and nocturnal sleep dysfunction. In fact, both PD and dopaminergic treatments for it can cause EDS. Intrinsic changes in sleep architecture include reduced SWS and reduced sleep spindles<sup>254,255</sup>.

To evaluate SXB in PD, Ondo *et al.*<sup>256</sup> evaluated 27 patients receiving a mean SXB dose of 7.8 g per night. Patients were titrated over the first 6 weeks, then maintained on a steady dose for another 12 weeks. From baseline to endpoint, mean overnight SWS time increased almost 90% (from  $41.3 \pm 33.2$  to  $78.0 \pm 61.2$  minutes) and subjective sleep quality improved by about 30% (Pittsburgh Sleep Quality Inventory score significantly lowered from  $10.9 \pm 4.0$  to  $6.6 \pm 3.9$ ). In response, subjective sleepiness scores improved by nearly 28% (Epworth Sleepiness Scale score dropped from  $15.6 \pm 4.2$  to  $9.0 \pm 5.0$ ) and fatigue improved by about 18% (Fatigue Severity Scale score dropped from  $42.9 \pm 13.2$  to  $36.3 \pm 14.3$ ). However, there were no significant changes in Unified Parkinson Disease Rating Scale scores. It was concluded that, given the robust efficacy and good tolerability of SXB in this patient sample, and the lack of effective treatment for EDS in patients with PD, controlled trials with SXB in PD using objective measures of daytime sleepiness are justified<sup>256</sup>.

#### **4.2.4**     *Alzheimer's Disease*

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder that comprises between 60-80% of all age-related dementias<sup>257</sup>. The disease has three primary groups of symptoms: cognitive symptoms, non-cognitive symptoms, and instrumental symptoms. The cognitive symptoms can include memory loss, language issues, and diminished executive functioning. The non-cognitive symptoms can include behavioral disturbances, depression, hallucinations, delusions, and agitation. The instrumental symptoms refer to issues that impair the ability to perform activities of daily life (e.g., driving, shopping, dressing, and eating unaided)<sup>258</sup>.

### *Pathogenesis of Alzheimer's Disease*

The neurological processes that give way to AD can take decades to occur<sup>259</sup>. It is believed that the accumulation and aggregation of amyloid beta (A $\beta$ ) peptides is the initial step in the complex series of biochemical and physiological processes, termed the 'amyloid cascade.' A $\beta$  accumulation is thought to result from an imbalance between its production via the amyloidogenic processing of the amyloid peptide precursor and its removal from the brain through various clearance pathways and enzyme-mediated degradation. Impaired clearance of A $\beta$  has been proposed to play a key role in the development of sporadic forms of AD<sup>260,261</sup>. Therefore, lowering A $\beta$  concentrations in the brain constitutes a potential therapeutic approach to protect against amyloidopathy and cognitive decline and/or delay the onset of AD<sup>262</sup>. During this preclinical period, the activity of several critical proteins, regulated by multiple inherited or environmental factors, participates in homeostasis of A $\beta$ .

Because the process by which AD develops can take years-to-decades, identifying factors that can even slightly alter the pathogenic process could lead to delayed onset and reduced disease severity.

### *Neurobiological Effects of GHB on AD Pathogenesis*

Pharmacologic doses of SXB have been shown to induce multiple effects that could have a beneficial impact on limiting AD pathophysiology, including HDACs inhibition, gene expression of A $\beta$ -degrading brain protease, neprilysin, and SWS induction<sup>263</sup>.

Research by Klein *et al.*<sup>264</sup> evaluated the effects of auto-administration of SXB to APPSWE mice who express high concentrations of the mutant A $\beta$ , develop significant amyloid plaques, and display memory deficits. SXB induced the overexpression of brain neprilysin, reduced cerebral A $\beta$  contents, counteracted phosphoramidon-induced brain neprilysin inhibition and A $\beta$  accumulation, and prevented cognitive deficits. Because SXB doses used in this study were clinically relevant, data suggested that chronic oral administration of GHB or its analogs may be considered for strategies against presymptomatic or established AD.

### *Sleep and AD*

Compared to cognitively normal older adults, sleep disruption is more pronounced in older individuals with mild cognitive impairment (MCI) or AD—with over 60% having at least one clinical sleep disorder—or in individuals at high genetic risk for developing AD (APOE4+ carrier)<sup>265,266</sup>.

Sleep disturbance appears to be one of the earliest observable symptoms of AD, often present even before diagnosis<sup>266</sup>. Mounting evidence indicates that sleep disruption has a causal and bi-directional relationship with AD pathophysiology. This is substantiated by the fact that insomnia and sleep apnea are simultaneously features of, and risk factors for MCI and AD, decreasing their onset age<sup>267,268</sup>, conversely, superior sleep quality or successful treatment of sleep disturbances

can protect cognitive function and decrease the risk of developing MCI and AD<sup>268–270</sup>. Importantly, poor sleep correlates with the severity of cortical A $\beta$  burden and with CSF measures of A $\beta$ , both in cognitively normal older adults and in MCI and AD patients<sup>271–273</sup>.

NREM sleep seems to be of special relevance to the pathophysiology of AD, since cognitive impairment in AD patients is most significantly associated with poor NREM sleep quality<sup>271,272</sup>. NREM SWS decline is accelerated in AD patients relative to age-matched controls, with the magnitude of sleep disruption and the severity of AD appearing to be correlated<sup>271,274,275</sup>. Also, the levels of tau and A $\beta$  protein in the CSF of AD patients predict the degree of reduced SWS time and the decreases in sleep efficiency and REM sleep<sup>271</sup>. Importantly, the experimental increase in cortical A $\beta$  was shown to lead to a fragmentation of NREM sleep<sup>276,277</sup>, while the experimental decrease in NREM sleep and increase in wake time was shown to escalate A $\beta$  production and its cortical deposition<sup>276</sup>.

NREM sleep promotes the clearance of extracellular A $\beta$  that accumulates during wakefulness<sup>278</sup>; disrupted NREM SWS and excess wakefulness may therefore increase A $\beta$  accumulation, which itself impairs NREM SWS, thereby promoting its own accumulation<sup>279</sup>. NREM sleep disturbance can also impair cellular repair processes and the regulation of oxidative stress, enhancing the metabolic distress and oxidative damage induced by wakefulness<sup>280,281</sup>; oxidative stress in turn promotes A $\beta$  accumulation<sup>282</sup>, which further promotes oxidative stress<sup>283</sup>, leading to an amplification of A $\beta$  accumulation through another positive feedback loop. A $\beta$  aggregation then triggers increased sleep disruption, further feeding this vicious cycle that accelerates AD pathogenesis. By being associated with A $\beta$  accumulation, sleep disruption can also be linked to the AD-associated progression of cognitive decline. CSF A $\beta$  (and tau and orexin) levels correlate with both sleep and cognitive measures, supporting the association between sleep, AD pathophysiology and cognitive decline<sup>271</sup>.

Although the precise mechanism through which A $\beta$  disrupts NREM sleep is unknown, it has been proposed that it may be associated with a disruption of NMDA and GABA<sub>A</sub> receptor function<sup>284–285</sup> leading to a reduction in the generation of low-frequency (<1 Hz) slow oscillations of NREM sleep<sup>286</sup>.

#### *Pharmacologically-Induced SWS to Ameliorate AD Pathogenesis*

Since sleep is a modifiable factor, it can also be a target for AD therapy. The increasingly evident association between sleep disturbances and AD pathophysiology suggests that therapeutic interventions aiming at restoring NREM SWS may be beneficial by increasing A $\beta$  clearance, decreasing metabolic distress, promoting cellular repair processes, regulating oxidative stress, and, consequently, delaying cognitive decline.

Given the growing evidence of the impact of sleep disturbances on AD pathophysiology, the ability of GHB to improve sleep quantity and quality in both healthy and clinical populations, particularly in promoting SWS and delta power<sup>173,174</sup>, may be of interest in the context of AD therapy. In humans, NREM

sleep induced by GHB treatment seems to be beneficial for the consolidation of declarative memory<sup>287</sup>. Also, pharmacological studies have shown that therapeutic doses of GHB elicit a substantial GABAergic potentiation in the brain<sup>288</sup>, which, in turn, has been shown to significantly attenuate the severe SWS changes observed in a mouse model of AD<sup>289</sup>.

The effects of GHB in increasing NREM SWS<sup>46</sup> could potentially attenuate the vicious cycle of A $\beta$  accumulation and AD progression promoted by poor NREM SWS<sup>278,279</sup>. However, this possibility still needs to be adequately studied so that a proper insight into the role of GHB in hampering AD pathogenesis can be obtained.

#### **4.2.5 Narcolepsy**

GHB has a three decade history and the most robust clinical dataset of all drugs assessed in the treatment of narcolepsy<sup>290,291</sup>. Broughton and Mamelak<sup>172</sup> and Mamelak *et al.*<sup>174</sup> initially hypothesized that GHB could reduce sleep fragmentation in narcolepsy through its known effects on increased sleep consolidation, SWS augmentation and REM sleep facilitation. Their seminal combined work in patients with narcolepsy led to the beginnings of an understanding of GHB as a potent treatment not only for improving sleep, but also for controlling cataplexy and enhancing daytime alertness<sup>174,290</sup>.

Further work by Mamelak *et al.*<sup>292</sup>, Scharf *et al.*<sup>293</sup>, Scrima *et al.*<sup>294</sup>, Lammers *et al.*<sup>295</sup>, and others provided long-term, controlled evidence supporting the effectiveness of GHB in narcolepsy. Recently, extensive research, through multiple large controlled and open-label studies—including a comparative study with modafinil in the treatment of daytime sleepiness—has led to an understanding of GHB as a primary and unparalleled treatment for the complex of narcolepsy symptoms: sleepiness, cataplexy and sleep fragmentation. The following sections focus only on the sleep and wakefulness (i.e., impact on excessive daytime sleepiness) data generated from the work of multiple researchers.

##### **4.2.5.1 Initial Exploration**

The initial exploration of GHB by Broughton and Mamelak<sup>290,291</sup> in 20 subjects with narcolepsy proved promising in improving sleep continuity and reducing daytime sleepiness in narcolepsy. PSG analysis revealed statistically significant changes in sleep, including increased SWS duration and sleep efficiency, and reduced stage 1 sleep, sleep fragmentation, REM sleep latency and REM sleep density with treatment compared with baseline. Additionally, daytime sleep duration and number of sleep episodes were significantly reduced.

Scharf *et al.*<sup>293</sup> conducted a treatment trial of GHB, enrolling 30 patients (17 women and 13 men) in an open-label study comparing pretreatment baseline measures with those after 1 month and 6 months of treatment. By the end of the first week of treatment, a statistically significant improvement over baseline was evident in daytime sleep attacks. Sleep attacks were reduced by 70% of baseline at this time. All symptoms were found to improve still further until the end of the 6-

month observation period. PSG at 1 month versus baseline revealed significantly increased total sleep time, sleep efficiency and percentage of time spent in SWS; significant reductions in total wake time, awakenings and REM sleep latency were also noted. Changes in nocturnal sleep observed at 4 weeks continued throughout the 6 months of treatment, as demonstrated by PSG in a subset of 12 patients who underwent repeat PSG at 6 months.

Long-term open-label data were collected in a combined effort by Mamelak, Scharf and Woods and reported in the mid-1980s<sup>292</sup>. This report included experience of 48 patients (21 men and 27 women) treated with GHB 4.5–9 g for durations of 6 months to 9 years. Thirty-six of the 48 patients (75%) became symptom free over time, with GHB given as monotherapy in some patients and the addition of low-dose dexamphetamine or methylphenidate (<30 mg/day) in others. An additional six patients experienced an incomplete but clinically meaningful response, while the remaining six patients reported a lack of response, which resulted in discontinuing treatment. No patients discontinued treatment because of adverse effects.

#### 4.2.5.2 Early Placebo-Controlled Trials

In 1989 and 1990, Scrima *et al.*<sup>294,296</sup> published the first reports of a blinded, placebo-controlled trial of low-dose GHB in narcolepsy. This U.S. government-sponsored study employed a 4-week (29-day) crossover design with a 6-day washout period. Twenty patients (ten men and ten women) were randomized to treatment with the relatively low dose of GHB (50 mg/kg) or placebo per night during the first period. After the washout period, patients were transferred to the contrasting treatment for 29 days in a double-blind fashion. By the end of week 1, subjective 'arousals from sleep' and hypnagogic hallucinations had decreased significantly in patients receiving GHB compared with in those receiving placebo ( $p=0.035$  and  $p=0.008$ , respectively). This improvement continued for the duration of treatment. Objective measures of sleep were also significantly influenced by GHB administration. Specifically, decreased stage 1 sleep ( $p=0.012$ ), increased stage 3 sleep ( $p=0.008$ ), increased delta (stages 3 and 4 combined) sleep ( $p=0.049$ ), fewer stage shifts ( $p=0.002$ ) and fewer awakenings ( $p=0.006$ ) occurred after GHB versus placebo.

Despite substantial effects on nocturnal sleep, low-dose GHB did not significantly improve measures of daytime alertness (both subjective and objective) compared with placebo in this study. These measures included the Stanford Sleepiness Scale (SSS), the number of sleep attacks per day, the number of naps per day and the multiple sleep-latency test (MSLT). However, a trend toward reduced sleepiness on the MSLT ( $p=0.074$ ) as well as a significant increase in MSLT wakefulness ( $p=0.03$ ) and a significant reduction in sleep-onset REM episodes ( $p=0.020$ ) during MSLT on the final day (day 29) of GHB versus placebo was seen.

Lammers *et al.*<sup>295</sup> initiated a similar blinded, placebo-controlled, crossover study of low-dose GHB in 24 patients (13 men and 11 women) with narcolepsy and cataplexy. Nocturnal sleep-related subjective and objective (PSG) measures

showed a response generally consistent with the previously mentioned controlled and uncontrolled studies. A marked reduction in hypnagogic hallucinations ( $p=0.008$ ), an increase in SWS (stages 3 and 4,  $p=0.053$ ), a reduction in REM-sleep awakenings ( $p=0.016$ ) and a decrease in time spent awake during REM sleep ( $p=0.007$ ) was observed during GHB treatment compared with placebo. Of interest, all parameters of subjective daytime sleepiness were substantially improved, despite the low dose of GHB used. Patient-reported number of sleep attacks ( $p=0.001$ ) and severity of daytime sleepiness ( $p=0.028$ ) were reduced. MSLT results, however, were not significantly different from placebo, although only seven subjects had adequate MSLT data for analysis.

The FDA solicited the participation of Orphan Medical in providing the necessary research and development work required for regulatory approval of GHB for medicinal use in narcolepsy. At the time, no medication had undergone the rigorous evaluation required for approval in cataplexy treatment. The following section summarizes the substantial exploration of GHB conducted under the direction of Orphan Medical in approximately 1000 patients with narcolepsy.

#### 4.2.5.3 Multicenter Clinical Trials

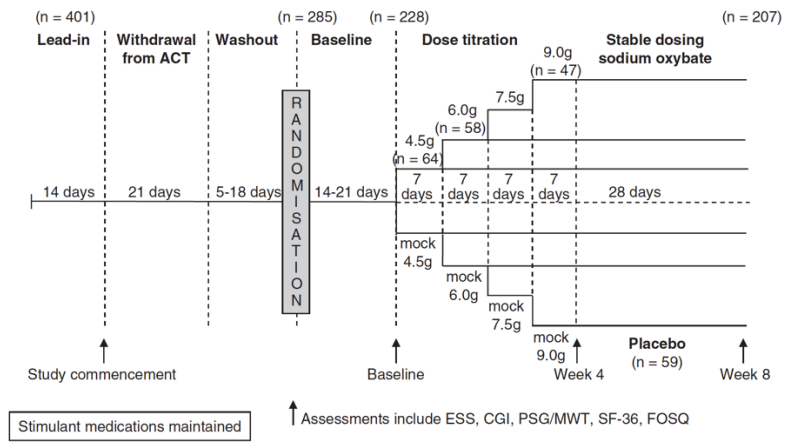
A large number of multicenter clinical trials of SXB in the treatment of narcolepsy have been sponsored by Orphan Medical in the past decade. The results of these trials provide definitive and essential information on the efficacy of this agent in excessive daytime sleepiness and nocturnal sleep fragmentation, as well as on its tolerability and safety profile. The results of the most relevant multicenter clinical trials, at the time of writing, are summarized in this section.

The assessment of the impact of SXB on subjective measures of daytime sleepiness was assessed in a 4-week placebo-controlled, multicenter trial in which 136 patients with narcolepsy were randomized to receive SXB 3, 6 or 9 g or placebo nightly for 4 weeks<sup>175</sup>. A clear dose-related improvement on all measures assessed, including the ESS and the number of inadvertent naps/sleep attacks, was observed. These effects reached statistical significance at the 9 g dose for both measures ( $p = 0.001$  and  $p = 0.012$ , respectively). An open-label extension trial monitored these patients for another 12 months after the initial 4-week double-blind period. This study included bi-monthly Epworth Sleepiness Scale (ESS) measures of sleepiness<sup>297</sup>. Mean ESS for patients before enrolling in the preceding placebo-controlled trial was 18 and was significantly improved to 13 ( $p < 0.001$ ) during the 1-year period, with little fluctuation over time and no evidence of tachyphylaxis.

To further explore the dose relationship of SXB-induced effects on night-time sleep and daytime sleepiness, a subsequent multicenter study subjected patients to a 'forced' dose titration of SXB<sup>173</sup>. Twenty-five patients (7 men and 18 women) were evaluated during a baseline period, then administered SXB 4.5 g for 4 weeks followed by 6 g for 2 weeks, then 7.5 g for 2 weeks, and then 9.0 g for 2 weeks. Measures assessed included PSG, ESS and the 20-minute Maintenance of Wakefulness Test (20-min MWT). The main findings of this trial included a robust, dose-related and statistically significant increase in SWS duration at doses of 7.5 g

and 9 g, and in delta power (a measure of the rate of occurrence of approximately 0.5–4Hz EEG activity coupled with the amplitude of the waves in this frequency range) across all doses compared with baseline. Additionally, REM sleep latency was significantly decreased, and REM sleep duration significantly increased during the first night of administration of SXB 4.5 g. In contrast, with longer administration, the shortened REM sleep latency abated and total REM sleep duration decreased in a dose-dependent manner, so that at a dose of 9 g, REM sleep duration was moderately but statistically significantly reduced relative to baseline. No consistent effects were seen on sleep latency, total sleep duration, stage 1 or 2 sleep duration, or the number of awakenings, across all doses, in this study.

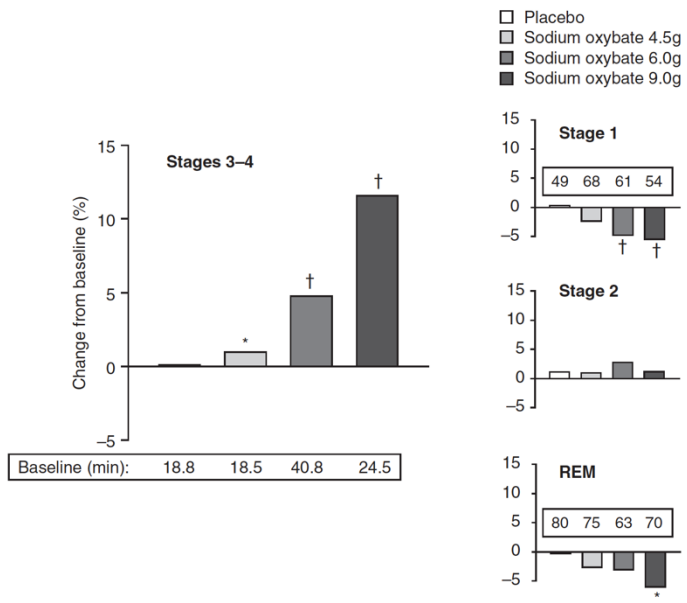
Subjective sleepiness showed significant improvement, as ESS group means decreased (improved) with all doses of SXB ( $p<0.001$ ) compared with baseline. The study participants, when receiving the 7.5 g ( $p<0.001$ ) and 9 g ( $p<0.001$ ) doses, showed significant improvement compared with pre-baseline assessment. Objective sleep-latency measures during the 20-min MWT also evidenced robust improvement with both 7.5 g ( $p<0.01$ ) and 9 g ( $p<0.001$ ) compared with baseline. MWT group means more than doubled at the 9 g dose (baseline = 4.5 minutes, 9 g = 10.6 minutes). Results of this study parallel those found by Broughton and Mamelak<sup>290</sup> and Mamelak *et al.*<sup>174</sup> almost 30 years ago. The effects of SXB on nocturnal sleep and excessive daytime sleepiness were again tested in a multicenter, placebo-controlled, parallel-group trial. This study evaluated 228 patients for 8 weeks<sup>298</sup>, with doses of 4.5 g, 6 g and 9 g (Figure 4).



**FIGURE 4.** Design of an 8-week randomised, double-blind, placebo-controlled, parallel-group trial comparing the effects of three doses (4.5, 6.0 and 9.0g nightly in two evenly-divided doses 2.5–4 hours apart) of orally administered sodium oxybate with placebo in 228 patients diagnosed with narcolepsy.<sup>298</sup>

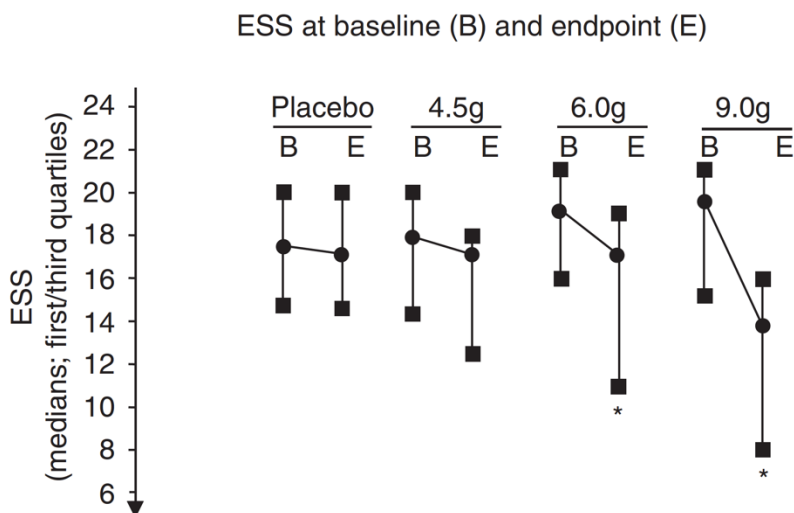
As expected from the results of prior studies, SXB had a significant and remarkable effect on nighttime sleep (Figure 5): SWS (4.5 g,  $p<0.05$ ; 6 g and 9 g,  $p<0.001$ ) and

delta power (4.5 g,  $p=0.006$ ; 6 g and 9 g,  $p<0.001$ ) were significantly increased at all doses of SXB compared with placebo. REM sleep duration was again moderately decreased ( $p<0.05$ ) at the 9 g dose only. A significant decrease in the number of awakenings ( $p<0.05$ ) and stage 1 sleep ( $p<0.001$ ) occurred at the 6 g and 9 g doses and a modest increase in total sleep duration ( $p<0.05$ ) was observed at the 9 g dose. Improvement in measures of sleepiness was again consistent with previous research.



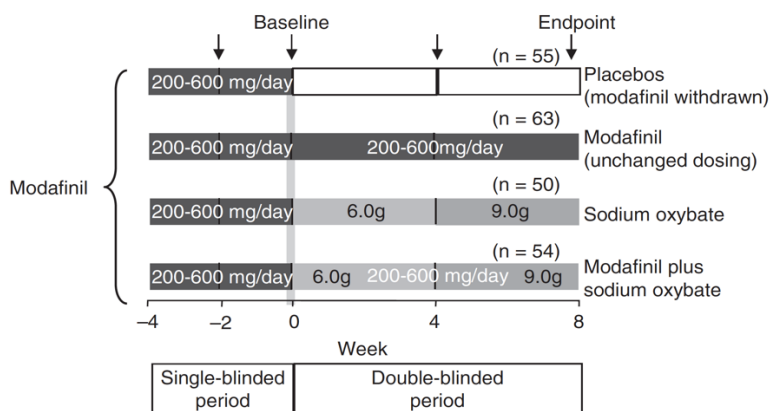
**FIGURE 5.** Effect of sodium oxybate on polysomnographically recorded sleep stages in 193 patients with narcolepsy.<sup>298</sup>

Subjective sleepiness showed significant improvement at both 6 g and 9 g via decreased (improved) ESS (Figure 6) and number of inadvertent naps/sleep attacks ( $p<0.001$  for both measures on both doses.). Additionally, sleepiness improved, with a 10-minute MWT increase from 7.6 minutes at baseline to 17.7 minutes at 9 g ( $p<0.001$ ); this magnitude of improvement for a drug taken at night on daytime sleepiness is unusual and unprecedented.



**FIGURE 6.** Effect of sodium oxybate on subjective sleepiness, as assessed using the Epworth Sleepiness Scale (ESS), in 228 patients with narcolepsy.<sup>298</sup>

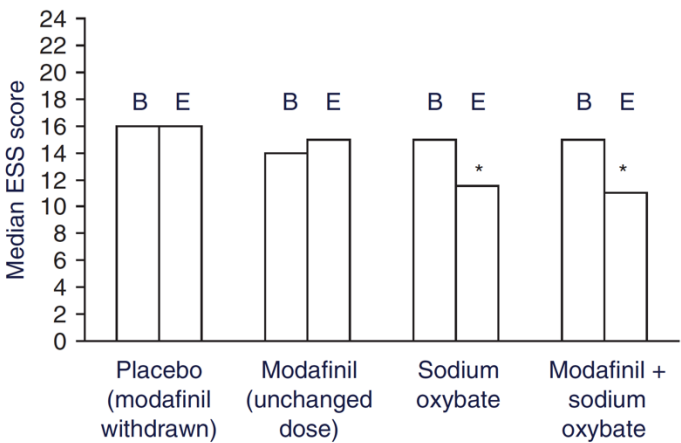
Ultimately, a comparative study was designed to evaluate the effect of SXB on daytime sleepiness compared with modafinil<sup>299</sup>. This double-blind placebo-controlled (double-dummy) trial compared SXB alone, modafinil alone, modafinil in combination with sodium oxybate, and placebo (Figure 7) in 222 patients with narcolepsy with or without comorbid cataplexy for an 8-week treatment period.



**FIGURE 7.** Design of an 8-week randomised, double-blind, double-dummy, placebo-controlled, parallel-group, multicentre trial (trial 4) comparing the effects of orally administered sodium oxybate and modafinil with placebo in the treatment of daytime sleepiness and sleep fragmentation in narcolepsy.<sup>299</sup>

Patients entering the study who had been receiving stable, effective and tolerable doses of modafinil for daytime sleepiness were randomized to one of these four

treatment groups. Statistically significant improvement in daytime sleepiness, as measured by ESS (Figure 8) and the number of inadvertent naps/sleep attacks, was found in the group of patients receiving SXB alone compared with modafinil alone. The combination therapy group had the greatest overall improvement in subjective and objective measures of sleepiness.



**FIGURE 8.** Mean change in sleep latency on the Epworth Sleepiness Scale (ESS) after 8 weeks of treatment with placebo, modafinil, sodium oxybate, or a combination of modafinil and sodium oxybate in patients with narcolepsy.<sup>299</sup>

Throughout these multicenter clinical trials, the most common reported adverse events in SXB recipients included dose-related headache, nausea, dizziness and somnolence.

**5. Conclusions**

**5.1 Neurobiology**

GHB is an endogenous short-chain fatty acid synthesized locally within the CNS, mostly from its parent compound GABA. Approximately 1–2% of GABA converts to GHB, which is relatively rapidly converted into CO<sub>2</sub> and H<sub>2</sub>O through the Krebs cycle. GHB for exogenous administration was first synthesized in the early 1960s and found to readily cross the blood-brain barrier into the CNS, where it displays distinct pharmacological effects. Evidence suggests a role for GHB as a neuromodulator/neurotransmitter, as GHB is synthesized in neurons heterogeneously distributed throughout the CNS, stored in vesicles, released via potassium-dependent depolarization into the synaptic cleft, and undergoes reuptake into the nerve terminal. Under endogenous conditions and concentrations, and depending on the cell group affected, GHB may increase or decrease neuronal activity by inhibiting the release of the primary co-localized neurotransmitter. For example, GHB may decrease neuronal activity when inhibiting the release of the excitatory neurotransmitter dopamine and increase neuronal activity when inhibiting the release of the inhibitory neurotransmitter GABA. After exogenous administration, it is likely that GHB acts at GHB binding

site(s) and GABA<sub>B</sub> receptors, although it appears that most of the behavioral effects are mediated through the GABA<sub>B</sub> receptor. On neurons, supraphysiological concentrations of GHB have a qualitatively different effect than endogenous GHB concentrations. These elevated levels, mostly acting through GABA<sub>B</sub> modulation on various neuron groups, decrease neuronal activity. On washout from supraphysiological concentrations, increased neuronal responsiveness has been observed. This activity may underlie the sleep modulation seen when GHB is administered before nighttime sleep onset and, conversely, the wakefulness stimulating effects observed during the day following nighttime administration.

## **5.2 Clinical Effects**

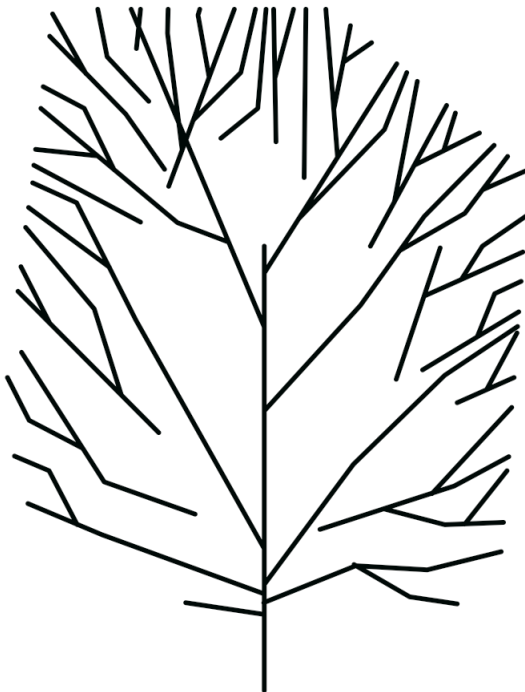
GHB, administered in the form of SXB, modulates sleep in healthy subjects, and sleep and wakefulness in clinical populations, including groups with insomnia, fibromyalgia and narcolepsy. In narcolepsy, the results of large, multicenter trials corroborate earlier work and demonstrate a consistent effect of SXB on SWS activity, yielding substantial, dose-related increases in SWS duration and delta power. Additionally, dose-related reductions in stage 1 sleep and number of awakenings are apparent in the larger studies, as well as modest increases in total sleep duration and reductions in REM sleep duration at a dose of 9 g. Multiple measures of daytime sleepiness demonstrated consistent short- and long-term improvement when SXB was administered in combination with stimulant therapy or as the only wake-promoting treatment. In addition, compared with modafinil, SXB as monotherapy appears to produce equal or greater improvement in daytime sleepiness in patients with narcolepsy with, or without, co-morbid cataplexy.

## **CHAPTER 3**

### **Sodium Oxybate for Excessive Daytime Sleepiness in Parkinson Disease - An Open-Label Polysomnographic Study**

*Archives of Neurology (2008) 65(10): 1337-1340*

**Authors:** William G. Ondo, Thomas Perkins, Todd Swick, Keith L. Hull Jr, J. Ernesto Jimenez, Tippy S. Garriss and Daniel Pardi



**Abstract**

|                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Background</b>                    | Many patients with Parkinson disease (PD) have excessive daytime sleepiness and numerous nocturnal sleep abnormalities.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Objective</b>                     | To determine the safety and efficacy of the controlled drug sodium oxybate in a multicenter, open-label, polysomnographic study in subjects with PD and sleep disorders.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Design, Setting, and Patients</b> | Inclusion required an Epworth Sleepiness Scale (ESS) score greater than 10 and any subjective nocturnal sleep concern, usually insomnia. An acclimation and screening polysomnogram was performed to exclude subjects with sleep-disordered breathing. The following evening, subjects underwent another polysomnogram, followed by an evaluation with the Unified Parkinson Disease Rating Scale (UPDRS) while practically defined off (“off”) PD medications, ESS (primary efficacy point), Pittsburgh Sleep Quality Inventory, and Fatigue Severity Scale. Subjects then started sodium oxybate therapy, which was titrated from 3 to 9 g per night in split doses (at bedtime and 4 hours later) across 6 weeks and returned for subjective sleep assessments. They then returned at 12 weeks after initiating therapy for a third polysomnogram, an off-medication UPDRS evaluation, and subjective sleep assessments. Data are expressed as mean (SD). |
| <b>Results</b>                       | We enrolled 38 subjects. At screening, 8 had sleep apnea (n=7) or depression (n= 1). Twenty-seven of 30 subjects completed the study. Three dropped out owing to dizziness (n=3) and concurrent depression (n = 1). The mean dose of sodium oxybate was 7.8 (1.7) g per night. The ESS score improved from 15.6 (4.2) to 9.0 (5.0) (P<.001); the Pittsburgh Sleep Quality Inventory score, from 10.9 (4.0) to 6.6 (3.9) (P<.001); and the Fatigue Severity Scale score, from 42.9 (13.2) to 36.3 (14.3) (P<.001). Mean slow-wave sleep time increased from 41.3 (33.2) to 78.0 (61.2) minutes (P = .005). Changes in off-medication UPDRS scores were not significant, from 28.3 (10.3) to 26.2 (9.6).                                                                                                                                                                                                                                                       |
| <b>Conclusion</b>                    | Nocturnally administered sodium oxybate improved excessive daytime sleepiness and fatigue in PD.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Trial Registration:</b>           | clinicaltrials.gov Identifier: NCT00641186                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

## Introduction

Parkinson's Disease (PD) is strongly associated with the following 2 broad categories of sleep abnormalities: excessive daytime sleepiness (EDS) and nocturnal sleep dysfunction. Excessive daytime sleepiness has been well demonstrated using the subjective Epworth Sleepiness Scale (ESS) and objective polysomnography (PSG), including multiple sleep latency testing. The consequences of EDS, however, are sometimes difficult to segregate clearly from fatigue, lethargy, and depression, all of which are also common in PD.

Excessive daytime sleepiness in PD has generally been associated with greater age, more advanced disease, and dopaminergic drug use.<sup>1-5</sup> Therefore, both PD and its treatment can cause EDS. Nocturnal sleep in PD is also markedly abnormal. Documented problems include fragmented sleep with multiple arousals and/or full awakenings associated with rigidity, dystonia, tremor, pain, sialorrhea, and nocturia<sup>6-8</sup>; rapid eye movement sleep behavior disorder<sup>9-11</sup>; periodic limb movements<sup>12,13</sup>; restless legs syndrome<sup>14</sup>; and sleep apnea.<sup>15</sup> Some of these may precede the motor symptoms of PD by many years. Intrinsic changes in sleep architecture are less marked but include reduced slow-wave sleep (SWS) and reduced sleep spindles.<sup>16,17</sup> Although it seems intuitive, studies have not confirmed a correlation between nocturnal sleep dysfunction and EDS in PD.<sup>16,18</sup> Relatively little therapeutic research has addressed these problems. Modafinil<sup>19-21</sup> has been demonstrated to have some benefit for EDS in patients with PD, although the improvement was modest. To our knowledge, no reported therapeutic studies have carefully evaluated a specific treatment for nocturnal sleep problems in the PD population.

Sodium oxybate (Xyrem; Jazz Pharmaceuticals, Inc.) is a unique compound approved by the US Food and Drug Administration for the treatment of cataplexy and EDS in patients with narcolepsy.<sup>22,23</sup> Owing to the potential for abuse, especially when mixed with alcohol (this is a salt of gamma-hydroxybutyrate, the "date rape drug"), use of sodium oxybate is restricted through a central pharmacy registry. Sodium oxybate is a metabolite of 7-aminobutyric acid, although it may be an independent endogenous neurotransmitter, with a very short half-life and short clinical effect, usually 2.5 to 4.0 hours. Therefore, 2 doses are used to achieve a typical full night of sleep, one at initiation of sleep and the other 4 hours later. Polysomnographic studies show a consistent increase in SWS in subjects with normal sleep<sup>24</sup> and in those with sleep abnormalities.<sup>25</sup> We evaluated the use of sodium oxybate for EDS in subjects with PD in a multicenter, open-label, PSG study.

## Methods

Subjects were recruited from the Baylor College of Medicine Parkinson Disease Center and Movement Disorder Clinic and Raleigh Neurology Associates. The protocol was approved by the Baylor College of Medicine Institutional Review Board and the Western Institutional Review Board. All subjects signed informed consent.

We enrolled subjects with PD, aged 30 to 75 years, with Hoehn and Yahr stages 1.5 to 4.0 during periods while practically defined off ("off") medication, Mini-Mental State Examination scores of greater than 24, ESS scores of greater than 10, and a patient report of unsatisfactory sleep. This could include any sleep concern, but always resulted in some insomnia. The subjects could not be taking medications with known central nervous system-depressant properties and had been receiving stable PD medications for at least 30 days before and throughout the study. We excluded subjects with serious medical conditions, including renal insufficiency or congestive heart failure, depression (Beck Depression Inventory score, > 16), or known sleep apnea or narcolepsy.

The subjects underwent a screening/acclimation PSG. They were subsequently excluded if they had more than mild sleep apnea (apnea/hypopnea index >15) and oxygen desaturation levels consistently below 90%. Obstructive apneas were scored as 10 seconds of more than 90% airflow reduction with continued respiratory effort. Obstructive hypopneas were scored as 10-second epochs of more than 30% airflow reduction associated with either a 3% oxygen desaturation or an electro-encephalographic arousal.

Within 7 days, the subjects underwent the entry PSG. They returned to the clinic the following morning without taking their usual PD medications (off-medication state) and underwent assessment with the Unified Parkinson Disease Rating Scale (UPDRS). After taking their PD medications, they completed the Fatigue Severity Scale (FSS), Pittsburgh Sleep Quality Inventory (PSQI), the 36-Item Short Form Health Survey quality-of-life assessment, and the ESS (primary efficacy point). The subjects then started sodium oxybate therapy, 4.5 g per night, to be taken in 2 equally divided doses: 2.25 g (4.5 mL) at bedtime, and 2.25 g (4.5 mL) 2.5 to 4.0 hours later. They woke naturally or set an alarm for their second dose. A follow-up telephone call 1 week later reviewed medication adherence and any possible adverse effects. The subjects were examined after 2 weeks of therapy with reevaluations of the ESS score, vital signs, and adverse events. The dose was increased to 6 g per night, to be taken in 2 equally divided doses. After another follow-up telephone call and according to the clinical judgment of site investigators, the dose was increased weekly by 1.5-g increments to a maximum nightly dose of 9.0 g. In the event that adverse effects developed with the higher doses, the dose could be reduced to a tolerated level for the remainder of the trial. The final clinic visit (study day 56) included a final PSG, followed by an off-medication UPDRS evaluation, then a repeated battery of tests after PD medication therapy was restored. The subjects returned all unused drug.

The primary efficacy point was change in the ESS score (daytime sleepiness). Other measures of daytime symptoms (the ESS score) and nocturnal symptoms (the polysomnogram and PSQI) were secondary measures. Statistics included descriptive calculations and paired, 2-tailed t tests. Significance was set at  $P < .05$ . Study design, data management, database design, statistical analysis, and manuscript drafting were all performed by the primary investigator (W.G.O.), who maintains ownership of the data. The primary investigator received an

Investigational New Drug exemption from the US Food and Drug Administration for the study. Unless otherwise specified, data are expressed as mean (SD).

## Results

Thirty-eight subjects with PD were enrolled to achieve 30 successful screenings. We excluded 8 subjects at screening secondary to sleep apnea criteria ( $n = 7$ ) and depression ( $n = 1$ ). Three subjects dropped out after randomization for dizziness ( $n = 3$ ) and concurrent depression ( $n = 1$ ); two of these dropped out before any follow-up data were collected and were therefore excluded from the efficacy analysis. The 6-week subjective sleep data in the third subject were included as the last observation carried forward.

The mean age of the 30 subjects (of whom 24 were men) was 61.5 (8.7) years, and the duration of PD was 8.6 (5.5) years (range, 1-25 years). The Hoehn and Yahr stages were 2.0 ( $n = 14$ ), 2.5 ( $n = 11$ ), and 3.0 ( $n = 5$ ). Twenty-seven subjects were white, 2 were Hispanic, and 1 was Asian. The mean entry Mini-Mental State Examination score was 29.1 (1.3) (range, 25-30). All subjects were treated with a dopamine agonist without levodopa ( $n = 8$ ), levodopa without a dopamine agonist ( $n = 3$ ), or levodopa and a dopamine agonist ( $n = 19$ ). Six of the subjects taking levodopa and a dopamine agonist also took a monoamine oxidase type B inhibitor; 10 subjects took a catechol-O-methyl-transferase inhibitor; and 11 subjects took amantadine hydrochloride.

The mean final dose of sodium oxybate was 7.8 (1.7) g per night. The final nightly doses were 3.0 g ( $n = 2$ ), 4.5 g ( $n = 1$ ), 6.0 g ( $n = 6$ ), 7.5 g ( $n = 4$ ), and 9.0 g ( $n = 17$ ).

The ESS, PSQI, and FSS scores improved significantly. Changes in the 36-Item Short Form Health Survey score were not significant (**Table 1**).

Slow-wave sleep time increased in 27 subjects, ( $P = .005$ ) (**Table 2**), whereas rapid eye movement sleep time was modestly reduced. Total apneas mildly increased, but the mean and maximum oxygen desaturation values did not change. No other PSG features changed significantly. Increased SWS time (in minutes) did not correlate with reduced ESS scores ( $r = 0.10$ ;  $P = .09$ ).

Mean off-medication morning UPDRS motor scores were stable in 27 subjects, changing from 28.4 (10.3) to 26.2 (9.6) (NS). No subject subjectively reported that there was any meaningful change in his or her motor symptoms.

Adverse events probably or definitely related to the drug included dizziness ( $n = 3$ ), nocturia/enuresis ( $n = 3$ ), nausea ( $n = 1$ ), daytime sleepiness ( $n = 1$ ), reduced alertness ( $n = 1$ ), and rebound morning tremor ( $n = 1$ ). One subject reported increased morning tremor. Additional adverse events that were considered not related to the study drug included constipation ( $n = 1$ ), 1 delusions ( $n = 1$ ), and, in a single subject, bradycardia, anxiety, depression, and edema. Twenty-two of 30 subjects (73%) reported no adverse events.

At the study's conclusion, 18 of 27 subjects (67%) completed application forms for the central distribution pharmacy to continue sodium oxybate therapy.

**TABLE 1.** Sleep and Fatigue Results<sup>a</sup>

| Instrument | Mean (SD) Score               |                              | P Value |
|------------|-------------------------------|------------------------------|---------|
|            | Before Sodium Oxybate Therapy | After Sodium Oxybate Therapy |         |
| ESS        | 15.6 (4.2)                    | 9.0 (5.0)                    | <.001   |
| PSQI       | 10.9 (4.0)                    | 6.6 (3.9)                    | <.001   |
| FSS        | 42.9 (13.2)                   | 36.3 (14.3)                  | <.001   |
| SF-36      | 95.7 (7.1)                    | 92.3 (5.1)                   | .71     |

Abbreviations: ESS, Epworth Sleepiness Scale; FSS, Fatigue Severity Scale; PSQI, Pittsburgh Sleep Quality Inventory; SF-36, 36-Item Short Form Health Survey.

<sup>a</sup>Findings are reported for 28 subjects.

**TABLE 2.** PSG Results in 27 Subjects with Parkinson Disease

|                                          | Mean (SD) Findings            |                              | P Value |
|------------------------------------------|-------------------------------|------------------------------|---------|
|                                          | Before Sodium Oxybate Therapy | After Sodium Oxybate Therapy |         |
| Total sleep time, min                    | 363 (65)                      | 353 (74)                     | .11     |
| Stage 1, min                             | 40 (45)                       | 34 (44)                      | .23     |
| Stage 2, min                             | 215 (88)                      | 197 (78)                     | .27     |
| Stages 3-4, min                          | 41 (33)                       | 78 (61)                      | .005    |
| REM sleep, min                           | 57 (39)                       | 37 (24)                      | .002    |
| Sleep efficiency, %                      | 75 (14)                       | 74 (15)                      | .29     |
| Total No. of PLMs                        | 14 (14)                       | 22 (30)                      | .13     |
| Total apnea /hypopnea index <sup>a</sup> | 7 (6)                         | 13 (12)                      | .004    |
| Mean oxygen saturation level, %          | 93.9 (2.5)                    | 94.1 (2.3)                   | .68     |
| Minimum oxygen saturation level, %       | 88.3 (3.5)                    | 87.0 (3.6)                   | .44     |
| No. of awakenings                        | 57 (42)                       | 59 (77)                      | .69     |

Abbreviations: PLMs, periodic limb movements; PSG, polysomnography; REM, rapid eye movement. <sup>a</sup>Calculation of the index is explained in the "Methods" section.

## Comment

Overall, nocturnally administered sodium oxybate was well tolerated, increased SWS, and improved subjective nighttime and daytime sleep problems and daytime fatigue in subjects with PD. Improvements in ESS were similar to or better than those found when the drug is used as therapy for narcolepsy.<sup>22,23</sup> The PD motor features were unchanged.

The mechanism by which sodium oxybate improves EDS is not known. It is known to increase SWS;<sup>26</sup> however, our study did not show a significant correlation between improved SWS and ESS scores. The SWS change in our study compared only two nights and is variable. In addition, our study was not powered in any way to specifically address this question, so we cannot entirely exclude the possibility of SWS variations. Sodium oxybate has also been postulated to improve EDS by decreasing sleep fragmentation in narcolepsy trials<sup>25</sup>; however, we found improved EDS without reduced awakenings in this PD study. Furthermore, deep brain stimulation of the subthalamic nucleus improves sleep fragmentation associated with nocturnal motor abnormalities in subjects with PD but has not improved EDS in a small number of studied subjects.<sup>27</sup>

As an alternative explanation, nocturnal sodium oxybate use may result in the rebound vigilant state observed during the day after nighttime administration. The short half-life of sodium oxybate allows for complete washout by the morning, when increased release of stored dopamine and norepinephrine may occur and contribute to the observed enhanced wakefulness.<sup>28,29</sup> Dopamine release is actually inhibited while the drug is active, which may account for the nonsignificant increase in periodic limb movements.<sup>30</sup> We did not believe that the slight increase in apnea was clinically meaningful, but this needs to be monitored. There was no evidence of abuse.

This study has all of the shortcomings of any open-label trial. We intentionally designed broad inclusion criteria and did not exclude subjects with restless legs syndrome or rapid eye movement sleep behavior disorder, neither of which appears to affect EDS in PD.<sup>14</sup> Given the robust efficacy and good tolerability of the study drug and the lack of effective treatment for EDS in patients with PD, we believe that controlled trials using objective measures of daytime sleepiness are justified.

## Author Contributions

Study concept and design: Ondo. Acquisition of data: Ondo, Perkins, Swick, Hull, Jimenez, Garriss, and Pardi. Analysis and interpretation of data: Ondo. Drafting of the manuscript: Ondo. Critical revision of the manuscript for important intellectual content: Ondo, Perkins, Swick, Hull, Jimenez, Garriss, and Pardi. Statistical analysis: Ondo. Administrative, technical, and material support: Ondo, Perkins, Swick, Hull, Jimenez, Garriss, and Pardi. Obtained funding: Ondo. Study supervision: Ondo.

## Financial Disclosure

Dr. Ondo has received research grant support from Jazz Pharmaceuticals, Inc; is a member of the speaker's bureau for Allergan, Boehringer Ingelheim, GlaxoSmithKline, TEVA, UCB Pharma, and Valeant; and has received research funding from Allergan, Boehringer Ingelheim, Forest, Schwartz Pharmaceuticals, and Valeant. Dr. Perkins is a member of the speaker's bureau for Boehringer Ingelheim, Cephalon, GlaxoSmithKline, and Jazz Pharmaceuticals, Inc; and has received research support and has served on the professional advisory board for Jazz Pharmaceuticals, Inc. Dr. Swick is a member of the speaker's bureau for Boehringer Ingelheim, Cephalon, GlaxoSmithKline, Jazz Pharmaceuticals, Inc, Sanofi-Aventis, Sepracor, and Takeda Pharmaceuticals; has received research support from Cephalon, GlaxoSmithKline, Jazz Pharmaceuticals, Inc, Merck, Pfizer, Sanofi-Aventis, Somazon, and Takeda; and has served on the professional advisory board for Jazz Pharmaceuticals, Inc. Mr. Pardi is an employee of Jazz Pharmaceuticals, Inc, and provided assistance in the analysis of the data and the review of the manuscript. Funding/Support: This study was supported by an unrestricted research grant and supply of the study drug from Jazz Pharmaceuticals, Inc.

## References

1. Arnulf I. Excessive daytime sleepiness in parkinsonism. *Sleep Med Rev.* 2005;9 (3):185-200.
2. Hobson DE, Lang AE, Martin WR, Razmy A, Rivest J, Fleming J. Excessive daytime sleepiness and sudden-onset sleep in Parkinson disease: a survey by the Canadian Movement Disorders Group. *JAMA.* 2002;287(4):455-463.
3. Ondo WG, Dat Vuong K, Khan H, Atassi F, Kwak C, Jankovic J. Daytime sleepiness and other sleep disorders in Parkinson's disease. *Neurology.* 2001;57 (8):1392-1396.
4. Tandberg E, Larsen JP, Karlsen K. Excessive daytime sleepiness and sleep benefit in Parkinson's disease: a community-based study. *Mov Disord.* 1999;14 (6):922-927.
5. Rye DB. The two faces of Eve: dopamine's modulation of wakefulness and sleep. *Neurology.* 2004;63(8)(suppl 3):S2-S7.
6. Askenasy JJ, Yahr MD. Parkinsonian tremor loses its alternating aspect during non-REM sleep and is inhibited by REM sleep. *J Neurol Neurosurg Psychiatry.* 1990;53(9):749-753.
7. Lees AJ, Blackburn NA, Campbell VL. The nighttime problems of Parkinson's disease. *Clin Neuropharmacol.* 1988;11(6):512-519.

8. van Hilten B, Hoff JJ, Middelkoop HA, et al. Sleep disruption in Parkinson's disease: assessment by continuous activity monitoring. *Arch Neurol.* 1994;51(9):922-928.
9. Boeve BF, Silber MH, Parisi JE, et al. Synucleinopathy pathology and REM sleep behavior disorder plus dementia or parkinsonism. *Neurology.* 2003; 61(1):40-45.
10. Comella CL, Nardine TM, Diederich NJ, Stebbins GT. Sleep-related violence, injury, and REM sleep behavior disorder in Parkinson's disease. *Neurology.* 1998; 51(2):526-529.
11. Wetter TC, Trenkwalder C, Gershanik O, Högl B. Polysomnographic measures in Parkinson's disease: a comparison between patients with and without REM sleep disturbances. *Wien Klin Wochenschr.* 2001;113(7-8):249-253.
12. Högl B, Rothdach A, Wetter TC, Trenkwalder C. The effect of cabergoline on sleep, periodic leg movements in sleep, and early morning motor function in patients with Parkinson's disease. *Neuropsychopharmacology.* 2003;28(10):1866-1870.
13. Wetter TC, Collado-Seidel V, Pollmacher T, Yassouridis A, Trenkwalder C. Sleep and periodic leg movement patterns in drug-free patients with Parkinson's disease and multiple system atrophy. *Sleep.* 2000;23(3):361-367.
14. Ondo WG, Vuong K, Jankovic J. Exploring the relationship between Parkinson disease and restless legs syndrome. *Arch Neurol.* 2002;59(3):421-424.
15. Diederich NJ, Vaillant M, Leischen M, et al. Sleep apnea syndrome in Parkinson's disease: a case-control study in 49 patients. *Mov Disord.* 2005;20(11): 1413-1418.
16. Rye DB, Bliwise D, Dihenia B, Gurecki P. Daytime sleepiness in Parkinson's disease. *J Sleep Res.* 2000;9(1):63-69.
17. Rye DB, Jankovic J. Emerging views of dopamine in modulating sleep/wake state from an unlikely source: PD. *Neurology.* 2002;58(3):341-346.
18. Arnulf I, Konofal E, Merino-Andreu M, et al. Parkinson's disease and sleepiness: an integral part of PD. *Neurology.* 2002;58(7):1019-1024.
19. Adler CH, Caviness JN, Hentz JG, Lind M, Tiede J. Randomized trial of modafinil for treating subjective daytime sleepiness in patients with Parkinson's disease. *Mov Disord.* 2003;18(3):287-293.

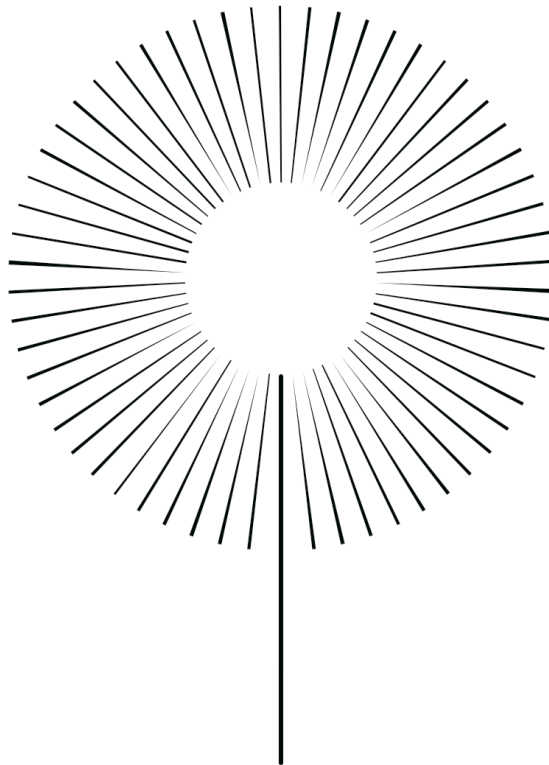
20. Högl B, Saletu M, Brandauer E, et al. Modafinil for the treatment of daytime sleepiness in Parkinson's disease: a double-blind, randomized, crossover, placebo-controlled polygraphic trial. *Sleep*. 2002;25(8):905-909.
21. Ondo WG, Fayle R, Atassi F, Jankovic J. Modafinil for daytime somnolence in Parkinson's disease: double blind, placebo controlled parallel trial. *J Neurol Neurosurg Psychiatry*. 2005;76(12):1636-1639.
22. U.S. Xyrem Multicenter Study Group. A 12-month, open-label, multicenter extension trial of orally administered sodium oxybate for the treatment of narcolepsy. *Sleep*. 2003;26(1):31-35.
23. Black J, Houghton WC. Sodium oxybate improves excessive daytime sleepiness in narcolepsy. *Sleep*. 2006;29(7):939-946.
24. Lapierre O, Montplaisir J, Lamarre M, Bedard MA. The effect of gamma-hydroxybutyrate on nocturnal and diurnal sleep of normal subjects: further considerations on REM sleep-triggering mechanisms. *Sleep*. 1990;13(1):24-30.
25. Mamelak M, Black J, Montplaisir J, Ristanovic R. A pilot study on the effects of sodium oxybate on sleep architecture and daytime alertness in narcolepsy. *Sleep*. 2004;27(7):1327-1334.
26. Boero JA, Duntley SP, Jennifer M. Sodium oxybate improves slow wave sleep and daytime sleepiness in narcolepsy [abstract]. *Sleep*. 2006;29(suppl):A229.
27. Lyons KE, Pahwa R. Effects of bilateral subthalamic nucleus stimulation on sleep, daytime sleepiness, and early morning dystonia in patients with Parkinson disease. *J Neurosurg*. 2006;104(4):502-505.
28. Hechler V, Gobaille S, Bourguignon JJ, Maitre M. Extracellular events induced by gamma-hydroxybutyrate in striatum: a microdialysis study. *J Neurochem*. 1991; 56(3):938-944.
29. Szabo ST, Gold MS, Goldberger BA, Blier P. Effects of sustained gamma-hydroxybutyrate treatments on spontaneous and evoked firing activity of locus coeruleus norepinephrine neurons. *Biol Psychiatry*. 2004;55(9):934-939.
30. Abril B, Carlander B, Touchon J, Dauvilliers Y. Restless legs syndrome in narcolepsy: a side effect of sodium oxybate? *Sleep Med*. 2007;8(2):181-183.

## **CHAPTER 4**

### **The Nightly Administration of Sodium Oxybate Results in Significant Reduction in the Nocturnal Sleep Disruption of Patients with Narcolepsy**

*Sleep Medicine (2009) 10(8): 829-835*

**Authors:** Jed Black, Daniel Pardi, Carl S. Hornfeldt and Neil Inhaber



**Abstract**

|                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Background</b> | Previous studies indicate that nightly sodium oxybate administration reduces nocturnal sleep disruption in narcolepsy. The present study provided an opportunity to further characterize these sleep-related effects in patients with narcolepsy during treatment with sodium oxybate as monotherapy or in combination with modafinil.                                                                                                                                                                                                                |
| <b>Methods</b>    | This double-blind, placebo-controlled study enrolled 278 patients with narcolepsy taking modafinil 200–600 mg daily for the treatment of excessive daytime sleepiness (EDS). Following a baseline polysomnogram (PSG) and Maintenance of Wakefulness Test (MWT), patients were randomized to receive treatment with: (1) placebo, (2) sodium oxybate, (3) modafinil, or (4) sodium oxybate + modafinil. PSGs and MWTs were repeated after 4 and 8 weeks. Other efficacy measures included Epworth Sleepiness Scale scores and daily diary recordings. |
| <b>Results</b>    | After 8 weeks, significant changes in sleep architecture among patients receiving sodium oxybate and sodium oxybate/modafinil included a median increase in Stage 3 and 4 sleep (43.5 and 24.25 min, respectively) and delta power and a median decrease in nocturnal awakenings (6.0 and 9.5, respectively). No significant changes in PSG parameters were noted in patients treated with placebo or modafinil alone.                                                                                                                                |
| <b>Conclusion</b> | In addition to its established efficacy for the treatment of cataplexy and EDS, nightly sodium oxybate administration significantly reduces measures of sleep disruption and significantly increases slow-wave sleep in patients with narcolepsy.                                                                                                                                                                                                                                                                                                     |

## **Introduction**

Early descriptions of narcolepsy included symptoms of excessive daytime sleepiness (EDS) and attacks of muscle weakness. The symptom subset was later expanded to include the so-called “tetrad” of symptoms, consisting of EDS, cataplexy, hypnagogic hallucination, and sleep paralysis [1,2]. Disrupted nocturnal sleep has long been recognized as a common clinical finding in these patients [3] and was formally added to the clinical description of narcolepsy in 1975 [4].

Investigations using nocturnal polysomnography (PSG) have consistently demonstrated pathological changes in the nocturnal sleep of patients with narcolepsy, including sleep-onset REM periods, increased Stage 1 sleep, diminished Stage 3 and 4 (slow wave) sleep, numerous and sometimes prolonged awakenings after sleep onset, and frequent stage shifts [3,5-8]. Fragmented sleep patterns are also observed in canine [9,10] and rodent [11,12,13] models of narcolepsy.

Therapies for disrupted nocturnal sleep in narcolepsy have consisted primarily of short-acting benzodiazepines [14] and sedating antidepressants [15]. These provide limited benefit and do not improve the daytime symptoms of the disease. Further, traditional pharmacotherapy for the daytime symptoms of narcolepsy has provided little benefit for disrupted nocturnal sleep [16].

In contrast, several investigators over many years have observed that the nightly administration of sodium oxybate improves subjective and objective measures of nocturnal sleep, as well as daytime symptoms, in patients with narcolepsy [6,17-21]. These observations led to larger controlled evaluations of sodium oxybate for the treatment of nocturnal sleep disruption, in addition to cataplexy and EDS, in patients with narcolepsy [22-26].

One of these studies demonstrated that 8 weeks of nightly sodium oxybate administration resulted in robust increases in Stage 3 and 4 sleep and delta power, while the frequency of nocturnal awakenings significantly decreased [26]. These changes in nocturnal sleep quality were associated with significant decreases in the severity and frequency of cataplexy and EDS [25,26].

While the primary efficacy measure in the present study was the change in EDS as measured by the Maintenance of Wakefulness Test (MWT), this study permitted further characterization of the effects of nightly sodium oxybate administration on PSG parameters over an 8-week period. The beneficial effect of sodium oxybate administration on EDS in these patients has been published elsewhere [27].

## **Methods**

### *Subjects*

Enrollment criteria included age 18 years or older, current diagnosis of narcolepsy [28], stable therapy with modafinil (200–600 mg/day) for the treatment

of EDS for  $\geq 1$  month prior to the trial, willingness to forego driving or other hazardous activities if recommended by the investigator, and willingness to complete the study by signing an informed consent. Female patients were enrolled if they were surgically sterile, two years post-menopausal, or agreed to use a medically accepted method of birth control during the trial.

Specific exclusion criteria included the use of sodium oxybate or an investigational therapy  $\leq 30$  days prior to the trial; diagnosis of sleep apnea disorder or any other cause of daytime sleepiness; a physical or psychiatric illness that placed patients at risk or compromised the objectives of the trial; a history of a substance abuse disorder; serum creatinine  $>2.0$  mg/dL, AST or ALT  $>2$  times the upper limit of normal, or bilirubin  $>1.5$  times the upper limit of normal; a seizure disorder, head trauma, or past invasive intracranial surgery; or an occupation requiring rotating shifts or routine night shifts.

### *Trial medications*

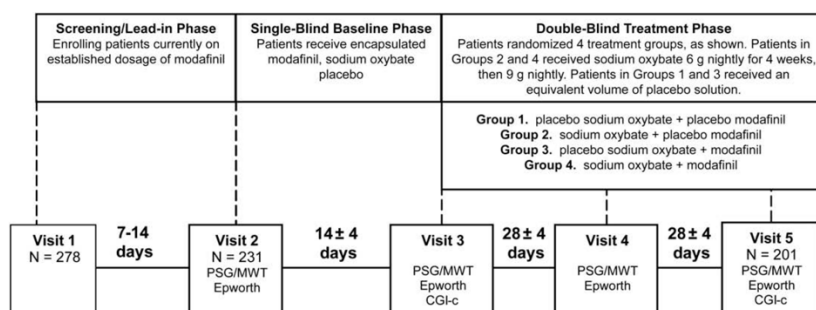
Trial medications included a concentrated solution of sodium oxybate 500 mg/mL (Xyrem<sup>®</sup>, Jazz Pharmaceuticals, Inc., Palo Alto, CA); sodium oxybate placebo consisted of a sodium citrate solution that was equimolar to sodium oxybate with respect to sodium. Modafinil tablets 200 mg (Provigil<sup>®</sup>, Cephalon Inc., West Chester, PA) were enclosed in lactose-filled gelatin capsules, and the modafinil placebo consisted of identical capsules containing lactose only. Prior testing demonstrated that modafinil tablet encapsulation did not alter the dissolution characteristics of the drug (Jazz Pharmaceuticals, Inc., data on file). Patients were cautioned about the use of potentially sedating medications, including alcoholic beverages, and were encouraged to discuss the use of all medications with the investigator.

### *Study design*

The study design is outlined in Figure 1. Trial candidates were evaluated for inclusion at Visit 1. During this visit, inclusion/exclusion criteria were reviewed; medical history, physical examination, and vital sign information were recorded; and samples were obtained for clinical laboratory testing. After providing informed consent, enrolled patients began daily diary training.

Clinic Visit 2 occurred 1–2 weeks later when overnight polysomnography (PSG) was performed, followed by the Maintenance of Wakefulness Test (MWT). PSGs were scored the following day to identify any co-morbid conditions causing daytime sleepiness, such as obstructive sleep apnea. Patients meeting inclusion criteria entered the 2-week single-blind baseline period and continued taking modafinil at their customary doses (between 200 and 600 mg/day). At this time, patients began taking single-blind placebo sodium oxybate solution in two equally divided doses: the first dose at bedtime, and the second 2.5–4 h later. At the end of the 2-week baseline period (Visit 3), baseline PSG and MWT measures were performed on all patients prior to beginning the treatment phase according to prior double-blind randomization:

- Group 1. *Placebo group*: placebo sodium oxybate + placebo modafinil
- Group 2. *Sodium oxybate group*: sodium oxybate + placebo modafinil
- Group 3. *Modafinil group*: placebo sodium oxybate + modafinil
- Group 4. *Sodium oxybate/modafinil group*: sodium oxybate + modafinil



**FIGURE 1.** Study design. At Visit 1, patients were evaluated for inclusion into the trial. After 1–2 weeks to allow review of hematology and blood chemistry results as well as diary training, patients returned to the clinic for Visit 2. While on stable doses of modafinil, patients underwent a PSG and MWT. The overnight PSG was immediately scored to rule out other conditions that could be primary causes of daytime sleepiness. Subsequently, each patient entered a 2-week baseline phase, remaining on modafinil while receiving placebo sodium oxybate solution in single-blind fashion. At Visit 3, patients were randomized into the four treatment groups as shown. The dose of sodium oxybate (or placebo equivalent) was increased from 6 g nightly to 9 g nightly after week 4. The efficacy and safety measures were performed as indicated, except patient diaries, which were maintained throughout the trial.

Patients randomized to Groups 3 and 4 continued to receive their unchanged modafinil dosage. Patients randomized to Groups 2 and 4 received sodium oxybate at a dose of 6 g nightly, administered in two equally divided doses (at bedtime and 2.5–4 h later) for the initial 4-week period of the study. Patients in Groups 1 and 3 received an equivalent volume of placebo sodium oxybate solution. After 4 weeks, patients returned for PSG and MWT (Visit 4). Patients then continued taking their prescribed modafinil dose; however, the dose of sodium oxybate was increased to 9 g nightly in two equally divided doses. Patients assigned to placebo sodium oxybate increased their placebo solution by an equivalent volume. All patients continued taking their assigned drug regimen for an additional 4 weeks before returning to the clinic for final efficacy and safety assessments at Visit 5. Patients were permitted to remain on stable doses of drug therapy for cataplexy, if needed, throughout the trial.

Efficacy and safety assessments were performed at Visits 2 through 5 and included the Epworth Sleepiness Scale (ESS) and a nocturnal overnight PSG followed by

MWT. The Clinical Global Impression of Severity (CGI-s) and the Clinical Global Impression of Change (CGI-c) scales were completed at Visits 3 and 5, respectively.

Patient diaries, used to collect information about the incidence of inadvertent daytime naps, trial medication use, concomitant medication use, and adverse events (AEs), were reviewed at each clinic visit.

### *Safety measures*

Safety assessments at Visits 1 and 5 included a physical examination and measurement of vital signs. Clinical laboratory tests were performed at a central laboratory and included hematology and clinical chemistry measures and a serum pregnancy test, if applicable. Clinically significant laboratory parameters that were outside the reference range of the central laboratory were repeated. An electrocardiogram was performed at Visit 1 and repeated at Visit 5 if clinically indicated according to the investigator.

All reported AEs were followed until resolution of the event. Any patient who received trial medication during the double-blind phase, but later chose to withdraw from the trial, provided a final measurement of vital signs and blood sample for clinical laboratory tests before discontinuation. If the early termination was due to an AE, the patient was followed until satisfactory resolution of the event occurred.

### *Statistics*

The primary endpoint analysis was conducted on the intent-to-treat (ITT) population. The primary analysis was an ITT analysis at Visit 5. The primary pair-wise comparisons of sodium oxybate and sodium oxybate/modafinil versus placebo were obtained using Dunnett's test. The secondary pair-wise comparisons of modafinil versus placebo did not include an adjustment for multiple comparisons. If data were unavailable for a patient, last observation carried forward analysis was used with last post-baseline observation available for that patient. Two-sided *p*-values were reported, and the level of significance was tested at 0.05.

In the analysis of safety data, AEs were summarized by treatment group, and their incidence was compared using Fisher's exact test. For laboratory data, the mean changes from baseline were compared across treatment groups using ANOVA. The significance of changes from baseline in laboratory parameters within each treatment group was evaluated with paired *t*-tests.

### *Ethics*

The study was conducted at 44 sites in the United States, Canada, Czech Republic, France, Germany, Netherlands, Switzerland, and United Kingdom. The protocol used in this study was approved by the Institutional Review Board/Ethics Committee of each participating trial center. Written informed consent was

obtained from each patient prior to initiation of the study. This study was conducted in accordance with the Helsinki Declaration, revised 1997.

## Results

Of 278 patients enrolled in the study, 231 were randomly assigned to one of the four treatment groups. The intent-to-treat (ITT) population consisted of 222 patients who received at least one dose of double-blind medication and provided efficacy data at Visit 3 (baseline), Visit 4, and/or Visit 5. Patient demographics of the ITT population are provided in Table 1.

**TABLE 1.** Patient demographics by treatment group (ITT population)

|                     | Placebo<br>(N = 55) | Sodium<br>oxybate<br>(N = 50) | Modafinil<br>(N = 63) | Sodium oxybate +<br>Modafinil<br>(N = 54) | Total<br>(N = 222) |
|---------------------|---------------------|-------------------------------|-----------------------|-------------------------------------------|--------------------|
| <b>Gender, N(%)</b> |                     |                               |                       |                                           |                    |
| <b>Male</b>         | 24 (44)             | 26 (52.0)                     | 32 (51)               | 25 (46)                                   | 107 (48)           |
| <b>Female</b>       | 31 (56)             | 24 (48.0)                     | 31 (49)               | 29 (54)                                   | 115 (52)           |
| <b>Race, N(%)</b>   |                     |                               |                       |                                           |                    |
| <b>White</b>        | 43 (78)             | 47 (94.0)                     | 57 (90)               | 48 (88)                                   | 195 (88)           |
| <b>Black</b>        | 11 (20)             | 2 (4.0)                       | 5 (8)                 | 5 (6)                                     | 21 (11)            |
| <b>Asian</b>        | 0                   | 1 (2.0)                       | 0                     | 0                                         | 1 (1)              |
| <b>Other</b>        | 1 (2)               | 0                             | 1 (2)                 | 3 (6)                                     | 5 (2)              |
| <b>Age (years)</b>  |                     |                               |                       |                                           |                    |
| <b>Mean (SD)</b>    | 41.0 (13.4)         | 35.1 (12.9)                   | 38.9 (15.6)           | 38.9 (15.9)                               | 38.6<br>(14.6)     |
| <b>Weight (kg)</b>  |                     |                               |                       |                                           |                    |
| <b>Mean (SD)</b>    | 84.7 (19.9)         | 81.8 (17.9)                   | 80.6 (15.1)           | 79.4 (16.9)                               | 81.6<br>(17.4)     |

### *Polysomnography parameters*

After 4 weeks of treatment (Visit 4), there was no significant change in total sleep time in either the sodium oxybate group or the sodium oxybate/modafinil group. The sodium oxybate group demonstrated significant increases in Stage 3 and 4 sleep ( $p = 0.030$ ) and decreases in REM sleep compared to placebo ( $p = 0.004$ ) (Table 2). The sodium oxybate/modafinil group demonstrated significant increases in Stage 3 and 4 sleep ( $p = 0.007$ ) as well as total non-REM sleep ( $p = 0.042$ ) and delta power ( $p = 0.012$ ) compared to placebo. The increase in total non-REM sleep closely matched the observed decreases in REM sleep ( $p = 0.005$ ) and Stage 1 sleep ( $p = 0.012$ ) for this treatment group versus the placebo group. In addition, there was a significant decrease in nocturnal awakenings in the sodium

oxybate/modafinil group compared to placebo ( $p = 0.029$ ). In the modafinil group, no changes in sleep parameters were observed.

**TABLE 2.** Measures of polysomnographic parameters<sup>a,b,c</sup> (Week 4, ITT population; median change)

|                                  | Placebo<br>(N = 55) | Sodium oxybate 6 g<br>(N = 50) | Modafinil<br>(N = 63) | Sodium oxybate 6 g +<br>Modafinil<br>(N= 54) |
|----------------------------------|---------------------|--------------------------------|-----------------------|----------------------------------------------|
| <b>Total sleep time (min)</b>    |                     |                                |                       |                                              |
| Baseline                         | 408                 | 427.5                          | 419                   | 407.5                                        |
| Endpoint                         | 408                 | 412.75                         | 419.75                | 411.5                                        |
| Change                           | -5.5                | -5                             | 3                     | 6.5                                          |
|                                  | –                   | NS                             | NS                    | NS                                           |
| <b>Total non-REM sleep (min)</b> |                     |                                |                       |                                              |
| Baseline                         | 327                 | 340                            | 332.75                | 324                                          |
| Endpoint                         | 332                 | 346                            | 330                   | 350                                          |
| Change                           | 0.25                | 15                             | 2.5                   | 22.5                                         |
|                                  | –                   | NS                             | NS                    | $p = 0.042$                                  |
| <b>Total REM sleep (min)</b>     |                     |                                |                       |                                              |
| Baseline                         | 73                  | 80.5                           | 78.75                 | 68.5                                         |
| Endpoint                         | 79                  | 54.25                          | 78.5                  | 54                                           |
| Change                           | 6.25                | -14.5                          | 1.5                   | -11.5                                        |
|                                  | –                   | $p = 0.004$                    | NS                    | $p = 0.005$                                  |
| <b>Stage 1 sleep (min)</b>       |                     |                                |                       |                                              |
| Baseline                         | 41.5                | 38.5                           | 40.5                  | 42.5                                         |
| Endpoint                         | 48.5                | 31.75                          | 37                    | 29.5                                         |
| Change                           | 3.25                | -9.5                           | -2                    | -11.5                                        |
|                                  | –                   | NS                             | NS                    | $p = 0.012$                                  |
| <b>Stage 2 sleep (min)</b>       |                     |                                |                       |                                              |
| Baseline                         | 252.5               | 267                            | 241.75                | 219                                          |
| Endpoint                         | 241                 | 253                            | 241.5                 | 222.5                                        |
| Change                           | -5.25               | 0.5                            | -1.5                  | 10                                           |
|                                  | –                   | NS                             | NS                    | NS                                           |
|                                  |                     |                                |                       |                                              |

|                                                            | Placebo<br>(N = 55) | Sodium oxybate 6 g<br>(N = 50) | Modafinil<br>(N = 63) | Sodium oxybate 6 g +<br>Modafinil<br>(N= 54) |
|------------------------------------------------------------|---------------------|--------------------------------|-----------------------|----------------------------------------------|
| <b>Stage 3 and 4 sleep (min)</b>                           |                     |                                |                       |                                              |
| Baseline                                                   | 18.5                | 13.5                           | 29                    | 43.75                                        |
| Endpoint                                                   | 24                  | 40                             | 26.5                  | 74.5                                         |
| Change                                                     | 0                   | 11                             | 1.5                   | 11.5                                         |
|                                                            | –                   | <i>p</i> = 0.030               | <i>NS</i>             | <i>p</i> = 0.007                             |
| <b>Delta power (<math>\mu V^2</math> / Hz)<sup>d</sup></b> |                     |                                |                       |                                              |
| Baseline                                                   | 77,166              | 86,801                         | 90,134                | 85,175                                       |
| Endpoint                                                   | 76,620              | 94,698                         | 81,197                | 108,548                                      |
| Change                                                     | –2326               | 10,474                         | –965                  | 12,292                                       |
|                                                            | –                   | <i>NS</i>                      | <i>NS</i>             | <i>p</i> = 0.012                             |
| <b>Nocturnal awakenings</b>                                |                     |                                |                       |                                              |
| Baseline                                                   | 30                  | 27                             | 30                    | 26.5                                         |
| Endpoint                                                   | 26                  | 24.5                           | 33.5                  | 22                                           |
| Change                                                     | –0.5                | –1                             | 1                     | –4                                           |
|                                                            | –                   | <i>NS</i>                      | <i>NS</i>             | <i>p</i> = 0.029                             |

*NS*, not significant. <sup>a</sup>Statistical significance was established compared to placebo.

<sup>b</sup>Expressed as medians following transformation of non-normal data. <sup>c</sup>Missing data were imputed using last observation carried forward. <sup>d</sup>Median average.

Following 8 weeks of treatment (Visit 5), there were no significant changes in total sleep time noted in any treatment group; however, compared to the placebo group, the sodium oxybate and sodium oxybate/modafinil groups each demonstrated significant increases in total non-REM sleep (for each, *p* < 0.001), specifically sleep Stage 3 and 4 (for each, *p* < 0.001) (Table 3). These increases again corresponded with reciprocal decreases in Stage 1 sleep for the sodium oxybate group (*p* < 0.001) and sodium oxybate/modafinil group (*p* = 0.004) and decreased total REM sleep in both groups (for each, *p* < 0.001). In addition, both groups displayed significant increases in delta power (for each, *p* < 0.001) and significant decreases in the number of nocturnal awakenings in sodium oxybate-treated (*p* = 0.008) and sodium oxybate/modafinil-treated patients (*p* = 0.014). Compared to placebo-treated patients, no changes in any measured parameter were observed in the patients randomized to receive continued modafinil treatment.

**TABLE 3.** Measures of polysomnographic parameters<sup>a,b,c</sup> (Week 8, ITT population; median change)

|                                  | Placebo<br>(N = 55) | Sodium oxybate 9 g (N = 50) | Modafinil<br>(N = 63) | Sodium<br>oxybate 9 g<br>+ Modafinil<br>(N= 54) |
|----------------------------------|---------------------|-----------------------------|-----------------------|-------------------------------------------------|
| <b>Total sleep time (min)</b>    |                     |                             |                       |                                                 |
| Baseline                         | 408                 | 427.5                       | 419                   | 407.5                                           |
| Endpoint                         | 411                 | 416.75                      | 410.5                 | 416.25                                          |
| Change                           | -0.5                | -4.5                        | 1                     | 7                                               |
|                                  | –                   | NS                          | NS                    | NS                                              |
| <b>Total non-REM sleep (min)</b> |                     |                             |                       |                                                 |
| Baseline                         | 327                 | 340                         | 332.75                | 324                                             |
| Endpoint                         | 324                 | 370.75                      | 328.5                 | 365.75                                          |
| Change                           | -1                  | 38                          | -3                    | 42.75                                           |
|                                  | –                   | $p < 0.001$                 | NS                    | $p < 0.001$                                     |
| <b>Total REM sleep (min)</b>     |                     |                             |                       |                                                 |
| Baseline                         | 73                  | 80.5                        | 78.75                 | 68.5                                            |
| Endpoint                         | 79                  | 43.75                       | 78.5                  | 48.75                                           |
| Change                           | 10                  | -38.5                       | 0.75                  | -26.5                                           |
|                                  | –                   | $p < 0.001$                 | NS                    | $p < 0.001$                                     |
| <b>Stage 1 sleep (min)</b>       |                     |                             |                       |                                                 |
| Baseline                         | 41.5                | 38.5                        | 40.5                  | 42.5                                            |
| Endpoint                         | 40.5                | 20                          | 40.5                  | 25.25                                           |
| Change                           | 1.5                 | -16                         | -0.5                  | -17                                             |
|                                  | –                   | $p < 0.001$                 | NS                    | $p = 0.004$                                     |
| <b>Stage 2 sleep (min)</b>       |                     |                             |                       |                                                 |
| Baseline                         | 252.5               | 267                         | 241.75                | 219                                             |
| Endpoint                         | 235.5               | 240.5                       | 251                   | 211.5                                           |
| Change                           | -8.25               | 3.5                         | -1.75                 | 9.5                                             |
|                                  | –                   | NS                          | NS                    | NS                                              |
| <b>Stage 3 and 4 sleep (min)</b> |                     |                             |                       |                                                 |
| Baseline                         | 18.5                | 13.5                        | 29                    | 43.75                                           |
| Endpoint                         | 25                  | 74                          | 37                    | 89.25                                           |

|                                                          | Placebo<br>(N = 55) | Sodium oxybate 9 g (N = 50) | Modafinil<br>(N = 63) | Sodium<br>oxybate 9 g<br>+ Modafinil<br>(N= 54) |
|----------------------------------------------------------|---------------------|-----------------------------|-----------------------|-------------------------------------------------|
| Change                                                   | 0                   | 43.5                        | 0.25                  | 24.25                                           |
|                                                          | –                   | $p < 0.001$                 | NS                    | $p < 0.001$                                     |
| <b>Delta power (<math>\mu V^2/Hz</math>)<sup>d</sup></b> |                     |                             |                       |                                                 |
| Baseline                                                 | 77,166              | 86,801                      | 90,134                | 85,175                                          |
| Endpoint                                                 | 74,833              | 105,910                     | 84,911                | 123,182                                         |
| Change                                                   | –3221               | 18,443                      | –639.9                | 21496                                           |
|                                                          | –                   | $p < 0.001$                 | NS                    | $p < 0.001$                                     |
| <b>Nocturnal awakenings</b>                              |                     |                             |                       |                                                 |
| Baseline                                                 | 30                  | 27                          | 30                    | 26.5                                            |
| Endpoint                                                 | 30                  | 21                          | 32                    | 18.5                                            |
| Change                                                   | –0.5                | –6                          | 1.5                   | –9.5                                            |
|                                                          | –                   | $p = 0.008$                 | NS                    | $p = 0.014$                                     |

NS, not significant. <sup>a</sup>Statistical significance was established compared to placebo.

<sup>b</sup>Expressed as medians following transformation of non-normal data. <sup>c</sup>Missing data were imputed using last observation carried forward. <sup>d</sup>Median average.

#### *Maintenance of Wakefulness Test and Epworth Sleepiness Score*

Patients who had been randomized to placebo demonstrated a significant decrease in MWT sleep latency at 8 weeks ( $p < 0.001$ ) once they had been switched to placebo following stable chronic modafinil treatment. Conversely, oxybate/modafinil-treated patients demonstrated a significant increase in MWT sleep latency ( $p < 0.001$ ), compared to baseline modafinil treatment. Patients assigned to receive either modafinil or sodium oxybate alone demonstrated no significant change in MWT sleep latency, compared to baseline modafinil treatment.

Only slight worsening of EDS, as indicated by increased ESS scores, was noted in placebo-treated patients ( $p = 0.011$ ) after discontinuing baseline modafinil, and ESS scores continued unchanged in the group that was randomized to continue modafinil treatment; however, sodium oxybate-treated patients and sodium oxybate/modafinil-treated patients experienced significant improvements in ESS scores (for each,  $p < 0.001$ ). There was no change in ESS scores in the group maintained on modafinil alone. A detailed discussion of the effects of sodium oxybate, modafinil, and sodium oxybate/modafinil on narcolepsy symptoms in this study has been previously reported [27].

## *Safety*

At least one AE was reported by 151 of 231 patients (65.4%) who entered the double-blind treatment phase of the study. Compared to the incidence of AEs reported in the sodium oxybate (60%), modafinil (54.0%), or placebo groups (69.6%), a somewhat greater number of AEs were reported in the combination sodium oxybate/modafinil group (78.9%). Among all patients, the most common treatment-emergent AEs were headache (15.2%), nausea (11.7%), dizziness (9.1%), nasopharyngitis (6.1%), vomiting (6.1%), and somnolence (5.6%). Of these, nausea, vomiting, and dizziness were statistically significantly different between treatment groups.

Nausea and vomiting occurred with the highest frequency in the sodium oxybate groups, while the incidence of dizziness was highest in the sodium oxybate/modafinil group. Statistically significant differences in AEs between treatment groups were also noted with tremor (4.8%) and paresthesia (2.6%), occurring more often in patients receiving sodium oxybate or sodium oxybate/modafinil, and upper respiratory tract infections (2.2%) occurring primarily in the placebo group.

The number of patients (*N*) who withdrew from the study early as the result of a treatment-emergent AE was greatest in the sodium oxybate/modafinil group (6) compared to the sodium oxybate (4), modafinil (2), or placebo groups (1). Serious AEs were reported by three patients and included abdominal pain (modafinil group), palpitations (placebo group), and a psychotic disorder due to a general medical condition (narcissistic personality disorder; sodium oxybate/modafinil group); however, the only event considered drug-related was the psychotic disorder. One patient receiving placebo reported a pregnancy (protocol deviation) after completing the trial.

## **Discussion**

A growing body of evidence indicates that nightly administration of sodium oxybate to patients with narcolepsy reduces nocturnal sleep disruption, a common clinical finding in this patient population. The therapeutic effects of sodium oxybate on sleep quality, initially shown to occur clinically in eight patients suffering from insomnia [29], were later demonstrated in thirty patients with narcolepsy [6,17]. These changes generally coincided with significant improvements in excessive daytime sleepiness and REM-related narcolepsy symptoms [18], and the findings have been replicated in small placebo-controlled clinical trials [19,21].

The results of a placebo-controlled study in 228 narcolepsy patients revealed that the administration of sodium oxybate at doses of 4.5, 6, or 9 g nightly for 8 weeks significantly increased the duration of Stage 3 and 4 sleep, corresponding with significant, dose-related decreases in Stage 1 sleep and REM sleep. Compared to placebo-treated patients, delta power was significantly increased in all dose-

groups [30]. These changes in nocturnal sleep were associated with significant improvements in daytime narcolepsy symptoms [25,26].

The current trial was designed to evaluate the efficacy of sodium oxybate, alone and in combination with modafinil, for the treatment of EDS in patients with narcolepsy. Although compared to placebo, sodium oxybate and modafinil each improved EDS as measured by MWT, and overall clinical condition as measured by clinical global impression of change [27], modafinil alone did not significantly impact nocturnal sleep architecture. In contrast, patients treated with sodium oxybate, either alone or in combination with modafinil, demonstrated significant increases in Stage 3 and 4 sleep after 4 weeks of treatment. In addition, sodium oxybate/modafinil-treated patients demonstrated significant increases in total non-REM sleep and delta power, while Stage 1 sleep and nocturnal awakenings decreased. Following an additional 4 weeks of treatment with sodium oxybate at the 9 g/night dose, the increases in Stage 3 and 4 sleep, total non-REM sleep, and delta power became even more robust and were statistically significant in both sodium oxybate groups, suggesting either a dose-dependent, time-dependent, or dose- and time-dependent effect.

Results from a separate study [unpublished observations] suggest that sodium oxybate may impact sleep in both a dose-dependent and time-on-drug dependent fashion. This study, however, was not designed to characterize any dose-related effects or treatment-duration effects of sodium oxybate on sleep. It remains unclear whether the observed changes in sleep architecture in this study are related to the dose (6 or 9 g/night) or duration (4 or 8 weeks) of sodium oxybate treatment or both. Additionally, it is not known if the observed impact of sodium oxybate on sleep-EEG activity represents pharmacologically-induced alterations in true sleep-related activity, effects representing anesthetic-like changes, or epiphenomenal EEG activity unrelated to either sleep or anesthesia.

The AEs reported by the patients enrolled in the current study were consistent with the known AE profiles of sodium oxybate and modafinil. As might be expected, the incidence of AEs occurred with greater frequency in patients randomized to receive sodium oxybate/modafinil, although this is a common treatment strategy in the clinical setting when neither agent alone adequately addresses EDS [31,32].

## **Conclusion**

This trial represents the first controlled study performed to evaluate sodium oxybate as a single agent for the treatment of excessive daytime sleepiness in narcolepsy. In addition to improvements in EDS, nocturnal PSG data revealed that the nightly administration of sodium oxybate was associated with changes in nighttime sleep suggestive of reduced nocturnal sleep disruption and improved sleep continuity, as indicated by significant decreases in nighttime awakenings and increases in Stage 3 and 4 sleep. While the daytime administration of stimulants improves daytime functioning by increasing alertness, they have not been shown to provide beneficial effects on nocturnal sleep. In addition to improving daytime

symptoms of EDS and cataplexy in patients with narcolepsy, sodium oxybate reduces the nocturnal sleep disruption of narcolepsy.

## **Disclosures**

Jed Black, MD is an employee of Actelion Pharmaceuticals, Ltd and has received research support from ALZA, Cephalon, GlaxoSmithKline, Jazz Pharmaceuticals, Merck, and Takeda and honoraria from Jazz Pharmaceuticals, GlaxoSmithKline, Boehringer-Ingelheim, Takeda, and UCB.

Carl S. Hornfeldt, PhD is a consultant to Jazz Pharmaceuticals. Neil Inhaber, MD is an employee of Jazz Pharmaceuticals and own shares of stock and stock options in the company. Daniel Pardi, MS is a former employee of Jazz Pharmaceuticals and owns shares of stock in the company.

All data were collected independently by the respective sites participating in this study. Data entry into the study database was performed by external contract services and the sponsor. Additional independent analysis of the data presented in this manuscript was performed by the authors.

## **Acknowledgments**

This study was sponsored by Jazz Pharmaceuticals, Palo Alto, CA, and conducted by the members of the Xyrem<sup>®</sup> International Study Group: Joshua Aaron, MD, Union Hospital Sleep Center, Elkton, MD; Arshad Abassi, MD, Ingham Center for Sleep & Alertness, Lansing, MI; Mansoor Ahmed, MD, Cleveland Sleep Center, Middlebrook Heights, OH; Isabelle Arnulf, MD, Federation des Pathologies du Sommeil, CHU Pitie Salpetriere, France; Claudio Bassetti, MD, Neurologische Poliklinik, Universitäts Spital Zürich, Zürich, Switzerland; Jed Black, MD, Stanford Sleep Disorders Clinic, Stanford, CA; Richard K. Bogan, MD, Palmetto Baptist Medical Center, Sleep Disorders Center, Columbia, SC; Brian J. Bohner, MD, Pulmonary and Critical Care Associates of Baltimore, Towson, MD; Collier, MD, Regional Center for Sleep Disorders, Sunrise Hospital & Medical Center, Las Vegas, NV; James Cook, MD, The Center for Sleep & Wake Disorders, Danville, IN; Yves Dauvilliers, MD, Service de Neurologie B, Hopital Gui de Chauliac, Montpellier, France; Bhupesh Dihenia, MD, Neurology & Sleep Center, Lubbock, TX; Karl Doghramji, MD, Thomas Jefferson University, Philadelphia, PA; Stephen Duntley, MD, Washington University Sleep Medicine Center, St. Louis, MO; Helene Emsellem, MD, Center for Sleep and Wake Disorders, Chevy Chase, MD; Milton Erman, MD and Roza Hayduk, MD, Pacific Sleep Medicine Services, San Diego, CA; Neil T. Feldman, MD, St. Petersburg Sleep Disorders Center, St. Petersburg, FL; H. Kenneth Fisher, MD, Pacific Sleep Medicine Services, Los Angeles, CA; June Fry, MD, PhD, Center for Sleep Medicine, Lafayette Hill, PA; Peter Geisler, MD, Psychiatrische Universitätsklinik, Regensburg, Germany; Steven Gorman, MD, Vermont Medical Sleep Disorders Center, Inc., Essex Junction, VT; Donald Greenblatt, MD, Strong Sleep Disorder Center, Rochester, NY; Christopher Hanning, MD, Leicester General Hospital, Leicester, United Kingdom; James Hansbrough, MD, PhD, Graves Gilbert Clinic, Bowling Green, KY; Dennis L. Hill, MD, Central Carolina Neurology & Sleep, Salisbury, NC; William C. Houghton, MD, and

Carl S. Hornfeldt, PhD, Orphan Medical, Inc., Minnetonka, MN; Robert Israel, MD, Sleep Disorder Center of Rochester, Rochester, NY; Thomas Kaelin, DO, Lowcountry Lung and Critical Care PA, Charleston, SC; Gert Jan Lammers MD PhD, Leiden University Medical Center, Leiden, the Netherlands; Bernard Levine, MD, Pulmonary Associates, PA, Phoenix, AZ; Mortimer Mamelak, MD, Brain & Sleep Diagnostic Center, Toronto, ON; Geert Mayer, MD, Hephata Klinik, Schwalmstadt-Treysa, Germany; David Mayleben, PhD, CRMA, Cincinnati, OH; Sona Nevsimalova, MD, DSc and Karel Sonka, MD, DSc, Charles University, Prague, Czech Republic; Ralph Pascualy, MD, Swedish Sleep Medicine Institute, Seattle, WA; A. Thomas Perkins, MD, PhD, Raleigh Neurology Associated PA, Raleigh, NC; Justine Petrie, MD, St. Jude Medical Center, Fullerton, CA; John Schneerson, MD, Papworth Hospital, Papworth Everard, Cambridge, United Kingdom; Richard Shubin, MD, Neuro-Therapeutics, Inc., Pasadena, CA; John Baldwin Smith, MD, Summit Sleep Disorder, Winston-Salem, NC; Veronica Sosa, MD, Sleep Disorders Research Center, Henry Ford Hospital, Detroit, MI; James C. Stevens, MD, Fort Wayne Neurology Center, Fort Wayne, IN; Richard Strobel, MD, Lehigh Valley Hospital Sleep Disorders Centers, Allentown, PA; Leon Sweer, MD, Capital Region Sleep Disorder Center of Carlisle, Carlisle, PA; Todd Swick, MD, The Houston Sleep Center, Houston, TX; David A. Thomas, MD, Sioux Valley Clinic, Sioux Falls, SD; Nora L. Walker, MD, Sleep Walker Sleep Disease Center & Diagnostics, San Antonio, TX; David Walker, DO, Sleep Diagnostics of Northern Michigan, Traverse City, MI; Timothy J. Walter, MD, Grove City Sleep Diagnostic Center, Grove City, OH; Arthur Walters, MD, Seton Hall University School of Graduate Medical Education, New Jersey Neuroscience Institute, JFK Medical Center, Edison, NJ; Patrick E. Whitten, MD, Peoria Pulmonary Associates, Peoria, IL; Adrian Williams, MD, St. Thomas Hospital, London, United Kingdom; David H. Winslow, MD, Sleep Medicine Specialists, Louisville, KY.

## References

1. Yoss R.E., and Daly D.D.: Criteria for the diagnosis of the narcolepsy syndrome. Proc Staff Meet Mayo Clin 1957; 32: pp. 320-328
2. Fromherz S., and Mignot E.: Narcolepsy research: past, present, and future perspectives. Arch Ital Biol 2004; 142: pp. 479-486
3. Rechtschaffen A., Wolpert E.A., Dement W.C., Mitchell S.A., and Fisher C.: Nocturnal sleep of narcoleptics. Electroencephalogr Clin Neurophysiol 1963; 15: pp. 599-609
4. Plazzi G., Serra L., and Ferri R.: Nocturnal aspects of narcolepsy with cataplexy. Sleep Med Rev 2008; 12: pp. 109-128
5. Montplaisir J., Billiard M., Takahashi S., Bell I.R., Guilleminault C., and Dement W.C.: Twenty-four-hour recording in REM-narcoleptics with special reference to nocturnal sleep disruption. Biol Psychiatry 1978; 13: pp. 73-89

6. Broughton R., and Mamelak M.: Effects of nocturnal gamma-hydroxybutyrate on sleep/waking patterns in narcolepsy–cataplexy. *Can J Neurol Sci* 1980; 7: pp. 23-31
7. Baker T.L., Guilleminault C., Nino-Murcia G., and Dement W.C.: Comparative polysomnographic study of narcolepsy and idiopathic central nervous system hypersomnia. *Sleep* 1986; 9: pp. 232-242
8. Folkerts M., Rosenthal L., Roehrs T., Krstevska S., Murlidhar A., Zorick F., et al: The reliability of the diagnostic features in patients with narcolepsy. *Biol Psychiatry* 1996; 40: pp. 208-214
9. Nishino S., Tafti M., Sampathkumaran R., Dement W.C., and Mignot E.: Circadian distribution of rest/activity in narcoleptic and control dogs: assessment with ambulatory activity monitoring. *J Sleep Res* 1997; 6: pp. 120-127
10. Nishino S., Riehl J., Hong J., Kwan M., Reid M., and Mignot E.: Is narcolepsy a REM sleep disorder? Analysis of sleep abnormalities in narcoleptic Dobermans. *Neurosci Res* 2000; 38: pp. 437-446
11. Beuckmann C.T., Sinton C.M., Williams S.C., Richardson J.A., Hammer R.E., Sakurai T., et al: Expression of a poly-glutamine-ataxin-3 transgene in orexin neurons induces narcolepsy-cataplexy in the rat. *J Neurosci* 2004; 24: pp. 4469-4477
12. Mieda M., Willie J.T., Hara J., Sinton C.M., Sakurai T., and Yanagisawa M.: Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. *Proc Natl Acad Sci USA* 2004; 101: pp. 4649-4654
13. Zhang S., Lin L., Kaur S., Thankachan S., Blanco-Centurion C., Yanagisawa M., et al: The development of hypocretin deficiency in ataxin-hypocretin transgenic rats. *Neuroscience* 2007; 148: pp. 34-43
14. Overeem S., Mignot E., van Dijk J.G., and Lammers G.J.: Narcolepsy: clinical features, new pathophysiologic insights and future perspectives. *J Clin Neurophysiol* 2001; 18: pp. 78-105
15. Hublin C.: Narcolepsy. Current drug treatment options. *CNS Drugs* 1996; 5: pp. 426-436
16. Rogers A.E., Aldrich M.S., and Caruso C.C.: Patterns of sleep and wakefulness in treated narcoleptic subjects. *Sleep* 1994; 17: pp. 590-597

17. Broughton R., and Mamelak M.: The treatment of narcolepsy–cataplexy with nocturnal gamma-hydroxybutyrate. *Can J Neurol Sci* 1979; 6: pp. 1-6
18. Scharf M.B., Brown D., Woods M., Borwn L., and Hirschowitz J.: The effects and effectiveness of  $\gamma$ -hydroxybutyrate in patients with narcolepsy. *J Clin Psychiatry* 1985; 46: pp. 222-225
19. Scrima L., Hartman P.G., Johnson F.H., and Hiller F.C.: Efficacy of gamma-hydroxybutyrate versus placebo in treating narcolepsy–cataplexy: double blind subjective measures. *Biol Psychiatry* 1989; 26: pp. 331-343
20. Scrima L., Hartman P.G., Johnson F.H., Thomas E.E., and Hiller F.C.: The effects of  $\gamma$ -hydroxybutyrate on the sleep of narcolepsy patients: a double-blind study. *Sleep* 1990; 13: pp. 479-490
21. Lammers G.J., Arends J., Declerck A.C., Ferrari M.D., Schouwink G., and Troost J.: Gamma-hydroxybutyrate and narcolepsy: a double-blind placebo-controlled study. *Sleep* 1993; 16: pp. 216-220
22. US Xyrem Multicenter Study Group. A randomized, double blind, placebo-controlled multicenter trial comparing the effects of three doses of orally administered sodium oxybate with placebo for the treatment of narcolepsy. *Sleep* 2002;25:42–9.
23. US Xyrem Multicenter Study Group. A 12-month, open-label, multi-center extension trial of orally administered sodium oxybate for the treatment of narcolepsy. *Sleep*, 2003;26:31–5.
24. US Xyrem Multicenter Study Group. Sodium oxybate demonstrates long-term efficacy for the treatment of cataplexy in patients with narcolepsy. *Sleep Med* 2004;5:119–23.
25. Xyrem International Study Group. Further evidence supporting the use of sodium oxybate for the treatment of cataplexy: a double-blind, placebo-controlled study in 228 patients. *Sleep Med* 2005;6:415–21.
26. Xyrem International Study Group. A double-blind, placebo-controlled, study demonstrates the nightly administration of sodium oxybate significantly improves excessive daytime sleepiness in narcolepsy patients taking concurrent stimulant medications. *J Clin Sleep Med* 2005;1:391–7.
27. Black J., and Houghton W.C.: Sodium oxybate improves excessive daytime sleepiness in narcolepsy. *Sleep* 2006; 29: pp. 939-946

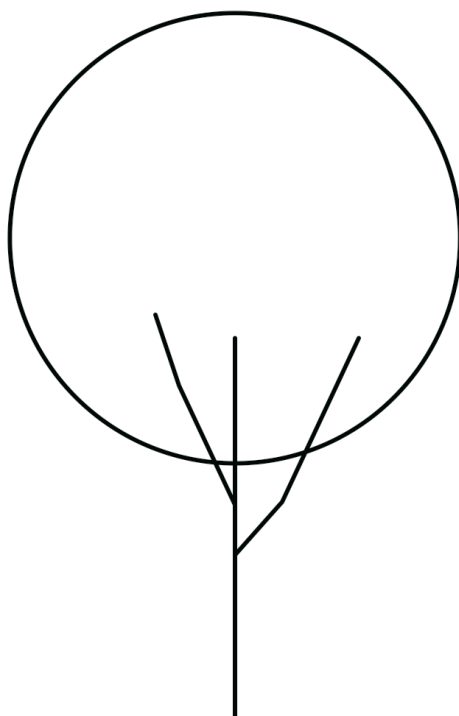
28. International classification of sleep disorder. 2nd ed. Diagnostic and coding manual. Rochester, MN: American Academy of Sleep Medicine; 2005.
29. Mamelak M., Escriu J.M., and Stokan O.: The effects of gamma-hydroxybutyrate on sleep. *Biol Psychiatry* 1977; 12: pp. 273-288
30. Black J, Pardi D, Hornfeldt C, Inhaber N. The nightly use of sodium oxybate is associated with significant improvements in nocturnal sleep: a double-blind, placebo-controlled study in patients with narcolepsy. Unpublished manuscript.
31. Wise M.S., Arand D.L., Auger R.R., Brooks S.N., and Watson N.F.: Treatment of narcolepsy and other hypersomnias of central origin. *Sleep* 2007; 30: pp. 1712-1727
32. Thorpy M.: Therapeutic advances in narcolepsy. *Sleep Med* 2007; 8: pp. 427-440

# **CHAPTER 5**

## **Illicit *gamma*-Hydroxybutyrate (GHB) and Pharmaceutical Sodium Oxybate (Xyrem®): Differences in Characteristics and Misuse.**

*Drug and Alcohol Dependence (2009) 104(1-2):1-10.*

**Authors:** Lawrence P. Carter, Daniel Pardi, Jane Gorsline, Roland R. Griffiths



## Abstract

There are distinct differences in the accessibility, purity, dosing, and misuse associated with illicit *gamma*-hydroxybutyrate (GHB) compared to pharmaceutical sodium oxybate. *Gamma*-hydroxybutyrate sodium and sodium oxybate are the chemical and drug names, respectively, for the pharmaceutical product Xyrem® (sodium oxybate) oral solution. However, the acronym GHB is also used to refer to illicit formulations that are used for non-medical purposes. This review highlights important differences between illicit GHB and sodium oxybate with regard to their relative abuse liability, which includes the likelihood and consequences of abuse. Data are summarized from the scientific literature; from national surveillance systems in the U.S., Europe, and Australia (for illicit GHB); and from clinical trials and post-marketing surveillance with sodium oxybate (Xyrem). In the U.S., the prevalence of illicit GHB use, abuse, intoxication, and overdose has declined from 2000, the year that GHB was scheduled, to the present and is lower than that of most other licit and illicit drugs. Abuse and misuse of the pharmaceutical product, sodium oxybate, has been rare over the 5 years since its introduction to the market, which is likely due in part to the risk management program associated with this product. Differences in the accessibility, purity, dosing, and misuse of illicit GHB and sodium oxybate suggest that risks associated with illicit GHB are greater than those associated with the pharmaceutical product sodium oxybate.

## Introduction

*Gamma*-hydroxybutyric acid is an endogenous compound and putative neurotransmitter that differs from the primary inhibitory neurotransmitter *gamma*-aminobutyric acid (GABA) by the substitution of a hydroxyl group in place of the amino group of the GABA molecule (Maitre, 1997; Pardi and Black, 2006). Sodium *gamma*-hydroxybutyrate or sodium 4-hydroxybutyrate (GHB) is the International Union of Pure and Applied Chemistry chemical name for the sodium salt of *gamma*-hydroxybutyric acid, whereas sodium oxybate is the international drug name for the identical compound (Hillebrand et al., 2008). Sodium oxybate is marketed as Xyrem® in the U.S., Canada, and Europe by Jazz Pharmaceuticals, Valeant Pharmaceuticals International, and UCB, respectively. It is approved for the treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy in the U.S., for the treatment of narcolepsy with cataplexy in adult patients in Europe, and for the treatment of cataplexy with narcolepsy in Canada. Sodium oxybate is approved in Germany as an anesthetic, Somsanit® (Dr. F. Köhler Chemie), and is approved in Austria and Italy for the treatment of opioid and alcohol withdrawal as Alcover® (Laboratorio Farmaceutico; Hillebrand et al., 2008). Clinical development programs are also under way to study the clinical efficacy and safety of sodium oxybate for the treatment of conditions such as fibromyalgia (Russell et al., 2009) and essential tremor (Frucht et al., 2005). For the purposes of this report, sodium oxybate will be used to refer to the government-approved drug or pharmaceutical product. GHB will be used to refer to endogenous *gamma*-hydroxybutyric acid and chemical grade *gamma*-hydroxybutyrate. Illicit GHB will be used to refer to illicitly manufactured *gamma*-hydroxybutyric acid or *gamma*-hydroxybutyrate and street drug products that are purported to be GHB and might contain GHB or other compounds of unknown dose and purity.

GHB was legally manufactured and widely available as a nutritional supplement (to induce sleep or increase muscle mass) in the 1980s until reports of abuse as a “club drug” (a drug used in a club or party setting for its euphoric effects; e.g., Sumnall et al., 2008) and “date-rape drug” (a drug used for drug-facilitated sexual assault; e.g., Chin et al., 1992) led to the scheduling of GHB as a controlled substance. As of March 2000, GHB and sodium oxybate were placed in a unique bifurcated Federal schedule in the U.S. GHB for non-medical use is Schedule I, the most restrictive schedule of the Controlled Substances Act (2008). When used as prescribed for medical purposes (e.g., the treatment of narcolepsy), it is a Schedule III substance.

In March 2001, the Commission on Narcotic Drugs of the United Nations, at the recommendation of the World Health Organization, added GHB to Schedule IV of the 1971 Convention on Psychotropic Substances, with GHB subject to scheduling or control in all Member States of the European Union. Some Member States (e.g., Italy, Latvia, and Sweden) subsequently also placed controls on one or both of the GHB precursors *gamma*-butyrolactone (GBL) and 1,4-butanediol (1,4-BD; Hillebrand et al., 2008). In the U.S., GBL is a List I chemical (a chemical that is used in, and important to, the manufacture of a controlled substance) and is subject to regulatory controls; 1,4-BD is neither controlled nor listed at the Federal level

(Controlled Substances Act, 2008), but is controlled in some U.S. states under State Law (e.g., Hawaii, Nevada; Hawaii Revised Statute, 2008; Nevada Administrative Code, 2008). Canada lists sodium oxybate/GHB and all salts as Schedule III.

Distinguishing between illicit GHB and licit GHB or sodium oxybate from clinical case reports of abuse and dependence is difficult. Many of the epidemiological studies or case reports that describe the effects of illicit GHB refer to the molecule simply as GHB (e.g., Kim et al., 2008). Without forensic analysis of the substance consumed or of a biological sample from the consumer, extrapolating effects reported after the administration of illicit GHB or a GHB precursor to the effects of chemical grade GHB or sodium oxybate requires several assumptions to be made, such as the illicit formulation contained GHB, the illicit formulation was not contaminated or adulterated by other chemicals, and the effects were not caused by a co-administered drug or chemical.

However, studies examining the effects of GHB are applicable to sodium oxybate. All pharmaceutical products have chemical names (e.g., sodium *gamma*-hydroxybutyrate), non-proprietary pharmaceutical names (e.g., sodium oxybate), and trade names (e.g., Xyrem). Clinical studies that were conducted with pharmaceutical grade GHB prior to the development of sodium oxybate as a commercial product (e.g., Broughton and Mamelak, 1979; Scharf et al., 1985) use the chemical name GHB and are applicable to the pharmaceutical product. The use of the chemical name in the scientific literature has likely persisted because of the availability and use of chemical grade GHB for non-human studies (e.g., Carter et al., 2003; Goodwin et al., 2005).

It is important, however, to recognize that illicit GHB and sodium oxybate have different risks or liabilities of abuse and using “GHB” to refer to both illicit GHB and sodium oxybate has blurred this distinction in the scientific literature and in the popular press. The purpose of this review is to summarize the differences between the relative abuse liability of sodium oxybate and that of illicit GHB, with a specific focus on the availability and prevalence of non-medical use, and the risks and consequences of misuse and abuse. Relative abuse liability includes both a drug’s liability for abuse (likelihood that the drug will be abused) and its liability of abuse (consequences of abuse; Griffiths et al., 2003). Information on sodium oxybate, GHB, and illicit GHB from three types of sources are presented in this review: data from the peer-reviewed scientific literature; data from national surveys of drug use, abuse, and law enforcement activity; and data from Jazz Pharmaceuticals on the rates of abuse, diversion, drug-facilitated sexual assault, and deaths associated with sodium oxybate.

## **1. Characteristics of illicit GHB compared to those of sodium oxybate**

### **1.1. Availability**

#### **1.1.1. Availability of illicit GHB.**

After GHB was scheduled in the U.S. in 2000 and became an illegal drug, it continued to be sold as a dietary supplement under a variety of different names,

as a GHB alternative, or more covertly, as a solvent not recommended for human consumption (Maxwell and Spence, 2005). Chemistry kits, reagents, and recipes to convert GHB precursors into GHB also became available for purchase over the internet; however, the availability of these kits, reagents, and recipes is thought to have diminished in recent years (Nicholson and Balster, 2001; Mason and Kerns, 2002; European Monitoring Centre for Drugs and Drug Addiction Annual Report, 2007; National Drug Intelligence Center U.S. Department of Justice, 2008). Epidemiological data show that illicit GHB remains accessible to individuals in the U.S., Europe, and Australia (Degenhardt et al., 2005; Barker et al., 2007; Sumnall et al., 2008). International restrictions on the production and sale of GHB are thought to have shifted recreational use from GHB toward the GHB precursors GBL and 1,4-BD in the U.S., Europe, and Australia (Winickoff et al., 2000; Zvosec et al., 2001; Dupont and Thornton, 2001; Caldicott et al., 2004; Palmer, 2004; European Monitoring Centre for Drugs and Drug Addiction Annual Report, 2008; Hillebrand et al., 2008; Knudsen et al., 2008; Wood et al., 2008). GBL and 1,4-BD are ingested for recreational use presumably because they are converted to GHB in the body (Doherty and Roth, 1978; Lettieri and Fung, 1978), but they can also be chemically converted to illicit GHB prior to consumption.

Several surveys and data collection methods have been used in different countries to assess the relative availability and reported prevalence of use of illicit GHB (Table 1). In the U.S., the relative availability of illicit GHB can be assessed by examining the reported prevalence of use by individuals and the prevalence of drug confiscations by law enforcement. Data regarding the use of illicit GHB by adults in the U.S. are limited since GHB was not added to the U.S. National Survey on Drug Use and Health (formerly the National Household Survey on Drug Abuse) until 2006 (Substance Abuse and Mental Health Services Administration, Office of Applied Studies, 2008b). However, data from the Monitoring the Future Study indicate that the estimated annual rates of non-medical use of GHB by 8th, 10th, and 12th grade students are low (0.5–1.2%) and have declined from 2000 (the first year in which GHB use was queried in the survey) to 2008 (Johnston et al., 2008). Reported rates of non-medical use of GHB among each of the grade levels in the Monitoring the Future Study in 2007 were lower than those of other sedatives, tranquilizers, Vicodin®, Oxycontin®, Ritalin®, and over-the-counter cough and cold medicines (Johnston et al., 2008). Similarly, the rate of confiscations of illicit GHB/GBL by U.S. law enforcement is also low, relative to that of other drugs, and has declined in recent years. Data from the U.S. Drug Enforcement Administration's National Forensic Laboratory Information System (NFLIS) indicate that the percentage of items that tested positive for GHB or GBL decreased by 83% from 2000 to 2007 (Fig. 1, top panel; The National Forensic Laboratory Information System, 2001; The National Forensic Laboratory Information System, 2002; Strom et al., 2003, 2004; Weimer et al., 2004, 2006, 2007; Office of Diversion Control, 2008). Indeed, the 2009 National Drug Threat Assessment authored by the U.S. Department of Justice states that GHB has been a very low threat and low priority for law enforcement for the last several years (National Drug Intelligence Center U.S. Department of Justice, 2008).

**TABLE 1.** Sources of data for reported availability and use of illicit GHB

|           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| U.S.      | <p><b>U.S. National Survey on Drug Use and Health (formerly the National Household Survey on Drug Abuse)</b></p> <ul style="list-style-type: none"> <li>• Annual survey by the Substance Abuse and Mental Health Services Administration (SAMHSA).</li> <li>• Assesses the prevalence and incidence of illicit drug, alcohol, and tobacco use, substance abuse and dependence, and mental health problems in the civilian, non-institutionalized population ages 12+ in the U.S.</li> <li>• N ~70,000.</li> </ul> <p><b>Monitoring the Future Study</b></p> <ul style="list-style-type: none"> <li>• Annual survey carried out by the University of Michigan Institute for Social Research and supported by the National Institute on Drug Abuse, a part of the National Institutes of Health.</li> <li>• Assesses self-reported behavior, attitudes, and values of secondary school students (8th, 10th, and 12th grade), college students, and young adults.</li> <li>• N ~50,000.</li> </ul> <p><b>National Forensic Laboratory Information System (NFLIS)</b></p> <ul style="list-style-type: none"> <li>• Program sponsored by the U.S. Drug Enforcement Administration (DEA) in partnership with 274 federal, state, and local forensic laboratories.</li> <li>• Systematic collection (annual, mid-year, and special reports) of results from drug analyses conducted by state and local forensic laboratories of substances seized in law enforcement operations across the country.</li> <li>• Resource for monitoring illicit drug availability, including the diversion of legally manufactured drugs into illegal markets.</li> </ul> <p><b>Drug Abuse Warning Network (DAWN)</b></p> <ul style="list-style-type: none"> <li>• Public health surveillance system that is operated by the Substance Abuse and Mental Health Services Administration (SAMHSA).</li> <li>• Monitors drug-related hospital emergency department visits and drug-related deaths to track the impact of drug use, misuse, and abuse in the U.S.</li> </ul> <p><b>National Poison Database</b></p> <ul style="list-style-type: none"> <li>• Comprehensive poisoning surveillance database system in the U.S. operated by the American Association of Poison Control Centers.</li> <li>• Information included in its annual reports reflects the information submitted by the regional Poison Control Centers.</li> </ul> |
| Europe    | <p><b>European Monitoring Center for Drugs and Drug Addiction (EMCDDA)</b></p> <ul style="list-style-type: none"> <li>• Annual report on the state of the drugs problem in Europe; comprehensive information on drugs and drug addiction in Europe.</li> <li>• Works with network of ~30 national monitoring centers (Reitox network).</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Australia | <p><b>National Drug Strategy Household Survey</b></p> <ul style="list-style-type: none"> <li>• National survey conducted by the Australian Institute of Health and Welfare (AIHW) on drug use patterns, attitudes and behaviors in individuals ages 12+ in Australia.</li> <li>• Conducted every 2–3 years</li> <li>• N ~30,000.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

In Europe, the European Monitoring Centre for Drugs and Drug Addiction is the central source of comprehensive information on drugs and drug addiction. The

European Monitoring Centre for Drugs and Drug Addiction 2007 and 2008 Annual Reports and thematic paper on GHB and GBL showed that the prevalence of illicit GHB use in Europe is low, with levels of use limited to specific subpopulations of drug users (Bellis et al., 2003; European Monitoring Centre for Drugs and Drug Addiction Annual Report, 2007; European Monitoring Centre for Drugs and Drug Addiction Annual Report, 2008; Hillebrand et al., 2008). In Australia, data from the National Drug Strategy Household Survey conducted by the Australian Institute of Health and Welfare estimated that in 2007 a total of 0.1% of the Australian population over 14 years of age reported using GHB in the previous 12 months (Australian Institute of Health and Welfare, 2008). This figure is the same as that reported in 2004 for GHB (Degenhardt and Dunn, 2008), and is considerably lower than the reported use of other illicit drugs, such as marijuana/cannabis (9.1%) and ecstasy (3.5%), as well as non-medical use of pain-killers (2.5%) and sleeping pills during the same year (1.4%; Australian Institute of Health and Welfare, 2008). In contrast to the national reports, a recent retrospective study from a metropolitan area in Australia reported that GHB-related, non-fatal ambulance calls increased from 2001 to 2005, suggesting that use of illicit GHB in Australia might also be limited to specific sub populations of drug users (Dietze et al., 2008).

#### *1.1.2. Availability of sodium oxybate.*

Sodium oxybate is available by prescription in the U.S., Canada, and Europe. In the U.S., an extensive risk- management program called the Xyrem Success Program® was specifically designed to prevent diversion and misuse of sodium oxybate by limiting distribution of the drug and by educating physicians and patients on proper use of the drug. In the U.S., Xyrem is manufactured at a single source and is distributed through a central pharmacy. The Xyrem Success Program also includes physician and patient registries, whereby prescriptions are verified before being filled (in addition to confirming that the prescriber and the prescription are valid, the central pharmacy calls the physician to identify the physician's name and DEA and state license numbers, confirms that the physician's DEA number is valid, and confirms that the physician is registered with the central pharmacy, a one-time process in which the physician must attest that he/she has read specific educational materials that include information regarding the approved indications and doses). The central pharmacy also calls each patient to confirm that they have received and understood the Patient Success Program materials explaining the risks and proper use of Xyrem (and sends or re-sends the materials if they have not been received). Once it is documented that the patient has read and understood the educational materials, the drug is shipped overnight to the patient; if a patient or a patient's designee does not sign for the drug, it is returned to the central pharmacy (see Fuller et al., 2004; Wedin et al., 2006).

The central pharmacy tracks all instances of potential diversion, theft, and loss of drug (e.g., reports of misplaced, spilt, or damaged bottles; delivery of drug to an incorrect address). From the approximately 26,000 patients that have received drug and the approximately 600,000 bottles of Xyrem distributed worldwide (approximately 5 million defined daily doses, based on the modal prescribed dose of 9g/night) from market introduction through March 2008, there have been 5 reported instances of drug diversion (drug was used or intended to be used by

someone other than the patient), 6 instances of possible diversion (drug was reported to have been stolen with no information about its subsequent use), 9 instances of coincidental theft (drug was part of a general theft including other items; e.g., burglary, theft of a backpack), and 22 instances in which the drug was lost or missing and theft was not suspected (e.g., drug was delivered to the wrong address and was not recovered, drug was left in a hotel room by the patient). Given the five reported instances of drug diversion, the estimated rate of diversion of Xyrem is approximately one instance per 5200 patients treated (approximately 0.019%), or one instance per 120,000 bottles of drug shipped (less than 0.0009%; Wang et al., 2009).

## **1.2. Product identity, purity, and dosing**

### **1.2.1. Identity, purity, and dosing of illicit GHB.**

As with any illicit drug, there are no standards governing the production or sale of illicit GHB. A powder or solution sold as GHB might or might not contain GHB or other substances. Similarly, illicitly synthesized GHB might contain toxic contaminants or residual reagents from the synthesis. As such, case reports that describe instances of illicit GHB intoxication and unintentional overdose without forensic confirmation that GHB and no other drugs was present in a biological sample, assume that GHB was actually ingested and that illicit GHB, and not a contaminant of synthesis or another drug(s) or chemical(s), was responsible for the observed effects.

GHB used for non-medical purposes is most commonly synthesized illicitly from GBL. As such, less than careful chemistry can result in a final product that contains GBL. GBL is metabolized to GHB in the body (Lettieri and Fung, 1978); however, in a number of different species, including mice, rats, pigeons, and baboons, GBL has been shown to be more potent than GHB (cf., Weertsetal., 2005; Goodwin et al., 2006), which is likely due to pharmacokinetic differences between GHB and GBL (see Carter et al., 2009 for review). Thus, the use of illicitly synthesized GHB can result in greater harm if GBL is present since GBL is more potent than GHB across species and might increase the risk of unintentional overdose.

In addition, GBL or other drugs such as 1,4-BD might be sold as illicit GHB. For example, reported use of GHB by patients admitted to the emergency department in a recent study in the U.K. was substantially higher than data from analyzed substances would suggest. In one study that included 158 patients who reported use of either GHB, GBL, or 1,4-BD after being admitted to the emergency department, 95% of the patients reported using GHB. However, an analysis of substances seized from clubs in the same area showed that 38% of seized substances contained GHB, whereas 62% contained GBL (Wood et al., 2008).

Although both GBL and 1,4-BD are metabolized to GHB in vivo following oral administration (Doherty and Roth, 1978; Lettieri and Fung, 1978), the toxic effects of these compounds might differ from those of GHB. In one study in mice, 1,4-BD produced loss of righting and resulted in 25% lethality (LD25) at a dose of 1780 mg/kg, whereas lethality was not observed after doses of GHB up to 3200mg/kg (Carter et al., 2005). A study in human subjects reported that there were no serious

adverse effects observed after a single relatively low dose of 1,4-BD (25 mg/kg; Thai et al., 2007); however, the relative toxicity of 1,4-BD in humans has been suggested to be quite high (Zvosec et al., 2001).

Recreational users of illicit GHB have typically reported self-administering one or more “capfuls” of liquid. Depending on the size of the cap and the concentration of the solution, a capful of liquid could contain 5 g of GHB, or approximately 70 mg/kg for a 70 kg person (based on an approximate concentration of 1g/mL of pure GHB solution; Miotto et al., 2001; Barker et al., 2007; Sumnall et al., 2008). In a few studies, users of illicit GHB reported taking the drug 1–6 times per week, 1–3 times per day, often in the evenings (but not immediately before bed), and most frequently on the weekends (Miotto et al., 2001; Barker et al., 2007; Sumnall et al., 2008). In addition, case reports of illicit GHB abuse and withdrawal often describe use that has escalated to larger doses and frequent dosing throughout the day and night (e.g., Craig et al., 2000; Wojtowicz et al., 2008).

The adverse consequences of illicit GHB abuse have been postulated to be worse than those of other sedative/hypnotic drugs because the dose–effect curve for GHB appears to be steep and there is a narrow range between doses that are used for recreational purposes and those that can result in loss of consciousness (Griffiths and Johnson, 2005). As noted in the following Section 2.2.2, studies conducted with sodium oxybate under controlled conditions have shown that the pharmacokinetics are nonlinear, with, for instance, a doubling of the dose resulting in a greater than 2-fold increase in blood levels of the drug. In surveys of illicit GHB users in the U.S. and Australia, more than half of the respondents reported experiencing some degree of unintentional loss of consciousness as a result of their illicit GHB use (Miotto et al., 2001; Degenhardt et al., 2003).

#### 1.2.2. *Identity, purity, and dosing of sodium oxybate.*

Sodium oxybate is manufactured in liquid form to precise specifications (500 mg sodium oxybate per mL of solution), and doses are measured by patients using a calibrated device. Under controlled conditions and using known doses of sodium oxybate, the dose–effect curve, across a wide range of doses, was found to be relatively steep and the relative potency of the sedative effects somewhat variable across participants (Abanades et al., 2006, 2007; Carter et al., 2006). This finding is consistent with the nonlinear pharmacokinetics of sodium oxybate, in which blood levels increased 3.7-fold as the dose was doubled from 4.5 to 9 g per day (Palatini et al., 1993; Scharf et al., 1998).

The recommended starting dose of sodium oxybate for treating cataplexy and excessive daytime sleepiness associated with narcolepsy is 4.5 g/night divided into two equal doses of 2.25 g to be taken at bedtime and 2.5–4 h later. The dose can be increased to a maximum of 9g/night (two doses of 4.5g/night) in increments of 1.5 g/night (0.75 g per dose; Xyrem Prescribing Information, 2008). The doses under evaluation in Phase II and Phase III trials for fibromyalgia are 4.5 and 6 g/night (Russell et al., 2009; data on file, Jazz Pharmaceuticals).

## **2. Substance Abuse, Substance Dependence, and misuse with illicit GHB compared to sodium oxybate**

### **2.1. Substance Abuse and Substance Dependence with illicit GHB**

In cases of intoxication or unintentional overdose, the dose or doses of illicit GHB are often unknown; however, surveys of users of illicit GHB provide some information about the effects of acute illicit GHB intoxication. In surveys of recreational users of illicit GHB in the U.S., U.K., and Australia, individuals reported using GHB for euphoric-, sociable-, and aphrodisiac-like effects (Miotto et al., 2001; Degenhardt et al., 2002; Barker et al., 2007; Sumnall et al., 2008). In some of the same studies, more than half of the respondents also reported that acute adverse effects of illicit GHB intoxication included confusion, dizziness, blurred vision, hot/cold flushes, profuse sweating, vomiting, and loss of consciousness (Miotto et al., 2001; Degenhardt et al., 2002). In one survey, users who had experienced an unintentional, non-fatal overdose with illicit GHB reported that the most frequent reason for the unintentional overdose was taking “too much GHB” (37% of respondents), followed by a concentration of illicit GHB “stronger than usual” (21% of respondents; Degenhardt et al., 2003).

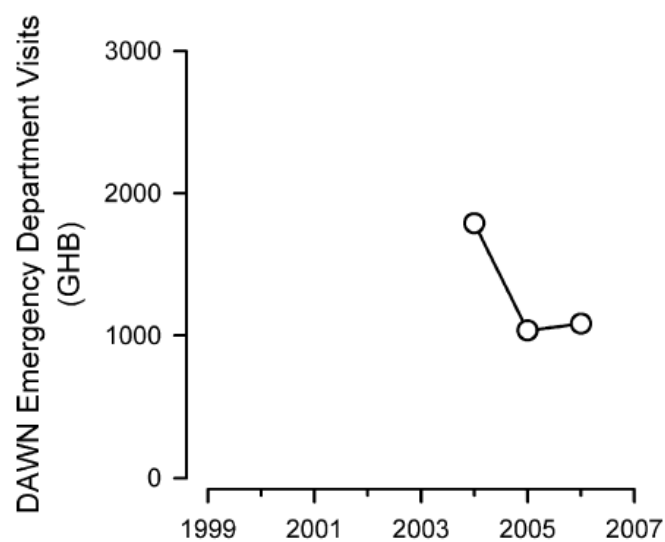
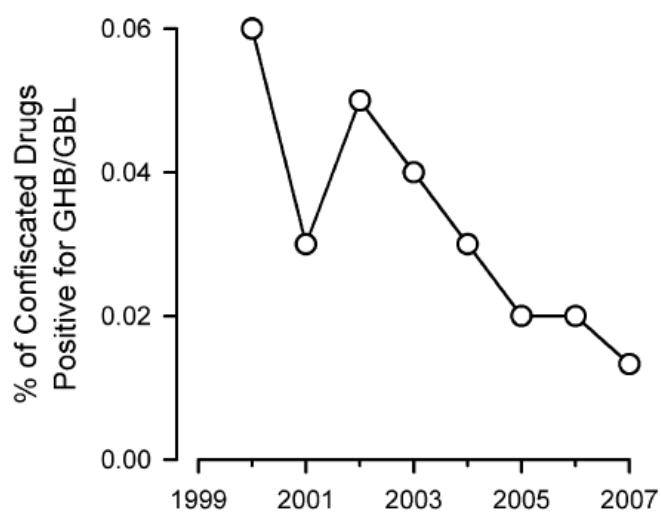
Epidemiological information on the prevalence of illicit GHB abuse and intoxication includes data from the Drug Abuse Warning Network (DAWN; Substance Abuse and Mental Health Services Administration, Office of Applied Studies, 2008a) and the American Association of Poison Control Centers, which receive reports of drug-related hospital emergency department visits and calls or case reports of poisonings, respectively (Table 1). Data from the DAWN surveillance system estimate that the number of emergency department visits in which GHB or a GHB precursor was mentioned decreased from 2004 to 2006 (Fig. 1, middle panel); however, the number of emergency department visits reported in 2006 was not statistically different than those reported in 2004 or 2005 (Substance Abuse and Mental Health Services Administration, Office of Applied Studies, 2008b; Dr. Elizabeth Crane, personal communication). Annual Reports of the American Association of Poison Control Centers also suggest that poisonings attributed to illicit GHB, GHB analogs, and GHB precursors have declined in recent years, with the number of GHB exposures decreasing by 73% from a total of 1916 exposures (including six deaths) in 2001 to 518 exposures (including no deaths) in 2007 (Fig. 1, bottom panel; Litovitz et al., 2002; Watson et al., 2003, 2004, 2005; Lai et al., 2006; Bronstein et al., 2007, 2008). Similarly, a retrospective review of cases presented to the California Poison Control System showed that from 1999 to 2003 there was a 76% decrease in case reports of GHB exposure to the California Poison Control System (Anderson et al., 2006).

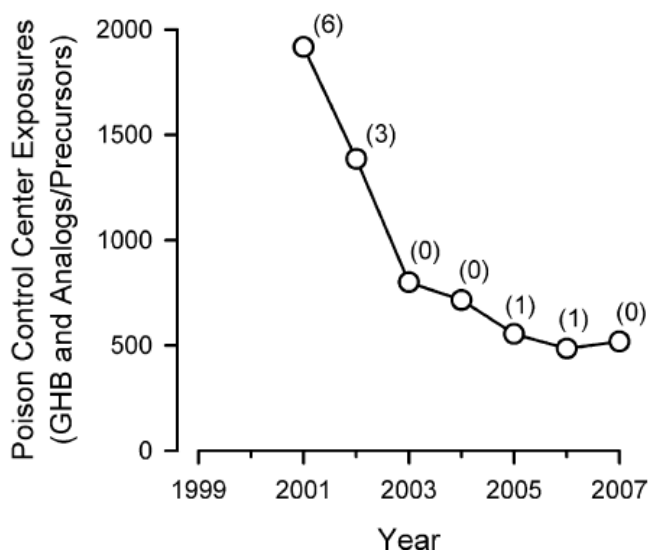
The actual prevalence of death from illicit GHB overdose is difficult to determine because of the lack of objective assessments, such as sensitive, specific assays for measuring GHB in biological matrices; the high prevalence of concomitant drug use; and differences in reporting practices within the medical community. Nausea and vomiting can occur after GHB administration and increase the risk for aspiration if an individual is unconscious. Cardiac and respiratory depression can

lead to bradycardia, bradypnea, and apnea. Myoclonic seizure-like activity and loss of consciousness have also been described (Centers for Disease Control, 1991; Dyer, 1991; Chin et al., 1992). The clinical course of illicit GHB overdose is thought to be relatively short, with most people awakening within 3–4h and recovering within 5–8h of ingestion (Chin et al., 1998; Van Sassenbroeck et al., 2007; but see Strickland et al., 2005). The concomitant use of other sedative-hypnotics, including alcohol, has been reported to prolong the time it takes to recover (Williams, 1998; Thai et al., 2006).

Fatalities due to illicit GHB intoxication both alone and together with other drugs have been reported (Fig. 1, bottom panel; also see Ferrara et al., 1995; Caldicott et al., 2004; Knudsen et al., 2008). The relationship between adverse events and the consumption of illicit GHB is often not straightforward. Studies conducted in the U.S., Europe, and Australia have shown that the majority of individuals co-administer illicit GHB with at least one other substance (Miotto et al., 2001; Degenhardt et al., 2002, 2003; Barker et al., 2007; Kim et al., 2007; Dietze et al., 2008; Sumnall et al., 2008). In addition, cases of intentional or unintentional overdose from drugs other than GHB might be mistaken for illicit GHB overdose (Couper et al., 2004; Wood et al., 2008).

There are case reports that describe individuals who appear to fulfill DSM-IV criteria for a diagnosis of Substance Dependence upon (i.e., addiction to) illicit GHB (Galloway et al., 1997; Craig et al., 2000; McDaniel and Miotto, 2001; Degenhardt et al., 2002). There are also case reports (including those above) that describe individuals who have become physically dependent (evidenced by a withdrawal syndrome) upon illicit GHB as a result of frequent administration of the drug (every 1–3h), resulting in daily doses of 43–144 g/day (e.g., Price, 2000; Dyer et al., 2001; Miotto et al., 2001; Glasperet al., 2005). In many cases, DSM-IV Substance Dependence and physical dependence on illicit GHB are associated with administration of supratherapeutic doses of GHB in an around-the-clock manner (Dyer et al., 2001; Tarabar and Nelson, 2004). Surveys of recreational users of illicit GHB have reported a prevalence of physical dependence ranging from 4% to 21%, with a higher prevalence in surveys in which participants reported more frequent use of illicit GHB (Miotto et al., 2001; Degenhardt et al., 2002).





**FIGURE 1**

## **2.2. Substance Abuse and Substance Dependence with sodium oxybate**

Human abuse liability studies of supratherapeutic doses of sodium oxybate have shown that although subjects report positive subjective effects comparable to those of alcohol or a benzodiazepine, they also report greater negative subjective effects such as nausea and gastrointestinal distress (Carter et al., 2006; Abanades et al., 2007). Data from post-marketing surveillance indicate that abuse of sodium oxybate by patients and/or recreational drug users is rare. All reported cases of sodium oxybate abuse from spontaneous reporting and the central pharmacy were reviewed for fulfilling the DSM-IV criteria for Substance Abuse. Ten cases fulfilling DSM-IV criteria for Substance Abuse with sodium oxybate have been reported from market introduction through March 2008 in the U.S., Europe, and Canada (Wang et al., 2009). As shown in Table 2, there has been approximately one case of Substance Abuse reported for every 2600 patients treated (a rate of approximately 0.039%). Also noted in Table 2, the majority of cases that fulfilled DSM-IV criteria for Substance Abuse involved use of sodium oxybate in a potentially hazardous situation.

A total of 21 deaths have been reported in patients who were likely or known to be currently taking sodium oxybate (out of 26,000 patients who took sodium oxybate) since market introduction in the U.S., Europe, and Canada (Wang et al., 2009). In 6 of the 21 cases, a physician deemed that the cause of death was not related to sodium oxybate. The causes of death in the remaining 15 cases include unknown causes (seven cases), drug overdose (three cases total, in two of the three cases, the overdose itself did not involve sodium oxybate; all three overdoses involved more than one drug), accidental drowning (one case), suicide (one case), renal failure (one case), cardiac arrest (one case), and metastatic lung cancer (one case).

Since market introduction in the U.S., Europe, and Canada, four cases fulfilling a DSM-IV diagnosis of Substance Dependence (i.e., addiction) on sodium oxybate (three cases also fulfilled the criterion for physical dependence) have been documented by the central pharmacy and from spontaneous reporting (Table 2). Of the cases that fulfilled DSM-IV criteria for Substance Dependence, the criteria of tolerance (four cases), withdrawal (three cases), and using larger amounts of the drug than intended (four cases) were the most common criteria endorsed (Table 2). An additional five cases fulfilled the criterion for physical dependence but did not fulfill DSM-IV criteria for a Substance Dependence diagnosis. Thus, reported rates of Substance Dependence per DSM-IV criteria and physical dependence, respectively, are one case for every 6500 patients treated and one case for every 3300 patients treated, or approximately 0.015% and 0.031% (Wang et al., 2009).

The effects of abrupt discontinuation of therapeutic doses of sodium oxybate were examined in a retrospective analysis of clinical trial data. In a study in which 29 of 55 narcoleptic patients who had been taking nightly doses of 3–9g of sodium oxybate for 7–44 months were (re)randomized to placebo treatment under double-blind conditions, 5 instances of possible withdrawal symptoms (e.g., anxiety, dizziness, insomnia, and somnolence) were reported (U.S. Xyrem Multi-Center Study Group, 2003).

**TABLE 2.** DSM-IV criteria fulfilled in the post-marketing cases of DSM-IV Substance Abuse and Substance Dependence on Xyrem<sup>a</sup>

| Cases of DSM-IV Substance Abuse <sup>b</sup>      | DSM-IV Substance Abuse Criteria Fulfilled <sup>c</sup>      | Physical dependence | Patient history of Substance Abuse |
|---------------------------------------------------|-------------------------------------------------------------|---------------------|------------------------------------|
| Case #2                                           | B – use in physically hazardous situation                   | No                  | Yes                                |
| Case #3                                           | B – use in physically hazardous situation                   | No                  | Yes                                |
| Case #4                                           | D – continued use despite interpersonal problems            | No                  | Yes                                |
| Case #5                                           | B – use in physically hazardous situation                   | No                  | Yes                                |
| Case #7                                           | A – failure to fulfill major role obligations               | No                  | Unknown                            |
| Case #8                                           | B – use in physically hazardous situation                   | No                  | Unknown                            |
| Case #10                                          | B – use in physically hazardous situation                   | No                  | No                                 |
| Case #12                                          | B – use in physically hazardous situation                   | No                  | Yes                                |
| Case #13                                          | B – use in physically hazardous situation                   | No                  | No                                 |
| Case #40                                          | B – use in physically hazardous situation                   | No                  | Unknown                            |
|                                                   |                                                             |                     |                                    |
| Cases of DSM-IV Substance Dependence <sup>d</sup> | DSM-IV Substance Dependence Criteria Fulfilled <sup>e</sup> | Physical dependence | Patient history of Substance Abuse |

|          |                                                             |     |     |
|----------|-------------------------------------------------------------|-----|-----|
| Case #9  | E – tolerance; F – withdrawal; G – larger amounts           | Yes | No  |
| Case #21 | E – tolerance; F – withdrawal; G – larger amounts           | Yes | Yes |
| Case #22 | E – tolerance; G – larger amounts; K – use despite problems | No  | Yes |
| Case #31 | E – tolerance; F – withdrawal; G – larger amounts           | Yes | Yes |

<sup>a</sup>The 10 cases of Substance Abuse and the four cases of Substance Dependence were identified using case reports from the approximately 26,000 patients that received Xyrem worldwide from its introduction to the market in the U.S. in 2002, in Europe in 2005, and in Canada in 2007, through March 31, 2008. Thus, the rate of Substance Abuse and Substance Dependence in this population of approximately 26,000 patients is 0.039% and 0.015%, respectively.

<sup>b</sup>A case was designated as DSM-IV Substance Abuse if one or more of four criteria for Substance Abuse were fulfilled in the same 12-month period and symptoms did not meet criteria for DSM-IV Substance Dependence.

<sup>c</sup>Letters designate which of the four criteria for Substance Abuse listed below were fulfilled in each case. In all ten cases, only one criterion was fulfilled.

(A) Recurrent substance use resulting in a failure to fulfill major role obligations at work, school, or home (e.g., repeated absences or poor work performance related to substance use; substance-related absences, suspensions, or expulsions from school; neglect of children or household).

(B) Recurrent substance use in situation in which it is physically hazardous (e.g., driving an automobile or operating a machine when impaired by substance use).

(C) Recurrent substance-related legal problems (e.g., arrests for substance-related disorderly conduct).

(D) Continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance (e.g., arguments with spouse about consequences of intoxication, physical fights).

<sup>d</sup>A case was designated as DSM-IV Substance Dependence if three or more of the seven Substance Dependence diagnostic criteria were fulfilled in the same 12-month period.

<sup>e</sup>Letters designate which of the seven criteria for Substance Dependence listed below were fulfilled in each case. In all four cases, only three criteria were fulfilled.

(E) Tolerance, as defined by either of the following: a need for markedly increased amounts of the substance to achieve intoxication or desired effect or markedly diminished effect with continued use of the same amount of the substance.

(F) Withdrawal, as manifested by either of the following: the characteristic withdrawal syndrome for the substance or the same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms.

(G) The substance is often taken in larger amounts or over a longer period than was intended.

(H) There is a persistent desire or unsuccessful efforts to cut down or control substance use.

(I) A great deal of time is spent in activities necessary to obtain the substance (e.g., visiting multiple doctors or driving long distances), use the substance (e.g., chain-smoking), or recover from its effects.

(J) Important social, occupational, or recreational activities are given up or reduced because of substance use.

(K) The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance (e.g., current cocaine use despite recognition of cocaine-induced depression or continued drinking despite recognition that an ulcer was made worse by alcohol consumption).

### **2.3. Drug-facilitated sexual assault with illicit GHB**

A major concern in the area of illicit GHB misuse and abuse has been its use for incapacitating victims for the purpose of sexual assault or “date rape.” It has been suggested that some effects attributed to illicit GHB, such as a rapid onset of sedation, anterograde amnesia, and increased libido and suggestibility, might specifically lend illicit GHB for use in drug-facilitated sexual assault, particularly when combined with alcohol (Marwick, 1997; ElSohly and Salamone, 1999; Varela et al., 2004). This aspect of illicit GHB abuse has drawn much attention from the media and criminal justice systems in the U.S., Europe, and Australia. As is the case with voluntary non-medical use of illicit GHB, determining whether illicit GHB was associated with drug-facilitated sexual assault is often confounded by the presence of other drugs of abuse (e.g., cannabis and/or alcohol), the difficulty in confirming that GHB was in fact administered due to its relatively short (40–50 min) half-life (Scharf et al., 1998; Borgen et al., 2004; Abanades et al., 2006), the lack of readily available specific assays for detecting GHB, and the difficulty of differentiating between levels of endogenous GHB and exogenously administered illicit GHB (Slaughter, 2000).

GHB is an endogenous compound and human urine and other biological specimens often contain measurable amounts of GHB in the absence of exogenous GHB administration (Doherty and Roth, 1978; LeBeau et al., 2006, 2007). Thus, failure to distinguish between exogenous and endogenous concentrations of GHB in biological specimens, as well as the documented post-mortem increase of GHB levels in biological specimens (LeBeau et al., 2001, 2007), might have resulted in the reporting of false-positive test results for illicit GHB prior to the development of sensitive and specific analytical methods and established cut-off values for normal endogenous levels of GHB. Specific and sensitive analytical methods for measuring GHB in biological matrices were developed during the clinical development of sodium oxybate as a pharmaceutical product (Scharf et al., 1998) and subsequent methodology has continued to address the need for more accurate assessments in forensic toxicology (e.g., LeBeau et al., 2006).

Forensic analyses of cases of suspected drug-facilitated sexual assault have typically reported a lower prevalence of illicit GHB use compared to other drugs. In two studies conducted in the U.S., forensic analysis showed that one or more drugs were detected in approximately 40–60% of biological samples from cases of suspected drug-facilitated sexual assault (ElSohly and Salamone, 1999; Slaughter, 2000; Juhascik et al., 2007). Of the samples in which only one drug was detected, GHB was found in 0–3%, whereas alcohol, tetrahydrocannabinol (THC; the primary active compound in marijuana), or a benzodiazepine was found in 8–69% of samples (ElSohly and Salamone, 1999; Slaughter, 2000). Of the samples in which one or more drugs were detected, GHB was found in 3–4%, whereas alcohol, THC, or a benzodiazepine was found in 19–56% of samples (ElSohly and Salamone, 1999; Slaughter, 2000). The ability to detect a drug in a biological sample depends on the rate of elimination of a drug or drug metabolite, and the window of detection for some drugs (e.g., THC, benzodiazepines) is typically longer than for others (e.g., alcohol, GHB). Such differences can reduce the likelihood of detecting drugs with a shorter half-life in blood or urine. Each of the studies mentioned above included only samples collected within 72h of the assault and acknowledged that the detection of GHB in urine or plasma is limited by the relatively rapid elimination of GHB from the body and its metabolism to carbon dioxide and water (ElSohly and Salamone, 1999; Slaughter, 2000; Juhascik et al., 2007). These issues complicate the interpretation of these forensic data and have prompted the development of toxicological analyses able to detect GHB or specific GHB metabolites in biological specimens for greater periods of time after administration.

#### **2.4. Drug-facilitated sexual assault with sodium oxybate**

In ten clinical trials evaluating the effects of sodium oxybate in different disease states in a total of 781 patients, one instance of drug-facilitated sexual assault was reported (~0.128%), which involved the assault of a patient (Wanget al., 2009). Two confirmed cases (~0.008%) of drug-facilitated sexual assault were reported from the approximately 26,000 patients treated with sodium oxybate (one case involved a patient, one case did not) since market introduction through March 2008 in the U.S., Europe, and Canada. One additional suspected case of drug-facilitated sexual assault not included in the numbers above involved a report of a

drug-facilitated sexual assault of a patient made to the central pharmacy by a prescribing physician; however, the central pharmacy had neither received a prescription for, nor shipped Xyrem to, this physician's patient (Wang et al., 2009).

Administration of known supratherapeutic doses of sodium oxybate can produce a state in which the patient is largely unresponsive, and with larger doses, a state of unconsciousness (Carter et al., 2006). However, in human studies with drug users (Carter et al., 2006) and non-drug users (Carter et al., 2007), administration of sodium oxybate did not produce the same magnitude of anterograde amnesic effects on the encoding of episodic memory that were observed after comparable doses of the benzodiazepine triazolam.

### **3. Discussion and conclusions**

There are important differences between illicit GHB and the pharmaceutical product sodium oxybate in accessibility, purity, and dosing, as well as in the prevalence and potential consequences of misuse and abuse. Illicit GHB and GHB precursors are more widely available than sodium oxybate, which is manufactured and distributed under strict conditions that allow the prospective monitoring of potential cases of abuse and dependence. Illicit GHB is synthesized and sold in a manner in which the purity and dose of the illicit formulation are often unknown to the consumer, whereas sodium oxybate is produced and stored according to Good Manufacturing Practice standards. Illicit GHB is often consumed at frequent intervals and together with other illicit drugs, whereas sodium oxybate is specifically prescribed to be taken in bed and not to be taken with alcohol or other CNS depressants.

Despite confounding factors (e.g., co-administration of other substances, misreporting, challenges in detection, and generic use of the term GHB to refer to any drug that has caused sedation or has been used for sexual assault), data from national surveys of drug use and abuse, law enforcement activity, emergency department visits, and poison control center exposures and deaths suggest that the rate of GHB abuse has remained low over the past several years, even as sodium oxybate was introduced to the market. These data, together with the extremely low rates of diversion of sodium oxybate, support the conclusion that market introduction has not substantially contributed to the rates of GHB abuse or misuse. There are several possible reasons for the decreases in production, availability, and use of illicit GHB. International restrictions on the production and sale of illicit GHB have been suggested to have shifted use away from illicit GHB and toward GHB precursors GBL and 1,4-BD (Zvosec et al., 2001; Wood et al., 2008). The relative risk of overdose with illicit GHB, possibly due to a narrow dose range for abuse, impurities of illicit synthesis, or consumption with other drugs, is thought to lessen its desirability as a drug of abuse. Moreover, in studies conducted in the U.S. and Australia more than half of recreational GHB users report that the acute effects of illicit GHB intoxication include confusion, dizziness, blurred vision, hot/cold flushes, profuse sweating, vomiting, and loss of consciousness (Miotto et al., 2001; Degenhardt et al., 2002).

Rates of DSM-IV Substance Abuse and Substance Dependence, physical dependence, and diversion of sodium oxybate have been extremely low since its introduction to the market in the U.S., Europe, and Canada. The product's risk-management program has played an important role in prospectively identifying instances of sodium oxybate misuse, abuse, or diversion and discontinuing access to the drug in those cases. Prospective monitoring of reports of lost or stolen drug and requests for early refills by the central pharmacy reduces the likelihood that patients can develop a chronic problem undetected. In fact, in 2007 Barker et al. reported that in a survey of 51 individuals in the U.S. who reported using illicit GHB for an average of 4.3 years, "only a handful of people in the study, those working in healthcare, had ever heard of the drug Xyrem .... No one had ever received or attempted to procure a prescription for this substance from a physician" (Barker et al., 2007). Data also suggest that the likelihood of developing physical dependence to sodium oxybate at therapeutic doses is low (Tarabar and Nelson, 2004). For the relatively small population of narcoleptic patients, prospective monitoring by the central pharmacy is feasible and has likely limited and prevented potential cases of abuse, dose escalation, and dependence.

Some of the surveillance systems described in this review are limited by their reliance on the spontaneous reporting of events (i.e., events might go unreported or different types of cases might be more likely to be reported than others) and the inability to specifically differentiate between the abuse of illicit GHB and the abuse of the pharmaceutical product. Prospective post-marketing surveillance methodologies have been developed that monitor the non-medical use of drugs in drug-using populations and that can distinguish between the abuse of illicit and licit formulations of drugs (e.g., Arfken et al., 2003). Future investigations that prospectively differentiate abuse of illicit GHB from abuse of sodium oxybate would speak directly to some of the issues presented in this review.

Data from the scientific literature, national surveillance systems, and the manufacturer support the conclusion that there are substantial differences in the availability, purity, and dosing, as well as in the prevalence and potential consequences of misuse and abuse of illicit GHB compared to the pharmaceutical product sodium oxybate. These differences result in a greater liability of abuse of illicit GHB compared to sodium oxybate, which is reflected in the additional controls, restrictions, and penalties associated with illicit GHB in the U.S. Although GHB and sodium oxybate are the chemical and non-proprietary drug names for the identical compound, recognition of the different liabilities of abuse for illicit GHB and sodium oxybate will help to ensure that patients who benefit from the pharmaceutical product will have continued unimpeded access to their medication.

**Role of funding source**

Support to prepare an initial draft of this manuscript was provided to Jane Gorsline under a consulting agreement with Jazz Pharmaceuticals. Support for Roland Griffiths' involvement was provided in part by grant R01 DA03889 from the National Institute on Drug Abuse.

**Contributors**

Daniel Pardi and Jane Gorsline were involved in the initial literature search and the writing of the first draft. Lawrence Carter and Roland Griffiths reviewed data from the national surveillance systems and Jazz Pharmaceuticals and were involved in the writing of subsequent drafts. All authors contributed to and have approved the final manuscript.

**Conflict of interest**

Lawrence Carter is a former employee of Jazz Pharmaceuticals and owns company stock. Daniel Pardi is a former employee of Jazz Pharmaceuticals, owns company stock, and has served as a paid consultant for UCB. Jane Gorsline has served as a paid consultant for Jazz Pharmaceuticals, Neurocrine Biosciences, and Somaxon Pharmaceuticals. Roland Griffiths is principal investigator on grants R01 DA03889 and R01 DA03890 from the National Institute on Drug Abuse and is co-investigator on a contract and several other grants from the National Institute on Drug Abuse. During the past 3 years, on issues about drug abuse liability, he has been a consultant for or has received grants from the following pharmaceutical companies: Abbott Laboratories, Alexza Pharmaceuticals, Bristol-Myers Squibb, Forest Laboratories, Jazz Pharmaceuticals, Merck & Co., Neurocrine Biosciences, Novartis, Pharmacia Corporation, Pfizer, Sanofi-Aventis, Somaxon Pharmaceuticals, Takeda Pharmaceuticals North America, TransOral Pharmaceuticals, Wyeth Pharmaceuticals.

**Acknowledgements**

We thank Grace Wang who provided data from Jazz Pharmaceuticals for analysis, Elizabeth Crane who provided guidance regarding the use of DAWN data, and Susanna Grzeschik, Diane Guinta, and Deborah Waxman for helpful editorial comments. Drs. Wang, Guinta, and Ms. Waxman are current employees of Jazz Pharmaceuticals and own company stock and hold company stock options. Dr. Grzeschik is a former employee of Jazz Pharmaceuticals and owns company stock.

## References

- Abanades, S., Farré, M., Barral, D., Torrens, M., Closas, N., Langohr, K., Pastor, A., de la Torre, R., 2007. Relative abuse liability of *gamma*-hydroxybutyric acid, flunitrazepam, and ethanol in club drug users. *J. Clin. Psychopharmacol.* 27, 625-638.
- Abanades, S., Farré, M., Segura, M., Pichini, S., Barral, D., Pacifici, R., Pellegrini, M., Fonseca, F., Langohr, K., De La Torre, R., 2006. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. *Ann. N.Y. Acad. Sci.* 1074, 559-576.
- Anderson, I.B., Kim, S.Y., Dyer, J.E., Burkhardt, C.B., Iknoian, J.C., Walsh, M.J., Blanc, P.D., 2006. Trends in gamma-hydroxybutyrate (GHB) and related drug intoxication: 1999 to 2003. *Ann. Emerg. Med.* 47,177-183.
- Arfken, C.L., Schuster, C.R., Johanson, C.E., 2003. Postmarketing surveillance of abuse liability of sibutramine. *Drug Alcohol Depend.* 69,169-173.
- Australian Institute of Health and Welfare, 2008. 2007 National Drug Strategy Household Survey: first results. Drug Statistics Series number 20.Cat. no. PHE98. Canberra: AIHW. <<http://www.aihw.gov.au/publications/index.cfm/title/10579>> (accessed 8.10.08.).
- Barker, J.C., Harris, S.L., Dyer, J.E., 2007. Experiences of gamma hydroxybutyrate (GHB) ingestion: a focus group study. *J. Psychoactive Drugs.* 39,115-129.
- Bellis, M.A., Hughes, K., Bennett, A., Thomson, R., 2003. The role of an international nightlife resort in the proliferation of recreational drugs. *Addiction* 98, 1713-1721.
- Borgen, L.A., Okerholm, R.A., Lai, A., Scharf, M.B., 2004. The pharmacokinetics of sodium oxybate oral solution following acute and chronic administration to narcoleptic patients. *J. Clin. Pharmacol.* 44, 253-257.
- Bronstein, A.C., Spyker, D.A., Cantilena, L.R., Green, J., Rumack, B.H., Heard, S.E., 2007. 2006 Annual Report of the American Association of Poison Control Centers' National Poison Data System. *Clin. Toxicol.* 45, 815-917.
- Bronstein, A.C., Spyker, D.A., Cantilena, J.R., Louis, R., Green, J.L., Rumack, B.H., Heard, S.E., 2008. 2007 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 25th Annual Report. *Clin. Toxicol.* 46, 927-1057.
- Broughton, R., Mamelak, M., 1979. The treatment of narcolepsy-cataplexy with nocturnal gamma-hydroxybutyrate. *Can. J. Neurol. Sci.* 6,1-6.

- Caldicott, D.G., Chow, F.Y., Burns, B.J., Felgate, P.D., Byard, R.W., 2004. Fatalities associated with the use of 7-hydroxybutyrate and its analogues in Australasia. *Med. J. Aust.* 181, 310-313.
- Carter, L.P., Flores, L.R., Wu, H., Chen, W., Unzeitig, A.W., Coop, A., France, C.P., 2003. The role of GABAB receptors in the discriminative stimulus effects of 7-hydroxybutyrate in rats: time course and antagonism studies. *J. Pharmacol. Exp. Ther.* 305, 668-674.
- Carter, L.P., Griffiths, R.R., Mintzer, M.Z., 2007. Psychomotor, subjective, and cognitive effects of GHB and triazolam in healthy volunteers. Presented at the 69th Annual Meeting of the College on Problems of Drug Dependence. <<http://www.cpdd.vcu.edu/Pages/Meetings/MeetProgAbSearchAll.html>> (accessed 17.07.08.).
- Carter, L.P., Koek, W., France, C.P., 2009. Behavioral analyses of GHB: receptor mechanisms. *Pharmacol. Ther.* 121,100-114.
- Carter, L.P., Richards, B.D., Mintzer, M.Z., Griffiths, R.R., 2006. Relative abuse liability of GHB in humans: a comparison of psychomotor, subjective, and cognitive effects of supratherapeutic doses of triazolam, pentobarbital, and GHB. *Neuropsychopharmacology* 31, 2537-2551.
- Carter, L.P., Wu, H., Chen, W., Matthews, M.M., Mehta, A.K., Hernandez, R.J., Thomson, J.A., Ticku, M.K., Coop, A., Koek, W., France, C.P., 2005. Novel gamma-hydroxybutyric acid (GHB) analogs share some, but not all behavioral effects of GHB and GABAB receptor agonists. *J. Pharmacol. Exp. Ther.* 313,1314-1323.
- Centers for Disease Control, 1991. Multistate outbreak of poisonings associated with illicit use of gamma hydroxy butyrate. *JAMA* 265,447-448.
- Chin, M.Y., Kreutzer, R.A., Dyer, J.E., 1992. Acute poisoning from 7-hydroxybutyrate in California. *West. J Med.* 156,380-384.
- Chin, R.L., Sporer, K.A., Cullison, B., Dyer, J.E., Wu, T.D., 1998. Clinical course of gamma-hydroxybutyrate overdose. *Ann. Emerg. Med.* 31, 716-722.
- Couper, F.J., Thatcher, J.E., Logan, B.K., 2004. Suspected GHB overdoses in the emergency department. *J. Anal. Toxicol.* 28, 481-484.
- Craig, K., Gomez, H.F., McManus, J.L., Bania, T.C., 2000. Severe gamma-hydroxybutyrate withdrawal: a case report and literature review. *J. Emerg. Med.* 18, 65-70.
- Degenhardt, L., Copeland, J., Dillon, P., 2005. Recent trends in the use of "club drugs": an Australian review. *Subst. Use Misuse* 40,1241-1256.

- Degenhardt, L., Darke, S., Dillon, P., 2002. GHB use among Australians: characteristics, use patterns and associated harm. *Drug Alcohol Depend.* 67, 89-94.
- Degenhardt, L., Darke, S., Dillon, P., 2003. The prevalence and correlates of gamma-hydroxybutyrate (GHB) overdose among Australian users. *Addiction* 98, 199-204.
- Degenhardt, L., Dunn, M., 2008. The epidemiology of GHB and ketamine use in an Australian household survey. *Int. J. Drug Policy* 19,311-316.
- Dietze, P.M., Cvetkovski, S., Barratt, M.J., Clemens, S., 2008. Patterns and incidence of gamma-hydroxybutyrate (GHB)-related ambulance attendances in Melbourne. *Victoria Med. J. Aust.* 188, 709-711.
- Doherty J.D., Roth R.H., 1978. Metabolism of 7-hydroxy-[1-14C] butyrate by rat brain: relationship to the Krebs cycle and metabolic compartmentation of aminoacids. *J. Neurochem.* 30,1305-1309.
- Dupont, P., Thornton, J., 2001. Near-fatal gamma-butyrolactone intoxication—first report in the UK. *Hum. Exp. Toxicol.* 20,19-22.
- Dyer, J.E., 1991.  $\gamma$ -Hydroxybutyrate: a health-food product producing coma and seizure-like activity. *Am. J. Emerg. Med.* 9,321-324.
- Dyer, J.E., Roth, B., Hyma, B.A., 2001. Gamma-hydroxybutyrate withdrawal syndrome. *Ann. Emerg. Med.* 37,147-153.
- ElSohly, M.A., Salamone, S.J., 1999. Prevalence of drugs used in cases of alleged sexual assault. *J. Anal. Toxicol.* 23,141-146.
- European Monitoring Centre for Drugs and Drug Addiction, Annual report 2007: the state of the drugs problem in Europe. Luxembourg: Office for Official Publications of the European Communities. <<http://www.emcdda.europa.eu/html.cfm/index44682EN.html>> (accessed 17.07.08.).
- European Monitoring Centre for Drugs and Drug Addiction, Annual report 2008: the state of the drugs problem in Europe. Luxembourg: Office for Official Publications of the European Communities. <[http://www.emcdda.europa.eu/attachements.cfm/att\\_64227\\_EN\\_EMCDAR08\\_en.pdf](http://www.emcdda.europa.eu/attachements.cfm/att_64227_EN_EMCDAR08_en.pdf)> (accessed 6.02.09.).
- Ferrara, S.D., Tedeschi, L., Frison, G., Rossi, A., 1995. Fatality due to gamma-hydroxybutyric acid (GHB) and heroin intoxication. *J. Forensic Sci.* 40,501-504.

- Frucht, S.J., Houghton, W.C., Bordelon, Y., Greene, P.E., Louis, E.D., 2005. A single-blind, open-label trial of sodium oxybate for myoclonus and essential tremor. *Neurology* 65, 1967-1969.
- Fuller, D.E., Hornfeldt, C.S., Kelloway, J.S., Stahl, P.J., Anderson, T.F., 2004. The Xyrem risk management program. *Drug Saf.* 27, 293-306.
- Galloway, G.P., Frederick, S.L., Staggers Jr., F.E., Gonzales, M., Stalcup, S.A., Smith, D.E., 1997. Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. *Addiction* 92, 89-96.
- Glasper, A., McDonough, M., Bearn, J., 2005. Within-patient variability in clinical presentation of gamma-hydroxybutyrate withdrawal: a case report. *Eur. Addict. Res.* 11, 152-154.
- Goodwin, A.K., Froestl, W., Weerts, E.M., 2005. Involvement of gamma-hydroxybutyrate (GHB) and GABAB receptors in the acute behavioral effects of GHB in baboons. *Psychopharmacology* 180,342-351.
- Goodwin, A.K., Griffiths, R.R., Brown, P.R., Froestl, W., Jakobs, C., Gibson, K.M., Weerts, E.M., 2006. Chronic intragastric administration of gamma-butyrolactone produces physical dependence in baboons. *Psychopharmacology* 189, 71-82.
- Griffiths, R.R., Bigelow, G.E., Ator, N.A., 2003. Principles of initial experimental drug abuse liability assessment in humans. *Drug Alcohol Depend.* 70 (Suppl. 3), S41-54.
- Griffiths, R.R., Johnson, M.W., 2005. Relative abuse liability of hypnotic drugs: a conceptual framework and algorithm for differentiating among compounds. *J. Clin. Psychiatry* 66 (Suppl.), 31-41.
- Hawaii Revised Statute, Chapter 329 - Uniformed Controlled Substances Act. <<http://hawaii.gov/dcca/areas/pvl/main/hrs/>> (accessed 1.10.08.).
- Hillebrand, J., Olszewski, D., Sedefov, R., 2008. EMCDDA Thematic Papers—GHB and its precursor GBL: an emerging trend case study. European Monitoring Centre for Drugs and Drug Addiction. <<http://www.emcdda.europa.eu/html.cfm/index50526EN.html>> (accessed 17.07.08.).
- Johnston, L.D., O'Malley, P.M., Bachman, J.G., Schulenberg, J.E., 2008. Various stimulant drugs show continuing gradual declines among teens in 2008, most illicit drugs hold steady, University of Michigan News Service: Ann Arbor, MI. <<http://monitoringthefuture.org/>> (accessed 23.01.09.).
- Juhascik, M.P., Negrusz, A., Faugno, D., Ledray, L, Greene, P., Lindner, A., Haner, B., Gaensslen, R.E., 2007. An estimate of the proportion of drug-facilitation of sexual assault in four U.S. localities. *J. Forensic Sci.* 52,1396-1400.

- Kim, S.Y., Anderson, I.B., Dyer, J.E., Barker, J.C., Blanc, P.D., 2007. High-risk behaviors and hospitalizations among gamma hydroxybutyrate (GHB) users. *Am. J. Drug Alcohol Abuse* 33, 429-438.
- Kim, S.Y., Barker, J.C., Anderson, I.B., Dyer, J.E., Earnest, G., Blanc, P.D., 2008. Systematic assessment of gamma hydroxybutyrate (GHB) effects during and after acute intoxication. *Am. J. Addict.* 17,312-318.
- Knudsen, K., Greter, J., Verdicchio, M., 2008. High mortality rates among GHB abusers in Western Sweden. *Clin. Toxicol. (Phila)* 46,187-192.
- Lai, M.W., Klein-Schwartz, W, Rodgers, G.C., Abrams, J.Y., Haber, D.A., Bronstein, A.C., Wruk, K.M., 2006. 2005 Annual Report of the American Association of Poison Control Centers' National Poisoning and Exposure Database. *Clin. Toxicol.* 44, 803-932.
- LeBeau, M.A., Miller, M.L., Levine, B., 2001. Effect of storage temperature on endogenous GHB levels in urine. *Forensic Sci. Int.* 15,161-167.
- LeBeau, M.A., Montgomery, M.A., Morris-Kukoski, C, Schaff, J.E., Deakin, A., 2007. Further evidence of in vitro production of gamma-hydroxybutyrate (GHB) in urine samples. *Forensic Sci. Int.* 169,152-156.
- LeBeau, M.A., Montgomery, M.A., Morris-Kukoski, C, Schaff, J.E., Deakin, A., Levine, B., 2006. A comprehensive study on the variations in urinary concentrations of endogenous gamma-hydroxybutyrate (GHB). *J. Anal. Toxicol.* 30, 98-105.
- Lettieri, J., Fung, H., 1978. Improved pharmacological activity via pro-drug modification: comparative pharmacokinetics of sodium 7-hydroxybutyrate and 7-butyrolactone. *Res. Commun. Chem. Pathol. Pharmacol.* 22,107-118.
- Litovitz, T.L., Klein-Schwartz, W, Rodgers, G.C., Cobaugh, D.J., Youniss, J., Omslaer, J.C, May, M.E., Woolf, A.D., Benson, B.E., 2002. 2001 Annual Report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. *Am. J. Emerg. Med.* 20, 391-452.
- Maitre, M., 1997. The 7-hydroxybutyrate signaling system in brain: organization and functional implications. *Prog. Neurobiol.* 51,337-361.
- Marwick, C, 1997. Coma-inducing drug GHB may be reclassified. *JAMA* 277, 1505-1506.
- Mason, P.E., Kerns 2nd, W.P., 2002. Gamma hydroxybutyric acid (GHB) intoxication. *Acad. Emerg. Med.* 9, 730-739.

- Maxwell, J.C., Spence, R.T., 2005. Profiles of club drug users in treatment. *Subst. Use Misuse* 40,1409-1426.
- McDaniel, C.H., Miotto, K.A., 2001. Gamma hydroxybutyrate (GHB) and gamma butyrolactone (GBL) withdrawal: five case studies. *J. Psychoactive Drugs* 33, 143-149.
- Miotto, K.A., Darakjian, J., Basch, J., Murray, S., Zogg, J., Rawson, R., 2001. Gamma-hydroxybutyric acid: patterns of use, effects and withdrawal. *Am. J. Addict.* 10, 232-241.
- National Drug Intelligence Center US. Department of Justice, 2008. National Drug Threat Assessment 2009. <<http://www.usdoj.gov/ndic/pubs31/31379/index.htm>> (accessed 23.01.09.).
- Nevada Administrative Code, Chapter 453 - Controlled Substances. <<http://www.leg.state.nv.us/NAC/NAC-453.html#NAC453Sec510>> (accessed 1.10.08.).
- Nicholson, K.L., Balster, R.L., 2001. GHB: a new and novel drug of abuse. *Drug Alcohol Depend.* 63,1-22.
- Office of Diversion Control, 2008. National Forensic Laboratory Information System: Year 2007 Annual Report. Washington, DC: U.S. Drug Enforcement Administration. <http://www.deadiversion.usdoj.gov/nflis/index.html> (accessed 6.02.09.).
- Palatini, P., Tedeschi, L, Frison, G., Padriani, R., Zordan, R., Orlando, R., Gallimberti, L, Gessa, G.L., Ferrara, S.D., 1993. Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. *Eur. J. Clin. Pharmacol.* 45, 353-356.
- Palmer, R.B., 2004. Gamma-butyrolactone and 1,4-buandiol: abused analogues of gamma-hydroxybutyrate. *Toxicol. Rev.* 23, 21-31.
- Pardi, D., Black, J., 2006. Gamma-hydroxybutyrate/sodium oxybate: neurobiology, and impact on sleep and wakefulness. *CNS Drugs* 20,993-1018.
- Price, G., 2000. In-patient detoxification after GHB dependence. *Br.J. Psychiatry* 177, 181.
- Russell, I.J., Perkins, A.T., Michalek, J.E., Oxybate SXB-26 Fibromyalgia Syndrome Study Group, 2009. Sodium oxybate relieves pain and improves function in fibromyalgia syndrome: a randomized, double-blind, placebo-controlled, multicenter clinical trial. *Arthritis Rheum.* 60, 299-309.

- Scharf, M.B., Brown, D., Woods, M., Brown, L., Hirschowitz, J., 1985. The effects and effectiveness of gamma-hydroxybutyrate in patients with narcolepsy. *J. Clin. Psychiatry* 46, 222-225.
- Scharf, M.B., Lai, A.A., Branigan, B., Stover, R., Berkowitz, D.B., 1998. Pharmacokinetics of gamma-hydroxybutyrate (GHB) in narcoleptic patients. *Sleep* 21,507-514.
- Slaughter, L., 2000. Involvement of drugs in sexual assault. *J. Reprod. Med.* 45, 425-430.
- Strickland, R.M., Felgate, P., Caldicott, D.G., 2005. Survival of massive gamma-hydroxybutyrate/1,4-butanediol overdose. *Emerg. Med. Australas.* 17, 281-283.
- Strom, K.J., Wong, L., Fornnarino, L., Eicheldinger, C., Bethke, A., Ancheta, J., Rachal, V., 2003. The National Forensic Laboratory Information System: 2002 Annual Report. Washington, DC: U.S. Drug Enforcement Administration. <<http://www.deadiversion.usdoj.gov/nflis/index.html>> (accessed 17.07.08.).
- Strom, K.J., Wong, L., Fornnarino, L., Eicheldinger, C., Bethke, A., Ancheta, J., Rachal, V., 2004. The National Forensic Laboratory Information System: 2003 Annual Report. Washington, DC: U.S. Drug Enforcement Administration. <<http://www.deadiversion.usdoj.gov/nflis/index.html>> (accessed 17.07.08.).
- Substance Abuse and Mental Health Services Administration, Office of Applied Studies, 2008a. Drug Abuse Warning Network, 2006: National Estimates of Drug-Related Emergency Department Visits. DAWN Series D-30, DHHS Publication No. (SMA) 08-4339, Rockville, MD. <<http://dawninfo.samhsa.gov/pubs/edpubs/default.asp>> (accessed 6.02.09.).
- Substance Abuse and Mental Health Services Administration, Office of Applied Studies, 2008b. Results from the 2007 National Survey on Drug Use and Health: National Findings. NSDUH Series H-34, DHHS Publication No. (SMA) 08-4343, Rockville, MD. <<http://oas.samhsa.gov/nsduh/2k7nsduh/2k7Results.pdf>> (accessed 6.02.09.).
- Sumnall, H.R., Woolfall, K., Edwards, S., Cole, J.C., Beynon, CM., 2008. Use, function, and subjective experiences of gamma-hydroxybutyrate (GHB). *Drug Alcohol Depend.* 92, 286-290.
- Tarabar, A.F., Nelson, L.S., 2004. The gamma-hydroxybutyrate withdrawal syndrome. *Toxicol. Rev.* 23, 45-49.
- Thai, D., Dyer, J.E., Benowitz, N.L., Haller, C.A., 2006. Gamma-hydroxybutyrate and ethanol effects and interactions in humans. *J. Clin. Psychopharmacol.* 26, 524-529.

- Thai, D., Dyer, J.E., Jacob, P., Haller, C.A., 2007. Clinical pharmacology of 1,4-butanediol and gamma-hydroxybutyrate after oral 1,4-butanediol administration in healthy volunteers. *Clin. Pharmacol. Ther.* 81,178-184.
- The National Forensic Laboratory Information System, 2001. Year 2000 Annual Report. U.S. Drug Enforcement Administration, Office of Diversion Control. Washington, D.C. <<http://www.deadiversion.usdoj.gov/nflis/index.html>> (accessed 17.07.08.).
- The National Forensic Laboratory Information System, 2002. Year 2001 Annual Report. U.S. Drug Enforcement Administration, Office of Diversion Control. Washington, D.C. <<http://www.deadiversion.usdoj.gov/nflis/index.html>> (accessed 17.07.08.).
- U.S. Controlled Substances Act. Title 21, U.S.C. 802(34). <http://www.deadiversion.usdoj.gov/21cfr/21usc/802.htm#34> (accessed 4.09.08.).
- U.S. Xyrem Multi-Center Study Group, 2003. The abrupt cessation of therapeutically administered sodium oxybate (GHB) does not cause withdrawal symptoms. *J. Toxicol. Clin. Toxicol.* 41,131-135.
- Van Sassenbroeck, D.K., De Neve, N., De Paepe, P., Belpaire, F.M., Verstraete, A.G., Calle, P.A., Buylaert, W.A., 2007. Abrupt awakening phenomenon associated with gamma-hydroxybutyrate use: a case series. *Clin. Toxicol. (Phila)* 45,533-538.
- Varela, M., Nogue, S., Oros, M., Miro, O., 2004. Gamma hydroxybutyrate use for sexual assault. *Emerg. Med.J.* 21, 255-256.
- Wang, Y.G., Swick, T.J., Carter, L.P., Thorpy, M.J., Benowitz, N.L. Safety overview of post-marketing and clinical experience of sodium oxybate (Xyrem®): abuse, misuse, dependence, and diversion. *J. Clin. Sleep Med*, in press.
- Watson, W.A., Litovitz, T.L., Rodgers, G.C., Klein-Schwartz, W., Reid, N., Youniss, J., Flanagan, A., Wruk, K.M., 2005. 2004 Annual Report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. *Am. J. Emerg. Med.* 23, 589-666.
- Watson, W.A., Litovitz, T.L., Rodgers, G.C., Klein-Schwartz, W., Youniss, J., Reid, N., Rouse, W.G., Rembert, R.S., Borys, D., 2004. 2003 Annual Report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. *Am. J. Emerg. Med.* 22, 335-404.
- Watson, W.A., Litovitz, T.L., Rodgers, G.C., Klein-Schwartz, W., Youniss, J., Rose, S.R., Borys, D., May, M.E., 2003. 2002 Annual Report of the American Association

of Poison Control Centers Toxic Exposure Surveillance System. *Am. J. Emerg. Med.* 21,353-421.

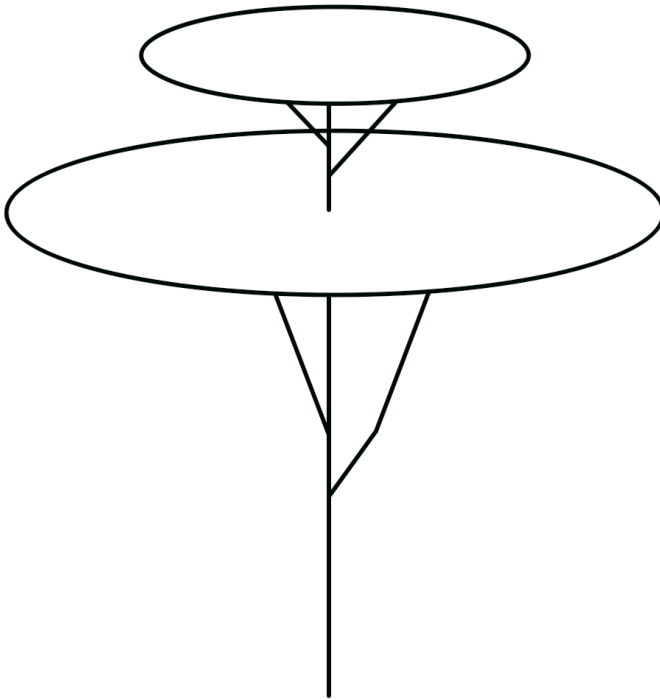
- Wedin, G.P., Hornfeldt, C.S., Ylitalo, L.M., 2006. The clinical development of gamma-hydroxybutyrate (GHB). *Curr. Drug Safety* 1, 99-106.
- Weerts, E.M., Goodwin, A.K., Griffiths, R.R., Brown, P.R., Froestl, W., Jakobs, C., Gibson, K.M., 2005. Spontaneous and precipitated withdrawal after chronic intragastric administration of gamma-hydroxybutyrate (GHB) in baboons. *Psychopharmacology* 179, 678-687.
- Weimer, B.J., Peters, D., Sannerud, C., Eicheldinger, C., Ancheta, J., Strom, K., Rachal, V., 2007. The National Forensic Laboratory Information System: 2006 Annual Report. Washington, DC: U.S. Drug Enforcement Administration. <<http://www.deadiversion.usdoj.gov/nflis/index.html>> (accessed 17.07.08.).
- Weimer, B.J., Wong, L., Sannerud, C., Eicheldinger, C., Ancheta, J., Strom, K., Rachal, V., 2006. The National Forensic Laboratory Information System: 2005 Annual Report. Washington DC: U.S. Drug Enforcement Administration. <<http://www.deadiversion.usdoj.gov/nflis/index.html>> (accessed 17.07.08.).
- Weimer, B.J., Wong, L., Strom, K., Forti, A., Eicheldinger, C., Bethke, A., Ancheta, J., Rachal, V., 2004. The National Forensic Laboratory Information System: Year 2004 Annual Report. Washington, DC: U.S. Drug Enforcement Administration. <<http://www.deadiversion.usdoj.gov/nflis/index.html>> (accessed 17.07.08.).
- Williams, S.R., 1998. Gamma-hydroxybutyric acid poisoning. *West. J. Med.* 168, 187-188.
- Winickoff, J.P., Houck, C.S., Rothman, E.L., Bauchner, H., 2000. Verve and Jolt: deadly new internet drugs. *Pediatrics* 106, 829-830.
- Wojtowicz, J.M., Yarema, M.C., Wax, P.M., 2008. Withdrawal from gamma-hydroxybutyrate, 1,4-butanediol and gamma-butyrolactone: a case report and systematic review. *CJEM* 10, 69-74.
- Wood, D.M., Warren-Gash, C., Ashraf, T., Greene, S.L., Shather, Z., Trivedy, C., Clarke, S., Ramsey, J., Holt, D.W., Dargan, P.I., 2008. Medical and legal confusion surrounding gamma-hydroxybutyrate (GHB) and its precursors gamma-butyrolactone (GBL) and 1,4-butanediol (1,4BD). *QJM* 101, 23-29.
- Xyrem Prescribing Information, 2008. Jazz Pharmaceuticals, Inc. <<http://www.xyrem.com/prescribing-information.php>> (accessed 17.07.08.).
- Zvosec, D.L., Smith, S.W., McCutcheon, J.R., Spillane, J., Hall, B.J., Peacock, E.A., 2001. Adverse events, including death, associated with the use of 1,4-butanediol. *New Engl. J. Med*

## **CHAPTER 6**

### **The Nightly Use of Sodium Oxybate Is Associated with a Reduction in Nocturnal Sleep Disruption: A Double-Blind, Placebo-Controlled Study in Patients with Narcolepsy**

*Journal of Clinical Sleep Medicine (2010) 6(6): 596-602.*

**Authors:** Jed Black, Daniel Pardi, Carl S. Hornfeldt and Neil Inhaber



**Abstract**

|                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Objective</b>  | To further explore the effects of sodium oxybate (SXB) administration on nocturnal sleep in narcolepsy patients during a double-blind, placebo-controlled, parallel group study conducted with 228 adult patients with narcolepsy/cataplexy in the United States, Canada, and Europe.                                                                                                                                                                                                                                                                                                        |
| <b>Methods</b>    | Patients were withdrawn from antidepressants and sedative/hypnotics, and then randomized to receive 4.5, 6, or 9 g SXB or placebo nightly for 8 weeks. Patients receiving 6 and 9 g/night doses were titrated to their final dose in weekly 1.5 g increments, while patients receiving placebo were randomized to undergo a similar mock dose titration. The use of stimulant therapy continued unchanged. Changes in sleep architecture were measured using centrally scored nocturnal polysomnograms. Daily diaries were used to record changes in narcolepsy symptoms and adverse events. |
| <b>Results</b>    | Following 8 weeks of SXB treatment, study patients demonstrated significant dose-related increases in the duration of stage 3 and 4 sleep, reaching a median increase of 52.5 minutes in patients receiving 9 g nightly. Compared to placebo-treated patients, delta power was significantly increased in all dose groups. Stage 1 sleep and the frequency of nocturnal awakenings were each significantly decreased at the 6 and 9 g/night doses. The changes in nocturnal sleep coincided with significant decreases in the severity and frequency of narcolepsy symptoms.                 |
| <b>Conclusion</b> | The nightly administration of SXB to narcolepsy patients significantly impacts measures of slow wave sleep, wake after sleep onset, awakenings, total sleep time, and stage 1 sleep in a dose-related manner. The frequency and severity of narcolepsy symptoms decreased with treatment.                                                                                                                                                                                                                                                                                                    |

**Keywords:** Narcolepsy, polysomnography, sodium oxybate, sleep architecture, delta power

**Citation:** Black J; Pardi D; Hornfeldt CS; Inhaber N. The nightly use of sodium oxybate is associated with a reduction in nocturnal sleep disruption: a double-blind, placebo-controlled study in patients with narcolepsy.

## Brief Summary

**Current Knowledge/Study Rationale:** The efficacy of sodium oxybate (SXB) for the treatment of cataplexy and excessive daytime sleepiness in patients with narcolepsy has been previously demonstrated in randomized controlled trials and may be due, in part, to SXB-related improvement in disrupted nocturnal sleep. The present study is the first, large, randomized, controlled, parallel group trial in patients with narcolepsy examining the impact of nightly administration of SXB on sleep architecture and narcolepsy symptoms.

**Study Impact:** The nocturnal administration of SXB to narcolepsy results in significant dose-related changes in sleep architecture resulting in decreased sleep disruption and increased slow wave sleep. These findings are consistent with improvement in measures of sleep continuity and suggest SXB may promote some amelioration of the sleep fragmentation that is common in narcolepsy.

## Introduction

The efficacy of nightly administered sodium oxybate (SXB) for the treatment of cataplexy and excessive daytime sleepiness (EDS) in patients with narcolepsy has been well established.<sup>1-5</sup> While the mechanism whereby SXB diminishes the diurnal symptoms of narcolepsy is unknown, it has been observed that SXB also has pharmacodynamic effects on nocturnal sleep, which is frequently disrupted in patients with narcolepsy.<sup>6</sup> Of particular interest, SXB has been shown to consistently increase the duration of stage 3 and 4 (slow wave or delta) sleep.<sup>7-10</sup>

An initial 10-week pilot study in 21 patients with narcolepsy tested the hypothesis that nightly SXB administration produces dose-related changes in sleep architecture. After withdrawing patients from antidepressants and sedative-hypnotics, the administration of SXB decreased nightly awakenings and increased the duration of sleep stages 3 and 4. In addition, delta power was significantly increased at all doses tested. The duration of REM sleep increased initially, and then decreased modestly over the duration of the 10-week trial.<sup>11</sup> As the patients in this preliminary study were titrated from 4.5 to 9 g nightly, it was not possible to determine whether the effects of SXB were dependent upon the dose used, the 10-week duration of therapy, or a combination of both.

The following double-blind, placebo-controlled, parallel group study, designed to assess the efficacy of SXB for the treatment of excessive daytime sleepiness in narcolepsy, permitted further examination of the effects of nightly SXB administration on sleep architecture. Specifically, changes in nocturnal polysomnography (PSG) parameters were measured, providing additional information on the effects of SXB on nocturnal sleep. Other measures of efficacy have been published elsewhere.<sup>1-5,12</sup>

## **Methods**

### **Subjects**

Patients included in the trial were  $\geq 16$  years of age and met the following criteria: diagnosis of narcolepsy based on an overnight PSG and multiple sleep latency test (MSLT)<sup>13</sup>, and current symptoms of narcolepsy, including excessive daytime sleepiness, cataplexy, and recurrent sleep attacks for  $> 3$  months (all patients met current ICSD-2 criteria for narcolepsy with cataplexy). Additional criteria included: willingness to forgo operating a car or heavy machinery if indicated by the investigator; and willingness to complete the entire trial as described in the protocol by signing an informed consent. Women of child-bearing potential agreed to use a medically accepted method of birth control, unless surgically sterile or two years post-menopausal.

The following criteria were used to exclude patients from the trial: use of SXB or investigational drug therapy within 30 days of trial entry; sleep apnea or any other cause of daytime sleepiness; use of hypnotics, anxiolytics, or any other sedating medications; any unstable disease that might place the patient at risk during the study or might compromise the study objectives; history of a substance abuse disorder; serum creatinine  $> 2.0$  mg/dL, liver function tests more than twice the normal upper limit, serum bilirubin  $> 1.5$  times the normal upper limit, or an ECG demonstrating clinically significant arrhythmias; history of myocardial infarction within 6 months; an occupation requiring changing shifts or routine night shifts; or history of seizure disorder, head trauma, or invasive intracranial surgery. Patients were also excluded from the study if the initial PSG study revealed the presence of moderate to severe sleep apnea syndrome, defined as an apnea index of  $> 10/h$ , or apnea/ hypopnea index  $> 15/h$ , or any sleep disorder except narcolepsy.

### **Dosing and Administration of Study Drug**

Trial medication consisted of a concentrated oral solution containing 500 mg/mL SXB; placebo consisted of a sodium citrate solution that was equimolar to the study drug with respect to sodium. Previous taste tests confirmed the placebo is indistinguishable from SXB solution (Jazz Pharmaceuticals, Inc., data on file). Study drug or placebo was administered in two equally divided doses each night. Patients participating in the trial were instructed to take the second dose of SXB 2.5 to 4 h following the first dose, when dosing the medication at home. However, during the in-lab PSG nights, the total PSG-recording duration was set at exactly 8 h and the 8-h night was split into 2 consecutive 4-h periods. SXB was dosed at the beginning of each 4-h period.

Overall, 78% of patients were taking CNS stimulants for the treatment of EDS; the dosage of these medications was held constant throughout the trial. A post hoc analysis revealed the use of stimulant medications was uniformly distributed across placebo and active drug groups (range 74.6% to 83.6%) (Jazz Pharmaceuticals, Inc., data on file). Patients were cautioned against the use of alcoholic beverages and potentially sedating medications such as opiate analgesics

or skeletal muscle relaxants at any time during the trial and were required to discuss the use of all medicines with a study investigator.

## Study Design

The study design, including visit number and frequency, is illustrated in **Figure 1**. Following clinic Visit 1, patients recorded narcolepsy symptoms and adverse events associated with current narcolepsy treatments in daily diaries during a 14-day lead-in period. Following clinic Visit 2, patients were gradually tapered from antidepressants or any other medication used for the treatment of cataplexy during a 21-day withdrawal period. This was followed by a washout period lasting 5 days or 5 times the half-life of the discontinued drug, whichever was longer, but not exceeding 18 days. Withdrawal from fluoxetine was initiated at clinic Visit 1 due to its very long half-life. If withdrawal from an antidepressant was not required, patients entered a mock 5-day washout period.

Following the washout period, patients entered a 14-day baseline period and received placebo in single-blind fashion. During this phase of the trial, patients were acclimated to the use of daily diaries, and baseline assessments of narcolepsy symptoms were recorded. The 14-day baseline period was extended to 21 days if, in the opinion of the investigator, the frequency of cataplexy attacks had not stabilized. To remain eligible for the double-blind phase of the trial, each patient was required to record a minimum average of 8 cataplexy attacks per week during the baseline period. Patients were kept unaware of this requirement. To be classified as cataplexy for this trial, the event must have had sudden onset, been localized to a specific muscle group(s) or part of the body in a bilateral manner, and occurred while the patient was lucid (i.e., not experiencing a sleep attack or microsleep).

The dose-titration phase (Visit 5) was conducted in randomized, double-blind fashion as follows:

- *Week 1:* One-fourth of the study patients received placebo, while three-fourths received 4.5 g SXB nightly.
- *Week 2:* One-third of patients receiving SXB remained at the 4.5 g/night dose, while two-thirds increased their dose to 6 g nightly; two-thirds of placebo patients increased the volume of their placebo dose by an equivalent amount to match the 6 g/night dose.
- *Week 3:* One-half of patients taking SXB at the 6 g/night dose remained at this dose, while the remaining half increased their dose to 7.5 g nightly; one-half of the placebo patients taking the mock 6 g/night dose continued taking the same volume, while the remaining half increased their volume of their placebo dose to match the 7.5 g/night dose.
- *Week 4:* All patients taking the 7.5 g/night SXB dose increased their dose to 9 g nightly, while the placebo patients taking the mock 7.5 g/night dose increased the volume of their dose by an equivalent amount.

Each subject returned to the clinic at Visit 6 when study measures for efficacy were conducted and safety assessments were made. Patients then continued at their

assigned dose for the remaining 28 days of the study before returning for the final efficacy and safety assessments at Visit 7. PSG and MWT were performed at Visits 2, 5, 6, and 7; and awakenings, defined as the average number of awakenings during the night per week based on diary data from the 2 weeks immediately preceding the visit, were recorded.

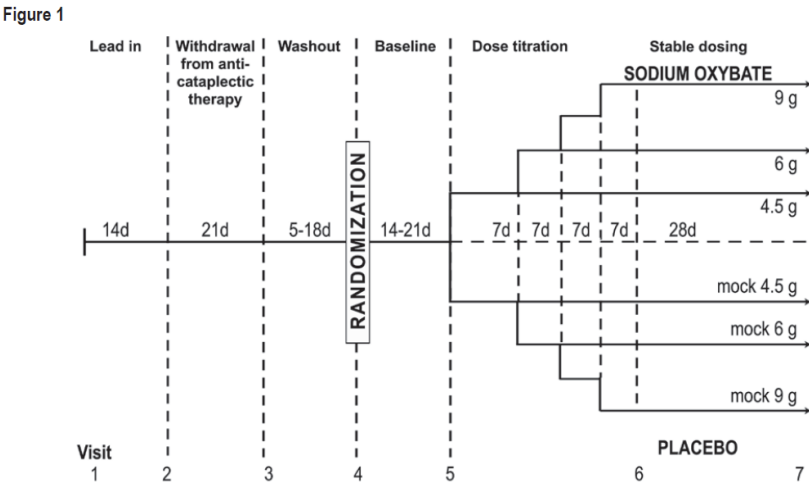


Figure 1 – Study design

**Polysomnographic Recordings**

The PSG data were obtained as described elsewhere<sup>11</sup> using previously described techniques.<sup>14</sup> Briefly, data from electro encephalographic (EEG) and other parameters, including eye movements (EOG), submental muscle tone (chin-EMG), electrocardiogram (ECG), and right and left anterior tibialis muscle activity (leg-EMG) as well as nasal air flow, thoracic and abdominal effort, and oxygen saturation, were digitally recorded and manually scored by trained registered polysomnographers in a blind manner using a validated software program. The following variables were measured during each half of the night, corresponding with the first and second doses of SXB, and were subsequently added together for the entire night: sleep latency; total sleep time (TST); wake after sleep onset (WASO); duration of stages 1, 2, 3 and 4, and REM sleep; sleep stage shifts per hour; and nocturnal awakenings. REM density was defined as the percent of 2-sec REM epochs containing one or more rapid eye movements; REM sleep latency was examined for the first half of the night only; delta power was defined as the accumulated EEG signal power for all frequencies between and including 0.5 Hz and 4 Hz.

## Data Analysis

All computations were performed using the Statistical Analysis System (SAS; SAS Institute Inc., Cary, NC) and were performed on an intent-to-treat basis using patients who received trial drug and completed at least one post-treatment evaluation visit. All analyses were based on the change from baseline to endpoint. Baseline was defined as the PSG measures at Visit 5. Endpoint was defined as the PSG measures at Visit 7, or in the absence of Visit 7 data, Visit 6. The test for a normal distribution of data was performed using the Wilks-Shapiro test, and homogeneity of the variability was examined graphically. If the data were found to be normal and homogeneous in distribution, an analysis of variance (ANOVA) model was used to analyze the data. Factors in the ANOVA model included treatment group, trial site, and the interaction between treatment and trial site. If the interaction was not found to be statistically significant ( $p \geq 0.10$ ) the term was dropped from the model. If a significant interaction effect was found, the nature of the interactions and the impact on the study conclusion was assessed. If a significant difference between treatment groups was found, each of the treatment groups was compared to placebo using the Dunnett test. If the data were not normally distributed, nonparametric tests were used. The Kruskal-Wallis test was used to assess the differences between treatment groups in the change from baseline. The Wilcoxon signed rank test was used to assess the change from baseline within treatment groups, and the Mann-Whitney test was used for pairwise comparisons of active treatment versus placebo. Interpretation and generalization of the study results in this multi-center study were assessed by examining the treatment by trial site interactions. In the data analysis involving ANOVA models, treatment by trial site interaction was tested and if the interaction was significant, the impact on the study results was evaluated. This interaction was modeled as a fixed effect, although modeling as a random effect may further increase the ability to generalize these results. The dose-response relationship in the PSG measures was assessed by testing the slope of simple linear regression model of individual PSG measure and dose, using data from the 3 active treatment groups. Statistical significance was accepted if the adjusted p-value was  $< 0.05$ . The significance of the mean change from baseline for each treatment group was determined using a paired *t*-test.

## Ethics

This trial was conducted at 42 sites between November 2000 and March 2004 in the United States, Canada, United Kingdom, Germany, France, Switzerland, Netherlands, and the Czech Republic, and was approved by the institutional review board/ethics committee of each participating trial center. Written informed consent was obtained from each patient prior to initiation of the study. This study was conducted in accordance with the ethical principles delineated in the Helsinki Declaration of 1975, as revised in 1997.

## Results

Of 401 patients who signed informed consent and entered the screening phase, 353 were enrolled, 285 were randomized to treatment, 246 received at least one dose of study drug, and 228 entered the double-blind phase of the trial. The intent-to-treat (ITT) population included these 228 patients who received at least one dose of study drug and had baseline PSG efficacy data and week 4 (Visit 6) and/or week 8 (Visit 7) PSG data. Of the patients randomized to treatment (285), 78% were taking stimulants, 14.7% (42/285) were taking tricyclic antidepressants (TCAs), and 14% (40/285) were taking serotonin selective reuptake inhibitors (SSRIs).

Of the ITT population, 149 (65.4%) were female and 79 (34.6%) were male. The average age was 40.5 years (range 16–75); the average height was 168.1 cm (range 139.7–202.0); the average weight was 85.7 kg (range 46.3–170.6). A total of 196 patients were Caucasian, 25 were of African descent, 2 were Asian, 2 were Hispanic, and 2 were of other ethnic origins. An analysis across all treatment groups indicated that patients were evenly distributed with respect to the above demographic parameters. The study was completed by 206 patients and polysomnographic data were available at weeks 4 and 8 for 191 and 193 patients, respectively. The duration of REM sleep and NREM sleep were found to be normally distributed and were analyzed with ANOVA models. For the other variables, nonparametric methods were used.

### Effect of Sodium Oxybate on Nocturnal Polysomnography Variables

#### *Sleep Latency and Total Sleep Time*

Sleep latency was not significantly different among the treatment groups after the initial dose or after the 2nd dose (data not shown). TST was increased after 8 weeks of treatment, reaching significance at the 9 g/night dose (**Table 2**), but no change was seen at 4 weeks (**Table 1**). At 8 weeks, there was a significant relationship between dose and increased TST ( $p = 0.0127$ )

#### *Awakenings and Wake after Sleep Onset*

The number of nocturnal awakenings was significantly decreased in all dose groups at 4 weeks (**Table 1**) and in the 6 g/night and 9 g/night groups at 8 weeks (**Table 2**). There was a significant relationship between increased dose and decreased number of awakenings at 8 weeks ( $p = 0.0444$ ). WASO was significantly decreased in the 9 g/night group at 8 weeks (**Table 2**). There was a significant dose relationship for the decrease in WASO at 8 weeks as well ( $p = 0.0075$ ). Sleep stage shifts per hour was not different between the treatment groups at either time point.

### *Stage 1 and 2 Sleep*

The duration of Stage 1 sleep was significantly decreased in all SXB treatment groups at 4 weeks (**Table 1**) and in the 6 g/night and 9 g/night groups at 8 weeks of treatment (**Table 2**). There was a significant dose relationship for the decrease in stage 1 sleep ( $p = 0.0355$ ). The duration of stage 2 sleep was not significantly different among the treatment groups at either 4 or 8 weeks.

### *Stage 3 and 4 Sleep*

The duration of stage 3 and 4 sleep was significantly increased for the 6 g/night and 9 g/night groups at 4 weeks (**Table 1**) and for all 3 SXB treatment groups at 8 weeks (**Table 2**). As shown in **Tables 1** and **2**, substantial increases occurred during both halves of the night. This increase in stage 3 and 4 sleep was significantly dose-related at both 4 weeks ( $p < 0.0001$ ) and 8 weeks ( $p < 0.0001$ ).

### *Delta Power*

Median delta power was significantly increased in all SXB treatment groups at both 4 weeks (**Table 1**) and 8 weeks (**Table 2**). These increases were proportionately greater during the second half of the night (**Tables 1** and **2**). A significant dose relationship was not observed ( $p = 0.1353$  at 4 weeks and  $p = 0.1323$  at 8 weeks) due to high variability.

### *REM Sleep Latency and Duration*

Sodium oxybate administration had no appreciable effect on REM sleep. The duration of REM sleep was significantly decreased in the 9 g/night group at 4 weeks (**Table 1**) and 8 weeks (**Table 2**).

### *Effect of Sodium Oxybate on the Symptoms of Narcolepsy*

Data reported elsewhere<sup>4,5</sup> indicate that the nightly administration of 4.5, 6, and 9 g/night doses of SXB resulted in significant decreases in median weekly cataplexy attacks. Patients also experienced significant improvements in both subjective and objective measures of excessive daytime sleepiness (Epworth Sleepiness Scale; 40-min maintenance of wakefulness test) and quality of life,<sup>12</sup> as well as significant improvements in the clinical investigator-rated evaluation of disease severity.

### *Safety*

Twenty-one patients discontinued the trial due to an adverse event, with most occurring in the 9 g/night dose group; 15 unique events occurred overall with a frequency significantly greater than placebo. Nausea, headache, dizziness, nasopharyngitis, and enuresis occurred with an overall incidence greater than 5%. Of these, only nausea and dizziness reached a level of statistical significance compared with placebo. These appeared to be dose related and occurred in 34/186 (18.3%) and 31/186 (16.7%) of the SXB-treated subjects. There were no deaths.

Six serious adverse events were reported during the study. Three of these occurred in patients receiving placebo. Of the remaining 3 events, an episode of pneumonitis was reported in one patient receiving 6 g of SXB and was reported to be un-related to drug. Another patient receiving 9 g of SXB suffered a fractured ankle following an accidental fall during the night. In this case, the relationship to study medication was reported as unknown. The third patient receiving 4.5 g of SXB demonstrated abnormal amino alanine transferase (ALT) and aspartate amino transferase (AST) at the conclusion of the trial, which returned to normal approximately 8 months later. This event was reported to be possibly due to study medication. These events are described in greater detail in another report.<sup>5</sup>

**TABLE 1.** Changes in polysomnographic parameters following 4 weeks of treatment\*† (change from baseline; median)

|                                | Placebo | SXB 4.5g | SXB 6g | SXB 9g  |                                                | Placebo  | SXB 4.5g | SXB 6g   | SXB 9g   |
|--------------------------------|---------|----------|--------|---------|------------------------------------------------|----------|----------|----------|----------|
|                                | N=48    | N=59     | N=49   | N=35    |                                                | N=48     | N=59     | N=49     | N=35     |
| <b>Total Sleep Time (min)</b>  |         |          |        |         | <b>Stage 3 and 4 Sleep (min)</b>               |          |          |          |          |
| 1 <sup>st</sup> Half           | 1.50    | 1.75     | -0.25  | -1.50   | 1 <sup>st</sup> Half                           | 0.00     | 1.00     | 9.75     | 27.50    |
| 2 <sup>nd</sup> Half           | 0.00    | 0.00     | 9.25   | 9.00    | 2 <sup>nd</sup> Half                           | 0.00     | 0.50     | 5.75     | 34.00    |
| <b>TOTAL</b>                   | 1.75    | 1.00     | 9.25   | 7.00    | <b>TOTAL</b>                                   | 0.00     | 3.25     | 3.25     | 71.00    |
|                                | -       | NS       | NS     | NS      |                                                | -        | NS       | P=0.002  | P<0.001  |
| <b>Total NREM Sleep (min)</b>  |         |          |        |         | <b>Wake After Sleep Onset (min)</b>            |          |          |          |          |
| 1 <sup>st</sup> Half           | 9.25    | 6.75     | 0.50   | 8.50    | 1 <sup>st</sup> Half                           | -2.50    | -3.75    | -2.50    | -0.50    |
| 2 <sup>nd</sup> Half           | -0.50   | 10.00    | 15.50  | 38.50   | 2 <sup>nd</sup> Half                           | 1.25     | 2.25     | -8.50    | -8.00    |
| <b>TOTAL</b>                   | 4.25    | 16.25    | 13.00  | 41.50   | <b>TOTAL</b>                                   | -3.75    | -0.25    | -13.75   | -7.50    |
|                                | -       | NS       | NS     | p=0.001 |                                                | -        | NS       | NS       | NS       |
| <b>Total REM Sleep (min)</b>   |         |          |        |         | <b>Sleep Stage Shifts Per Hour</b>             |          |          |          |          |
| 1 <sup>st</sup> Half           | -5.25   | -0.25    | -3.50  | -9.50   | 1 <sup>st</sup> Half                           | 0.07     | -1.32    | 1.59     | -4.08    |
| 2 <sup>nd</sup> Half           | -1.75   | 1.00     | -5.50  | -19.00  | 2 <sup>nd</sup> Half                           | -3.13    | -1.67    | -2.64    | -1.05    |
| <b>TOTAL</b>                   | -8.50   | -0.50    | -8.00  | -22.00  | <b>AVERAGE</b>                                 | -0.59    | -0.55    | -0.20    | -1.86    |
|                                | -       | NS       | NS     | p=0.010 |                                                | -        | NS       | NS       | NS       |
| <b>REM Sleep Latency (min)</b> |         |          |        |         | <b>Delta Power (microvolts<sup>2</sup>/Hz)</b> |          |          |          |          |
|                                | -0.75   | -3.25    | -9.00  | 1.00    | 1 <sup>st</sup> Half                           | -3720.20 | 5320.09  | 14868.09 | 26498.77 |
|                                | -       | NS       | NS     | NS      | 2 <sup>nd</sup> Half                           | 1142.55  | 7889.88  | 17058.46 | 28035.86 |

|                            |       |         |         |         |                             |         |         |          |          |
|----------------------------|-------|---------|---------|---------|-----------------------------|---------|---------|----------|----------|
|                            |       |         |         |         | <b>AVERAGE</b>              | -782.46 | 4842.03 | 14812.20 | 29629.76 |
|                            |       |         |         |         |                             | -       | p=0.026 | p<0.001  | p<0.001  |
| <b>Stage 1 Sleep (min)</b> |       |         |         |         | <b>Nocturnal Awakenings</b> |         |         |          |          |
| 1 <sup>st</sup> Half       | -1.50 | -6.75   | -6.00   | -9.00   | 1 <sup>st</sup> Half        | 0.00    | -3.50   | -3.00    | -4.00    |
| 2 <sup>nd</sup> Half       | 0.00  | -7.50   | -13.00  | -12.50  | 2 <sup>nd</sup> Half        | -1.50   | -6.00   | -6.00    | -9.00    |
| <b>TOTAL</b>               | -0.50 | -11.75  | -17.00  | -22.50  | <b>TOTAL</b>                | -1.00   | -7.50   | -10.50   | -15.00   |
|                            | -     | p=0.002 | p=0.002 | p<0.001 |                             | -       | p=0.011 | p=0.011  | p=0.015  |
| <b>Stage 2 Sleep (min)</b> |       |         |         |         |                             |         |         |          |          |
| 1 <sup>st</sup> Half       | 0.00  | 9.00    | -1.00   | 3.00    |                             |         |         |          |          |
| 2 <sup>nd</sup> Half       | 1.50  | 11.75   | 7.00    | 6.00    |                             |         |         |          |          |
| <b>TOTAL</b>               | -0.25 | 11.00   | 4.25    | 11.00   |                             |         |         |          |          |
|                            | -     | NS      | NS      | NS      |                             |         |         |          |          |

\*Statistical significance was established compared to placebo. NS = not significant.

†Expressed as medians following transformation of non-normal data.

## Discussion

One of the earliest reports describing the use of sodium oxy-bate (also known as γ-hydroxybutyrate sodium; GHB sodium) for the treatment of narcolepsy indicated that doses of 50 mg/kg (approximately 3.75–6.25 g nightly) significantly increased the duration of nocturnal slow wave sleep at the expense of stage 1 sleep and generally improved the continuity of nocturnal sleep.<sup>8</sup> These changes in nocturnal sleep coincided with patient reports of improvements in the quality of sleep, diminished daytime drowsiness and decreased cataplexy.<sup>15</sup>

Subsequent investigations into the use of SXB have yielded similar results on nocturnal sleep in patients with narcolepsy.<sup>9-11,16,17</sup> Collectively, these studies demonstrate that the nightly administration of SXB decreases nocturnal awakenings and increases slow wave sleep in a dose-dependent fashion, as well as imparting effects on REM sleep. These studies also demonstrate that the changes in nighttime sleep are accompanied by improvements in other clinical manifestations of narcolepsy, including cataplexy, subjective and objective measures of excessive daytime sleepiness, hypnagogic hallucinations, and sleep paralysis.

The present study is the largest controlled study to compare multiple doses of SXB to placebo performed to date, permitting a more thorough evaluation of impact on nighttime sleep and narcolepsy symptoms following the nightly administration

of SXB in patients with narcolepsy. As predicted by a previous open-label pilot study,<sup>11</sup> the nocturnal administration of SXB produced significant dose-related increases in both slow wave sleep and total sleep time. Robust increases in stage 3 and 4 sleep and delta power occurred in association with corresponding decreases in stage 1 sleep, REM sleep, and number of nocturnal awakenings, while stage 2 sleep remained unaffected. These findings are consistent with previously published SXB studies.<sup>9,10,16,17</sup>

Whether the observed impact of SXB on stages 3 and 4 sleep and on delta power represents a true sleep effect, delta-wave effects similar to those seen with CNS anesthetic agents, or an epiphenomenon unrelated to either sleep or anesthesia is unknown. With the data available, it is difficult to evaluate this question as it is currently not possible to investigate this issue meaningfully through characterization of EEG spectral features alone. One may hypothesize that an altered pattern of increased delta power activity may suggest a pharmacological effect that is not representative of sleep. Yet, such a conclusion may be inaccurate as this variant pattern of EEG activity may reflect an alteration of some aspects of sleep but not others, or an impact on all aspects of sleep, but weighted in a fashion distinct from normal physiological sleep. Taken together, the observed impacts of SXB on sleep, coupled with the observed improvements in daytime symptoms, are consistent with the hypothesis that sodium oxybate enhances SWS processes. Further work to better characterize the nature of the increased delta activity may be of interest.

Similar to the initial pilot study,<sup>11</sup> changes in sleep architecture following the nightly administration of SXB in the present study coincided with significant improvements in narcolepsy symptoms, including significant dose-related reductions in median number of weekly cataplexy attacks; a significant dose-related improvement in EDS, as measured with the Epworth Sleepiness Scale, the maintenance of wakefulness test, and incidence of inadvertent naps; and significant dose-related improvements in the CGI-c. These results are reported elsewhere.<sup>5</sup> Although some of the changes in sleep architecture reported here are significant only at the highest dose, the relationship between sleep architecture and subjective outcomes remains unclear and warrants further study.

**TABLE 2.** Changes in polysomnographic parameters following 8 weeks of treatment\*† (change from baseline; median)

|                         | Place<br>bo | SXB<br>4.5g | SXB<br>6g | SXB<br>9g                 |                      | Place<br>bo | SXB<br>4.5g | SXB 6g | SXB 9g |
|-------------------------|-------------|-------------|-----------|---------------------------|----------------------|-------------|-------------|--------|--------|
|                         | N=50        | N=59        | N=49      | N=35                      |                      | N=50        | N=59        | N=49   | N=35   |
| Total Sleep Time (min)  |             |             |           | Stage 3 and 4 Sleep (min) |                      |             |             |        |        |
| 1 <sup>st</sup><br>Half | 0.50        | -0.50       | 2.00      | 3.00                      | 1 <sup>st</sup> Half | -0.25       | 1.00        | 10.00  | 20.50  |
| 2 <sup>nd</sup><br>Half | -1.75       | 5.00        | 4.50      | 10.50                     | 2 <sup>nd</sup> Half | 0.00        | 0.50        | 4.50   | 26.50  |
| <b>TOT<br/>AL</b>       | 0.25        | 0.00        | 13.00     | 18.00                     | <b>TOTAL</b>         | 0.00        | 3.00        | 21.00  | 52.50  |

|                                |       |         |         |         |                                                |          |         |          |          |
|--------------------------------|-------|---------|---------|---------|------------------------------------------------|----------|---------|----------|----------|
|                                | -     | NS      | NS      | p=0.049 |                                                | -        | p=0.015 | p<0.001  | p<0.001  |
| <b>Total NREM Sleep (min)</b>  |       |         |         |         | <b>Wake After Sleep Onset (min)</b>            |          |         |          |          |
| 1 <sup>st</sup> Half           | -2.75 | -1.50   | 4.00    | 15.50   | 1 <sup>st</sup> Half                           | -0.50    | 0.25    | -0.25    | -1.00    |
| 2 <sup>nd</sup> Half           | 1.75  | 15.00   | 17.00   | 40.50   | 2 <sup>nd</sup> Half                           | -2.00    | -1.50   | -2.75    | -9.50    |
| <b>TOTAL</b>                   | 2.00  | 10.00   | 24.00   | 51.50   | <b>TOTAL</b>                                   | 2.00     | -5.75   | -3.75    | -22.00   |
|                                | -     | NS      | p=0.010 | p<0.001 |                                                | -        | NS      | NS       | p=0.052  |
| <b>Total REM Sleep (min)</b>   |       |         |         |         | <b>Sleep Stage Shifts Per Hour</b>             |          |         |          |          |
| 1 <sup>st</sup> Half           | -0.75 | -1.00   | -3.50   | -5.50   | 1 <sup>st</sup> Half                           | -1.82    | -1.51   | -1.18    | -1.91    |
| 2 <sup>nd</sup> Half           | 2.00  | -5.00   | -4.50   | -17.00  | 2 <sup>nd</sup> Half                           | -0.88    | -0.45   | -0.85    | 0.00     |
| <b>TOTAL</b>                   | -1.00 | -6.00   | -7.00   | -22.00  | <b>AVERAGE</b>                                 | -1.03    | -1.36   | -1.11    | -0.68    |
|                                | -     | NS      | NS      | p=0.026 |                                                | -        | NS      | NS       | NS       |
| <b>REM Sleep Latency (min)</b> |       |         |         |         | <b>Delta Power (microvolts<sup>2</sup>/Hz)</b> |          |         |          |          |
|                                | 0.00  | -1.00   | -1.50   | 6.00    | 1 <sup>st</sup> Half                           | 85.42    | 8413.11 | 14624.92 | 25164.43 |
|                                | -     | p=0.521 | p=0.203 | p=0.547 | 2 <sup>nd</sup> Half                           | -3538.83 | 7725.27 | 19726.61 | 32951.43 |
|                                |       |         |         |         | <b>AVERAGE</b>                                 | -1102.98 | 7166.20 | 14736.32 | 28796.65 |
|                                |       |         |         |         |                                                | -        | p=0.006 | p<0.001  | p<0.001  |
| <b>Stage 1 Sleep (min)</b>     |       |         |         |         | <b>Nocturnal Awakenings</b>                    |          |         |          |          |
| 1 <sup>st</sup> Half           | -2.75 | -3.00   | -5.00   | -7.50   | 1 <sup>st</sup> Half                           | -1.50    | -2.00   | -3.00    | -3.00    |
| 2 <sup>nd</sup> Half           | 1.25  | -1.50   | -9.50   | -14.00  | 2 <sup>nd</sup> Half                           | -0.00    | -3.00   | -5.00    | -9.00    |
| <b>TOTAL</b>                   | -2.25 | -9.50   | -13.50  | -22.50  | <b>TOTAL</b>                                   | -0.50    | -5.00   | -8.00    | -12.00   |
|                                | -     | p=0.086 | p<0.001 | p<0.001 |                                                | -        | NS      | p=0.005  | p=0.009  |
| <b>Stage 2 Sleep (min)</b>     |       |         |         |         |                                                |          |         |          |          |
| 1 <sup>st</sup> Half           | 1.50  | -1.00   | -5.00   | -4.50   |                                                |          |         |          |          |
| 2 <sup>nd</sup> Half           | 0.25  | 12.00   | 13.50   | 24.00   |                                                |          |         |          |          |
| <b>TOTAL</b>                   | 3.50  | 9.50    | 13.00   | 31.50   |                                                |          |         |          |          |
|                                | -     | NS      | NS      | NS      |                                                |          |         |          |          |

\*Statistical significance was established compared to placebo. NS = not significant.

†Expressed as medians following transformation of non-normal data.

A shortcoming of the initial pilot study was the lack of a control for the dose and duration of SXB therapy.<sup>11</sup> An open-label 12-month extension study of SXB in the treatment of narcolepsy demonstrated sustained improvement in cataplexy and EDS.<sup>2</sup> The present study was not designed to detect differences in sleep architecture variables at different time points with steady doses of SXB. These matters may merit further study. Although the greatest percentage of changes occurred within the initial 4-week treatment period, some parameters of sleep were further impacted during the subsequent 4 weeks of stable-dose treatment including total sleep time, total NREM sleep, SWS, and number of awakenings. The further changes from week 4 to week 8 may suggest a time-on-drug effect for these parameters, but this cannot be clarified with these data.

## **Conclusion**

The nocturnal administration of SXB to narcolepsy patients in two equally divided doses results in significant dose-related changes in sleep architecture, including an increase in slow wave sleep and TST and a decrease in stage 1 sleep, wake after sleep onset, and number of awakenings. These findings are consistent with improvement in measures of sleep continuity and suggest SXB may promote some amelioration of the sleep fragmentation that is common in narcolepsy. At SXB doses of 4.5 g, 6 g, and 9 g/night, dose-related improvements in cataplexy and overall change in severity of patient's disease state and at doses of 6 g and 9 g/night, decreases in excessive daytime sleepiness were noted as previously reported.<sup>4,5</sup>

## **Acknowledgements**

This study was sponsored by Jazz Pharmaceuticals, Inc., Palo Alto, CA and conducted by the members of the Xyrem International Study Group who are: Mansoor Ahmed, MD, Cleveland Sleep Center, Middlebrook Heights, OH; Philip Becker, MD, Sleep Medicine Association of Texas, Plano, TX; Michael Biber, MD, Center for Sleep Diagnostics, Newton, MA; Jed Black, MD, Stanford Sleep Disorder Clinic, Stanford, CA; Richard Bogan, MD, Palmetto Baptist Medical Center, Columbia, SC; Andrew Chesson Jr, MD, Louisiana State University Sleep Disorders Center, Shreveport, LA; James Cook, MD, The Center for Sleep & Wake Disorders, Danville, IN; Stephen Duntley, MD, Washington University Medical Center, St. Louis, MO; Helene Emsellem, MD, Center for Sleep & Wake Disorders, Chevy Chase, MD; Milton Erman, MD and Roza Hayduk, MD, Pacific Sleep Medicine Services, La Jolla, CA; Neil Feldman, MD, St. Petersburg Sleep Disorders Center, St. Petersburg, FL; John Fleming, MD, Vancouver Hospital, Vancouver, British Columbia, Canada; Peter Geisler, MD, Psychiatrische Universitätsklinik, Regensburg, Germany; Martha Hagaman, MD, Children's Sleep Institute, Nashville, TN; Dennis Hill, MD, Central Carolina Neurology & Sleep, Salisbury, NC; William C. Houghton, MD and Carl S. Hornfeldt, PhD, Orphan Medical, Inc., Minnetonka, MN; Aatif M. Husain, MD, Duke Health Center at Morreene Road, Durham, NC; Thomas Kaelin, MD, Lowcountry Lung & Critical Care PA, Charleston, SC; Gert Jan Lammers MD, PhD, Leiden University Medical Center, Leiden, the Netherlands; D. Alan Lankford, PhD, Sleep Disorders Center of Georgia, Atlanta, GA; Judith Leech, MD, The Ottawa Hospital Sleep Centre, Ottawa, Ontario, Canada; Mortimer Mamelak,

MD, Brain & Sleep Diagnostic Center, Toronto, Ontario, Canada; Geert Mayer, MD, Hepatic Klinik, Schwalmstadt-Treysa, Germany; Harvey Moldofsky, MD, Center for Sleep and Chronobiology, Toronto, Ontario, Canada; Jacques Montplaisir, MD, Sleep Disorder Centre, Montreal, Quebec, Canada; Rachel Morehouse, MD, Saint John Regional Hospital, Saint John, New Brunswick, Canada; Adam Moscovitch, MD, Canadian Sleep Institute, Calgary, Alberta, Canada; Sonka Nevsimalova, MD, Charles University, Prague, Czech Republic; William Orr, PhD, Lynn Health Science Institute, Oklahoma City, OK; Ralph Pascualy, MD, Swedish Sleep Medicine Institute, Seattle, WA; Vernon Pegg, PhD, Sleep Disorders Center of Alabama, Birmingham, AL; Thomas Perkins, MD, PhD, Raleigh Neurology Associates PA, Raleigh, NC; Jayant Phadke, MD, St. Vincent Hospital, Worcester, MA; Ruzica Ristanovic, MD, Evanston Hospital Sleep Disorders Center, Evanston, IL; John Shneerson, MD, Papworth Hospital, Cambridge, England; James Stevens, MD, Fort Wayne, IN; Todd Swick, MD, The Houston Sleep Center, Houston, TX; Joyce Walsleben, PhD, Bellevue Hospital Sleep/Wake Center, New York, NY; Timothy Walter, MD, Grove City Sleep Diagnostic Center, Grove City, OH; J. Catesby Ware, PhD, Sleep Disorders Center, Norfolk, VA; Patrick Whitten, MD, Peoria, IL; David Winslow, MD, Sleep Medicine Specialists, Louisville, KY. The authors wish to thank the Stanford Sleep Disorders Clinic (Oscar Carillo) for assistance in data collection and data analysis. The authors also acknowledge Howard Liang, MS, and Chinglin Lai, PhD, (Jazz Pharmaceuticals, Inc.) in performing statistical analyses and Teresa Steininger, PhD, and Chinglin Lai, PhD, (Jazz Pharmaceuticals, Inc.) for editorial assistance and helpful suggestions.

### **Disclosure Statement**

This study was sponsored by Jazz Pharmaceuticals, Inc., Palo Alto, CA and conducted by the members of the Xyrem International Study Group (see Acknowledgments for names of the group). Dr. Black is an employee of Actelion Pharmaceuticals, LTD and has received research support from Cephalon, GlaxoSmithKline, Jazz Pharmaceuticals, Merck, and Takeda, and honoraria from Boehringer-Ingelheim, Takeda, and UCB. Dr. Hornfeldt is a paid consultant to Jazz Pharmaceuticals. Dr. Inhaber and Mr. Pardi are former employees of Jazz Pharmaceuticals and own shares of stock.

### **References**

1. U.S. Xyrem Multicenter Study Group. A randomized, double blind, placebo controlled multicenter trial comparing the effects of three doses of orally administered sodium oxybate with placebo for the treatment of narcolepsy. *Sleep* 2002;25:42-9.
2. U.S. Xyrem Multicenter Study Group. A 12-month, open-label, multicenter extension trial of orally administered sodium oxybate for the treatment of narcolepsy. *Sleep* 2003;26:31-5.
3. U.S. Xyrem Multicenter Study Group. Sodium oxybate demonstrates long-term efficacy for the treatment of cataplexy in patients with narcolepsy. *Sleep Med* 2004;5:119-123.

4. Xyrem International Study Group. A double-blind, placebo-controlled, study demonstrates sodium oxybate is effective for the treatment of excessive daytime sleepiness in narcolepsy. *J Clin Sleep Med* 2005; 1:289-95.
5. Xyrem International Study Group. Further evidence supporting the use of sodium oxybate for the treatment of cataplexy: a double-blind, placebo-controlled study in 228 patients. *Sleep Med* 2005;6:415-21.
6. Overeem S, Mignot E, van Dijk JG, Lammers GJ. Narcolepsy: Clinical features, new pathophysiologic insights, and future perspectives. *J Clin Neurophysiol* 2001;18:78-105.
7. Mamelak M, Escriu JM, Stokan O. The effects of gamma-hydroxybutyrate on sleep. *Biol Psychiatry* 1977;12:273-88.
8. Broughton R, Mamelak M. Effects of nocturnal gamma-hydroxybutyrate on sleep waking patterns in narcolepsy-cataplexy. *Can J Neurol Sci* 1980;7:23-30.
9. Scrima L, Hartman PG, Johnson FH, Thomas EE, Hiller FC. The effects of  $\gamma$ -hydroxybutyrate on the sleep of narcolepsy patients: A double blind study. *Sleep* 1990;13:479-90.
10. Lammers GJ, Arends J, Declerck AC, Ferrari MN, Schouwink G, Troost, J. Gamma-hydroxybutyrate and narcolepsy: a double blind placebo controlled study. *Sleep* 1993;16:216-20.
11. Mamelak M, Black J, Montplaisir J, Ristanovic R. A dose response study on the effects of sodium oxybate on sleep architecture and daytime alertness in narcolepsy. *Sleep* 2004;27:1327-34.
12. Weaver TE, Cuellar N. A randomized trial evaluating the effectiveness of sodium oxybate therapy on quality of life in narcolepsy. *Sleep* 2006;29:1189-94.
13. American Academy of Sleep Medicine. International classification of sleep disorders: diagnostic and coding manual 2nd ed. Westchester, IL: American Academy of Sleep Medicine; 2005.
14. Rechtschaffen A, Kales A (eds). A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Public Health Service, US Government Printing Office, 1968.
15. Broughton R, Mamelak M. The treatment of narcolepsy-cataplexy with nocturnal gamma-hydroxybutyrate. *Can J Neurol Sci* 1979;6:1-6.
16. Scharf MB, Brown D, Woods M, Brown L, Hirschowitz J. The effects and effectiveness of gamma-hydroxybutyrate in patients with narcolepsy. *J Clin Psychiatry* 1985;46:222-5.

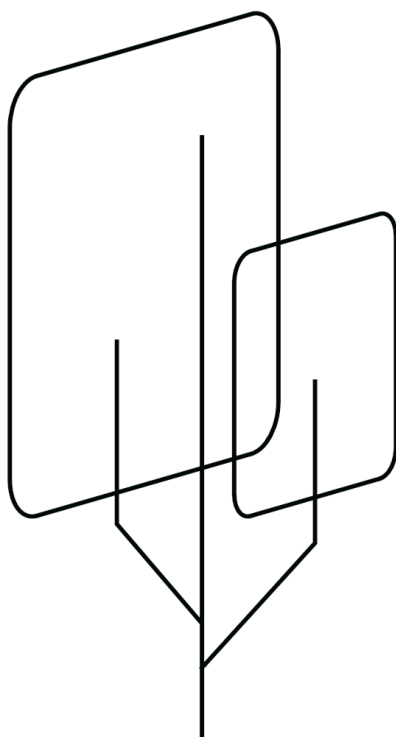
17. Lapierre O, Montplaisir J, Lamarre M, Bedard MA. The effect of gamma-hydroxybutyrate on nocturnal and diurnal sleep of normal subjects: further considerations on REM sleep. *Sleep* 1990;13:24-30.

# **CHAPTER 7**

## **Plasma Total Ghrelin and Leptin Levels in Human Narcolepsy and Matched Healthy Controls: Basal Concentrations and Response to Sodium Oxybate**

*Journal of Clinical Sleep Medicine (2013) 9(8):797-803*

**Authors:** Daniel Pardi, Claire E.H.M. Donjacour, N. Ahmad Aziz, Marijke Frölich, Ferdinand Roelfsema, Sebastiaan Overeem, Hanno Pijl and Gert Jan Lammers



**Abstract**

|                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Study Objective</b> | Narcolepsy is caused by a selective loss of hypocretin neurons and is associated with obesity. Ghrelin and leptin interact with hypocretin neurons to influence energy homeostasis. Here, we evaluated whether human hypocretin deficiency, or the narcolepsy therapeutic sodium oxybate, alter the levels of these hormones.                                                                                                                                                     |
| <b>Methods</b>         | Eight male, medication free, hypocretin deficient, narcolepsy with cataplexy patients, and 8 healthy controls matched for age, sex, body mass index (BMI), waist-to-hip ratio, and body fat percentage were assessed. Blood samples of total ghrelin and leptin were collected over 24 hours at 60 and 20-min intervals, respectively, during two study occasions: baseline, and during the last night of 5 consecutive nights of sodium oxybate administration (2 x 3.0g/night). |
| <b>Results</b>         | At baseline, mean 24-h total ghrelin ( $936 \pm 142$ vs. $949 \pm 175$ pg/mL, $p = 0.873$ ) and leptin ( $115 \pm 5.0$ vs. $79.0 \pm 32$ mg/L, $p = 0.18$ ) levels were not different between hypocretin deficient narcolepsy patients and controls. Furthermore, sodium oxybate did not significantly affect the plasma concentration of either one of these hormones.                                                                                                           |
| <b>Conclusion</b>      | The increased BMI of narcolepsy patients is unlikely to be mediated by hypocretin deficiency-mediated alterations in total ghrelin or leptin levels. Thus, the effects of these hormones on hypocretin neurons may be mainly unidirectional. Although sodium oxybate may influence body weight, the underlying mechanism is unlikely to involve changes in total ghrelin or leptin secretion.                                                                                     |

## Introduction

The hypocretin system, also known as the orexin system, is of major importance in the regulation of sleep and sustained wakefulness. Moreover, hypocretin neurons are responsive to metabolites and hormones helping to translate signals of metabolic state into adaptive levels of activity and consciousness.<sup>1,2,3</sup> Hypocretin deficiency leads to narcolepsy, a sleep-wake disorder characterized by excessive daytime sleepiness, cataplexy, and disrupted nocturnal sleep. Obesity is associated with the disorder,<sup>3</sup> yet the cause of the increased body weight has been challenging to discern due to inconsistent findings on the hormonal and metabolic characteristics of this population.<sup>4-9</sup> However, altered ingestive behavior has been consistently observed in these patients,<sup>7, 10-12</sup> suggesting hypocretin deficiency may dysregulate feeding behavior, and possibly energy homeostasis.

Ghrelin is a peptide hormone mainly produced by endocrine cells in the stomach and gastrointestinal tract, and is an important endogenous regulator of energy balance and growth hormone (GH) secretion.<sup>13</sup> Its expression is complex<sup>14</sup> and influenced by sympathetic nervous system activity.<sup>15</sup> Across the wake period, plasma concentration wax and wane episodically providing an orexigenic signal to the brain.<sup>16, 24</sup> During sleep, ghrelin levels increase in the early part of the night and decrease towards morning, however, this nocturnal increase is blunted during sleep deprivation.<sup>17</sup> Hypocretin neurons directly sense and are excited by ghrelin and an interaction between these two systems has been shown to be involved in ingestive behavior.<sup>18</sup> A study by Toshinai et al.,<sup>19</sup> first identified this connection. In that study, ghrelin-induced feeding was attenuated in rats pretreated with anti-hypocretin-1 IgG and anti-hypocretin-2 IgG and suppressed in hypocretin-knockout mice. Later, it was demonstrated that ghrelin plays a key role in the rewarding aspects of eating,<sup>20</sup> but it requires the presence of intact hypocretin signaling to impart this effect.

Leptin is another peptide hormone involved in energy homeostasis, the dominant role of which is to signal energy deficiency to the brain.<sup>21</sup> It is an adipokine produced primarily by subcutaneous white adipose tissue and its expression is stimulated by various hormones, sympathetic outflow, energy intake and output.<sup>20, 21, 22</sup> Under normal conditions, blood levels display circadian variation as levels rise across the day and peak in the middle of the night.<sup>23</sup> During sleep deprivation, blood leptin levels show a reduced and flattened profile.<sup>24</sup> Receptors for leptin are found on hypocretin cells<sup>25</sup> and leptin can directly inhibit the expression of isolated hypocretin neurons.<sup>18</sup> Indirectly, leptin can affect the activity of hypocretin cells via energy-regulating neurons in the arcuate nucleus of the hypothalamus.<sup>26</sup> Conversely, because the hypocretin system greatly influences autonomic control,<sup>1</sup> it is plausible that hypocretin deficiency may alter leptin expression via inhibited sympathetic activity. Indeed, obese hypocretin deficient mice have lowered sympathetic vasoconstrictor outflow, while greater heart rate variability has been observed in hypocretin deficient narcolepsy patients.<sup>27, 28</sup> Thus, leptin and hypocretin may interact to affect levels of physical activity and wakefulness in response to energy needs, and the loss of hypocretin neurons may dysregulate leptin expression and signaling.

While ghrelin levels have not been previously reported in hypocretin-deficient narcoleptic patients, abnormal leptin levels have been observed.<sup>4, 5</sup> It is unknown if the associations between hypocretin and total ghrelin or leptin are uni-or-bidirectional. Because hypocretin influences sympathetic outflow, and sympathetic nervous system activity effects the expression of both leptin and ghrelin, hypocretin deficiency may lead to altered levels of these hormones. This study of hypocretin-deficient narcoleptic patients provides a unique opportunity to further explore the nature of these relationships. We hypothesize both total ghrelin and leptin levels will be abnormal in hypocretin-deficient narcolepsy patients, which may help explain the increased BMI and abnormal ingestive behavior seen in this population.<sup>5, 7, 8, 29-31</sup>

Additionally, we explored if the narcolepsy therapeutic,<sup>48</sup> sodium oxybate, has an effect on these hormones. In a narcolepsy population, sodium oxybate improves disrupted nocturnal sleep, impaired wakefulness, and cataplexy, and promotes weight loss.<sup>32, 33</sup> Like ghrelin, sodium oxybate administration also stimulates GH release.<sup>34</sup> We hypothesize that its administration will alter total ghrelin levels, the effect of which may be involved in its GH-promoting effects.

Here, we investigate whether total blood ghrelin or leptin levels are altered in hypocretin-deficient narcoleptic patients compared to controls, and whether total ghrelin or leptin levels are influenced by sodium oxybate.

## **Materials and methods**

### **Subjects**

We included eight medication free, male hypocretin deficient narcolepsy with cataplexy patients and eight healthy male controls, matched for age, BMI and body fat percentage. Hypocretin measurement was performed according to international standards.<sup>35</sup> Body fat percentage was measured with bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK). Two patients were drug naive, one patient was tapered from antidepressants at least two weeks prior to the study, and two patients had prior history with sodium oxybate therapy; however, no subject took sodium oxybate within 20 days of study initiation. The other patients did not take any medication for at least several months prior to beginning the study.

Subjects were eligible for participation after exclusion of chronic conditions, with particular attention to the absence of sleep disorders in control subjects, hypertension, pituitary disease, and weight change (>4% kg weight gain or loss within the last 3 months) as assessed by structured clinical interview. None of the participants had previously undergone gastrectomy. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of the Leiden University Medical Center.

## **Clinical protocol**

All subjects were admitted to the Clinical Research Center for 24-h blood sampling before and after 5 days of sodium oxybate administration. A cannula was inserted into an antecubital vein at least 45 minutes before the start of blood sampling at 1200 h. Blood samples were collected with S-monovetten (Sarstedt, Etten-Leur, The Netherlands) from a three-way stopcock that was attached to a 0.9% NaCl and heparin (1 U/ml) infusion (750 ml/24 h) to keep the cannula from clotting. For total ghrelin measurements, blood was collected in EDTA tubes at 60-min intervals and these tubes were immediately put on ice. Ghrelin samples were acidified with 50  $\mu$ l of 1 N HCL. Within 5 minutes of sampling, tubes were centrifuged at 1250 g at 4 °C for 20 minutes. For Leptin measurements, blood was collected at 20-min intervals. After clotting, the blood was centrifuged within 30 minutes of sampling (20 minutes, 1250 g, 4 °C). Serum was then stored at -80 °C until hormonal assays. Three standardized meals were served at 0830, 1300, and 1800 h (Nutridrink, 1.5 kcal/ml, 2100 kcal/d; macronutrient composition per 100 ml: protein, 6 g; fat, 5.8 g; carbohydrate, 18.4 g; Nutricia, Zoetermeer, The Netherlands). Subjects were asked to complete each meal provided. Food-induced suppression of total ghrelin release was defined as the ratio between total ghrelin levels one hour postprandially to the levels immediately before the meal (lunch and dinner) or 30 min postprandially to 30 min before the meal (breakfast). Subjects remained sedentary except for bathroom visits. In both study occasions, lights were switched off (dark period) at 2300 h and then switched on at 0730 h.

## **Sodium oxybate**

In the drug-intervention study occasion, sodium oxybate was administered in a total nightly dose of 6 grams per night for 5 consecutive nights in both the narcoleptic patients and the controls. Each night, 3 grams of sodium oxybate were administered orally at 2300 h and 0300 h. Lights were turned off after ingestion of the first dose.

## **Sleep recordings**

During the 24-hour sampling periods, polysomnographic recordings were performed using an ambulant EEG-recording system (Embletta X100, Embla) and a standard EEG/EMG montage to allow sleep scoring according to the AASM-criteria. Using a marker-tool, the start of the sampling protocol was registered to synchronize sleep-recordings with hormone measurements. Sleep recordings were scored by an experienced technician, blinded for the subject under study.

## **Assays**

Plasma total ghrelin and leptin levels were measured by radioimmunoassay (LINCO Research, St. Charles, MO, USA) with a detection limit of 93 pg/mL, and an interassay variation ranging from 14.7 to 17.8% for total ghrelin and a detection limit of 0.5  $\mu$ g/L and an interassay variation ranging from 3.0 to 5.1% for leptin. Samples from each patient and matched control were handled in the same run.

**Deconvolution analysis**

Leptin concentration time series were analyzed via a recently developed automated deconvolution method, empirically validated using hypothalamo-pituitary sampling and simulated pulsatile time series.<sup>36</sup> The MATLAB-based algorithm first detrends the data and normalizes concentrations to the unit interval [0, 1]. Second, the program creates multiple successive potential pulse-time sets, each containing one fewer burst via a smoothing process (a nonlinear adaptation of the heat-diffusion equation). Third, a maximum-likelihood expectation estimation method computes all secretion and elimination parameters simultaneously conditional on each of the multiple candidate pulse-time sets. The fast half-life was represented as 3.4 min constituting 19% of the decay amplitude. The slow half-life was estimated as an unknown variable between 6 and 70 min. Here we present only results for pulse frequency (pulses per 24 h), basal secretion, pulsatile secretion and total secretion per 24 h, all expressed as µg per liter distribution volume.

**Data analysis and statistics**

Results are expressed as mean ± standard deviation (SD). Unpaired t-tests were used to assess differences in means between the two groups, while paired t-tests were applied to assess changes in means within each group. All tests were two-tailed, and significance level was set at p < 0.05. Statistical analyses were performed using SPSS for Windows (release 17.0, SPSS, Inc., Chicago, IL).

**Results**

**Subjects**

Patients and controls did not differ with respect to age, BMI, waist-to-hip ratio, and body fat percentage (Table 1). Sodium oxybate was well tolerated by all participants. Apart from mild drowsiness, no other side-effects were reported during the study.

**TABLE 1.** Demographics, body composition, baseline parameters

|                          | <b>Patients</b> | <b>Controls</b> | <b>P</b> |
|--------------------------|-----------------|-----------------|----------|
| Age (yrs)                | 38.0 ± 13.4     | 37.9 ± 11.6     | 0.984    |
| BMI (kg/m <sup>2</sup> ) | 28.1 ± 4.6      | 27.4 ± 4.0      | 0.742    |
| Waist/hip ratio          | 0.92 ± 0.10     | 0.90 ± 0.04     | 0.579    |
| Body fat (%)             | 23.6 ± 6.0      | 23.4 ± 4.8      | 0.946    |

Data are shown as mean ± standard deviation.

**Sleep and wakefulness differences**

When compared to controls, during baseline conditions and after sodium oxybate administration, narcolepsy patients spent significantly less time awake across a 24 h period, and during the day (defined as the lights-on period between 0730 h-2300

h) they spent less time awake and more time in slow wave sleep (SWS) ( $p = 0.004$  and  $p = 0.005$ , respectively) (Table 2).

**TABLE 2.** Sleep patterns before and after sodium oxybate administration

|                      | Narcolepsy  |            | Controls    |             |                                    |                               |                  |                                 |
|----------------------|-------------|------------|-------------|-------------|------------------------------------|-------------------------------|------------------|---------------------------------|
|                      | Baseline    | SXB        | Baseline    | SXB         | Narcolepsy vs. controls (baseline) | Narcolepsy vs. controls (SXB) | Treatment effect | Interaction (group × treatment) |
| Wake total (%)       | 60.8 ± 2.9  | 60.8 ± 2.2 | 68.7 ± 2.0  | 70.1 ± 2.4  | 0.044*                             | 0.013*                        | 0.58             | 0.57                            |
| Wake day (%)         | 79.4 ± 4.2  | 82.9 ± 3.2 | 95.6 ± 2.1  | 97.3 ± 1.0  | 0.004**                            | 0.001**                       | 0.098            | 0.60                            |
| Wake night (%)       | 25.8 ± 5.7  | 19.2 ± 4.3 | 18.4 ± 4.0  | 19.2 ± 5.8  | 0.31                               | 1.00                          | 0.40             | 0.087                           |
| Stage I/II total (%) | 29.1 ± 1.4  | 26.3 ± 1.4 | 25.0 ± 2.4  | 21.1 ± 2.2  | 0.16                               | 0.063                         | 0.011*           | 0.62                            |
| Stage I/II day (%)   | 14.6 ± 3.0  | 11.1 ± 2.5 | 2.5 ± 1.6   | 1.6 ± 1.0   | 0.003**                            | 0.005**                       | 0.038*           | 0.23                            |
| Stage I/II night (%) | 55.1 ± 2.5  | 53.5 ± 3.7 | 65.6 ± 5.7  | 56.4 ± 5.3  | 0.11                               | 0.65                          | 0.056            | 0.13                            |
| SWS total (%)        | 3.7 ± 0.7   | 7.6 ± 1.2  | 2.5 ± 0.7   | 6.6 ± 0.9   | 0.24                               | 0.53                          | 0.001**          | 0.90                            |
| SWS day (%)          | 2.1 ± 0.6   | 2.7 ± 1.1  | 0.03 ± 0.03 | 0.05 ± 0.05 | 0.005**                            | 0.041*                        | 0.49             | 0.56                            |
| SWS night (%)        | 6.5 ± 1.9   | 16.5 ± 3.0 | 7.1 ± 1.9   | 18.5 ± 2.4  | 0.84                               | 0.61                          | 0.001**          | 0.76                            |
| REM total (%)        | 6.3 ± 1.8   | 4.7 ± 1.0  | 3.7 ± 0.8   | 2.1 ± 0.8   | 0.19                               | 0.070                         | 0.009**          | 0.93                            |
| REM day (%)          | 2.9 ± 1.4   | 1.2 ± 0.5  | 0.8 ± 0.5   | 0.0 ± 0.0   | 0.20                               | 0.032*                        | 0.041*           | 0.51                            |
| REM night (%)        | 12.6 ± 3.0  | 10.8 ± 2.1 | 8.8 ± 1.8   | 5.8 ± 2.3   | 0.31                               | 0.13                          | 0.063            | 0.53                            |
| No. of awakenings    | 50.5 ± 10.5 | 35.0 ± 4.8 | 35.5 ± 7.1  | 15.3 ± 1.7  | 0.26                               | 0.005**                       | 0.002**          | 0.85                            |
| Sleep efficiency (%) | 66.9 ± 7.0  | 81.5 ± 4.9 | 81.2 ± 4.0  | 81.9 ± 6.0  | 0.10                               | 0.96                          | 0.082            | 0.06                            |

Percentages of sleep stages during the 24 hours of study, before and after SXB administration. Data are shown as mean ± SEM. Unpaired t tests were used to assess differences between the two groups. Mixed-effects models were applied to assess the effect of treatment and potential interaction effects between group (i.e. narcolepsy or control) and treatment. \*  $p < 0.05$  and \*\*  $p < 0.01$ .<sup>37</sup>

**TABLE 3.** Plasma ghrelin concentrations and deconvolution of leptin levels before and after administration of sodium oxybate in both narcoleptic patients and controls

|                                                                              | Baseline    |             |       | Sodium Oxybate |             |       |
|------------------------------------------------------------------------------|-------------|-------------|-------|----------------|-------------|-------|
|                                                                              | Patients    | Controls    | P     | Patients       | Controls    | P     |
| <b>Ghrelin</b>                                                               |             |             |       |                |             |       |
| <b>24-h total integrated concentration (pg/mL)</b>                           | 936 ± 142   | 949 ± 175   | 0.873 | 920 ± 142      | 886 ± 150   | 0.642 |
| <b>Dark period<sup>a</sup>(pg/mL)</b>                                        | 1012 ± 156  | 1009 ± 196  | 0.973 | 983 ± 163      | 910 ± 211   | 0.449 |
|                                                                              |             |             |       |                |             |       |
| <b>Food induced suppression of ghrelin concentration<sup>b</sup> (pg/mL)</b> |             |             |       |                |             |       |
| <b>Lunch</b>                                                                 | 0.83 ± 0.10 | 0.86 ± 0.09 | 0.413 | 0.87 ± 0.08    | 0.88 ± 0.16 | 0.920 |
| <b>Dinner</b>                                                                | 0.93 ± 0.16 | 0.83 ± 0.17 | 0.301 | 0.89 ± 0.20    | 0.80 ± 0.10 | 0.261 |
| <b>Breakfast</b>                                                             | 1.05 ± 0.10 | 1.01 ± 0.09 | 0.437 | 0.98 ± 0.12    | 0.98 ± 0.06 | 0.880 |
| <b>Postprandial total ghrelin<sup>c</sup> (pg/mL)</b>                        | 0.93 ± 0.08 | 0.90 ± 0.11 | 0.540 | 0.91 ± 0.08    | 0.88 ± 0.06 | 0.428 |
|                                                                              |             |             |       |                |             |       |
| <b>Leptin</b>                                                                |             |             |       |                |             |       |
| <b>Total 24-h secretion (µg/Lx24h)</b>                                       | 115 ± 98    | 79.0 ± 88   | 0.18  | 100 ± 113      | 64.0 ± 35   | 0.58  |
| <b>Basal 24-h secretion (µg/Lx24h)</b>                                       | 64.7 ± 63   | 37.9 ± 30   | 0.96  | 56.0 ± 70      | 47.6 ± 63   | 0.94  |
| <b>Pulsatile 24-h secretion (µg/Lx24h)</b>                                   | 50.3 ± 36   | 25.6 ± 11   | 0.11  | 43.8 ± 46      | 31.0 ± 27   | 0.29  |
| <b>Pulse frequency (no/24h)</b>                                              | 18.5 ± 2.7  | 15.3 ± 4.8  | 0.04  | 19.8 ± 2.4     | 19.0 ± 3.0  | 0.04  |

<sup>a</sup> In both study occasions, lights were switched off (dark period) at 2300 h and then switched on at 0730 h

<sup>b</sup> Expressed as the ratio between post- to preprandial ghrelin concentration

<sup>c</sup> Averaged over three occasions

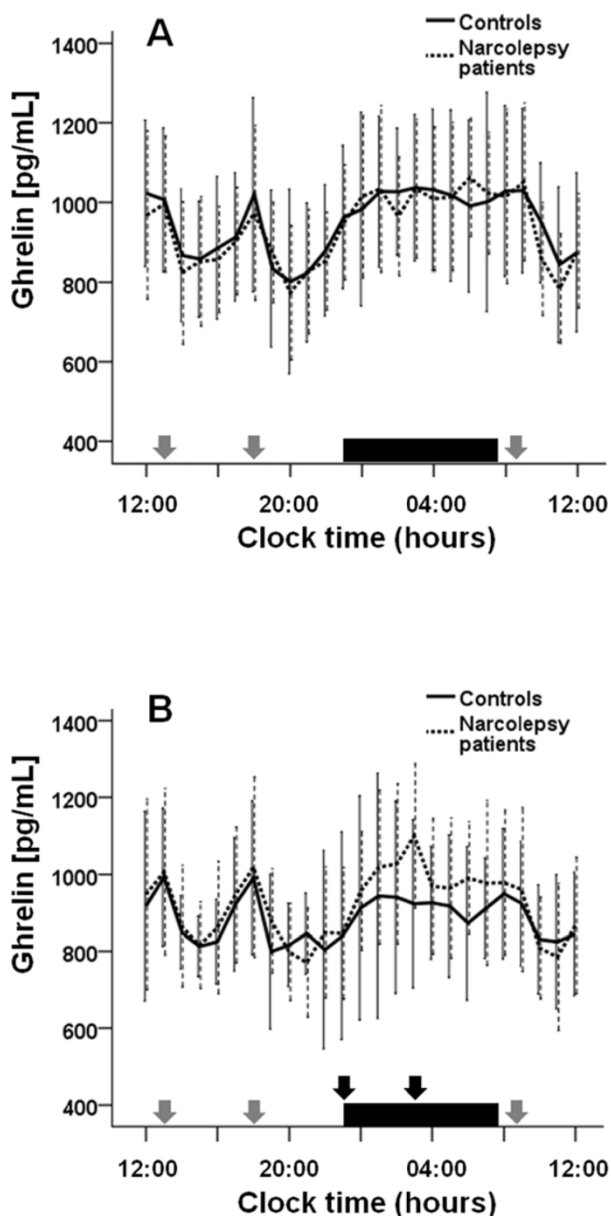
### Effect of sodium oxybate administration on sleep and wakefulness

In both groups, administration of sodium oxybate resulted in a significant decrease in stages I/II non-rapid eye movement (REM) and REM sleep over 24 hours ( $p = 0.011$  and  $p = 0.009$ , respectively), while at night, awakenings were significantly reduced ( $p = 0.002$ ) and the percentage of SWS more than doubled (narcolepsy:  $6.5 \pm 5.5\%$  vs.  $16.5 \pm 8.4\%$ , controls:  $7.1 \pm 5.5\%$  vs.  $18.5 \pm 6.4\%$ ;  $p = 0.001$  for administration effect). During the day, time spent in stages I/II non-REM and REM

sleep ( $p = 0.038$  and  $p = 0.041$ , respectively) was reduced, while there was a trend towards longer periods of wakefulness ( $p = 0.098$ ).

### **Baseline total ghrelin levels**

Mean 24-h total ghrelin levels at baseline were virtually identical between narcolepsy patients and controls ( $p = 0.873$ ; Fig. 1A). Mean total ghrelin levels were also not different between the two groups when the analyses were restricted to the dark period ( $p = 0.973$ ). In fact, at no single time-point an intergroup difference could be detected (all  $p \geq 0.232$ ). Food induced suppression of total ghrelin concentration (expressed as the ratio between post- to preprandial total ghrelin concentration) was similar in the two groups (lunch:  $p = 0.413$ , dinner:  $p = 0.301$ , breakfast:  $p = 0.437$ , and mean postprandial total ghrelin levels averaged over the three occasions ( $p = 0.540$ ) (Table 3).



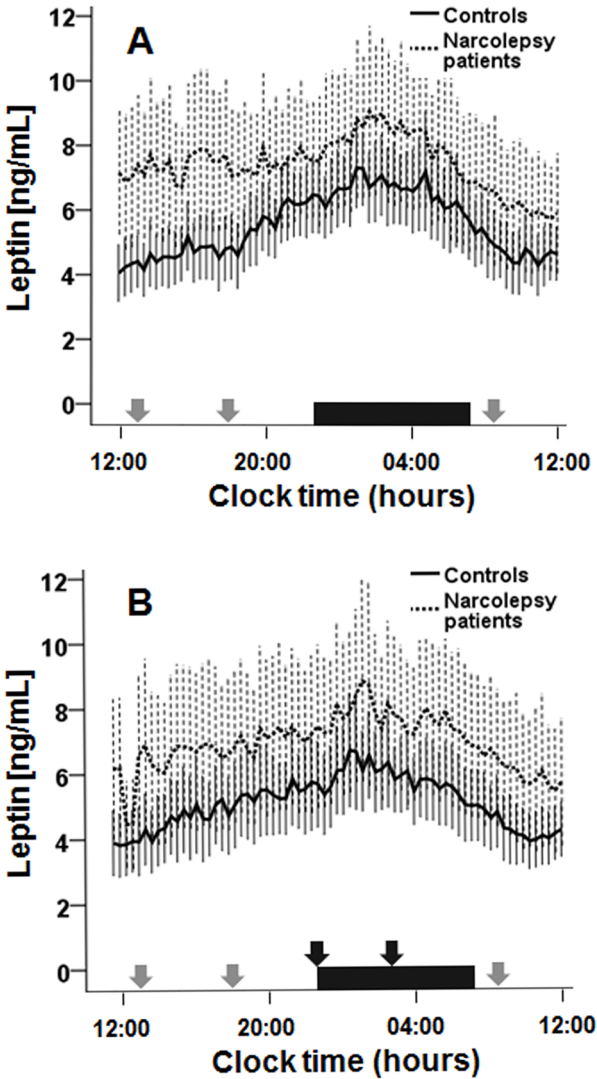
**FIGURE 1.** Mean 24 h ghrelin levels in narcolepsy patients and matched controls. The diurnal plasma ghrelin levels, as well as food induced suppression of ghrelin release were not significantly different between narcolepsy patients and matched controls, either during basal conditions (A) or after five days of sodium oxybate administration (B). Hourly blood sampling started at noon and continued for 24 hours. The black bar on the abscissa indicates the dark period (2300-0730 h). The grey arrows indicate the timings of the lunch, dinner and breakfast at 1300 h, 1800 h and 0830 h, respectively. The black arrows indicate the timings of sodium oxybate administrations during the second study occasion at 2300 h and 0300 h.

Error bars show the means  $\pm$  standard deviation.

### Effect of sodium oxybate on total ghrelin levels

Twenty-four hour mean total ghrelin levels during sodium oxybate treatment were not different between narcolepsy patients and controls ( $p = 0.642$ ; Fig. 1B). Similar to baseline, mean total ghrelin levels during the dark period did not differ between the two groups ( $p = 0.449$ ), and at no single time-point a difference could be

detected between groups (all  $p \geq 0.05$ ). Postprandial total ghrelin suppression, as defined above, was also similar between the two groups after sodium oxybate administration: lunch ( $p = 0.920$ ), dinner ( $p = 0.261$ ), and breakfast ( $p = 0.880$ ); mean postprandial total ghrelin levels averaged over the three occasions ( $p = 0.428$ ) (Table 3). The average change in 24 h total ghrelin levels between the second and first occasion amounted to  $-15 \pm 72$  pg/ml in narcolepsy patients and  $-63 \pm 87$  pg/ml in controls but was not significantly different from zero in either group (paired t-tests:  $p = 0.56$  and  $p = 0.078$ , respectively).



**FIGURE 2.** Mean 24-h plasma leptin concentration  $\pm$  SD, before (A) and during sodium oxybate administration (B) in narcolepsy patients and matched controls. The black horizontal bar on the abscissa indicates the lights off period. The grey arrows indicate the timing of meals and the black arrows indicate timing of sodium oxybate administration (B).

### Baseline leptin levels

Mean 24-h total leptin levels at baseline were not significantly different between narcolepsy patients and controls ( $p = 0.18$ ; Fig. 2A). Mean pulse frequency was different between the two groups ( $p = 0.04$ ) but mean 24-h basal and pulsatile secretion levels were not different ( $p = 0.96$ ;  $p = 0.11$ , respectively) (Table 3).

### Effect of sodium oxybate on leptin levels

Mean 24-h total leptin levels during sodium oxybate treatment were not significantly different between narcolepsy patients and controls ( $p = 0.58$ ; Fig. 2B) and neither were mean 24-h basal and pulsatile secretion rates ( $p = 0.94$ ;  $p = 0.29$ , respectively). Mean pulse frequency was different between the two groups ( $p = 0.04$ ) (Table 3).

### Discussion

We found no differences in mean 24-h total plasma ghrelin levels or food-induced suppression of ghrelin concentrations between narcolepsy patients and controls, nor any influence of 5 days of sodium oxybate administration in both groups. In view of the capacity of ghrelin to stimulate growth hormone secretion, it is worth noting that a report from this same research protocol showed no differences in mean hourly GH levels between patients and controls, supporting our conclusion that total ghrelin levels are not altered with hypocretin deficiency.<sup>37</sup>

Despite the excitatory influence of ghrelin on hypocretin neurons, and the interaction of the ghrelin-hypocretin systems to influence food reinforcement, our finding did not show the total ghrelin level to be influenced by hypocretin deficiency, suggesting a unidirectional relationship. These findings also suggest that disturbed ingestive behavior is unlikely mediated by an altered total ghrelin level in narcolepsy patients. Notably, we measured total ghrelin levels and not the biologically active, octanoylated-ghrelin fraction. While there is a high correlation between the total and octanoylated fraction ghrelin level,<sup>38</sup> it remains possible that the active fraction may be altered in this population.

In contrast to earlier reports,<sup>4, 5</sup> more recent, larger, controlled studies have not demonstrated an abnormal leptin level in humans with hypocretin deficiency.<sup>6, 39</sup> Similar to the recent research on this subject, we found that the mean 24-h total leptin level, and basal and pulsatile secretion levels were not significantly different between narcolepsy patients and controls. The mean leptin pulse frequency was slightly but significantly higher in narcolepsy patients in both conditions, but the clinical relevance of this finding is unclear. Because sleep disruption and insulin resistance<sup>40</sup> have been shown to affect leptin levels, it is plausible that previous investigations showing decreased leptin in narcolepsy may have resulted from a study sample of narcoleptic patients with relatively poor sleep or a difference in insulin sensitivity compared to the control group.

There were several limitations to the study. The small number of patients and controls raise the possibility of a type II statistical error. However, the intergroup

differences were very small therefore a large sample size would be needed to detect a difference if present. Since sleep-wake state instability is intrinsic to hypocretin-deficiency, standardizing research parameters such as study environment, meal timing and composition, and predefined bed times may have created a setting not representative of real-life conditions for these patients. Therefore, although we did not find alterations in total ghrelin and leptin concentrations in this controlled and standardized environment, it remains possible that the release of these hormones is affected by the altered sleep, wake, and eating patterns described in this population.

As expected, in both groups' nighttime administration of sodium oxybate increased SWS and reduced awakenings, and the narcoleptic-patient group showed a trend towards increased wakefulness the following day. As demonstrated in other studies,<sup>48, 51</sup> sodium oxybate administration corresponds with a significant increase in GH release.<sup>34, 37, 53</sup> However, we found no evidence that the GH-elevating effect is mediated through an influence on total ghrelin secretion. Although, the difference in total ghrelin levels between patients and controls after sodium oxybate administration was not significant, it is possible that significant differences would be seen with higher doses, prolonged periods of nightly administration or in a larger group of subjects. Lastly, we did not see an effect of sodium oxybate on the leptin level and to our knowledge, an interaction between this drug and hormone has not been reported elsewhere.

Therefore, mechanisms underlying increased BMI and altered ingestive behavior in narcolepsy, and the effects of sodium oxybate administration on GH release and weight loss, are unlikely to involve changes in total plasma ghrelin or leptin concentrations. Future investigations should further evaluate if the sleep-wake instability intrinsic to hypocretin-deficient narcolepsy promotes ingestive and activity patterns that promote positive energy balance.

## **Acknowledgements**

We thank E.J.M. Ladan-Eygenraam, M. van Dijk-Besling, H.G. Haasnoot-van der Bent, J. van Vliet-de Regt and P.J. van Someren for technical assistance throughout the study.

## **Support**

This study was supported by an unrestricted grant from UCB Europe.

## Disclosures

- Claire E.H.M. Donjacour, MD has no relevant financial relationships or relations with a commercial interest.
- Daniel Pardi, MS was previously employed with Jazz Pharmaceuticals and has consulted with UCB Europe.
- N. Ahmad Aziz, MSc has no relevant financial relationships or relations with a commercial interest.
- Marijke Frölich, MD, PhD has no relevant financial relationships or relations with a commercial interest.
- Ferdinand Roelfsema, MD, PhD has no relevant financial relationships or relations with a commercial interest.
- Sebastiaan Overeem, MD, PhD has consulted for and received honoraria as a speaker from UCB Europe
- Hanno Pijl, MD, PhD has no relevant financial relationships or relations with a commercial interest.
- Gert Jan Lammers, MD, PhD is member of the international advisory board on narcolepsy for UCB and received honoraria as a speaker from UCB

## References

1. Burdakov D, Gonzalez JA. Physiological functions of glucose-inhibited neurones. *Acta Physiol (Oxf)* 2009;195:71-8.
2. Sakurai T. Roles of orexins and orexin receptors in central regulation of feeding behavior and energy homeostasis. *CNS Neurol Disord Drug Targets* 2006;5:313-25.
3. Kok SW, Overeem S, Visscher TL, et al. Hypocretin deficiency in narcoleptic humans is associated with abdominal obesity. *Obes Res* 2003;11:1147-54.
4. Schuld A, Blum WF, Uhr M, et al. Reduced leptin levels in human narcolepsy. *Neuroendocrinology* 2000;72:195-8.
5. Kok SW, Meinders AE, Overeem S, et al. Reduction of plasma leptin levels and loss of its circadian rhythmicity in hypocretin (orexin)-deficient narcoleptic humans. *J Clin Endocrinol Metab* 2002;87:805-9.
6. Arnulf I, Lin L, Zhang J, et al. CSF versus serum leptin in narcolepsy: is there an effect of hypocretin deficiency? *Sleep* 2006;29:1017-24.
7. Chabas D, Foulon C, Gonzalez J, et al. Eating disorder and metabolism in narcoleptic patients. *Sleep* 2007;30:1267-73.

8. Poli F, Plazzi G, Di Dalmazi G, et al. Body mass index-independent metabolic alterations in narcolepsy with cataplexy. *Sleep* 2009;32:1491-7.
9. Dahmen N, Tonn P, Messroghli L, Ghezel-Ahmadi D, Engel A. Basal metabolic rate in narcoleptic patients. *Sleep* 2009;32:962-4.
10. Kotagal S, Krahn LE, Slocumb N. A putative link between childhood narcolepsy and obesity. *Sleep Med* 2004;5:147-50.
11. Dahmen N, Becht J, Engel A, Thommes M, Tonn P. Prevalence of eating disorders and eating attacks in narcolepsy. *Neuropsychiatr Dis Treat* 2008;4:257-61.
12. Fortuyn HA, Swinkels S, Buitelaar J, et al. High prevalence of eating disorders in narcolepsy with cataplexy: a case-control study. *Sleep* 2008;31:335-41.
13. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000;407:908-13.
14. Yin X, Li Y, Xu G, An W, Zhang W. Ghrelin fluctuation, what determines its production? *Acta Biochim Biophys Sin (Shanghai)* 2009;41:188-97.
15. Hosoda H, Kangawa K. The autonomic nervous system regulates gastric ghrelin secretion in rats. *Regul Pept* 2008;146:12-8.
16. Toshinai K, Mondal MS, Nakazato M, et al. Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem Biophys Res Commun* 2001;281:1220-5.
17. Dzaja A, Dalal MA, Himmerich H, Uhr M, Pollmacher T, Schuld A. Sleep enhances nocturnal plasma ghrelin levels in healthy subjects. *Am J Physiol Endocrinol Metab* 2004;286:E963-7.
18. Yamanaka A, Beuckmann CT, Willie JT, et al. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 2003;38:701-13.
19. Toshinai K, Date Y, Murakami N, et al. Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* 2003;144:1506-12.
20. Perello M, Sakata I, Birnbaum S, et al. Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. *Biol Psychiatry* 2010;67:880-6.

21. Ahima RS, Prabakaran D, Mantzoros C, et al. Role of leptin in the neuroendocrine response to fasting. *Nature* 1996;382:250-2.
22. Spiegel K, Leproult R, L'Hermite-Baleriaux M, Copinschi G, Penev PD, Van Cauter E. Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. *J Clin Endocrinol Metab* 2004;89:5762-71.
23. Licinio J, Mantzoros C, Negrao AB, et al. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nat Med* 1997;3:575-9.
24. Chaput JP, Despres JP, Bouchard C, Tremblay A. Short sleep duration is associated with reduced leptin levels and increased adiposity: Results from the Quebec family study. *Obesity (Silver Spring)* 2007;15:253-61.
25. Hakansson M, de Lecea L, Sutcliffe JG, Yanagisawa M, Meister B. Leptin receptor- and STAT3-immunoreactivities in hypocretin/orexin neurones of the lateral hypothalamus. *J Neuroendocrinol* 1999;11:653-63.
26. Flier JS. Clinical review 94: What's in a name? In search of leptin's physiologic role. *J Clin Endocrinol Metab* 1998;83:1407-13.
27. Ferguson AV, Samson WK. The orexin/hypocretin system: a critical regulator of neuroendocrine and autonomic function. *Front Neuroendocrinol* 2003;24:141-50.
28. Zhang W, Sakurai T, Fukuda Y, Kuwaki T. Orexin neuron-mediated skeletal muscle vasodilation and shift of baroreflex during defense response in mice. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R1654-63.
29. Okun ML, Lin L, Pelin Z, Hong S, Mignot E. Clinical aspects of narcolepsy-cataplexy across ethnic groups. *Sleep* 2002;25:27-35.
30. Nishino S, Ripley B, Overeem S, et al. Low cerebrospinal fluid hypocretin (Orexin) and altered energy homeostasis in human narcolepsy. *Ann Neurol* 2001;50:381-8.
31. Schuld A, Hebebrand J, Geller F, Pollmacher T. Increased body-mass index in patients with narcolepsy. *Lancet* 2000;355:1274-5.
32. Pardi D, Black J. gamma-Hydroxybutyrate/sodium oxybate: neurobiology, and impact on sleep and wakefulness. *CNS Drugs* 2006;20:993-1018.

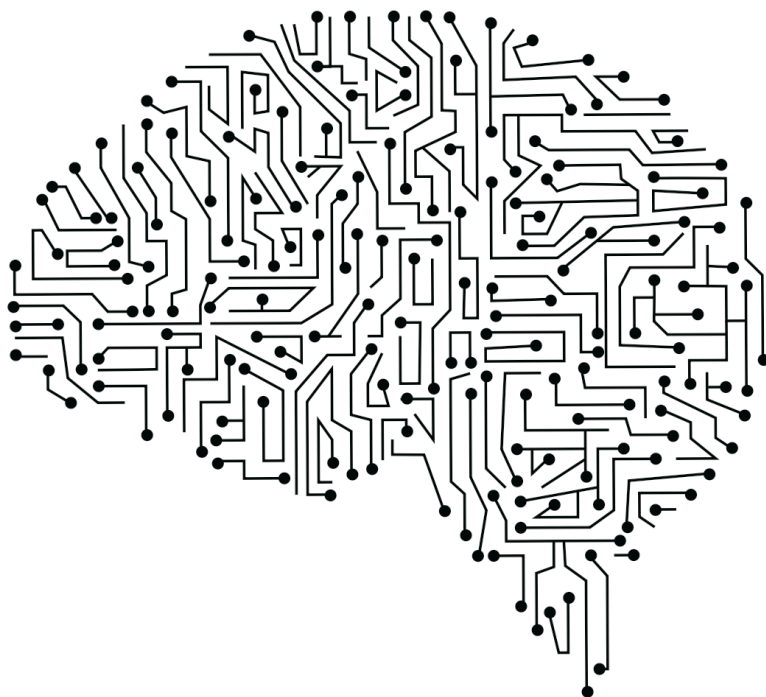
33. Husain AM, Ristanovic RK, Bogan RK. Weight loss in narcolepsy patients treated with sodium oxybate. *Sleep Med* 2009;10:661-3.
34. Van Cauter E, Plat L, Scharf M, et al. Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in normal young Men. *Journal of Clinical Investigation* 1997;100:745.
35. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* 2000;355:39-40.
36. Liu PY, Keenan DM, Kok P, Padmanabhan V, O'Byrne KT, Veldhuis JD. Sensitivity and specificity of pulse detection using a new deconvolution method. *Am J Physiol Endocrinol Metab* 2009;297:E538-44.
37. Donjacour CE, Aziz NA, Roelfsema F, et al. The effect of sodium oxybate on growth hormone secretion in narcolepsy patients and healthy controls. *American journal of physiology. Endocrinology and metabolism* 2011.
38. Marzullo P, Verti B, Savia G, et al. The relationship between active ghrelin levels and human obesity involves alterations in resting energy expenditure. *J Clin Endocrinol Metab* 2004;89:936-9.
39. Dahmen N, Engel A, Helfrich J, et al. Peripheral leptin levels in narcoleptic patients. *Diabetes Technol Ther* 2007;9:348-53.
40. Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol* 2003;149:331-5.

# **PART II**

## **Sleep, Eating and Metabolism**

# **CHAPTER 8**

## **Introduction to Sleep, Eating, and Metabolism**



## CHAPTER 8 CONTENTS

|       |                                                                |         |
|-------|----------------------------------------------------------------|---------|
| 1.    | Sleep and Weight                                               | - 157 - |
| 1.1   | Obesity Rates Have Risen Drastically                           | - 157 - |
| 1.2   | Obesity is a Major Public Health Concern                       | - 157 - |
| 1.3   | Obesity Is Multifactorial and Related To Sleep                 | - 157 - |
| 1.4   | Sleep Times Are Reduced                                        | - 158 - |
| 2.    | Sleep Times Impact Body Weight                                 | - 158 - |
| 2.1   | Reduced Sleep, Weight, and Energy Regulations                  | - 158 - |
| 2.1.1 | Epidemiological Evidence                                       | - 158 - |
| 2.1.2 | Actigraphy and PSG Evidence                                    | - 159 - |
| 2.2   | Sleep Elongation, Weight, and Energy Regulation                | - 160 - |
| 3.    | Potential Causes of Weight Gain with Sleep Disturbance         | - 161 - |
| 3.1   | Metabolic, Endocrine, Immune, and Autonomic Relationships      | - 161 - |
| 3.1.1 | Glucose Metabolism                                             | - 161 - |
| 3.1.2 | Fatty Acid Metabolism                                          | - 163 - |
| 3.1.3 | Hormones                                                       | - 163 - |
| 3.1.4 | Endocannabinoids                                               | - 165 - |
| 3.1.5 | Immune System                                                  | - 166 - |
| 3.1.6 | Energy Expenditure and Temperature                             | - 166 - |
| 3.2   | Chronobiology                                                  | - 167 - |
| 3.2.1 | Circadian Introduction                                         | - 167 - |
| 3.2.2 | Circadian Metabolism                                           | - 167 - |
| 3.3   | Altered Energy Intake and Expenditure                          | - 168 - |
| 3.3.1 | Energy Intake                                                  | - 168 - |
| 3.3.2 | Energy Expenditure                                             | - 169 - |
| 3.3.3 | Altered Energy Regulation Under Calorie Restriction            | - 170 - |
| 4.    | Sleep, Brain Processing, and Energy Regulation                 | - 170 - |
| 4.1   | Arousal, Attention, Cognition, Affective Processing, and Sleep | - 170 - |
| 4.1.1 | Vigilance Regulation                                           | - 170 - |
| 4.1.2 | Compensatory Sleep                                             | - 171 - |
| 4.1.3 | Executive Functioning                                          | - 172 - |
| 4.2   | Sleep Disturbance, Brain Processing, and Energy Regulation     | - 175 - |
| 4.2.1 | Altered Inhibitory Control and Energy Regulation               | - 175 - |
| 4.2.2 | Altered Memory and Energy Regulation                           | - 176 - |
| 4.2.3 | Altered Mood and Energy Regulation                             | - 176 - |
| 4.2.4 | Altered Reward Processing and Energy Regulation                | - 176 - |

## **1. Sleep and Weight**

### **1.1 Obesity Rates Have Risen Drastically**

The regulation of energy stores in the human body is maintained by a complex regulatory system involving multiple physiological pathways. In this system, peripheral signals coordinate with neural circuits to affect physiological processes and behaviors to maintain body weight within a narrow range<sup>300–303</sup>. Obesity, categorized as BMI equal to or over 30.0 kg/m<sup>2</sup>, is a condition that takes place when energy intake exceeds expenditure over a prolonged period of time manifesting in excess adiposity from this chronic, positive energy balance. In the United States, between 1976 and 2008, the prevalence of obesity increased by an alarming amount<sup>304,305</sup>. In 1990, among the states participating in the Behavioral Risk Factor Surveillance System, 10 states had a prevalence of obesity less than 10% and no states had prevalence equal to or greater than 15%. In comparison, in 2009, only nineteen years later, only Colorado and the District of Columbia had a prevalence of obesity less than 20%. Thirty-three states had a prevalence equal to or greater than 25%; nine of these states had a prevalence of obesity equal to or greater than 30%<sup>306</sup>. While the prevalence of obesity varies by age and sex, and by race-ethnic groups<sup>306,307</sup>, in 2007–2008 the overall rate of obesity in the U.S. was 32.2% among adult men and 35.5% among adult women. Despite this alarming trend, Flegal *et al.*<sup>305</sup> recently reported that in women and possibly in men, the increases in the prevalence of obesity did not appear to be rising at the same rate over the past 10 years and it had in years prior.

### **1.2 Obesity is a Major Public Health Concern**

A higher body weight is associated with increased incidence of a number of conditions, including diabetes mellitus, kidney disease, cardiovascular disease, nonalcoholic fatty liver disease, an increased risk of disability and decreased health-related quality of life<sup>308–311</sup>. The report *Healthy People 2010*<sup>312</sup> identified the condition of being overweight or greater as one of the ten leading indicators for health, and chronic obesity at middle age significantly increases lifetime Medicare costs relative to those who remain at normal weight<sup>313</sup>. Thus, the current proportion of obese people in the U.S. is alarmingly high and rates continue to rise. Furthermore, obesity increases disability and morbidity from a variety of other conditions and has important social and healthcare costs.

### **1.3 Obesity is Multifactorial and Related to Sleep**

The etiology of obesity is believed to comprise genetic, metabolic, environmental, behavioral, and sociodemographic factors<sup>314</sup>. The recent rise in obesity suggests behavioral and environmental changes are the cause of the current epidemic<sup>315,316</sup>. In an analysis conducted by Chaput *et al.*<sup>317</sup>, nine risk factors for obesity were evaluated from a six-year period of data collection. They found that sleep duration and eating behavior (i.e., high disinhibition and restraint eating) significantly predicted weight gain while, perhaps surprisingly, energy intake and physical activity did not.

The association between chronic sleep restriction and obesity is likely to be bidirectional and circular as symptoms of obesity such as pain and discomfort and comorbid conditions such as obstructive sleep apnea have been shown to impair and disrupt sleep<sup>318</sup>. Indeed, it has been reported that approximately 50% of obese people complain about the quality of their sleep<sup>319</sup>. While there are many factors proposed to contribute to energy imbalance in humans, evidence suggests that in those with weight pathology, even moderate weight loss (~10%) can be beneficial in reducing levels of many co-morbid risk factors<sup>320</sup>. Given the alarming increase and significant burden of obesity, the identification and quantification of salient factors that either promote or decrease energy imbalance is of critical importance.

## **1.4 Sleep Times Are Reduced**

Over the past 40 years, self-reported sleep duration in the U.S. has decreased by almost 2 hours<sup>52,321–324</sup>. In 1982, a study from the American Cancer Society queried 1.12 million Americans and found that average sleep duration per night was distributed approximately normally (e.g., 52.4% = <7.5h; 19.7% = <6.5h; 4.0% = <5.5h)<sup>325</sup>. In contrast to this study, a 2005 Gallup poll found that among 1,500 U.S. adults, the average self-reported sleep duration was 6.8 h on weekdays and 7.4 h on weekends<sup>326</sup>. In corroboration of this finding, a U.S.-based survey conducted by the National Sleep Foundation in 2009 found that, compared to 2001, the amount of Americans that report average sleep time of less than 6 h per night has significantly increased (from 13% to 20%), while the amount who report 8 h or more has significantly decreased (from 38% to 28%)<sup>327</sup>. It is likely that a multitude of factors contribute to chronic sleep restriction, including chronic physical and mental health status (including sleep disorders), environment, sociodemographic status, and lifestyle<sup>318,328</sup>. Additionally, in America, both work and commute times are extending, which may encourage Americans to voluntarily curtail sleep duration in order to have more available wake time for non-work activities<sup>52</sup>.

## **2. Sleep Times Impact Body Weight**

### **2.1 Reduced Sleep, Weight, and Energy Regulations**

#### **2.1.1 Epidemiological Evidence**

Reduced average sleep times could be critical to the rising rates of obesity as sleep duration has been shown to be closely associated with body weight<sup>324,325</sup>. Large questionnaire-based, population samples have shown an either an inverse or curvilinear dose-response relationship between sleep duration and BMI in adults<sup>324,325,329–340</sup>.

In a study by Watson *et al.*<sup>341</sup> from the University of Washington Twin Registry (average age 36.9 years; 69% female), the researchers used a multivariate adjusted analysis on data from 1,224 monozygotic (423), dizygotic (143), and indeterminant (46) pair samples to showed that, within twins, shorter sleepers (<7 h/n) had a higher BMI (25.8 kg/m<sup>2</sup>) than the BMI (24.9 kg/m<sup>2</sup>) of the twin who slept longer (7-

8.9 h/night;  $p = 0.02$ )<sup>341</sup>. This finding stresses that environmental factors such as sleeping behavior are likely important indicators for body weight.

Epidemiological evidence has also shown a similar effect in children. Persistently short sleep duration (<10 h) during early childhood significantly increases the risk of excess weight or obesity in childhood, and appears to be independent of other obesogenic factors<sup>342–345</sup>, even when adjusting for physical maturation and socio-economic status<sup>346</sup>. Importantly, sleep curtailment during youth has been associated with future weight gain<sup>333,346</sup>. To test associations between daytime and nighttime sleep duration and subsequent obesity in children and adolescents, Bell *et al.*<sup>347</sup> used a prospective cohort from a Panel Survey of Income Dynamics Child Development supplements (1997 and 2002) from U.S. children, ages 0 to 13 years ( $n=1930$ ). The authors found that insufficient nighttime sleep among infants and preschool-aged children may be a lasting risk factor for subsequent obesity and that napping does not appear to be a substitute for nighttime sleep in terms of obesity prevention.

To help further assess this field of study, systematic literature review and analyses have taken place. Nielsen *et al.*<sup>348</sup> performed a systematic literature review of 71 original studies investigating the association between short sleep and weight gain. Overall, they found short sleep duration to be consistently associated with the development of obesity in children and young adults, but not consistently so in older adults<sup>348</sup>. Cappuccio *et al.*<sup>338</sup> also performed a systematic literature review and identified 696 studies, of which 45 met the inclusion criteria (19 in children and 26 in adults) and 30 (12 and 18, respectively) were pooled in the meta-analysis for a total of 36 population samples. Together, this analysis assessed data from 634,511 global subjects (30,002 children and 604,509 adults) across all ages (2 to 102 yrs) and genders. The pooled odds ratio (OR) for short duration of sleep and obesity was 1.89 ( $p<0.0001$ ) for children and 1.55 ( $p<0.0001$ ) for adults. In adults, the pooled beta for short sleep duration was -0.35 unit change in BMI per hour of sleep change. In total, this analysis found that cross-sectional studies from around the world show a consistent increased risk of obesity amongst short sleepers in both children and adults<sup>338</sup>.

### **2.1.2 Actigraphy and PSG Evidence**

Several reports have shown that self-report sleep duration may not accurately assess actual sleep duration<sup>345,349</sup>. In addition to questionnaire-based reports, studies utilizing objective measures, such as actigraphy and PSG, have been performed to assess the sleep-weight relationship<sup>323</sup>. In the Coronary Artery Risk Development in Young Adults (CARDIA) Sleep Study (2000–2006), researchers used several nights of wrist actigraphy to measure sleep among a cohort of 612 middle-aged subjects to examine whether average sleep duration is associated with BMI, and 5-year change in BMI. In their cross-sectional analysis, shorter sleep was strongly associated with higher BMI and the association was very strong in persons who reported snoring, but weak in those who did not. Importantly, there were no

longitudinal associations between sleep measurements and change in BMI over the 5-year assessment period<sup>323,349,350</sup>.

In a study that assessed the connection between sleep duration and BMI in older adults (aged 67-99 years), Patel *et al.* used wrist actigraphy to evaluate 6097 older men and women. Adjusting for sleep apnea, insomnia and daytime sleepiness, the authors compared relatively short sleepers to those sleeping an average of 7-8 h per night. A sleep duration of less than 5 h was associated with higher average BMI and greater odds of obesity, for both men (BMI=2.5 kg/m<sup>2</sup> greater; obesity=3.7-fold greater) and women (BMI=1.8 kg/m<sup>2</sup> greater; obesity=2.3-fold greater), respectively. In addition, short sleep was also associated with central body fat distribution and increased percentage of body fat<sup>350</sup>. A similar study using wrist actigraphy in community-dwelling elderly population (mean age 68.4±6.9 years, range, 57-97) showed that sleep duration had a U-shaped relationship with BMI and obesity, such that higher BMI was seen in both short (<6 h) and long sleepers (≥8 h), compared to subjects who slept 7 to <8 h<sup>349</sup>.

Studies utilizing PSG have also shown a similar relationship between sleep duration and BMI. A population-based longitudinal study with 1,024 volunteers from the Wisconsin Sleep Cohort also showed a U-shaped curvilinear relationship between sleep duration and BMI where the minimum BMI was predicted at an average of 7.7 h of sleep per night. The authors further commented that in persons sleeping less than 8 h, an increase in BMI was proportional to decreased sleep<sup>324</sup>. Another study utilizing PSG in a population of Swedish women showed an inverse relationship between short sleep duration and anthropometric measurement of central obesity, even after adjusting for confounders such as age and lifestyle parameters like exercise, smoking and alcohol consumption. Additionally, the duration of SWS and REM sleep were both inversely related to waist circumference suggesting that the loss of specific sleep stages may be important factors in the association between sleep loss and central obesity<sup>351</sup>. However, the results of this study are cross-sectional and do not allow any inference about causality.

Therefore, while inconsistent findings have been reported, there does appear to be a relationship between sleep duration and BMI. This relationship may not be monotonic but rather curvilinear, such that higher BMI associates with both shorter and longer sleep durations. However, the influence of absolute sleep duration and abnormal BMI may depend on age. For example, 10 h of sleep in adolescents appears to pose no sleep-related obesogenic risk, whereas, for adults and the elderly, that same absolute sleep duration appears to be associated with a greater risk for increased BMI. Thus, over the course of a lifespan, there may be increased risk for weight gain if a person is to get less or more sleep than what is considered average for their age.

## **2.2 Sleep Elongation, Weight, and Energy Regulation**

One notable recent pediatric study showed for the first time that experimental sleep elongation reduced caloric intake. To our knowledge, this is the first time that

such an effect like has been shown. This work done by Hart *et al.*<sup>352</sup> used a within-subjects, counterbalanced, crossover design, with 37 children aged 8 to 11 years old in a three-week study. Participants achieved a 2 hour, 21-minute difference in the actigraph-defined sleep period time between the increase and decrease sleep conditions ( $p < 0.001$ ). Compared with the decrease sleep condition, during the increase condition, children reported consuming an average of 134 kcal/day less ( $p < 0.05$ ) and exhibited lower fasting morning leptin values ( $p < 0.05$ ). However, it should also be noted that this study utilized food recall questionnaires, which is not an accurate way to measure actual food intake.

### **3. Potential Causes of Weight Gain with Sleep Disturbance**

#### **3.1 Metabolic, Endocrine, Immune, and Autonomic Relationships**

Sleep plays an important role in neuroendocrine and metabolic functioning. There is abundant evidence from epidemiologic studies and well-controlled laboratory studies in both animals and humans that indicate that sleep curtailment and sleep disruption have adverse effects on metabolic parameters involved in energy regulation and, thus, body weight. The direction of these associations has not yet been confirmed, but it appears that the abnormal sleep patterns can increase the risk of weight gain. For instance, impaired sleep has been associated with alterations in appetite-regulating hormones and with glucose and adipose regulation, which indicate that fat accumulation may be promoted. Additionally, changes in energy expenditure, core body temperature, and circadian rhythms synchronization may also arise, promoting a condition for greater metabolic efficiency and increased risk of weight gain.

##### **3.1.1 Glucose Metabolism**

Reduced sleep and disrupted sleep have been associated with both metabolic abnormalities and metabolic disorders related to glucose regulation. Individuals who sleep either less than six hours or more than nine hours have been shown to have a higher incidence of diabetes mellitus and impaired glucose tolerance compared to those who sleep seven to either hours per night<sup>353</sup>. Epidemiological studies have shown an independent association between sleep-disordered breathing and both decreased insulin sensitivity<sup>354</sup> and incident diabetes<sup>355,356</sup>.

Interventional, partial sleep restriction studies in humans have also shown a connection between reduced or disrupted sleep and impaired glucose tolerance and decreased insulin sensitivity in both healthy subjects<sup>357</sup> and in subjects with type 1 diabetes<sup>358</sup>. In 1997, Eve Van Cauter and colleagues<sup>359</sup> first documented aberrant glucose regulation after sleep restriction. The study compared a group getting 4 hours of time-in-bed to a group getting 12 hours of time-in-bed over the course of 6 days and found that the group with restricted time for sleep expressed a glucoregulatory pattern similar to pre-diabetes mellitus. Sleep-restricted patients had a 30% decrease in glucose effectiveness, decreased glucose tolerance, and a 40% slower rate of glucose clearance, but no significant differences in insulin

sensitivity. Further evidence of a detrimental effect of aberrant sleep on glucose control came from a study that evaluated 400 females aged 20-70 years with one full night of PSG and found obstructive sleep apnea to be independently associated with decreased insulin sensitivity, indicating that sleep quality is important in glucoregulatory dynamics<sup>351</sup>.

Animal studies have also supported these findings. A study with rats assessed the effects of 1 or 8 days of experimental sleep disturbance on parameters of glucose homeostasis. It was found that both moderate and severe sleep disturbance produced hyperglycemia and decreased insulin levels during intravenous glucose tolerance tests<sup>360</sup>.

### *Sleep Stage Suppression*

Following up on these findings, several studies probed this relationship further by looking at whether suppression of certain sleep stages impacts glucose regulation. It has been observed that the initiation of SWS temporally associates with changes in physiological parameters that could affect glucose homeostasis, such as decreased brain glucose utilization, stimulation of GH release, inhibition of corticotropic activity, decreased sympathetic nervous system activity, and increased vagal tone. In a study by Tasali *et al.*<sup>361</sup>, nine healthy young subjects underwent 3 consecutive nights of selective suppression of SWS by acoustic stimuli. SWS suppression resulted in marked decreases in insulin sensitivity without adequate compensatory increase in insulin release, leading to reduced glucose tolerance. Furthermore, the magnitude of the decrease in insulin sensitivity was strongly correlated with the magnitude of the reduction in SWS. Similarly, Hertzog and colleagues<sup>362</sup> reported that, after SWS suppression through acoustic stimulation, a morning oral glucose tolerance test showed a decrease of up to 20% in insulin sensitivity as determined by the Matsuda Index. However, selective suppression of REM sleep did not affect next-day glucose homeostasis.

Interestingly, biopsies from human adipose tissue taken after interventional sleep restriction have been shown to exhibit insulin resistance<sup>363,364</sup>.

### *Sleep, Appetite and Glucose Control*

These findings provide strong evidence that glucose homeostasis is sensitive to changes in sleep duration and quality. Over time, altered glucose regulation may contribute to diabetes and weight gain. According to the Glucostatic Theory of Appetite Control, glucose plays an important role in the regulation of satiety and appetite<sup>365,366</sup>, whereby low brain glucose utilization leads to the perception of hunger, whereas higher glucose utilization in these same areas promotes satiation<sup>366</sup>. In support of this theory, lower blood glucose concentrations at the end of an oral glucose tolerance test predicted weight gain over a 6-year period<sup>367</sup>. Additionally, impaired insulin sensitivity is one of the major defects underlying the development of type 2 diabetes, which is a disorder closely related in its occurrence to the occurrence of obesity. Thus, it is possible that chronic sleep

restriction contributes to obesity by disrupting the regulation of glucose in a manner that promotes increased food intake.

### **3.1.2 Fatty Acid Metabolism**

While more research has looked at sleep loss on sugar metabolism, there is research indicating sleep loss has a negative impact on fatty acid metabolism, as well.

The enzyme stearoyl-CoA desaturase 1 (SCD1) plays an important role in lipid biosynthesis and in regulating mitochondrial fatty acid oxidation. The expression of this enzyme may meaningfully influence the composition of fatty acids in lipid pools. One night of TSD has been shown to elevate levels of hepatic SCD1 expression and de-novo fatty acid synthesis. These changes were associated with increased DNA methylation at key sites regulating SCD1 expression, and therefore, at least some of the negative effects of sleep loss on fatty acid pools are thought to be epigenetically driven<sup>368</sup>.

### **3.1.3 Hormones**

#### **3.1.3.1 Cortisol and Glucagon**

The changes observed between reduced or disrupted sleep and impaired glucose tolerance may at least be partially explained by observed changes in glucoregulatory hormones. Activation of both the hypothalamic–pituitary–adrenal (HPA) axis and hypocretin neurons have been observed during sleep deprivation and sleep restriction and severe sleep restriction results in elevated evening cortisol levels<sup>369</sup>. The deprivation of REM sleep appears to activate the HPA axis via increased levels of corticotropin-releasing hormone, adrenocorticotrophic hormone and corticosterone<sup>370</sup>. Importantly, elevated cortisol levels have been shown to promote increased food intake and the accumulation of visceral fat in humans<sup>371,372</sup>. Reduced sleep also associates with reduced circulating glucagon levels<sup>373</sup>.

Relative to the well-rested condition, Guyon and colleagues observed that after sleep loss the response to CRH from ACTH decreased by 27% and the response from cortisol decreased by 21%. In this study, the cortisol response showed reduced reactivity and slower recovery to the CRH indicating decreased adrenal sensitivity after sleep loss<sup>374</sup>.

#### **3.1.3.2 Leptin and Ghrelin**

Leptin is another peptide hormone involved in energy homeostasis, the dominant role of which is to signal energy deficiency to the brain<sup>375</sup>. It is an adipokine produced primarily by subcutaneous white adipose tissue and its expression is stimulated by various hormones, sympathetic outflow, energy intake and output<sup>375,376</sup>. Under normal conditions, blood levels display circadian variation as

levels rise across the day and peak in the middle of the night<sup>377</sup>. During sleep deprivation, blood leptin levels show a reduced and flattened profile<sup>378</sup>.

Ghrelin is a peptide hormone mainly produced by endocrine cells in the stomach and gastrointestinal tract, and is an important endogenous regulator of energy balance and GH secretion<sup>201</sup>. Its expression is complex<sup>379</sup> and influenced by sympathetic nervous system activity<sup>380</sup>. Across the wake period, plasma concentration wax and wane episodically providing an orexigenic signal to the brain<sup>381</sup>. During sleep, ghrelin levels increase in the early part of the night and decrease towards morning, however, this nocturnal increase is blunted during sleep deprivation<sup>382</sup>.

### *Leptin, Ghrelin and Sleep*

The association between leptin, ghrelin and sleep has been assessed in multiple studies. In a population-based study that evaluated 1,024 volunteers from the Wisconsin Sleep Cohort, morning fasted blood samples were analyzed for serum levels of leptin and ghrelin. Compared to individuals with a habitual sleep time of 8 hours, those sleeping only 5 hours were predicted to have 15.5% lower leptin and 14.9% higher ghrelin, independently of BMI. This finding suggested that, regardless of BMI, short sleep duration may alter appetite regulating hormones in a way that can potentially increase the risk for weight gain<sup>324</sup>.

Acute interventional studies have also shown a connection between sleep reduction and appetite regulating hormones. In the first study to report this connection in humans, Mullington *et al.*<sup>383</sup>, evaluated leptin levels in response to 88 h of total sleep deprivation in 10 healthy men. They found evidence that sleep influenced the nocturnal leptin profile such that the diurnal amplitude of leptin was reduced during sleep deprivation and returned toward normal during the period of recovery sleep. A subsequent study by Spiegel *et al.*<sup>321</sup> evaluated 12 healthy young men with normal BMI under conditions of less severe sleep restriction. In this randomized, crossover clinical study, subjects underwent 2 days of both sleep restriction (4 h time in bed) and sleep elongation (10 h time in bed) while being controlled for caloric intake and physical activity. Similar to Mullington's total sleep deprivation study, sleep restriction also associated with a significant decrease in leptin (-18%;  $p=0.04$ ). This study also showed a significant increase in ghrelin (+28%;  $p<0.04$ ), hunger (+24%;  $p<0.01$ ) and appetite (+23%;  $p=0.01$ ), especially for foods of high caloric-density with high carbohydrate content (+33% to 45%;  $p=0.02$ ).

The reduction of leptin levels seen with acute sleep restriction have been associated with elevated sympathovagal balance, and altered cortisol and thyroid stimulating hormone profiles<sup>376</sup>. Additionally, in overweight individuals undergoing controlled, reduced calorie consumption, 14 days of interventional sleep restriction to 5.5 hours/night also appeared to promote perturbations in appetite regulating hormones, such that body weight adjusted 24-hour ghrelin concentrations, and the ratio of ghrelin to leptin in the circulation, were significantly increased<sup>384</sup>.

Of note, several studies failed to show a connection between sleep reduction and changes in leptin and ghrelin levels. These studies cite increased activation of the hypocretin system to account for changes induced by sleep deprivation, especially hyperphagia<sup>369</sup>. Other studies have also noted a decrease in daytime physical activity as the potentially important behavioral mechanism for the health-impairing influence of sleep loss<sup>385</sup>.

### **3.1.4 Endocannabinoids**

#### *Endocannabinoid System and Feeding*

The endocannabinoid system is a key component of pathways involved in modulating appetite, food intake, and energy homeostasis. The endocannabinoid receptor, CB1, is found in hypothalamic nuclei involved in energy homeostasis and in the mesolimbic system, including the nucleus accumbens and ventral tegmental area<sup>386,387</sup>. Activation of CB1 receptors promotes hunger, whereas CB1 agonists suppress appetite<sup>388,389</sup>. In particular, endocannabinoid binding to CB1 receptors on dopamine and opioid pathways evoke a preference for highly palatable rewarding food<sup>389–391</sup>.

#### *Endocannabinoids and Sleep*

In regard to energy regulation, there's notable overlap between the effects of activation of the endocannabinoid system and the effect of disrupted sleep. Similar to what is observed with sleep loss, increased activity of the CB1 receptor promotes feeding behavior in excess of energy need, reduces glucose tolerance, tends to reduce leptin levels and to promote ghrelin release, and stimulates reward centers. It has been shown that decreased sleep enhances the daily rhythm of the endocannabinoid 2-Arachidonoylglycerol (2-AG)<sup>392</sup>, which naturally leads to the hypothesis that altered endocannabinoid activity during sleep restriction mediates sleep loss-induced changes in energy homeostasis.

To explore this, Hanlon and colleagues<sup>392</sup> conducted a randomized crossover study comparing 4 days of bedtime restriction to 4.5 hours per night to regular 8.5-hour bedtimes in non-obese healthy individuals. In the sleep debt condition, these researchers found that the amplitude of the daily rhythm of circulating concentrations of 2-AG was amplified due to higher and extended peak afternoon concentrations, without change in the level or timing of the nocturnal nadir. This suggested that the early afternoon drive for hedonic eating may be stronger and last longer in a state of sleep debt. Concurrent with the altered endocannabinoid levels, sleep restricted participants, despite reporting fullness after a normal-sized meal, reported higher scores for hunger, desire to eat, quantity of food that could be eaten, and appetite. The authors hypothesized that these increases in peripheral endocannabinoid concentrations could be a mechanism by which recurrent sleep restriction results in excessive food intake, particularly in the form of snacks, despite minimal increases in energy need.

### **3.1.5 Immune system**

Shorter sleep duration and certain sleep disorders like insomnia and sleep apnea are associated with increased markers of inflammation, including C-reactive protein (CRP) and interleukin-6 (IL-6). Epidemiological evidence shows that poor sleep associates with elevated inflammation<sup>393</sup>. Studies have also shown that a full night or partial night sleep deprivation leads to next day activation of inflammatory signaling pathways<sup>394,395</sup>. Importantly, systemic inflammation is associated with insulin resistance and with effectors of the adipostat<sup>396</sup>. Furthermore, inflammatory signals have been shown to be soporific, as IL-6 can promote sleepiness. It is plausible that sleepiness can have an effect on activity levels, which may further affect sleep and metabolic sensitivity to energy substrates and hormones. Observational studies have shown that shift workers have increases in circulating levels of inflammatory markers<sup>397</sup>, as do older adults, who have poorer sleep than younger adults<sup>398</sup>.

### **3.1.6 Energy Expenditure and Temperature**

#### *Energy Expenditure*

It is well known that individuals vary considerably in terms of rate of energy expenditure, even when corrected for differences in fat free mass. Total daily energy expenditure is primarily comprised of activities that take place during waking, such as one's basal metabolic rate which results from such things as homeothermy and maintenance cellular functions, and activity-and-diet induced thermogenesis. Additionally, sleep metabolic rate is estimated to comprise roughly 25% of total daily energy expenditure<sup>399</sup>. Interestingly, sleep reduction has been shown to influence next day energy expenditure in inconsistent ways. In rats, sleep deprivation between 24 to 96 h and sleep restricted for 21 days rapidly increased energy expenditure and led to a negative energy balance. These changes in energy expenditure were mostly attributed to activation of the hypocretin system<sup>369</sup>.

The prolonged suppression of REM sleep has been associated with increased activation of the HPA axis, and with loss of body weight despite an increase of food intake. This apparent paradox between loss of body weight and increased energy consumption could be a result of an increase in energy expenditure. In 2009, Galvao *et al.*<sup>370</sup> examined the mechanism involved in the influence of REM sleep deprivation on metabolism, feeding behavior and stress response. REM sleep deprivation resulted in increased diurnal food intake, however, produced no significant changes in food intake over a 24-hour period.

In human studies, compared to baseline standards, sleep restriction has produced highly inconsistent results related to alterations in daytime physical activity and resting metabolic rates<sup>384,385,400</sup>.

## *Temperature*

Core body temperature may also be an important factor influencing energy expenditure and, therefore, energy balance. It is known that a decrease in body temperature occurs at night in relation to the sleep cycle in human populations<sup>401</sup>. It has also been hypothesized that lower body temperatures may contribute to decreased energy expenditure of the obese state<sup>402,403</sup>. However, a study that restricted sleep over a 14-day period found no significant decrease in core body temperature from sleep restriction<sup>404</sup>. Further research is necessary to know if chronic sleep reduction is accompanied by a reduction in core body temperature that would promote greater metabolic efficiency and increase risk of weight gain.

## **3.2 Chronobiology**

### **3.2.1 *Circadian Introduction***

Nearly every physiological and biochemical function of the body shows rhythmic circadian variations. These circadian rhythms are biological events that constantly repeat in a 24-hour period and are generated by endogenous mechanisms, or clocks, that allow the organism to predictively adapt to changes in its environment<sup>405</sup>. The optimization of the organism's time-of-day responses depends on both the synchronization between external environmental cycles and signals with the clock system intrinsic to the organism, and the synchronization of central (suprachiasmatic nucleus) and peripheral (local, tissue specific) clocks within the organism.

### **3.2.2 *Circadian Metabolism***

Energy homeostasis is impacted by cellular and behavioral chronobiology. The adipocyte generates patterns of signals across the 24-hour period that influence satiety, cellular differentiation and proliferation. Behaviorally, time of day influences food intake patterns, and, conversely, food intake timing, frequency, regularity and composition may affect chronobiological patterns of both the central clock and peripheral clocks, as in adipose tissue<sup>406</sup>. Chronic desynchronization of the circadian system may promote energy efficiency, and thus, obesity<sup>407</sup>. Work from the Turek lab has evaluated the role of the circadian phase of food consumption and how food consumption influences the mammalian clock. For example, in one study, mice fed a high-fat diet only during the 12-hour light phase gained significantly more weight than mice fed only during the 12-hour dark phase<sup>408</sup>. In separate studies, they demonstrated how the consumption of a high-calorie diet alters the function of the mammalian circadian clock<sup>409</sup> and that the circadian clock gene network plays an important role in mammalian energy balance both at the behavioral and molecular level<sup>410</sup>.

Shift workers have a chronic desynchronization of endogenous rhythms due to frequent alterations in the sleep/wake pattern. Shift work is associated with a variety of metabolic disturbances, including decreased HDL cholesterol and increases in fasting glucose, triglycerides, free fatty acids, arterial blood pressure,

abdominal circumference, and BMI<sup>411,412</sup>. Shift work has also been associated with greater incidence of cardiovascular disease, diabetes, and obesity<sup>413</sup>. This chronic desynchronization of endogenous rhythms may also alter eating behaviors and it has been shown that shift workers eat more meals during the evening and night<sup>412</sup>. Thus, it appears probable that circadian desynchronization and other behaviors such as eating timing can alter clock genes and rhythms and can interfere with the complex mechanism of metabolic and hormonal patterns, contributing to diseases such as energy imbalance (i.e., obesity) and energy-system dysregulation (i.e., diabetes)<sup>406</sup>.

### **3.3 Altered Energy Intake and Expenditure**

#### **3.3.1 Energy Intake**

##### *Appetite and hunger*

Sleep restriction has been shown to increase appetite and the preference for meals that contain high carbohydrate content<sup>321</sup>. In one study, ten young lean healthy subjects were presented with an assortment of *ad lib* food tailored to meet their dietary preferences, including a buffet lunch and dinner and unrestricted access to snacks, after 4 consecutive nights of 4.5 hours in bed. Compared to an 8.5-hour sleep baseline condition, sleep restriction associated with a ~15% increase in total caloric intake and ~10% increase in carbohydrate intake<sup>414</sup>. In another study, when compared to a group of control subjects who received 8 hours of sleep, after one night of sleep restriction to 4 hours, subjects consumed 22% ( $p<0.01$ ) more energy and had greater preprandial hunger before breakfast ( $p<0.001$ ) and dinner ( $p<0.05$ ) without a change in the perceived pleasantness of the foods or in the subjective desire to eat the foods. Interestingly, despite being significantly more sleepy, the sleep restriction group was also significantly more physically active between the hours of 12:15 and 20:15<sup>400</sup>.

##### *Calorie intake*

Previous research showed that insufficient sleep leads to increased calorie intake. Work by Calvin *et al.*<sup>415</sup> comparing the caloric intake in conditions of usual sleep versus a sleep restriction of two-thirds of normal sleep time, for 8 days/8 nights, in a hospital-based clinical research unit. Caloric intake in the sleep-restricted group increased by +559 kcal/day ( $p=0.006$ ) and decreased in the control group by -118 kcal/day ( $p=0.51$ ) for a net change of +677 kcal/day ( $p=0.0149$ ).

##### *Snacking*

Research by Spaeth *et al.*<sup>416</sup> and Markwald *et al.*<sup>417</sup> supports indicated that chronically sleep-restricted adults with late bedtimes seem more susceptible to weight gain due to greater daily caloric intake, and that much of this surplus may take place in the hours immediately prior to bedtime, when sleep deprived people consume highly palatable, calorically-dense foods.

In the largest, most diverse healthy sample studied to date under controlled laboratory conditions, Spaeth *et al.*<sup>416</sup> assessed body weight at admittance and discharge in 225 subjects, and evaluated the time-course caloric intake and meal timing following 2 baseline nights, 5 nights of sleep restriction (4 h time in bed) and 2 recovery nights, or following control conditions (10 h time in bed/night). Compared to control subjects, sleep-restricted subjects gained more weight than control subjects ( $p=0.007$ ) and consumed extra calories ( $p=0.003$ ). The increased daily caloric intake was due to more meals and the consumption of  $552.9 \pm 265.8$  additional calories between 22:00-03:59. The percentage of calories derived from fat was greater during late-night hours compared to daytime and evening hours ( $p < 0.05$ ).

In other research, Hogenkamp *et al.*<sup>418</sup> used a randomized within-subject design ( $n=16$ ) to compare portion size choice after a night of 8 hours sleep and a night of sleep loss. In the morning after sleep loss, subjects had increased plasma ghrelin levels (13%,  $p=0.04$ ), increased self-reported hunger ( $p<0.01$ ), and chose larger portions (14%,  $p=0.02$ ), irrespective of the type of food, as compared to the sleep condition. Additionally, following breakfast, sleep-deprived subjects chose larger portions of snacks (16%,  $p=0.02$ ), yet meal items did not differ between interventions. The researchers concluded that after sleep loss, overeating in the morning is driven by both homeostatic and hedonic factors, and that portion size choice after sleep loss depends on both an individual's hunger status, and the type of food offered. In a separate paper from the same study<sup>419</sup>, this research group reported on food purchasing behaviors after sleep deprivation. They found that independently of both type of food offered and food price, sleep-deprived men purchased significantly more calories (+9%) and grams (+18%) of food than they did after one night of sleep (both  $p<0.05$ ).

It must be considered that sleep reduction may increase food intake, not only by alterations in homeostatic mechanisms that control hunger, but also due to the increased time of exposure to food. Also, sleep loss may affect decision making processes involved in food choices and eating behaviors.

### **3.3.2 Energy Expenditure**

In contrast, other studies have shown that acute sleep restriction did not increase food intake but decreased next-day physical activity. In one study<sup>385</sup>, the authors postulated that the observed decrease in daytime physical activity could be a salient behavioral mechanism for the health-impairing influence of sleep loss.

Many studies have shown that alterations in energy regulating hormones, like leptin and ghrelin, correlate with sleep deprivation. However, these changes may not represent a unique effect of sleep deprivation on their metabolism. Alterations in the levels of these hormones would be expected due to increases in energy expenditure, which take place as a consequence of the additional hours of wakefulness, by opposition to the reduced metabolic rate of the sleep state. A report by Markwald *et al.*<sup>417</sup> showed that after 5 days of sleep restriction (5 hours

of time in bed), insufficient sleep increased total daily energy expenditure by ~5% when compared to a 9-hour time in bed control condition. The increase in energy expenditure corresponded with increased food intake, which the authors noted is a normal physiological adaptation to provide the energy needed to sustain additional wakefulness. However, especially in women, insufficient sleep reduced dietary restraint, and energy intake was in excess of energy needed to maintain energy balance, especially at night after dinner. Interestingly, they also found that when subjects transitioned from an insufficient to adequate/recovery sleep schedule, energy intake—especially of fats and carbohydrates—decreased and led to weight loss ( $-0.03 \pm 0.50$  kg).

### **3.3.3 Altered Energy Regulation Under Calorie Restriction**

It has also been demonstrated that sleep restriction interferes with the beneficial effects of a reduced-calorie diet on excess body weight and adiposity. In one such study, overweight individuals underwent caloric restriction to 90% of their resting metabolic rate for a two-week period, while under two different sleep conditions, separated three months apart. While weight loss during each treatment remained similar, the composition of the weight loss differed markedly. In the 8.5-hour bedtime condition, fat constituted 57% of the lost weight. However, in the sleep restricted condition (5.5-hour bedtime), fat constituted only 26% of the weight loss and showed increased weight loss from lean body mass<sup>384</sup>. Together, these results show that sleep restriction may increase food intake, craving for carbohydrates, either increase or decrease physical activity, and reduce the beneficial effects of a reduced-calorie diet in those with excess adiposity.

## **4. Sleep, Brain Processing, and Energy Regulation**

### **4.1 Arousal, Attention, Cognition, Affective Processing, and Sleep**

#### **4.1.1 Vigilance Regulation**

In the two process model for sleep and wake offered by Borbely *et al.*<sup>420</sup>, vigilance is affected by two, mutually exclusive processes that combine to influence the human sleep-wake cycle: 1) a homeostatic process that builds up pressure for sleep during wakefulness and dissipates this pressure during sleep and 2) a wake-and sleep promoting circadian rhythm that oscillates in intensity over a 24-hour period<sup>421</sup>.

Sleep can be reduced in a variety of ways, including partial or total sleep loss over a period of time. Sleep can also be reduced through fragmentation, which disrupts sleep architecture and can limit the actual amount of sleep achieved during a period of time in bed. In addition, selectively, a sleep stage can be reduced or eliminated while total sleep time remains intact. Voluntary sleep curtailment is a type of partial sleep deprivation in which individuals voluntarily reduce the amount of sleep they get on a chronic basis<sup>422</sup>.

When sleep is reduced, vigilance performance deteriorates progressively over days and is dependent on total sleep lost during the observation period. Remarkably, 14 consecutive days of sleep restriction to 4-6 hours sleep periods impair behavioral alertness to the same degree as seen after 1-3 nights of total sleep deprivation, suggesting that the effects of sleep restriction accumulate over time<sup>423,424</sup>. As with vigilance performance, cumulative neurobehavioral deficits are also seen in other cognitive functions, including impairments in executive functioning, decision making, working memory, and emotional states<sup>425-443</sup>. In studies of total sleep deprivation, there is a monotonic relationship between deficits in neurocognitive performance and subjective ratings of sleepiness. Interestingly, the accumulation of objective cognitive performance deficits seen with nightly sleep restriction below 8 hours is not paralleled by equivalent subjective ratings of sleepiness<sup>424,444</sup>. This mismatch between subjective perception of sleepiness and actual cognitive impairment may lead people to underestimate the actual degree to which they are cognitively impaired and overestimate their readiness to perform tasks<sup>445</sup>. Notably, there are also considerable individual differences in the degree of vulnerability to performance impairment from sleep loss, and these differences represent a trait<sup>425,446-450</sup>.

Vigilance performance decrements in response to sleep loss also show a time-of-day effect such that, during periods of sleep restriction, significant differences in vigilance performance and subjective sleepiness<sup>451</sup> will be seen at various time-points across a 24-hour period. Compared to diurnal periods, worse performance tends to take place during habitually entrained night periods<sup>421</sup>; however, additional variation takes place within diurnal and nocturnal time frames. For example, Mollicone *et al.* showed that following 8 days of sleep restriction to 4 hours/day, diurnal vigilance errors were worst at 08:00 and became progressively smaller across the hours of the day, especially between 16:00 and 20:00<sup>451</sup>. Remarkably, subjects averaged 8.3 more Psychomotor Vigilance Task performance lapses at 08:00 than at 18:00<sup>451</sup>. It has been suggested that circadian wake processes facilitate a period of relatively protected alertness in the late afternoon and early evening hours when nocturnal sleep is chronically restricted<sup>451</sup>.

#### **4.1.2 Compensatory Sleep**

A compensatory mechanism to support vigilance performance appears to exist to withstand acute reductions in sleep. As homeostatic pressure for sleep builds up higher across prolonged wakefulness, the rate of dissipation of that pressure during subsequent sleep is enhanced exponentially, so that even brief periods of sleep provide significant performance recuperation<sup>421</sup>. Depending on the timing and duration of sleep, and the number of days it is reduced relative to normal, average sufficient sleep of 8 hours per night, some aspects of sleep are conserved, occur sooner, or intensify, while other aspects of sleep are diminished<sup>423,424,452,453</sup>. Banks *et al.*<sup>454</sup> showed that after 5 nights of sleep restriction to 4 h per night, sleep parameters such as total sleep time, stage 2, REM sleep and NREM slow wave energy, and the objective Maintenance of Wakefulness Test, increase monotonically across an ascending dose of sleep recovery time that ranged from 4

to 10 hours. However, other neurobehavioral deficits induced by sleep restriction, such as decrements in vigilance and subjective sleepiness (as measured by the Psychomotor Vigilance Task and Karolinska Sleep Scale, respectively), improved exponentially after recovery sleep. It is important to note that one night of sleep recovery up to 10 hours of time in bed was insufficient to abolish all vigilance, subjective sleepiness and mood deficits caused by the 5 nights of sleep reduction in this experiment<sup>454</sup>. The authors concluded that complete recovery from such sleep restriction may require a longer sleep period during one night, and/or multiple nights of recovery sleep<sup>454</sup>.

### **4.1.3 Executive Functioning**

#### **4.1.3.1 Introduction**

Sleep deprivation adversely affects the ability to perform cognitive tasks. However, it is uncertain if sleep deprivation selectively reduces the capacity of specific cognitive functions, such as the executive functions, or if the observed cognitive decline is the result of a global cognitive impairment due to reduced stability in attentional networks<sup>455</sup>.

An executive act is any act toward oneself intended to influence future outcomes via self-regulated behavior and is therefore instrumental to purposive, intentional behavior<sup>456</sup>. The executive functions are thought to be processes that allow for the development of a temporally remote goal and the ability to work towards that goal. This requires retaining the goal in memory, maintaining attention to the goal to inhibit distractions and competing responses, and modifying original plans to meet expected outcomes<sup>457</sup>. Together, these cognitive abilities are thought to be instrumental in the execution of complex tasks such as interpersonal communication, creative problem solving, and decision making<sup>458</sup>. It has been hypothesized that these abilities may have developed as an adaptation to environmental pressure associated with group and social living<sup>456</sup>. It is also believed that the executive functions strengthen from birth to adulthood as demonstrated by the remarkable shift over the first three decades of life toward a greater performance on functions such as delaying gratification versus selecting an immediate reward but at a cost for its immediacy<sup>459</sup>. Furthermore, executive functions have been shown to be distinct from other cognitive functions, like intelligence<sup>460</sup>.

Component cognitive functions that comprise the executive function abilities include attention and concentration, memory (working and verbal), behavioral flexibility, planning, and response inhibition. Additionally, it has been hypothesized that emotional responses—triggered by environmental cues—provide initiative and energizing behavior for goal attainment and therefore influence the decision making process in an adaptive manner<sup>461</sup>. Brain areas relevant to these related functions include specific parts of the cortex (i.e., the anterior cingulate, dorsolateral prefrontal, and orbital frontal) and hippocampus<sup>462</sup>, with the addition of limbic nuclei and the inferior medial frontal region of the cortex (anterior cingulate) for emotional processing<sup>461</sup>. To assess aspects of brain activity related

to various cognitive demands, Duncan *et al.*<sup>463</sup> reviewed functional neuroimaging patterns of frontal-lobe activation, including aspects of perception, response selection, executive control, working memory, episodic memory, and problem solving. They found strong evidence for regional specialization of function within prefrontal cortex indicating same brain regions may subserve different functions in different behaviors. However, this specialization poses a methodological problem for studying distinct components since a specific frontal-lobe network is consistently recruited for diverse cognitive problems<sup>463</sup>.

#### 4.1.3.2 Sleep and Executive Functioning

It has been hypothesized that sleep restriction directly impairs executive functions and performance on tasks that rely on prefrontal cortical function more than non-executive task performance<sup>464</sup>. This stems from various findings that show total sleep deprivation selectively alters theta power density<sup>16, 17</sup> in the frontal cortex and metabolism in the prefrontal cortex<sup>428</sup> during wake periods. While research on the effect of sleep restriction on executive function has been studied extensively<sup>436,443,464–477</sup>, a clear association has been hard to discern due to inconsistent findings and task impurity problems<sup>473,475,476,478</sup>. One study that did find an association between sleep reduction and executive impairment assigned 106 new parents to either a sleep-curtailed group (<7 hours/night) and a non-sleep-curtailed group (≥7 hours/night) based on self-reported nighttime sleep duration from a 6-month period immediately preceding the study. In this study, the ability to flexibly implement a task goal was significantly impaired in the sleep curtailed group<sup>479</sup>. Contrary to this finding, Tucker *et al.* studied the effects of sleep deprivation on executive functions using a task battery that allowed dissociation of some important executive processes from non-executive components of cognition. While performance on the control task battery was considerably degraded during sleep deprivation<sup>477</sup>, working memory scanning efficiency, resistance to proactive interference, and dissociated executive processes of phonemic verbal fluency performance were not significantly impaired compared to baseline. These results challenge the view that executive functions are especially vulnerable to sleep loss<sup>477</sup>.

Alternatively, Dinges and colleagues hypothesized that sleep-deprivation induced cognitive impairments result from instability in the wake state<sup>449</sup>. Similar to the two process sleep wake model from Borbely *et al.*<sup>420</sup>, the homeostatic drive for sleep and the circadian drive for wakefulness—with the addition of a compensatory effort to perform—interact to determine the net state of vigilance and cognitive performance. Furthermore, during conditions of sleep loss, there is an increase in moment-to-moment variability of attention which then impairs a wide variety of cognitive tasks, including executive functions that are necessary for goal-directed activities<sup>449</sup>. Thus, according to this model, sleep deprivation does not necessarily cause selective impairments in executive functions due to discriminant prefrontal cortex vulnerability to sleep loss. Rather, sleep deprivation affects cognitive performance globally, at least in part, due to deficits in the ability to sustain attention.

While sleep deprivation may not affect the executive functions more than other cognitive functions, executive abilities may still be impacted in a vigilance-impaired condition. Due to the prefrontal cortex specialization for a variety of discrete behavioral abilities, various instruments used to assess executive functions are complicated by task impurity<sup>480</sup>, as executive functions both operate on, and are operated on by other cognitive processes. Therefore, any task that targets executive functions also likely implicates non-executive cognitive processes. An impaired non-executive process serving as a weak link in the executive system may diminish the overall executive functions score; however, this low score would not necessarily arise from impairment of the target executive functions<sup>481</sup>. Even then, research on sleep restriction can assess functional loss of abilities related to executive function, but it is still difficult to claim that any observed effects would be a result of impairment primarily in executive systems and not the non-executive systems they influence or that influence them.

#### 4.1.3.3 Behavioral Choice Theory and Delay Discounting

A theoretical approach to assess executive functioning is behavioral choice theory, or behavioral economics. This paradigm combines research from a variety of disciplines to help understand how people make decisions and has been extended to health behaviors, such as eating, physical activity, and obesity<sup>482</sup>. Choice research uses various models to assess choice decisions across concurrent alternative reinforcers or choice alternatives that vary temporally<sup>483</sup>. As an example of the second model of choice, the Delay Discounting paradigm assesses the degree to which a commodity fluctuates in reward value as a function of time. The measurement assesses the willingness to postpone receiving an immediate reward in order to gain additional benefits after a time delay for acquisition. Delay discounting is thought to assess a variety of hallmark executive functions such as risk/benefit calculation, inhibitory control, and delayed gratification<sup>456,484</sup>.

Delay discounting scores have been shown to predict the likelihood to engage in what are thought to be a range of health-related behaviors. For example, steep time discounting decreases the probability of engagement in anti-obesogenic behavioral habits, such as regular exercise and healthful eating patterns<sup>485,486</sup> and predicts higher energy intake in food reinforcement paradigms<sup>483</sup>. An analysis performed by Ikeda *et al.* on data from 2,987 respondents (average age = 49 years; 47% male) from the Japan Household Survey on Consumer Preferences and Satisfaction 2005 (JH05) show that obesity results in part from temporal decision biases<sup>487</sup>. Future reward discounting was positively associated with BMI, impatience, and inclination toward procrastination. A one-unit increase in the degree of procrastination was associated with a 2.81 percentage-point increase in the probability of being obese. Alternatively, respondents exhibiting the sign effect—where future negative payoffs are discounted at a lower rate than future positive payoffs (less risky)—show a 3.69 percentage-point lower probability of being obese<sup>487</sup>. In another study, obese and healthy-weight age-matched subjects completed Delay Discounting tasks assessing various monetary rewards over various time scales. Compared to controls, greater delay discounting was seen in obese women ( $p < 0.02$ ) but not in obese men. Subsequent analyses showed that

these differences between obese women and healthy-weight age-matched controls were not related to differences in IQ or income<sup>488</sup>. Thus, differences in a person's preference for the present over the future may substantially influence their propensity to adopt a healthy lifestyle and affect weight gain.

#### **4.1.3.4 Risk Taking and Working Memory**

The reduced ability to delay gratification for a future positive outcome can be thought of as a form of impulsivity and risk-taking behavior. To study how sleep deprivation affects risk decisions with a potential loss-bearing outcome, Venkatremam *et al.*<sup>476</sup> deprived subjects of sleep for 24 h and then had subjects perform a gambling task while undergoing neuroimaging. Interestingly, they found that sleep deprivation modulated activation of various brain regions associated with risky decision making and emotional processing. For example, following sleep deprivation, riskier choices on the gambling task concurrently invoked a neural response indicative of elevated expectation for a higher reward and a diminished loss aversion. Neuroanatomically, this was represented by greater activation in the right nucleus accumbens and reduced insular and orbitofrontal cortices, respectively<sup>476</sup>.

## **4.2 Sleep Disturbance, Brain Processing, and Energy Regulation**

It has been reported that we make nearly 200 decisions per day about food<sup>489</sup>. Additionally, our awareness of a multitude of environmental factors can significantly influence what we eat and how much we eat. It has also been shown that certain environmental factors can lead to a misperception in the amount of actual energy consumed at a meal. For example, in *ad libitum* food environments, people eat different amounts depending on their awareness of how much they have already eaten<sup>490</sup>. Indeed, the quantity of food consumed by subjects in experimental procedures has been shown to be influenced by such things as: the size of food packaging, plate size and shape, meal portion size<sup>491,492</sup>, ambient lighting and noise/music element of the room<sup>493</sup>, temperature of eating environment<sup>494–496</sup>, the number of people and the behaviors of those people whom we eat with<sup>497,498</sup>, the variety of food types available to us, and the proximity and visibility of the food types in our environment<sup>499</sup>. Many of these environmental parameters serve to either establish social norms<sup>500–502</sup>, which suggest appropriate consumption quantity, or distract from an awareness of actual energy consumed and internal cues of satiety<sup>490,503</sup>. In fact, environmental influences on eating behaviors, like portion size<sup>504,505</sup>, have been shown to have increased in parallel with the rise in the prevalence of obesity<sup>506–509</sup>. Thus, the act of self-monitoring both actual consumption volume and satiety in diverse environmental settings requires awareness to resist excessive caloric intake.

### **4.2.1 Altered Inhibitory Control and Energy Regulation**

The susceptibility to overeat has also been shown to be a product of disinhibitory control over the action to stop eating. Studies have shown that high susceptibility to overeat combined with low restriction is associated with higher body weight<sup>510–</sup>

<sup>513</sup>. For example, in a cross-sectional study using survey data in Danish women, risky eating, in part defined as a self-rating of inhibitory control to stop eating, showed that this type of behavior was a risk factor for obesity<sup>514</sup>. Furthermore, this study showed that those with a BMI of 25 or more had an increased risk of maladaptive eating behaviors, including reduced inhibitory control, by approximately 2.5 times. In a model of dietary restraint proposed by Polivy and Hermann<sup>515</sup>, the authors suggest how dieting itself might lead to weight gain by reducing cognitive control over the physiological cues of hunger. Notably, hypoglycemia and sleep loss have both been implicated in decreased neurocognitive function; however, in one study, one night of total sleep deprivation deteriorated neurocognitive function but did not do so in a synergistic manner to aggravate the impairing influence of acute hypoglycemia<sup>516</sup>.

#### **4.2.2    *Altered Memory and Energy Regulation***

Additionally, what a person eats may also be influenced by their memory of what they had previously eaten in a day. In a study by Higgs *et al.*<sup>517</sup>, unrestrained eaters were examined to see how much they would eat after being cued or not cued of a recent eating episode. While subjective ratings of hunger, fullness, and desire to eat did not vary as a function of cue type, subjects who were cued about a recent meal ate less than those who received no cue. This result suggests that memory of recent eating is an important cognitive factor influencing food intake.

#### **4.2.3    *Altered Mood and Energy Regulation***

As discussed, emotions and mood are hypothesized to play a key role in the decision making process<sup>461</sup> and have also been shown to be affected by sleep loss<sup>518</sup>. With food, emotional states have also been shown to impact eating quantity. For example, a study by Turner *et al.*<sup>519</sup> showed that positive mood resulted in consuming significantly less calories in those with a controlled eating style. By contrast, among those who presented an uncontrolled eating style, positive mood enhancement led to greater calorie consumption<sup>519</sup>. In addition, the suppression of emotion has been shown to lead to increased food intake<sup>520</sup>.

#### **4.2.4    *Altered Reward Processing and Energy Regulation***

Work by St-Onge *et al.*<sup>521</sup> showed that, compared to controls, sleep-restricted subjects had greater neuronal activity in response to food stimuli and consumed an average of 296 kcal more per day when sleep deprived compared to when they were well rested. Similarly, Benedict *et al.*<sup>522</sup> found that acute sleep loss enhances hedonic stimulus processing in the brain, leading to an increased drive to consume food and coinciding with increased self-reported hunger. Other studies by St-Onge *et al.*<sup>523</sup> and Greer *et al.*<sup>524</sup> provided possible models of neuronal mechanisms that relate short sleep to altered appetitive drive increasing the risk for calorie surplus and the development of obesity. The St-Onge study<sup>523</sup> used fMRI in 25 normal weight subjects after a period of five nights of either 4 or 9 hours in bed to determine whether specific neural systems are preferentially activated after sleep

loss in response to unhealthy compared with healthy foods. After sleep restriction, viewing unhealthy foods led to greater activation in brain reward and food-sensitive centers such as the superior and middle temporal gyri, middle and superior frontal gyri, left inferior parietal lobule, orbitofrontal cortex, and right insula, compared with healthy foods. Further, food intake increased in association with a relative decreased activity observed in the right insula. The Greer *et al.* study<sup>524</sup> used a food-desire task in combination with fMRI to characterize the impact of sleep loss on the brain mechanisms governing appetitive food desire. Subjects (23 normal weight men and women) underwent a repeated-measures, counterbalanced, crossover-design trial involving a night of normal rested sleep (avg. 8h sleep) and a night of monitored total sleep deprivation (avg. 24h awake), separated by 7+ days. In this study, during food desirability choices after sleep deprivation there was decreased activity in appetitive evaluation regions within the human frontal cortex and insular cortex, and amplified activity within the amygdala. This brain activity pattern associated with a significant increase in the desire for high-calorie foods, the extent of which is predicted by the subjective severity of sleep loss across participants. The authors argued that this mechanism may explain how insufficient sleep leads to the development/maintenance of obesity through diminished activity in higher-order cortical evaluation regions, combined with excess subcortical limbic responsivity, resulting in the selection of foods most capable of triggering weight-gain. Together, these reports, and the previously reported work by St-Onge *et al.*<sup>521</sup> and Benedict *et al.*<sup>522</sup>, illuminate a consistent association with short-term sleep loss and patterns of neuronal activity in brain centers involved in food reward and evaluation indicative of enhanced hedonic drive towards foods that are of high risk for maintaining a calorie surplus necessary for weight gain.

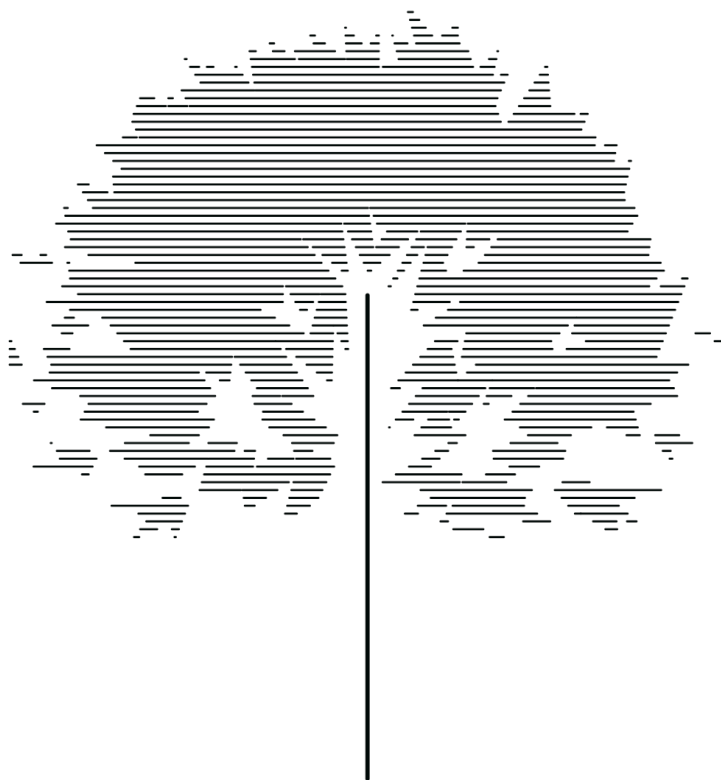
In the recent past, multiple important studies were reported evaluating calorie intake after insufficient sleep. While increased calorie intake would be expected after increased energy expenditure due to extended wakefulness, sleep deprivation may lead to a calorie intake that exceeds the expenditure differences between the sleep deprived vs. full sleep condition. While this surplus energy expenditure can occur from high calorie consumption at different parts of the day, there are several studies now that show the time immediately prior to sleep as particularly vulnerable for the consumption of high calorie meals that are comprised of calorie-dense foods. A potential mechanism that explains these observations is developed from imaging work that shows a pattern of neuronal activity after insufficient sleep in brain centers involved with food reward and evaluation. This pattern indicates that sleep loss enhances hedonic drive towards fattening foods. Together, these findings suggest that insufficient sleep does typically manifest in surplus energy intake and this surplus may be mediated by increased hedonic drive towards fattening foods, increased impulsivity, and increased willingness to eat foods that are believed by the subject to be unhealthy.

## **CHAPTER 9**

### **Eating Decisions Based on Alertness Levels after a Single Night of Sleep Manipulation: A Randomized Clinical Trial**

*Sleep (2016) Oct 20. pii: sp-00425-16*

**Authors:** Daniel Pardi, Matthew Buman, Jed Black, Gert Jan Lammers, and Jamie M. Zeitzer



**Abstract**

|                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Study Objective</b>           | To determine the relationship between an ecologically-relevant change in sleep behavior and its subsequent effects on daytime alertness and feeding behavior.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Methods</b>                   | Fifty healthy, young participants (10 male, 40 female) completed two 3-hour study sessions that were at least five days apart. The first session was a baseline evaluation. On the night prior to Session 2, the amount of time in bed was manipulated to be 60-130% of the individual's habitual sleep time. Within both sessions, subjective (Stanford Sleepiness Scale) and objective (Psychomotor Vigilance Test) alertness were measured. During the middle of each session, a 40-minute ad libitum meal opportunity allowed participants to eat from eight different food items. Food healthfulness, caloric density, distribution and number of calories were measured and compared to alertness levels. |
| <b>Results</b>                   | The induced variation in time in bed resulted in induced variation in both subjective and objective ( $p<0.05$ ) measures of alertness. Decreased subjective alertness was associated with increased total caloric consumption ( $p<0.05$ ), and a greater number of calories consumed from less healthy food ( $p<0.05$ ), as rated by both the investigators and by the participant. Decreased objective alertness was associated with less healthy food choices ( $p<0.05$ ), and the consumption of more food from the calorically-dense items ( $p<0.05$ ).                                                                                                                                                |
| <b>Conclusion</b>                | Ecologically-relevant impairments in subjective and objective alertness are associated with increased caloric intake and dysfunctional eating decisions. People experiencing reduced alertness after modest sleep loss may be more willing to eat food they recognize as less healthful and appear to prefer more calorically dense foods.                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Statement of Significance</b> | Both laboratory-based and epidemiologic research demonstrate a relationship between reduced sleep and increased body weight. The laboratory-based studies, however, have relied on fairly extreme models of sleep loss in order to observe substantive changes in metabolic measures. Our research demonstrates that even small, ecologically-relevant changes in daytime alertness are associated with changes in food choices and eating behaviors.                                                                                                                                                                                                                                                           |

## Introduction

Substantial epidemiological evidence shows significant association between reduced sleep and increased body weight and a multitude of energy-regulation mechanisms have been explored to better understand this association<sup>1-3</sup>. Cross-sectional and prospective data show that short-duration sleepers have modified eating behaviors, including altered within-day eating timing,<sup>4,5</sup> increased snacking behavior,<sup>4,6</sup> and increased calories from beverages<sup>4</sup>. Controlled, prospective research demonstrates an increase in caloric intake on the day following one<sup>7</sup> to several nights<sup>5,8,9</sup> of partial sleep restriction in normal weight adults. While it is common for self-reported hunger to increase after sleep deprivation,<sup>10,11</sup> some studies show no difference in hunger between the sleep-rested and sleep-deprived conditions, despite difference in food selection behavior<sup>12</sup>. The increase in caloric intake may in part be explained by reduced satiety<sup>13</sup>.

Some increase in caloric intake after sleep loss would be expected to accommodate for increased energy expenditure from additional wake time<sup>5</sup>. Several studies show that, indeed, homeostatic factors contribute to increased caloric intake after sleep loss;<sup>5,11</sup> although, this increased caloric intake seems to exceed the level expected to accommodate for energy expenditure associated with the additional time awake<sup>14</sup>. It has been argued that altered hedonic-valuation factors increase portion size and alter food selection after sleep loss<sup>15</sup>.

A well-described and consistent response to sleep loss is impaired alertness, which can be observed in multiple ways, including decreases in both subjective and objective alertness measurements<sup>16</sup>. Moreover, it has been hypothesized that sleep deprivation-induced cognitive impairments, such as reduced alertness and attention, result from instability in the wake state<sup>17</sup> and that such an increase in moment-to-moment variability of attention impairs a wide variety of cognitive tasks, including goal-directed activities<sup>17</sup>. Eating is one such goal-directed activity with food decisions being made 200 times or more each day<sup>18</sup>. Eating is influenced by a wide variety of factors. Internal factors include metabolic state, health beliefs and objectives, emotional state, and the behavioral and metabolic consequences of dietary habits<sup>19-21</sup>. External influences include food presentation and environmental conditions<sup>22-29</sup>. External factors can shift awareness away from internal drivers of food intake, potentially causing diminished accordance with health goals and internal signals of satiety<sup>30</sup>. Thus, the act of self-monitoring food intake volume and food type, as well as satiety requires awareness, which is influenced by alertness, which is influenced by sleep.

We hypothesized that lowered alertness would lead to less surveillance in both total caloric intake and decisions about the types of food one eats. In the current study, we examined whether experimentally induced changes in subjective or objective alertness were associated with changes in total calorie consumption, and calorie consumption based on several categorizations, including the healthfulness, the tempting nature, and the caloric density of the offered foods.

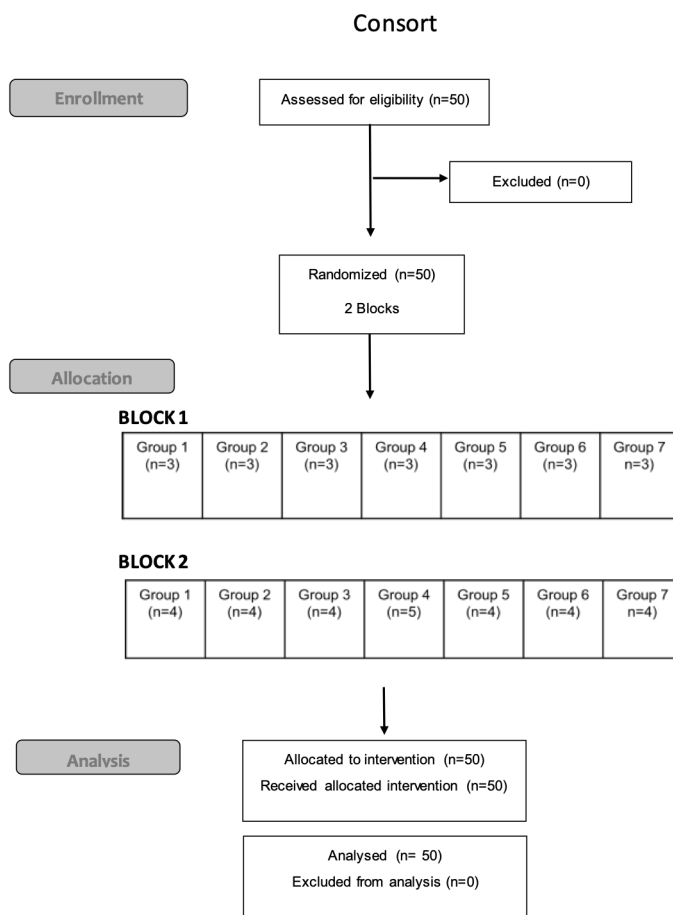
## Methods

### Design

Using a within-participant design, we examined whether, compared to baseline, changes in alertness are related to changes in food consumption. To induce changes in alertness, participants were randomized to receive more, less, or the same duration of time in bed relative to their habitual sleep time for a single night immediately prior to their second visit. The first visit served as a baseline assessment. The study described in this manuscript was approved by the Stanford University Institutional Review Board (IRB) on May 15<sup>th</sup>, 2011 and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Each participant provided written informed consent prior to enrollment. The trial was registered with ClinicalTrials.gov (registration number NCT02484846). The authors confirm that all ongoing and related trials for this intervention are registered.

### Recruitment

A group of healthy adult men ( $n = 10$ ) and women ( $n = 40$ ) were recruited via Facebook Ads for a research study of sleep and decision making, cognition, and mood (Figure 1). Participants needed to be aged 18-45 years, with a BMI between 20-29 kg/m<sup>2</sup>, eat breakfast at least five days per week, have a wake time between 5 and 10 AM at least 5 days per week and a time in bed allocated for sleep of 5-10 hours at least 5 days per week. Potential participants were excluded from the study if they had dietary restrictions that prohibited them from selecting any of the food options offered in the study (e.g., gluten allergies, diabetes, vegan diet, dairy avoidance, allergies to the respective food choices available), were currently on a calorie-restricted diet, or were currently at a weight that was less than 20% of their highest weight within the last three years. Other exclusion criteria included participation in shift work within seven nights of the experiment, being diagnosed as having a sleep disorder (including moderate or severe sleep apnea syndrome, narcolepsy, chronic insomnia) or an eating disorder, having an active serious health condition, regularly taking CNS-acting medication (e.g., methylphenidate), or typically consuming more than 600 mg of caffeine or smoking more than 10 cigarettes per day. Four females, one obese and three underweight, were included in the study despite being outside the range of our inclusion criterion for BMI. The study staff determined these individuals to be appropriate candidates based on their stable weight and positive health status. *Post hoc* examination of caloric intake (during both sessions as well as the difference between the two sessions) of these four participants indicated that it was within the 95% confidence interval of the remaining subjects. We also ran a parallel analysis in which we examined caloric intake normalized for subject bodyweight and found that caloric intake in these four subjects was statistically indistinguishable from the remaining subjects.



**FIGURE 1.** Consort diagram.

As participants were recruited for a study on sleep and cognitive function, the recruited participants were not made aware that the study would measure eating behaviors. As much as possible, the study was designed to not draw attention to the fact that their eating behavior would be evaluated as a part of the study. For that reason, there was no randomization for the order of the sessions and subjects were only asked to complete the Food Health and Liking Assessment Battery (FH-LAB) at the end of the second session. All participants completed the study within a six-month time frame between September 2011 and March 2012 in the San Francisco Bay Area. The research was conducted at two different study locations (Palo Alto and San Francisco, California); the configuration was identical at both sites and set-up by the same person (DP).

### Study Outline

The study evaluated participants across two sessions that occurred at least five days apart: a baseline session after an unmodified night of sleep (Session 1) and a

second session that immediately followed a night of sleep during which the time in bed was experimentally manipulated (Session 2). Given the experimentally-induced sleep restriction in some of the participants prior to Session 2 (see below), we requested that all participants be driven to or take public transportation to the laboratory on Session 2. On the morning of both study sessions, participants were asked to rise at their self-reported average wake time and arrive at the testing center 1.5 hours later. Between waking and arrival, participants were instructed not to exercise or consume food, beverages or cigarettes. Participants were allowed access to water throughout the duration of the study (approximately three hours) and to a restroom between study sections. Testing took place in a dimly lit room. The conditions between the two sessions were identical except that participants were consented during Session 1 and experienced an alteration of their time in bed on the night immediately prior to Session 2.

### **Randomization**

To induce variability in the independent variable, participants were randomly assigned (by S. Pardi) to one of seven sleep intervention groups: 60% (Group 1, n=7), 70% (Group 2, n=7), 80% (Group 3, n=7), 90% (Group 4, n=8), 100% (Group 5, n=7), 115% (Group 6, n=7), and 130% (Group 7, n=7) of average habitual time in bed, as captured from self-report from the five nights immediately prior to Session 1. We used a 2-block randomization procedure to assign half of the participants in each block. Three subjects were assigned to each group in block 1. Four to five subjects were allocated to each group in block 2. We did the allocation procedure in this manner to ensure that the distribution of participants was spread out more evenly across the entirety of the enrollment period. All investigators were blind to participant allocation until all study procedures were completed, and all data were entered, and the database was locked.

### **At-Home Sleep Monitoring**

At-home sleep patterns were recorded by participants using the Consensus Sleep Diary<sup>31</sup> for 5-7 days prior to Session 1 and the night prior to Session 2. The data collected prior to Session 1 were used to establish an average amount of time in bed for each participant. Target time in bed duration on the intervention night prior to Session 2 was calculated as the self-reported average nightly time in bed duration from the five nights prior to Session 1, multiplied by the randomization Group percentage. For example, a participant randomized to Group 3 (80%) who normally spent 7 hours in bed would be scheduled to be in bed for 5 hours 36 minutes for the night prior to Session 2. Sleep data collected prior to Session 2 were used to examine compliance with the randomization procedure. The wake time documented for morning of Session 1 was prescribed as the wake time for Session 2. The bedtime for the intervention night of sleep was calculated by subtracting the prescribed sleep time from established wake time. This way, between sessions, wake times were fixed and time-to-bed times and total time in bed were adjusted.

## **Alertness Testing**

### *Psychomotor Vigilance Test (PVT)*

Performance on a sustained vigilance test was assessed using the PVT-192 (Ambulatory Monitoring, Ardsley, NY). The PVT is a commonly used objective measure of alertness<sup>32</sup> in which users are asked to hit a button as soon as they see a light (LED millisecond timer) appear on the device. This is followed by a variable delay between 2,000 and 10,000 milliseconds and a subsequent light stimulus. The PVT has been shown to be sensitive in testing sessions lasting up to 20 minutes<sup>33</sup>. In this iteration of the PVT, we had participants continue for 15 minutes. The median reaction time (mRT) on the PVT was used as the main measure of objective alertness, with an increase in mRT indicating a decrease in alertness.

### *Stanford Sleepiness Scale*

Current subjective alertness was measured using the Stanford Sleepiness Scale (SSS). The SSS is a 7-point scale in which each point has a descriptive label, ranging from 1 — ‘feeling active and vital; alert or wide awake,’ to 7 — ‘No longer fighting sleep, sleep onset soon; having dream-like thoughts’<sup>34</sup>. The SSS has been found to be sensitive to changes in alertness due to both circadian variations and sleep loss<sup>35</sup>. Additionally, it has been shown that performance scores for the SSS are more sensitive when taken after, instead of before, a PVT<sup>35</sup>.

## **Food, Eating, and Nutrition Quantitation**

During the approximately 40-minute eating opportunity, eight food items were placed between the participant and a video screen. While the participant ate, two different videos from TED.com (TED Conferences LLC, New York, NY), both approximately 20 minutes in length, played back to back. The two videos from Session 1 (‘Seth Berkley on HIV and Flu – The Vaccine Strategy’ and ‘Hans Rosling Shows the Best Stats You’ve Ever Seen’) differed from the two videos played during Session 2 (‘Dan Dennett on The Illusion of Consciousness’ and ‘William McDonough on Cradle to Cradle Design’) but the total video time for both sessions was identical (41 minutes 53 seconds). The purpose of these videos was to distract the participants while they ate, in an attempt to engender less mindfulness about their eating.

To eliminate possible inter-brand bias in food selection, all food items provided were from the Whole Foods Market brand (Whole Foods Market, Inc., Austin, TX). Each item was provided in large enough quantities so that item availability was not a limiting factor in consumption volume. Available items were: Butter Toffee Peanuts, Whole Almonds (raw, unsalted), Apple Rings (dried apple slices), Cinnamon and Sugar Glazed Walnuts, Fig Bars, Turkish Apricots, Chocolate Cherry Trail Mix, and Agave Gummy Bears. We aimed to provide participants with food items that ranged in perceived healthfulness. Food containers were weighed before and after the meal sections and measured in grams to the nearest 0.1 g. Participants were not informed that their choice of food was part of the experimental protocol. The foods were placed as a “snack” between cognitive

testing periods and all participants, who had fasted since the previous evening, were told that it was important that they eat before the last cognitive testing section. At the start of the eating opportunity, the exact phrase “*it is better to be closer to full than still hungry*” was used with each participant and during each session. Consumed gram totals were multiplied by individual calorie-per-gram amounts provided on the back of the food packaging to yield total calories consumed per food item.

### **Food Health and Liking Assessment Battery**

The Food Health and Liking Assessment Battery (FH-LAB) was created by our research group for this study. This questionnaire assesses two areas related to food consumption recall and assessment. The first part measures subjective liking of each of the eight food items provided. Two questions are asked per item: (1) Do you like this food item? (2) Do you regularly eat this food item? Responses are collected on a bipolar visual analog scale (100 mm, with 1 mm segmenting). For the former, the ends are anchored by “dislike strongly” (0) and “enjoy maximally” (100). For the latter, the ends are anchored by “never eat it” (0) and “eat this everyday” (100). The second part measures a subjective assessment of the healthfulness of each individual food item on a bipolar visual analog scale (100 mm, with 1 mm segmenting), anchored by “unhealthy” (0) and “most healthy” (100).

We evaluated consumption in several ways. *Experimenter rating.* We divided the eight food options into two categories: ‘good’ and ‘bad.’ The ‘good’ category included the four foods that the experimenters considered more healthful on the 8-item list (apple rings, apricot, almonds, and fig bars). The ‘bad’ category included the four foods that the experimenters estimated less healthful on the 8-item food list (gummy bears, cinnamon-sugar walnut, toffee peanuts, and sweetened trail mix). *Participant rating.* Scores of participant-rated food healthiness were obtained from the FH-LAB. To obtain a healthfulness session value, we took the fraction of calories eaten per item multiplied by the participant’s healthiness rating of that item and summed these per-item values across each of the eight items to get a session health score.

### **Calories consumed based on four distinct decision types**

Food decisions were also evaluated by combining the participants rating of two components of the FH-LAB: liking of each food item and healthfulness of each food item. Participants faced four possible decision types:

1. Low like, low health (lowest temptation)
2. Low like, high health (characteristic of some health decisions)
3. High like, high health (easiest choice)
4. High like, low health (tempting, hedonic-driven choice)

The ratio of liking to health (L:H) was calculated for each food in each participant. We evaluated the relationship between objective and subjective alertness on the various decision types, with highest interest in how alertness influenced decisions in category 4: high like, low health (food choices characterized by a polarization of

low health and high liking, thus a more hedonically-driven choice). On a per participant basis, we determined this by taking the fraction of calories eaten per food option multiplied by the participant's L:H ratio per food.

### **Calories consumed based on caloric density per food item**

To assess caloric consumption based on the caloric density of each food item, we first divided number of grams per serving by calories per serving to yield a caloric density score per item. Each food item was assigned a percentage of total grams consumed within a session. This was multiplied by the caloric density and the sum of each of these (for each of the eight food items) generated a caloric density score for the session.

### **Neurocognitive and Psychological Testing**

We captured information on delayed discounting (Kirby Monetary Choice Questionnaire), mood (Profile of Mood States), and working memory (N-Back Working Memory test). The results of these measures are not reported in this paper.

### **Data Analyses**

We used linear regression, Spearman correlation, t-test, and  $\chi^2$  test to test the relationship between alertness and food. Linear regression and Spearman correlations were calculated using OriginPro (v.8.0891, OriginLab Corporation, Northampton MA), t-tests were calculated using Microsoft Excel (v.12.0.6683.5002, Microsoft Corporation, Redmond WA), and  $\chi^2$  tests were calculated using the java script found on [www.physics.csbsju.edu/stats/contingency.html](http://www.physics.csbsju.edu/stats/contingency.html) (accessed 09-15-14). Outlier detection was done using the Extreme Studentized Deviate, calculated using the java script found on [graphpad.com/quickcalcs/Grubbs1.cfm](http://graphpad.com/quickcalcs/Grubbs1.cfm) (accessed 03-23-15).

### **Results**

All 50 of the individuals who were recruited completed this study, though there are instances of missing data. Of the 50, 10 were men (aged 23-43,  $30 \pm 6.3$  years), 40 were women (aged 21-40,  $27 \pm 4.2$  years), and 10 were smokers, all of whom reported smoking four cigarettes per day or fewer. Among the men, 5 were healthy weight (BMI = 18.5-24.9 kg/m<sup>2</sup>) and 5 were overweight (BMI = 25-29.9 kg/m<sup>2</sup>). Among the women, 29 were healthy weight, 7 were overweight, 1 was obese (BMI > 30 kg/m<sup>2</sup>), and 3 were underweight (BMI < 18.5 kg/m<sup>2</sup>). There was no difference between the BMI of male ( $24.3 \pm 2.69$ ) and female ( $22.4 \pm 3.52$ ) participants ( $p = 0.12$ , t-test), nor was there a sex difference in the categorical BMI distribution ( $p = 0.16$ ,  $\chi^2$  test). At baseline, participants had time in bed (TIB) of  $7.73 \pm 0.875$  hrs (7.33-8.50 hrs, Q1-Q3).

## **Randomization and Sleep Intervention**

On the night immediately prior to Session 2, participants had a TIB of  $6.78 \pm 2.00$  hrs (5.25-8 hrs, Q1-Q3). In comparing the targeted change in TIB to the self-reported TIB preceding Session 2, participants were generally compliant, having an actual TIB within  $0.4\% \pm 12.6\%$  of the assigned TIB. This change in TIB had a concurrent evoked change in both objective and subjective measures of alertness. For objective alertness, mRT during Session 1 was  $260 \text{ ms} \pm 35.2$  (range 192 – 406 ms) and for Session 2 it was  $259 \text{ ms} \pm 35.0$  ms (range 196 – 371 ms) with a change between session of  $-2.6 \text{ ms} \pm 21$  ms (range -61 – 72 ms). For subjective alertness, SSS for Session 1 was  $3 \pm 0$  IQR (range 2 – 6) and for Session 2 it was  $3 \pm 2$  IQR (range 1 – 6) with a change between session of  $0 \pm 2$  IQR (range -4 - 3). Change in TIB was linearly associated with change in scores on the SSS, such that individuals who got less than their usual TIB were less subjectively alert during Session 2 ( $r = -0.45$ ,  $p < 0.01$ , Spearman correlation). Change in TIB was also linearly associated with change in mRT on the PVT such that individuals who got less than their usual TIB had greater mRT (less objectively alert) during Session 2 ( $r = -0.38$ ,  $p < 0.05$ , linear regression).

## **Subjective but not objective alertness associated with calories consumed**

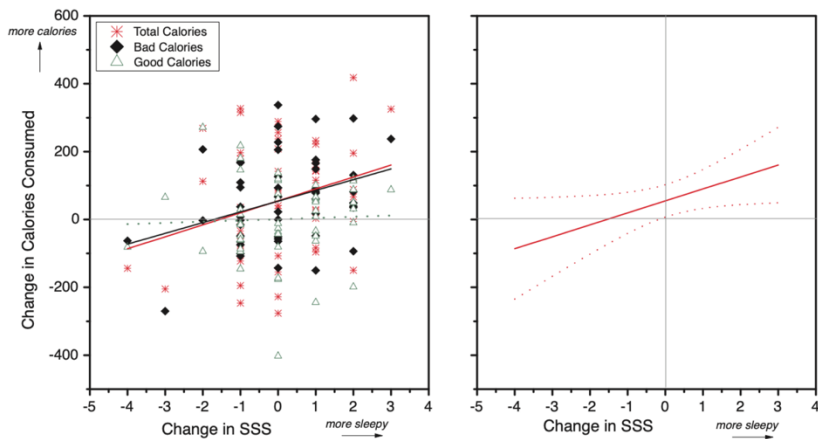
There was a linear relationship between change in subjective alertness and change in total number of calories consumed ( $r = 0.29$ ,  $p < 0.05$ , linear regression) and total number of calories consumed relative to body weight ( $r = 0.31$ ,  $p < 0.05$ , linear regression), such that when subjects were less subjectively alert than during Session 1, they ate more calories (Figure 2). There was, however, no relationship between change in mRT on the PVT and change in the total number of calories consumed ( $p = 0.93$ , linear regression) or mRT and change in total number of calories consumed relative to body weight ( $p = 0.80$ , linear regression).

## **Alertness associated with calories from investigator- or participant- determined 'bad' foods**

We found a positive association between change in subjective alertness and caloric intake from 'bad' choices as defined by the investigators ( $p < 0.05$ , linear regression), such that when individuals were less subjectively alert than during Session 1, they ate more calories from 'bad' choices (Figure 2). We, however, found no linear association between subjective alertness and calories from 'good' choices ( $p = 0.78$ , linear regression), indicating that as subjective alertness changed between Session 1 and Session 2, there was no associated change in the amount of 'good' calories eaten (Figure 2). There was no association between objective alertness (mRT) and either 'bad' ( $p = 0.27$ , linear regression) or 'good' ( $p = 0.26$ , linear regression) choices.

We also found a negative association between subjective alertness and self-rated food healthiness such that when participants rated themselves less alert they chose to eat foods that they rated less healthy ( $p < 0.05$ , linear regression). In addition, we also found a negative association between objective alertness and

self-rated food healthiness such that when participants had higher mRT (less alert), they chose to eat foods that they rated less healthy ( $p < 0.05$ , linear regression).



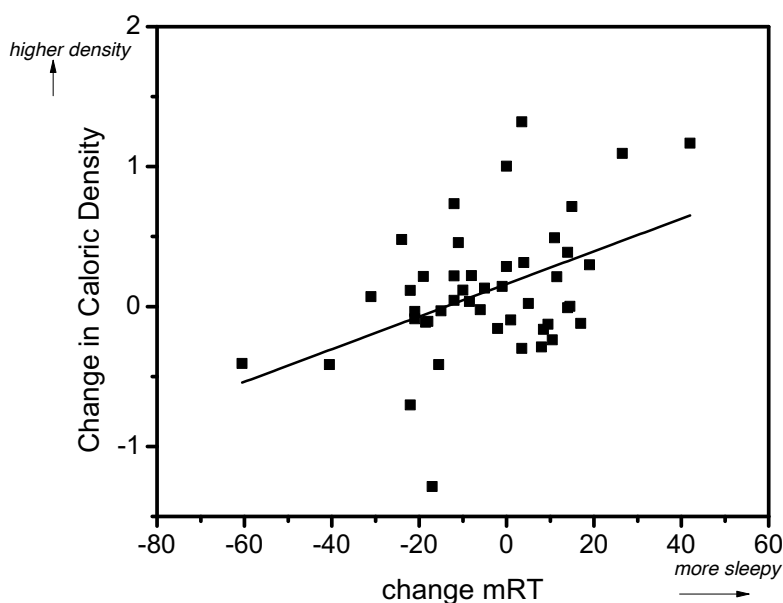
**FIGURE 2.** [Left] Change in total (red), investigator-rated 'bad' (black), and investigator-rated 'good' (green) calories as compared with change in subjective alertness (SSS, Stanford Sleepiness Scale). There was a significant linear relationship between the change in subjective sleepiness and change in both total and bad calories consumed, but no such relationship with good calories consumed. Significant linear fits are indicated by the appropriately colored solid line; the non-significant linear fit is indicated by the dotted colored line. [Right] The relationship between change in total calories and change in objective alertness is re-plotted with 95% confidence intervals (dotted lines). The 95% confidence interval crosses  $y=0$  (no change in calories) for all negative changes in SSS score (i.e., increased alertness) indicating that increased calories are associated with decreased alertness but the converse is not true.

**Calories consumed based on four distinct decision types**

The absolute level of subjective alertness correlated with greater number of calories consumed from 'tempting, hedonic foods' ( $r = -0.23$ ,  $p < 0.05$ , Spearman). We did not see a within-participant difference between sessions.

**Calories consumed based on caloric density per food item**

While changes in caloric density were not associated with changes in subjective alertness ( $r = 0.14$ ,  $p = 0.19$ , linear regression), they were associated with changes in objective alertness such that when subjects had decreases in objective alertness (i.e., higher mRT), they were more likely to eat more calorically-dense foods ( $r = 0.32$ ,  $p < 0.05$ ) (Figure 3).



**FIGURE 3.** Change in caloric density as a function of change in median reaction time (mRT) on the PVT. There was a linear relationship between an increase in mRT, indicating slower responses commensurate with increased sleepiness, and an increase in the caloric density of foods elected to be consumed ( $r = 0.32$ ,  $p < 0.05$ ). A point in the lower right of the graph ( $x = 72$ ,  $y = -0.24$ ) was found to be an outlier (Extreme Studentized Deviate test,  $p < 0.05$ ), but the correlation was stronger ( $p < 0.01$ ,  $r = 0.45$ ) when removed.

## Discussion

This study suggests that reductions in alertness that follow a single night of modest sleep loss have an impact on eating behaviors, and that some of these altered behaviors depend on whether alertness is changed subjectively (i.e., feeling sleepier) or objectively (i.e., reacting more slowly). The subjective feeling of sleepiness correlated with total calories consumed and the caloric intake from foods categorized as ‘bad’ by the investigators and ‘less healthy’ by the participants. Additionally, the absolute level of subjective sleepiness positively correlated with the intake of calories from ‘tempting, hedonic foods.’

These findings suggest several things. First, when a person feels less alert, the hedonic processing of tempting foods may be intensified. Previous work using functional magnetic resonance imaging (fMRI) has observed that one night of total sleep deprivation induced an amplification of subcortical areas that code salience for food decisions, and that these neural changes are associated with a greater desire for caloric density<sup>12</sup>. Similar to our findings, Greer et al.,<sup>12</sup> showed that after

sleep deprivation, subjective sleepiness positively correlated with the percentage of overall calories wanted by participants classified as high-calorie foods. Interestingly, a recent study simulating one night of shift work under experimental conditions demonstrated a similar response: compared to the control condition, shift work participants ate significantly more high-fat breakfast items<sup>36</sup>.

Another possibility is that when people feel less alert, they may relax personal standards resulting in the consumption of foods that they ordinarily may try to avoid or limit. It's been shown that sleep loss promotes effort discounting, which is when a person is less likely to make an effort to gain something he or she values as important or desirable. In our study, this could mean that participants with impaired alertness might have eaten foods they would otherwise avoided if they were more alert<sup>23</sup>. We did not, however, capture information about the participant's eating standards, so we were unable to assess whether choosing 'high like, low health' foods constituted a defection from a person's typical health pattern. This should be studied in future research as it is an important component to the multiplex relationship of sleep, eating, and weight control.

Similar to the effects of decreased subjective alertness, we found a negative association between reaction time and caloric intake related to self-rated food healthiness, such that when participants were less alert, a greater fraction of their caloric intake came from foods they rated as less healthy. While it is possible that the mild sleep deprivation may have altered the *post hoc* assessment of food-option healthiness by participants who had shorter time in bed, such a mild sleep loss is unlikely to reverse a person's characterization of an item<sup>37</sup>. For example, it is unlikely that a person who typically perceives cookies as unhealthy will view them as healthy when mildly sleep deprived. It should be noted, however, that during sleep loss, economic preferences can be altered to produce an 'optimism bias' where individuals become increasingly focused on potential gains and decreasingly focused on potential losses<sup>38</sup>. Translating this finding to health choices and food consumption, a sleep-deprived individual might be more focused on taste and less focused on health consequences of a 'high like, low health' food. Additionally, because mood and optimism can change after sleep loss, it is possible that the food ratings themselves were altered by state to be more or less generous in perceived healthfulness<sup>38</sup>. Future research should evaluate this question directly.

While there was some overlap between the effects of subjective and objective alertness on food intake, there were also differences. When reaction time slowed, participants ate more calories per session from denser food options; an observation unique to objective alertness. This finding is consistent with a recent study by Fang et al.,<sup>39</sup> that showed that compared to the day following baseline sleep, after a night of total sleep deprivation, participants consumed a greater percentage of calories from the more calorically-dense macronutrient (fat) compared to the less calorically-dense macronutrient (carbohydrate). Evaluation by fMRI revealed that after sleep deprivation, brain regions core to the food-salience network positively correlated with the percentage of calories consumed

from fat and negatively correlated with the percentage of calories consumed from carbohydrates. These findings also align with the previously-mentioned Greer et al.,<sup>12</sup> study that showed that the highest calorie foods accrued the largest increase in desirability ratings following sleep loss.

The changes in subjective and objective alertness observed in this study occurred after degrees of sleep loss commonly experienced in modern society<sup>40, 41</sup>. Most previous studies examining the relationship between sleep deprivation and food intake, or appetitive processing, utilized much greater degrees of sleep loss, evoking larger impairments in alertness, but perhaps yielding findings that are not as ecologically relevant. While losing an entire night of sleep, or experiencing sleep curtailment closer to a common sleep-restriction protocol (e.g., only 4-6 hrs of sleep per night for five continuous nights<sup>42</sup>), undoubtedly occurs with unfortunate regularity in modern life, typical sleep curtailment is much less severe<sup>41</sup>. As such, our data are directly relevant to types of changes in food-intake patterns that occur under conditions of modern society and are therefore notably germane to the consistent epidemiological findings inversely connecting sleep loss to weight gain.

It must be noted that while we manipulated the amount of time people spent in bed, we did not objectively record sleep with polysomnography or actigraphy or use the amount of self-reported sleep as the main predictor in our analyses. Rather, we examined the *consequence* of the change in sleep, that is, a change in daytime alertness - and its variability - as our main predicting variable. By not having participants endure an extended, significant curtailment of sleep we limited the change in daytime alertness to a more restricted - likely more typical - range. This probably diminished the statistical power in our correlation analyses, but while previous work has explored the capacity of sleep loss to modify eating behaviors and metabolism, we aimed to probe sensitivity of the system. In other words, by not having dichotomous groups on two ends of the alertness spectrum (i.e., normally alert vs. very tired), we limited our ability to detect correlations between alertness and our food-intake outcomes. Despite this imposed restriction, we did detect significant and meaningful correlations between small changes in alertness - changes typically experienced by normal people day-by-day - and changes in food choices. It should be noted, however, that only worsening of alertness between sessions correlated with the changes in eating behaviors observed as improvements in alertness did not yield changes (Figure 2). Because sleep restricted participants were asked to stay awake past their normal bedtimes on the night prior to Session 2, it is possible that light at night, during the wake extension period, could have caused a small shift in circadian phase. Such a shift could modify eating behavior independent of changes in alertness. It is also important to point out that 80% of our participants are female. Future research should look to confirm these findings in males. Additionally, it would be useful for subsequent investigations into this topic to include objective measurements of sleep so as to further explore the relationship between sleep loss *per se* and eating behaviors.

In Western societies, many people regularly experience varying degrees of sleep-deprivation, and it is common to be surrounded by palatable, energy-dense foods. Regardless of accuracy, people have opinions on the healthfulness of foods, and may be more likely to consume foods recognized as unhealthy after mild sleep curtailment. Because alertness is a measure of the functionality of attentional networks in the central nervous system - and because attention is a requirement of many goal-directed activities - reduced alertness, therefore, could impair health-goal directed behavior related to food choice. The ability to purposefully avoid foods perceived as unhealthy is likely an important factor in maintaining a consistent level of adiposity. Reduced alertness, therefore, is possibly an important contributor to weight gain in our society. Future research should examine whether variability in alertness leads to meaningful net-differences in eating behaviors over extended periods of time.

### **Acknowledgements**

We would like to thank Paul Stowers who helped with data collection and Samara Pardi who assisted with subject scheduling and randomization.

### **Competing Interests**

DP is the founder of humanOS.me, a commercial health technology organization that supports healthy lifestyle practices. He is the majority shareholder of the organization but does not receive a salary from it. The other authors have declared that no competing interests exist.

### **Conflicts of Interest**

The authors report no conflicts of interest.

### **Author Contributions**

Conceived and designed the experiments: DP, JB, GJ, JZ. Performed the experiments: DP. Analyzed the data: DP, JZ, MB. Wrote the paper: DP, JZ, MB, GJL.

### **References**

1. Hanlon EC, Van Cauter E. Quantification of sleep behavior and of its impact on the cross-talk between the brain and peripheral metabolism. *Proc Natl Acad Sci USA*. 2011;108:15609-16.
2. Leproult R, Van Cauter E. Role of sleep and sleep loss in hormonal release and metabolism. *Endo Dev*. 2010;17:11.
3. Van Cauter E, Knutson KL. Sleep and the epidemic of obesity in children and adults. *Eur J Endocrinol*. 2008;159:S59-S66.

4. Kant AK, Graubard BI. Association of self-reported sleep duration with eating behaviors of American adults: NHANES 2005–2010. *Amer J Clin Nutr.* 2014;100:938-47.
5. Markwald RR, Melanson EL, Smith MR, et al. Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain. *Proc Natl Acad Sci USA.* 2013;110:5695-700.
6. Nedeltcheva AV, Kilkus JM, Imperial J, Kasza K, Schoeller DA, Penev PD. Sleep curtailment is accompanied by increased intake of calories from snacks. *Amer J Clin Nutr.* 2009;89:126-33.
7. Brondel L, Romer MA, Nougues PM, Touyarou P, Davenne D. Acute partial sleep deprivation increases food intake in healthy men. *Amer J Clin Nutr.* 2010;91:1550-9.
8. Calvin AD, Carter RE, Adachi T, et al. Effects of experimental sleep restriction on caloric intake and activity energy expenditure. *Chest.* 2013;144:79-86.
9. Spaeth AM, Dinges DF, Goel N. Effects of experimental sleep restriction on weight gain, caloric intake, and meal timing in healthy adults. *Sleep* 2013;36:981-90.
10. Benedict C, Brooks SJ, O'Daly OG, et al. Acute sleep deprivation enhances the brain's response to hedonic food stimuli: an fMRI study. *J Clin Endocrinol Metab.* 2012;97:E443-7.
11. Hogenkamp PS, Nilsson E, Nilsson VC, et al. Acute sleep deprivation increases portion size and affects food choice in young men. *Psychoneuroendocrinol.* 2013;38:1668-74.
12. Greer SM, Goldstein AN, Walker MP. The impact of sleep deprivation on food desire in the human brain. *Nature Comm.* 2013;4:2259.
13. McNeil J, Drapeau V, Gallant AR, Tremblay A, Doucet E, Chaput JP. Short sleep duration is associated with a lower mean satiety quotient in overweight and obese men. *Eur J Clin Nutr.* 2013;67:1328-30.

14. Shechter A, Rising R, Albu JB, St-Onge MP. Experimental sleep curtailment causes wake-dependent increases in 24-h energy expenditure as measured by whole-room indirect calorimetry. *Am J Clin Nutr*. 2013;98:1433-9.
15. Chapman CD, Nilsson EK, Nilsson VC, et al. Acute sleep deprivation increases food purchasing in men. *Obesity (Silver Spring)*. 2013;21:E555-60.
16. Durmer JS, Dinges DF. Neurocognitive consequences of sleep deprivation. *Semin Neurol*. 2005;25:117-29.
17. Doran SM, Van Dongen HP, Dinges DF. Sustained attention performance during sleep deprivation: evidence of state instability. *Arch Ital Biol*. 2001;139:253-67.
18. Wansink B, Sobal J. Mindless eating the 200 daily food decisions we overlook. *Environ Behav*. 2007;39:106-23.
19. Dar-Nimrod I, Cheung BY, Ruby MB, Heine SJ. Can merely learning about obesity genes affect eating behavior? *Appetite*. 2014;81:269-76.
20. Sellayah D, Sikder D. Food for thought: understanding the multifaceted nature of orexins. *Endocrinol*. 2013;154:3990-9.
21. Zheng H, Lenard NR, Shin AC, Berthoud HR. Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals. *Intl J Obesity*. 2009;33 Suppl 2:S8-13.
22. Brobeck JR. Food intake as a mechanism of temperature regulation. *Yale J Biol Medi*. 1948;20:545.
23. Libedinsky C, Massar SA, Ling A, Chee W, Huettel SA, Chee MW. Sleep deprivation alters effort discounting but not delay discounting of monetary rewards. *Sleep*. 2013;36:899-904.

24. Rolls BJ, Roe LS, Kral TV, Meengs JS, Wall DE. Increasing the portion size of a packaged snack increases energy intake in men and women. *Appetite*. 2004;42:63-9.
25. Stroebele N, De Castro JM. Effect of ambience on food intake and food choice. *Nutrition*. 2004;20:821-38.
26. Wansink B, Painter JE, Lee YK. The office candy dish: proximity's influence on estimated and actual consumption. *Intl J Obesity*. 2006;30:871-5.
27. Wansink B, Painter JE, North J. Bottomless bowls: why visual cues of portion size may influence intake. *Obesity Res*. 2005;13:93-100.
28. Wansink B, van Ittersum K, Painter JE. Ice cream illusions bowls, spoons, and self-served portion sizes. *Am J Prev Med*. 2006;31:240-3.
29. Westerterp-Plantenga MS. Effects of extreme environments on food intake in human subjects. *Proc Nutr Soc*. 1999;58:791-8.
30. Polivy J, Herman CP, Hackett R, Kuleshnyk I. The effects of self-attention and public attention on eating in restrained and unrestrained subjects. *J Pers Soc Psychol*. 1986;50:1253-60.
31. Carney CE, Buysse DJ, Ancoli-Israel S, et al. The consensus sleep diary: standardizing prospective sleep self-monitoring. *Sleep*. 2012;35:287-302.
32. Lim J, Dinges DF. Sleep deprivation and vigilant attention. *Ann N Y Acad Sci*. 2008;1129:305-22.
33. Van Dongen HP, Dinges DF. Sleep, circadian rhythms, and psychomotor vigilance. *Clin Sports Med*. 2005;24:237-49, vii-viii.
34. Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC. Quantification of sleepiness: a new approach. *Psychophysiol*. 1973;10:431-6.

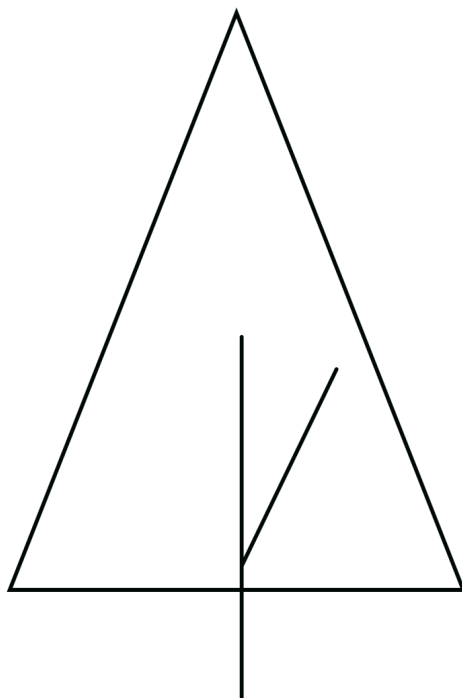
35. Babkoff H, Caspy T, Mikulincer M. Subjective sleepiness ratings: the effects of sleep deprivation, circadian rhythmicity and cognitive performance. *Sleep*. 1991;14:534-9.
36. Cain SW, Filtner AJ, Phillips CL, Anderson C. Enhanced preference for high-fat foods following a simulated night shift. *Scand J Work Environ Health*. 2015.
37. Killgore WD, Kahn-Greene ET, Lipizzi EL, Newman RA, Kamimori GH, Balkin TJ. Sleep deprivation reduces perceived emotional intelligence and constructive thinking skills. *Sleep Med*. 2008;9:517-26.
38. Venkatraman V, Huettel SA, Chuah LY, Payne JW, Chee MW. Sleep deprivation biases the neural mechanisms underlying economic preferences. *J Neurosci*. 2011;31:3712-8.
39. Fang Z, Spaeth AM, Ma N, et al. Altered salience network connectivity predicts macronutrient intake after sleep deprivation. *Sci Rep*. 2015;5:8215.
40. "Sleep in America - Teens and Sleep" poll. 2006 [accessed 2015 March 1st]; Available from: <http://sleepfoundation.org/sleep-polls-data/sleep-in-america-poll/2006-teens-and-sleep>
41. "Sleep in America - Sleep and Pain" poll. 2015 [accessed 2015 March 1st]; Available from: <http://sleepfoundation.org/sleep-polls-data/2015-sleep-and-pain>
42. Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. *J Clin Sleep Med*. 2007;3:519-28.

## **CHAPTER 10**

### **Back to the Future. Metabolic Effects of a 4-Day Outdoor Trip Under Simulated Paleolithic Conditions – New Insights from The Eifel Study**

*Journal of Evolution and Health (2016) 1(1):16.*

**Authors:** Jens Freese, Daniel J. Pardi, Begonia Ruiz-Núñez, Sebastian Schwarz, Regula Heynck, Robert Renner and Philipp Zimmer



**Abstract**

|                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Background</b> | <p>The observation that the emergence of common Western diseases takes place with much greater prevalence as societies migrate from natural-living cultures to modernized societies, has been well documented. For approximately 84,000 generations humans lived under hunter-gatherer conditions but recently endured dramatic change from our native lifestyle with the occurrence of the agricultural, industrial, and digital revolutions. The massive technological advancement that occurred within a relatively recent timeframe enabled humans to live in manner that is remarkably different than our pre-agricultural past. Consequently, the shift from a natural to a modern lifestyle likely promotes a gene-environment mismatch which causes metabolic dysregulation which causes disease.</p> |
| <b>Methods</b>    | <p>Using a within-participant design, we examined whether, compared to baseline, changes in lifestyle towards a more Paleolithic-style pattern, for a four-day and four-night period related to changes in a variety of metabolic parameters. Two groups of 14 volunteers were isolated for a period of four days and four nights in the natural park Südeifel on the borders between Germany and Luxembourg. Participants lived outdoors without tents. The daily hiking performance was 16.4 km (<math>\approx</math> 24963 steps/day) and the daily activity time 5.49 h/day by a mean caloric intake of 1747 kcal/day.</p>                                                                                                                                                                                |
| <b>Results</b>    | <p>After four days of simulated Paleolithic conditions, body weight (-2.9%), body mass index (-2.7%), body fat (-10.4%), visceral fat (-13.6%) and waist-hip-ratio (-2.2%) significantly decreased, while muscle mass significantly increased (+2,3%). Additionally, fasting glucose (-6.5%), basal insulin (-44.4%), homeostasis model assessment-index (-49.3%) and fatty liver index (-41%) significantly dropped. In contrast, C-reactive protein, significantly increased (+67.1%).</p>                                                                                                                                                                                                                                                                                                                  |
| <b>Conclusion</b> | <p>Our study indicates that a short nature trip, where modern humans adjust their behavioral patterns to simulate a more Paleolithic-like condition, could serve as an effective strategy to help prevent or improve modern metabolic disease. Particularly, the major findings of an expeditious reduction of homeostasis model assessment-index and fatty liver index scores in only four days reveal the potential for meaningful benefits with such an intervention, even when compared to the effects of longer-term, single-intervention studies such as dietary or fitness programs on similar metabolic parameters.</p>                                                                                                                                                                               |

## **Background**

The observation that the emergence of common Western diseases (WD) – from obesity to coronary heart disease to cancers [1-3] – takes place with much greater prevalence as societies migrate from natural-living cultures to those that increasingly assume the characteristics of wealthier, modernized societies, has been well documented [4-8]. This is highlighted clearly by, for example, the drastically increased prevalence of obesity and diabetes in recently urbanized vs rural-living indigenous peoples [9, 10]. For instance, in Nauru, since the 1920<sup>s</sup>, royalties for the natural resource phosphate has allowed these people to become one of the world's richest per capita. This wealth, however, has also afforded a rapid change in lifestyle. In this population, the first case of type 2 diabetes (T2D) was noted only in 1925. Now, however, the Nauruans are the world's most obese people (92.8%), have the highest blood pressure in the Western Pacific region, and two-thirds of their population over age 55 suffer from T2D [11, 12].

For approximately 84,000 generations humans lived under hunter and gatherer conditions [13,14] but recently humans have endured dramatic change from their native lifestyle with the occurrence of the agricultural, industrial, and digital revolutions [15-18]. Despite the massive technological innovation that has taken place during these revolutions, they have all occurred within a relatively recent timeframe. These innovations have enabled humans to live in a manner that is discordant with expectancies of our genes, which were largely established during our pre-agricultural past.

The metabolic dysregulation that appears to accompany the rural-modern lifestyle transition is supported by an abundance of evidence indicting elements of the modern lifestyle as causative in WD. These include but are not limited to: overnutrition [19], low dietary fiber intake [20], sugar-rich diet [21], physical inactivity [22], vitamin D deficiency [23], psychosocial stress [24, 25], sleep deprivation and circadian rhythms disturbances [26, 27], and more. Therefore, the shift from a natural to a modern lifestyle likely promotes a gene-environment mismatch [28, 29] which causes metabolic dysregulation which causes disease.

In contrast to single-intervention studies, our study aimed to have participants emulate a modern-day, Paleolithic-like lifestyle pattern during a short nature trip – which included multiple alterations from the default lifestyle pattern of modern living – to assess signs of favorable metabolic changes. We hypothesize that adopting a more Paleolithic-like lifestyle pattern will yield favorable and observable effects on metabolism, even in the short term.

## **Methods**

### **Design**

Using a within-participant design, we examined whether, compared to baseline, changes in lifestyle towards a more natural-living pattern, “Paleolithic-style pattern”, for a four-day and four-night period related to changes in a variety of metabolic parameters. Two groups of 14 volunteers were isolated for a period of

four days and four nights in the natural park Südeifel on the borders between Germany and Luxembourg. The protocol is in accordance with the declaration of Helsinki and was approved by the ethics committee of the German Sports University of Cologne.

### Participants & Recruitment

Participants were recruited from advanced training courses of the German Trainer Academy in Cologne. Eligibility was determined by completion of a pre-admission questionnaire. The pool of participants comprised personal trainers and health professionals who were healthy and non-obese. People were excluded from the study if they were using any medication chronically or had acute injuries or psychiatric disorders. All participants accepted Jens Freese and Sebastian Schwarz as the coordinators of this study and provided written informed consent prior to their participation.

### Baseline

All participants completed a 60-minute on-site introductory seminar about the main principles of our study design and were then randomly divided into two cohorts. The intervention for both cohorts was identical. Each cohort had designated guides who had participated in the pilot study one year before, and were therefore experienced in the procedure, hiking tracks, and region.

### Interventions

#### *Diet*

For the duration of the study, all participants followed a “Paleolithic” diet according to guidelines proposed by Cordain *et al.* [6, 17, 30-33] (Figure 1). The diet included lean meat, fish, eggs, vegetables, fruit, nuts and herbs. Foods were provided in limited quantity. For a complete list of food choices see Table 3. Seasonal, organic foods were bought locally in order to achieve optimal freshness. All processed, packaged foods, and all foods not in accordance with the Cordain guidelines for the Paleolithic diet, were excluded. Water was the only beverage allowed during the study period and was provided *ad libitum*. To quantify the total caloric intake and macronutrient ratios, we applied the USDA Nutrient Interactive Database [34].



**FIGURE 1.** A typical dinner of the intervention group according to the Paleo diet recommendations [6, 17, 31].

*Eating Schedule*

In order to simulate what is estimated to be a more Paleolithic pattern of eating [6, 17, 31] food intake was only twice a day. The timing of the food availability was between noon and before sunset. Hence, each day the participants were provided no breakfast, snacks in the form of nuts and fruit after noon, and one main meal at dinner (Figure 2).

Groups hiked apart from each other, not knowing the direction, times of rest and times of food intake. Every participant carried a small day package of fruits and nuts with the instruction not to eat before noon. The intention of the present study was to guarantee an intermittent fasting period of at least 15 hours a day from last meal of the day to first meal of the next.

Recipes changed day by day. Before cooking, all foods were measured with a digital kitchen scale (Söhnle Food Control Easy, Leifheit AG, Nassau, Germany) by weight (grams). Each afternoon, coordinators prepared dinner in a nearby hotel and delivered the meals to the participants by car. Dinner plates and waste were collected after each meal by one of the study coordinators for disposal.

| Foods Distribution |  |        |              |
|--------------------|--|--------|--------------|
|                    |  |        |              |
| Before Noon        |  | Noon   | Evening      |
|                    |  |        |              |
| Spring Water       |  | Fruits | Paleo Dinner |
|                    |  | Nuts   |              |

**FIGURE 2.** Foods distribution during the intervention.

*Physical Activity and Sleep*

To mimic foraging conditions, participants hiked for four hours each day starting after sunrise. Sleep period occurred during the natural day-night cycle, and morning awakening of participants happened naturally without the use of an alarm clock. Participants were also instructed not to expose themselves to artificial light. For the quantification of the daily hiking distance, we used the portable navigation system Etrex Vista HCX (Garmin International, Olathe, USA). Comprised with a high-sensitive GPS receiver, the group’s position was monitored in their respective environments. A built-in compass was used by the group leaders for navigation in the woods.

Measurement of the participants’ daily energy expenditure and sleep behavior were collected with the SenseWear® armbands (BodyMedia Inc., Pittsburgh, USA). Due to the fact that the individuals within the two groups were together day and night throughout the intervention, we utilized armbands with only two participants to estimate physical activity and sleep for all participants. One male and one female subject wore an armband on the back of the upper left arm, facing upwards

towards the shoulder with the sensors touching the skin. The SenseWear® system records physiological parameters and uses algorithms to report the average daily activity and sleeping duration. Of the two samples, mean values were calculated and divided by the number of days in order to identify the approximate daily average for each measurement. Over the course of the intervention, participants lived outdoors without tents, but were provided tarps to protect participants from moisture at night.

### **Other Components of the Lifestyle Intervention**

We also attempted to simulate non-food and activity-related Paleolithic conditions. As such, we implemented these conditions during the intervention:

- 24 hours in an open air, wooded environment
- Spending time with a tribe of 14 people
- Cut off from technology and modern-style work stress (e.g., notifications from mobile phones, email, time pressure, etc.)
- Exposure to natural 24-hour temperature variability (only modest amount of clothing was allowed; a sleeping bag was made available)

### **Blood Sample Collection and Anthropometric Data**

Blood samples (lithium-heparin and potassium oxalate/sodium fluoride tubes) and EDTA-anticoagulated blood tests were drawn by venipuncture from fasted subjects the first morning and on day four immediately after the morning hike and during fasting conditions. Blood samples were drawn by a medical doctor and drained into three tubes: Sarstedt S-Monovette Serum-Gel (clinical chemistry), Sarstedt S-Monovette Kalium-EDTA (blood panel) and Sarstedt-S-Monovette Natrium-Fluorid (glucose). The tubes were stored in a cooling bag and immediately driven to the Dr. Quade & Kollegen laboratories in Cologne. Complete blood cell-count was analyzed with the XN 2000 Sysmex (Sysmex GmbH, Norderstedt, Germany). Quantitative and proportional analysis of the examined blood components were determined by electric impedance, laser light dispersion and dye binding. Gamma-glutamyltransferase, triglycerides, cholesterol, high density- and low-density lipoprotein were determined with the ADVIA 1800 Siemens (Siemens Healthcare GmbH, Erlangen, Germany) by the IFCC-method. Glucose was measured by the hexokinase reaction. Insulin was analyzed by chemical luminescence immunoassay reaction and high-sensitive C-reactive protein (CRP) by latex-enhanced-immunturbidimetric assay with the ADVIA 1800 Siemens. Body composition was measured by bioelectrical impedance analysis (BIA), using the Tanita Weight Management System MC 780MA S (Tanita Europe B.V, Amsterdam, The Netherlands) and carried out immediately after blood tests. BIA measurements were also drawn on the first and the last day of the intervention. Food was measured by the digital kitchen scale Söhnle Food Control Easy (Leifheit AG, Nassau, Germany). The homeostasis model assessment-index (HOMA) scores were calculated by the laboratory. To determine the fatty liver index (FLI), a diagnostic tool used to estimate the likelihood of fatty liver, we used the calculation method developed by Bedogni et al. [35]

## Statistical Analysis

Statistical analysis of all measurements were made using exploratory student t-tests for dependent samples using Microsoft Excel 2013 and SPSS (version 16.0). P-values shown are uncorrected. For all cases,  $p < 0.05$  was considered statistically significant.

## Results

### *Demographic Profile*

A total of 28 participants were enrolled in the study. Three participants dropped out after one night due to the perceived stress load concerning the study's program design. In total, 25 participants (12 females; 13 males) completed the protocol. Subjects were classified as exercise-trained if they regularly performed three hours per week of anaerobic and aerobic exercise with moderate to high intensity. Collectively, subjects were relatively healthy, and did not currently take any prescription drugs. For additional participant demographics, please see Table 1.

**TABLE 1.** Demographic and anthropometric features of all participants

| Items                                        | Participants    |
|----------------------------------------------|-----------------|
| Race                                         | Caucasian       |
| N                                            | 25              |
| Female                                       | 12 (48%)        |
| Male                                         | 13 (52%)        |
| Age (years)                                  | 40 ( $\pm 13$ ) |
| Normal weight BMI $< 25$ ( $\text{kg m}^2$ ) | 20 (80%)        |
| Overweight BMI $> 25$ ( $\text{kg m}^2$ )    | 5 (20%)         |
| Exercised trained ( $> 3$ h/week)            | 12 (48%)        |
| Not exercised trained ( $< 3$ h/week)        | 13 (52%)        |
| Smoker                                       | 0               |

### *Food and Calorie Profile*

Daily energy intake for participants averaged 1747 kcal/day. The ratio of macronutrient intake during the intervention were calculated to be 26% carbohydrates, 49% fat and 25% protein. The available foods, and the total amount eaten from all participants across the duration of the intervention is shown in Table 3.

**TABLE 2.** Offered and consumed foods over 4 days

| <b>Foods</b>         | <b>Weight (kg)</b> | <b>Kcal</b> | <b>Protein (g)</b> | <b>Fat (g)</b> | <b>Carbo-hydrate (g)</b> |
|----------------------|--------------------|-------------|--------------------|----------------|--------------------------|
| Almond               | 1.5                | 8685        | 317.25             | 748.95         | 323.25                   |
| Apple                | 19.9               | 1040        | 5.2                | 3.4            | 276.2                    |
| Apple Cider Vinegar  | 0.3                | 63          | 0                  | 0              | 2.79                     |
| Apricot              | 4.9                | 2352        | 68.6               | 19.11          | 544.88                   |
| Eggplant             | 3.45               | 1225        | 29.05              | 8.05           | 305.55                   |
| Avocado              | 0.4                | 640         | 8                  | 58.64          | 34.12                    |
| Banana               | 16.13              | 14356       | 175.82             | 53.23          | 3684.09                  |
| Broccoli             | 1.4                | 476         | 2.57               | 5.18           | 92.96                    |
| Canola Oil           | 0.65               | 5693        | 0                  | 644            | 0                        |
| Carrot               | 13.5               | 5535        | 125.5              | 32.4           | 1293.3                   |
| Celery               | 0.6                | 96          | 4.14               | 1.02           | 17.82                    |
| Cashew               | 0.4                | 2212        | 72.88              | 175.4          | 120.76                   |
| Chicken thigh        | 10.12              | 23664       | 2372.52            | 1500.42        | 0                        |
| Cabbage              | 5                  | 1250        | 64                 | 5              | 290                      |
| Dark chocolate       | 0.8                | 4632        | 48.96              | 306.48         | 419.36                   |
| Eggs                 | 100                | 7150        | 625.5              | 473.5          | 35.5                     |
| Ginger               | 0.1                | 80          | 1.82               | 0.75           | 17.77                    |
| Ground Beef          | 6                  | 13800       | 1707               | 722.4          | 0                        |
| Honey                | 0.22               | 1216        | 1.2                | 0              | 329.6                    |
| Leek                 | 2                  | 620         | 16.2               | 4              | 152.4                    |
| Beef, lean, cooked   | 6.28               | 13608       | 1924.02            | 658.98         | 0                        |
| Mushrooms            | 3.6                | 792         | 111.24             | 12.24          | 117.36                   |
| Nut mix              | 4.87               | 24255       | 1568               | 1862           | 539                      |
| Olive Oil            | 0.95               | 7638        | 0                  | 864            | 0                        |
| Onion                | 3.9                | 1560        | 42.9               | 3.9            | 364.26                   |
| Brazil nut           | 0.8                | 5248        | 114.56             | 531.44         | 98.16                    |
| Peach                | 3                  | 1170        | 27.3               | 7.5            | 286.2                    |
| Pepper sweet         | 2.3                | 713         | 22.77              | 6.9            | 138.69                   |
| Radishes             | 3.2                | 512         | 21.76              | 3.2            | 108.8                    |
| Salmon, wild, cooked | 6                  | 11040       | 1641.6             | 450            | 0                        |
| Spinach              | 1                  | 230         | 28.6               | 3.9            | 36.3                     |
| Strawberry           | 1.9                | 608         | 12.73              | 5.7            | 145.92                   |
| Swiss Chard          | 0.35               | 70          | 6.58               | 0.28           | 14.46                    |

|                      |      |                |                    |                |                         |
|----------------------|------|----------------|--------------------|----------------|-------------------------|
| Turnip               | 4.7  | 1316           | 42.3               | 4.7            | 302.21                  |
| Walnut               | 1.27 | 8034           | 312.78             | 767            | 128.83                  |
| Watermelon           | 24   | 7200           | 146.4              | 36             | 1812                    |
| Zucchini, squash     | 4    | 760            | 40.4               | 10.8           | 155.2                   |
| kcal/total (4 days)  |      | <b>179,539</b> |                    |                |                         |
| kcal/person/day      |      | <b>1,747</b>   |                    |                |                         |
| Macronutrient ratios |      |                | <b>Protein 25%</b> | <b>Fat 49%</b> | <b>Carbohydrate 26%</b> |

All foods were measured by weight (grams). To estimate total caloric intake and macronutrient ratios, we used the USDA Nutrient Interactive Database [34].

#### *Physical Activity and Sleep*

Measurements revealed that the participants averaged 5.49 h of physical activity (non-sedentary time), 24,962 steps, and 16.21 km of hiking per day. At night, it is estimated that participants averaged 7.15 h time in bed for sleep.

#### *Anthropometric and Biochemical Measurements*

After 4 days of simulated Paleolithic conditions we found decreased body weight (-2.9 kg), BMI (-2.7%), body fat (-10.4%), visceral fat (-13.6%) and waist/hip-ratio (-2.2%) (see Table 3). Muscle mass increased (+2,3%), although participants performed mainly low impact movement and their daily caloric intake (1747 kcal/day) was above the basal metabolic rate. (Table 4). We also observed decreases in the following metabolic parameters: total cholesterol (-6.1%), LDL/HDL-Quotient (-16.1), fasting glucose (-6,5%), basal insulin (-44.4%), HOMA (-49.3%), FLI (-41%). In contrast to all measured metabolic parameters, CRP, which represents the first immunological response to inflammatory conditions, increased (+67.1%) significantly (see Figures 3-7).

**TABLE 3.** Changes in anthropometric data over the course of the intervention

| Body Composition     | Pre             | Post            | Change           | p        |
|----------------------|-----------------|-----------------|------------------|----------|
| Body Fat (%)         | 20.17 (± 7.38)  | 18.07 (± 7.99)  | -2.10 (- 10.4 %) | < 0.001* |
| Visceral Fat (cm²)   | 4.58 (± 2.9)    | 3.96 (± 2.77)   | -0.63 (- 13.6 %) | < 0.001* |
| Weight (kg)          | 74.55 (± 13.5)  | 72.42 (± 13.01) | -2.13 (- 2.9 %)  | < 0.001* |
| BMI (kg/m²)          | 23.68 (± 2.82)  | 23.03 (± 2.72)  | -0.65 (- 2.7 %)  | < 0.001* |
| Fat Free Mass (kg)   | 59.43 (± 11.77) | 59.35 (± 12.43) | -0.08 (- 0.1 %)  | 0.83     |
| Muscle Mass (%)      | 75.64 (± 6.78)  | 77.83 (± 7.63)  | 2.18 (+2.3 %)    | < 0.001* |
| Total Body Water (%) | 56.73 (± 5.29)  | 58.54 (± 6.06)  | 1.81 (+ 3.2 %)   | < 0.001* |
| Waist (cm)           | 83.67 (± 9.32)  | 81.79 (± 9.22)  | -1.88 (- 2.2 %)  | < 0.001* |
| Waist/Hip-Ratio      | 0.83 (± 0.08)   | 0.814 (± 0.08)  | -0.02 (- 2.2 %)  | < 0.001* |

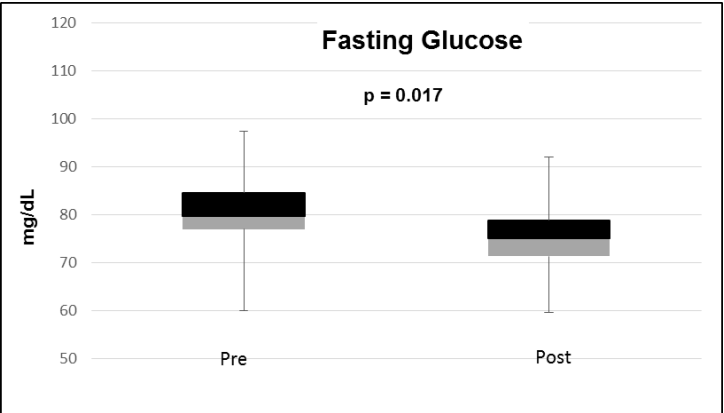
Values represent the mean ± SD. P-values shown are uncorrected. \*Significant difference between the values before and after the intervention. Abbreviations: BMI (body mass index), ICW (intracellular water), ECW (extracellular water)

**TABLE 4.** Changes in biochemical data over the course of the intervention

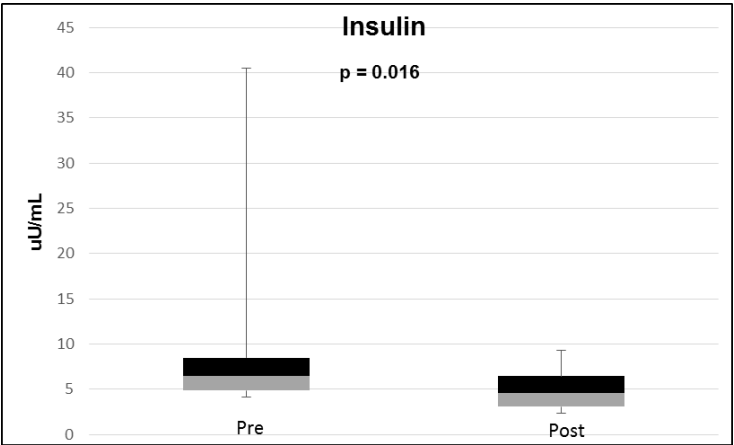
| Biochemical Data          | Pre                   | Post                  | Change            | p           |
|---------------------------|-----------------------|-----------------------|-------------------|-------------|
| Leukocytes (nl)           | 6.11 ( $\pm$ 1.54)    | 5.91 ( $\pm$ 1.49)    | -0.20 (- 3.2 %)   | 0.558       |
| Erythrocytes (PL)         | 4.85 ( $\pm$ 0.36)    | 4.67 ( $\pm$ 0.41)    | -0.18 (- 3.7 %)   | <<br>0.001* |
| Hemoglobin (g/dl)         | 14.82 ( $\pm$ 0.93)   | 14.20 ( $\pm$ 1.02)   | -0.62 (- 4.2%)    | <<br>0.001* |
| Hematocrit (%)            | 42.63 ( $\pm$ 2.47)   | 41.00 ( $\pm$ 2.98)   | -1.63 (- 3.8 %)   | <<br>0.001* |
| MCV (fl)                  | 88.10 ( $\pm$ 3.5)    | 88.00 ( $\pm$ 3.59)   | -0.09 (- 0.1 %)   | 0.729       |
| MCH (pg)                  | 30.61 ( $\pm$ 1.42)   | 30.50 ( $\pm$ 1.45)   | -0.12 (- 0.4 %)   | 0.328       |
| MCHC (g/dl)               | 34.76 ( $\pm$ 0.66)   | 34.65 ( $\pm$ 0.72)   | -0.10 (- 0.3 %)   | 0.289       |
| RDW (%)                   | 12.78 ( $\pm$ 0.68)   | 12.53 ( $\pm$ 0.7)    | -0.25 (- 2 %)     | <<br>0.001* |
| Thrombocytes (nl)         | 240.13 ( $\pm$ 47.34) | 242 ( $\pm$ 48.39)    | 1.88 (+ 0.8 %)    | 0.672       |
| Neutrophils (%)           | 54.83 ( $\pm$ 8.81)   | 58.58 ( $\pm$ 6.69)   | 3.74 (+ 6.8 %)    | 0.031*      |
| Lymphocytes (%)           | 33.27 ( $\pm$ 8.14)   | 29.69 ( $\pm$ 6.46)   | -3.58 (- 10.8 %)  | 0.022*      |
| Monocytes (%)             | 8.54 ( $\pm$ 1.94)    | 9.11 ( $\pm$ 1.79)    | 0.58 (+ 6.8 %)    | 0.088       |
| Eosinophils (%)           | 2.67 ( $\pm$ 1.93)    | 1.93 ( $\pm$ 1.18)    | -0.73 (- 27.5 %)  | 0.014*      |
| Basophils (%)             | 0.70 ( $\pm$ 0.24)    | 0.69 ( $\pm$ 0.29)    | -0.01 (- 1.2 %)   | 0.865       |
| Fasting glucose (mg/dl)   | 80.34 ( $\pm$ 8.84)   | 75.10 ( $\pm$ 7.87)   | -5.25 (- 6.5 %)   | 0.017*      |
| Gamma GT (U/L)            | 20.71 ( $\pm$ 15.22)  | 20.13 ( $\pm$ 12.64)  | -0.58 (- 2.8 %)   | 0.365*      |
| Total cholesterol (mg/dl) | 200.50 ( $\pm$ 32.93) | 188.25 ( $\pm$ 33.21) | -12.25 (- 6.1 %)  | <<br>0.001* |
| HDL (mg/dl)               | 75.57 ( $\pm$ 21.4)   | 78.30 ( $\pm$ 19.83)  | 2.73 (+ 3.6 %)    | 0.056*      |
| Triglycerides (mg/dl)     | 95.63 ( $\pm$ 78.71)  | 49.04 ( $\pm$ 18.7)   | -46.58 (- 48.7 %) | 0.004*      |
| LDL (mg/dl)               | 113.11 ( $\pm$ 28.28) | 101.53 ( $\pm$ 23.43) | -11.58 (- 10.2 %) | <<br>0.001* |
| LDL/HDL-Quotient          | 1.61 ( $\pm$ 0.58)    | 1.36 ( $\pm$ 0.4)     | -0.24 (- 15.1 %)  | 0.002*      |
| CRP high sensitive (mg/l) | 1.334 ( $\pm$ 2.62)   | 2.23 ( $\pm$ 2.96)    | 0.895 (+ 67.1%)   | 0.121       |
| Insulin (uU/ml)           | 8.943 ( $\pm$ 7.52)   | 4.97 ( $\pm$ 2.11)    | -3.97 (- 44.4 %)  | 0.016*      |
| HOMA-Index                | 1.847 ( $\pm$ 1.83)   | 0.94 ( $\pm$ 0.46)    | -0.91 (- 49.3 %)  | 0.022*      |
| Fatty Liver Index         | 20.51 ( $\pm$ 23.9)   | 12.10 ( $\pm$ 15.3)   | -8.41 (- 41 %)    | <<br>0.001* |

Values represent the mean  $\pm$  SD. P-values shown are uncorrected. \*Significant difference between the values before and after the intervention. Abbreviations: MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red blood cell distribution), Gamma GT (gamma-glutamyl transpeptidase)

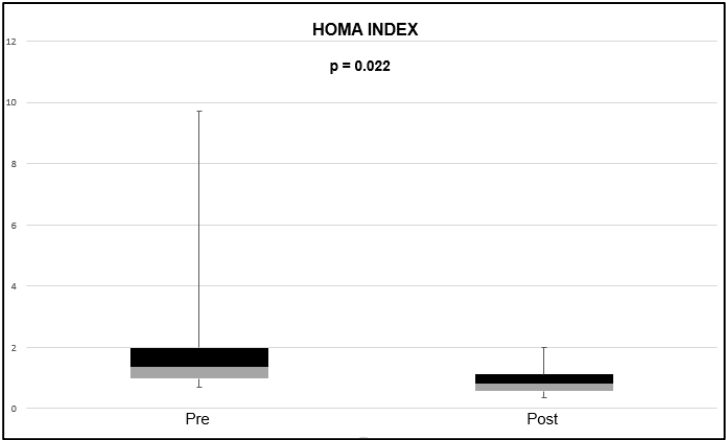
**FIGURE 3.** Boxplots showing fasting glucose at baseline (Pre) and post intervention (Post).



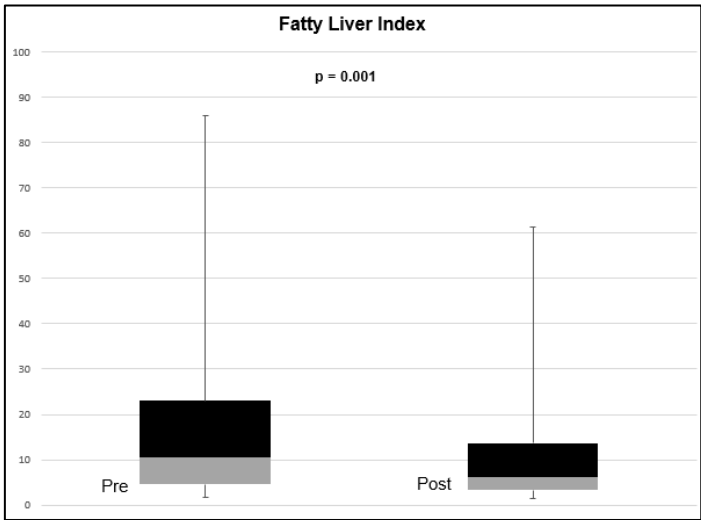
**FIGURE 4.** Boxplots showing insulin at baseline (Pre) and post intervention (Post).



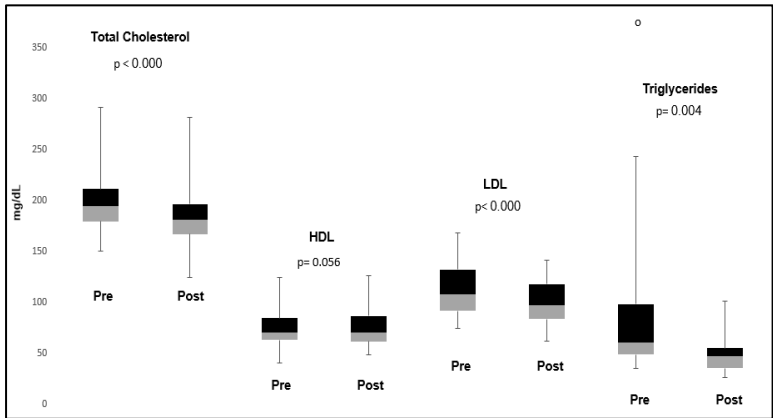
**FIGURE 5.** Boxplots showing HOMA-Index at baseline (Pre) and post intervention (Post).



**FIGURE 6.** Boxplots showing Fatty Liver Index at baseline (Pre) and post intervention (Post).



**FIGURE 7.** Boxplots showing total cholesterol, HDL, LDL and triglycerides at baseline (Pre) and post intervention (Post).



## Discussion

The aim of our study was to investigate if a 4-day period simulating a modern day version of Paleolithic conditions has the potential to demonstrate signs of favorable metabolic and inflammatory effects on already healthy and fit adults. In 2012, the first author of this paper followed a hunter and gatherer-type lifestyle over 10 days in the Spanish Pyrenees. Afterwards, our study group initiated the current Eifel study starting with a small pilot group in 2013 [36] and followed by this larger 25-person cohort in this follow-up study presented here. For this study, we estimated that the 10-day intervention performed by Freese in 2012 would be too demanding for sedentary modern people not acclimated to living in this manner. Additionally, considering that the Asian forest bath studies [37-40] impressively impacted several health markers after short hiking trips in woodlands compared to walks in a city, we estimated that favorable effects on metabolic and inflammation parameters would be observable after a shorter intervention period.

Although humans' metabolic system depends on a sufficient replenishment of macro- and micronutrients, it is flexible to produce sufficient energy under multiple environmental circumstances. The range of challenges to produce sufficient energy include everything from seasonal abundance and deprivation of food(s), to even sustaining of energy over long journeys without food to the rapid production of energy to escape predators [41, 42]. Hence, over millennia, humans evolved to develop metabolic flexibility to survive the variety of situations likely faced over the span of time required to successfully reproduce.

The organ demanding the most protection from metabolism is the human brain [43, 44]. Neurons, however, do not store energy substrates but do show an extraordinary flexibility in producing energy from a variety of substrates including glucose, their primary fuel source, ketone bodies, and lactate. This flexibility guarantees adequate energy provision in the face of fluctuating environment and physiological circumstances. Thus, the human brain does not depend on continuous fueling with a single source of fuel.

Today, overnutrition is a major issue affecting health [19, 45]. Due to the learned ability of humans to create foods that leverage the preferences of our central nervous system', we are not only constantly surrounded by food in our modern environment, but we are also surrounded by foods that encourage us to eat in the absence of hunger [46]. Therefore, the obesogenic environment of modern humans has led to, among other things, an internal condition with impaired capability to allocate energy from alternative fuel sources other than glucose, especially for the needs of neurons. This is due to the fact that these alternative energy systems are rarely, if ever, required to be the dominant source of energy provisions [47, 48]. In affluent societies, food is not only consumed in order to maintain energy balance but also for hedonic attributes independent of energy status, and this might be one of the reasons for calorie excess [49, 50]. This situation is then compounded by prevalent physical inactivity [22, 51] and sleep disturbance [52], all combining to lead to severe metabolic disturbances such as metabolic syndrome and T2D mellitus over time [21, 53].

**Calories**

It has been hypothesized that human body composition and selected metabolic parameters are able to return to a more ancestral-like state, when diet and lifestyle resemble hunter-gatherer conditions. More than 30 years ago, O’Dea *et al.* showed that returning diabetic, urbanized Aboriginal Australians [41] to their natural habitat for 7-weeks of a hunter-gatherer lifestyle could normalize glucose metabolism and reverse insulin resistance. The favorable health outcomes of that study, however, were likely impacted by the substantial negative energy balance observed in participants making it hard to determine if it was the lifestyle, the caloric restriction, or both that were the cause of the metabolic improvements.

In our study, participants were asked to imitate life as a hunter-gatherer. With this goal in mind, our protocol implemented a daily shorted eating window (i.e., intermittent fast) allowing only two meals per day. All provided foods complied with the tenets of the Paleo diet offered by Cordain and colleagues [6, 17, 31]. Physical activity, most of which was conducted under fasting conditions, also aimed to emulate the amount, intensity, and modality of hunter-gatherers [14, 54]. Lastly, the environment with which the study was conducted was also considered natural. The participants were kept in the wild, observing plants and animals, and exposed to the natural vicissitudes of environmental light and temperature. Modern day technologies that minimize the fluctuations in these natural signals – such as sunglasses during the day, artificial light at night, a range thermo-neutralizing clothing – were also excluded or minimized (Figure 3).

**TABLE 6.** Simulated Paleolithic conditions over the course of the 4-day intervention

| Conditions    | Activities                           |
|---------------|--------------------------------------|
| Physical      | Hiking                               |
|               | Swimming in a river                  |
|               | Climbing                             |
|               | Collecting and lifting wood          |
| External      | Reduced calories                     |
|               | Less frequent food intake            |
|               | Intense sunlight                     |
|               | Exposure to bugs                     |
|               | High temperatures during the day     |
|               | Cool temperatures morning and nights |
| Physiological | Thirst                               |
|               | Hungry                               |
|               | Sweating and freezing                |
|               | Aching muscles                       |
| Other         | Building a fire                      |

|  |                                   |
|--|-----------------------------------|
|  | Group socializing                 |
|  | Searching for suitable night camp |
|  | Orientating in the forest         |
|  | Looking for wild foods            |
|  | Watching wildlife                 |
|  | Sunbathing                        |

In our study, the average daily food allotment per person per day was 1,747 kcal. Because the participants in our study were given a definitive amount of food, and because of the high degree of physical activity per day, we do not know how much food these people would have eaten if food was provided ad libitum. Thus, like the O’Dea study mentioned above, our study suffered from the same limitation of not knowing whether our findings were a results of negative calorie balance or other aspects of the lifestyle intervention we implemented. Additionally, it is likely that our daily calorie total is artificially high. As mentioned, 28 participants were originally enrolled but only 25 participants completed the protocol. Daily calorie intake, however, was estimated based on the total amount of food provided to participants over the four day – without the benefit of any food not consumed being factored into the calculation – divided by the number of participants who completed the study. The three participants who dropped out did consume one daily fruit pack and one dinner before dropping out. Those calories have been factored into our equation for average daily energy intake per participant.

### **Body Measurements**

The average change in body fat was -10.4% and the average change in visceral fat was -13.6%. While the changes in these measurements are impressive, it is critical to note that our methods of measuring both parameters, bioelectrical impedance, is subject to large fluctuations depending upon hydration status and is generally not considered accurate when compared to gold standard measurement methods. It is also likely that our subjects were less hydrated after the four-day intervention which would explain some or all change in both parameters.

We also observed a 48.7% reduction in triglycerides and a 41% reduction in the FLI. Given that 10-35% of Western people suffer from a non-alcoholic fatty liver disease [55], the remarkable reduction of the triglyceride and FLI in only four days intrigues us that brief outdoor trips based on humans’ primal behavior patterns, should be investigated further to help the spreading metabolic epidemic.

### **Insulin**

Previous research by Lindeberg observed a lower mean insulin (-50%) concentration in primal living Kitava islanders aged between 50-74 years when compared to a sample of age-matched Swedish people characterized by a typical Western lifestyle pattern [15]. In line with those findings, our data show a 44.4% decrease in mean fasting insulin from baseline to the end of the intervention (from

8.94 ± 7.52 uU/ml to 4.97 ± 2.11 uU/ml;  $p=0.016$ ), as well as a 6.5% decrease in mean fasting glucose. Together, this led to a remarkable reduction of HOMA (-49.3%; from 1.85 ± 1.83 to 0.94 ± 0.46;  $p=0.022$ ). Also notable is the reduced variance in the post-intervention values, compared to baseline, for both fasting insulin and HOMA scores. This reduction in value variance indicates that most of the people in the study clustered around the lower, presumably healthier values for both measures in response to the intervention. Similarly, a study by Frassetto et al. [55] in people following a Paleolithic diet for three days also showed markedly reduced mean values and reduced variance in response to the intervention for both fasting insulin and HOMA. In the light of a worldwide expanding T2D epidemic, it is striking that such a short intervention presented here, even with healthy subjects, has yielded comparable figures to the findings by Lindeberg in the natural habitat and Frassetto in a clinical setting [15, 55].

## Inflammation

Intriguingly, the acute phase protein CRP increased by 67.1%. This protein is produced by the liver in response to elevated concentrations of interleukin-6 which is distributed by macrophages and adipocytes in order to orchestrate pathogen-induced inflammation, among other functions. There are several plausible explanations for this large increase in this inflammatory signal.

The radical change from a more sterile modern environment into a wild habitat – replete with bacteria, parasites, fungi, and phytoncides (wood essential oils) – could have stimulated a response of the innate immune system [36, 57, 58], possibly explaining the large increase in CRP observed in our study. Recently, a study by Gurven et al. [59] of the Tsimane, a Bolivian tribe in the Amazon, showed that these forager-horticulturalists have a level of white blood cells that is ten times the level of the US population. This is unsurprising given the fact that approximately 70% of the Tsimane people are infected by parasitic helminthes. We did not test our participants for parasitic infections, but these infections are rare in our study population. Future studies should evaluate whether people who are exposed to nature on a regular basis show lower inflammatory reactions during a nature trip similar to what was used in our study. Indeed, evidence suggests that those who take regular walks in forested areas show reduced inflammatory cytokines [39].

Despite the fact that our participants were healthy and fit, it is possible that the amount and type of physical work – mostly under fasting conditions – could have stimulated a significant stress response and cell damage. Animals in starvation or under severe stress load show elevated uric acid levels [60] – a by-product of cell destruction – which can promote the release of CRP as a part of an acute immune response [61-63], which further stimulates the production of antibodies from B-lymphocytes to fight against pathogens [64]. This stress marker has also been shown to increase after long-distance runs [65, 66]. While our study did not measure uric acid levels, future studies should monitor this marker directly and do a correlation analysis with it and CRP.

## Conclusion

At this stage, our case and pilot studies indicate that there could be a positive health effect during a short-term implementation of a Paleo-like nature trip. We believe our findings justify more advanced investigations into this method to influence health. Future studies should aim to use better controls to investigate several things. First, what aspects of this lifestyle, if any, are most impactful in promoting health? Second, are the effects of a short-term Paleo-like nature trip durable? Third, are there meaningful side effects to such an intervention, and what populations might benefit most? Fourth, what is the ideal time number of days need to maximize the benefit of this style of health intervention?

Different components of a lifestyle pattern – food, physical activity, sleep, etc. – all independently influence the internal metabolic milieu. Assessing attribution of potential effects from a multifactorial health program, given the complexities of eliminating confounds, does generate important questions as to what factor, factors or combination of factors is having an influence. We must acknowledge, however, that potential synergies exist in improving several health influences simultaneously, even when it's hard to determine why. Additionally, focusing on the net impact of a dynamic lifestyle pattern on health and disease - even if its implementation is only short term, like with our protocol – still has great merit. This study indicates that a short trip, where modern humans adjust their behavioral patterns to simulate a more Paleolithic-like condition, could serve as an effective strategy to help prevent the dangers of metabolic diseases. Particularly, the major findings of an expeditious reduction of HOMA and FLI in only four days may reveal the potential of exceptional benefits even when compared to long-term, single interventions such as dietary or fitness programs.

## Abbreviations

BIA: bioelectrical impedance analysis; CRP: High-sensitive C-reactive protein; FLI: Fatty liver index; HOMA: Homeostasis model assessment-index; T2D: Type 2 diabetes; WD: Western diseases.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## Authors' contributions

JF conceived and designed the experiment. JF, SS performed the experiment. JF, RH, BR analyzed the data and performed statistical analysis. JF and DP wrote the manuscript. All authors read and approved the final manuscript.

## References

1. Sieck, G. (2014). Physiology in perspective: The burden of obesity. *Physiology (Bethesda, Md.)*, 29(2), 86-7. doi:10.1152/physiol.00004.2014
2. Rodríguez-Hernández, H., Simental-Mendía, L. E., Rodríguez-Ramírez, G., Reyes-Romero, M. A. (2013). Obesity and inflammation: Epidemiology, risk

- factors, and markers of inflammation. *International Journal of Endocrinology*, 2013, 678159. doi:10.1155/2013/678159
3. Grant, R. W., Dixit, V. D. (2015). Adipose tissue as an immunological organ. *Obesity (Silver Spring, Md.)*. doi:10.1002/oby.21003
  4. Schulz, L. O., Bennett, P. H., Ravussin, E., Kidd, J. R., Kidd, K. K., Esparza, J., Valencia, M. E. (2006). Effects of traditional and western environments on prevalence of type 2 diabetes in Pima Indians in Mexico and the U.S. *Diabetes Care*, 29(8), 1866-71. doi:10.2337/dc06-0138
  5. Lindeberg, S. (2010). *Food and Western disease health and nutrition from an evolutionary perspective*. Oxford; Ames, Iowa: Wiley-Blackwell.
  6. Lindeberg, S. (2012). Paleolithic diets as a model for prevention and treatment of western disease. *American Journal of Human Biology: The Official Journal of the Human Biology Council*, 24(2), 110-5. doi:10.1002/ajhb.22218
  7. Egger, G., Dixon, J. (2011). Non-nutrient causes of low-grade, systemic inflammation: Support for a 'canary in the mineshaft' view of obesity in chronic disease. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity*, 12(5), 339-45. doi:10.1111/j.1467-789X.2010.00795.x
  8. Esparza-Romero, J., Valencia, M. E., Urquidez-Romero, R., Chaudhari, L. S., Hanson, R. L., Knowler, W. C., Schulz, L. O. (2015). Environmentally driven increases in type 2 diabetes and obesity in Pima Indians and non-Pimas in Mexico over a 15-year period: The Maycoba Project. *Diabetes Care*, 38(11), 2075-82. doi:10.2337/dc15-0089
  9. Foliaki, S., Pearce, N. (2003). Prevalence and causes of diabetes in pacific people. *Pacific Health Dialog*, 10(2), 90-98.
  10. Celis-Morales, C. A., Perez-Bravo, F., Ibañez, L., Sanzana, R., Hormazabal, E., Ulloa, N., Gill, J. M. (2011). Insulin resistance in Chileans of European and indigenous descent: Evidence for an ethnicity x environment interaction. *PLoS ONE*, 6(9), e24690. doi:10.1371/journal.pone.0024690
  11. WHO: Obesity and Overweight. Fact sheet N°311 (updated January 2015). [www.who.int/mediacentre/factsheets/fs311/en](http://www.who.int/mediacentre/factsheets/fs311/en)].
  12. Chan, J. C., Cho, N. H., Tajima, N., Shaw, J. (2014). Diabetes in the Western pacific region - past, present and future. *Diabetes Research and Clinical Practice*, 103(2), 244-55. doi:10.1016/j.diabres.2013.11.012
  13. Fenner, J. N. (2005). Cross-cultural estimation of the human generation

- interval for use in genetics-based population divergence studies. *American Journal of Physical Anthropology*, 128(2), 415-23. doi:10.1002/ajpa.20188
14. O'Keefe, J. H., Vogel, R., Lavie, C. J., Cordain, L. (2010). Organic fitness: Physical activity consistent with our hunter-gatherer heritage. *The Physician and Sports Medicine*, 38(4), 11-8. doi:10.3810/psm.2010.12.1820
  15. Lindeberg, S., Eliasson, M., Lindahl, B., Åhrén, B. (1999). Low serum insulin in traditional pacific islanders – the Kitava study. *Metabolism: Clinical and Experimental*, 48(10), 1216-1219.
  16. Marlowe, F. (2002). Why the Hadza are still hunter-gatherers. *Ethnicity, Huntergatherers, and the Other*, Ed. S. Kent, 247-81. Smithsonian Institution Press, Washington D.C.
  17. Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., Brand-Miller, J. (2005). Origins and evolution of the western diet: Health implications for the 21st century. *The American Journal of Clinical Nutrition*, 81(2), 341-54.
  18. Pontzer, H., Raichlen, D. A., Wood, B. M., Mabulla, A. Z., Racette, S. B., Marlowe, F. W. (2012). Hunter-gatherer energetics and human obesity. *PLoS ONE*, 7(7), e40503. doi:10.1371/journal.pone.0040503
  19. Cai, D. (2013). Neuroinflammation and neurodegeneration in overnutrition-induced diseases. *Trends in Endocrinology and Metabolism: TEM*, 24(1), 40-7. doi:10.1016/j.tem.2012.11.003
  20. Carrera-Bastos, P., Fontes, O'Keefe, Lindeberg, Cordain. (2011). The western diet and lifestyle and diseases of civilization. *Research Reports in Clinical Cardiology*, 15. doi:10.2147/RRCC.S16919
  21. Bremer, A. A., Mietus-Snyder, M., Lustig, R. H. (2012). Toward a unifying hypothesis of metabolic syndrome. *Pediatrics*, 129(3), 557-70. doi:10.1542/peds.2011-2912
  22. Lee, I., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N., Katzmarzyk, P. T. (2012). Effect of physical inactivity on major non-communicable diseases worldwide: An analysis of burden of disease and life expectancy. *Lancet*, 380(9838), 219-229. doi:10.1016/s0140-6736(12)61031-9
  23. Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine*, 357(3), 266-281.
  24. Hamer, M., Stamatakis, E. (2008). Inflammation as an intermediate pathway in the association between psychosocial stress and obesity. *Physiology & Behavior*, 94(4), 536-9. doi:10.1016/j.physbeh.2008.03.010

25. Schmidt, D., Reber, S. O., Botteron, C., Barth, T., Peterlik, D., Uschold, N., Lechner, A. (2010). Chronic psychosocial stress promotes systemic immune activation and the development of inflammatory the cell responses. *Brain, Behavior, and Immunity*, 24(7), 1097-104. doi:10.1016/j.bbi.2010.04.014
26. Cizza, G., Requena, M., Galli, G., de Jonge, L. (2011). Chronic sleep deprivation and seasonality: Implications for the obesity epidemic. *Journal of Endocrinological Investigation*, 34(10), 793-800. doi:10.3275/7808
27. Challet, E. (2013). Circadian clocks, food intake, and metabolism. *Progress in Molecular Biology and Translational Science*, 119, 105-35. doi:10.1016/B978-0-12-396971-2.00005-1
28. Nesse, R. M., Ganten, D., Gregory, T. R., Omenn, G. S. (2012). Evolutionary molecular medicine. *Journal of Molecular Medicine (Berlin, Germany)*, 90(5), 509-22. doi:10.1007/s00109-012-0889-9
29. Varki, A. (2012). Nothing in medicine makes sense, except in the light of evolution. *Journal of Molecular Medicine (Berlin, Germany)*, 90(5), 481-94. doi:10.1007/s00109-012-0900-5
30. Eaton, S. (1995). An evolutionary perspective enhances understanding of human nutritional requirements. *Evolutionary Perspectives of Human Nutrition*.
31. Eaton, B. S., Cordain, L. (1997). Evolutionary aspects of diet: Old genes, new fuels. In *Nutrition and fitness: Evolutionary aspects, children's health, programs and policies world rev nutr diet* (Vol. 81, pp. 26-37). Karger, Basel.
32. Osterdahl, M., Kocturk, T., Koochek, A., Wändell, P. E. (2008). Effects of a short-term intervention with a paleolithic diet in healthy volunteers. *European Journal of Clinical Nutrition*, 62(5), 682-5. doi:10.1038/sj.ejcn.1602790
33. Frassetto, L. A., Schloetter, M., Mietus-Synder, M., Morris, R. C., Sebastian, A. (2009). Metabolic and physiologic improvements from consuming a paleolithic, hunter-gatherer type diet. *European Journal of Clinical Nutrition*, 63(8), 947-55. doi:10.1038/ejcn.2009.4
34. National Nutrient Database for Standard Reference, 2013, Release 26, USDA, Agricultural Research Service United States Department of Agriculture, <http://ndb.nal.usda.gov/ndb/foods> on August, 27.2014
35. Bedogni, G., Bellentani, S., Miglioli, L., Masutti, F., Passalacqua, M., Castiglione, A., Tiribelli, C. (2006). The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol*, 6, 33. doi:10.1186/1471-230X-6-33

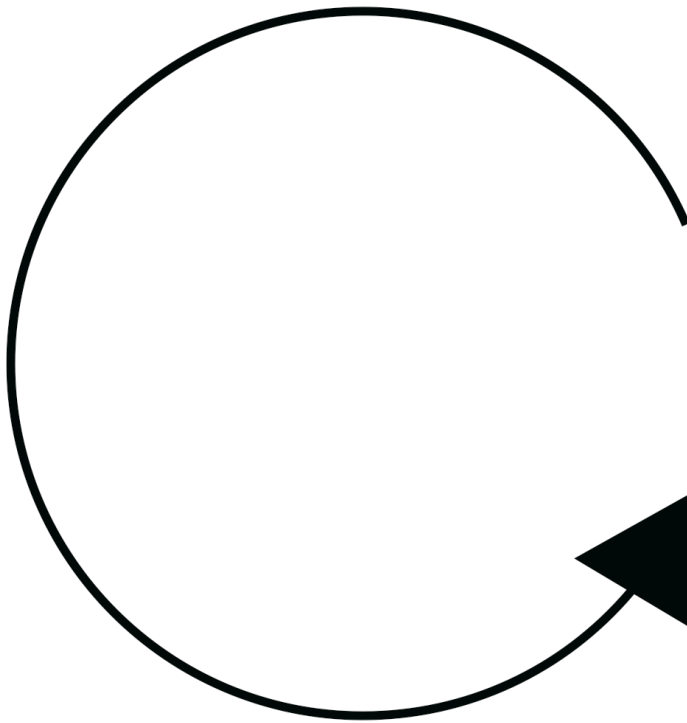
36. Freese J, Ruiz-Núñez B, Heynck R, Schwarz S, Pruimboom L, Renner R, Lötzerich H. To restore health, “do we have to go back to the future?” The impact of a 4-day Paleolithic lifestyle change on human metabolism – a pilot study. *Journal of Evolution and Health* 2016, Mar 2;1(1).
37. Li, Q. (2010). Effect of forest bathing trips on human immune function. *Environmental Health and Preventive Medicine*, 15(1), 9-17. doi:10.1007/s12199-008-0068-3
38. Li, Q., Otsuka, T., Kobayashi, M., Wakayama, Y., Inagaki, H., Katsumata, M., Kagawa, T. (2011). Acute effects of walking in forest environments on cardiovascular and metabolic parameters. *European Journal of Applied Physiology*, 111(11), 2845-53. doi:10.1007/s00421-011-1918-z
39. Park, B. J., Tsunetsugu, Y., Kasetani, T., Kagawa, T., Miyazaki, Y. (2010). The physiological effects of shinrin-yoku (taking in the forest atmosphere or forest bathing): Evidence from field experiments in 24 forests across japan. *Environmental Health and Preventive Medicine*, 15(1), 18-26. doi:10.1007/s12199-009-0086-9
40. Mao, G. X., Lan, X. G., Cao, Y. B., Chen, Z. M., He, Z. H., Lv, Y. D., Yan, J. (2012). Effects of short-term forest bathing on human health in a broad-leaved evergreen forest in Zhejiang province, china. *Biomedical and Environmental Sciences*, 25(3), 317-324.
41. O'dea, K. (1984). Marked improvement in carbohydrate and lipid metabolism in diabetic Australian aborigines after temporary reversion to traditional lifestyle. *Diabetes*, 33(6), 596-603.
42. Wells, J. C. (2006). The evolution of human fatness and susceptibility to obesity: An ethological approach. *Biological Reviews of the Cambridge Philosophical Society*, 81(2), 183-205. doi:10.1017/S1464793105006974
43. Peters, A., Schweiger, U., Pellerin, L., Hubold, C., Oltmanns, K. M., Conrad, M., Fehm, H. L. (2004). The selfish brain: Competition for energy resources. *Neuroscience and Biobehavioral Reviews*, 28(2), 143-80. doi:10.1016/j.neubiorev.2004.03.002
44. Peters, A., Langemann, D. (2009). Build-ups in the supply chain of the brain: On the neuroenergetic cause of obesity and type 2 diabetes mellitus. *Frontiers in Neuroenergetics*, 1, 2. doi:10.3389/neuro.14.002.2009
45. Chopra, M., Galbraith, S., Darnton-Hill, I. (2002). A global response to a global problem: The epidemic of overnutrition. *Bulletin of the World Health Organization*, 80(12), 952-8.

46. Alsiö, J., Olszewski, P. K., Levine, A. S., Schiöth, H. B. (2012). Feed-forward mechanisms: Addiction-like behavioral and molecular adaptations in overeating. *Frontiers in Neuroendocrinology*, 33(2), 127-39.
47. Hitze, B., Hubold, C., van Dyken, R., Schlichting, K., Lehnert, H., Entringer, S., Peters, A. (2010). How the selfish brain organizes its supply and demand. *Frontiers in Neuroenergetics*, 2, 7. doi:10.3389/fnene.2010.00007
48. Klement, J., Hubold, C., Cords, H., Oltmanns, K. M., Hallschmid, M., Born, J., Peters, A. (2010). High-calorie glucose-rich food attenuates neuroglycopenic symptoms in patients with addison's disease. *The Journal of Clinical Endocrinology and Metabolism*, 95(2), 522-8. doi:10.1210/jc.2009-1752
49. Adam, T. C., Epel, E. S. (2007). Stress, eating and the reward system. *Physiology & Behavior*, 91(4), 449-58. doi:10.1016/j.physbeh.2007.04.011doi:10.1016/j.yfrne.2012.01.002
50. Kenny, P. J. (2011). Reward mechanisms in obesity: New insights and future directions. *Neuron*, 69(4), 664-79. doi:10.1016/j.neuron.2011.02.016
51. Hamilton, M. T., Hamilton, D. G., Zderic, T. W. (2014). Sedentary behavior as a mediator of type 2 diabetes. *Medicine and Sport Science*, 60, 11-26. doi:10.1159/000357332
52. Hirotsu, C., Tufik, S., Andersen, M. L. (2015). Interactions between sleep, stress, and metabolism: From physiological to pathological conditions. *Sleep Sci*, 8(3), 143-52. doi:10.1016/j.slsi.2015.09.002
53. Fulop, T., Tessier, D., Carpentier, A. (2006). The metabolic syndrome. *Pathologie-biologie*, 54(7), 375-86. doi:10.1016/j.patbio.2006.07.002
54. O'Keefe, J. H., Vogel, R., Lavie, C. J., Cordain, L. (2011). Exercise like a hunter-gatherer: A prescription for organic physical fitness. *Progress in Cardiovascular Diseases*, 53(6), 471-9. doi:10.1016/j.pcad.2011.03.009
55. Frassetto, L. A., Schloetter, M., Mietus-Synder, M., Morris, R. C., Sebastian, A. (2009). Metabolic and physiologic improvements from consuming a paleolithic, hunter-gatherer type diet. *European Journal of Clinical Nutrition*, 63(8), 947-55. doi:10.1038/ejcn.2009.4
56. Marino, L., Jornayvaz, F. R. (2015). Endocrine causes of nonalcoholic fatty liver disease. *World Journal of Gastroenterology*, 21(39), 11053-76. doi:10.3748/wjg.v21.i39.11053
57. Ruiz-Núñez, B., Pruimboom, L., Dijck-Brouwer, D. A., Muskiet, F. A. (2013). Lifestyle and nutritional imbalances associated with western diseases: Causes and consequences of chronic systemic low-grade inflammation in an

- evolutionary context. *The Journal of Nutritional Biochemistry*, 24(7), 1183-201. doi:10.1016/j.jnutbio.2013.02.009
58. Gurven, M. D., Trumble, B. C., Stieglitz, J., Yetish, G., Cummings, D., Blackwell, A. D., Pontzer, H. (2016). High resting metabolic rate among Amazonian forager-horticulturalists experiencing high pathogen burden *American Journal of Physical Anthropology*. doi:10.1002/ajpa.23040
  59. Serhan, C. N., Savill, J. (2005). Resolution of inflammation: The beginning programs the end. *Nature Immunology*, 6(12), 1191-7. doi:10.1038/ni1276
  60. Fox, I. H., Palella, T. D., Kelley, W. N. (1987). Hyperuricemia: A marker for cell energy crisis. *The New England Journal of Medicine*, 317(2), 111-2. doi:10.1056/NEJM198707093170209
  61. Kanellis, J., Watanabe, S., Li, J. H., Kang, D. H., Li, P., Nakagawa, T., Johnson, R. J. (2003). Uric acid stimulates monocyte chemoattractant protein-1 production in vascular smooth muscle cells via mitogen-activated protein kinase and cyclooxygenase-2. *Hypertension*, 41(6), 1287-93. doi:10.1161/01.HYP.0000072820.07472.3B
  62. Shi, Y., Evans, J. E., Rock, K. L. (2003). Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature*, 425(6957), 516-521.
  63. Chen, C. -J., Kono, H., Golenbock, D., Reed, G., Akira, S., Rock, K. L. (2007). Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nature Medicine*, 13(7), 851-856.
  64. Behrens, M. D., Wagner, W. M., Krco, C. J., Erskine, C. L., Kalli, K. R., Krempski, J., Knutson, K. L. (2008). The endogenous danger signal, crystalline uric acid, signals for enhanced antibody immunity. *Blood*, 111(3), 1472-1479.
  65. Arakawa, K., Hosono, A., Shibata, K., Ghadimi, R., Fuku, M., Goto, C., Tokudome, S. (2016). Changes in blood biochemical markers before, during, and after a 2-day ultramarathon. *Open Access Journal of Sports Medicine*, 7, 43-50. doi:10.2147/OAJSM.S97468
  66. Shin, K. A., Park, K. D., Ahn, J., Park, Y., & Kim, Y. J. (2016). Comparison of changes in biochemical markers for skeletal muscles, hepatic metabolism, and renal function after three types of long-distance running: Observational study. *Medicine*, 95(20), e3657. doi:10.1097/MD.0000000000003657

# **CHAPTER 11**

## **Summary, Conclusions & Future Perspectives**



## Summary and conclusions

### Part I - Neurobiological and Clinical Effects of Sodium Oxybate

GHB is an endogenous short-chain fatty acid synthesized locally within the CNS, mostly from its parent compound GABA. Approximately 1–2% of GABA converts to GHB, which is relatively rapidly converted into CO<sub>2</sub> and H<sub>2</sub>O through the Krebs cycle. GHB for exogenous administration was first synthesized in the early 1960s and found to readily cross the blood-brain barrier into the CNS, where it displays distinct pharmacological effects. Evidence suggests a role for GHB as a neuromodulator/neurotransmitter, as GHB is synthesized in neurons heterogeneously distributed throughout the CNS, stored in vesicles, released via potassium-dependent depolarization into the synaptic cleft, and undergoes reuptake into the nerve terminal. Under endogenous conditions and concentrations, and depending on the cell group affected, GHB may increase or decrease neuronal activity by inhibiting the release of the primary co-localized neurotransmitter. For example, GHB may decrease neuronal activity when inhibiting the release of the excitatory neurotransmitter dopamine and increase neuronal activity when inhibiting the release of the inhibitory neurotransmitter GABA.

Sodium oxybate is the sodium salt of GHB used for its exogenous oral administration. The behavioral effects induced by SXB appear to be mediated by GHB acting as a neuromodulator/neurotransmitter at GABA<sub>B</sub> receptors. After exogenous administration, it is likely that GHB acts at GHB binding site(s) and GABA<sub>B</sub> receptors, although it appears that most of the behavioral effects are mediated through the GABA<sub>B</sub> receptor. On neurons, supraphysiological concentrations of GHB have a qualitatively different effect than endogenous GHB concentrations. These elevated levels, mostly acting through GABA<sub>B</sub> modulation on various neuron groups, decrease neuronal activity. On washout from supraphysiological concentrations, increased neuronal responsiveness has been observed. This activity may underlie the sleep modulation seen when GHB is administered before nighttime sleep onset and, conversely, the wakefulness stimulating effects observed during the day following nighttime administration.

A review of the pharmacology and physiological actions of GHB and SXB is presented in the first part of Chapter 2. In the second part of the same chapter, I review the evidence supporting a modulatory effect of GHB and SXB on sleep and wakefulness, both in healthy and in clinical populations. In Chapter 3, I examine the safety and efficacy of SXB in individuals with PD and sleep disorders. In Chapter 4, I analyze the effect of nightly SXB administration on nocturnal sleep disruption in narcolepsy patients, a subject to which I return in Chapter 6. In Chapter 5, I review and compare the accessibility, purity, dosing, and misuse of illicit GHB and pharmaceutical SXB. In Chapter 7, I evaluate a possible association between narcolepsy, hypocretin neurons, the hormones ghrelin and leptin, and SXB.

### *GHB and SXB modulate sleep and wakefulness in healthy and in clinical populations*

GHB has shown a dose-dependent effect in decreasing sleep onset latency, promoting delta activity and enhancing sleep maintenance. These effects have been reported in both healthy and clinical populations. A review of these effects is presented in the second part of Chapter 2.

In healthy subjects, GHB has been shown to decrease sleep onset latency, promote delta activity, and enhance SWS and sleep maintenance<sup>14,46,184,239</sup>. Similar effects have been described in clinical contexts. Evidence indicates that GHB/SXB may improve sleep in patients with insomnia<sup>47,246</sup>. Patients with fibromyalgia have also benefited from similar effects, with GHB/SXB being effective in decreasing not only sleep disruption, but also pain, fatigue and overall multidimensional function<sup>37,38,228,229,250</sup>. The beneficial effects of GHB/SXB in modulating sleep also extend to patients with neurodegenerative diseases. In the context of Alzheimer's disease, an association between NREM sleep impairment and disease pathogenesis has been revealed<sup>267,268</sup>, with poor sleep correlating with the severity of cortical A $\beta$  burden in Alzheimer's disease patients<sup>272,273</sup>. Given the effects of GHB in increasing NREM SWS<sup>46</sup>, this establishes a therapeutic potential for GHB on Alzheimer's disease pathogenic processes.

In narcolepsy, the results of large, multicenter trials corroborate earlier work and demonstrate a consistent effect of SXB on SWS activity, yielding substantial, dose-related increases in SWS duration and delta power. Additionally, dose-related reductions in stage 1 sleep and number of awakenings are apparent in the larger studies, as well as modest increases in total sleep duration and reductions in REM sleep duration at a dose of 9 g. Multiple measures of daytime sleepiness demonstrated consistent short- and long-term improvement when SXB was administered in combination with stimulant therapy or as the only wake-promoting treatment. In addition, compared with modafinil, SXB as monotherapy appears to produce equal or greater improvement in daytime sleepiness in patients with narcolepsy with, or without, co-morbid cataplexy<sup>174,290,292–295</sup>.

### *SXB can decrease excessive daytime sleepiness and fatigue in Parkinson's disease*

Excessive daytime sleepiness and nocturnal sleep dysfunction associated with Parkinson's disease have been well documented. However, a correlation between them had not been confirmed, and no specific treatments for nocturnal sleep problems in the Parkinson's disease population had been explored. In chapter 3, the possibility of using SXB for EDS in subjects with Parkinson's disease was evaluated in a multicenter, open-label, polysomnographic study<sup>1</sup>. It was hypothesized that using SXB as a treatment for nocturnal sleep dysfunction could also have a therapeutic effect in Parkinson's disease-associated EDS.

Twenty-seven subjects with Parkinson's disease completed the study. The subjects started SXB therapy at a dose of 4.5 g per night, taken in 2 equal doses of 2.25 g, at bedtime and 2.5 to 4 hours later. After 2 weeks, the dose was increased to 6 g per night, and then increased weekly by 1.5 g to a maximum nightly dose of 9 g

(mean dose of 7.8 g SXB per night for 6 weeks). ESS scores were used as the primary efficacy point. The Fatigue Severity Scale, the Pittsburgh Sleep Quality Inventory, and PSG were assessed as secondary measures of daytime symptoms (FSS) and nocturnal symptoms (PSQI and PSG).

Overall, nightly administration of SXB increased SWS, decreased subjective nighttime and daytime sleep problems, and reduced daytime fatigue in individuals with Parkinson's disease. Improvements in the subjective ESS were similar to or better than those observed while using SXB as therapy for narcolepsy<sup>297,299</sup>. SXB was generally well tolerated.

These results indicate that nightly SXB administration can have beneficial effects on EDS and fatigue associated with Parkinson's disease. These findings also highlight the potential relevance of SXB as a therapeutic tool for Parkinson's disease-associated sleep dysfunctions.

*SXB can reduce measures of sleep disruption and increase SWS in patients with narcolepsy.*

PSG studies have repeatedly demonstrated pathological changes in the nocturnal sleep of patients with narcolepsy<sup>525–527</sup>. Therapeutic approaches for narcolepsy-associated nocturnal sleep disruption have provided limited benefit in improving daytime symptoms. Likewise, therapies for daytime symptoms of narcolepsy have provided little benefit for disrupted nocturnal sleep<sup>528</sup>.

Multiple studies have reported improvements in subjective and objective measures of nocturnal sleep and daytime symptoms in patients with narcolepsy after nightly administration of SXB<sup>172,291,294,295</sup>. One such study demonstrated that 8 weeks of nightly SXB administration robustly increased stage 3 and 4 sleep and delta power, while the frequency of nocturnal awakenings significantly decreased<sup>298</sup>, with these changes being associated with significant improvements in daytime narcolepsy symptoms<sup>49,298</sup>.

Chapter 4 aims at further characterizing the efficacy of SXB for the treatment of EDS in patients with narcolepsy. A double-blind, placebo-controlled study was conducted in patients with narcolepsy undergoing stable therapy with modafinil (200–600 mg/day) for the treatment of EDS<sup>2</sup>. The effect of SXB was assessed both as monotherapy and in combination with modafinil. The intent-to-treat population consisted of 222 patients randomized to receive treatment with placebo (n=55), SXB (n=50), modafinil (n=63), or SXB + modafinil (n=54).

Patients receiving modafinil maintained their previous dosage. Patients receiving SXB started the trial at a dose of 6 g/night, administered in two equal doses (at bedtime and 2.5–4 h later) for the first 4 weeks; the dose of SXB was then increased to 9 g/night for an additional 4 weeks. Treatment efficacy was assessed using overnight PSG, ESS and Maintenance of Wakefulness Test scores, and daily diary recordings.

After 4 weeks of treatment, patients treated with SXB, either alone or in combination with modafinil, showed significant increases in stage 3 and 4 sleep. SXB/modafinil-treated patients also demonstrated significant increases in total NREM sleep and delta power, along with decreased stage 1 sleep and nocturnal awakenings. After an additional 4 weeks of treatment with SXB at the 9 g/night dose, these changes became even more robust and were statistically significant in both SXB groups. It remained unclear whether this increased robustness of effects was related to the dose (6 or 9 g/night), the duration of SXB treatment (4 or 8 weeks), or both.

MWT sleep latency was significantly increased in SXB/modafinil-treated patients, compared to baseline modafinil treatment, whereas patients receiving either modafinil or SXB alone showed no significant change in MWT sleep latency. SXB-treated patients and SXB/modafinil-treated patients also experienced significant improvements in ESS scores, as had been previously reported in detail<sup>299</sup>.

The results from this trial, the first controlled study evaluating SXB as a single agent for the treatment of EDS in narcolepsy, suggested that, in addition to improving EDS, the nightly administration of SXB was associated with reduced nocturnal sleep disruption and improved sleep continuity, as indicated by the observed decreases in nighttime awakenings and increases in stage 3 and 4 sleep.

#### *SXB has less risk of misuse and abuse than illicit GHB*

Gamma-hydroxybutyrate sodium is the chemical name for SXB, but the acronym GHB also refers to the illicit formulations of the drug. Reports of abuse of illicit GHB as a “club drug” and “date-rape drug” have led to the scheduling of GHB as a controlled substance. The use of the chemical name ‘GHB’ to refer to both illicit GHB and to SXB has blurred the distinction between them and has clouded the notion that illicit GHB and SXB have different risks or liabilities of abuse.

In Chapter 5, I address this issue by means of a review that aims at summarizing the differences in accessibility, purity, dosing, and relative abuse liability of pharmaceutical SXB (Xyrem®) and illicit GHB, focusing on the availability and prevalence of non-medical use, and the risks and consequences of misuse and abuse<sup>3</sup>.

This review draws information from three types of sources: data from the peer-reviewed scientific literature; data from national surveys of drug use, abuse, and law enforcement activity in the U.S., Europe, and Australia; and data from clinical trials and post-marketing surveillance from Jazz Pharmaceuticals on the rates of abuse, diversion, drug-facilitated sexual assault, and deaths associated with SXB.

Data presented in this review supports the conclusion that there are substantial differences in the availability, purity, and dosing of illicit GHB compared to pharmaceutical SXB, and that the risks associated with illicit GHB are greater than those associated with pharmaceutical SXB. This review shows that the prevalence of illicit GHB use, abuse, intoxication and overdose has declined in the U.S. since it

became illegal, and that the abuse and misuse of pharmaceutical SXB has been rare since its introduction to the market.

*SXB can improve sleep fragmentation associated with narcolepsy*

In Chapter 6, I extend the studies from Chapter 4 by further analyzing the effects of nightly SXB administration on nocturnal sleep in narcolepsy patients. Chapter 6 describes the first large randomized, double-blind, placebo-controlled, parallel group trial examining the impact of SXB on sleep architecture and narcolepsy symptoms<sup>4</sup>. The data presented in this chapter focus on the changes in nocturnal PSG parameters, providing additional information on the effects of SXB on nocturnal sleep.

The trial was conducted with 228 adult patients with narcolepsy/cataplexy in the U.S., Canada, and Europe. Patients received either 4.5, 6, or 9 g/night of SXB or placebo, administered in 2 equally divided doses each night for 8 weeks. Following randomization, patients were started on placebo in single-blind fashion and recorded baseline cataplexy occurrences over a 14-day period. After the baseline analysis, patients started receiving SXB or placebo, and were titrated to their final dose during the first 4 weeks of treatment. Patients were then maintained at their assigned dose for the remaining 4 weeks of the study, before returning for the final efficacy and safety assessments. PSG and MWT were performed, and changes in narcolepsy symptoms and adverse events were recorded in daily diaries.

Results showed that sleep latency was not significantly altered at any dose or treatment time. Total sleep time was significantly increased at the 8<sup>th</sup> week of treatment with the 9 g/night dose. The number of nocturnal awakenings significantly decreased at 4 weeks with all doses and remained so with the 6 and 9 g/night doses at 8 weeks. Wake after sleep onset significantly decreased in the 9 g/night group at 8 weeks. There was a significant association between dose and increased total sleep time, decreased number of awakenings, and decreased wake after sleep onset at 8 weeks.

The duration of stage 1 sleep was significantly decreased with all SXB doses at 4 weeks and remained so with the 6 and 9 g/night doses at 8 weeks; a significant dose association for the decrease in stage 1 sleep was found. The duration of stage 2 sleep was unaltered. The duration of stage 3 and 4 sleep was significantly increased with the 6 g/night and 9 g/night groups at 4 weeks and with all SXB doses at 8 weeks, being significantly dose-dependent at both 4 weeks and 8 weeks. Median delta power was significantly increased with all SXB doses at both 4 and 8 weeks, but a significant dose relationship was not observed. The duration of REM sleep was significantly decreased with the 9 g/night dose at 4 and 8 weeks.

Other measures of efficacy, reported elsewhere, indicated that the nightly administration of 4.5, 6, and 9 g/night doses of SXB significantly decreased cataplexy attacks, and significantly improved subjective and objective measures of EDS and quality of life<sup>49,298,529</sup>.

These results indicated that SXB induces dose-related improvements in measures of sleep continuity and that SXB may improve the sleep fragmentation that is commonly associated with narcolepsy. The continued improvements from week 4 to week 8 also suggest a possible time-dependent effect.

*SXB's influence on BMI is unlikely to involve changes in the secretion of ghrelin or leptin*

Ghrelin and leptin, two hormones with important roles in regulating energy homeostasis<sup>201,375,376,381</sup>, can be directly sensed by hypocretin neurons, and their interaction with the hypocretin system has been shown to be involved in ingestive behavior<sup>202</sup>.

Because hypocretin influences sympathetic nervous system activity, which in turn can affect the expression of both leptin and ghrelin, hypocretin deficiency may lead to altered levels of these hormones, potentially affecting ingestive behavior and energy metabolism.

In narcolepsy patients, altered ingestive behavior and obesity are commonly observed and have been associated with hypocretin deficiency<sup>530–532</sup>. Since the hypocretin system has a key role in the regulation of sleep and wakefulness, with hypocretin deficiency also being associated with narcolepsy, it is possible that hypocretin deficiency may dysregulate feeding behavior and energy homeostasis. Therefore, in Chapter 7, I examine the link between narcolepsy, hypocretin neurons, the hormones ghrelin and leptin, and SXB, aiming at evaluating whether human hypocretin deficiency or SXB can alter the levels of these hormones, which could help explain the altered ingestive behavior and increased BMI seen in narcolepsy patients<sup>5</sup>. We investigated whether total blood ghrelin or leptin levels are altered in hypocretin-deficient narcoleptic patients compared to controls, and whether total ghrelin or leptin levels are influenced by SXB.

Eight medication-free, male hypocretin-deficient narcolepsy with cataplexy patients and 8 healthy male controls, matched for age, BMI, and body fat percentage were included in this study. Plasma total ghrelin and leptin levels were assessed at baseline and after 5 consecutive nights of SXB treatment at a total dose of 6 g/night, administered in two equal doses of 3 g, 4 hours apart. PSG recordings were also performed.

Both in controls and in narcolepsy patients, administration of SXB resulted in a significant decrease in stages 1/2 NREM and REM sleep over 24 hours, while at night, awakenings were significantly reduced and the percentage of SWS increased more than 2-fold. During the day, time spent in stages 1/2 NREM and REM sleep was reduced, and a trend towards longer periods of wakefulness was observed. No differences in ghrelin or leptin levels nor any effects of SXB on the plasma levels of either hormone were found.

Even though a small number of patients was included in this study, the small intergroup differences indicate that the increased BMI of narcolepsy patients is

unlikely to be mediated by hypocretin deficiency-mediated changes in total ghrelin or leptin levels, and that SXB's influence on body weight is unlikely to involve changes in the secretion of the hormones.

## **Part II: Sleep, Eating, and Metabolism**

An overview of the epidemiological evidence linking sleep and obesity is presented in Chapter 8. In addition, I discuss how sleep affects metabolic, endocrine, immune, and circadian processes, how brain-processing circuits and functions are affected by sleep loss, and how this altered brain function can influence eating behavior. Chapter 9 discusses how manipulation of a single night of sleep may influence food preferences in humans. Chapter 10 examines how a short, outdoor excursion under Paleolithic-like eating, living, and sleeping conditions improves physiological and metabolic parameters in the body.

### *Epidemiology shows a correlation between sleep loss and obesity*

The first part of Chapter 8 addresses the question of whether there is an epidemiological relationship between sleep loss and obesity. In 2012, 70 million U.S. adults reported getting less than six hours of sleep at night<sup>533</sup>. Sleep is a major public health concern, and insufficient sleep is related to motor vehicle crashes, industrial accidents, and medical errors<sup>534</sup>. Epidemiology studies show that there is a relationship between sleep duration and body weight, and show that sleep disruption impacts metabolism, immune function, and circadian rhythms. Because obesity rates are rising worldwide in adults and children<sup>305,535</sup>, it will be important to understand how sleep duration and quality affect human health.

### *Objective measures of sleep reveal that total sleep time has not decreased over the last 50 years*

The second part of Chapter 8 addresses the question of whether actual sleep time has decreased in the last 50 years. A literature review found that sleep duration increased in some countries (Bulgaria, Poland, Canada, France, Britain, Korea, and the Netherlands), decreased in others (Japan, Russia, Finland, Germany, Belgium, and Austria), and was inconsistent in the U.S. and Sweden<sup>536</sup>, and later reports have shown that the number of individuals sleeping 6 hours or less has increased<sup>537,538</sup>. However, these studies cannot differentiate between people reporting that they sleep less versus people actually sleeping less. Objective measures of sleep duration can only be observed in a sleep laboratory under controlled conditions using sleep-recording techniques like PSG and actigraphy. Researchers first made use of this type of data in a meta-analysis of 65 studies over 40 years to determine that sleep duration decreases with age<sup>539</sup>. Similarly, other researchers have used this type of data to determine that sleep duration has not decreased over the past 50 years<sup>540</sup>.

### *Sleep manipulation can drive food preferences in humans*

Many laboratory studies and epidemiologic research have shown a connection between reduced sleep and increased weight. However, laboratory studies have used fairly extreme models of sleep in order to observe substantial changes in

metabolic parameters. Chapter 9 discusses how lowered alertness by a moderate change in sleep restriction might drive an individual's food preferences and total calorie consumption.

Fifty healthy, young participants completed two 3-hour study sessions. The first session was a baseline evaluation after an unmodified night of sleep. On the night prior to the second session, the amount of time in bed was manipulated to be 60-130% of an individual's sleep time. Changes in time in bed were linearly associated with changes in scores on the Stanford Sleepiness Scale, so that individuals who had less time in bed were less subjectively alert during the second session. During the middle of each session, participants were allowed to eat from eight different food items with varying degrees of healthfulness, caloric density and distribution, and number of calories.

There was a linear relationship between a change in subjective alertness and a change in total calories consumed and total calories consumed relative to body weight. In addition, there was a positive correlation between subjective alertness and the number of calories consumed from "bad" food choices (i.e., gummy bears, cinnamon-sugar walnuts, toffee peanuts, and sweetened trail mix), but no correlation with the number of calories consumed from "good" food choices (i.e., apple rings, apricots, almonds, and fig bars). There was also a negative association between subjective alertness and the food quality rated by the participants, such that when participants rated themselves less alert, they ate foods that they rated less healthy.

The study showed that manipulation of next-day alertness via the manipulation of sleep for a single night can have a detrimental impact on eating behaviors. Increased subjective feelings of sleepiness correlated with an increase in total calories consumed and with an increase in calories categorized as "bad" by the investigators and "less healthy" by the participants. This study suggests that when a person feels less alert, the hedonic processing for tempting foods may be increased. Previous studies have shown using fMRI that a night of sleep deprivation amplifies regions of the brain responsible for food decisions, and that these changes are associated with a greater desire for caloric density<sup>524</sup>. In addition, simulation of shift work under experimental conditions increases the likelihood of participants eating high-fat breakfast items compared to that of the control condition<sup>541</sup>. This agrees with a study showing that participants consumed a greater percentage of calories from fat compared to carbohydrates the day after a night of total sleep deprivation compared to a day following baseline sleep<sup>542</sup>.

Alternatively, sleep loss might relax personal inhibitions against unhealthy foods. Sleep deprivation alters effort discounting, a principle that suggests that the value attached to a reward is inversely related to the amount of effort required to obtain it<sup>543</sup>. Perhaps participants with impaired alertness in our study ate unhealthy foods they might have otherwise avoided because they were less likely to make an effort as a result of their sleep deprivation. Sleep loss may also have shifted the focus of participants to foods that subjectively taste better, which correlates with less

healthy foods, from a focus on eating healthier foods. This type of bias has been reported in the context of economic preferences for monetary gambling where sleep deprivation favors the pursuit of large rewards, and reduces minimization of loss<sup>544</sup>. In our study, monetary gains would correspond to the pleasure of eating unhealthy foods, and losses would correspond to the detrimental effects of eating less healthy foods. Thus, sleep-deprived participants may have eaten more of the unhealthy food options because they were more pleasurable and discounted the negative effects of those unhealthy foods.

Importantly, our study examined moderate impairments in sleep loss rather than total sleep deprivation. Insufficient sleep is a major health problem and related to an increase in chronic diseases, such as diabetes, depression, obesity, cancer, increased mortality, and reduced quality of life<sup>534</sup>. In addition, it has been shown that several consecutive days of chronic sleep restriction below 7 hours results in significant cognitive impairments that accumulate to levels comparable to that after a night of total sleep deprivation<sup>445</sup>. Thus, our study is relevant to food preferences and sleep impairments in modern society and consistent with epidemiological studies that show a relationship between sleep loss and weight gain<sup>338,545,546</sup>.

*A short outdoor excursion under Paleolithic living conditions improves metabolic function and increases weight loss*

For more than 2.5 million years, humans have relied on foraging and gathering to supply food. Abundant, regular physical activity under natural lighting and temperature conditions to forage and hunt for food, and large meals in the evening, were the norm. The evolutionarily recent shift to readily available and calorically dense foods has contributed to a wave of 'Western diseases,' such as diabetes and obesity. Permanent food availability, increased meal frequency, and high glycemic foods have resulted in alternating peaks in blood sugar and elevated basal insulin levels<sup>547,548</sup>, which leads to visceral obesity, glucose intolerance, persistent elevated insulin, and low-grade inflammation<sup>549–551</sup>. As a result, the incidence of type 2 diabetes has been rising worldwide for decades<sup>552</sup>. Furthermore, obesity is caused a chronic imbalance between energy intake and energy expenditure and results in persistent low-grade inflammation throughout the body, such as elevated tumor-necrosis factor alpha (TNF- $\alpha$ ), interleukin-1-beta (IL-1 $\beta$ ), and macrophage counts in visceral adipose tissue<sup>553,554</sup>. TNF- $\alpha$  in cooperation with IL-1 $\beta$  enhances insulin resistance<sup>555</sup>, and experimentally induced hyperglycemia increases TNF- $\alpha$  and other pro-inflammatory cytokines, such as IL-6 and C-reactive protein<sup>556–558</sup>. While pancreatic insulin secreted from elevated glucose levels suppresses inflammation<sup>559–561</sup>, this anti-inflammatory effect is reduced in a state of chronic insulin resistance.

Early in our evolutionary history, caloric intake was counterbalanced by its seasonal availability, physical efforts, and knowledge of the surrounding environment<sup>562,563</sup>. Exercise before eating lowers postprandial inflammation and produces non-inflammatory molecules, such as lactoferrin, immunoglobulin A (IgA), and lysozyme<sup>564</sup>. These molecules are absent or reduced in overweight

individuals<sup>565</sup> and they have increased postprandial inflammation, which leads to the development of cardiovascular disease, obesity, insulin resistance, and chronic low-grade inflammation<sup>565–567</sup>. Animal experiments have revealed that caloric restriction and intermittent fasting can suppress weight-gain-related illness and extend lifespan. In mice, caloric restriction increases lifespan by 30–40% by reducing levels of CRP and TNF- $\alpha$ <sup>568–570</sup>. Human studies have also begun to reveal the beneficial effects of caloric restriction<sup>571</sup>.

In Chapter 10, I describe a study that examines participants on an outdoor nature trip for 4 days under Paleolithic-like living conditions. Individuals lived outdoors without tents and were required to hike throughout the day to simulate the activity level of gathering food. A small snack was provided after noon to mimic the delayed time to gather food, and a meal without modern, processed foods was provided at dinner time. This relatively moderate lifestyle change over a period of 4 days resulted in dramatic improvements in physiological and metabolic parameters. Body weight, body fat, BMI, and visceral fat area all decreased as expected because of reduced caloric intake and increased exercise. Fasting glucose, insulin and HOMA also decreased significantly and CRP, the main indicator of low-grade inflammation, increased. Previously, it has been shown that trips into the forest stimulates human immune function and improves cardiovascular parameters<sup>572–574</sup>, perhaps as anticipatory protection from bacteria, viruses, insects, or other predators. Natural living in our study may have had similar effects.

This study shows that a short intervention under Paleolithic living conditions can dramatically improve physiological and metabolic parameters, which may aid in the prevention of obesity and type 2 diabetes. The individual factors responsible for these improvements are difficult to parse without further studies that isolate caloric restriction, outdoor activity, and intermittent fasting, but likely a combination of all three were partially responsible for the beneficial effects.

## Future perspectives

In Chapter 3, we evaluated the possibility of using SXB for EDS in subjects with Parkinson's disease. Our results provided an indication that nightly SXB administration can have beneficial effects on EDS and fatigue associated with Parkinson's disease. These putative therapeutic effects of SXB are worth pursuing in controlled trials using objective measures of daytime sleepiness. Confirming these results could establish SXB as an important therapeutic tool for Parkinson's disease, with the capacity to improve patients' quality of life.

In Chapter 4, we studied the efficacy of SXB for the treatment of EDS in patients with narcolepsy. The results from this first controlled study evaluating SXB as a single agent for the treatment of EDS in narcolepsy suggested that, in addition to improving EDS, the nightly administration of SXB was associated with reduced nocturnal sleep disruption and improved sleep continuity, as indicated by the decreases in nighttime awakenings and increases in stage 3 and 4 sleep. This study was extended in Chapter 6 by further analyzing the effects of nightly SXB administration on nocturnal sleep in narcolepsy patients. The results indicated that SXB induces dose-related improvements in measures of sleep continuity and that SXB may improve the sleep fragmentation that is commonly associated with narcolepsy.

Although a dose-dependent effect was observed, it remained unclear whether the changes in sleep architecture of narcolepsy patients induced by SXB are related only to the dose or also to the duration of SXB treatment. The continued improvements from week 4 to week 8 suggest a possible time-dependent effect that warrants further clarification.

Additionally, it would be valuable to understand if the observed impact of SXB on sleep EEG activity represents pharmacologically-induced alterations in true sleep-related activity, effects representing anesthetic-like changes, or epiphenomenal EEG activity unrelated to either sleep or anesthesia.

In Chapter 7, we examined the link between narcolepsy, hypocretin neurons, the hormones ghrelin and leptin, and SXB, aiming at evaluating whether human hypocretin deficiency or SXB can alter the levels of these hormones, which could help explain the altered ingestive behavior and increased BMI observed in narcolepsy patients. Given that no differences in ghrelin or leptin levels nor any effects of SXB on the plasma levels of either hormone were found, it is unlikely that changes in total plasma ghrelin or leptin concentrations underlie the increased BMI and altered ingestive behavior in narcolepsy, as well as the effects of SXB administration on BMI.

The small number of patients included in this study calls for further future investigations to confirm these findings and to further evaluate whether or not the sleep-wake instability intrinsic to hypocretin-deficiency drives the altered energy balance associated with narcolepsy.

In Chapter 8, we detailed the epidemiological evidence for the impact of sleep on human health. Future epidemiological studies will need to continue to monitor the rising rates of obesity, and how reduced sleep and impaired sleep quality affect this growing problem. Importantly, sleep is also related to depression, immune function, cancer, circadian rhythms, and other physiological processes. Because sleep is so interconnected to other processes, and loss of sleep has both human health and economic consequences, understanding how to improve and increase sleep will be central to happy workers and healthy economies.

In Chapter 9, we showed that moderate manipulation of alertness via a sleep intervention for a single night can have a detrimental impact on eating behaviors including increased total caloric intake and increased caloric intake from unhealthy foods. However, we did not objectively record sleep with polysomnography or actigraphy, nor was manipulation of sleep controlled in a sleep laboratory. Furthermore, instead of relying on self-reported sleep as the main predictor in our analysis, we used the consequence of sleep loss—subjective daytime sleepiness. Future studies could measure and manipulate sleep loss in a more controlled fashion in a sleep laboratory to determine if objective measures of sleep and impairment correspond to our findings. In addition, because sleep curtailment was relatively mild, this probably reduced the statistical power of our correlation analysis compared to that of studies of severely impaired sleep or total sleep deprivation. Nevertheless, we could detect significant and meaningful correlations between small changes in alertness, typical of sleep disruptions in modern society, and changes in food preferences.

Another caveat of our study is that we could not distinguish between changes in alertness and disruptions in an individual's circadian rhythm. Participants were asked to delay their bedtime, which may have caused a small shift in circadian phase. Future studies could examine melatonin levels in participants to understand the relationship between a participant's circadian rhythms, reduced alertness, and changes in food preferences. In addition, we only analyzed data from 50 participants and 40 of the participants were women. To understand if there are differences between men and women, or to examine other demographic differences, such as ethnicity and age, future studies would need to include a larger sample size.

In Chapter 10, we showed that a 4-day Paleolithic lifestyle change improved many bioelectric and biochemical parameters in study participants, including body weight, body fat, BMI, visceral fat area, fasting glucose, fasting insulin, and HOMA. C-reactive protein, which is a major indicator of low-grade inflammation, increased by an average of approximately 170%. However, we did not distinguish among the effects of caloric restriction, increased exercise, and outdoor living. Future studies could isolate these individual variables to determine which has the most impact on a participant's health. Moreover, an increased number of participants as well as control subjects that do not undergo the intervention would improve the statistical robustness of these preliminary findings.

Another caveat is that the duration of the intervention lasted for only 4 days. It's unclear if the participants' metabolic parameters would return to where they were before the intervention as they re-adapt to modern society. Another possibility is that extended periods of living under Paleolithic-like conditions may cause unintended or unforeseen harm. For example, increased exposure to parasites, bacteria, etc., or other increased environmental stresses could be detrimental to an individual's wellbeing. Future studies could extend the trial intervention to longer periods of time or repeat the intervention at some periodic interval to assess if occasional, short-term natural trips have a longer-term, beneficial impact on health. In any case, this study provides an entry point to examine how simple lifestyle interventions can have dramatic improvements on an individual's health.

## References

1. Ondo WG, Perkins T, Swick T, et al. Sodium oxybate for excessive daytime sleepiness in Parkinson disease: an open-label polysomnographic study. *Arch Neurol* 2008; 65: 1337–1340.
2. Black J, Pardi D, Hornfeldt CS, et al. The nightly administration of sodium oxybate results in significant reduction in the nocturnal sleep disruption of patients with narcolepsy. *Sleep Med* 2009; 10: 829–835.
3. Carter LP, Pardi D, Gorsline J, et al. Illicit gamma-hydroxybutyrate (GHB) and pharmaceutical sodium oxybate (Xyrem®): Differences in characteristics and misuse. *Drug Alcohol Depend* 2009; 104: 1–10.
4. Black J, Pardi D, Hornfeldt CS, et al. The nightly use of sodium oxybate is associated with a reduction in nocturnal sleep disruption: a double-blind, placebo-controlled study in patients with narcolepsy. *J Clin Sleep Med* 2010; 6: 596–602.
5. Donjacour CE, Pardi D, Aziz NA, et al. Plasma total ghrelin and leptin levels in human narcolepsy and matched healthy controls: basal concentrations and response to sodium oxybate. *J Clin Sleep Med* 2013; 9: 797–803.
6. Pardi D, Buman M, Black J, et al. Eating Decisions Based on Alertness Levels After a Single Night of Sleep Manipulation: A Randomized Clinical Trial. *Sleep*; 40. Epub ahead of print February 2017. DOI: 10.1093/sleep/zsw039.
7. Freese J, Pardi DJ, Ruiz-Núñez B, et al. Back to the Future. Metabolic Effects of a 4-Day Outdoor Trip Under Simulated Paleolithic Conditions – New Insights from The Eifel Study. *J Evol Heal*; 1. Epub ahead of print October 2016. DOI: 10.15310/2334-3591.1035.
8. Bessman SP, Fishbein WN. Gamma-hydroxybutyrate, a normal brain metabolite. *Nature* 1963; 200: 1207–1208.
9. Roth RH, Giarman NJ. Conversion in vivo of gamma-aminobutyric to gamma-hydroxybutyric acid in the rat. *Biochem Pharmacol* 1969; 18: 247–250.
10. Fishbein WN, Bessman SP. Gamma-Hydroxybutyrate in Mammalian Brain. Reversible Oxidation by Lactic Dehydrogenase. *J Biol Chem* 1964; 239: 357–361.
11. Doherty JD, Hattox SE, Snead OC, et al. Identification of endogenous gamma-hydroxybutyrate in human and bovine brain and its regional distribution in human, guinea pig and rhesus monkey brain. *J Pharmacol Exp Ther* 1978; 207: 130–139.
12. Snead 3rd OC, Furner R, Liu CC. In vivo conversion of gamma-aminobutyric acid and 1,4-butanediol to gamma-hydroxybutyric acid in rat brain. Studies using stable isotopes. *Biochem Pharmacol* 1989; 38: 4375–4380.
13. Laborit H. Sodium 4-Hydroxybutyrate. *Int J Neuropsychopharmacol* 1964; 32: 433–451.
14. Helrich M, McAslan TC, Skolnik S, et al. Correlation of Blood Levels of 4-Hydroxybutyrate with State of Consciousness. *Anesthesiology* 1964; 25: 771–775.

15. Blumenfeld M, SRG and HMM. Sodium gamma-hydroxybutyric acid: a new anaesthetic adjuvant. . *Anesth Analg* 1962; 41: 721–726.
16. Laborit G, Larcen A, Kind A. [Electrocardiographic study of sodium 4-hydroxybutyrate.]. *Agressologie* 1963; 4: 77–88.
17. Kleinschmidt S, Grundmann U, Knocke T, et al. Total intravenous anaesthesia with gamma-hydroxybutyrate (GHB) and sufentanil in patients undergoing coronary artery bypass graft surgery: a comparison in patients with unimpaired and impaired left ventricular function. *Eur J Anaesthesiol* 1998; 15: 559–564.
18. Solway J, Sadove MS. 4-Hydroxybutyrate: a clinical study. *Anesth Analg* 1965; 44: 532–539.
19. Metcalf DR, Emde RN, Stripe JT. An EEG-behavioral study of sodium hydroxybutyrate in humans. *Electroencephalogr Clin Neurophysiol* 1966; 20: 506–512.
20. Yamada Y, Yamamoto J, Fujiki A, et al. Effect of butyrolactone and gamma-hydroxybutyrate on the EEG and sleep cycle in man. *Electroencephalogr Clin Neurophysiol* 1967; 22: 558–562.
21. Miller JD, Piper IR, Dearden NM. Management of intracranial hypertension in head injury: matching treatment with cause. *Acta Neurochir Suppl* 1993; 57: 152–159.
22. Strong AJ. gamma-Hydroxybutyric acid and intracranial pressure. *Lancet* 1984; 1: 1304.
23. Kolin A, Brezina A, Mamelak M, et al. Cardioprotective action of sodium gamma-hydroxybutyrate against isoproterenol induced myocardial damage. *Int J Exp Pathol* 1993; 74: 275–281.
24. Ueki Y. [Effects of gamma-hydroxybutyrate on monoamine metabolism and protein synthesis after transient global cerebral ischemia]. *No Shinkei Geka* 1992; 20: 937–946.
25. Geldenhuys FG, Sonnendecker EW, De Klerk MC. Experience with sodium-gamma-4-hydroxybutyric acid (gamma-OH) in obstetrics. *J Obs Gynaecol Br Commonw* 1968; 75: 405–413.
26. Ferrara SD, Giorgetti R, Zancaner S, et al. Effects of single dose of gamma-hydroxybutyric acid and lorazepam on psychomotor performance and subjective feelings in healthy volunteers. *Eur J Clin Pharmacol* 1999; 54: 821–827.
27. Addolorato G, Castelli E, Stefanini GF, et al. An open multicentric study evaluating 4-hydroxybutyric acid sodium salt in the medium-term treatment of 179 alcohol dependent subjects. GHB Study Group. *Alcohol Alcohol* 1996; 31: 341–345.
28. Biggio G, Cibin M, Diana M, et al. Suppression of voluntary alcohol intake in rats and alcoholics by gamma-hydroxybutyric acid: a non-GABAergic mechanism. *Adv Biochem Psychopharmacol* 1992; 47: 281–288.
29. Bowles TM, Sommi RW, Amiri M. Successful management of prolonged gamma-hydroxybutyrate and alcohol withdrawal. *Pharmacotherapy* 2001; 21: 254–257.

30. Ferrara SD, Zotti S, Tedeschi L, et al. Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. *Br J Clin Pharmacol* 1992; 34: 231–235.
31. Gallimberti L, Canton G, Gentile N, et al. Gamma-hydroxybutyric acid for treatment of alcohol withdrawal syndrome. *Lancet* 1989; 2: 787–789.
32. Gessa GL, Gallimberti L. Gamma-hydroxybutyric acid in the treatment of alcohol dependence. *Clin Neuropharmacol* 1992; 15 Suppl 1: 303A–304A.
33. Maremmani I, Lamanna F, Tagliamonte A. Long-term therapy using GHB (sodium gamma hydroxybutyrate) for treatment-resistant chronic alcoholics. *J Psychoact Drugs* 2001; 33: 135–142.
34. Nimmerrichter AA, Walter H, Gutierrez-Lobos KE, et al. Double-blind controlled trial of gamma-hydroxybutyrate and clomethiazole in the treatment of alcohol withdrawal. *Alcohol Alcohol* 2002; 37: 67–73.
35. Gallimberti L, Cibir M, Pagnin P, et al. Gamma-hydroxybutyric acid for treatment of opiate withdrawal syndrome. *Neuropsychopharmacology* 1993; 9: 77–81.
36. Gallimberti L, Spella MR, Soncini CA, et al. Gamma-hydroxybutyric acid in the treatment of alcohol and heroin dependence. *Alcohol* 2000; 20: 257–262.
37. Scharf MB, Baumann M, Berkowitz D V. The effects of sodium oxybate on clinical symptoms and sleep patterns in patients with fibromyalgia. *J Rheumatol* 2003; 30: 1070–1074.
38. Scharf MB, Hauck M, Stover R, et al. Effect of gamma-hydroxybutyrate on pain, fatigue, and the alpha sleep anomaly in patients with fibromyalgia. Preliminary report. *J Rheumatol* 1998; 25: 1986–1990.
39. Rinaldi F, Puca FM, Mastrosimone F, et al. [On the use of gamma-hydroxybutyrate of sodium in psychiatric therapy]. *Acta Neurol* 1967; 22: 21–41.
40. Martellotta MC, Balducci C, Fattore L, et al. Gamma-hydroxybutyric acid decreases intravenous cocaine self-administration in rats. *Pharmacol Biochem Behav* 1998; 59: 697–702.
41. Vescovi PP, Di Gennaro C. Failure of gammahydroxy butyric acid to stimulate growth hormone secretion in cocaine addicts. *Neuropeptides* 1997; 31: 459–462.
42. Frucht SJ, Bordelon Y, Houghton WH. Marked amelioration of alcohol-responsive posthypoxic myoclonus by gamma-hydroxybutyric acid (Xyrem). *Mov Disord* 2005; 20: 745–751.
43. Frucht SJ, Bordelon Y, Houghton WH, et al. A pilot tolerability and efficacy trial of sodium oxybate in ethanol-responsive movement disorders. *Mov Disord* 2005; 20: 1330–1337.
44. Frucht SJ, Houghton WC, Bordelon Y, et al. A single-blind, open-label trial of sodium oxybate for myoclonus and essential tremor. *Neurology* 2005; 65: 1967–1969.
45. Rubin BA, Giarmann NJ. The therapy of experimental influenza in mice with antibiotic lactones and related compounds. *Yale J Biol Med* 1947; 19:

- 1017–1023.
46. Lapierre O, Montplaisir J, Lamarre M, et al. The effect of gamma-hydroxybutyrate on nocturnal and diurnal sleep of normal subjects: further considerations on REM sleep-triggering mechanisms. *Sleep* 1990; 13: 24–30.
  47. Mamelak M, Escriu JM, Stokan O. Sleep-inducing effects of gammahydroxybutyrate. *Lancet* 1973; 2: 328–329.
  48. Fuller DE, Hornfeldt CS, Kelloway JS, et al. The Xyrem risk management program. *Drug Saf* 2004; 27: 293–306.
  49. Xyrem International Study G. Further evidence supporting the use of sodium oxybate for the treatment of cataplexy: a double-blind, placebo-controlled study in 228 patients. *Sleep Med* 2005; 6: 415–421.
  50. US Drug Enforcement Administration. Gamma hydroxybutyric acid (GHB, liquid X, Goop, Georgia Home Boy)[http://www.usdoj.gov/dea/pubs/pressrel/pr031300\\_01.htm](http://www.usdoj.gov/dea/pubs/pressrel/pr031300_01.htm).
  51. Galloway GP, Frederick SL, Staggers Jr. FE, et al. Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. *Addiction* 1997; 92: 89–96.
  52. American Time Use Survey: Sleep Time and Its Relationship to Waking Activities. 2007; 1–11.
  53. Woolverton WL, Rowlett JK, Winger G, et al. Evaluation of the reinforcing and discriminative stimulus effects of gamma-hydroxybutyrate in rhesus monkeys. *Drug Alcohol Depend* 1999; 54: 137–143.
  54. McMahon LR, Coop A, France CP, et al. Evaluation of the reinforcing and discriminative stimulus effects of 1,4-butanediol and gamma-butyrolactone in rhesus monkeys. *Eur J Pharmacol* 2003; 466: 113–120.
  55. Carter LP, Richards BD, Mintzer MZ, et al. Relative abuse liability of GHB in humans: A comparison of psychomotor, subjective, and cognitive effects of supratherapeutic doses of triazolam, pentobarbital, and GHB. *Neuropsychopharmacology* 2006; 31: 2537–2551.
  56. Anderson I, Kim S, Dyer J, et al. Trends in  $\gamma$ -Hydroxybutyrate (GHB) and Related Drug Intoxication: 1999 to 2003. *Ann Emerg Med* 2006; 47: 177–183.
  57. Drug Abuse Warning Network, 2003: Interim National Estimates of Drug-Related Emergency Department Visits. 2004; 1–104.
  58. Slaughter L. Involvement of drugs in sexual assault. *J Reprod Med* 2000; 45: 425–430.
  59. Doherty JD, Snead OC, Roth RH. A sensitive method for quantitation of gamma-hydroxybutyric acid and gamma-butyrolactone in brain by electron capture gas chromatography. *Anal Biochem* 1975; 69: 268–277.
  60. Ehrhardt JD, Vayer P, Maitre M. A rapid and sensitive method for the determination of gamma-hydroxybutyric acid and trans-gamma-hydroxycrotonic acid in rat brain tissue by gas chromatography/mass spectrometry with negative ion detection. *Biomed Env Mass Spectrom* 1988; 15: 521–524.

61. Snead 3rd OC. gamma-Hydroxybutyric acid in subcellular fractions of rat brain. *J Neurochem* 1987; 48: 196–201.
62. Snead 3rd OC, Morley BJ. Ontogeny of gamma-hydroxybutyric acid. I. Regional concentration in developing rat, monkey and human brain. *Brain Res* 1981; 227: 579–589.
63. Nelson T, Kaufman E, Kline J, et al. The extraneural distribution of gamma-hydroxybutyrate. *J Neurochem* 1981; 37: 1345–1348.
64. Gold BI, Roth RH. Kinetics of in vivo conversion of gamma-[3H]aminobutyric acid to gamma-[3H]hydroxybutyric acid by rat brain. *J Neurochem* 1977; 28: 1069–1073.
65. Weissmann-Nanopoulos Belin, M.F., Mandel, P. and Maitre, M. D. Immunocytochemical evidence for the presence of enzymes synthesizing GABA and GHB in the same neuron. . *Neurochem Intl* 1984; 6: 333–338.
66. Rumigny JF, Cash C, Mandel P, et al. Evidence that a specific succinic semialdehyde reductase is responsible for gamma-hydroxybutyrate synthesis in brain tissue slices. *FEBS Lett* 1981; 134: 96–98.
67. Maitre M. The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. *Prog Neurobiol* 1997; 51: 337–361.
68. Eli M, Cattabeni F. Endogenous gamma-hydroxybutyrate in rat brain areas: postmortem changes and effects of drugs interfering with gamma-aminobutyric acid metabolism. *J Neurochem* 1983; 41: 524–530.
69. Rumigny JF, Cash C, Mandel P, et al. Ontogeny and distribution of specific succinic semialdehyde reductase apoenzyme in the rat brain. *Neurochem Res* 1982; 7: 555–561.
70. Rumigny JF, Maitre M, Cash C, et al. Regional and subcellular localization in rat brain of the enzymes that can synthesize gamma-hydroxybutyric acid. *J Neurochem* 1981; 36: 1433–1438.
71. Hearl WG, Churchich JE. A mitochondrial NADP<sup>+</sup>-dependent reductase related to the 4-aminobutyrate shunt. Purification, characterization, and mechanism. *J Biol Chem* 1985; 260: 16361–16366.
72. Cho Song, M.S., Kim, G.Y., Kang, W.D., Choi, E.Y. and Choi, S.Y. SW. Kinetics and mechanism of an NADPH-dependent succinic semialdehyde reductase from bovine brain. . *Eur J Biochem* 1993; 211: 757–762.
73. Barker SA, Snead OC, Poldrugo F, et al. Identification and quantitation of 1,4-butanediol in mammalian tissues: an alternative biosynthetic pathway for gamma-hydroxybutyric acid. *Biochem Pharmacol* 1985; 34: 1849–1852.
74. Roth RH Giarman NJ. LR. Dependence of rat serum lactonase upon calcium. . *Biochem Pharmacol* 1967; 16: 596–598.
75. Doherty JD, Stout RW, Roth RH. Metabolism of (1-14C)gamma-hydroxybutyric acid by rat brain after intraventricular injection. *Biochem Pharmacol* 1975; 24: 469–474.
76. Mohler H, Patel AJ, Balazs. Gamma-hydroxybutyrate degradation in the brain in vivo: negligible direct conversion to GABA. *J Neurochem* 1976; 27: 253–258.

77. Kaufman EE, Nelson T. Evidence for the participation of a cytosolic NADP<sup>+</sup>-dependent oxidoreductase in the catabolism of gamma-hydroxybutyrate in vivo. *J Neurochem* 1987; 48: 1935–1941.
78. Kaufman EE, Nelson T, Goochee C, et al. Purification and characterization of an NADP<sup>+</sup>-linked alcohol oxido-reductase which catalyzes the interconversion of gamma-hydroxybutyrate and succinic semialdehyde. *J Neurochem* 1979; 32: 699–712.
79. Vayer P, Schmitt M, Bourguignon JJ, et al. Evidence for a role of high Km aldehyde reductase in the degradation of endogenous gamma-hydroxybutyrate from rat brain. *FEBS Lett* 1985; 190: 55–60.
80. Kaufman EE, Relkin N, Nelson T. Regulation and properties of an NADP<sup>+</sup> oxidoreductase which functions as a gamma-hydroxybutyrate dehydrogenase. *J Neurochem* 1983; 40: 1639–1646.
81. Kaufman EE, Nelson T. Kinetics of coupled gamma-hydroxybutyrate oxidation and D-glucuronate reduction by an NADP<sup>+</sup>-dependent oxidoreductase. *J Biol Chem* 1981; 256: 6890–6894.
82. Kaufman EE, Nelson T. An overview of gamma-hydroxybutyrate catabolism: the role of the cytosolic NADP(+)-dependent oxidoreductase EC 1.1.1.19 and of a mitochondrial hydroxyacid-oxoacid transhydrogenase in the initial, rate-limiting step in this pathway. *Neurochem Res* 1991; 16: 965–974.
83. Taberner P V, Rick JT, Kerkut GA, et al. The action of gamma-hydroxybutyric acid on cerebral glucose metabolism. *J Neurochem* 1972; 19: 245–254.
84. Snead OC, Bearden LJ, Pegram V, et al. Effect of acute and chronic anticonvulsant administration on endogenous gamma-hydroxybutyrate in rat brain. *Neuropharmacology* 1980; 19: 47–52.
85. Gibson KM. Gamma-hydroxybutyric aciduria: a biochemist's education from a heritable disorder of GABA metabolism. *J Inherit Metab Dis* 2005; 28: 247–65.
86. Crunelli V, Emri Z, Leresche N. Unravelling the brain targets of gamma-hydroxybutyric acid. *Curr Opin Pharmacol* 2006; 6: 44–52.
87. Kaufman EE, Nelson T, Miller D, et al. Oxidation of gamma-hydroxybutyrate to succinic semialdehyde by a mitochondrial pyridine nucleotide-independent enzyme. *J Neurochem* 1988; 51: 1079–1084.
88. Margolis RK. The effect of gamma-hydroxybutyric acid on amino acid levels in brain. *Biochem Pharmacol* 1969; 18: 1243–1246.
89. Mitoma C, Neubauer SE. Gamma-hydroxybutyric acid and sleep. *Experientia* 1968; 24: 12–13.
90. Vayer P, Mandel P, Maitre M. Conversion of gamma-hydroxybutyrate to gamma-aminobutyrate in vitro. *J Neurochem* 1985; 45: 810–814.
91. Vayer P, Dessort D, Bourguignon JJ, et al. Natural occurrence of trans-gamma hydroxycrotonic acid in rat brain. *Biochem Pharmacol* 1985; 34: 2401–2404.
92. Walkenstein SS, Wiser R, Gudmundsen C, et al. Metabolism of Gamma-Hydroxybutyric Acid. *Biochim Biophys Acta* 1964; 86: 640–642.

93. Maitre M, Cash C, Weissmann-Nanopoulos D, et al. Depolarization-evoked release of gamma-hydroxybutyrate from rat brain slices. *J Neurochem* 1983; 41: 287–290.
94. Maitre M, Mandel P. [Calcium-dependent liberation of gamma-hydroxybutyrate after depolarization of rat brain slices]. *C R Seances Acad Sci III* 1982; 295: 741–743.
95. Vayer P, Maitre M. Regional differences in depolarization-induced release of gamma-hydroxybutyrate from rat brain slices. *Neurosci Lett* 1988; 87: 99–103.
96. Benavides J, Rumigny JF, Bourguignon JJ, et al. High affinity binding sites for gamma-hydroxybutyric acid in rat brain. *Life Sci* 1982; 30: 953–961.
97. Hechler V, Bourguignon JJ, Wermuth CG, et al. gamma-Hydroxybutyrate uptake by rat brain striatal slices. *Neurochem Res* 1985; 10: 387–396.
98. Maitre M, Rumigny JF, Benavides J, et al. High affinity binding site for gamma-hydroxybutyric acid in rat brain. *Adv Biochem Psychopharmacol* 1983; 37: 441–453.
99. Snead 3rd OC, Liu CC. Gamma-hydroxybutyric acid binding sites in rat and human brain synaptosomal membranes. *Biochem Pharmacol* 1984; 33: 2587–2590.
100. Ratomponirina C, Hode Y, Hechler V, et al. gamma-Hydroxybutyrate receptor binding in rat brain is inhibited by guanyl nucleotides and pertussis toxin. *Neurosci Lett* 1995; 189: 51–53.
101. Mamelak M. HD. *Gamma-Hydroxybutyrate and oxidative stress*. . Taylor & Francis, 2002.
102. Hechler V, Gobaille S, Maitre M. Selective distribution pattern of gamma-hydroxybutyrate receptors in the rat forebrain and midbrain as revealed by quantitative autoradiography. *Brain Res* 1992; 572: 345–348.
103. Hechler V, Weissmann D, Mach E, et al. Regional distribution of high-affinity gamma-[3H]hydroxybutyrate binding sites as determined by quantitative autoradiography. *J Neurochem* 1987; 49: 1025–1032.
104. Kaupmann K, Cryan JF, Wellendorph P, et al. Specific gamma-hydroxybutyrate-binding sites but loss of pharmacological effects of gamma-hydroxybutyrate in GABA(B)(1)-deficient mice. *Eur J Neurosci* 2003; 18: 2722–2730.
105. Kawaguchi Y, Wilson CJ, Augood SJ, et al. Striatal interneurons: chemical, physiological and morphological characterization. *Trends Neurosci* 1995; 18: 527–535.
106. Maitre M, Ratomponirina C, Gobaille S, et al. Displacement of [3H] gamma-hydroxybutyrate binding by benzamide neuroleptics and prochlorperazine but not by other antipsychotics. *Eur J Pharmacol* 1994; 256: 211–214.
107. Kemmel V, Taleb O, Perard A, et al. Neurochemical and electrophysiological evidence for the existence of a functional gamma-hydroxybutyrate system in NCB-20 neurons. *Neuroscience* 1998; 86: 989–1000.
108. Cash CD, Gobaille S, Kemmel V, et al. Gamma-hydroxybutyrate receptor

- function studied by the modulation of nitric oxide synthase activity in rat frontal cortex punches. *Biochem Pharmacol* 1999; 58: 1815–1819.
109. Kozhechkin SX. Microiontophoretic study of the mechanism of action of gamma-hydroxybutyric acid. *Bull Expl Biol Med* 1979; 88: 1293–1296.
  110. Olpe HR, Koella WP. Inhibition of nigral and neocortical cells by gamma-hydroxybutyrate: a microiontophoretic investigation. *Eur J Pharmacol* 1979; 53: 359–364.
  111. Mathivet P, Bernasconi R, De Barry J, et al. Binding characteristics of gamma-hydroxybutyric acid as a weak but selective GABAB receptor agonist. *Eur J Pharmacol* 1997; 321: 67–75.
  112. Bernasconi R, Lauber J, Marescaux C, et al. Experimental absence seizures: potential role of gamma-hydroxybutyric acid and GABAB receptors. *J Neural Transm Suppl* 1992; 35: 155–177.
  113. Madden TE, Johnson SW. Gamma-hydroxybutyrate is a GABAB receptor agonist that increases a potassium conductance in rat ventral tegmental dopamine neurons. *J Pharmacol Exp Ther* 1998; 287: 261–265.
  114. Ocana M, Cendan C, Cobos E, et al. Potassium channels and pain: present realities and future opportunities. *Eur J Pharmacol* 2004; 500: 203–219.
  115. Lingenhoehl K, Brom R, Heid J, et al. Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. *Neuropharmacology* 1999; 38: 1667–1673.
  116. Engberg G, Nissbrandt H. gamma-Hydroxybutyric acid (GHBA) induces pacemaker activity and inhibition of substantia nigra dopamine neurons by activating GABAB-receptors. *Naunyn Schmiedebergs Arch Pharmacol* 1993; 348: 491–497.
  117. Williams SR, Turner JP, Crunelli V. Gamma-hydroxybutyrate promotes oscillatory activity of rat and cat thalamocortical neurons by a tonic GABAB, receptor-mediated hyperpolarization. *Neuroscience* 1995; 66: 133–141.
  118. Xie X, Smart TG. Gamma-hydroxybutyrate hyperpolarizes hippocampal neurones by activating GABAB receptors. *Eur J Pharmacol* 1992; 212: 291–294.
  119. Godbout R, Pivik RT. EEG and behavioral effects of gamma-hydroxybutyrate in the rabbit. *Life Sci* 1982; 31: 739–748.
  120. Snead 3rd OC. Evidence for a G protein-coupled gamma-hydroxybutyric acid receptor. *J Neurochem* 2000; 75: 1986–1996.
  121. Vayer P, Gobaille S, Mandel P, et al. 3'-5' cyclic-guanosine monophosphate increase in rat brain hippocampus after gamma-hydroxybutyrate administration. Prevention by valproate and naloxone. *Life Sci* 1987; 41: 605–610.
  122. Ito Y, Ishige K, Zaitse E, et al. gamma-Hydroxybutyric acid increases intracellular Ca<sup>2+</sup> concentration and nuclear cyclic AMP-responsive element- and activator protein 1 DNA-binding activities through GABAB receptor in cultured cerebellar granule cells. *J Neurochem* 1995; 65: 75–83.
  123. Vayer P, Maitre M. Gamma-hydroxybutyrate stimulation of the formation

- of cyclic GMP and inositol phosphates in rat hippocampal slices. *J Neurochem* 1989; 52: 1382–1387.
124. Lettieri JT, Fung HL. Dose-dependent pharmacokinetics and hypnotic effects of sodium gamma-hydroxybutyrate in the rat. *J Pharmacol Exp Ther* 1979; 208: 7–11.
  125. Shumate JS, Snead 3rd OC. Plasma and central nervous system kinetics of gamma-hydroxybutyrate. *Res Commun Chem Pathol Pharmacol* 1979; 25: 241–256.
  126. Nissbrandt H, Elverfors A, Engberg G. Pharmacologically induced cessation of burst activity in nigral dopamine neurons: significance for the terminal dopamine efflux. *Synapse* 1994; 17: 217–224.
  127. Roth RH, Doherty JD, Walters JR. Gamma-hydroxybutyrate: a role in the regulation of central dopaminergic neurons? *Brain Res* 1980; 189: 556–560.
  128. Bustos G, Kuhar MJ, Roth RH. Effect of gamma-hydroxybutyrate and gamma-butyrolactone on dopamine synthesis and uptake by rat striatum. *Biochem Pharmacol* 1972; 21: 2649–2652.
  129. Bustos G, Roth RH. Release of monoamines from the striatum and hypothalamus: effect of -hydroxybutyrate. *Br J Pharmacol* 1972; 46: 101–115.
  130. Broxterman HJ, Noach EL, van Valkenburg CF, et al. Cross-tolerance of dopamine metabolism to baclofen, gamma-butyrolactone and HA-966 in the striatum and olfactory tubercle of the rat. *Life Sci* 1981; 28: 973–981.
  131. Da Prada M, Keller HH. Baclofen and gamma-hydroxybutyrate: similar effects on cerebral dopamine neurones. *Life Sci* 1976; 19: 1253–1263.
  132. Kelly PH, Moore KE. Dopamine concentrations in the rat brain following injections into the substantia nigra of baclofen, gamma-aminobutyric acid, gamma-hydroxybutyric acid, apomorphine and amphetamine. *Neuropharmacology* 1978; 17: 169–174.
  133. Waldmeier PC, Fehr B. Effects of baclofen and gamma-hydroxybutyrate on rat striatal and mesolimbic 5-HT metabolism. *Eur J Pharmacol* 1978; 49: 177–184.
  134. Aghajanian GK, Roth RH. Gamma-hydroxybutyrate-induced increase in brain dopamine: localization by fluorescence microscopy. *J Pharmacol Exp Ther* 1970; 175: 131–138.
  135. Dyck LE, Kazakoff CW. Acceleration of the biosynthesis of rat striatal dopamine by incubation and by administration of gamma-butyrolactone. *J Neurosci Res* 1982; 8: 57–65.
  136. Gessa Vargiu, L., Crabai, F., Boero, G.C., Caboni, F., Camba, R. GL. *Selective increase of brain dopamine induced by gamma hydroxybutyrate*. . 1966.
  137. Gessa GL, Crabai F, Yargiu L, et al. Selective increase of brain dopamine induced by gamma-hydroxybutyrate: study of the mechanism of action. *J Neurochem* 1968; 15: 377–381.
  138. Lundborg P, Hedner T, Engel J. Catecholamine concentration in the developing rat brain after gamma-hydroxybutyric acid. *J Neurochem* 1980;

- 35: 425–429.
139. Pericic D, Walters JR. Dopamine in substantia nigra and cortex after gamma-butyrolactone treatment. *J Pharm Pharmacol* 1976; 28: 527–530.
  140. Spano PF, Tagliamonte A, Tagliamonte P, et al. Stimulation of brain dopamine synthesis by gamma-hydroxybutyrate. *J Neurochem* 1971; 18: 1831–1836.
  141. Hedner T, Lundborg P. Effect of gamma-hydroxybutyric acid on catecholamine synthesis and utilization in the developing rat brain. *J Neural Transm* 1982; 54: 19–28.
  142. Roth RH, Nowicky MC, Walters JR, et al. gamma-Hydroxybutyrate: effects on nonstriatal dopaminergic neurons. *Adv Biochem Psychopharmacol* 1977; 16: 483–488.
  143. Roth RH, Suhr Y. Mechanism of the gamma-hydroxybutyrate-induced increase in brain dopamine and its relationship to 'sleep'. *Biochem Pharmacol* 1970; 19: 3001–3012.
  144. Walters JR, Roth RH. Dopaminergic neurons: drug-induced antagonism of the increase in tyrosine hydroxylase activity produced by cessation of impulse flow. *J Pharmacol Exp Ther* 1974; 191: 82–91.
  145. Walters JR, Roth RH. Dopaminergic neurons - alteration in the sensitivity of tyrosine hydroxylase to inhibition by endovenous dopamine after cessation of impulse flow. *Biochem Pharmacol* 1976; 25: 649–654.
  146. Zivkovic B, Guidotti A, Costa E. The regulation of striatal tyrosine hydroxylase. Effects of gamma hydroxybutyric acid and haloperidol. *Naunyn Schmiedeberg Arch Pharmacol* 1975; 291: 193–200.
  147. Arluison M, Javoy-Agid F, Feuerstein C, et al. Histofluorescence analysis of several systems of catecholaminergic nerve fibres within the rat neostriatum revealed by either restricted lesions of the substantia nigra or gamma-hydroxybutyrate. *Brain Res Bull* 1982; 9: 355–365.
  148. Tunnicliff G. Significance of gamma-hydroxybutyric acid in the brain. *Gen Pharmacol* 1992; 23: 1027–1034.
  149. Chéramy A, Nieoullon A, Glowinski J. Stimulating effects of gamma-hydroxybutyrate on dopamine release from the caudate nucleus and the substantia nigra of the cat. *J Pharmacol Exp Ther* 1977; 203: 283–293.
  150. Hechler V, Gobaille S, Bourguignon JJ, et al. Extracellular events induced by gamma-hydroxybutyrate in striatum: a microdialysis study. *J Neurochem* 1991; 56: 938–944.
  151. Biggio G, Casu M, Corda MG, et al. Effect of muscimol, a GABA-mimetic agent, on dopamine metabolism in the mouse brain. *Life Sci* 1977; 21: 525–531.
  152. Snead 3rd OC, Bearden LJ. Naloxone overcomes the dopaminergic, EEG, and behavioral effects of gamma-hydroxybutyrate. *Neurology* 1980; 30: 832–838.
  153. Dingledine R, Iversen LL, Breuker E. Naloxone as a GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies. *Eur J Pharmacol* 1978; 47: 19–27.

154. Schmidt-Mutter C, Muller C, Zwiller J, et al. Gamma-hydroxybutyrate and cocaine administration increases mRNA expression of dopamine D1 and D2 receptors in rat brain. *Neuropsychopharmacology* 1999; 21: 662–669.
155. Miguez I, Aldegunde M, Duran R, et al. Effect of low doses of gamma-hydroxybutyric acid on serotonin, noradrenaline, and dopamine concentrations in rat brain areas. *Neurochem Res* 1988; 13: 531–533.
156. Spano E. PF and P. Stimulation of serotonin synthesis by anesthetic and non-anesthetic doses of gamma-hydroxybutyrate. . *Pharmacol Res Commun* 1973; 5: 55–69.
157. Boadle-Biber MC. *Biosynthesis of serotonin*. . New York.: N.N. Osborne. Wiley, 1982.
158. Gerra G, Caccavari R, Fontanesi B, et al. Naloxone and metergoline effects on growth hormone response to gamma-hydroxybutyric acid. *Int Clin Psychopharmacol* 1995; 10: 245–250.
159. Delitala G, Masala A, Alagna S, et al. Growth hormone and prolactin release in acromegalic patients following metergoline administration. *J Clin Endocrinol Metab* 1976; 43: 1382–1386.
160. Lason W, Przewlocka B, Przewlocki R. The effect of gamma-hydroxybutyrate and anticonvulsants on opioid peptide content in the rat brain. *Life Sci* 1983; 33 Suppl 1: 599–602.
161. Prodynorphin and proenkephalin mRNAs are increased in rat brain after acute and chronic administration of gamma-hydroxybutyrate. 1999; 1–4.
162. Vayer P, Mandel P, Maitre M. Gamma-hydroxybutyrate, a possible neurotransmitter. *Life Sci* 1987; 41: 1547–1557.
163. Crosby G, Ito M, Kaufman E, et al. Naloxone pretreatment alters the local cerebral metabolic effect of gamma-hydroxybutyrate in rats. *Brain Res* 1983; 275: 194–197.
164. Devoto P, Colombo G, Cappai F, et al. Naloxone antagonizes ethanol- but not gamma-hydroxybutyrate-induced sleep in mice. *Eur J Pharmacol* 1994; 252: 321–324.
165. Gobaille S, Schmidt C, Cupo A, et al. Characterization of methionine-enkephalin release in the rat striatum by in vivo dialysis: effects of gamma-hydroxybutyrate on cellular and extracellular methionine-enkephalin levels. *Neuroscience* 1994; 60: 637–648.
166. Sethy VH, Roth RH, Walters JR, et al. Effect of anesthetic doses of gamma-hydroxybutyrate on the acetylcholine content of rat brain. *Naunyn Schmiedebergs Arch Pharmacol* 1976; 295: 9–14.
167. Nava F, Carta G, Bortolato M, et al. gamma-Hydroxybutyric acid and baclofen decrease extracellular acetylcholine levels in the hippocampus via GABA(B) receptors. *Eur J Pharmacol* 2001; 430: 261–263.
168. Shefner SA, Osmanovic SS. GABAA and GABAB receptors and the ionic mechanisms mediating their effects on locus coeruleus neurons. *Prog Brain Res* 1991; 88: 187–195.
169. Persson B, Henning M. Central cardiovascular effects of gamma-hydroxybutyric acid: interactions with noradrenaline, serotonin, dopamine

- and acetylcholine transmission. *Acta Pharmacol Toxicol* 1980; 47: 335–346.
170. Szabo ST, Gold MS, Goldberger BA, et al. Effects of sustained gamma-hydroxybutyrate treatments on spontaneous and evoked firing activity of locus coeruleus norepinephrine neurons. *Biol Psychiatry* 2004; 55: 934–939.
  171. Sleep Neurobiology for the Clinician. 2004; 1–10.
  172. Broughton R, Mamelak M. Effects of nocturnal gamma-hydroxybutyrate on sleep/waking patterns in narcolepsy-cataplexy. *Can J Neurol Sci* 1980; 7: 23–31.
  173. A Pilot Study on the Effects of Sodium Oxybate on Sleep Architecture and Daytime Alertness in Narcolepsy. 2004; 1–8.
  174. Mamelak M Stokan O. EJM. The effects of gamma-hydroxybutyrate on sleep. . *Biol Psychiatry* 1977; 12: 273–288.
  175. Xyrem. A randomized, double blind, placebo-controlled multicenter trial comparing the effects of three doses of orally administered sodium oxybate with placebo for the treatment of narcolepsy. *Sleep* 2002; 25: 42–49.
  176. Sodium oxybate demonstrates long-term efficacy for the treatment of cataplexy in patients with narcolepsy. *Sleep Med* 2004; 5: 119–123.
  177. Grou USXM-CS. The Abrupt Cessation of Therapeutically Administered Sodium Oxybate (GHB) Does Not Cause Withdrawal Symptoms. *Clin Toxicol* 2003; 41: 131–135.
  178. Galloway GP, Frederick-Osborne SL, Seymour R, et al. Abuse and therapeutic potential of gamma-hydroxybutyric acid. *Alcohol* 2000; 20: 263–269.
  179. Ferraro L, Tanganelli S, O'Connor WT, et al. gamma-Hydroxybutyrate modulation of glutamate levels in the hippocampus: an in vivo and in vitro study. *J Neurochem* 2001; 78: 929–939.
  180. Berton F, Brancucci A, Beghe F, et al. Gamma-Hydroxybutyrate inhibits excitatory postsynaptic potentials in rat hippocampal slices. *Eur J Pharmacol* 1999; 380: 109–116.
  181. King MA, Thinschmidt JS, Walker DW. Gammahydroxybutyrate (GHB) receptor ligand effects on evoked synaptic field potentials in CA1 of the rat hippocampal slice. *J Neural Transm* 1997; 104: 1177–1193.
  182. Banerjee PK, Snead 3rd OC. Presynaptic gamma-hydroxybutyric acid (GHB) and gamma-aminobutyric acidB (GABAB) receptor-mediated release of GABA and glutamate (GLU) in rat thalamic ventrobasal nucleus (VB): a possible mechanism for the generation of absence-like seizures induced by GH. *J Pharmacol Exp Ther* 1995; 273: 1534–1543.
  183. Oyama T, Takiguchi M. Effects of gamma-hydroxybutyrate and surgery on plasma human growth hormone and insulin levels. *Agressologie* 1970; 11: 289–298.
  184. Van Cauter E, Plat L, Scharf MB, et al. Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in

- normal young Men. *J Clin Invest* 1997; 100: 745–753.
185. Van Cauter E, Copinschi G. Interrelationships between growth hormone and sleep. *Growth Horm IGF Res* 2000; 10 Suppl B: S57-62.
  186. Gerra G, Caccavari R, Fontanesi B, et al. Flumazenil effects on growth hormone response to gamma-hydroxybutyric acid. *Int Clin Psychopharmacol* 1994; 9: 211–215.
  187. Volpi R, Chiodera P, Caffarra P, et al. Different control mechanisms of growth hormone (GH) secretion between gamma-amino- and gamma-hydroxy-butyrac acid: neuroendocrine evidence in Parkinson's disease. *Psychoneuroendocrinology* 1997; 22: 531–538.
  188. Pathophysiology of the Neuroregulation of Growth Hormone Secretion in Experimental Animals and the Human\*. 1998; 1–81.
  189. Volpi R, Chiodera P, Caffarra P, et al. Muscarinic cholinergic mediation of the GH response to gamma-hydroxybutyric acid: neuroendocrine evidence in normal and parkinsonian subjects. *Psychoneuroendocrinology* 2000; 25: 179–185.
  190. Overeem S, Kok SW, Lammers GJ, et al. Somatotrophic axis in hypocretin-deficient narcoleptic humans: altered circadian distribution of GH-secretory events. *Am J Physiol Metab* 2003; 284: E641–E647.
  191. Donjacour CEHM, Aziz NA, Roelfsema F, et al. The effect of sodium oxybate on growth hormone secretion in narcolepsy patients and healthy controls. *Am J Physiol Metab* 2011; 300: E1069–E1075.
  192. Barbaccia ML, Carai MA, Colombo G, et al. Endogenous gamma-aminobutyric acid (GABA)(A) receptor active neurosteroids and the sedative/hypnotic action of gamma-hydroxybutyric acid (GHB): a study in GHB-S (sensitive) and GHB-R (resistant) rat lines. *Neuropharmacology* 2005; 49: 48–58.
  193. Freeman ME, Kanyicska B, Lerant A, et al. Prolactin: structure, function, and regulation of secretion. *Physiol Rev* 2000; 80: 1523–1631.
  194. Bole-Feysot C, Goffin V, Edery M, et al. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev* 1998; 19: 225–268.
  195. Carter LP, Koek W, France CP. Behavioral analyses of GHB: receptor mechanisms. *Pharmacol Ther* 2009; 121: 100–114.
  196. Donjacour CEHM, Aziz NA, Frölich M, et al. Sodium oxybate increases prolactin secretion in narcolepsy patients and healthy controls. *Eur J Endocrinol* 2011; 164: 363–370.
  197. Zeitzer JM, Duffy JF, Lockley SW, et al. Plasma melatonin rhythms in young and older humans during sleep, sleep deprivation, and wake. *SLEEP-NEW YORK THEN WESTCHESTER* 2007; 30: 1437.
  198. Reiter RJ, Tan D-X, Fuentes-Broto L. Melatonin: a multitasking molecule. *Prog Brain Res* 2010; 181: 127–151.
  199. Appelbaum L, Wang GX, Maro GS, et al. Sleep–wake regulation and hypocretin–melatonin interaction in zebrafish. *Proc Natl Acad Sci* 2009; 106: 21942–21947.

200. Donjacour CEHM, Kalsbeek A, Overeem S, et al. Altered circadian rhythm of melatonin concentrations in hypocretin-deficient men. *Chronobiol Int* 2012; 29: 356–362.
201. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; 407: 908–913.
202. Hypothalamic Orexin Neurons Regulate Arousal According to Energy Balance in Mice. 2003; 1–14.
203. Gao Z, Yin J, Zhang J, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009; 58: 1509–1517.
204. Pimpin L, Wu JHY, Haskelberg H, et al. Is butter back? A systematic review and meta-analysis of butter consumption and risk of cardiovascular disease, diabetes, and total mortality. *PLoS One* 2016; 11: e0158118.
205. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; 143: 913–916. e7.
206. Hartstra A V, Bouter KEC, Bäckhed F, et al. Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care* 2015; 38: 159–165.
207. Christensen DP, Dahllöf M, Lundh M, et al. Histone deacetylase (HDAC) inhibition as a novel treatment for diabetes mellitus. *Mol Med* 2011; 17: 378.
208. Klein C, Kemmel V, Taleb O, et al. Pharmacological doses of gamma-hydroxybutyrate (GHB) potentiate histone acetylation in the rat brain by histone deacetylase inhibition. *Neuropharmacology* 2009; 57: 137–147.
209. Johannsson G, Mårin P, Lönn L, et al. Growth Hormone Treatment of Abdominally Obese Men Reduces Abdominal Fat Mass, Improves Glucose and Lipoprotein Metabolism, and Reduces Diastolic Blood Pressure 1. *J Clin Endocrinol Metab* 1997; 82: 727–734.
210. Junnila RK, List EO, Berryman DE, et al. The GH/IGF-1 axis in ageing and longevity. *Nat Rev Endocrinol* 2013; 9: 366–376.
211. Donjacour CE, Aziz NA, Overeem S, et al. Glucose and fat metabolism in narcolepsy and the effect of sodium oxybate: a hyperinsulinemic-euglycemic clamp study. *Sleep* 2014; 37: 795–801.
212. Saper CB, Lowell BB. The hypothalamus. *Curr Biol* 2014; 24: R1111–R1116.
213. Romeijn N, Raymann RJEM, Møst E, et al. Sleep, vigilance, and thermosensitivity. *Pflügers Arch J Physiol* 2012; 463: 169–176.
214. Kräuchi K, Cajochen C, Werth E, et al. Physiology: warm feet promote the rapid onset of sleep. *Nature* 1999; 401: 36–37.
215. Van Someren EJW. Mechanisms and functions of coupling between sleep and temperature rhythms. *Prog Brain Res* 2006; 153: 309–324.
216. Yetish G, Kaplan H, Gurven M, et al. Natural sleep and its seasonal variations in three pre-industrial societies. *Curr Biol* 2015; 25: 2862–2868.
217. Fronczek R, Overeem S, Lammers GJ, et al. Altered skin-temperature regulation in narcolepsy relates to sleep propensity. *Sleep* 2006; 29: 1444–

- 1449.
218. Van Someren EJW. Sleep propensity is modulated by circadian and behavior-induced changes in cutaneous temperature. *J Therm Biol* 2004; 29: 437–444.
  219. Kaufman EE, Porrino LJ, Nelson T. Pyretic action of low doses of gamma-hydroxybutyrate in rats. *Biochem Pharmacol* 1990; 40: 2637–2640.
  220. Spatz M, Abe K, Smialek M, et al. Evaluation of gamma-hydroxybutyrate treatment in experimental cerebral ischemia. In: *Pathophysiology and pharmacotherapy of cerebrovascular disorders*. Wilzrock Baden Baden, 1980, pp. 286–289.
  221. Yosunkaya A, Ak A, Bariskaner H, et al. Effect of gamma-hydroxybutyric acid on lipid peroxidation and tissue lactate level in experimental head trauma. *J Trauma* 2004; 56: 585–590.
  222. Chin RL, Sporer KA, Cullison B, et al. Clinical course of gamma-hydroxybutyrate overdose. *Ann Emerg Med* 1998; 31: 716–22.
  223. Krul J, Girbes ARJ. Gamma-hydroxybutyrate: Experience of 9 years of gamma-hydroxybutyrate (GHB)-related incidents during rave parties in The Netherlands. *Clin Toxicol* 2011; 49: 311–315.
  224. Queva C, Bremner-Danielsen M, Edlund A, et al. Effects of GABA agonists on body temperature regulation in GABA(B(1))<sup>-/-</sup> mice. *Br J Pharmacol* 2003; 140: 315–322.
  225. Lin MT, Chern YF, Wang HS, et al. Effects of gamma-hydroxybutyric acid on metabolic, respiratory and vasomotor activities and body temperature in rats. *J Pharmacol Exp Ther* 1979; 211: 167–170.
  226. van der Heide A, Werth E, Donjacour CE, et al. Core Body and Skin Temperature in Type 1 Narcolepsy in Daily Life; Effects of Sodium Oxybate and Prediction of Sleep Attacks. *Sleep* 2016; 39: 1941.
  227. Mease PJ, Clauw DJ, Christensen R, et al. Toward development of a fibromyalgia responder index and disease activity score: OMERACT module update. *J Rheumatol Suppl* 2011; 38: 1487–1495.
  228. Russell IJ, Holman AJ, Swick TJ, et al. Sodium oxybate reduces pain, fatigue, and sleep disturbance and improves functionality in fibromyalgia: results from a 14-week, randomized, double-blind, placebo-controlled study. *Pain* 2011; 152: 1007–1017.
  229. Spaeth M, Bennett RM, Benson BA, et al. Sodium oxybate therapy provides multidimensional improvement in fibromyalgia: results of an international phase 3 trial. *Ann Rheum Dis* 2012; annrheumdis-2011-200418.
  230. Rechtschaffen A, Gilliland MA, Bergmann BM, et al. Physiological correlates of prolonged sleep deprivation in rats. *Science (80- )* 1983; 221: 182–184.
  231. Walsh JK, Hall-Porter JM, Griffin KS, et al. Enhancing slow wave sleep with sodium oxybate reduces the behavioral and physiological impact of sleep loss. *Sleep* 2010; 33: 1217–1225.
  232. McNay DEG, Speakman JR. High fat diet causes rebound weight gain. *Mol Metab* 2013; 2: 103–108.

233. Fisler JS, Shimizu H, Bray GA. Brain 3-hydroxybutyrate, glutamate, and GABA in a rat model of dietary obesity. *Physiol Behav* 1989; 45: 571–577.
234. Borbély AA, Huston JP. Effects of Gamma-Butyrolactone on Body Temperature and Evoked Potentials in the Rat1. In: *Sleep* 1972. Karger Publishers, 1973, p. 355a–359.
235. Jouvet M, Cier A, Mounier D, et al. [Effect of sodium 4-hydroxybutyrate and 4-butyrolactone on cat EEG and behavior.]. *C R Seances Soc Biol Fil* 1961; 155: 1313–1316.
236. Laborit H. Correlations between protein and serotonin synthesis during various activities of the central nervous system (slow and desynchronized sleep, learning and memory, sexual activity, morphine tolerance, aggressiveness, and pharmacological action of sodium gam. *Res Commun Chem Pathol Pharmacol* 1972; 3: 51–81.
237. Godschalk M, Dzoljic MR, Bonta IL. Slow wave sleep and a state resembling absence epilepsy induced in the rat by gamma-hydroxybutyrate. *Eur J Pharmacol* 1977; 44: 105–111.
238. Winters WD, Spooner CE. Various Seizure Activities Following Gamma-Hydroxybutyrate. *Int J Neuropharmacol* 1965; 31: 197–200.
239. Marcus RJ, Winters WD, Mori K, et al. EEG and behavioral comparison of the effects of gamma-hydroxybutyrate, gamma-butyrolactone and short chain fatty acids in the rat. *Int J Neuropharmacol* 1967; 6: 175–185.
240. Bosch OG, Eisenegger C, Gertsch J, et al. Gamma-hydroxybutyrate enhances mood and prosocial behavior without affecting plasma oxytocin and testosterone. *Psychoneuroendocrinology* 2015; 62: 1–10.
241. Bosch OG, Havranek MM, Baumberger A, et al. Neural underpinnings of prosexual effects induced by gamma-hydroxybutyrate in healthy male humans. *Eur Neuropsychopharmacol*.
242. Laborit H, Jouany JM, Gerard J, et al. [Generalities concerning the experimental study and clinical use of gamma hydroxybutyrate of Na.]. *Agressologie* 1960; 1: 397–406.
243. Laborit H, Jouany JM, Gerard J, et al. [Summary of an experimental and clinical study on a metabolic substrate with inhibitory central action: sodium 4-hydroxybutyrate.]. *Press Med* 1960; 68: 1867–1869.
244. Laborit H, Buchard F, Laborit G, et al. [Use of sodium 4-hydroxybutyrate in anesthesia and resuscitation.]. *Agressologie* 1960; 1: 549–560.
245. Jouany JM, Gerard J, Weber B, et al. [Interference of various pharmacological agents with the establishment and character of sleep induced by 4-hydroxybutyric acid.]. *Agressologie* 1961; 2: 45–47.
246. Reder Mednick AS, Brown P, Spire JP, Van Cauter E, Wollmann RL, Cervenakova L, Goldfarb LG, Garay A, Ovsiew F, et al. AT. Clinical and genetic studies of fatal familial insomnia. . *Neurology* 1995; 45: 1068–1075.
247. Moldofsky H. Sleep and musculoskeletal pain. *Am J Med* 1986; 81: 85–89.
248. Moldofsky H, Scarisbrick P, England R, et al. Musculoskeletal symptoms and non-REM sleep disturbance in patients with ‘fibrositis syndrome’ and healthy subjects. *Psychosom Med* 1975; 37: 341–351.

249. Lentz MJ, Landis CA, Rothermel J, et al. Effects of selective slow wave sleep disruption on musculoskeletal pain and fatigue in middle aged women. *J Rheumatol* 1999; 26: 1586–1592.
250. Moldofsky H, Inhaber NH, Guinta DR, et al. Effects of sodium oxybate on sleep physiology and sleep/wake-related symptoms in patients with fibromyalgia syndrome: a double-blind, randomized, placebo-controlled study. *J Rheumatol* 2010; 37: 2156–2166.
251. Russell IJ, Perkins AT, Michalek JE, et al. Sodium oxybate relieves pain and improves function in fibromyalgia syndrome: a randomized, double-blind, placebo-controlled, multicenter clinical trial. *Arthritis Rheum* 2009; 60: 299–309.
252. Roizenblatt S, Moldofsky H, Benedito-Silva AA, et al. Alpha sleep characteristics in fibromyalgia. *Arthritis Rheum* 2001; 44: 222–230.
253. Vijayan S, Klerman EB, Adler GK, et al. Thalamic mechanisms underlying alpha-delta sleep with implications for fibromyalgia. *J Neurophysiol* 2015; 114: 1923–1930.
254. Rye DB, Bliwise DL, Dihenia B, et al. FAST TRACK: daytime sleepiness in Parkinson's disease. *J Sleep Res* 2000; 9: 63–69.
255. Rye DB, Jankovic J. Emerging views of dopamine in modulating sleep/wake state from an unlikely source: PD. *Neurology* 2002; 58: 341–346.
256. Ondo WG, Perkins T, Swick T, et al. Nocturnal sodium oxybate for daytime sedation and fatigue in Parkinson's disease, a polysomnogram trial. *World Congress of Movement Disorders*.
257. Burns A, Iliffe S. Alzheimer's disease. *BMJ* 2009; 338: b158.
258. Burns A, Jacoby R, Levy R. Psychiatric phenomena in Alzheimer's disease. IV: Disorders of behaviour. *Br J Psychiatry* 1990; 157: 86–94.
259. Maitre M, Klein C, Mensah-Nyagan AG. A proposed preventive role for Gamma-hydroxybutyrate (Xyrem(R)) in Alzheimer's disease. *Alzheimers Res Ther* 2016; 8: 37.
260. Mawuenyega KG, Sigurdson W, Ovod V, et al. Decreased clearance of CNS  $\beta$ -amyloid in Alzheimer's disease. *Science (80- )* 2010; 330: 1774.
261. Miners JS, Barua N, Kehoe PG, et al. A $\beta$ -degrading enzymes: potential for treatment of Alzheimer disease. *J Neuropathol Exp Neurol* 2011; 70: 944–959.
262. Nalivaeva NN, Beckett C, Belyaev ND, et al. Are amyloid-degrading enzymes viable therapeutic targets in Alzheimer's disease? *J Neurochem* 2012; 120: 167–185.
263. Mamelak M. Alzheimer's disease, oxidative stress and gammahydroxybutyrate. *Neurobiol Aging* 2007; 28: 1340–1360.
264. Klein C, Mathis C, Leva G, et al.  $\gamma$ -Hydroxybutyrate (Xyrem) ameliorates clinical symptoms and neuropathology in a mouse model of Alzheimer's disease. *Neurobiol Aging* 2015; 36: 832–844.
265. Guarnieri B, Adorni F, Musicco M, et al. Prevalence of Sleep Disturbances in Mild Cognitive Impairment and Dementing Disorders: A Multicenter Italian Clinical Cross-Sectional Study on 431 Patients. *Dement Geriatr Cogn*

- Disord* 2012; 33: 50–58.
266. Hita-Yanez E, Atienza M, Gil-Neciga E, et al. Disturbed Sleep Patterns in Elders with Mild Cognitive Impairment: The Role of Memory Decline and ApoE &#949; 4 Genotype. *Curr Alzheimer Res* 2012; 9: 290–297.
  267. Osorio RS, Pirraglia E, Agüera-Ortiz LF, et al. Greater risk of Alzheimer’s disease in older adults with insomnia. *J Am Geriatr Soc* 2011; 59: 559–62.
  268. Osorio RS, Gumb T, Pirraglia E, et al. Sleep-disordered breathing advances cognitive decline in the elderly. *Neurology* 2015; 84: 1964–1971.
  269. Lim ASP, Kowgier M, Yu L, et al. Sleep Fragmentation and the Risk of Incident Alzheimer’s Disease and Cognitive Decline in Older Persons. *Sleep* 2013; 36: 1027–1032.
  270. Ancoli-Israel S, Palmer BW, Cooke JR, et al. Cognitive Effects of Treating Obstructive Sleep Apnea in Alzheimer’s Disease: A Randomized Controlled Study. *J Am Geriatr Soc* 2008; 56: 2076–2081.
  271. Liguori C, Romigi A, Nuccetelli M, et al. Orexinergic System Dysregulation, Sleep Impairment, and Cognitive Decline in Alzheimer Disease. *JAMA Neurol* 2014; 71: 1498.
  272. Mander BA, Marks SM, Vogel JW, et al.  $\beta$ -amyloid disrupts human NREM slow waves and related hippocampus-dependent memory consolidation. *Nat Neurosci* 2015; 18: 1051–1057.
  273. Sprecher KE, Bendlin BB, Racine AM, et al. Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults. *Neurobiol Aging* 2015; 36: 2568–2576.
  274. Ancoli-Israel S, Klauber MR, Butters N, et al. Dementia in Institutionalized Elderly: Relation to Sleep Apnea. *J Am Geriatr Soc* 1991; 39: 258–263.
  275. Prinz PN, Vitaliano PP, Vitiello M V., et al. Sleep, EEG and mental function changes in senile dementia of the Alzheimer’s type. *Neurobiol Aging* 1982; 3: 361–370.
  276. Kang J-E, Lim MM, Bateman RJ, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science* 2009; 326: 1005–7.
  277. Roh JH, Huang Y, Bero AW, et al. Disruption of the sleep-wake cycle and diurnal fluctuation of  $\beta$ -amyloid in mice with Alzheimer’s disease pathology. *Sci Transl Med* 2012; 4: 150ra122.
  278. Xie L, Kang H, Xu Q, et al. Sleep Drives Metabolite Clearance from the Adult Brain. *Science (80- )* 2013; 342: 373–377.
  279. Ju Y-ES, Lucey BP, Holtzman DM. Sleep and Alzheimer disease pathology—a bidirectional relationship. *Nat Rev Neurol* 2013; 10: 115–119.
  280. Everson CA, Henchen CJ, Szabo A, et al. Cell Injury and Repair Resulting from Sleep Loss and Sleep Recovery in Laboratory Rats. *Sleep* 2014; 37: 1929–1940.
  281. Villafuerte G, Miguel-Puga A, Murillo Rodríguez E, et al. Sleep Deprivation and Oxidative Stress in Animal Models: A Systematic Review. *Oxid Med Cell Longev* 2015; 2015: 1–15.
  282. Misonou H, Morishima-Kawashima M, Ihara Y. Oxidative Stress Induces

- Intracellular Accumulation of Amyloid  $\beta$ -Protein (A $\beta$ ) in Human Neuroblastoma Cells. *Biochemistry* 2000; 39: 6951–6959.
283. Yatin SM, Varadarajan S, Link CD, et al. In vitro and in vivo oxidative stress associated with Alzheimer's amyloid beta-peptide (1-42). *Neurobiol Aging* 1999; 20: 325–30-42.
  284. Kurup P, Zhang Y, Xu J, et al. A -Mediated NMDA Receptor Endocytosis in Alzheimer's Disease Involves Ubiquitination of the Tyrosine Phosphatase STEP61. *J Neurosci* 2010; 30: 5948–5957.
  285. Steriade M, Nuñez A, Amzica F. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci* 1993; 13: 3252–65.
  286. Mander BA, Winer JR, Jagust WJ, et al. Sleep: A Novel Mechanistic Pathway, Biomarker, and Treatment Target in the Pathology of Alzheimer's Disease? *Trends Neurosci* 2016; 39: 552–566.
  287. Kaestner EJ, Wixted JT, Mednick SC. Pharmacologically Increasing Sleep Spindles Enhances Recognition for Negative and High-arousal Memories. *J Cogn Neurosci* 2013; 25: 1597–1610.
  288. Maitre M, Klein C, Mensah-Nyagan AG. Mechanisms for the Specific Properties of  $\gamma$ -Hydroxybutyrate in Brain. *Med Res Rev* 2016; 36: 363–388.
  289. Busche MA, Kekuš M, Adelsberger H, et al. Rescue of long-range circuit dysfunction in Alzheimer's disease models. *Nat Neurosci* 2015; 18: 1623–1630.
  290. Broughton and Mamelak M. R. *Gamma-hydroxybutyrate in the treatment of compound narcolepsy: a preliminary report. I.* Spectrum, New York., 1976.
  291. Broughton R, Mamelak M. The treatment of narcolepsy-cataplexy with nocturnal gamma-hydroxybutyrate. *Can J Neurol Sci* 1979; 6: 1–6.
  292. Mamelak M, Scharf MB, Woods M. Treatment of narcolepsy with gamma-hydroxybutyrate. A review of clinical and sleep laboratory findings. *Sleep* 1986; 9: 285–289.
  293. Scharf MB, Brown D, Woods M, et al. The effects and effectiveness of gamma-hydroxybutyrate in patients with narcolepsy. *J Clin Psychiatry* 1985; 46: 222–225.
  294. Scrima L, Hartman PG, Johnson Jr. FH, et al. Efficacy of gamma-hydroxybutyrate versus placebo in treating narcolepsy-cataplexy: double-blind subjective measures. *Biol Psychiatry* 1989; 26: 331–343.
  295. Lammers GJ, Arends J, Declerck AC, et al. Gammahydroxybutyrate and narcolepsy: a double-blind placebo-controlled study. *Sleep* 1993; 16: 216–220.
  296. Scrima L, Hartman PG, Johnson Jr. FH, et al. The effects of gamma-hydroxybutyrate on the sleep of narcolepsy patients: a double-blind study. *Sleep* 1990; 13: 479–490.
  297. Group USXMS. A 12-month, open-label, multicenter extension trial of orally administered sodium oxybate for the treatment of narcolepsy. *Sleep* 2003; 26: 31–35.

298. A Double-Blind, Placebo-Controlled Study Demonstrates Sodium Oxybate Is Effective for the Treatment of Excessive Daytime Sleepiness in Narcolepsy. 2005; 1–7.
299. Sodium Oxybate Improves Excessive Daytime Sleepiness in Narcolepsy. 2006; 1–8.
300. Blundell JE, Gillett A. Control of food intake in the obese. *Obes Res* 2001; 9 Suppl 4: 263S–270S.
301. Blundell JE, Goodson S, Halford JC. Regulation of appetite: role of leptin in signalling systems for drive and satiety. *Int J Obes Relat Metab Disord* 2001; 25 Suppl 1: S29–34.
302. Saper CB. Staying awake for dinner: hypothalamic integration of sleep, feeding, and circadian rhythms. *Prog Brain Res* 2006; 153: 243–252.
303. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. *Cell* 2001; 104: 531–543.
304. Flegal KM, Carroll MD, Kuczmarski RJ, et al. Overweight and obesity in the United States: prevalence and trends, 1960–1994. *Int J Obes Relat Metab Disord* 1998; 22: 39–47.
305. Prevalence and Trends in Obesity Among US Adults, 1999–2008. 2010; 1–8.
306. BRFSS. BRFSS, Behavioral Risk Factor Surveillance System <http://www.cdc.gov/brfss/> (2010).
307. *Vital Signs: State-Specific Obesity Prevalence Among Adults —United States 2009*. 2010.
308. Fontaine KR, Barofsky I. Obesity and health-related quality of life. *Obes Rev* 2001; 2: 173–182.
309. Flegal KM. Estimating the impact of obesity. *Soz Präventivmed* 2005; 50: 73–74.
310. Flegal KM, Graubard BI, Williamson DF, et al. Cause-specific excess deaths associated with underweight, overweight, and obesity. *JAMA* 2007; 298: 2028–2037.
311. Flegal KM, Williamson DF, Graubard BI. Obesity and cancer. *N Engl J Med* 2003; 349: 502–504.
312. Maiese DR. Healthy people 2010—leading health indicators for women. *Womens Heal Issues* 2002; 12: 155–164.
313. Cai L, Lubitz J, Flegal KM, et al. The predicted effects of chronic obesity in middle age on medicare costs and mortality. *Med Care* 2010; 48: 510–517.
314. Stein CJ, Colditz GA. The epidemic of obesity. *J Clin Endocrinol Metab* 2004; 89: 2522–2525.
315. Hill JO, Wyatt HR, Reed GW, et al. Obesity and the environment: where do we go from here? *Science (80- )* 2003; 299: 853–855.
316. World Health Organization. *Obesity: Preventing and Managing the Global Epidemic*. Geneva, Switzerland: World Health Organization, 2000.
317. Chaput JP, Leblanc C, Perusse L, et al. Risk factors for adult overweight and obesity in the Quebec Family Study: have we been barking up the wrong

- tree? *Obes (Silver Spring)* 2009; 17: 1964–1970.
318. Magee CA, Iverson DC, Huang XF, et al. A link between chronic sleep restriction and obesity: methodological considerations. *Public Health* 2008; 122: 1373–1381.
  319. Obesity and Sleep: A Bidirectional Association? 2010; 1–2.
  320. Ogden CL, Yanovski SZ, Carroll MD, et al. The epidemiology of obesity. *Gastroenterology* 2007; 132: 2087–2102.
  321. Spiegel K, Tasali E, Penev P, et al. Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Ann Intern Med* 2004; 141: 846–850.
  322. 2005 ‘Sleep in America’ Poll <http://www.sleepfoundation.org/product.nsf-2005-sleep-america-poll> (2005).
  323. Lauderdale DS, Knutson KL, Rathouz PJ, et al. Cross-sectional and Longitudinal Associations Between Objectively Measured Sleep Duration and Body Mass Index: The CARDIA Sleep Study. *Am J Epidemiol* 2009; 170: 805–813.
  324. Taheri S, Lin L, Austin D, et al. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLoS Med* 2004; 1: e62.
  325. Kripke DF, Garfinkel L, Wingard DL, et al. Mortality associated with sleep duration and insomnia. *Arch Gen Psychiatry* 2002; 59: 131–136.
  326. Foundation NS. "Sleep in America" Poll.
  327. Foundation NS. Sleep for America Poll. *Summ Find*.
  328. Knutson KL, Spiegel K, Penev P, et al. The metabolic consequences of sleep deprivation. *Sleep Med Rev* 2007; 11: 163–178.
  329. Locard E, Mamelie N, Billette A, et al. Risk factors of obesity in a five year old population. Parental versus environmental factors. *Int J Obes Relat Metab Disord* 1992; 16: 721–729.
  330. Vioque J, Torres A, Quiles J. Time spent watching television, sleep duration and obesity in adults living in Valencia, Spain. *Int J Obes Relat Metab Disord* 2000; 24: 1683–1688.
  331. von Kries R, Toschke AM, Wurmser H, et al. Reduced risk for overweight and obesity in 5- and 6-y-old children by duration of sleep--a cross-sectional study. *Int J Obes Relat Metab Disord* 2002; 26: 710–716.
  332. Gupta NK, Mueller WH, Chan W, et al. Is obesity associated with poor sleep quality in adolescents? *Am J Hum Biol* 2002; 14: 762–768.
  333. Hasler G, Buysse DJ, Klaghofer R, et al. The association between short sleep duration and obesity in young adults: a 13-year prospective study. *Sleep* 2004; 27: 661–666.
  334. Heslop P, Smith GD, Metcalfe C, et al. Sleep duration and mortality: The effect of short or long sleep duration on cardiovascular and all-cause mortality in working men and women. *Sleep Med* 2002; 3: 305–314.
  335. Park SE, Kim HM, Kim DH, et al. The association between sleep duration

- and general and abdominal obesity in Koreans: data from the Korean National Health and Nutrition Examination Survey, 2001 and 2005. *Obesity* 2009; 17: 767–771.
336. Taheri S. The link between short sleep duration and obesity: we should recommend more sleep to prevent obesity. *Arch Dis Child* 2006; 91: 881–884.
  337. Gangwisch JE, Malaspina D, Boden-Albala B, et al. Inadequate sleep as a risk factor for obesity: analyses of the NHANES I. *Sleep* 2005; 28: 1289–1296.
  338. Cappuccio FP, Taggart FM, Kandala NB, et al. Meta-analysis of short sleep duration and obesity in children and adults. *Sleep* 2008; 31: 619–626.
  339. Patel SR, Blackwell T, Redline S, et al. The association between sleep duration and obesity in older adults. *Int J Obes* 2008; 32: 1825–1834.
  340. Association of Short Sleep Duration with Weight Gain and Obesity at 1-Year Follow-Up: A Large-Scale Prospective Study. 2010; 1–10.
  341. A Twin Study of Sleep Duration and Body Mass Index. 2010; 1–7.
  342. Associations Between Sleep Duration Patterns and Overweight/Obesity at Age 6. 2008; 1–8.
  343. Padez C, Mourao I, Moreira P, et al. Long sleep duration and childhood overweight/obesity and body fat. *Am J Hum Biol* 2009; 21: 371–376.
  344. Ozturk A, Mazicioglu MM, Poyrazoglu S, et al. The relationship between sleep duration and obesity in Turkish children and adolescents. *Acta Paediatr* 2009; 98: 699–702.
  345. Knutson KL, Lauderdale DS. Sleep Duration and Overweight in Adolescents: Self-reported Sleep Hours Versus Time Diaries. *Pediatrics* 2007; 119: e1056–e1062.
  346. Danielsen Y, Pallesen S, Stormark K, et al. The relationship between school day sleep duration and body mass index in Norwegian children (aged 10–12). *Int J Pediatr Obes* 2010; 5: 214–220.
  347. Shortened Nighttime Sleep Duration in Early Life and Subsequent Childhood Obesity. 2010; 1–6.
  348. Nielsen LS, Danielsen K V, Sørensen TIA. Short sleep duration as a possible cause of obesity: critical analysis of the epidemiological evidence. *Obes Rev* 2011; 12: 78–92.
  349. Van Den Berg JF, Neven AK, Tulen JHM, et al. Actigraphic sleep duration and fragmentation are related to obesity in the elderly: the Rotterdam Study. *Int J Obes* 2008; 32: 1083–1090.
  350. Patel SR, Ayas NT, Malhotra MR, et al. A prospective study of sleep duration and mortality risk in women. *Sleep* 2004; 27: 440–444.
  351. Theorell-Haglow J, Berne C, Janson C, et al. Associations between short sleep duration and central obesity in women. *Sleep* 2010; 33: 593–598.
  352. Hart CN, Carskadon MA, Considine R V, et al. Changes in children’s sleep duration on food intake, weight, and leptin. *Pediatrics* 2013; 132: e1473–80.

353. Association of Sleep Time With Diabetes Mellitus and Impaired Glucose Tolerance. 2005; 1–6.
354. Shin C, Kim J, Lee S, et al. Association of habitual snoring with glucose and insulin metabolism in nonobese Korean adult men. *Am J Respir Crit Care Med* 2005; 171: 287–291.
355. Elmasry A, Janson C, Lindberg E, et al. The role of habitual snoring and obesity in the development of diabetes: a 10-year follow-up study in a male population. *J Intern Med* 2000; 248: 13–20.
356. Al-Delaimy WK, Manson JE, Willett WC, et al. Snoring as a risk factor for type II diabetes mellitus: a prospective study. *Am J Epidemiol* 2002; 155: 387–393.
357. Donga E, van Dijk M, van Dijk JG, et al. A single night of partial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects. *J Clin Endocrinol Metab* 2010; 95: 2963–2968.
358. Donga E, van Dijk M, van Dijk JG, et al. Partial sleep restriction decreases insulin sensitivity in type 1 diabetes. *Diabetes Care* 2010; 33: 1573–1577.
359. Van Cauter E, Polonsky KS, Scheen AJ. Roles of circadian rhythmicity and sleep in human glucose regulation. *Endocr Rev* 1997; 18: 716–738.
360. Barf RP, Meerlo P, Scheurink AJ. Chronic sleep disturbance impairs glucose homeostasis in rats. *Int J Endocrinol* 2010; 2010: 819414.
361. Slow-wave sleep and the risk of type 2 diabetes in humans. 2008; 1–6.
362. Herzog N, Jauch-Chara K, Hyzy F, et al. Selective slow wave sleep but not rapid eye movement sleep suppression impairs morning glucose tolerance in healthy men. *Psychoneuroendocrinology* 2013; 38: 2075–2082.
363. Broussard J, Brady MJ. The impact of sleep disturbances on adipocyte function and lipid metabolism. *Best Pract Res Clin Endocrinol Metab* 2010; 24: 763–773.
364. Broussard J, Day A, Brady M, et al. Experimental Reduction on Sleep Duration or Quality is Associated with Impaired Insulin Signaling in the Adipocyte. *Sleep*; Suppl.
365. Chaput JP, Despres JP, Bouchard C, et al. Association of sleep duration with type 2 diabetes and impaired glucose tolerance. *Diabetologia* 2007; 50: 2298–2304.
366. Chaput JP, Tremblay A. The glucostatic theory of appetite control and the risk of obesity and diabetes. *Int J Obes* 2009; 33: 46–53.
367. Boule NG, Chaput JP, Doucet E, et al. Glucose homeostasis predicts weight gain: prospective and clinical evidence. *Diabetes Metab Res Rev* 2008; 24: 123–129.
368. Skuladottir GV, Nilsson EK, Mwinyi J, et al. One-night sleep deprivation induces changes in the DNA methylation and serum activity indices of stearoyl-CoA desaturase in young healthy men. *Lipids Health Dis* 2016; 15: 137.
369. Martins PJ, Marques MS, Tufik S, et al. Orexin activation precedes increased NPY expression, hyperphagia, and metabolic changes in response to sleep deprivation. *Am J Physiol Endocrinol Metab* 2010; 298:

E726-34.

- 370. Galvao Mde O, Sinigaglia-Coimbra R, Kawakami SE, et al. Paradoxical sleep deprivation activates hypothalamic nuclei that regulate food intake and stress response. *Psychoneuroendocrinology* 2009; 34: 1176–1183.
- 371. Bjorntorp P. Do stress reactions cause abdominal obesity and comorbidities? *Obes Rev* 2001; 2: 73–86.
- 372. Bjorntorp P, Rosmond R. Obesity and cortisol. *Nutrition* 2000; 16: 924–936.
- 373. Schmid SM, Jauch-Chara K, Hallschmid M, et al. Mild sleep restriction acutely reduces plasma glucagon levels in healthy men. *J Clin Endocrinol Metab* 2009; 94: 5169–5173.
- 374. Guyon A, Morselli LL, Balbo ML, et al. Effects of Insufficient Sleep on Pituitary-Adrenocortical Response to CRH Stimulation in Healthy Men. *Sleep*.
- 375. Ahima RS, Prabakaran D, Mantzoros C, et al. Role of leptin in the neuroendocrine response to fasting. *Nature* 1996; 382: 250–252.
- 376. Spiegel K, Leproult R, L'Hermite-Baleriaux M, et al. Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. *J Clin Endocrinol Metab* 2004; 89: 5762–5771.
- 377. Licinio J, Mantzoros C, Negrao AB, et al. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nat Med* 1997; 3: 575–579.
- 378. Chaput JP, Despres JP, Bouchard C, et al. Short sleep duration is associated with reduced leptin levels and increased adiposity: Results from the Quebec family study. *Obes (Silver Spring)* 2007; 15: 253–261.
- 379. Yin X, Li Y, Xu G, et al. Ghrelin fluctuation, what determines its production? *Acta Biochim Biophys Sin* 2009; 41: 188–197.
- 380. Hosoda H, Kangawa K. The autonomic nervous system regulates gastric ghrelin secretion in rats. *Regul Pept* 2008; 146: 12–18.
- 381. Toshinai K, Mondal MS, Nakazato M, et al. Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem Biophys Res Commun* 2001; 281: 1220–1225.
- 382. Dzaja A, Dalal MA, Himmerich H, et al. Sleep enhances nocturnal plasma ghrelin levels in healthy subjects. *Am J Physiol Endocrinol Metab* 2004; 286: E963-7.
- 383. Sleep Loss Reduces Diurnal Rhythm Amplitude of Leptin in Healthy Men. 2003; 1–4.
- 384. Nedeltcheva A, Kilkus J, Imperial J, et al. Sleep restriction can compromise the beneficial effect of diet-induced weight loss on total body adiposity. In: David F. Dinges P (ed) *Associated Professional Sleep Societies (APSS)*. Seattle, WA: SLEEP, 2009, p. A126.
- 385. Schmid SM, Hallschmid M, Jauch-Chara K, et al. Short-term sleep loss decreases physical activity under free-living conditions but does not increase food intake under time-deprived laboratory conditions in healthy

- men. *Am J Clin Nutr* 2009; 90: 1476–1482.
386. Di Marzo V, Goparaju SK, Wang L, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 2001; 410: 822–825.
  387. Kola B, Farkas I, Christ-Crain M, et al. The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. *PLoS One* 2008; 3: e1797.
  388. Cota D, Tschöp MH, Horvath TL, et al. Cannabinoids, opioids and eating behavior: the molecular face of hedonism? *Brain Res Rev* 2006; 51: 85–107.
  389. Melis T, Succu S, Sanna F, et al. The cannabinoid antagonist SR 141716A (Rimonabant) reduces the increase of extra-cellular dopamine release in the rat nucleus accumbens induced by a novel high palatable food. *Neurosci Lett* 2007; 419: 231–235.
  390. Kirkham TC, Williams CM, Fezza F, et al. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol* 2002; 136: 550–557.
  391. De Luca MA, Solinas M, Bimpisidis Z, et al. Cannabinoid facilitation of behavioral and biochemical hedonic taste responses. *Neuropharmacology* 2012; 63: 161–168.
  392. Hanlon EC, Tasali E, Leproult R, et al. Sleep restriction enhances the daily rhythm of circulating levels of endocannabinoid 2-arachidonoylglycerol. *Sleep* 2016; 39: 653–664.
  393. Liukkonen T, Rasanen P, Ruokonen A, et al. C-reactive protein levels and sleep disturbances: observations based on the Northern Finland 1966 Birth Cohort study. *Psychosom Med* 2007; 69: 756–761.
  394. Irwin MR, Wang M, Campomayor CO, et al. Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Arch Intern Med* 2006; 166: 1756–1762.
  395. Irwin MR, Olmstead R, Valladares EM, et al. Tumor necrosis factor antagonism normalizes rapid eye movement sleep in alcohol dependence. *Biol Psychiatry* 2009; 66: 191–195.
  396. Rolland YM, Perry 3rd HM, Patrick P, et al. Leptin and adiponectin levels in middle-aged postmenopausal women: associations with lifestyle habits, hormones, and inflammatory markers--a cross-sectional study. *Metabolism* 2006; 55: 1630–1636.
  397. Zheng H, Patel M, Hryniewicz K, et al. Association of extended work shifts, vascular function, and inflammatory markers in internal medicine residents: a randomized crossover trial. *JAMA* 2006; 296: 1049–1050.
  398. Vgontzas AN, Zoumakis M, Bixler EO, et al. Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: physiologic and therapeutic implications. *J Clin Endocrinol Metab* 2003; 88: 2087–2095.
  399. Ravussin E, Lillioja S, Anderson TE, et al. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J*

- Clin Invest* 1986; 78: 1568–1578.
400. Brondel L, Romer MA, Nougues PM, et al. Acute partial sleep deprivation increases food intake in healthy men. *Am J Clin Nutr* 2010; 91: 1550–1559.
  401. Weinert D, Waterhouse J. The circadian rhythm of core temperature: effects of physical activity and aging. *Physiol Behav* 2007; 90: 246–256.
  402. Landsberg L, Young JB, Leonard WR, et al. Do the obese have lower body temperatures? A new look at a forgotten variable in energy balance. *Trans Am Clin Clim Assoc* 2009; 120: 287–295.
  403. Landsberg L, Young JB, Leonard WR, et al. Is obesity associated with lower body temperatures? Core temperature: a forgotten variable in energy balance. *Metabolism* 2009; 58: 871–876.
  404. Nedeltcheva A V, Kessler L, Imperial J, et al. Exposure to recurrent sleep restriction in the setting of high caloric intake and physical inactivity results in increased insulin resistance and reduced glucose tolerance. *J Clin Endocrinol Metab* 2009; 94: 3242–3250.
  405. Albrecht U, Eichele G. The mammalian circadian clock. *Curr Opin Genet Dev* 2003; 13: 271–277.
  406. Zanutta MM, Correa-Giannella ML, Monteiro MB, et al. Body weight, metabolism and clock genes. *Diabetol Metab Syndr* 2010; 2: 53.
  407. Ekmekcioglu C, Touitou Y. Chronobiological aspects of food intake and metabolism and their relevance on energy balance and weight regulation. *Obes Rev* 2010; 12: 14–25.
  408. Arble DM, Bass J, Laposky AD, et al. Circadian timing of food intake contributes to weight gain. *Obesity* 2009; 17: 2100–2102.
  409. Kohsaka A, Laposky AD, Ramsey KM, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab* 2007; 6: 414–421.
  410. Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science (80- )* 2005; 308: 1043–1045.
  411. Biggi N, Consonni D, Galluzzo V, et al. Metabolic syndrome in permanent night workers. *Chronobiol Int* 2008; 25: 443–454.
  412. Esquirol Y, Bongard V, Mabile L, et al. Shift work and metabolic syndrome: respective impacts of job strain, physical activity, and dietary rhythms. *Chronobiol Int* 2009; 26: 544–559.
  413. Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup Env Med* 2001; 58: 747–752.
  414. Tasali E, Broussard J, Day A, et al. Sleep curtailment in healthy young adults is associated with increased ad lid food intake. *Sleep*.
  415. Calvin AD, Carter RE, Adachi T, et al. Effects of experimental sleep restriction on caloric intake and activity energy expenditure. *Chest* 2013; 144: 79–86.
  416. Spaeth AM, Dinges DF, Goel N. Effects of Experimental Sleep Restriction on Weight Gain, Caloric Intake, and Meal Timing in Healthy Adults. *Sleep* 2013; 36: 981–990.

417. Markwald RR, Melanson EL, Smith MR, et al. Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain. *Proc Natl Acad Sci U S A* 2013; 110: 5695–5700.
418. Hogenkamp PS, Nilsson E, Nilsson VC, et al. Acute sleep deprivation increases portion size and affects food choice in young men. *Psychoneuroendocrinology* 2013; 38: 1668–1674.
419. Chapman CD, Nilsson EK, Nilsson VC, et al. Acute sleep deprivation increases food purchasing in men. *Obes (Silver Spring)* 2013; 21: E555–60.
420. Borbély AA. A two process model of sleep regulation. *Hum Neurobiol* 1982; 1: 195.
421. Van Dongen HPA, Dinges DF. Sleep, circadian rhythms, and psychomotor vigilance. *Clin Sport Med* 2005; 24: 237–249.
422. Van Dongen HPA, Rogers NL, Dinges DF. Understanding sleep debt: theoretical and empirical issues. *Sleep Biol Rhythm* 2003; 1: 4–12.
423. Belenky G, Wesensten NJ, Thorne DR, et al. Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: a sleep dose-response study. *J Sleep Res* 2003; 12: 1–12.
424. Van Dongen HP, Maislin G, Mullington JM, et al. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep* 2003; 26: 117–126.
425. Harrison Y, Horne JA. Sleep loss and temporal memory. *Q J Exp Psychol A* 2000; 53: 271–279.
426. Chee MW, Chuah LY, Venkatraman V, et al. Functional imaging of working memory following normal sleep and after 24 and 35 h of sleep deprivation: Correlations of fronto-parietal activation with performance. *Neuroimage* 2006; 31: 419–428.
427. Chee MW, Choo WC. Functional imaging of working memory after 24 hr of total sleep deprivation. *J Neurosci* 2004; 24: 4560–4567.
428. Thomas M, Sing H, Belenky G, et al. Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *J Sleep Res* 2000; 9: 335–352.
429. Dinges DF, Pack F, Williams K, et al. Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep* 1997; 20: 267–277.
430. Drummond SP, Brown GG. The effects of total sleep deprivation on cerebral responses to cognitive performance. *Neuropsychopharmacology* 2001; 25: S68–73.
431. Drummond SP, Gillin JC, Brown GG. Increased cerebral response during a divided attention task following sleep deprivation. *J Sleep Res* 2001; 10: 85–92.
432. Drummond SP, Brown GG, Gillin JC, et al. Altered brain response to verbal learning following sleep deprivation. *Nature* 2000; 403: 655–657.
433. Drummond SP, Brown GG, Salamat JS, et al. Increasing task difficulty

- facilitates the cerebral compensatory response to total sleep deprivation. *Sleep* 2004; 27: 445–451.
434. Drummond SP, Brown GG, Stricker JL, et al. Sleep deprivation-induced reduction in cortical functional response to serial subtraction. *Neuroreport* 1999; 10: 3745–3748.
  435. Drummond SP, Meloy MJ, Yanagi MA, et al. Compensatory recruitment after sleep deprivation and the relationship with performance. *Psychiatry Res* 2005; 140: 211–223.
  436. Drummond SP, Paulus MP, Tapert SF. Effects of two nights sleep deprivation and two nights recovery sleep on response inhibition. *J Sleep Res* 2006; 15: 261–265.
  437. McKenna JT, Tartar JL, Ward CP, et al. Sleep fragmentation elevates behavioral, electrographic and neurochemical measures of sleepiness. *Neuroscience* 2007; 146: 1462–1473.
  438. Stricker JL, Brown GG, Wetherell LA, et al. The impact of sleep deprivation and task difficulty on networks of fMRI brain response. *J Int Neuropsychol Soc* 2006; 12: 591–597.
  439. Chee MW, Chuah YM. Functional neuroimaging and behavioral correlates of capacity decline in visual short-term memory after sleep deprivation. *Proc Natl Acad Sci U S A* 2007; 104: 9487–9492.
  440. Turner TH, Drummond SP, Salamat JS, et al. Effects of 42 hr of total sleep deprivation on component processes of verbal working memory. *Neuropsychology* 2007; 21: 787–795.
  441. Cote KA, Milner CE, Smith BA, et al. CNS arousal and neurobehavioral performance in a short-term sleep restriction paradigm. *J Sleep Res* 2009; 18: 291–303.
  442. Franzen PL, Buysse DJ, Dahl RE, et al. Sleep deprivation alters pupillary reactivity to emotional stimuli in healthy young adults. *Biol Psychol* 2009; 80: 300–305.
  443. Mu Q, Mishory A, Johnson KA, et al. Decreased brain activation during a working memory task at rested baseline is associated with vulnerability to sleep deprivation. *Sleep* 2005; 28: 433–446.
  444. Banks S, Catcheside P, Lack L, et al. Low levels of alcohol impair driving simulator performance and reduce perception of crash risk in partially sleep deprived subjects. *Sleep* 2004; 27: 1063–1067.
  445. Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. *J Clin Sleep Med* 2007; 3: 519–528.
  446. Leproult R, Colecchia EF, Berardi AM, et al. Individual differences in subjective and objective alertness during sleep deprivation are stable and unrelated. *Am J Physiol Regul Integr Comp Physiol* 2003; 284: 280.
  447. Van Dongen HP. Brain activation patterns and individual differences in working memory impairment during sleep deprivation. *Sleep* 2005; 28: 386–388.
  448. Russo M, Thomas M, Thorne D, et al. Oculomotor impairment during chronic partial sleep deprivation. *Clin Neurophysiol* 2003; 114: 723–736.

449. Doran SM, Van Dongen HP, Dinges DF. Sustained attention performance during sleep deprivation: evidence of state instability. *Arch Ital Biol* 2001; 139: 253–267.
450. Durmer JS, Dinges DF. Neurocognitive consequences of sleep deprivation. *Semin Neurol* 2005; 25: 117–129.
451. Mollicone DJ, Van Dongen H, Rogers NL, et al. Time of Day Effects on Neurobehavioral Performance During Chronic Sleep Restriction. *Aviat Space Environ Med* 2010; 81: 735–744.
452. Brunner DP, Dijk DJ, Borbely AA. Repeated partial sleep deprivation progressively changes in EEG during sleep and wakefulness. *Sleep* 1993; 16: 100–113.
453. Guilleminault C, Powell NB, Martinez S, et al. Preliminary observations on the effects of sleep time in a sleep restriction paradigm. *Sleep Med* 2003; 4: 177–184.
454. Neurobehavioral Dynamics Following Chronic Sleep Restriction: Dose-Response Effects of One Night for Recovery. 2010; 1–17.
455. Ratcliff R, Van Dongen H. Sleep deprivation affects multiple distinct cognitive processes. *Psychon Bull Rev* 2009; 16: 742.
456. The Executive Functions and Self-Regulation: An Evolutionary Neuropsychological Perspective. 2001; 1–29.
457. Barkley RA. Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychol Bull* 1997; 121: 65.
458. Overtom C. Employability skills: An update. *ERIC Dig*; 220.
459. Green L, Myerson J, Lichtman D, et al. Temporal discounting in choice between delayed rewards: The role of age and income. *Psychol Aging* 1996; 11: 79–84.
460. Schuck SEB, Crinella FM. Why children with ADHD do not have low IQs. *J Learn Disabil* 2005; 38: 262.
461. Bechara A. Decision making, impulse control and loss of willpower to resist drugs: A neurocognitive perspective. *Nature Neuroscience* 2005; 8: 1458–1463.
462. Johnson A, van der Meer MAA, Redish AD. Integrating hippocampus and striatum in decision-making. *Curr Opin Neurobiol* 2007; 17: 692–697.
463. Common regions of the human frontal lobe recruited by diverse cognitive demands. 2000; 1–9.
464. Harrison Y, Horne JA. The Impact of Sleep Deprivation of Decision Making: A Review. *J Exp Psychol Appl* 2000; 6: 236–249.
465. Chuah YML, Venkatraman V, Dinges DF, et al. The Neural Basis of Interindividual Variability in Inhibitory Efficiency after Sleep Deprivation. *J Neurosci* 2006; 26: 7156–7162.
466. Binks PG, Waters WF, Hurry M. Short-term total sleep deprivations does not selectively impair higher cortical functioning. *Sleep* 1999; 22: 328.
467. Choo WC, Lee WW, Venkatraman V, et al. Dissociation of cortical regions

- modulated by both working memory load and sleep deprivation and by sleep deprivation alone. *Neuroimage* 2005; 25: 579–587.
468. Gottselig JM, Adam M, Rétey J V, et al. Random number generation during sleep deprivation: effects of caffeine on response maintenance and stereotypy. *J Sleep Res* 2006; 15: 31–40.
  469. Habeck C, Rakitin BC, Moeller J, et al. An event-related fMRI study of the neurobehavioral impact of sleep deprivation on performance of a delayed-match-to-sample task. *Cogn Brain Res* 2004; 18: 306–321.
  470. Heuer H, Kohlisch O, Klein W. The effects of total sleep deprivation on the generation of random sequences of key-presses, numbers and nouns. *Q J Exp Psychol Sect A* 2005; 58: 275–307.
  471. Jennings JR, Monk TH, Van der Molen MW. Sleep deprivation influences some but not all processes of supervisory attention. *Psychol Sci* 2003; 14: 473.
  472. Killgore WDS, Balkin TJ, Wesensten NJ. Impaired decision making following 49 h of sleep deprivation. *J Sleep Res* 2006; 15: 7–13.
  473. The effects of one night of sleep deprivation on known-risk and ambiguous-risk decisions. 2007; 1–9.
  474. Nilsson JP, Söderström M, Karlsson AU, et al. Less effective executive functioning after one night's sleep deprivation. *J Sleep Res* 2005; 14: 1–6.
  475. Sagaspe P, Sanchez-Ortuno M, Charles A, et al. Effects of sleep deprivation on Color-Word, Emotional, and Specific Stroop interference and on self-reported anxiety. *Brain Cogn* 2006; 60: 76–87.
  476. Sleep Deprivation Elevates Expectation of Gains and Attenuates Response to Losses Following Risky Decisions. 2007; 1–7.
  477. Tucker AM, Whitney P, Belenky G, et al. Effects of Sleep Deprivation on Dissociated Components of Executive Functioning. *Sleep* 2010; 33: 47–57.
  478. Jones K, Harrison Y. Frontal lobe function, sleep loss and fragmented sleep. *Sleep Med Rev* 2001; 5: 463.
  479. Plessow F, Kiesel A, Petzold A, et al. Chronic sleep curtailment impairs the flexible implementation of task goals in new parents. *J Sleep Res*. Epub ahead of print 2010. DOI: JSR878 [pii]10.1111/j.1365-2869.2010.00878.x.
  480. Phillips LH. Do 'frontal tests' measure executive function? Issues of assessment and evidence from fluency tests. *Methodol Front Exec Funct* 1997; 191–213.
  481. Whitney P, Jameson T, Hinson JM. Impulsiveness and executive control of working memory. *Pers Individ Dif* 2004; 37: 417–428.
  482. Epstein LH, Leddy JJ, Temple JL, et al. Food reinforcement and eating: a multilevel analysis. *Psychol Bull* 2007; 133: 884–906.
  483. Epstein LH, Salvy SJ, Carr KA, et al. Food reinforcement, delay discounting and obesity. *Physiol Behav* 2010; 100: 438–445.
  484. Mazur JE. Predicting the strength of a conditioned reinforcer: Effects of delay and uncertainty. *Curr Dir Psychol Sci* 1993; 2: 70–74.
  485. Daugherty JR, Brase GL. Taking time to be healthy: Predicting health

- behaviors with delay discounting and time perspective. *Pers Individ Dif* 2010; 48: 202–207.
486. Bradford WD. The Association Between Individual Time Preferences and Health Maintenance Habits. *Med Decis Mak* 2009; 30: 99–112.
  487. Ikeda S, Kang M-I, Ohtake F. Hyperbolic discounting, the sign effect, and the body mass index. *J Health Econ* 2010; 29: 268–284.
  488. Weller RE, Cook 3rd EW, Avsar KB, et al. Obese women show greater delay discounting than healthy-weight women. *Appetite* 2008; 51: 563–569.
  489. Wansink B, Sobal J. Mindless eating: The 200 daily food decisions we overlook. *Environ Behav* 2007; 39: 106–123.
  490. Wansink B, Payne CR. Counting bones: environmental cues that decrease food intake. *Percept Mot Ski* 2007; 104: 273–276.
  491. The influence of food portion size and energy density on energy intake: implications for weight management. 2005; 1–6.
  492. Ledikwe JH, Ello-Martin JA, Rolls BJ. Portion sizes and the obesity epidemic. *J Nutr* 2005; 135: 905.
  493. Lavin JG, Lawless HT. Effects of color and odor on judgments of sweetness among children and adults. *Food Qual Pref* 1998; 9: 283–89.
  494. Brobeck JR. Food intake as a mechanism of temperature regulation. *Yale J Biol Med* 1948; 20: 545–552.
  495. Westerterp-Plantenga MS, Westerterp-Plantenga MS. Effects of extreme environments on food intake in human subjects. *Proc Nutr Soc* 1999; 58: 791–798.
  496. Murray R. The effects of consuming carbohydrate-electrolyte beverages on gastric emptying and fluid absorption during and following exercise. *Sport Med* 1987; 4: 322–351.
  497. Clendenen VI, Herman CP, Polivy J. Social facilitation of eating among friends and strangers. *Appetite* 1994; 23: 1–13.
  498. Pliner P, Bell R, Kinchla M, et al. Time to eat? The impact of time facilitation and social facilitation on food intake. 2003, pp. 20–24.
  499. Wansink B, Painter JE, Lee YK. The office candy dish: proximity's influence on estimated and actual consumption. *Int J Obes* 2006; 30: 871–875.
  500. Wansink B. ENVIRONMENTAL FACTORS THAT INCREASE THE FOOD INTAKE AND CONSUMPTION VOLUME OF UNKNOWING CONSUMERS\*. *Annu Rev Nutr* 2004; 24: 455–479.
  501. Wansink - Bad Popcorn in big buckets portion size can influence intake as much as taste. 2005; 1–5.
  502. Wansink B, Painter JE, North J. Bottomless bowls: why visual cues of portion size may influence intake. *Obes Res* 2005; 13: 93–100.
  503. Stroebele N, De Castro JM. Effect of ambience on food intake and food choice. *Nutrition* 2004; 20: 821–838.
  504. Rolls BJ, Roe LS, Kral T V, et al. Increasing the portion size of a packaged snack increases energy intake in men and women. *Appetite* 2004; 42: 63–69.

505. Wansink B, Wansink CS. The largest Last Supper: depictions of food portions and plate size increased over the millennium. *Int J Obes* 2010; 1–2.
506. Nielsen SJ, Popkin BM. Patterns and trends in food portion sizes, 1977–1998. *JAMA* 2003; 289: 450.
507. Smicklas-Wright H, Mitchell DC, Mickle SJ, et al. Foods commonly eaten in the United States, 1989–1991 and 1994–1996: Are portion sizes changing? *J Am Diet Assoc* 2003; 103: 41–47.
508. Matthiessen J, Fagt S, Biloft-Jensen A, et al. Size makes a difference. *Public Health Nutr* 2007; 6: 65–72.
509. Young LR, Nestle M. The contribution of expanding portion sizes to the US obesity epidemic. *Am J Public Health* 2002; 92: 246.
510. Westenhoefer J, Broeckmann P, Munch AK, et al. Cognitive control of eating behaviour and the disinhibition effect. *Appetite* 1994; 23: 27–41.
511. Westenhoefer J, Pudel V, Maus N. Some restrictions on dietary restraint. *Appetite* 1990; 14: 133–137.
512. Lawson OJ, Williamson DA, Champagne CM, et al. The association of body weight, dietary intake, and energy expenditure with dietary restraint and disinhibition. *Obes Res* 1995; 3: 153–161.
513. Dykes J, Brunner EJ, Martikainen PT, et al. Socioeconomic gradient in body size and obesity among women: the role of dietary restraint, disinhibition and hunger in the Whitehall II study. *Int J Obes Relat Metab Disord* 2004; 28: 262–268.
514. Waadegaard M, Davidsen M, Kjoller M. Obesity and prevalence of risk behaviour for eating disorders among young Danish women. *Scand J Public Health* 2009; 37: 736–743.
515. Polivy J, Herman CP. Diagnosis and treatment of normal eating. *J Consult Clin Psychol* 1987; 55: 635–644.
516. Jauch-Chara K, Hallschmid M, Schmid SM, et al. Sleep loss does not aggravate the deteriorating effect of hypoglycemia on neurocognitive function in healthy men. *Psychoneuroendocrinology* 2010; 35: 624–628.
517. Higgs S. Memory for recent eating and its influence on subsequent food intake. *Appetite* 2002; 39: 159–166.
518. Meerlo P, Mistlberger RE, Jacobs BL, et al. New neurons in the adult brain: the role of sleep and consequences of sleep loss. *Sleep Med Rev* 2009; 13: 187–194.
519. Turner SA, Luszczynska A, Warner L, et al. Emotional and uncontrolled eating styles and chocolate chip cookie consumption. A controlled trial of the effects of positive mood enhancement. *Appetite* 2010; 54: 143–149.
520. Evers C, Marijn Stok F, de Ridder DTD. Feeding Your Feelings: Emotion Regulation Strategies and Emotional Eating. *Personal Soc Psychol Bull* 2010; 36: 792–804.
521. St-Onge M-P, McReynolds A, Trivedi ZB, et al. Sleep restriction leads to increased activation of brain regions sensitive to food stimuli. *Am J Clin Nutr* 2012; 95: 818–24.

522. Benedict C, Brooks SJ, O'Daly OG, et al. Acute sleep deprivation enhances the brain's response to hedonic food stimuli: an fMRI study. *J Clin Endocrinol Metab* 2012; 97: E443-7.
523. St-Onge MP, Wolfe S, Sy M, et al. Sleep restriction increases the neuronal response to unhealthy food in normal-weight individuals. *Int J Obes*. Epub ahead of print 2013. DOI: 10.1038/ijo.2013.114.
524. Greer S, Goldstein A, Walker M. The impact of sleep deprivation on food desire in the human brain. *Nat Commun* 2013; 4: 2259.
525. Rechtschaffen A, Wolpert EA, Dement WC, et al. Nocturnal Sleep of Narcoleptics. *Electroencephalogr Clin Neurophysiol* 1963; 15: 599–609.
526. Montplaisir J, Billiard M, Takahashi S, et al. Twenty-four-hour recording in REM-narcoleptics with special reference to nocturnal sleep disruption. *Biol Psychiatry* 1978; 13: 73–89.
527. Baker TL, Guilleminault C, Nino-Murcia G, et al. Comparative polysomnographic study of narcolepsy and idiopathic central nervous system hypersomnia. *Sleep* 1986; 9: 232–242.
528. Rogers AE, Aldrich MS, Caruso CC. Patterns of sleep and wakefulness in treated narcoleptic subjects. *Sleep* 1994; 17: 590–597.
529. A Randomized Trial Evaluating the Effectiveness of Sodium Oxybate Therapy on Quality of Life in Narcolepsy. 2006; 1–6.
530. Hypocretin Deficiency in Narcoleptic Humans Is Associated with Abdominal Obesity. 2003; 1–8.
531. Eating Disorder and Metabolism in Narcoleptic Patients. 2007; 1–7.
532. Fortuyn HA, Swinkels S, Buitelaar J, et al. High prevalence of eating disorders in narcolepsy with cataplexy: a case-control study. *Sleep* 2008; 31: 335–341.
533. Ford ES, Cunningham TJ, Croft JB. Trends in Self-Reported Sleep Duration among US Adults from 1985 to 2012. *Sleep* 2015; 38: 829–832.
534. Institute of Medicine (US) Committee on Sleep Medicine and Research X. *Sleep Disorders and Sleep Deprivation*. Washington, D.C.: National Academies Press. Epub ahead of print September 2006. DOI: 10.17226/11617.
535. Lobstein T, Baur L, Uauy R, et al. Obesity in children and young people: a crisis in public health. *Obes Rev* 2004; 5 Suppl 1: 4–104.
536. Bin YS, Marshall NS, Glozier N. Secular trends in adult sleep duration: a systematic review. *Sleep Med Rev* 2012; 16: 223–230.
537. Bin YS, Marshall NS, Glozier N. Sleeping at the limits: the changing prevalence of short and long sleep durations in 10 countries. *Am J Epidemiol* 2013; 177: 826–833.
538. Knutson KL, Van Cauter E, Rathouz PJ, et al. Trends in the prevalence of short sleepers in the USA: 1975-2006. *Sleep* 2010; 33: 37–45.
539. Ohayon MM, Carskadon MA, Guilleminault C, et al. Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan.

- Sleep* 2004; 27: 1255–1273.
540. Youngstedt SD, Goff EE, Reynolds AM, et al. Objective measures of sleep quality have not declined over the last 50 years. *Sleep Med Rev* 2016; 30: 108–109.
  541. Cain SW, Filtress AJ, Phillips CL, et al. Enhanced preference for high-fat foods following a simulated night shift. *Scand J Work Env Heal*. Epub ahead of print 2015. DOI: 10.5271/sjweh.3486.
  542. Fang Z, Spaeth AM, Ma N, et al. Altered salience network connectivity predicts macronutrient intake after sleep deprivation. *Sci Rep* 2015; 5: 8215.
  543. Libedinsky C, Massar SA, Ling A, et al. Sleep deprivation alters effort discounting but not delay discounting of monetary rewards. *Sleep* 2013; 36: 899–904.
  544. Venkatraman V, Huettel SA, Chuah LY, et al. Sleep deprivation biases the neural mechanisms underlying economic preferences. *J Neurosci* 2011; 31: 3712–3718.
  545. Cappuccio FP, D’Elia L, Strazzullo P, et al. Quantity and Quality of Sleep and Incidence of Type 2 Diabetes: A systematic review and meta-analysis. *Diabetes Care* 2010; 33: 414–420.
  546. Magee L, Hale L. Longitudinal associations between sleep duration and subsequent weight gain: a systematic review. *Sleep Med Rev* 2012; 16: 231–241.
  547. Tsatsoulis A, Mantzaris MD, Bellou S, et al. Insulin resistance: an adaptive mechanism becomes maladaptive in the current environment - an evolutionary perspective. *Metabolism* 2013; 62: 622–633.
  548. Sellayah D, Cagampang FR, Cox RD. On the evolutionary origins of obesity: a new hypothesis. *Endocrinology* 2014; 155: 1573–1588.
  549. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol* 2008; 8: 923–934.
  550. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol* 2011; 29: 415–445.
  551. Calay ES, Hotamisligil GS. Turning off the inflammatory, but not the metabolic, flames. *Nat Med* 2013; 19: 265–267.
  552. Chan JC, Cho NH, Tajima N, et al. Diabetes in the Western Pacific Region--past, present and future. *Diabetes Res Clin Pr* 2014; 103: 244–255.
  553. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; 115: 1111–1119.
  554. Johnson AR, Milner JJ, Makowski L. The inflammation highway: metabolism accelerates inflammatory traffic in obesity. *Immunol Rev* 2012; 249: 218–238.
  555. Clark I, Atwood C, Bowen R, et al. Tumor necrosis factor-induced cerebral insulin resistance in Alzheimer’s disease links numerous treatment rationales. *Pharmacol Rev* 2012; 64: 1004–1026.
  556. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine

- concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 2002; 106: 2067–2072.
557. Black PH. The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med Hypotheses* 2006; 67: 879–891.
  558. Rodriguez-Hernandez H, Simental-Mendia LE, Rodriguez-Ramirez G, et al. Obesity and inflammation: epidemiology, risk factors, and markers of inflammation. *Int J Endocrinol* 2013; 2013: 678159.
  559. Ebrahimi A, Nabipour I, Vahdat K, et al. High sensitivity C-reactive protein is associated with the metabolic syndrome independent to viral and bacterial pathogen burden. *Diabetes Res Clin Pr* 2009; 84: 296–302.
  560. Dandona P, Chaudhuri A, Ghanim H, et al. Proinflammatory effects of glucose and anti-inflammatory effect of insulin: relevance to cardiovascular disease. *Am J Cardiol* 2007; 99: 15B–26B.
  561. Dandona P, Chaudhuri A, Ghanim H, et al. Insulin as an anti-inflammatory and antiatherogenic modulator. *J Am Coll Cardiol* 2009; 53: S14–20.
  562. O’Keefe JH, Vogel R, Lavie CJ, et al. Organic fitness: physical activity consistent with our hunter-gatherer heritage. *Phys Sport* 2010; 38: 11–18.
  563. O’Keefe JH, Vogel R, Lavie CJ, et al. Exercise like a hunter-gatherer: a prescription for organic physical fitness. *Prog Cardiovasc Dis* 2011; 53: 471–479.
  564. Fernandez-Real JM, Garcia-Fuentes E, Moreno-Navarrete JM, et al. Fat overload induces changes in circulating lactoferrin that are associated with postprandial lipemia and oxidative stress in severely obese subjects. *Obes (Silver Spring)* 2010; 18: 482–488.
  565. Holmer-Jensen J, Karhu T, Mortensen LS, et al. Differential effects of dietary protein sources on postprandial low-grade inflammation after a single high fat meal in obese non-diabetic subjects. *Nutr J* 2011; 10: 115.
  566. Luchsinger JA. Diabetes, related conditions, and dementia. *J Neurol Sci* 2010; 299: 35–38.
  567. Spreadbury I. Comparison with ancestral diets suggests dense acellular carbohydrates promote an inflammatory microbiota, and may be the primary dietary cause of leptin resistance and obesity. *Diabetes Metab Syndr Obes* 2012; 5: 175–189.
  568. Speakman JR, Mitchell SE. Caloric restriction. *Mol Asp Med* 2011; 32: 159–221.
  569. Mattson MP. Energy intake, meal frequency, and health: a neurobiological perspective. *Annu Rev Nutr* 2005; 25: 237–260.
  570. Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J Nutr Biochem* 2005; 16: 129–137.
  571. Redman LM, Ravussin E. Caloric restriction in humans: impact on physiological, psychological, and behavioral outcomes. *Antioxid Redox Signal* 2011; 14: 275–287.
  572. Li Q. Effect of forest bathing trips on human immune function. *Environ*

- Health Prev Med* 2010; 15: 9–17.
573. Li Q, Otsuka T, Kobayashi M, et al. Acute effects of walking in forest environments on cardiovascular and metabolic parameters. *Eur J Appl Physiol* 2011; 111: 2845–2853.
  574. Park BJ, Tsunetsugu Y, Kasetani T, et al. The physiological effects of Shinrin-yoku (taking in the forest atmosphere or forest bathing): evidence from field experiments in 24 forests across Japan. *Env Heal Prev Med* 2010; 15: 18–26.
  575. Laposky AD, Bass J, Kohsaka A, et al. Sleep and circadian rhythms: key components in the regulation of energy metabolism. *FEBS Lett* 2008; 582: 142–151.
  576. Copinschi G. Metabolic and endocrine effects of sleep deprivation. *Essent Psychopharmacol* 2005; 6: 341–347.
  577. Patel SR, Hu FB. Short sleep duration and weight gain: a systematic review. *Obes (Silver Spring)* 2008; 16: 643–653.
  578. Van Cauter E, Spiegel K, Tasali E, et al. Metabolic consequences of sleep and sleep loss. *Sleep Med* 2008; 9 Suppl 1: S23–8.
  579. Krause AJ, Simon EB, Mander BA, et al. The sleep-deprived human brain. *Nat Rev Neurosci* 2017; 18: 404–418.
  580. Chen X, Beydoun MA, Wang Y. Is sleep duration associated with childhood obesity? A systematic review and meta-analysis. *Obes (Silver Spring)* 2008; 16: 265–274.
  581. Knutson KL, Van Cauter E. Associations between sleep loss and increased risk of obesity and diabetes. *Ann N Y Acad Sci* 2008; 1129: 287–304.
  582. Marshall NS, Glozier N, Grunstein RR. Is sleep duration related to obesity? A critical review of the epidemiological evidence. *Sleep Med Rev* 2008; 12: 289–298.
  583. Fatima Y, Doi SA, Mamun AA. Sleep quality and obesity in young subjects: a meta-analysis. *Obes Rev* 2016; 17: 1154–1166.
  584. Kim K, Shin D, Jung GU, et al. Association between sleep duration, fat mass, lean mass and obesity in Korean adults: the fourth and fifth Korea National Health and Nutrition Examination Surveys. *J Sleep Res* 2017; 26: 453–460.
  585. Carter PJ, Taylor BJ, Williams SM, et al. Longitudinal analysis of sleep in relation to BMI and body fat in children: the FLAME study. *BMJ* 2011; 342: d2712.
  586. Chaput JP, Despres JP, Bouchard C, et al. The Association between Short Sleep Duration and Weight Gain Is Dependent on Disinhibited Eating Behavior in Adults. *Sleep* 2011; 34: 1291–1297.
  587. Eisenmann JC, Ekkekakis P, Holmes M. Sleep duration and overweight among Australian children and adolescents. *Acta Paediatr* 2006; 95: 956–963.
  588. Nam GE, Han K, Kim DH, et al. Sleep duration is associated with body fat and muscle mass and waist-to-height ratio beyond conventional obesity parameters in Korean adolescent boys. *J Sleep Res* 2017; 26: 444–452.

589. Park YJ, Lee WC, Yim HW, et al. [The association between sleep and obesity in Korean adults]. *J Prev Med Public Heal* 2007; 40: 454–460.
590. Patel SR, Malhotra A, White DP, et al. Association between reduced sleep and weight gain in women. *Am J Epidemiol* 2006; 164: 947–954.
591. Rutters F, Gerver WJ, Nieuwenhuizen AG, et al. Sleep duration and body-weight development during puberty in a Dutch children cohort. *Int J Obes* 2010; 34: 1508–1514.
592. Snell EK, Adam EK, Duncan GJ. Sleep and the body mass index and overweight status of children and adolescents. *Child Dev* 2007; 78: 309–323.
593. Wang J, Adab P, Liu W, et al. Prevalence of adiposity and its association with sleep duration, quality, and timing among 9-12-year-old children in Guangzhou, China. *J Epidemiol*. Epub ahead of print 2017. DOI: 10.1016/j.je.2016.11.003.
594. Capers PL, Fobian AD, Kaiser KA, et al. A systematic review and meta-analysis of randomized controlled trials of the impact of sleep duration on adiposity and components of energy balance. *Obes Rev* 2015; 16: 771–782.
595. Akiyama M, Yuasa T, Hayasaka N, et al. Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-ablated mice. *Eur J Neurosci* 2004; 20: 3054–3062.
596. Nedeltcheva A V, Kilkus JM, Imperial J, et al. Sleep curtailment is accompanied by increased intake of calories from snacks. *Am J Clin Nutr* 2009; 89: 126–133.
597. Laposky AD, Bradley MA, Williams DL, et al. Sleep-wake regulation is altered in leptin-resistant (db/db) genetically obese and diabetic mice. *Am J Physiol Regul Integr Comp Physiol* 2008; 295: R2059-66.
598. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. *Lancet* 1999; 354: 1435–1439.
599. Sutcliffe JG, de Lecea L. The hypocretins: excitatory neuromodulatory peptides for multiple homeostatic systems, including sleep and feeding. *J Neurosci Res* 2000; 62: 161–168.
600. Leproult R, Van Reeth O, Byrne MM, et al. Sleepiness, performance, and neuroendocrine function during sleep deprivation: effects of exposure to bright light or exercise. *J Biol Rhythm* 1997; 12: 245–258.
601. Dinges DF, Douglas SD, Hamarman S, et al. Sleep deprivation and human immune function. *Adv Neuroimmunol* 1995; 5: 97–110.
602. Rogers NL, Szuba MP, Staab JP, et al. Neuroimmunologic aspects of sleep and sleep loss. *Semin Clin Neuropsychiatry* 2001; 6: 295–307.
603. Zager A, Andersen ML, Ruiz FS, et al. Effects of acute and chronic sleep loss on immune modulation of rats. *Am J Physiol Regul Integr Comp Physiol* 2007; 293: R504-9.
604. Vgontzas AN, Bixler EO, Lin HM, et al. IL-6 and its circadian secretion in humans. *Neuroimmunomodulation* 2005; 12: 131–140.
605. Easton A, Meerlo P, Bergmann B, et al. The suprachiasmatic nucleus regulates sleep timing and amount in mice. *Sleep* 2004; 27: 1307–1318.

- 606. Challet E, Turek FW, Laute M, et al. Sleep deprivation decreases phase-shift responses of circadian rhythms to light in the mouse: role of serotonergic and metabolic signals. *Brain Res* 2001; 909: 81–91.
- 607. Laposky A, Easton A, Dugovic C, et al. Deletion of the mammalian circadian clock gene BMAL1/Mop3 alters baseline sleep architecture and the response to sleep deprivation. *Sleep* 2005; 28: 395–409.
- 608. Sehgal A, Mignot E. Genetics of sleep and sleep disorders. *Cell* 2011; 146: 194–207.

## **Appendices**

**Acknowledgements**

**Curriculum Vitae**

## **Acknowledgements**

Science betters our world and I am honored to have the opportunity to be a scientist. As such, I have had many collaborators at Jazz Pharmaceuticals, Stanford University, Leiden University Medical Center and beyond who have contributed their expertise on large, human-oriented research projects of which I've been a part. I deeply appreciate all the connections I've made along the way, including the individuals who allow themselves to be studied so that we can advance knowledge. I am forever grateful to a select few who have provided extraordinary support along the way including Marie Pardi, John Pardi, Samara Pardi, David Levine, Jed Black, Hanno Pijl, and especially Jamie Zeitzer and Gert Jan Lammers.

# Curriculum Vitae

## NAME

Daniel Pardi, MS

## EDUCATION/TRAINING

| INSTITUTION AND LOCATION                  | DEGREE | YEAR(s) | FIELD OF STUDY         |
|-------------------------------------------|--------|---------|------------------------|
| University of San Francisco (USF).<br>USA | BS     | 1997    | Exercise<br>Physiology |
| Florida State University (FSU). USA       | MS     | 2000    | Exercise<br>Physiology |

## Positions and Honors

### Positions and Employment

|                 |                                                                                                                                                                  |
|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2010-present    | CEO, humanOS.me – A disease-prevention tool for the public based on novel tools related to education, skill development, and self-tracking health behaviors.     |
| 2011-2019       | Leiden University Medical Center. PhD Program.                                                                                                                   |
| 2006-2009       | The Investigator Initiated-Sponsored Research Associations ( <a href="http://www.IISRA.org">www.IISRA.org</a> ) – Chairman of the Board of Directors and Founder |
| 2008-2009       | Jazz Pharmaceuticals. Senior Manager, Medical and Scientific Affairs                                                                                             |
| 2007-2008       | Jazz Pharmaceuticals. Manager, Medical and Scientific Affairs                                                                                                    |
| 2004-2005       | Orphan Medical. Medical Science Liaison                                                                                                                          |
| 2002–2004       | Orphan Medical. Senior Specialty Sales Consultant                                                                                                                |
| 2000-2002       | DoubleTwist. Bioinformatics / Genomics Project Manager                                                                                                           |
| Jan–Nov<br>2000 | Preventative Medicine Research Institute. Research Assistant, Prostate Cancer Research Team                                                                      |
| May–Aug<br>1999 | Florida State University, School of Medicine. Gross Anatomy Assistant Instructor                                                                                 |

## Honors

- |      |                                                                                                                                                                             |
|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1998 | FSU Medical Gross Anatomy Student Elect: Nominated by Physiology department faculty (1 per year) to join medical students. Accepted by program and finished top 4 in class. |
| 1997 | USF Student Leadership Award                                                                                                                                                |
| 1997 | USF Department Honor Award (outstanding academic achievement - 1 of 5)                                                                                                      |
| 1997 | USF Student Counsel Department Nominee (Faculty elected - 1 of 2)                                                                                                           |
| 1997 | Who's Who in American Colleges and Universities (1 of 27 at USF)                                                                                                            |
| 1996 | USF Student Wellness Program founder                                                                                                                                        |

## **Selected peer-reviewed publications (in chronological order).**

Pardi D, Buman M, Black J, Lammers GJ, Zeitzer J. Eating Decisions Based on Alertness Levels after a Single Night of Sleep Manipulation: A Randomized Clinical Trial. **SLEEP** – Accepted, 2016.

Freese, Jens, et al. "Back to the Future. Metabolic Effects of a 4-Day Outdoor Trip Under Simulated Paleolithic Conditions—New Insights from The Eifel Study." **Journal of Evolution and Health** 1.1 (2016): 16.

Donjacour, C. E., Pardi, D., Aziz, N. A., Frölich, M., Roelfsema, F., Overeem, S., ... & Lammers, G. J. (2013). Plasma total ghrelin and leptin levels in human narcolepsy and matched healthy controls: basal concentrations and response to sodium oxybate. **Journal of Clinical Sleep Medicine: JCSM**, 9(8), 797. 2013.

Black J, Pardi D, Hornfeldt CS, Inhaber N. The nightly administration of sodium oxybate results in significant reduction in the nocturnal sleep disruption of patients with narcolepsy. **Journal of Clinical Sleep Medicine**, Vol. 6, No. 6, 2010.

Carter LP, Pardi D, Gorsline J, Griffiths RR. Illicit gamma-hydroxybutyrate (GHB) and pharmaceutical sodium oxybate (Xyrem): differences in characteristics and misuse. **Drug Alcohol Depend**. 2009;104(1-2):1-10.

Black J, Pardi D, Hornfeldt CS, Inhaber N. The nightly administration of sodium oxybate results in significant reduction in the nocturnal sleep disruption of patients with narcolepsy. **Sleep Med.** 2009.

Ondo WG, Perkins T, Swick T, Hull KL, Jr., Jimenez JE, Garriss TS, Pardi, D. Sodium oxybate for excessive daytime sleepiness in Parkinson disease: an open-label polysomnographic study. **Archives of Neurology.** 2008 Oct;65(10):1337-40.

Pardi D., Black J. Sodium Oxybate: Neurobiology and Clinical Efficacy. **Future Medicine**, 2006:721-35.

Pardi D., Black J. Gamma-Hydroxybutyrate / Sodium Oxybate: Neurobiology, and Impact on Sleep and Wakefulness. **CNS Drugs**, 2006;20(12):993-1018.

### **Book Chapters**

Xie X, Pardi D, Black J. Molecular and Cellular Actions of g-Hydroxybutyric Acid (GHB): Possible Mechanisms Underlying the Efficacy of GHB in Narcolepsy. In: Bassetti CL, Billiard M, Mignot E, eds. **Narcolepsy and Hypersomnia**: Informa Healthcare 2007:573-610.

### **Conference Posters and Presentations**

Donjacour, C., Pardi, D., Aziz, A., Overeem, S., Pijl, H., & Lammers, G. (2010, January). Normal 24 Hour Ghrelin Levels in Human Narcolepsy and in Response to Sodium Oxybate. In **SLEEP** (Vol. 33, pp. A269-A269).

Pardi, D., Patel, CB., Lammers, GJ. The Prevalence and Inter-Country Differences of Body Mass Index Classification in Narcolepsy with Cataplexy Patients in Five European Countries. **European Sleep Research Society**. Paris, 2010.

Pardi, D., Patel, CB., Lammers, GJ. Associations Between Body Mass Index and Sleep Parameters in Narcolepsy with Cataplexy Patients from the European Narcolepsy Network Database. **European Sleep Research Society**. Paris, 2010.

Black, J., Pardi, D., Hornfeldt, C. S., & Inhaber, N. (2010). The nightly use of sodium oxybate is associated with a reduction in nocturnal sleep disruption: a double-blind, placebo-controlled study in patients with narcolepsy. **J Clin Sleep Med**, 6(6), 596-602.

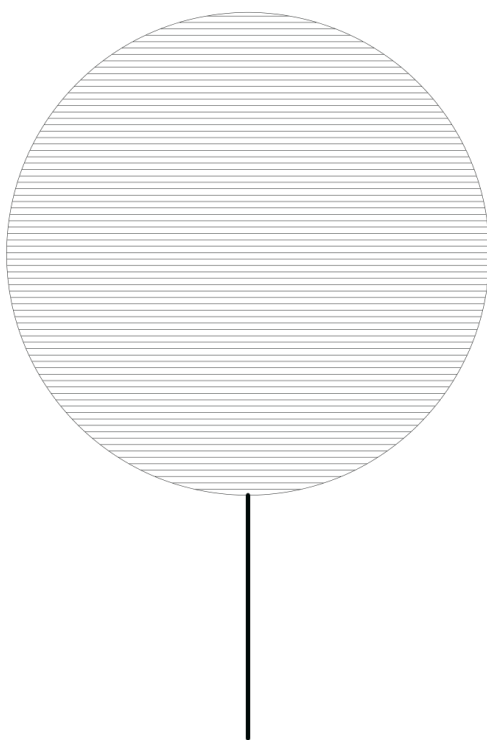
Carter, L and Pardi, D. Similarities and Differences Between Illicit GHB and Sodium Oxybate. **College on Problems of Drug Dependence**. Washington, DC. October 28-29<sup>th</sup>, 2008.

Ristanovic RK, Liang H, Hornfeldt CS, Lai C. Exacerbation of cataplexy following gradual withdrawal of antidepressants: Manifestation of probable protracted rebound cataplexy. **Sleep Med**. 2008 Aug 25. (Cited in acknowledgements)

Ondo WG, Perkins T, Swick T, Hull K, Jimenez E, Pardi D. Nocturnal Sodium Oxybate for Daytime Sedation and Fatigue in Parkinson's Disease: A Polysomnographic Study. **Associated Professional Sleep Societies** (Sleep), Minneapolis, Minnesota 2007.

Ondo WG, Perkins T, Swick T, Hull K, Jimenez E, Pardi D. Nocturnal Sodium Oxybate for Daytime Sedation and Fatigue in Parkinson's Disease: A Polysomnographic Study. **World Congress of Movement Disorders**. Kyoto, Japan 2006.

Black J, Perera P, Pardi D, Liang H. Sodium Oxybate-Induced Changes in Nocturnal Slow-Wave Sleep and Delta Power Correlate with Improvements in Measures of Daytime Alertness and Sleep Continuity in Patients with Narcolepsy. **European Sleep Research Society**. Innsbruck 2006.



End