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Title: Type 1 diabetes and sleep : implications for glucoregulation

Issue Date: 2015-06-18

TYPE 1 DIABETES MELLITUS AND SLEEP
Implications for glucoregulation

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The studies described in this thesis was performed at the department of Endocrinology and Metabolism, Leiden University Medical Center, Leiden, the Netherlands

Cover design: Leonie Esselink

Cover picture: ©Pablo Picasso, 'Jeune Fille Endormie', 1935, c/o Pictoright Amsterdam 2015

Layout and print: Off-page, Amsterdam, the Netherlands

ISBN: 978-94-6182-565-0

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This thesis was financially supported by Novo Nordisk B.V., Ipsen Farmaceutica B.V., Servier Nederland Farma B.V., Goodlife Healthcare B.V., and Rijnland Zorggroep B.V.

TYPE 1 DIABETES MELLITUS AND SLEEP
Implications for glucoregulation

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 18 juni 2015
Klokke 16.15 uur

Door

Marieke van Dijk
Geboren 29 oktober 1982
te Almelo

PROMOTIECOMMISSIE

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CHAPTER

1

GENERAL INTRODUCTION

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I. INTRODUCTION

Type 1 diabetes mellitus (T1DM) is characterized by absolute insulin-deficiency, resulting from immune-mediated destruction of insulin-producing β -cells of the pancreas.⁽¹⁾ Insulin replacement and frequent monitoring of blood glucose by patients themselves (SMBG) are the hallmarks of optimal treatment of T1DM.⁽²⁾

Despite advances in insulin therapy during the past 25 years, relatively few patients with T1DM are able to reach the glycemic and HbA_{1c} target levels, obtained by normal metabolic control in non-diabetic subjects⁽³⁾, and they generally have relatively large intra-individual variations in blood glucose levels. Although insulin analogues and insulin pumps enable a level of insulin replacement that approaches the finesses of normal physiological control, it still remains impossible to achieve fully normal glucose physiology. This inability might be related to normal intra-individual variations in physiological factors, which may interfere with glucoregulation in healthy subjects and also in patients with T1DM, e.g. food intake, physical activity, and stress.⁽⁴⁻⁷⁾

Voluntary sleep curtailment is common in our modern 24-h society. Increasing evidence exists for an important role of sleep as another physiological determinant of glucose metabolism in healthy people.⁽⁸⁻¹³⁾ Because altered sleep characteristics are known to affect glucoregulation in healthy controls, we hypothesized that this may also hold true for patients with T1DM. In this concept, altered sleep characteristics may be another factor challenging optimal glucoregulation in these patients. The effects of impaired sleep as a physiological determinant of impaired glucose regulation in patients with T1DM had not been studied previous to the studies documented in this thesis. Although there is only limited evidence that T1DM is associated with altered sleep patterns and sleep disorders⁽¹⁴⁻¹⁸⁾, metabolic dysregulation, frequently found in patients with T1DM, could still impair sleep characteristics; in turn, disturbed sleep could impair glucose metabolism, creating a vicious circle.

The studies described in this thesis focus on the interaction between sleep and glucose regulation in adult patients with T1DM. We studied effects of both shortened sleep duration and altered sleep composition on insulin sensitivity in adult patients with T1DM and healthy controls. In addition, we evaluated sleep patterns in T1DM, and studied the effect of hyperglycemia, induced by reduced insulin therapy, on sleep characteristics in patients with T1DM. Since T1DM was associated with cognitive impairment^(19;20), we also studied the effect of sleep characteristics on sustained attention in T1DM.

II. TYPE 1 DIABETES MELLITUS (T1DM)

1. Epidemiology

T1DM accounts for 5-10% of all cases of diabetes. Although it most commonly presents in patients younger than 16-18 years of age⁽³⁾ it may develop at any age.⁽²¹⁾ There is an enormous geographical variation in incidence of DM1 around the world, ranging from fewer than 4 cases per 100.000 children and adolescents (younger than 18 years) in much of Africa and Asia to more than 20 per 100.000 in Canada, Australia, and parts of Europe, with the highest reported incidences in Finland and Sardinia (about 50 and 37-45 cases per 100.000 children younger than 15 years,

respectively.⁽²²⁻²⁶⁾ The incidence of T1DM increases rapidly by 2% to 5% per year worldwide, especially in the youngest age group of who are less than 5 years of age.⁽²⁷⁾ If these trends continue, a doubling of new cases of T1DM in European children younger than 5 years is predicted to occur between 2005 and 2020. Likewise the number of cases younger than 15 years will rise by 70%.⁽²⁸⁾ There is no overall gender difference in the incidence of childhood DM1, but in select populations (e.g., older Europeans), there seems to be an increased risk for males (3:2 male to female ratio).⁽²⁹⁾

2. Pathogenesis

In the early 1980s Eisenbarth first describes the main phases of the pathogenesis of the immune form of T1DM.⁽³⁰⁾ Available evidence suggests a strong genetic component, the most important of which relates to the HLA class II locus on chromosome 6, and with lesser contribution from other non-HLA genes, including the insulin gene on chromosome 11^(31;32), and the cytotoxic T lymphocyte antigen 4 (CTLA4) on chromosome 2q33.⁽³³⁾ Genes located within the HLA class II region on chromosome 6p21 are considered to be the major susceptibility genes for T1DM and account for approximately 50% of the genetic susceptibility.⁽³⁴⁻³⁷⁾ Both susceptibility, associated with diabetes resistance, and protective HLA haplotypes, associated with protection from the disease, are identified.⁽³⁶⁾ The HLA haplotypes, *DR4-DQ8* and *DR3-DQ2*, contribute to the greatest risk and are present in more than 90% of the children with T1DM. About 30% of patients with T1DM have both haplotypes, which combination confers the greatest susceptibility.⁽³⁸⁾ These HLA susceptibility genes are thought to be important regulators of the immune response and have a major role in presentation of antigens involved in the pathogenesis of T1DM to antigen receptors on T-cells, the main effector cells of the destructive autoimmune process. The abnormal activation of the T-cell-mediated immune system by one or more environmental triggers in susceptible individuals leads to an inflammatory response in the islets of Langerhans, as well as to an humoral response with production of antibodies to β -cells antigens.⁽³⁾ Several autoantibodies have been identified that may play an important role in initiation or progression of auto-immune islet injury⁽³⁹⁾, including antibodies to insulin (IAA)⁽⁴⁰⁾, glutamic acid decarboxylase (GAD-65)⁽⁴¹⁾, protein tyrosine phosphatase IA2 (IA-2AA)⁽⁴²⁾, and zinc transporter (ZnT8).⁽⁴³⁾ The presence and persistence of these autoantibodies facilitates the likelihood of progression to clinical disease^(44;45), but there is no evidence that any of these antibodies have an active role in the pathogenesis of T1DM. The lifelong risk of DM1 is markedly increased in close relatives of patients with T1DM, averaging about 6 percent in their offspring, 5% in siblings, and 50% in identical twins (versus 0.4% in subjects with no family history).⁽⁴⁶⁻⁴⁸⁾ Environmental factors that trigger the onset of clinical disease are not well understood, but an interaction between genetic susceptibility and environmental factors is thought to be the fundamental element for the disease. Putative triggers include viruses (e.g., enteroviruses, coxsackie B, congenital rubella)⁽⁴⁹⁾, environmental toxins (e.g., nitrosamines)^(50;51), or foods (e.g., early exposure to cow's milk proteins, cereals, or gluten).⁽⁵²⁻⁵⁷⁾ Several pregnancy related and perinatal factors were associated with a small increase in risk of T1DM.⁽⁵⁸⁾ There is evidence for a protective role of omega-3 fatty acids⁽⁵⁹⁾ and vitamin D supplements⁽⁶⁰⁾ in the inflammatory response associated with autoimmune islet cell destruction.

3. Diagnosis

The symptoms of T1DM are caused by hyperglycemia and include polydipsia, meaning excessive drinking, polyuria, or excessive urination, and considerable weight loss. Other symptoms may include lethargy, polyphagia, frequent yeast infections and urinary tract infections, blurred vision, impotence, nausea, vomiting, and dehydration. Diagnostic criteria for diabetes described the WHO and American Diabetes Association (ADA) are a fasting plasma glucose >7.0 mmol/L, or a 2-hour glucose ≥ 11.1 mmol/L during a 75 gram oral glucose tolerance test (OGTT), confirmed by repeat testing on a different day in the absence of unequivocal hyperglycemia, or when classical symptoms of hyperglycemia are present and a random plasma glucose level is ≥ 11.1 mmol/L.⁽²⁾ The presence of T1DM is suggested by presence of serum auto-antibodies to glutamic acid decarboxylase (GAD-65), protein tyrosine phosphatase 1A2 (IA-2AA) and/or insulin (IAA).⁽³⁹⁾

4. Complications

The quality of long term glycemic control, as reflected in HbA_{1c} values, is associated with the onset and progression of the complications of T1DM.⁽⁶¹⁻⁶⁴⁾ Long-term diabetes-related complications are divided into microvascular disease – i.e. nephropathy, retinopathy, and peripheral neuropathy-, and macrovascular disease – i.e. cardiovascular, cerebrovascular, and peripheral vascular disease-, which account for most of the increased morbidity and mortality associated with the disease.⁽³⁾ Risk factors for diabetes-related complications include poor glycemic control, early onset and long duration of T1DM, genetic susceptibility, gender, hypertension, hyperlipidemia, and lifestyle factors (e.g., smoking, obesity, exercise).⁽³⁾ The mechanism by which poor glycemic control predisposes to vascular disease is not completely understood. Putative mechanisms, associated with hyperglycemia, include: 1) accumulation of advanced glycosylation end products that contribute to microvascular disease, 2) accumulation of sorbitol, which interferes with cellular metabolism, 3) end-organ response with activation of cytokines, profibrotic elements, vascular growth factors, inflammation and activation of protein kinase C. Genetic susceptibility is another important factor for the development of complications.^(62;65)

5. Management

Insulin therapy and frequent monitoring of blood glucose by patients themselves is fundamental to account for deficient endogenous insulin secretion and to attain normoglycemia.⁽²⁾ Long-term glycemic control is best measured by HbA_{1c} concentrations, which reflects the average glycemic concentration during the previous three months.⁽⁶⁶⁾ Most patients use basal-bolus approaches to insulin delivery with either multiple daily insulin injections (MDII) or continuous subcutaneous insulin pumps (CSII).^(67;68) There is a small but clinically significant improvement in HbA_{1c} levels with use of CSII compared to MDII.^(69;70) Despite advances in insulin therapy during the past 25 years, only a few individuals with T1DM are able to reach glycemic and HbA_{1c} target levels consistently, reflected in large intra-individual variations in blood glucose.⁽³⁾ Intervention studies on the effect of short treatment with CSII therapy showed some improvement of insulin resistance after CSII in patients with T1DM, but their insulin sensitivity remained lower than that of healthy subjects.⁽⁷¹⁻⁷⁴⁾ Other components of management of patients with T1DM

include nutritional planning and carbohydrate counting⁽⁷⁵⁻⁷⁷⁾, screening for and treatment of diabetes-related complications, screening for and treatment of diabetes –associated disorders, attention to psychological and psychosocial well-being, and avoidance or treatment of severe hypoglycemia and diabetic ketoacidosis.⁽³⁾ There is an inverse relation between glycemic control and the risk of hypoglycemic episodes. Repeated episodes of severe hypoglycemia are a risk factor for cognitive impairment in patients with T1DM.^(78;79)

III. SLEEP

1. Definition

Sleep is a natural and reversible periodic state, characterized by reduced or absent awareness and attention, limited sensory activity and inactivity of nearly all striated muscles. The reversible nature and predictable cycling of sleep stadia are features that distinguish sleep from pathological unconsciousness.⁽⁸⁰⁾

2. Sleep architecture and stadia

Normal sleep is composed of rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep, with NREM being subdivided into 3 stages representing a continuum of relative depth: stage N1, N2, and N3 with stage N3 designated as deep sleep or ‘slow-wave sleep’ (SWS).⁽⁸¹⁾ Each sleep stage has unique characteristics, including brain wave patterns on electroencephalography (EEG), eye movements and muscle tone. NREM and REM sleep stages alternate cyclically and display an ultradian rhythm, with a 90-120 minute cycle. Ultradian rhythms are recurrent periods or cycles repeated throughout a 24-hour circadian day. The cycle repeats 4-5 times throughout a normal 8-hour sleep period. Sleep begins in NREM and progresses through deeper NREM stages before the first episode of REM sleep begins approximately 90 minutes later. Although this cycle repeats through the night, the duration of REM sleep increases and the duration and depth of SWS decreases with each cycle. As a consequence of these shifts in duration, SWS predominates in the first third of the night and REM-sleep in the last third.^(82;83) Most of the entire sleep time (75-85%) is spent in NREM-sleep. About 1-5% of nocturnal sleep is accounted for by short normal periods of wakefulness.⁽⁸⁰⁾ Polysomnography describes the recording of various signals including the EEG, electro-oculography (EOG), and electromyography (EMG) used to distinguish between different sleep stages as well as to record additional signals such as respiratory or circulatory ones to diagnose sleep disorders.^(80;81)

NREM sleep

NREM sleep is defined as ‘a relatively inactive yet actively regulating brain in a movable body’.⁽⁸²⁾ During NREM sleep, cognitive activity is typically fragmented, and body activity periodically occurs as a person moves through the three stages of the NREM sleep. The three NREM stages roughly parallel a depth of sleep continuum with arousal thresholds generally lowest in N1 and highest in N3. Stage N1, drowsiness, is the transition from wakefulness to sleep and the lightest stage of sleep. It is characterized by relatively fast EEG frequencies with low amplitude. During

N1, muscle tone starts to decrease and attention and awareness of the external environment diminish. This stage accounts for 2 to 5 percent of the total sleep time (TST) in young adults. An increased proportion of stage N1 suggests sleep fragmentation due to a sleep disorder. Stage N2 is called 'intermediate' sleep and is characterized by a slowing of the main EEG frequency, the appearance of typical EEG phenomena such as sleep spindles and K-complexes, a further decrease in muscular activity, and loss of awareness of the external environment. Stage N2 sleep typically accounts for 40-50 percent of the total sleep time (TST). Benzodiazepines increase N2 sleep time at the expense of stage N3. The final NREM stage, N3 or SWS, is characterized by low frequency high-amplitude delta EEG waves, and accounts for 20 percent of the TST.

REM sleep

REM sleep is defined as a 'highly activated brain in a paralyzed body'.⁽⁸²⁾ It makes up about 20-25 percent of the total sleep time (TST) and is characterized by: 1) episodic bursts of rapid eye movements; 2) a low-voltage relatively fast EEG that resembles an active, awake pattern; 3) atonia of most skeletal muscles, excepting some such those involved in respiration; and 4) bursts of autonomic activity. REM sleep can be divided as having tonic and phasic periods, distinguished by short clusters of REM activity (*phasic*) that are followed by periods of relative inactivity (*tonic*).⁽⁸⁰⁾

3. Determinants of sleep

Sleep is thought to be regulated by several processes. The ultradian rhythm controls the NREM-REM cycle, whereas homeostatic drive (e.g. increased sleepiness follows longer periods of wakefulness) and circadian sleep-wake rhythms determine the amount and timing of sleep.^(80;82) Age, prior sleep history and the sleep environment, circadian rhythm disorders, external temperature, drugs, medical, neuropsychiatric, sleep and behavioral disorders are important factors which affect both sleep duration and stage distribution.^(82;84-86)

4. The function of sleep

Several theories have been proposed to describe the function of sleep, but the 'restorative theory of sleep' is most accepted. SWS is thought to be the most 'restorative' of all sleep stages.⁽⁸⁷⁾ Several important physiological activities occur during SWS, including reductions in heart rate, blood pressure, sympathetic nervous activity, while vagal tone increases.⁽⁸⁸⁾ SWS is also associated with a decrease in brain glucose metabolism.⁽⁸⁹⁾ Additionally, hormone release is modulated by SWS. The release of the hypothalamic-pituitary-adrenal (HPA) system is inhibited⁽⁹⁰⁾, whereas growth hormone (GH) and prolactin secretion are increased. The counterregulatory hormones GH and cortisol have both important roles in glucose metabolism and their levels are influenced by impaired sleep.⁽⁹¹⁾ Studies evaluating the effect of sleep deprivation indicated impairments in cognitive and physical performance^(92;93), and chronic sleep deprivation is associated with numerous cardiometabolic disturbances, including obesity, hypertension, dysfunction of glucose metabolism, increased diabetes risk, and even mortality.^(94;95)

IV. SLEEP AND GLUCOSEREGULATION

1. Effect of sleep characteristics on glucose regulation

In physiological conditions glucose metabolism shows a circadian rhythm with intra-individual daytime and nighttime variations in glucose tolerance.^(91;96;97) Various mechanisms operate during nocturnal sleep to maintain stable glucose levels during the night.⁽⁹¹⁾ In addition to circadian rhythmicity, sleep plays an important role in the regulation of glucose regulation during the night.^(97;98) During the first half of the sleep period glucose tolerance markedly decreases by diminished glucose utilization because of the predominance of SWS. The initiation of SWS is temporally associated with transient metabolic, hormonal, and neurophysiological changes, all of which could affect glucose metabolism, including a 30-40% decrease in cerebral glucose uptake, suppression of corticotropic and sympathetic activity, rapid anti-insulin-like effect by an increased growth hormone (GH) release, and decreased muscle tone.^(11;99) During the latter part of the sleep, plasma glucose levels progressively decrease towards pre-sleep values, reflecting increased glucose utilization due to the increase in awakenings and REM stages.⁽⁹⁸⁻¹⁰¹⁾

a. Sleep duration

Voluntary sleep curtailment has become a common behavior pattern in our modern '24-hour' society. There is strong evidence that partial sleep restriction affects glucose regulation. A pioneering study by Van Cauter and colleagues⁽¹⁰⁾ showed a 40% reduction in glucose tolerance, measured with the intravenous glucose tolerance test (ivGTT), and other endocrine and metabolic changes associated with diabetes, after six consecutive nights of only four hours sleep per night in 11 healthy young men. Other experimental⁽¹⁰²⁾ and epidemiological^(8;9;13;94;103;104) studies confirmed a strong association between (partial) sleep restriction during multiple nights and glucose tolerance in healthy subjects. Subsequent nights with partial sleep restriction result in impaired glucose tolerance in healthy controls, but the effect of *one single night* of partial sleep deprivation on *insulin sensitivity* had not been characterized in healthy controls. Moreover, previous studies did not include the assessment of insulin sensitivity by the hyperinsulinemic euglycemic clamp method, which is considered to be the gold standard for measurement of insulin sensitivity.⁽¹⁰⁵⁾ We therefore evaluated the effect of a single night of partial sleep restriction on parameters of insulin sensitivity, assessed by the hyperinsulinemic euglycemic clamp studies combined with tracer dilution of $[6,6\text{-}^2\text{H}_2]$ glucose in healthy controls (**Chapter 2**).

As said, glucose regulation cannot be normalized despite intensive insulin therapy in most patients with T1DM, apparent as large intra-individual variations in plasma glucose levels.⁽³⁾ Subtle intra-individual variations in glucoregulation and insulin sensitivity depend on variations in several physiological determinants, such as dietary factors, exercise and psychosocial factors.⁽⁴⁻⁷⁾ Since multiple nights of impaired sleep are known to impair glucose metabolism in healthy controls, we hypothesized that impaired sleep characteristics in patients with T1DM could contribute to the intra-individual variations in glucose regulation, and could be another physiological determinant of impaired glucose regulation seen in these patients. Therefore, we compared the effects of *one single night* of reduced sleep duration with those of a night of normal

sleep duration on hepatic and peripheral insulin sensitivity, as assessed by the hyperinsulinemic euglycemic clamp with stable isotopes, in adult patients with T1DM (**Chapter 3**).

b. Sleep composition

In addition to sleep duration, the composition of sleep in terms of sleep stages is also a determinant of glucose metabolism in healthy controls. Research has supported the clear role of SWS in the maintenance of normal glucose metabolism.^(11;91;96;98) Tasali and colleagues⁽¹¹⁾ showed a markedly decreased insulin sensitivity and glucose tolerance measured with the ivGTT in young healthy controls after selective suppression of SWS, without a change in TST, during three consecutive nights. Selective SWS suppression was reached by delivering acoustic stimuli on varying frequency and intensity to speakers placed on each side on the bed of the subjects. However, the effects of *one single night* of controlled SWS suppression on *insulin sensitivity*, measured by the hyperinsulinemic euglycemic clamp method, have not been studied in healthy subjects before. Therefore, in **Chapter 4**, we compared the effects of one single night of impaired sleep quality – by selective SWS suppression – with those of a night of normal sleep on hepatic and peripheral insulin sensitivity, using a hyperinsulinemic euglycemic clamp with stable isotopes ($[6,6\text{-}^2\text{H}_2]$ glucose), in healthy controls. We hypothesized that selective suppression of SWS would decrease insulin sensitivity in healthy controls, which in turn may contribute to the intra-individual variations in plasma glucose in patients with T1DM.

2. Sleep characteristics in patients with T1DM

The interaction between T1DM and sleep characteristics has not been extensively studied. Jauch-Chara and colleagues reported altered sleep architecture in 14 non-hypoglycemic adult patients compared with healthy controls. The patients spent more time in lighter sleep stages and less time in SWS.^(16;106) The authors suggested that T1DM was associated with disruption of the sleep-wake cycle. A pilot study of Borel and colleagues⁽¹⁴⁾ showed a high prevalence of obstructive sleep apnea (OSA) in 37 non-obese adults with T1DM. Available evidence showed that children with T1DM have a more disrupted sleep with more frequent and longer awakenings, and more sleep disorders, such as more frequent and longer lasting apneas and restless legs syndrome, than healthy children.^(15;17;18) These studies⁽¹⁵⁻¹⁸⁾ investigated relatively few subjects or children with T1DM. The relation between sleep characteristics and glucoregulation has not been studied in adult patients with T1DM. Therefore, in **Chapter 5** we compared subjective sleep characteristics measured by validated sleep questionnaires, between a large cohort of adult patients with T1DM with individually age, gender and BMI matched healthy controls. In addition, we related these sleep characteristics to the quality of glycemic control, *i.e.* HbA_{1c} values, and assessed other possible risk factors for impaired sleep characteristics in adult patients with T1DM. Our primary hypothesis was that adult patients with T1DM have alterations in subjective sleep characteristics compared with individually matched healthy controls. In addition, we hypothesized that subjective sleep disturbances would be associated with impaired glucoregulation. If T1DM indeed causes disruptions of sleep patterns, this may in turn impair glucose regulation, creating a vicious circle.

3. Consequences of impaired glucose regulation on sleep characteristics

Various aspects of diabetes may be linked to disturbed sleep, including physical complications of the disease, metabolic fluctuations, psychological factors, and high prevalence of sleep disorders.^(106;107) A study of Perfect et al⁽¹⁰⁶⁾ showed an association between higher HbA_{1c} and reduced SWS in diabetic children with T1DM. We hypothesized that metabolic dysregulation induced by reduction in insulin therapy in patients with T1DM may affect sleep characteristics. If so, this may induce a vicious circle: metabolic dysregulation would impair sleep and that in turn results in impaired glucose tolerance and insulin resistance. Therefore, we evaluated in **Chapter 6** the effects of controlled hyperglycemia on sleep characteristics in well-controlled (HbA_{1c} below 8% during the year prior to the start of the study) adult patients with T1DM on stable continuous subcutaneous insulin infusion (CSII). In order to obtain and maintain hyperglycemia during the night with glucose levels ~15 mmol/L, the basal and bolus insulin infusions of the patients with T1DM were reduced by 50%, compared with the euglycemic occasion.

V. SLEEP AND COGNITION

Patients with T1DM perform less well on neuropsychological tests including intelligence, psychomotor speed, visual and sustained attention, cognitive flexibility, visual perception, and speed of information processing.^(19;20) Although various disease-related variables are associated with cognitive impairment – e.g. chronic hyperglycemia⁽¹⁰⁸⁾, microangiopathy^(109;110), age at onset^(109;111), disease duration⁽¹¹¹⁾, and structural brain defects^(112;113) – the pathophysiology of cognitive dysfunction in T1DM is not well understood. Studies on the effect of severe hypoglycemia on cognitive function reported conflicting results. Whereas some retrospective studies suggested an association in adult patients^(114;115), large prospective studies failed to conform this.^(116;117) Disturbed sleep might well be another determinant of impaired cognitive function in patients with T1DM. Disturbed sleep impairs daytime functioning, one expression of which is a decline in sustained attention, measurable as prolongation of reaction time (RT), *i.e.* speed, and an increased number of errors, *i.e.* accuracy.⁽¹¹⁸⁾ Decreased sleep duration was associated with decreased speed and the beginning of accuracy failure in healthy controls.^(118;119) Hence, disturbed sleep characteristics might contribute to cognitive problems in T1DM. Deficits in sustained attention have already in part been reported in previous studies of patients with T1DM.⁽¹²⁰⁻¹²²⁾ Those studies investigated sustained attention in children⁽¹²¹⁾, in a controlled setting to investigate the effect of acute hypo- or hyperglycemia⁽¹²⁰⁾, or used tests like the digit vigilance test⁽¹²²⁾, the lottery ticket test⁽¹²⁰⁾ or the elevator counting test.⁽¹²¹⁾ The Sustained Attention to Response Task (SART)⁽¹²³⁾ that assesses sustained attention, has been shown to be sensitive to sleep deprivation in healthy controls⁽¹²⁴⁾ and to reflect excessive sleepiness in sleep-disordered patients.⁽¹²⁵⁾ Sustained attention and its relation with sleep characteristics, have, however, not been studied in adult patients with T1DM. The SART takes a short time to perform, and is easy to administer, which make the test useful in a clinical setting. Therefore, we examined sustained attention – *i.e.* RT and number of omission and commission errors – in adult patients with T1DM, compared to healthy controls, and explored if sleep related factors are a risk factor for sustained attention.. The results of this study are described in **Chapter 7**.

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CHAPTER

2

A SINGLE NIGHT OF PARTIAL SLEEP DEPRIVATION INDUCES INSULIN RESISTANCE IN MULTIPLE METABOLIC PATHWAYS IN HEALTHY SUBJECTS

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J Clin Endocrinol Metab 2010; 95 (6): 2963-68

ABSTRACT

Background

Subsequent nights with partial sleep restriction result in impaired glucose tolerance, but the effects on insulin sensitivity have not been characterized.

Objective

The aim of this study was to evaluate the effect of a single night of partial sleep restriction on parameters of insulin sensitivity.

Research design and methods

Nine healthy subjects (five men, four women) were studied once after a night of normal sleep duration (sleep allowed from 2300 to 730 h), and once after a night of 4 hours of sleep (sleep allowed from 0100 to 0500 h). Sleep characteristics were assessed by polysomnography. Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp studies (from 1130 to 1430 h) with infusion of [6,6-²H₂]glucose.

Results

sleep duration was shorter in the night with sleep restriction than in the unrestricted night (226±11 vs. 454±9 min, $P < 0.0001$). Sleep restriction did not affect basal levels of glucose, non-esterified fatty acids, insulin, or endogenous glucose production. Sleep restriction resulted in increased endogenous glucose production during the hyperinsulinemic clamp study compared to the unrestricted night (4.4±0.3 vs. 3.6±0.2 $\mu\text{mol} \times \text{kg lean body mass}^{-1} \cdot \text{min}^{-1}$, $P=0.017$), indicating hepatic insulin resistance. In addition, sleep restriction decreased the glucose disposal rate during the clamp (32.5±3.6 vs. 40.7±5.1 $\mu\text{mol} \times \text{kg lean body mass}^{-1} \cdot \text{min}^{-1}$, $P=0.009$), reflecting decreased peripheral insulin sensitivity. Accordingly, sleep restriction decreased the rate of glucose infusion by approximately 25% ($P=0.001$). Sleep restriction increased plasma nonesterified fatty acid levels during the clamp study (68±5 vs. 57±4 $\mu\text{mol/L}$, $P=0.005$).

Conclusions

Partial sleep deprivation during only a single night induces insulin resistance in multiple metabolic pathways in healthy subjects. This physiological observation may be of relevance for variations in glucoregulation in patients with type 1 and type 2 diabetes.

INTRODUCTION

Sleep plays a key role in the homeostasis of normal glucose metabolism.^(1,2) In physiological circumstances, glucose metabolism shows a diurnal pattern with intra-individual variations in glucose tolerance: glucose utilization is highest during wake and lowest during non-REM sleep.⁽³⁾ Reductions in sleep duration result in impaired glucose tolerance. Epidemiological studies documented a strong association between partial sleep restriction and impaired glucose tolerance.⁽⁴⁻⁶⁾ In accordance with these findings, experimental studies showed that sleep restriction to only 4 h of sleep during two or more nights reduced glucose tolerance by 40%, and reduced the acute insulin response to glucose in healthy subjects by 30%.^(7,8) However, the effects of only a single night of partial sleep restriction on insulin sensitivity are unknown. Moreover, previous studies did not include the assessment of insulin sensitivity by the hyperinsulinemic euglycemic clamp method, which is considered to be the golden standard for measurement of insulin sensitivity. We hypothesized that even a single night of partial sleep deprivation might induce insulin resistance in healthy subjects. Therefore, we compared the effects of a single night of partial sleep restriction and of a night of normal sleep duration on hepatic and peripheral insulin sensitivity in hyperinsulinemic euglycemic clamp conditions combined with tracer dilution of [6,6-²H₂] glucose.

SUBJECTS AND METHODS

Subjects

Nine healthy lean subjects (five men and four women) were recruited by advertisement. Their weight had been stable for at least three months before participation in this study. Subjects were instructed not to alter life style habits during the whole study period. All premenopausal women were studied in the follicular phase of their menstrual cycle. Exclusion criteria were a body mass index >26 kg/m², sleep disorders, habitual sleep duration of less than 6 h or more than 9 h, psychiatric disorders, use of sleep medication or of medication affecting glucose metabolism. The study was approved by the medical ethical committee of Leiden University Medical Center and written informed consent was obtained from all subjects prior to the study.

Study design

Actigraphy (*Actiwatch AW7, Cambridge Neurotechnology, UK*) was performed to objectively measure habitual sleep duration during 7 d before to the actual study, including one weekend. In addition, self-reported sleep duration and sleep quality were assessed using validated questionnaires (Pittsburgh Sleep Quality Index, Epworth Sleepiness scale and Berlin Questionnaire).⁽⁹⁻¹¹⁾

The subjects were studied on 3 d, separated by intervals of at least 3 wk. Subjects kept a detailed diary of their diet and physical activity for 3 d before each study day and were asked to maintain a standardized schedule of bedtimes and mealtimes in accordance with their usual habits. They were admitted to our clinical research center the night before each study day, and spent 8.5 h in bed from 2300 h to 0730 h on all 3 occasions. Subjects fasted throughout these nights from 2200 h. The first study day was included to let the subjects become accustomed to

sleeping in our clinical research center. Subjects were randomly assigned to sleep deprivation on either the second ($n=4$) or third ($n=5$) occasion.

During the night of sleep restriction, subjects spent 8.5 h in bed, but were only allowed to sleep from 0100 h to 0500 h A.M. They were allowed to read or watch movies in an upward position during the awake hours, and their wakefulness was monitored and assured if necessary.

The rationale for essentially broken sleep deprivation from 2300 to 0100 h and from 0500-0730 h, as opposed to sleep deprivation from 2300 to 03.00 h, or 0300-0730 h, was that in both conditions, the time in bed was centered at the same time, i.e. approximately 0300h. Slow-wave sleep (i.e. stage III of non-REM sleep) is thought to play the most important role in metabolic, hormonal and neurophysiologic changes during sleep. Slow wave sleep mainly occurs during the first part of the night, whereas REM sleep predominantly occurs during the latter part of the night.⁽¹²⁾ We used broken sleep deprivation to achieve a more equal compression of both non-REM and REM sleep stages. Moreover, we used the same experimental conditions for partial sleep deprivation as previously used in other studies^(7;13) to enable comparison of the results.

Polysomnography

Sleep recordings were performed using a portable PSG recorder (*Titanium, Embla Systems, Inc, Broomfield, USA*). Sleep was visually scored for each of the three nights according to the guidelines of the American Association of Sleep Medicine.⁽¹⁴⁾ For this study, the duration of wake, stage I, II, III and REM sleep were noted. Total sleep duration was the sum of sleep stages I, II, III and REM.

Hyperinsulinemic euglycemic clamp studies

Hyperinsulinemic euglycemic clamp studies were performed the day after the second and third nights. After an overnight fast, a catheter was inserted into an antecubital vein for infusion of isotopes, glucose and insulin, and a sampling catheter was inserted into a dorsal hand vein of the contralateral arm. For all blood samples, the heated hand technique was used to obtain arterialized blood.⁽¹⁵⁾ A primed [$17.6\mu\text{mol.kg}^{-1}$], continuous [$0.22\mu\text{mol.kg}^{-1}.\text{min}^{-1}$] infusion of [$6,6\text{-}^2\text{H}_2$] glucose (*Cambridge Isotope Laboratory, Andover, MA*) was started at 0830 h, both after the night of normal sleep duration and after the night of partial sleep deprivation, after basal blood samples had been taken for determination of background glucose enrichment. Labeled glucose was infused by a Pilot C syringe pump (*Fresenius Vial, Fresenius Kabi, Brezins, France*). Blood samples were obtained after 160, 170 and 180 min of [$6,6\text{-}^2\text{H}_2$] glucose infusion for assessment of glucose kinetics in the basal state and concentrations of glucose, insulin and plasma non-esterified fatty acids (NEFA).

Subsequently, infusion of insulin was started, using the method of DeFronzo *et al.*⁽¹⁶⁾ while the infusion of [$6,6\text{-}^2\text{H}_2$] glucose continued. Briefly, the infusion of insulin consisted of a primed ($80\text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ for 5 min and subsequently $40\text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ for 5 min), followed by continuous ($20\text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) infusion of insulin (*Actrapid; Novonordisk; Alphen a/d Rijn; The Netherlands*), dissolved in sterile NaCl 0.9%, using a Pilot C syringe pump (*Fresenius Vial, France*). A variable infusion of glucose 20% enriched with 3% [$6,6\text{-}^2\text{H}_2$] glucose was started four minutes after the start of insulin infusion. Plasma glucose concentrations were measured with

intervals of 5 min with a bedside calibrated glucose analyzer (*Accu-Chek; Roche; Mannheim; Germany*) and the infusion rate of glucose 20% was adjusted in order to keep the plasma glucose levels constant at 5.0 mmol/liter during the clamp study. Blood samples were obtained after 160, 170 and 180 min of combined insulin and [6,6-²H₂] glucose infusion for assessment of glucose kinetics and of concentrations of glucose, insulin and plasma NEFA.

Assays

Serum concentrations of glucose were measured using a fully automated Modular P 800 analyzer (*Roche/Hitachi, Mannheim, Germany*) with intraassay variations of 1%. Serum insulin concentrations were measured by enzyme labeled chemiluminiscent immunometric assay (*Immulite 2500; Siemens; Germany*) with an intraassay coefficient of variation (CV) of 4%. Cortisol was analysed on a Modular E-170 immunoanalyser of Roche Diagnostics (*Mannheim, Germany*). Intra- and interassay CV were less than 5% at all measured levels. Glucagon was analysed using an immunoradio-metric assay of Millipore (*Billerica MA, formerly known as Linco*) with a functional sensitivity of 20 pg/ml. Intraassay CV was less than 12% and inter-assay CV was less than 15%. NEFA were determined spectrophotometrically by enzymatic colorimetric acyl-CoA synthase/acyl-CoA oxidase assay (*WAKO Chemicals, Neuss, Germany*) with intra-assay CV of 2.7%. Enrichment of plasma [6,6-²H₂] glucose was determined in a single analytical run, using gas chromatography coupled to mass spectrometry, as described previously.⁽¹⁷⁾ All isotope enrichments were measured on a gas chromatograph mass spectrometer (*model 6890/5973; Hewlett-Packard; Palo Alto; CA*).

Calculations

An isotopic steady state was achieved during the final 30 min of the basal period and the final 30 min of the hyperinsulinemic clamp study. Therefore, the rates of appearance (*Ra*) and disappearance (*Rd*) of glucose were calculated as the tracer infusion rates divided by the tracer-to-tracee ratios.⁽¹⁸⁾ Endogenous glucose production during the basal steady state is equal to *Ra* of glucose, whereas endogenous glucose production during the hyperinsulinemic clamp study was calculated as the difference between *Ra* and the glucose infusion rates.

Statistical analysis

Data are presented as mean ± SEM. Differences between the effects of the night of normal sleep duration and sleep restriction were analyzed by Student's *t* test for paired samples. All analyses were performed using SPSS for Windows version 16.0 (*SPSS, Chicago, IL*). Significance was accepted at *P* < 0.05.

RESULTS

Clinical characteristics

Mean age of the subjects (five men, four women) was 44.6 ± 4.9 yr; mean weight was 72.4 ± 2.6 kg; mean height was 174.6 ± 1.4 cm; and mean body mass index was 23.8 ± 0.8 kg/m². All subjects had normal results on validated questionnaires on sleep characteristics.⁽⁹⁻¹¹⁾ Self-reported sleep

duration and recorded habitual sleep duration by actigraphy were not different (450 ± 20 vs 476 ± 11 min, $P=0.19$).

The effects of partial sleep restriction on polysomnographic parameters (Table 1)

Measured sleep duration was considerably shorter in the night with partial sleep restriction, compared to the night with normal sleep duration ($P<0.001$). The proportion of stage III sleep was higher in the sleep-deprived night ($P=0.007$), whereas the subjects had relatively less stage II sleep ($P=0.006$). The percentages of REM-sleep and wake time during sleep did not differ significantly between nights.

The effects of partial sleep restriction on basal metabolic parameters (Table 1)

Compared to normal sleep, partial sleep deprivation did not alter basal levels of glucose, NEFA, insulin, glucagon or cortisol measured the following morning. In addition, sleep restriction did not affect basal endogenous glucose production assessed by primed, continuous infusion of [6,6-D2 glucose].

Table 1. The effects of a night of normal sleep duration and of a night with sleep restricted to 4 hours on sleep parameters, basal and insulin-stimulated glucose and fatty acid metabolism measured the following morning in 9 healthy subjects.

Sleep parameters	Normal sleep duration	Partial sleep deprivation	P-value
Total sleep time(min)	454 ± 9	226 ± 11	0.000
Stage I (%)	9.6 ± 1.1	10.7 ± 2.3	0.490
Stage II (S)	41.3 ± 2.5	34.5 ± 3.4	0.006
Stage III (SWS) (%)	25.4 ± 1.8	33.5 ± 3.1	0.007
REM sleep (S)	23.7 ± 1.7	21.3 ± 3.0	0.364
Wake time during sleep (%)	6.8 ± 1.5	6.8 ± 2.2	1.0
Basal values			
Glucose (mmol/liter)	4.8 ± 0.18	4.8 ± 0.18	0.32
Insulin (mU/liter)	3.1 ± 0.5	3.6 ± 0.6	0.18
NEFA ($\mu\text{mol/liter}$)	630 ± 50	620 ± 50	0.76
Endogenous glucose production ($\mu\text{mol.kgLBM}^{-1}.\text{min}^{-1}$)	16.7 ± 0.4	16.7 ± 0.5	0.87
Glucagon (pg/ml)	36 ± 4	35 ± 4	0.888
Cortisol (nmol/liter)	349 ± 59	395 ± 59	0.308
Hyperinsulinemic euglycemic clamp study			
Glucose (mmol/liter)	5.0 ± 0.11	4.9 ± 0.9	0.10
Insulin (mU/liter)	22.0 ± 1.5	21.4 ± 1.8	0.30
NEFA ($\mu\text{mol/liter}$)	57 ± 4	68 ± 5	0.005
Endogenous glucose production ($\mu\text{mol.kgLBM}^{-1}.\text{min}^{-1}$)	3.6 ± 0.2	4.4 ± 0.3	0.017
Glucose Rd ($\mu\text{mol.kgLBM}^{-1}.\text{min}^{-1}$)	40.7 ± 5.1	32.5 ± 3.6	0.009
Glucose infusion rate ($\mu\text{mol.kg LBM}^{-1}.\text{min}^{-1}$)	36.9 ± 5.1	27.8 ± 3.7	0.001
Glucagon (pg/ml)	24 ± 3	25 ± 3	0.590
Cortisol (nmol/liter)	353 ± 47	410 ± 48	0.321

Data are presented as mean $\pm$ SEM. Significant differences are shown in bold. LBM, Lean body mass.

The effects of partial sleep restriction on metabolic parameters during hyperinsulinemic euglycemic clamp conditions (Table 1 and figure 1)

Steady state glucose and insulin levels did not differ between the two clamp studies. Sleep restriction resulted in an increase of endogenous glucose production of approximately 22% ($P=0.017$) during the clamp conditions, indicating hepatic insulin resistance. In addition, sleep restriction decreased the rate of glucose disposal (R_d) during the clamp by approximately 20% ($P=0.009$), reflecting decreased peripheral insulin sensitivity. Accordingly, the rate of infusion of glucose, necessary to maintain plasma glucose levels during the hyperinsulinemic clamp study, was approximately 25% lower after the night of reduced sleep duration than after the night of normal sleep duration ($P=0.001$). Finally, the night of partial sleep restriction induced an increase of approximately 19% in plasma NEFA levels ($P=0.005$) during the clamp study, indicating decreased insulin sensitivity of lipolysis.

There were no differences in glucagon or cortisol levels between the steady state conditions of the both clamp studies.

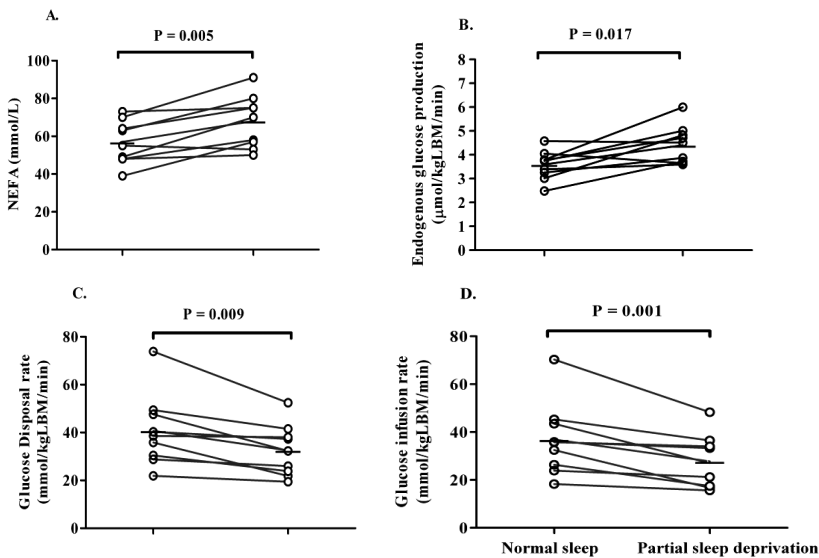


Figure 1. Individual values obtained during steady state of the hyperinsulinemic euglycemic clamp studies of nonesterified fatty acids (NEFA) (A), endogenous glucose production (B), glucose disposal rate (C), and the glucose infusion rate (D) after a night of normal sleep duration versus after a night of partial sleep deprivation in healthy subjects ($n=9$). Black horizontal lines represent the mean of the values of 9 subjects. There were significant differences ($P < 0.05$) for all these parameters after partial sleep deprivation.

DISCUSSION

The aim of the present study was to assess the effects of partial sleep deprivation during a single night on insulin sensitivity in healthy subjects. The results indicate that partial sleep restriction during only a single night reduces insulin sensitivity by 19-25% of hepatic and peripheral glucose

metabolism, as well as of peripheral lipolysis, reflected by NEFA levels. Therefore, a single night of sleep restriction to 4 h induces insulin resistance of multiple metabolic pathways in healthy subjects.

The results of the present study stress the importance of sleep duration as a physiological determinant for insulin sensitivity. The study is unique in several ways. First, the finding that a single night of shortened sleep is sufficient to reduce insulin sensitivity in healthy men and women is novel. Second, this is the first study detailing the effects of sleep restriction on parameters of insulin sensitivity documented during hyperinsulinemic euglycemic clamp conditions with isotope dilution of a glucose tracer. Third, we document that insulin sensitivity is reduced in different metabolic pathways, *i.e.* endogenous glucose production, glucose uptake and lipolysis.

The current study extends the observations of previous experimental studies on the effects of sleep deprivation on glucose metabolism, summarized in Table 2. These studies covered a range of sleep deprivation between 1 h of restriction of sleep during multiple subsequent nights, restriction to 4 h of sleep during subsequent nights and finally complete deprivation of sleep. There was no significant effect on glucose tolerance if sleep was reduced by only one h.⁽¹⁹⁾ More severe restriction of sleep duration to 4 h per night during multiple subsequent nights resulted in decreased glucose tolerance.^(7,20) Total sleep deprivation also decreased glucose tolerance.^(21,22) Apparently, there is a threshold of a minimum duration of sleep, which is required for maintenance of normal glucose tolerance. Moreover, based on our findings after only a single night of partial sleep deprivation, it is tempting to speculate that the negative effects of multiple nights of partial sleep restriction on glucose tolerance can be reproduced, at least in part, by only a single night of sleep deprivation.

Table 2. Summary of experimental studies on the effects of sleep deprivation on glucose metabolism

First author, year (Ref)	Sample size	Sleep deprivation	Insulin sensitivity	Results
VanHelder et al (1993) (22)	10 healthy males	Total sleep deprivation for 60 h	OGTT	No differences in plasma glucose response to OGTT 20% increase in resting plasma insulin levels after sleep deprivation
Spiegel et al (1999) (7)	11 healthy males Aged 18-27 yr	Partial sleep deprivation for 6 nights, bedtime 4 h/night	IVGTT 24 h profile glucose and hormone concentrations	40% decrease in glucose tolerance after sleep deprivation
González-Ortiz et al (2000)(21)	7 healthy males 7 healthy women	Total sleep deprivation for 24 h	Insulin suppression test with octreotide	18% increase in steady state glucose concentration after sleep deprivation
Zielinski et al (2008)(19)	40 healthy adults Aged 50-70 yr	Time in bed restriction for 8 weeks, total sleep time 1 h less/night	OGTT	No significant effect on glucose tolerance
Nedeltcheva et al (2009)(20)	6 healthy males 5 healthy females Mean age 39 yr	Partial sleep deprivation 14 nights, bedtime 5.5 h/night	OGTT IVGTT	10% increase in 2-h glucose levels and 17.5% decrease in insulin sensitivity
Present study	5 healthy men 4 healthy women Aged 23-62 yr	Partial sleep deprivation 1 night, bedtime 4h/night	Hyperinsulinemic euglycemic clamp [6,6-D2] glucose infusion	19-25% decrease in insulin sensitivity in multiple metabolic pathways

OGTT = oral glucose tolerance test; IVGTT = intravenous glucose tolerance test

Our data also indicate that insulin sensitivity is not fixed in healthy subjects, but, rather, depends on the duration of sleep in the preceding night. These observations extend previous studies, which documented consistent effects of short term variations in dietary macronutrient composition, dietary caloric content and short term fasting on insulin sensitivity in healthy subjects.^(17;23;24) Other studies have indicated effects of exercise in preceding days on insulin sensitivity.⁽²⁵⁾ Therefore, environmental factors including previous sleep duration, exercise, dietary macronutrient composition and content modulate insulin sensitivity in healthy subjects.

The current study was aimed at assessing the effects of a single night of sleep deprivation on parameters of insulin sensitivity. Unfortunately, the study was not designed to elucidate the underlying mechanisms of this effect of partial sleep deprivation. We observed no effects of partial sleep deprivation on cortisol and glucagon levels. Schmid *et al.*⁽²⁶⁾ documented that a single night of sleep restriction to 4.5 h reduced basal plasma glucagon and cortisol levels on the following morning, but did not affect basal glucose, insulin, C-peptide epinephrine, norepinephrine, or GH levels. Apparently, endocrine changes after a single night of partial sleep deprivation do not provide a simple explanation for the induction of insulin resistance. Spiegel *et al.*⁽⁷⁾ documented that subsequent nights of partial sleep deprivation increased cortisol levels during 24 h whereas Nedeltcheva *et al.*⁽²⁰⁾ did not find a major effect on cortisol levels. Nedeltcheva *et al.* documented that prolonged partial sleep deprivation induced a modest increase in 24-h epinephrine and nighttime norepinephrine levels. In accordance, Irwin *et al.*⁽²⁷⁾ documented that plasma levels of epinephrine increased in association with nocturnal awakening. Therefore, sequential nights of partial sleep deprivation induce modest changes in endocrine homeostasis and in glucose tolerance, but the relations between these phenomena are uncertain. Alternatively, it is possible that partial sleep deprivation decreases insulin sensitivity by altering the activity of the autonomous nervous system. However, this is a difficult area of investigation. Previous studies have documented an increased sympathetic tone derived from recordings of heart rate variability after sleep deprivation for a single or consecutive nights.^(13;28) However, it is not known to which extent partial sleep loss induces comparable increases in sympathetic activity at all peripheral sites. Consequently, the relationship between elevated sympathovagal balance at the level of the heart and the sympathetic outflow to liver, muscles, and adipose tissue is uncertain.⁽¹³⁾

What are the implications of the present observations? Sleep duration has shortened considerably in western societies in the past decades.^(5;29) Simultaneously, there has been an increase in the prevalence of insulin resistance and type 2 diabetes. The current and other studies (*Table 2*) indicate that shortened sleep duration is a factor that contributes to glucose intolerance and, even after a single night, to insulin resistance.

It is also tempting to speculate to which extent variations in sleep duration may be involved in the intraindividual variations in glucose levels that are present especially in patients with type 1 diabetes. At present it is unclear whether interventions aimed at optimization of sleep duration may be beneficial in stabilizing glucose levels in patients with diabetes.

In conclusion, the present study indicates that partial sleep restriction, even during a single night, decreases insulin sensitivity of multiple metabolic pathways in healthy subjects.

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CHAPTER

3

PARTIAL SLEEP RESTRICTION DECREASES INSULIN SENSITIVITY IN PATIENTS WITH TYPE 1 DIABETES

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Diabetes Care 2010; 33(7): 1573-77

ABSTRACT

Background

Sleep restriction results in decreased insulin sensitivity and glucose tolerance in healthy subjects. We hypothesized that sleep duration is a determinant of insulin sensitivity in patients with type 1 diabetes.

Research design and methods

we studied seven patients (three men, four women) with type 1 diabetes: mean age 44 ± 7 yr, body mass index (BMI) 23.5 ± 0.9 kg/m² and HbA_{1c} $7.6 \pm 0.3\%$. They were studied once after a night of normal sleep duration, and once after a night of only 4 h of sleep. Sleep characteristics were assessed by polysomnography. Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp studies with infusion of [6,6-²H₂] glucose.

Results

Sleep duration was shorter in the night with sleep restriction than in the unrestricted night (469 ± 8.5 vs. 222 ± 7.1 min, $P = 0.02$). Sleep restriction did not affect basal levels of glucose, non-esterified fatty acids (NEFAs) or endogenous glucose production. Endogenous glucose production during the hyperinsulinemic clamp was not altered during the night of sleep restriction compared with the night of unrestricted sleep (6.2 ± 0.8 vs. 6.9 ± 0.6 $\mu\text{mol.kg lean body mass}^{-1}.\text{min}^{-1}$, NS). In contrast, sleep restriction decreased the glucose disposal rate during the clamp (25.5 ± 2.6 vs. 22.0 ± 2.1 $\mu\text{mol.kg lean body mass}^{-1}.\text{min}^{-1}$, $P = 0.04$), reflecting decreased peripheral insulin sensitivity. Accordingly, sleep restriction decreased the rate of glucose infusion by $\sim 21\%$ ($P = 0.04$). Sleep restriction did not alter plasma NEFA levels during the clamp (143 ± 29 vs. 133 ± 29 $\mu\text{mol/l}$, NS).

Conclusions

Partial sleep deprivation during a single night induces peripheral insulin resistance in these seven patients with type 1 diabetes. Therefore, sleep duration is a determinant of insulin sensitivity in patients with type 1 diabetes.

INTRODUCTION

Intensive insulin therapy is essential for optimal glucoregulation in type 1 diabetes, because the degree and duration of hyperglycemia determine the incidence of complications.⁽¹⁾ However, glucoregulation cannot be normalized in patients with type 1 diabetes, as reflected in relatively large intra-individual variations of blood glucose levels. Subtle intra-individual variations in glucoregulation and insulin sensitivity in these patients depend on variations in physiological determinants, such as dietary factors and exercise.^(2,3) In contrast to healthy subjects, however, patients with type 1 diabetes mellitus are unable to compensate for these physiological variations, other than by variation of the dose of exogenous insulin.

Normal glucose homeostasis shows a diurnal pattern with variations in glucose tolerance, in which sleep characteristics plays a key role.⁽⁴⁾ In this respect, sleep duration may be particularly relevant because sleep curtailment is a common aspect of our modern 24-h society.^(5,6) A reduction in sleep duration impairs glucose tolerance in healthy subjects.⁽⁷⁾ The effects of reduction of sleep duration on insulin sensitivity have not been studied in patients with type 1 diabetes.

We hypothesize that partial sleep restriction decreases insulin sensitivity in patients with type 1 diabetes, which may contribute to intra-individual variations in glucoregulation. Therefore, we compared the effects of a single night of reduced sleep duration with those of a night of normal sleep duration on hepatic and peripheral insulin sensitivity, as assessed by hyperinsulinemic euglycemic clamp studies combined with tracer dilution of [6,6-²H₂] glucose in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Subjects

We included nine patients (five men and four women) with type 1 diabetes on stable continuous subcutaneous insulin pump therapy. Diagnostic criteria for type 1 diabetes were past or present positive antibodies against GAD or islet cells and plasma free C-peptide levels <0.3 nmol/l. All participants were screened in our outpatient clinic prior to the study. Their weight had been stable for at least 3 months prior to participation in this study. A1C levels had been below 8.5 % during the year prior to the start of the study. Exclusion criteria were a BMI >26 kg/m², clinical signs of autonomic neuropathy, known sleep disorders, habitual sleep duration of less than 6 h or more than 9 h, psychiatric disorders, use of sleep medication, β -blocking agents or prokinetic drugs. All patients had normal blood pressure and serum creatinine levels, and urinary microalbumin excretion rated below 30 mg/24 h. The study was approved by the medical ethical committee of Leiden University Medical Center and written informed consent was obtained from all subjects prior to the study.

The subjects were studied on 3 days, separated by intervals of at least 3 weeks. Subjects kept a detailed diary of their diet and physical activity for 3 days prior to each study day and were asked to maintain a standardized schedule of bedtimes and mealtimes in accordance with their usual habits. Actigraphy (*Actiwatch AW7, Cambridge Neurotechnology, U.K.*) was performed to objectively assess patterns of habitual active and inactive (sleep) periods for 7 days prior to the actual study, including one weekend. In addition, self-reported sleep duration and sleep

quality were assessed using validated questionnaires (Pittsburgh Sleep Quality Index, Epworth Sleepiness scale and Berlin Questionnaire).⁽⁸⁻¹⁰⁾

Subjects were admitted to our clinical research center the night preceding each study day, and spent 8.5 h in bed from 2300 h to 0730 h on all three occasions. Subjects fasted throughout these nights from 2200 h onwards. The first study day served to let subjects become accustomed to sleep conditions in a research setting. The optimal overnight infusion rate of insulin was determined in each subject prior to the start of the study. Subsequently, this same individualized infusion rate was used in both sleeping experiments. Subjects were instructed not to alter life style habits during the study period. Premenopausal women were studied in the follicular phase of their menstrual cycle.

Subjects were randomly assigned to partial sleep deprivation on either the second ($n=4$) or third ($n=5$) study occasion.

During the night of sleep restriction, subjects also spent 8.5 h in bed, but were only allowed to sleep from 0100 h to 0500 h. They were allowed to read or watch movies in an upward position, and their wakefulness was monitored and assured if necessary.

Polysomnography

Sleep recordings were visually scored for each of the three nights according to the guidelines of the American Association of Sleep Medicine (AASM).⁽¹¹⁾ In short, scoring of sleep stages depends on electroencephalography, eye movements, and submental muscle activity. To detect possible sleep disorders that might affect the study, respiratory movements were recorded by measurement of changes in nasal pressures and of truncal respiratory movements. Recordings were made using a portable polysomnography (PSG) recorder (*Titanium; Embla Systems, Broomfield, CO*). Sleep and wake stages were visually scored in consecutive epochs of 30 s, resulting in a list of epochs spent in wake, stage I (drowsiness), II, III and rapid-eye movement (REM) dream sleep. The time at which subjects went to bed and turned out the lights as well as times of getting out of bed were noted. The lists of stages were used to calculate the duration of time spent each night in the above-mentioned sleep and wake stages. These durations were also expressed as percentages of total sleep duration, defined as the summed duration of sleep stages I, II, III and REM.

Hyperinsulinemic euglycemic clamp studies

Hyperinsulinemic euglycemic clamp studies were performed the day following the second and third study occasions. After an overnight fast, a catheter was inserted into an antecubital vein for infusion of isotopes, glucose and insulin, and a sampling catheter was inserted into a dorsal hand vein of the contra lateral arm. For all blood samples, the heated hand technique was used to obtain arterialized blood.⁽¹²⁾ A primed [$17.6 \mu\text{mol/kg}^{-1}$] continuous [$0.22 \mu\text{mol.kg}^{-1}.\text{min}^{-1}$] infusion of [$6,6\text{-}^2\text{H}_2$] glucose (*Cambridge Isotope Laboratory, Andover, MA*) was started at 0830 h, after basal blood samples had been taken for determination of background glucose enrichment. Labeled glucose was infused by a Pilot C syringe pump (*Fresenius Vial, Brezins, France*). Blood samples were obtained after 160, 170 and 180 min of [$6,6\text{-}^2\text{H}_2$] glucose infusion for assessment of glucose kinetics in the basal state and concentrations of glucose and plasma non-esterified

fatty acids (NEFAs). Subsequently, administration of subcutaneous insulin was stopped and infusion of intravenous insulin was started, using the method of DeFronzo et al.⁽¹³⁾ Briefly, this consisted of a primed ($80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ for 5 minutes and subsequently $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ for 5 min), followed by continuous ($20 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) infusion of insulin (*Actrapid, Novo Nordisk, Alphen a/d Rijn, The Netherlands*), dissolved in sterile NaCl 0.9 %, using a Pilot C syringe pump (Fresenius Vial, France). A variable infusion of glucose 20% enriched with 3% $[6,6\text{-}^2\text{H}_2]$ glucose was started 4 min after the start of insulin infusion. Plasma glucose concentrations were measured with intervals of 5 min with a bedside calibrated glucose analyzer (*Accu-Chek, Roche, Mannheim, Germany*), and the infusion rate of glucose 20% was adjusted in order to keep the plasma glucose levels constant at 5.0 mmol/l during the clamp study. Blood samples were obtained after 150,160,170 and 180 min of combined insulin and $[6,6\text{-}^2\text{H}_2]$ glucose infusion for assessment of glucose kinetics and of concentrations of glucose, insulin and plasma NEFA.

Biochemical analysis

Serum concentrations of glucose were measured using a fully automated Modular P 800 analyzer (*Roche/Hitachi; Mannheim, Germany*) with intra-assay variations of 1%. Serum insulin concentrations were measured by enzyme labeled chemiluminescent immunometric assay (*Immulite 2500; Siemens; Bad Nauheim, Germany*) with an intra-assay coefficient of variation (CV) of 4%. NEFA were determined spectrophotometrically by enzymatic colorimetric acyl-CoA synthase/acyl-CoA oxidase assay (*WAKO Chemicals, Neuss, Germany*) with intra-assay CV of 2.7%. Enrichment of plasma $[6,6\text{-}^2\text{H}_2]$ glucose was determined in a single analytical run, using gas chromatography coupled to mass spectrometry, as described previously.⁽¹⁴⁾ All isotope enrichments were measured on a gas chromatograph mass spectrometer (*model 6890/5973, Hewlett-Packard, Palo Alto, CA*).

Calculations

Isotopic steady state was achieved during the final 30 min of the basal period and the final 30 min of the hyperinsulinemic clamp study. Therefore, the rates of appearance (R_a) and disappearance (R_d) of glucose were calculated as the tracer infusion rates divided by the tracer-to-tracee ratios. Endogenous glucose production (EGP) during the basal steady state is equal to R_a of glucose, whereas EGP during the hyperinsulinemic clamp study was calculated as the difference between R_a and the glucose infusion rates.

Statistical analysis

Data are presented as mean $\pm$ SEM. Differences between the effects of the night of normal sleep duration and sleep restriction were analyzed by the Wilcoxon signed rank test for paired samples. All analyses were performed using SPSS for Windows version 16.0 (*SPSS, Chicago, IL*). Significance was accepted at $p < 0.05$.

RESULTS

Clinical characteristics

The results of two of the nine patients were excluded, because of nocturnal hypoglycaemia and subsequent nocturnal hyperglycaemia during the study ($n=1$), and because of the presence of a previously undetected sleep apnea syndrome ($n=1$). Therefore, the analyses included the data of seven patients (three men) for analysis. Mean age of these seven subjects was 44.3 ± 6.6 years, mean weight 72.0 ± 4.0 kg, mean height 175 ± 3 cm and mean BMI 23.5 ± 0.9 kg/m². Mean A1C of the patients was $7.6 \pm 0.3\%$ and mean duration of diabetes was 23 ± 3.5 yr. All subjects had normal results on validated sleep questionnaires. Self-reported sleep duration and recorded habitual sleep duration by actigraphy were not different (475 ± 8 vs. 490 ± 7 min, $P=0.12$).

Effects of partial sleep restriction on polysomnographic parameters

Sleep duration was considerably shorter in the night with partial sleep restriction, compared to the night with normal sleep duration ($P=0.02$) (Table 1). Sleep in the sleep-deprived night showed a higher proportion of stage III sleep ($P=0.02$) and a lower proportion of REM sleep ($P=0.04$). The proportions of time spent in other stages did not differ between the conditions.

Table 1. The effects of a normal night of sleep and of a night with sleep restricted to 4 hours on sleep parameters assessed by polysomnography, basal and insulin stimulated glucose and fatty acid metabolism in 7 patients with type 1 diabetes.

	Normal sleep	Partial sleep deprivation	P value
Sleep parameters			
TST (min)	469 ± 8.5	222 ± 7.1	0.02
Stage 1 (% of TST)	10.6 ± 1.0	10.2 ± 2.1	0.49
Stage 2 (% of TST)	44.4 ± 2.8	40.4 ± 2.4	0.13
Stage 3 (% of TST)	22.7 ± 3.4	35.3 ± 4.4	0.02
REM sleep (% of TST)	22.2 ± 1.7	13.7 ± 2.3	0.04
Wake time during sleep (% of total sleep period)	4.2 ± 0.7	5.4 ± 2.3	0.87
Basal values			
Glucose (mmol/L)	7.2 ± 1.0	6.7 ± 1.4	0.87
NEFA (μmol/L)	673 ± 125	591 ± 99	0.35
Endogenous glucose production (μmol.kgLBM ⁻¹ .min ⁻¹)	17.6 ± 0.9	16.3 ± 1.0	0.31
Hyperinsulinemic euglycemic clamp study			
Glucose (mmol/l)	5.1 ± 0.2	5.2 ± 0.1	0.23
Insulin (mU/l)	24.0 ± 2.2	24.9 ± 1.5	0.40
NEFA (μmol/l)	143 ± 29	133 ± 29	0.69
EGP (μmol.kgLBM ⁻¹ .min ⁻¹)	6.2 ± 0.8	6.9 ± 0.6	0.74
Glucose Rd (μmol.kgLBM ⁻¹ .min ⁻¹)	25.5 ± 2.6	22.0 ± 2.1	0.04
Glucose infusion rate(μmol.kgLBM ⁻¹ .min ⁻¹)	19.0 ± 2.9	14.9 ± 2.1	0.04

Data are presented as mean $\pm$ SEM. Data in bold represent statistical significance. LBM, lean body mass; TST total sleep time.

Effects of partial sleep restriction on basal metabolic parameters

The mean overnight rate of subcutaneous infusion of insulin was 0.7 IE/h and was identical in both conditions (Table 1). Compared with normal sleep duration, partial sleep deprivation did not alter basal levels of glucose or NEFA measured the following morning. In addition, partial sleep restriction did not affect basal EGP assessed by primed, continuous infusion of [6,6- $^2\text{H}_2$ glucose].

The effects of partial sleep restriction on metabolic parameters during hyperinsulinemic euglycemic clamp conditions

Steady state glucose and insulin levels did not differ between the two clamp studies (Table 1 and Fig.1). Sleep restriction did not affect EGP during the clamp conditions. However, sleep restriction decreased the rate of glucose disposal (Rd) during the clamp by ~14% ($P=0.04$). Accordingly, the rate of infusion of glucose, necessary to maintain constant plasma glucose levels during the hyperinsulinemic clamp study, was ~21% lower after the night of reduced sleep duration than after the night of normal sleep duration ($p=0.04$), reflecting decreased peripheral insulin sensitivity. There was no significant effect of partial sleep restriction on plasma NEFA levels.

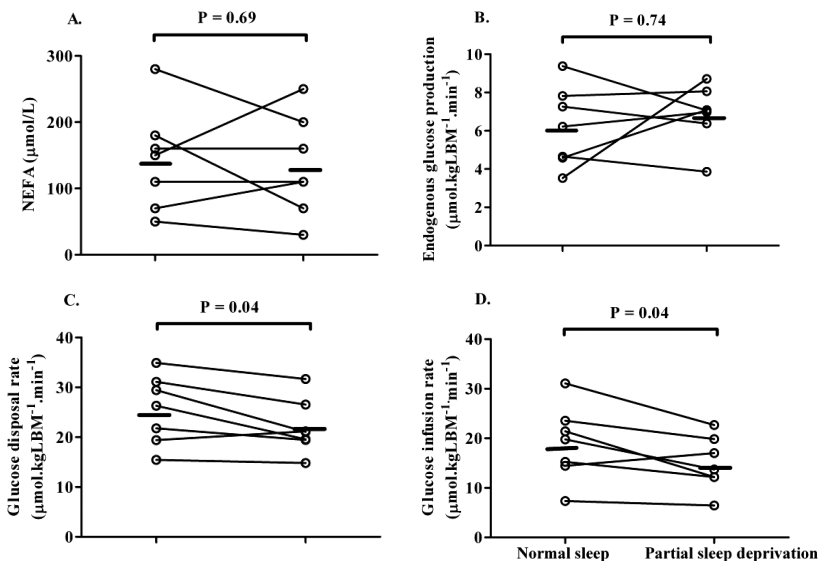


Figure 1. Individual values obtained during steady state of the hyperinsulinemic euglycemic clamp studies of NEFAs (A), EGP (B), glucose disposal rate (C), and the glucose infusion rate (D) after a night of normal sleep duration versus after a night of partial sleep deprivation in patients with type 1 diabetes ($n=7$). Black horizontal lines represent the mean of the values of seven subjects.

CONCLUSIONS

In this study, we assessed the effects of a single night of partial sleep restriction on insulin sensitivity in patients with type 1 diabetes. The results indicate that only a single night of partial sleep restriction

reduces insulin sensitivity of insulin stimulated glucose uptake by 14-21%. Partial sleep restriction did not significantly affect basal glucose metabolism. We conclude that sleep duration is a determinant of peripheral insulin sensitivity in patients with type 1 diabetes. In the current study, we included data of only seven patients with type 1 diabetes. The strictly controlled design of this pathophysiological study in combination with the fact that each subject served as his/her own control enabled us to establish subtle effects of partial sleep deprivation on parameters of insulin sensitivity. Nonetheless, larger numbers of subjects are required to assess the involvement of relevant patient characteristics such as sex, age, and antecedent glucoregulation on the effects of sleep restriction on insulin sensitivity.

This is the first study that documents an adverse effect of partial sleep restriction on insulin sensitivity in patients with type 1 diabetes. In healthy subjects, sleep restriction induces insulin resistance and reduces glucose tolerance.^(7;15) By analogy, it can be expected that sleep restriction increases postprandial glucose levels in patients with type 1 diabetes in the absence of concurrent adaptations of the dose of exogenous insulin. Insulin sensitivity is not static but varies over time within individuals. This is also reflected by the current study in patients with type 1 diabetes. Several epidemiological studies documented an association between chronic partial sleep restriction and development of insulin resistance and type 2 diabetes.^(5;16;17) Therefore, exposure to chronic sleep restriction might contribute to insulin resistance in patients with type 1 diabetes. In turn, insulin resistance is associated with an increased risk for microvascular and macrovascular complications in type 1 diabetes.⁽¹⁸⁾ Unfortunately, the current study was not designed to elucidate the mechanisms involved in the induction of insulin resistance by partial sleep deprivation. A single night of partial sleep restriction to 4.5 h does not cause endocrine changes that simply explain the induction of insulin resistance.⁽¹⁹⁾ Subsequent nights of partial sleep deprivation induce subtle changes in cortisol and catecholamine secretion.^(7;15;20) However, the relations between these effects of sleep deprivation on endocrine homeostasis and glucose tolerance are uncertain. Partial sleep deprivation for a single and subsequent nights increased the sympathetic tone based on recordings of heart rate variability after sleep deprivation.^(21;22) However, the relationship between elevated sympathovagal balance at the level of the heart and the sympathetic outflow to liver, muscles, and adipose tissue is uncertain.⁽²¹⁾

Interestingly, in addition to sleep duration, the composition of sleep in terms of sleep stages is also a determinant of insulin sensitivity. Selective suppression of slow-wave sleep, without any change in total sleep duration, decreased glucose tolerance.⁽²³⁾ The differential effects of altered sleep composition versus decrease total sleep duration on insulin sensitivity await further study.

Data on sleep physiology and sleep disturbances in patients with type 1 diabetes are rare. Jauch-Chara *et al.*⁽²⁴⁾ reported alterations in neuroendocrine sleep architecture and a trend towards less slow wave sleep in 14 patients with type 1 diabetes. Children with type 1 diabetes have a more disrupted sleep than healthy children.⁽²⁵⁾ If type 1 diabetes indeed causes disruption of sleep patterns, this may in turn impair glucose regulation, creating a vicious circle.

In conclusion, the present study indicates that partial sleep restriction decreases insulin sensitivity of insulin-mediated glucose uptake in patients with type 1 diabetes. It is important to further assess the relation between sleep physiology and glucoregulation in patients with type 1 diabetes. Sleep duration might become another therapeutic target in type 1 diabetes to improve glucoregulation in type 1 diabetes.

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CHAPTER

4

A SINGLE NIGHT OF SELECTIVE SUPPRESSION OF SLOW WAVE SLEEP DOES NOT AFFECT INSULIN SENSITIVITY IN HEALTHY SUBJECTS

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Submitted

ABSTRACT

Background

A single night of partial sleep deprivation reduced insulin sensitivity in healthy subjects. Curtailment of slow wave sleep (SWS) during subsequent nights reduces glucose tolerance in healthy subjects. We hypothesized that the effect of a single night of sleep restriction on insulin sensitivity could be due to a reduction in total duration of SWS.

Aim of the study

To assess the effects of one single night of selective SWS suppression on insulin sensitivity, assessed by the hyperinsulinemic euglycemic clamp, in healthy controls.

Methods

We assessed the effect of two nights of normal sleep duration, but with normal versus selectively reduced SWS on insulin sensitivity in 10 healthy controls. Mean age was 32 ± 4 yr, and mean BMI was 23.2 ± 0.5 kg/m². Sleep parameters were determined by polysomnography. Changes in autonomic balance were assessed by analysis of heart rate variability (HRV). SWS was suppressed by delivering acoustic tones of varying frequency, without awakening the subjects. The following morning, insulin sensitivity of glucose metabolism was assessed by hyperinsulinemic euglycemic clamp studies with infusion of [6,6-²H₂] glucose.

Results

The duration of SWS was decreased by 63% ($P < 0.001$), without reduction in total sleep duration. Nonetheless, selective suppression of SWS did not alter basal glucose levels or postabsorptive endogenous glucose production. During hyperinsulinemic clamp conditions, selective SWS suppression did not result in differences in glucose infusion rates or glucose disposal rates. In addition, SWS suppression did not alter plasma nonesterified fatty acid levels during hyperinsulinemia. SWS suppression increased the low frequency/ high frequency (LF/HF) ratio of HRV, indicating a shift towards sympathetic tone.

Conclusions

A single night of selective SWS suppression did not affect insulin sensitivity in healthy controls. Therefore, the reduction in insulin sensitivity induced by partial sleep deprivation in healthy subjects cannot merely be explained by a reduction in SWS.

INTRODUCTION

Shortening of sleep duration is associated with decreased insulin sensitivity in healthy subjects.⁽¹⁻³⁾ As selective disruption of slow-wave sleep (SWS) during subsequent nights decrease glucose tolerance by ~23% in healthy subjects⁽⁴⁾ and previous literature showed the clear restorative role of SWS in glucose metabolism⁽⁵⁻⁷⁾, SWS may be the most relevant sleep stage for the regulation of glucose metabolism. Human sleep is composed of rapid-eye movement (REM) sleep and 3 stages of non-rapid-eye movement (NREM) sleep. Stage 3 NREM sleep is also known as SWS.⁽⁸⁾ During SWS, changes occur in endocrine, metabolic and brain activity.^(9;10) The autonomic nervous system switches from sympathetic dominance during REM sleep to parasympathetic dominance in SWS sleep.⁽¹¹⁾ Disruptions in SWS are likely to alter the autonomic balance, in sense of increasing sympathetic tone. The autonomic nervous system could therefore be an essential pathway through which sleep affects endocrine and metabolic function, including glucose metabolism.

We previously showed that a single night of partial sleep deprivation decreased insulin sensitivity by ~20% in healthy subjects and patients with T1DM.^(2;12) We hypothesized that this could be, at least in part, due to a reduced duration of SWS. Therefore, we studied the effects of a single night of selective SWS suppression on insulin sensitivity and autonomic function in healthy subjects. Insulin sensitivity was assessed by hyperinsulinemic euglycemic clamp studies combined with tracer dilution of [6,6-²H₂] glucose.

SUBJECTS AND METHODS

Subjects

Eleven healthy subjects (6 men) were asked to participate in this study. All subjects were lean (BMI 20-26 kg/m²) and their weight had been stable for at least 3 months prior to participation in this study. Exclusion criteria were known sleep disorders, habitual sleep duration of less than 6h or more than 9h, shift work, psychiatric disorders, and use of sleep medication, β -blocking agents or pro-kinetic drugs. All healthy subjects had normal results on the following validated questionnaires, the Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Berlin Questionnaire and Hospital Anxiety and Depression Scale.⁽¹³⁻¹⁵⁾ We studied premenopausal women in the follicular phase of their menstrual cycle. This study was approved by the medical ethical committee of the Leiden University Medical Center, and written informed consent was obtained from all subjects prior to the study.

Study design

Subjects were studied on two days, with an interval of at least two weeks. Subjects kept a detailed diary of their diet and physical activity for three days prior to each study day and were asked to maintain a standardized schedule of bedtimes and mealtimes in accordance with their usual habits. Subjects were admitted to our clinical research center the night preceding each study day, and spent 8.5 hr in bed from 23:00 h to 07:30 h on both occasions. Subjects fasted throughout these nights from 22:00 h onwards. Subjects were randomly assigned to selective SWS suppression on either the first or second night. The same basal rate of subcutaneous insulin

infusion in patients with type 1 diabetes was used on both study occasions. To obtain glucose profiles during each night and to document euglycemia in patients with type 1 diabetes, a continuous glucose monitoring system (*iPro®2, Medtronic*) was inserted.

Polysomnography and SWS suppression

Sleep was visually scored by an experienced observer for each night according to the guidelines of the American Association of Sleep Medicine (AASM), that rely on electroencephalography, eye movements, and submental muscle activity. To detect as yet unknown sleep disorders, leg movements and respiratory movements were recorded according to the same guidelines.⁽¹⁶⁾ Recordings were made using a portable PSG recorder (*Titanium, Embla Systems, Inc, Broomfield, USA*). The times at which subjects went to bed and turned out the lights as well as times of getting out of bed were noted. The duration of time spent each night in each stage was noted in min and expressed as percentages of total sleep duration (i.e. the summed duration of REM and NREM sleep). During the night of SWS suppression, we aimed to substitute SWS with stage 1 or 2 NREM sleep, without changing total sleep duration. Awakenings were therefore carefully avoided. During the night of selective SWS suppression, acoustic stimuli of increasing intensity were delivered. Subjects slept in a room and were monitored by an experienced sleep technician in an adjacent room. An acoustic stimulus was delivered through two speakers in the patient's room, whenever two delta waves appeared in a 30 sec epoch. The equipment was designed to deliver sounds in increasing volume, starting with an acoustic tone of 40 dB and increasing with increments of 10 dB, if SWS did not disappear. If there was no response on the maximum acoustic tone of 110 dB, the laboratory worker knocked on the door or entered the room and tapped the subject on the shoulder.

Heart rate variability analysis

Changes in autonomic balance were assessed by analysis of heart rate variability (HRV). Heart rate (HR) (beats/min) was continuously recorded using a single lead ECG during the entire sleep period. HRV was estimated by calculating the mean and SD of consecutive R-R intervals; spectral analysis was done using a fast Fourier transformation using a Hamming window after cubic spline interpolating of RR intervals and resampling the signal at 3 Hz. Power was calculated for the low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.4 Hz) bands. Total power was calculated by adding the LF and HF power values. The LF/HF ratio was calculated and used as an index of sympathovagal balance. For each sleep stage, HR, LF and HF power and LF/HF ratio were calculated.

Hyperinsulinemic euglycemic clamp studies

Hyperinsulinemic euglycemic clamp studies were performed the next day after each night at 08:30 hr in each subject. After an overnight fast, a catheter was inserted into an antecubital vein for infusion of isotopes, glucose and insulin, and a sampling catheter was inserted into a dorsal hand vein of the contra lateral arm. For all blood samples, the heated hand technique was used to obtain arterialized blood.⁽¹⁷⁾ A primed [17.6 $\mu\text{mol.kg}^{-1}$] continuous [0.22 $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$] infusion of [6,6-²H₂] glucose (*Cambridge Isotope Laboratory, Andover,*

USA) was started at 08:30 h after basal blood samples had been taken for determination of background glucose enrichment. Labeled glucose was infused by a Pilot C syringe pump (Fresenius Vial, France). Blood samples were obtained after 160, 170 and 180 min of $[6,6\text{-}^2\text{H}_2]$ glucose infusion for assessment of glucose kinetics in the basal state. Subsequently, infusion of intravenous insulin was started, using the method of DeFronzo et al.⁽¹⁸⁾ Briefly, this consisted of a primed ($80 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ for 5 minutes and subsequently $40 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ for 5 min), followed by continuous ($20 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$) infusion of insulin (Actrapid, Novonordisk, Alphen a/d Rijn, The Netherlands), dissolved in sterile NaCl 0.9%, using a Pilot C syringe pump (Fresenius Vial, France). A variable infusion of glucose 20% enriched with 3% $[6,6\text{-}^2\text{H}_2]$ glucose was started four min after the start of insulin infusion. Plasma glucose concentrations were measured with intervals of 5 min with a bedside calibrated glucose analyzer (Accu-Check, Roche, Mannheim, Germany) and the infusion rate of glucose 20% was adjusted in order to keep the plasma glucose levels constant at 5.0 mmol/L during the clamp study. Blood samples were obtained after 150, 160, 170 and 180 min of combined insulin and $[6,6\text{-}^2\text{H}_2]$ glucose infusion for assessment of glucose kinetics and of concentrations of glucose and insulin.

Biochemical analysis

Serum concentrations of glucose were measured using a fully automated Modular P 800 analyzer (Roche/Hitachi, Mannheim, Germany) with intra-assay variations of 1%. Serum insulin concentrations were measured by enzyme labeled chemiluminescent immunometric assay (Immulite 2500, Siemens, Germany) with an intra-assay coefficient of variation (CV) of 4%. Enrichment of plasma $[6,6\text{-}^2\text{H}_2]$ glucose was determined in a single analytical run, using gas chromatography coupled to mass spectrometry, as described previously.⁽¹⁹⁾ All isotope enrichments were measured on a gas chromatograph mass spectrometer (model 6890/5973, Hewlett-Packard, Palo Alto, CA).

Calculations

Isotopic steady state was achieved during the final 30 min of the basal period and the final 30 min of the hyperinsulinemic euglycemic clamp study. Therefore, the rates of appearance (R_a) and disappearance (R_d) of glucose were calculated as the tracer infusion rates divided by the tracer-to-tracee ratios.⁽²⁰⁾ Endogenous glucose production (EGP) during the basal steady state is equal to R_a of glucose, whereas EGP during the hyperinsulinemic clamp study was calculated as the difference between R_a and the glucose infusion rates.

Statistical analysis

Data are presented as mean $\pm$ SEM. Differences in duration of sleep stages and parameters of insulin sensitivity between a night of normal sleep and a night with selective SWS suppression were analyzed by Student's t test for paired samples. All analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL, USA). Significance was accepted at $P < 0.05$.

RESULTS

Clinical characteristics

The results of one male control were excluded, because of a four-fold increase in wake time during the night of selective SWS suppression. Therefore, the analysis included data 10 healthy subjects (5 men). Mean age was 32 ± 4 yr; mean BMI was 23.2 ± 0.5 kg/m².

Effects of SWS suppression on sleep parameters (Figure 1)

In the night of SWS suppression, the duration of stage 3 NREM sleep decreased by 63% ($P < 0.001$), compared to the control night. There were no significant differences in total sleep duration and duration of nocturnal wake time between both nights. SWS suppression was associated with an increase in the duration of stage 2 NREM sleep by 51% ($P < 0.001$).

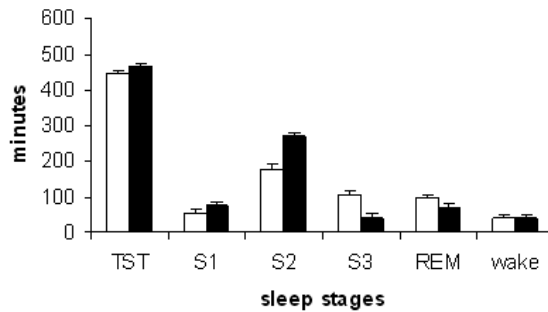


Figure 1. Effect of selective SWS suppression on sleep parameters in 10 healthy subjects. White bars represent duration of sleep phases during normal sleep and black bars represent duration of sleep phases during selective SWS suppression. TST, total sleep time; S1, NREM stage 1; S2, NREM stage 2; S3, NREM stage 3

Effects of SWS suppression on heart rate variability (Table 1)

During the night of SWS suppression, HRV was significantly elevated during wake, stage 3 NREM and REM sleep. Furthermore, SWS suppression increased the LF/HF ratio in stage 2 and 3 NREM sleep, indicating a shift towards sympathetic dominance during the night of SWS suppression.

Table 1. Effects of selective SWS suppression on heart rate variability in 10 healthy controls

Sleep stage	HR (beats/min)			LF/HF ratio		
	Normal sleep	SWS	P-value	Normal sleep	SWS	P-value
Wake	62 ± 1.1	67 ± 1.2	0.02	2.1 ± 0.9	1.9 ± 0.2	NS
Stage 1	58 ± 0.9	57 ± 0.9	NS	1.4 ± 0.2	2.0 ± 0.2	NS
Stage 2	55 ± 0.8	56 ± 0.9	NS	1.2 ± 0.2	1.9 ± 0.2	0.05
Stage 3(SWS)	55 ± 0.8	57 ± 0.9	0.05	0.8 ± 0.1	1.9 ± 0.2	0.001
REM	57 ± 0.9	59 ± 1.0	0.04	2.3 ± 0.3	2.4 ± 0.3	NS

Data are presented as mean ± SEM. HR, heart rate; LF, low frequency; HF, high frequency; SWS, slow wave sleep; NS, non-significant.

Effects of SWS suppression on basal and insulin-mediated glucose metabolism

Selective SWS suppression did not alter basal glucose levels nor basal endogenous glucose production (EGP), measured the following morning (Table 2). During clamp conditions, steady state glucose and insulin levels did not differ between the two occasions. During clamp conditions, selective SWS suppression did not result in significant differences in EGP, glucose infusion rates or glucose disposal rate.

Table 2. Effects of SWS suppression on basal and clamp metabolic parameters in 10 healthy controls

Metabolic parameters	Normal sleep	SWS suppression	p-value
Basal values			
Glucose (mmol/L)	4.9 ± 0.1	5.0 ± 0.1	0.1
EGP (μmol.kgLBM ⁻¹ .min ⁻¹)	19.7 ± 0.8	18.8 ± 0.8	0.3
Clamp conditions			
Glucose (mmol/L)	5.9 ± 0.1	6.0 ± 0.1	0.2
Insulin (pmol/L)	148 ± 12	147 ± 9	0.9
EGP (μmol.kgLBM ⁻¹ .min ⁻¹)	5.6 ± 0.6	5.1 ± 0.6	0.4
Glucose Rd (μmol.kgLBM ⁻¹ .min ⁻¹)	48.6 ± 4.5	45.1 ± 2.8	0.3
Glucose infusion rate (μmol.kgLBM ⁻¹ .min ⁻¹)	42.7 ± 4.4	39.7 ± 2.8	0.4

Data are presented as mean ± SEM. SWS, slow wave sleep; EGP, endogenous glucose production; LBM, lean body mass

DISCUSSION

In the present study, we assessed the effect of a single night of selective SWS suppression on insulin sensitivity in healthy controls. We previously showed that a single night of partial sleep deprivation induced insulin resistance in healthy subjects and patients with T1DM.^(2,12) In those studies, duration of total SWS was decreased by ~30% after partial sleep deprivation. The present study shows that SWS suppression during a single night did not influence insulin sensitivity, assessed by clamp studies, in healthy controls. Therefore, the negative effects of a single night of partial sleep restriction are not mediated through a reduction in duration of SWS.

In the previous study by Tasali *et al*⁽⁴⁾ selective suppression of SWS during multiple subsequent nights resulted in impaired glucose metabolism in healthy subjects. However, the design of their study differed from the present study in two main aspects. Firstly, SWS was suppressed for three consecutive nights versus only for a single night in our study. We cannot exclude the possibility that more nights of SWS suppression might provide different results. However, this does not explain why a single night of partial restriction of sleep duration already has major impact on insulin sensitivity in multiple tissues. Secondly, insulin sensitivity was assessed by an intravenous glucose tolerance test (ivGTT) rather than by hyperinsulinemic euglycemic clamp methods, like in our study. As the hyperinsulinemic euglycemic clamp method is the most sensitive method for measuring insulin sensitivity, it is unlikely that we have missed a major effect of SWS suppression on insulin sensitivity. Increased activity of the sympathetic nervous system is associated with insulin resistance.⁽²¹⁾ From the data obtained by analyses of heart rate variability, we conclude that suppression of SWS sleep did

affect the sympathovagal balance. A shift towards sympathetic predominance was observed during the night of SWS suppression, in accordance with the observations by Tasali *et al.*⁽⁴⁾ Although we measured this increase of relative sympathetic tone during the night of SWS suppression, it might be possible that this effect was not maintained during the following morning. In accordance with this notion, another study reported no significant changes in daytime HR and HRV after one night of selective SWS suppression.⁽²²⁾ Irrespective of the changes in sympathovagal balance during/after a night of SWS suppression, this did not affect insulin sensitivity the following morning. Irrespective of the results of the current study, our model of a single night of SWS suppression was a valid study design, since a single night of partial sleep deprivation induces clear insulin resistance in both healthy subjects and DM1 patients.^(2,12) Apparently, SWS suppression during a single night does not have major effects on insulin sensitivity.

Furthermore, it is well known that inter-individual differences are present in the amount of SWS.⁽²³⁾ The mean baseline duration of stage 3 NREM sleep was 111 min for healthy subjects, compared to ~80 min in the study by Tasali *et al.*⁽⁴⁾ A possible effect might be explained by the difference of absolute duration of remaining SWS sleep. In our study, absolute duration of NREM stage 3 sleep after SWS suppression was 30 min longer compared to the data by Tasali *et al.* Another study, designed to assess the effects of sleep continuity disruption, reported a 20 to 25% reduction in insulin sensitivity after 2 nights of sleep fragmentation.⁽²⁴⁾ In that study, micro-arousals were induced by acoustic and mechanical stimuli across all sleep stages. Although the total duration of SWS was considerably reduced after sleep fragmentation, other sleep stages were also affected, including less REM sleep. This argues for a notion that other sleep stages besides SWS play a role in reduction of insulin sensitivity of glucose metabolism after interruption of sleep.

In conclusion, the present study indicates that a night of selective suppression of SWS during a single night does not influence insulin sensitivity in healthy subjects. Therefore, the reduction in insulin sensitivity by partial sleep deprivation cannot be explained by a reduced duration of SWS.

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CHAPTER

5

DISTURBED SUBJECTIVE SLEEP CHARACTERISTICS IN ADULT PATIENTS WITH LONG STANDING TYPE 1 DIABETES MELLITUS

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Diabetologia 2011; 54(8):1967-76

ABSTRACT

Aims/hypothesis

Decreased sleep duration and/or impaired sleep quality negatively influence glucoregulation. The aim of this study was to assess subjective sleep variables in patients with type 1 diabetes, to relate sleep parameters to long-term glycaemic control, and to assess possible risk factors for impaired sleep.

Methods

We studied 99 adult patients with type 1 diabetes (55 men, 44 women, duration of diabetes 26.9 ± 1.2 yr) and 99 age-, sex-, and BMI- matched non-diabetic controls. Subjective sleep characteristics were assessed by validated questionnaires, *i.e.* Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, and the Berlin Questionnaire. Glucoregulation was assessed by HbA_{1c} -values. Clinical parameters were obtained from medical charts. Depression was assessed by the Hospital Anxiety and Depression Scale (HADS). Peripheral polyneuropathy was assessed by neurological examination and quantitative sensory testing.

Results

Of the patients with type 1 diabetes, 35% had subjective poor sleep quality compared to 20% of the control subjects ($P=0.021$). A higher proportion of the patients with type 1 diabetes were at increased risk for obstructive sleep apnoea (OSA) (17.2% vs. 5.1%, $P=0.012$). There was no significant association between individual sleep characteristics and HbA_{1c} -values. On logistic regression analysis, the HADS depression score, presence of peripheral polyneuropathy, habitual snoring, and other sleep disturbances -*e.g.* hypoglycaemia- were independently associated with poor sleep quality.

Conclusions/interpretation

Adult patients with longstanding type 1 diabetes mellitus have disturbed subjective sleep quality and a higher risk for OSA, compared to control subjects. Subjective sleep disturbances are part of the complex syndrome of longstanding type 1 diabetes.

INTRODUCTION

Patients with type 2 diabetes report sleep disturbances more frequently than individuals from the general population.^(1;2) This is relevant for glucoregulation, as epidemiological⁽³⁻⁵⁾ and experimental studies⁽⁶⁻⁸⁾ have shown that reduced sleep duration and/or decreased sleep quality markedly reduce glucose tolerance and insulin sensitivity.

There are only a few studies on sleep characteristics in patients with type 1 diabetes. Jauch-Chara et al.⁽⁹⁾ reported a trend towards less slow wave sleep in 14 non-hypoglycemic adult patients with type 1 diabetes. Children with type 1 diabetes had more disrupted sleep^(10;11) and more sleep disorders⁽¹²⁾ than healthy children. Conversely, in adult patients with type 1 diabetes, partial sleep deprivation, even during only a single night, reduced peripheral insulin sensitivity by 21%.⁽¹³⁾ Subjective sleep characteristics and their relation with glucoregulation have not been studied in adult patients with type 1 diabetes.

We hypothesized that adult patients with type 1 diabetes may have alterations in subjective sleep characteristics, assessed by validated sleep questionnaires, compared to healthy controls. In addition, we hypothesized that subjective sleep disturbances would be associated with impaired glucoregulation. Therefore, the aim of the present study was: 1) to assess subjective sleep characteristics by validated sleep questionnaires in adult patients with type 1 diabetes, compared to age-, sex-, and BMI- matched non-diabetic controls, 2) to relate sleep characteristics to the quality of glycemic control, i.e. HbA_{1c} values, and 3) to assess possible risk factors for impaired sleep in adult patients with type 1 diabetes.

METHODS

Participants

We included 99 consecutive patients with type 1 diabetes mellitus (55 men, 44 women) attending the outpatient clinic of the Leiden University Medical Center, and 99 age-, sex- and BMI-matched non-diabetic controls recruited by advertisement. Every patient with type 1 diabetes was individually matched with one non-diabetic healthy control for age, sex and BMI. Exclusion criteria for both groups were 1) previously diagnosed sleep disorders; 2) psychiatric disorders and/or use of psychotropic medication; 3) pregnancy or lactation; 4) working in nights shifts in the last 3 months; 5) travelling across time zones in the previous month; 6) age < 18 years; 7) other endocrine disorders; 8) neuropathy caused by other conditions than type 1 diabetes; 9) chronic co-morbidity, other than peripheral neuropathy, associated with pain; and 10) chronic use of glucocorticoids. The study was approved by the medical ethical committee of Leiden University Medical Center, and written informed consent was obtained from all subjects prior to the study.

Study design

Patients and controls were asked to complete 3 validated sleep questionnaires: the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and the Berlin Questionnaire (BQ), which provide data on sleep quality, daytime sleepiness, and the presence of sleeping

disorders.⁽¹⁴⁻¹⁶⁾ An additional questionnaire focused on duration and insulin management of type 1 diabetes, use of medication, co-morbidity, current smoking status, and use of alcohol and coffee. Four questions aimed to identify restless legs syndrome (RLS), using the minimum criteria defined by the International RLS Study group.⁽¹⁷⁾ As depressive feelings may also affect sleep characteristics, anxiety and depression scores were assessed by the Hospital Anxiety and Depression scale (HADS).⁽¹⁸⁾ Sleep disturbances were identified by the answers of the 10 possible encountered sleep disturbances of the PSQI- questionnaire. The option 'three times or more per week' was taken as the affirmative answer. Habitual snoring was defined as a 'yes' answer to the 3- item question 'Do you snore' in the BQ. The subjects who answered 'not known' to this question were classified as non-snorers. We also included additional questions: '*Has your sleep been disturbed by hypoglycemia in the past month? Has your sleep been disturbed by hyperglycemia in the past month?*'. The following four options were provided: 'never'; 'less than once per week'; 'once or twice per week'; 'three times or more per week'. The following data were obtained from medical records for the 12 months preceding the current study. Microvascular complications were defined by the presence of (1) retinopathy identified by retinal photography, detailed ophthalmologic examination, and/or previous laser therapy, (2) nephropathy identified by increased urinary albumin-to-creatinine ratios (men $>2.5 \mu\text{g}/\mu\text{mol}$, women $>3.5 \mu\text{g}/\mu\text{mol}$). Macrovascular complications were defined by objective documentation of coronary artery disease (diagnostic cardiac exercise test, coronary angiography, documented myocardial infarction and/or coronary artery bypass surgery or percutaneous coronary interventions), cerebral vascular disease (documented focal neurologic findings supported by appropriate imaging studies) or peripheral vascular disease defined by reduced ankle-arm index or angiography. Hypertension was defined as systolic blood pressure ≥ 135 mmHg or diastolic blood pressure ≥ 85 mmHg at multiple occasions or treatment with antihypertensive medication. All patients had HbA_{1c} data obtained within the previous 3 months. An HbA_{1c} value of 7.5% (58 mmol/mol) was taken as cut-off point dividing well controlled *versus* moderate to poorly controlled patients. The presence of peripheral polyneuropathy in the lower extremities was assessed by a single researcher (*M. van Dijk*), both in all type 1 diabetes patients and all controls, using the modified Toronto Clinical Neuropathy scale (mTCNs, see *below*)⁽¹⁹⁾ and a neurothesiometer (*Scientific laboratory supplies LTD, Nottingham, UK*). For the present study, peripheral polyneuropathy was considered to be present, using a 2.5% percentile cut-off point for abnormality using data from the matched healthy controls of the present study, when either mTCNs was >5 points or the vibration perception threshold was > 18.4 Volt (V).

Assessment of subjective sleep characteristics

Pittsburgh Sleep quality index

The validated PSQI is a 19-item self-rated questionnaire for assessing subjective sleep quality over the previous month. These questions generate seven 'components' scores, each weighted equally from 0 to 3: *subjective sleep quality; sleep duration, habitual sleep efficiency; sleep disturbances; use of sleep medication; and daytime dysfunction*. The global PSQI score ranges from 0 to 21, with higher scores indicating worse sleep quality.⁽¹⁴⁾

Epworth Sleepiness Scale

The ESS is a validated 8-item questionnaire, focusing on the likelihood of falling asleep in several common situations encountered in daily life on a scale of 0 to 3. Scores range from 0 (the least sleepy) to 24 (the most sleepy). Scores equal to, or above, 10 are interpreted as hypersomnolence.⁽¹⁵⁾

Berlin Questionnaire (BQ)

The BQ consists of 10 questions on risk factors for sleep apnoea, subdivided into three symptom categories: 1) snoring and apneas (6 points), 2) wake time sleepiness or fatigue (3 points), and 3) the presence of obesity or hypertension (2 points). If two or more categories are positive (category 1 and 2: two or more points, category 3: one or more points), a person is at high risk of having sleep apnea.⁽¹⁶⁾

Evaluation of depression

Hospital Anxiety and Depression Scale

The HADS consists of 14 items scoring anxiety and depression. Each item is measured on a four-point scale. Score for anxiety and depression subscale range from 0 to 21, and values of the total score range from 0 to 42. Higher scores indicate more severe anxiety or depression. A total score of 13 or more was considered to be increased.⁽¹⁸⁾ This questionnaire was provided to the subjects after the initial study day. Eighty-seven of 99 (78.8%) of the patients and 80 of the 99 healthy controls (80.8%) returned the HADS questionnaire. The other questionnaires were completed by all participating type 1 diabetic patients and controls.

Assessment of peripheral polyneuropathy

Modified Toronto Clinical Neuropathy scale

The mTCNs consists of a brief, easily administered semi-structured clinical interview and clinical sensory examination to assess symptoms and signs of diabetic sensorimotor polyneuropathy. The mTCNs rates individual symptoms (foot pain, numbness, tingling, weakness, imbalance, upper limb symptoms) as absent or present, and if present, grades them depending on interference with sense of well-being or activities of daily living. Symptoms without interference with sense of well-being or activities of daily living are graded as 1, those with interference with sense of well-being, but not with activities of daily living as 2, and those which interfere with both as 3. The clinical sensory examination was performed unilaterally. In case of previous trauma the unaffected leg was chosen, and otherwise the choice of the leg was random. Pinprick, temperature, light touch, vibration, and position sense were graded as (0) normal; (1) abnormal at the toes only; (2) between the toes and the ankle; or (3) above the ankle.⁽¹⁹⁾ For each of these modalities, reference stimuli were given to the dorsal site of one hand, after which testing was carried out on the sites described before, while subjects kept their eyes closed.

Neurothesiometer

Before testing vibration perception threshold on the toes, subjects were familiarised with vibration stimuli by applying these to the dorsum of one hand. Subsequently, vibration stimuli were applied

bilaterally to the distal pulp of both toes. Subjects were instructed to indicate when vibration was first perceived. Vibration intensity was gradually increased until vibration was first detected by the patient or until a maximum output was reached. Testing was carried out with the subject's eyes closed. Testing was carried out twice on each toe, and a mean of both values was calculated in Volts.

Statistical analysis

Data were analyzed using PASW Statistics version 17.0.2 (SPSS Inc., Chicago, IL, USA). Continuous variables were described as mean $\pm$ SEM; categorical variables were expressed as proportions. We used the paired *t* test and the McNemar test for differences in means and proportions for continuous and categorical paired variables, respectively, and the two-tailed independent *t* test and the χ^2 test for unpaired data.

In patients with type 1 diabetes, logistic regression analysis was performed to examine the association of each sleep characteristic with poor glycaemic control (yes/no) adjusting for age, sex, BMI, use of alcohol (>1 glass/day, yes/no), and anxiety and depression scores according to the HADS.

To investigate variables for impaired sleep quality (PSQI>5) in the diabetic population, we fitted logistic regressions separately for all possible variables that could affect sleep quality: total exogenous insulin dose (units per kilogram per day), use of beta-blockers (yes/no), use of ACE-inhibitors (yes/no), anxiety and depression score according to HADS, presence of hypertension (yes/no), nephropathy (yes/no), peripheral polyneuropathy (yes/no), macrovascular disease (yes/no), uncomfortable temperatures (yes/no), pain (yes/no), polyuria (yes/no), other sleep disturbances (e.g. metabolic dysregulation), habitual snoring (yes/no), high risk for OSA (yes/no), and RLS (yes/no). In these analyses, we adjusted for the confounders age, gender, and BMI by including them as covariates. Subsequently, we performed multivariate logistic regression analysis - including age, sex, BMI, HbA_{1c}, duration of the diabetes, and the risk factors that showed a *P*-value <0.2 in the preceding separate analyses.

RESULTS

Clinical characteristics

We included 99 patients with type 1 diabetes and 99 healthy non-diabetic controls matched for gender (55 men, 44 women), age (43.9 $\pm$ 1.3 vs. 44.1 $\pm$ 1.3 yr) and BMI (24.5 $\pm$ 0.3 vs. 24.5 $\pm$ 0.3 kg/m²). The two groups did not differ with respect to current smoking status, use of alcohol and coffee. Clinical characteristics are summarized in *Table 1*. Patients with type 1 diabetes used more frequently ACE-inhibitors, calcium antagonists, statins, angiotensin II receptor antagonists and anti-platelet agents. According to the HADS, both anxiety (5.0 $\pm$ 0.4 vs. 3.7 $\pm$ 0.3, *p*=0.004) and depression scores (3.3 $\pm$ 0.4 vs. 1.6 $\pm$ 0.2, *p*=0.001) were significantly higher in the patients with type 1 diabetes. Thirteen patients (13.1%) had elevated scores for anxiety and depression (total HADS score 13 or more) vs. 6 (6.1%) of the controls (*p*=0.267). The mean duration of the diabetes was 26.9 $\pm$ 1.2 yr. HbA_{1c} values were 7.8 $\pm$ 0.1% (32 $\pm$ 13 mmol/mol). Multiple daily insulin injections were used by 85 patients and the other 14 patients used continuous s.c. insulin infusion. The mean insulin dose was 0.70 $\pm$ 0.0 EH kg⁻¹day⁻¹. Microvascular complications were present in

Table 1. Clinical characteristics of the patients with type 1 diabetes and their non-diabetic matched controls.

	Patients with type 1 diabetes (n=99)	Controls (n=99)	P-value
Sex			
Male/female	55/ 44	55/ 44	1.000
Age (years)	43.9±1.3	44.1 ±1.3	0.472
BMI (kg/m ²)	24.5±0.3	24.5±0.3	0.986
BMI≥30 kg/m ²	4 (4.0)	6 (6.1)	0.727
HbA1c, %/ mmol/mol	7.8±0.1/62±1.3	NA	
HbA1c ≥ 7.5% (> 58 mmol/mol)	48 (48.5)		
Duration of diabetes (years)	26.9±1.2	NA	
Mean insulin dose (U kg ⁻¹ day ⁻¹)	0.7±0.0	NA	
Medications, n (%)			
Insulin		NA	
Continuous subcutaneous insulin infusion	14 (14.0)		
Insulin injections	85 (86.0)		
Beta-blockers	6 (6.1)	4 (4.0)	0.754
ACE-inhibitors	29 (29.3)	5 (5.1)	0.000
Calcium antagonists	9 (9.1)	2 (2.0)	0.039
Statins	39 (39.4)	6 (6.1)	0.000
Oral antidiabetics	1 (1.0)	0	1.000
Angiotensine II receptor antagonists	9 (9.1)	1 (1.0)	0.021
Diuretics	8 (8.1)	3 (3.0)	0.227
Oral contraceptives	7 (7.1)	8(8.1)	1.000
Antiplatelet agents	17 (17.2)	2 (2.0)	0.000
Proton pump inhibitors	6 (6.1)	2 (2.0)	0.289
Benzodiazepines	0	0	
Other	24 (24.2)	18 (18.2)	0.391
Diabetic complications, n (%)		NA	
Microvascular:	36 (36.4)		
Retinopathy	24 (24.2)		
Laser treatment	18 (18.2)		
Nephropathy	19 (19.2)		
Macrovascular	15 (15.0)		
Peripheral polyneuropathy	45 (45.5)	2 (2.0)	0.000
mTCNs-score	5.6±0.5	1.4±0.2	0.000
Neurothesiometer (Volt)	11.5±0.9	6.8±0.4	0.000
Hypertension, n (%)	24 (22.0)		
Current cigarette smokers, n (%)	18 (18.0)	11 (11.0)	0.210
Alcohol consumption ≥ 1 glass/day, n (%)	41 (41.4)	36 (36.4)	0.551
Coffee consumption ≥ 1 cup/day, n (%)	75 (75.8)	79(79.8)	0.307
Hospital Anxiety and Depression score (HADS)			
Anxiety score	5.0±0.4	3.7±0.3	0.004
Depression score	3.3±0.4	1.6±0.2	0.001
Total score	8.3±0.7	5.3±0.5	0.002
Score ≥ 13	13 (13.1)	6 (6.1)	0.267

Data are mean ± SEM for continuous variables and n (%) for categorical variables; mTCNs=modified Toronto Clinical Neuropathy scale; NA= not applicable

36 patients: 24 patients had retinopathy, and 19 patients had nephropathy. The criteria for peripheral polyneuropathy were met by 45 patients compared with two of the matched healthy controls ($p=0.000$). Peripheral polyneuropathy was considered to be present, using a 2.5% percentile cut-off point for abnormality using data from the matched healthy controls of the present study, when either mTCNs was >5 points or the vibration perception threshold was >18.4 V. Macrovascular complications were present in 15 patients.

Subjective sleep characteristics

Self-reported mean duration of sleep in the PSQI did not differ between patients and controls (7.2 ± 0.1 vs. 7.1 ± 0.1 hr, $p=0.372$; Table 2). In addition, the proportion of short sleepers (< 5 hr) did not differ significantly between the 2 groups (1 vs. 1%, $p=1.00$), and nor did the proportion of long sleepers (>9 hr: 1 vs. 1%, $p=1.00$). Although there were no significant differences in global PSQI scores between patients and controls, 35.4% of the patients had a total PSQI score >5 , compared to only 19.2% of the controls, indicating a higher number of patients with poor sleep quality ($p=0.021$). In the individual domains of the PSQI questionnaire, patients more often reported sleep disturbances ($p=0.001$) and daytime dysfunction ($p=0.031$). The following

Table 2. Subjective sleep characteristics of the patients with type 1 diabetes vs. matched non-diabetic controls.

	Patients with T1DM (n=99)	Controls (n=99)	P-value
Sleep duration			
Self-reported sleep duration, hr	7.2 ± 0.1	7.1 ± 0.1	0.372
Sleep quality			
Pittsburgh Sleep Quality Index (PSQI)			
Global score	4.6 ± 0.3	4.0 ± 0.2	0.079
PSQI > 5 , n (%)	36 (35.4)	20 (19.2)	0.021
PSQI Component-score			
1. Subjective sleep quality	0.88 ± 0.1	0.73 ± 0.1	0.096
2. Sleep latency	0.68 ± 0.1	0.74 ± 0.1	0.598
3. Sleep duration	0.73 ± 0.1	0.77 ± 0.1	0.781
4. Sleep efficiency	0.31 ± 0.1	0.22 ± 0.1	0.307
5. Sleep disturbances	1.12 ± 0.0	0.92 ± 0.0	0.001
6. Sleeping medication	0.13 ± 0.0	0.06 ± 0.0	0.239
7. Daytime dysfunction	0.75 ± 0.1	0.52 ± 0.1	0.031
Daytime sleepiness			
Epworth Sleepiness scale (ESS)			
Total score	5.9 ± 0.4	5.1 ± 0.4	0.192
Score ≥ 10 , n(%)	19 (19.2)	11(11.1)	0.152
Sleep disorders, n (%)			
Berlin Questionnaire (BQ)			
Habitual snoring	51 (51.0)	34 (34.0)	0.010
High risk OSA	17 (17.2)	5 (5.1)	0.012
Restless legs syndrome (RLS)	9 (9.3)	10 (10.1)	1.000

Data are mean $\pm$ SEM for continuous variables and n (%) for categorical variables. OSA= obstructive sleep apnoea

sleep disturbances occurred more often in patients than in controls: polyuria (42.4 vs. 24.2%, $p=0.004$); pain (8.1% vs. 0%, $p=0.008$); uncomfortable temperatures (8.1% vs. 1.0%, $p=0.021$); and habitual snoring (51% vs. 34%, $p=0.010$). The BQ indicated that more patients were at high risk for obstructive sleep apnoea (OSA) than controls (17.2 vs. 5.1%, $p=0.012$). Despite the reduction in sleep quality and more sleep disorders, there were no significant differences in reported daytime sleepiness between the 2 groups. Daytime hypersomnolence affected 19.2% of the patients versus 11.1% of the controls ($p=0.152$). Seventy-five patients answered the questions: 'Has your sleep been disturbed by hypoglycaemia in the past month? Has your sleep been disturbed by hyperglycaemia in the past month?' Forty-one patients indicated that their sleep was disturbed by hypoglycaemia in the past month; 34 patients indicated that their sleep was disturbed less than once per week, and 7 patients indicated impaired sleep by hypoglycaemia 1-2 times per week. Disturbed sleep due to hyperglycaemia was reported by 28 patients with type 1 diabetes; 24 patients indicated disturbed sleep less than once per week, 2 patients 1-2 times per week, and 2 patients reported a disturbed sleep by hyperglycaemia more than 3 times per week.

Well controlled vs. moderate to poorly controlled patients

Type 1 diabetes patients were divided in two groups: well controlled ($HbA_{1c} < 7.5\%$ [< 58 mmol/mol], $n=51$) vs. moderate to poorly controlled ($HbA_{1c} \geq 7.5\%$ [≥ 58 mmol/mol], $n=48$). There were no significant differences in clinical characteristics between both groups. We found no significant differences between both groups in subjective sleep characteristics, *i.e.* self-reported sleep duration, sleep quality, daytime sleepiness, or the prevalence of sleep disorders (*data not shown*). Logistic regression analysis was performed to assess the association between self-reported sleep characteristics and high HbA_{1c} values ($\geq 7.5\%$ [≥ 58 mmol/mol]). There were no significant associations between individual sleep characteristics and impaired glycaemic control (*Table 3*).

Assessment of risk factors of impaired sleep quality (PSQI > 5) in patients with type 1 diabetes

Logistic regression analyses for all possible variables that could affect sleep quality in patients with type 1 diabetes showed significant associations between impaired sleep quality and the presence of peripheral polyneuropathy (OR 2.98, 95% CI 1.05-8.46, $p=0.040$), habitual snoring (OR 4.19, 95% CI 1.47-11.97, $p=0.007$), uncomfortable temperatures (OR 7.89, 95% CI 1.31-47.31, $p=0.024$), other sleep disturbances, *e.g.* metabolic dysregulation (OR 9.52, 95% CI 1.03-88.17, $p=0.047$), HADS anxiety score (OR 1.20, 95% CI 1.02-1.40, $p=0.026$), and HADS depression score (OR 1.30, 95% CI 1.07-1.58, $p=0.009$; *Table 4*).

Multivariate logistic regression, including age, sex, BMI, HbA_{1c} , duration of the diabetes and the risk factors, that showed a p value < 0.2 in the preceding separate analyses confirmed that habitual snoring (OR 9.95, 95% CI 1.76-56.14, $p=0.009$), HADS depression score (OR 1.42, 95% CI 1.00-2.02, $p=0.048$), presence of peripheral polyneuropathy (OR 7.45, 95% CI 1.08-51.20, $p=0.041$) and other sleep disturbances (*e.g.* metabolic dysregulation (OR 27.78, 95% CI 1.11-697.48, $p=0.043$)) were still independent risk factors for poor sleep in type 1 diabetes. Peripheral

Table 3. Results of logistic regression analysis of the association between sleep characteristics and impaired glucoregulation in 99 patients with type 1 diabetes.

	HbA1c > 7.5% (>58 mmol/mol)	
	Odds Ratio (95% CI)	P-value
Sleep duration*		
Self-reported sleep duration (hr)	0.77 (0.48-1.24)	0.287
Sleep quality*		
PSQI		
Global PSQI-score	1.06 (0.89-1.27)	0.532
PSQI > 5	1.31 (0.47-3.63)	0.602
Daytime sleepiness*		
ESS		
Total ESS-score	1.04 (0.92-1.18)	0.521
ESS ≥ 10	2.82 (0.84-9.42)	0.093
Sleep disorders		
BQ		
Habitual snoring	1.24 (0.51-3.01)	0.639
High risk obstructive sleep apnea (OSA)	0.50 (0.15-1.59)	0.238
RLS	2.59 (0.59-11.41)	0.209

Each sleep characteristic was adjusted for age, gender, BMI, and alcohol consumption (> 1 glass/day). *Also adjusted for HADS.

polyneuropathy, clinically assessed according to strict criteria, was an independent risk factor for impaired sleep, even after adjusting for ‘pain’ and ‘uncomfortable temperatures’.

DISCUSSION

The aim of this study was to assess subjective sleep characteristics in adult patients with longstanding type 1 diabetes, and to relate sleep parameters to HbA_{1c} values. Although sleep duration did not differ between patients and controls, more patients had poor sleep quality compared to non-diabetic, age-, gender, and BMI-matched controls. Patients with type 1 diabetes reported more sleep disturbances and daytime dysfunction. A higher proportion of the patients with type 1 diabetes were at increased risk for OSA. There was no association between subjective sleep characteristics and impaired glucoregulation. These observations indicate that type 1 diabetes is associated with an increased prevalence of disturbed subjective sleep characteristics, which do not relate to glucoregulation.

Previous studies on the relation between diabetes and sleep characteristics mainly focussed on patients with type 2 diabetes.^(1,2) Only a few studies have assessed sleep characteristics in patients with type 1 diabetes.⁽⁹⁻¹²⁾ Those studies investigated relatively few subjects and children⁽¹⁰⁻¹²⁾ with type 1 diabetes. The present study extends those observations in showing that in a large group of adult patients with a long history of type 1 diabetes subjective sleep characteristics are impaired, compared to a carefully matched control group, controlling for potential confounding factors like age, sex and BMI.

Table 4. Results of logistic regression: possible risk factors for impaired sleep quality (PSQI>5) in 99 patients with type 1 diabetes

Characteristic	Poor sleep quality (PSQI>5)			
	minimally adjusted		maximally adjusted	
	(adjusted for age, sex, and BMI)			
	OR (95% CI)	P-value	OR (95% CI)	P-value
Clinical characteristics				
Use of medication				
Total insulin dose (EHk ⁻¹ day ⁻¹)	1.32 (0.21-8.31)	0.768	NI	
Use of beta-blockers	1.00 (0.17-5.92)	0.996	NI	
Use of ACE-inhibitors	0.42 (0.14-1.25)	0.118	0.85 (0.14-5.11)	0.859
Anxiety and depressive symptoms				
HADS Anxiety	1.20 (1.02-1.40)	0.026	0.91 (0.66-1.26)	0.572
HADS Depression	1.30 (1.07-1.58)	0.009	1.42 (1.00-2.02)	0.048
Diabetic complications				
Hypertension	0.41 (0.13-1.31)	0.133	0.38 (0.06-2.32)	0.292
Presence of nephropathy	0.88 (0.29-2.68)	0.828	*	
Macrovascular disease	0.78 (0.21-2.92)	0.707	*	
Presence of peripheral neuropathy	3.0 (1.05-8.46)	0.040	7.45 (1.08-51.20)	0.041
Sleep disturbances				
Uncomfortable temperatures	7.89 (1.31-47.31)	0.024	4.30 (0.27-69.45)	0.304
Presence of pain	3.38 (0.72-15.84)	0.122	6.50 (0.50-84.43)	0.152
Polyuria	1.49 (0.20-3.97)	0.405	*	
Other sleep disturbances, e.g. metabolic dysregulation	9.52 (1.03-88.17)	0.047	27.78(1.11-697.4)	0.043
Sleep disorders				
Habitual snoring	4.20 (1.47-11.97)	0.007	9.95 (1.76-56.14)	0.009
High risk OSA	1.27 (0.40-4.05)	0.682	*	
Restless legs syndrome	0.89 (0.20-3.97)	0.883	*	

HADS=Hospital Anxiety and Depression scale; OR=odds ratio; NI=Not included in final model because of P-value > 0.2

This decrease in sleep quality and increased prevalence of sleep disturbances in patients with longstanding type 1 diabetes may have important implications, since previous studies showed that reduction of sleep duration and/or decreased sleep quality impair glucose tolerance and reduce insulin sensitivity in healthy controls.⁽⁶⁻⁸⁾ Sleep disturbances might have a similar negative effect on glucose metabolism in patients with type 1 diabetes, resulting in worse diabetic control. However, this presumed relationship between sleep disturbances and impaired glucose metabolism, assessed by HbA_{1c} values, was not detectable in the current study. Nonetheless, it is still possible that disturbed sleep characteristics influence glucose metabolism in these patients. However, the effects of impaired sleep characteristics may not simply be reflected in HbA_{1c} values, because intensive glucose control and frequent, appropriate adjustments of insulin doses in patients at risk might have obtunded the effects of impaired sleep characteristics on glucoregulation. Various aspects of diabetes could be linked to disturbed sleep quality, including physical complications of the disease, psychological

factors, metabolic fluctuations, and high prevalence of sleep disorders. In the patients with type 1 diabetes in our study, disturbed sleep quality was independently associated with habitual snoring, higher depression scores according to the HADS questionnaire, presence of polyneuropathy, and other sleep disturbances, mainly by hypoglycaemia.

Previous studies showed a high prevalence of depression in diabetes⁽²⁰⁾ and chronic pain conditions.⁽²¹⁾ Although we excluded patients with a known depression, use of psychotropic drugs, and co-morbid disorders (other than neuropathy) associated with pain, in our study higher depression scores are independently associated with impaired sleep quality. Many patients with type 1 diabetes in our study used ACE-inhibitors, statins, and/or beta-blockers which might interfere with sleep characteristics. The effects of beta-blockers on sleep are not equivocal. A previous study showed that the use of beta-blockers could positively or negatively affect sleep.⁽²²⁾ A case-report by Cicolin *et al.* suggested that ACE-inhibitors may contribute to OSA by inducing upper airway inflammation.⁽²³⁾ Therefore, we have considered that the use of beta-blockers and/or ACE-inhibitors might affect sleep quality in patients with type 1 diabetes. However, in univariate logistic regression analysis we did not find an association between the use of beta-blockers and impaired sleep quality and between the use of ACE-inhibitors and impaired sleep quality. In univariate logistic regression analysis there was an association between the use of ACE-inhibitors and high risk of OSA. However, this association was no longer significant after correction for the confounders age, sex, BMI and hypertension. There are conflicting data on sleep disturbances in patients treated with statins. Some studies reported higher prevalence of sleep disturbances in patients treated with lipophilic statins than with pravastatin^(24;25) whereas other studies did not find an increased prevalence of sleep disturbances in patients treated with different statins compared to placebo.^(26;27) In the present study, there was no difference in the use of statins between patients with a poor sleep quality (PSQI > 5) and patients with a good sleep quality (PSQI ≤ 5). The use of statins was even higher in the group with good sleep quality. In accordance, in univariate analysis, use of statins was not associated with impaired sleep quality. Therefore, our conclusions are not likely to be merely explained by the use of medications in our patients. The clinical assessment of peripheral polyneuropathy according to strict criteria in subjects with type 1 diabetes is a major strength of our study, since our study shows diabetic polyneuropathy was a major determinant of impaired sleep. Polyneuropathy contributes to impaired sleep via several potential mechanisms. First, neuropathic pain may lead to disturbed sleep.⁽²⁸⁾ Second, polyneuropathy can impair thermoregulation. It has been proposed that autonomic changes in skin temperature modulate the neuronal activity of the thermosensitive neurons in the preoptic area/ anterior hypothalamus, which in turn regulate vigilance and sleepiness.⁽²⁹⁾ This hypothesis is supported by a report showing that diabetic patients, even those without evidence of clinical neuropathy, showed impaired thermoregulation during sleep.⁽³⁰⁾

The relatively high prevalence of type 1 diabetic patients with a high risk for OSA, according to the Berlin Questionnaire (BQ), suggests the potential of a high burden of unrecognised OSA in people with type 1 diabetes. This is a relatively new finding, in accordance with a recent pilot study of Borel *et al.*, which observed a prevalence of OSA of 40% in 37 non-obese adult patients with type 1 diabetes.⁽³¹⁾ In accordance with our data, this observation is remarkable, since BMI,

which is a risk factor for OSA in the general population, of our patients with type 1 diabetes was matched to that of the healthy controls. Several studies in patients with type 2 diabetes have shown that OSA is associated with the presence of autonomic neuropathy^(32;33), which might also be involved in patients with type 1 diabetes. Unfortunately, our current study was not designed to elucidate underlying mechanisms of disturbed sleep, and we did not include assessments of autonomic neuropathy. Nonetheless, there was no association between poor glycaemic control (HbA1c > 7.5% [≥ 58 mmol/mol]) and a 'high' risk for OSA in our study, despite the association between sleep disordered breathing, glucose intolerance and insulin resistance in patients with type 2 diabetes.^(34;35) There was also no association between the occurrence of hypoglycemia and the risk for OSA in our patients.⁽³⁶⁾

Sleep characteristics were assessed by validated questionnaires in the present study. The PSQI and the ESS have been developed to measure sleep quality and daytime sleepiness, respectively^(14;15), and reflect stable measures of sleep quality and sleepiness over the past year.⁽³⁷⁾ The PSQI has a diagnostic sensitivity 89.6% and specificity of 86.5% for identifying cases with poor sleep quality, using a cut-off score of 5. This questionnaire has been validated by polysomnographic measurements.⁽³⁸⁾ The BQ is a widely used screening tool to differentiate between 'high' and 'low' risk groups for OSA. This risk grouping was useful in the prediction of respiratory disturbances in consecutive participants, who visited internists for any reason. For example, being in the 'high-risk' group defined by the BQ, predicted more than respiratory events per hour with a sensitivity of 86%, and a specificity of 77%.⁽¹⁶⁾ In view of the current data, polysomnography is required to objectively assess the sleep quality and OSA in patients with type 1 diabetes mellitus at high risk for OSA according to the BQ. The current cross-sectional study was designed to assess subjective sleep parameters in patients with type 1 diabetes mellitus and, therefore, we cannot elucidate from the data which chain(s) of events lead from type 1 diabetes to disturbed sleep. In particular, the links between peripheral polyneuropathy and disturbed sleep and between type 1 diabetes and the risk of OSA are not fully clear. Another matter is whether disturbed sleep leads to further impairment of glucose metabolism, with the effect that sleep disturbances and glycaemic control can interact in a vicious circle. Additional studies with objective sleep measurements are warranted to assess these relations in more detail.

In conclusion, the present study demonstrated that adult patients with longstanding type 1 diabetes mellitus have altered self-reported sleep characteristics compared to gender-, age- and BMI matched non-diabetic controls. Therefore, disturbed subjective sleep characteristics are part of the complex syndrome of longstanding type 1 diabetes mellitus.

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CHAPTER

6

CONTROLLED HYPERGLYCEMIC DYSREGULATION DOES NOT SIGNIFICANTLY AFFECT OBJECTIVE SLEEP CHARACTERISTICS IN PATIENTS WITH TYPE 1 DIABETES

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ABSTRACT

Objective

Sleep restriction decreases insulin sensitivity in patients with type 1 diabetes, leading to hyperglycemia. We hypothesized that hyperglycemia might conversely impair sleep in well-controlled patients with type 1 diabetes on stable continuous subcutaneous insulin infusion therapy (CSII).

Research design and methods

We studied 7 well-controlled patients with type 1 diabetes on stable CSII on two occasions: once during a normoglycemic night and their habitual doses of insulin and once during a night of controlled hyperglycemia caused by reduction of the insulin infusion rates by 50%. Sleep characteristics were assessed by polysomnography.

Results

Reduction in insulin infusion rates increased plasma glucose by ~250% (6.5 ± 0.6 vs. 16.0 ± 0.6 mmol/l, $p < 0.001$). This did not significantly affect objective sleep characteristics, *i.e.* sleep duration, quality, latency, efficiency and the apnea-hypopnea index.

Conclusions

Reduction of insulin availability, resulting in hyperglycemic dysregulation, does not have significant effects on objective sleep characteristics in patients with type 1 diabetes.

INTRODUCTION

Patients with type 1 diabetes tend to have less slow wave sleep and more stadium 2 non-REM (NREM) sleep^(1,2), impaired sleep quality and more frequent obstructive sleep apnea⁽³⁾ compared with healthy non-diabetic controls. This is most likely related to complex interactions between metabolic control, physical complications, psychological factors, and high prevalence of sleep disorders.⁽²⁻⁴⁾

The alterations in sleep characteristics are relevant for metabolic regulation, since, for instance, sleep duration is a determinant of insulin sensitivity in these patients.⁽⁵⁾ Conversely, it is possible that hyperglycemic dysregulation, which is frequently present in these patients, might affect sleep characteristics. This might, in turn, impair glucose tolerance and insulin sensitivity, creating a vicious circle.

We hypothesized that a night of hyperglycemic dysregulation through reduction of insulin administration impairs sleep characteristics in adult patients with type 1 diabetes.

Therefore, the aim of the present study was to compare the effects of a night of controlled hyperglycemia, by reduction of the basal and bolus insulin infusion rates by 50%, versus a night of normoglycemia during habitual insulin administration on objective sleep characteristics in well-controlled patients with type 1 diabetes.

STUDY DESIGN AND METHODS

Patients

We included 7 well-controlled patients (4 men) with type 1 diabetes on stable continuous subcutaneous insulin pump therapy (CSII). Diagnostic criteria for type 1 diabetes were past or present positive antibodies against GAD or islet cells and plasma free C-peptide levels <0.3 nmol/l. All participants were screened at our outpatient clinic prior to the study. Exclusion criteria were: 1) age <18 or >65 yr; 2) previously diagnosed sleep disorders, or abnormal scores in one of three validated sleep questionnaires (Pittsburgh Sleep Quality Index⁽⁶⁾, Epworth Sleepiness Scale⁽⁷⁾, and Berlin Questionnaire⁽⁸⁾); 3) night shift work in the month before the study nights; 4) travelling across time zones in the previous month; 5) psychiatric disorders and/or use of psychotropic medication, or high total score, i.e. ≥ 13 , on the Hospital Anxiety and Depression Scale (HADS) (9) since depression is known to have an impact on sleep and cognitive performance; 6) chronic use of sleep medication and/or melatonin; 7) other endocrine diseases; 8) pregnancy or lactation; 9) clinical signs of (autonomic) neuropathy; 10) (chronic) co-morbidity associated with pain; 11) $HbA_{1c} > 8\%$ in the year prior to the study. The study was approved by the medical ethical committee of Leiden University Medical Center and written informed consent was obtained from all patients prior to the study.

Study design

Patients were studied on two days, separated by at least three weeks. They were asked to maintain a standardized schedule of meals and bedtimes the week before a study night and not to alter the insulin regimes during the study days. Self-reported sleep duration and quality were assessed using the Pittsburgh Sleep Quality Index.⁽⁶⁾ History of glucoregulation was obtained

from medical records and an additional questionnaire focusing on glycemic history. One or two days before each study day, a continuous glucose monitoring system (CGMS) sensor was inserted. Patients were admitted to our hospital the afternoon preceding each study night and spent 8.5 hr in bed from 11.00 PM to 7.30 AM the next morning on both occasions. The sequence of the euglycemic and hyperglycemic occasions were randomly assigned. The numbers of toilet visits during the nights were noted.

During the study day of hyperglycemia, patients were called at 8.00 AM by a research physician to reduce their basal and bolus insulin infusions with 50% during 24 hr aimed to obtain and maintain hyperglycemia with glucose levels between 15-20 mmol/L. Patients were instructed to check blood glucose levels at least four times a day. If it was ≥ 20 mmol/L or if they had complaints of hyperglycemia, they were instructed to consult one of the researchers (*M. van Dijk*) to adjust their insulin dose to prevent further increase of blood glucose levels. The patients were able to reach the research physician by telephone 24 hr/day.

Assessment of glucoregulation

Continuous glucose monitoring system (CGMS)

Each patient wore a CGMS (*Medtronic Minimed iPRO-2, Northridge, CA, US*) uninterruptedly for two or three days in order to obtain reliable blood glucose values during study nights and to document daily blood glucose fluctuations. The CGMS sensor was inserted into the abdominal area and provided a measurement of interstitial tissue fluid glucose once every 5 min. Counseling on sensors was given at the initial visit when the sensor was placed. While wearing the CGMS patients had to perform at least 4 capillary glycaemic tests per day (SMBG) with a calibrated digital glucometer (*Contour, Bayer, the Netherlands*) for sensor calibration and to obtain correlation coefficients between the SMBG and the CGMS values. The first capillary blood glucose value was measured after 60 min of using the CGMS. Each participant noted glucose values, meal times, insulin doses, and special events. The CGMS did not display actual glucose levels, leaving the patient blind to prevailing glucose values. At the end of the study the sensor was removed. Sensor data were uploaded to the Medtronic CareLink iPro Clinical Application via the internet after each study night.

Objective sleep parameters

Polysomnography

Sleep was visually scored for each of the 2 nights according to the guidelines of the American Association of Sleep Medicine (AASM) by a technician who was unaware of the study conditions.⁽¹⁰⁾ In short, scoring of sleep stages depends on electroencephalography (EEG), eye movements, and submental muscle activity. To detect possible sleep disorders that might affect the study, respiratory movements were recorded by measurements of changes in nasal pressures, of truncal respiratory movements and of leg movements. Apnea was scored as a complete or near complete cessation of airflow for at least 10 seconds followed by an arousal and/or 3% oxygen desaturation; hypopnea was scored as a 50% decrease in airflow for at least 10 seconds followed by an arousal and/or 3% oxygen desaturation. The apnea-hypopnea index (AHI) is an index of sleep apnea severity that

combines apneas and hypopneas. Recordings were made using a portable polysomnography (PSG) recorder (*Titanium; Embla Systems, Broomfield, CO*). Sleep and wake stages were visually scored in consecutive epochs of 30 s, resulting in a list of epochs spent in wake, stages I (drowsiness), II, III (slow-wave sleep), and rapid eye movement (REM) dream sleep. The times at which subjects went to bed and turned out the lights as well as times getting out of bed were noted.

Glycemic control questionnaire

History of glucoregulation was gathered from medical records and a short survey, focusing on age of diagnosis, diabetes duration, insulin management, frequency of severe hypoglycaemic episodes and/or hyperglycaemic episodes, and mean fasting plasma glucose values. Early-onset of disease was defined as diagnosis before the age of five years. Severe hypoglycemia was defined as any event requiring the assistance of another individual, including seizure and unconsciousness, with either blood glucose <2.78 mmol/l and/or subsequent reversal of symptoms with oral carbohydrate, glucagon injection, or intravenous glucose.^(m) If severe hypoglycemic episodes had occurred, their nature was established, i.e. their total number, and age at first episode.

Statistical analysis

Data were analyzed using PASW Statistics version 17.0.2 (SPSS Inc., Chigago, IL). Continuous variables were described as mean $\pm$ SEM; categorical variables were expressed as proportions. The analysis comprised the comparison of the polysomnographic parameters of the patients with type 1 diabetes of both study days. We used the non-parametric Wilcoxon signed rank test or the Students t-test for differences in means for continuous variables, and the Chi-square (χ^2) test for differences in proportions for categorical variables within patients. The mean $\pm$ SEM glucose values and variability during study nights were obtained from the Medtronic CareLink iPro Clinical Application (*Medtronic Minimed iPRO-2, Northridge, CA, US*). The level of significance was set at $P < 0.05$.

RESULTS

Patient characteristics (Table 1)

We studied 7 patients with well-controlled type 1 diabetes on stable continuous subcutaneous insulin infusion (CSII) (4 men, age 37.4 ± 5.0 yr, body mass index 25.1 ± 1.3 kg/m², HbA_{1c} $6.9 \pm 0.2\%$ (51.4 ± 2.2 mmol/mol), and mean insulin infusion rate 0.7 ± 0.0 units/kg/day). The mean disease duration was 20.6 ± 2.1 yr, and 1 patient (14.3%) had early-onset diabetes. Five patients (71.4%) had experienced 2.0 ± 0.3 severe hypoglycemic episodes per individual during their disease duration. The mean age at first episode was 30.6 ± 7.4 yr. None of the patients had micro- or macrovascular complications, except for one patient who was diagnosed with micro-albuminuria and used an ACE-inhibitor.

Effects of controlled hyperglycemia on polysomnographic parameters (Table 2)

Mean plasma glucose levels were 16.0 ± 0.5 mmol/l during the hyperglycemic night, considerably higher than during the euglycemic night (6.5 ± 0.6 mmol/l, $P < 0.001$), *Figure 1*. Glucose variability

Table 1. Clinical variables of 7 adult patients with type 1 diabetes mellitus on stable continuous subcutaneous insulin infusion (CSII)

Patients with type 1 diabetes (n=7)	
Gender (male/female)	4/3
Age (years)	37.4±5.0
BMI (kg/m ²)	25.1±1.3
Duration of diabetes (years)	20.6±2.1
HbA1c, % (mmol/mol)	6.9±0.2% (51.4±2.2)
Mean insulin infusion rate (Units/kg/day)	0.7±0.0
Duration of CSII therapy (years)	5.6±1.4
Education level#	5.0±1.4
Use of medication, n (%)	2 (25.0)
Beta-blockers	1 (12.5)
ACE-inhibitors	1 (12.5)
α2-adrenergic agonist	1 (12.5)
Age at diabetes onset (years)	16.0±4.7
Early onset of type 1 diabetes, n (%)*	1 (14.3)
History of SH events (n, %)	5 (71.4)
Frequency SH events**	2.0± 0.3
Age at first SH episode	30.6±7.4
Hypertension	0
Retinopathy	0
Nephropathy	1
Peripheral neuropathy	0
Macrovascular disease	0
Hypercholesterolaemia (n, %)	0
Hospital Anxiety and Depression score (HADS)	
Anxiety score	1.3±0.4
Depression score	2.7±1.4
Total score	4.0±1.5
Current cigarette smokers, n (%)	none
Head injury in the past with unconsciousness	none

Data are mean ± SD for continuous variables and n (%) for categorical variables;

*Early-onset of disease is defined as onset below 5 years of age

#Education level measured with a Dutch scoring system from 1 to 8, with 1 representing unfinished primary school and 8 representing university at master level.

** Averaged amount of self-reported severe hypoglycemic events per individual. SH: severe hypoglycaemia

was significantly higher during the normoglycemic night compared to the hyperglycemic night (17.1±2.7% vs. 7.6±1.0%, $P=0.006$).

There were no significant differences in the objective sleep parameters, *i.e.* sleep duration, latency, efficiency, proportions of time spent in the sleep or wake stages, or the presence of sleep disturbances between the hyperglycaemic nights compared with the normoglycemic nights. There was also no significant effect of glucose variability on the sleep parameters. There were not significantly more toilet visits during the hyperglycemic night compared to the normoglycaemic nights.

Table 2. The effect of hyperglycemic dysregulation versus a euglycaemic night on sleep parameters assessed by polysomnography (PSG) in 7 adult patients with type 1 diabetes.

Sleep parameters	Hyperglycaemic night	Euglycaemic night	P-value
Total sleep time, TST (min)	415.0±17.7	363.7±38.0	0.165
Sleep latency (min)	13.4±2.9	22.6±6.5	0.290
Sleep efficiency (%)	85.2±3.5	81.2±5.7	0.247
Stage 1 NREM(% TST)	14.8±1.5	12.3±1.9	0.131
Stage 1 NREM (min)	72.2 ±8.1	57.4±9.0	0.102
Stage 2 NREM (% TST)	35.6±4.8	39.4±4.6	0.073
Stage 2 NREM (min)	172.8±23.0	182.9±21.7	0.358
Stage 3 NREM (% TST)	20.1±1.9	18.4±2.8	0.515
Stage 3 NREM (min)	97.6±9.0	85.1±11.8	0.258
REM (% TST)	14.8±1.8	11.2±1.3	0.142
REM (min)	72.4±9.3	52.1±6.7	0.127
Wake time (% of sleep period)	14.8±3.5	18.8±5.7	0.247
Wake time (min)	72.1±16.9	86.5±25.9	0.288
Snoring (% of TST)	2.3±2.1	7.4±4.7	0.210
No. of Limb movements	171.3±104.7	84.7±58.3	0.159
Apnea/hypopnea index (/hr)	4.0±1.8	6.4±2.1	0.221

Data are mean ± SEM. TST: Total Sleep Time

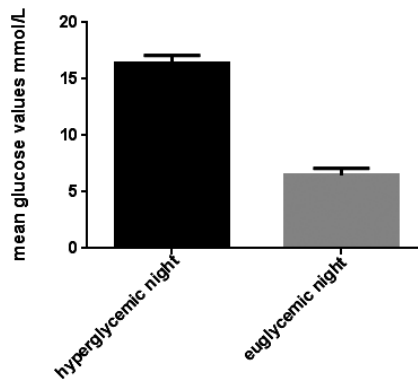


Figure 1. Mean glucose values (mmol/L) in 7 patients with type 1 diabetes during the night of hyperglycemic dysregulation vs. euglycemia. Data are mean ± SEM

DISCUSSION

The aim of the present study was to assess the impact of hyperglycemic dysregulation on objective sleep characteristics in well-controlled patients with type 1 diabetes. This study demonstrates that hyperglycemic dysregulation does not have major effects on objective sleep characteristics in otherwise well-controlled patients with type 1 diabetes.

This is the first study that documents the effects of short-term hyperglycemic dysregulation on objective sleep characteristics. Previous studies on the relation between sleep characteristics

and metabolic regulation focused on the impact of hypoglycemia on sleep architecture in patients with type 1 diabetes^(12;13) or the reverse effects of impaired sleep duration on glucose regulation and insulin sensitivity.⁽⁵⁾ We hypothesized that hyperglycemic dysregulation would affect objective sleep parameters, *i.e.* sleep duration, efficiency, latency, obstructive sleep apnea syndrome (OSAS), and composition of sleep and wake stages. However, a reduction of insulin infusion rates by 50% with resultant hyperglycemia did not significantly affect these parameters in this strictly controlled study design. Moreover, there were no significant differences in the number of toilet visits during both study nights, since hyperglycemia might have induced osmotic diuresis, which could have disturbed sleep.⁽¹⁴⁾

Intensive insulin therapy is the hallmark of treatment of type 1 diabetes. Although insulin analogues and insulin pumps enable more physiological insulin replacement approaches, it is impossible to achieve normal glucose physiology. Intra-individual variations in physiological factors may interfere with glucoregulation in patients with type 1 diabetes, *e.g.* partial sleep deprivation⁽⁵⁾, dietary macronutrient composition^(15;16), physical activity, and stress.⁽¹⁷⁻¹⁹⁾ Previous studies showed that nutrients, such as glucose, as well as the timing of meals, can reset the bodily rhythms of rodents.⁽²⁰⁾ Moreover, the timing of carbohydrate rich meals affects circadian rhythms⁽²¹⁾, but it is unknown whether this affects sleep patterns. Clinical interventions confirm cross-sectional observations that there is a relation between the intake of macronutrients and sleep. Carbohydrates and fats, at least in large quantities, may modulate sleep quality by influencing the ratio of REM and NREM sleep.⁽¹⁵⁾ Therefore, we speculated that the major increase in glucose levels, associated with a major reduction in insulin administration in patients with type 1 diabetes, might also affect objective sleep characteristics. However, despite a considerable increase in glucose levels, we could not establish any significant effect on objective sleep characteristics in patients with type 1 diabetes. This real life situation provides important knowledge for the care of patients with type 1 diabetes mellitus.

CONCLUSION

In conclusion, reduction of insulin availability, resulting in hyperglycemia, does not significantly disturb objective sleep parameters.

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CHAPTER

7

IMPAIRED SUSTAINED ATTENTION IN ADULT PATIENTS WITH TYPE 1 DIABETES RELATED TO DIABETES *PER SE*

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Diabetes Metab Res Rev. 2014;30 (2):132-9

ABSTRACT

Background

Patients with type 1 diabetes have altered sleep characteristics and are thought to have deficits in sustained attention. We compared the Sustained Attention to Response Task (SART) of patients with type 1 diabetes to that of healthy controls, and related results with sleep characteristics and disease-related factors.

Methods

The SART was applied in 122 patients and 109 controls. Glucoregulation was assessed by HbA_{1c} values and a questionnaire assessing glycemic history. Clinical parameters were obtained from medical charts. Polyneuropathy was assessed by neurological examination and quantitative sensory testing. Sleep characteristics were assessed with sleep questionnaires. Anxiety and depression scores were assessed by the Hospital Anxiety and Depression Scale.

Results

The SART reaction time (RT) was significant longer than in controls (327±5 ms vs. 285±3 ms, $p<0.001$), although there were no significant differences in error scores. Repeated measurement analyses showed that diabetes *per se* was associated with prolonged RT ($P<0.001$) and more commission errors ($p=0.010$). None of the sleep-related and diabetes-related factors were associated with these SART parameters.

Conclusions

Patients with type 1 diabetes had impaired sustained attention, which was associated with diabetes *per se*, but not with disturbed sleep characteristics.

INTRODUCTION

Patients with type 1 diabetes perform less well on neuropsychological tests, including psychomotor speed, sustained attention, and speed of information processing.^(1;2) Although various disease-variables are associated with cognitive impairment – e.g. chronic hyperglycemia⁽³⁾, microangiopathy^(4;5), age at onset^(4;6) or disease duration⁽⁶⁾, and structural brain defects^(7;8) – the pathophysiology of cognitive dysfunction is not well understood. Studies on the effect of severe hypoglycemia on cognitive function are conflicting; whereas some retrospective studies suggested an association in adult patients^(9;10), large prospective studies failed to conform this.^(11;12)

Disturbed sleep could be an alternate explanation, as patients with type 1 diabetes have altered subjective and objective sleep characteristics compared to controls^(13;14) and because disturbed sleep impairs cognitive functioning.^(15;16) The psychomotor vigilance test (PVT)⁽¹⁷⁾ and the Sustained Attention to Response Task (SART)⁽¹⁸⁾ assessed sustained attention, are sensitive to sleep deprivation in healthy controls⁽¹⁹⁾ or excessive sleepiness in sleep-disordered patients compared to controls, respectively.⁽²⁰⁾ Sleep characteristics and their relation with sustained attention have, however, not been studied in type 1 diabetes.

We hypothesized that type 1 diabetes is associated with impaired sustained attention, and that this would be associated with sleep disturbances or disease-related factors. We therefore aimed the following: 1) to compare sustained attention in patients with type 1 diabetes and controls; and 2) to assess if impaired sustained attention was associated with sleep or disease-related factors.

MATERIAL AND METHODS

Subjects

We included 122 consecutive patients with type 1 diabetes (74 men, 43.4±1.1 yr, 24.5±0.2 kg/m²) from our outpatient clinic of our hospital, and 109 comparable healthy controls (57 men, 42.7±1.3 yr, 24.5±0.3 kg/m²) through an advertisement in a local paper and through patients. Exclusion criteria were: 1) age <18 or >65 yr; 2) alcohol or drugs abuse; 3) psychiatric disorders or use of psychotropic medication; 4) history of cerebrovascular accident; 5) history of epilepsy or use of anti-epileptic drugs; 6) other endocrine disorders, including menopausal symptoms; 7) pregnancy or lactation; 8) chronic use of glucocorticoids or medication known to affect cognitive function; 9) visual impairment; 10) sleep disorders, shift work, travelling across time zones in the previous month, and chronic use of sleep medication; 11) polyneuropathy due to conditions other than diabetes; 12) pain not due to peripheral polyneuropathy.

The following data were obtained from medical records for the year preceding the study. Microvascular complications included (a) retinopathy (retinal photography, ophthalmologic examination, previous laser therapy), (b) nephropathy (increased urinary albumin-to-creatinine ratio (men >2.5 µg/umol, women >3.5 µg/umol)). Macrovascular complications were coronary artery disease (proven by cardiac exercise test, coronary angiography, myocardial infarction and/or coronary artery bypass surgery or percutaneous coronary interventions), or peripheral vascular disease (reduced ankle-arm index or angiography). Hypertension was defined as systolic blood pressure ≥135 mmHg or diastolic blood pressure ≥85 mmHg at multiple occasions, or use of

antihypertensive agents for the treatment of hypertension. Other macrovascular risk factors –i.e. current smoking status, obesity and hypercholesterolaemia - were assessed by questionnaire and medical records. HbA1c values had been obtained within the previous 3 months. Polyneuropathy in the feet was assessed using the modified Toronto Clinical Neuropathy scale (mTCNs) (21) and a neurothesiometer (*Scientific laboratory supplies LTD, Nottingham, UK*). For both measures the abnormality threshold was defined as the upper 2.5% percentile of data from the control group. Peripheral polyneuropathy was considered present when mTCNs was >5 points or the vibration perception threshold was >18.0 V. The study was approved by the medical ethical committee of Leiden University Medical Center, and informed consent was obtained from all subjects.

To evaluate any immediate effects of blood glucose levels on the SART, we measured glucose values just before the start of the first SART test in 10 patients with type 1 diabetes with a calibrated digital glucometer (Contour, Bayer, the Netherlands) and a continuous subcutaneous glucose sensor (iPro2, Medtronic, Northridge, CA, US).

Sustained attention to response task

SART is a go/no-go task in which the no-go target appears unpredictably and rarely. A number from 1 to 9 was shown 225 times in white on a black computer screen in a quiet room with dimmed lights. Each of the nine numbers was shown 25 times in a predetermined and quasi-random order. The font size was chosen at random from 26, 28, 36, or 72 points. Each number was presented for 250 ms, followed by a black screen for 900 ms. Subjects had to respond to the appearance of each number by pressing a button, except when the number was 3. Subjects were instructed that accuracy and speed were equally important. The SART was performed two times: the time in between was spent on an outpatient visit for the patients, and completion of the questionnaires and foot examination for the controls. The total error score consists of the number of times the button was pressed when a '3' was presented (*errors of commission*), plus the number of times when no key was pressed when it should have been (*errors of omission*). The reaction time (RT) is the average time in ms between the appearance of any number and the subject's appropriate presses.⁽¹⁸⁾ The subjects were not allowed to drink coffee 1 hour before and between the two tests.

Sleep questionnaires

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a 19-item questionnaire for assessing subjective sleep quality over the previous month. These questions generate 7 component scores, weighted equally from 0 to 3. Global PSQI score ranges from 0 to 21, with higher scores indicating worse sleep quality. PSQI scores >5 have a diagnostic sensitivity of 89.6% and a specificity of 86.5% to detect poor sleep quality.⁽²²⁾

Epworth Sleepiness Scale

The Epworth Sleepiness Scale is an eight-item questionnaire, concerning the likelihood of falling asleep in common situations on a scale of 0 to 3. Scores range from 0 to 24. Scores ≥ 10 are interpreted as hypersomnolence.⁽²³⁾

Berlin Questionnaire

The Berlin Questionnaire consists of ten questions on risk factors for obstructive sleep apnea (OSA), subdivided into three symptom categories: 1) snoring and apneas (6 points), 2) wake-time sleepiness or fatigue (3 points), and 3) presence of obesity or hypertension (2 points). Two or more categories positive (category 1 and 2: two or more points, category 3: one or more points) are interpreted as high risk for sleep apnea.⁽²⁴⁾

Glycaemic control questionnaire

History of glucoregulation was gathered from medical records and a questionnaire focusing on age of diagnosis, diabetes duration, insulin management, frequency of severe hypoglycaemic episodes and/or hyperglycaemic episodes, and mean fasting plasma glucose values. Early-onset of disease was defined as diagnosis before the age of 5 years. Severe hypoglycaemia was defined as any event requiring the assistance of another individual, including seizure and unconsciousness, with either blood glucose <2.78 mmol/l and/or subsequent reversal of symptoms with oral carbohydrate, glucagon injection, or intravenous glucose.⁽⁹⁾ If severe hypoglycaemic episodes had occurred, their nature was established, *i.e.*, their total number, and age at first episode. This questionnaire was mailed to the subjects afterwards, and was completed by 102 of 122 (83.6%) patients.

Hospital Anxiety Depression Scale

Because depression impacts on cognitive performance, the Hospital Anxiety Depression Scale (HADS) was used, consisting of 14 items scoring anxiety and depression. Each item is measured on a four-point scale. Scores for anxiety and depression subscale range from 0 to 21, and total score range from 0 to 42. Higher scores indicate more severe anxiety or depression. A total score higher than 13 points on both subscales together is used to characterize subjects as anxious or depressed.⁽²⁵⁾

Statistical analysis

Data were analyzed using PASW Statistics version 17.0.2 (SPSS Inc., Chicago, IL, USA). Continuous variables were described as mean $\pm$ SE; categorical ones as proportions. We used the independent *t*-test or Mann-Whitney U-test for differences in continuous variables, and the Chi-square (χ^2) test for proportions. We used a repeated-measurement analysis to explore differences between the two groups across time, and group by time interactions. We considered three models that were adjusted as follows: *model 1*) Unadjusted model; *model 2*) as model 1 plus RT (for errors of omission and commission); *model 3*) as model 2 plus total HADS scores, age, gender, BMI and the individual sleep characteristics, *i.e.* sleep quality, sleep duration, daytime sleepiness, and the risk for OSA. The β coefficients and *p*-values of these analyses were reported. Significance level was Bonferroni-corrected.

To investigate which variable, if any, affected the three parameters of the SART – *i.e.* RT, *errors of omission and commission*– we performed general linear model analysis for the following variables: diabetes duration (years), HbA_{1c}, early onset of diabetes, retinopathy, nephropathy, peripheral polyneuropathy, macrovascular disease, hypertension, one or

more other macrovascular risk factors- i.e. presence of smoking, and/or obesity, and/or hypercholesterolaemia -, and severe hypoglycaemia. The model was created from a saturated model including all disease related factors, followed by removing non-significant parameters as long as the model fit was not significantly impaired. We adjusted for age, sex, BMI, HADS scores, test, and RT, by including them as covariates. The β -coefficients, standard errors of the mean and the p-values of these analyses were reported. Significance level was Bonferroni corrected. Missing variables of the HADS were replaced with means of the controls or the patients.

RESULTS

Subject characteristics (Table 1)

We included 122 patients with type 1 diabetes (74 men, 43.4 ± 1.1 yr, 24.5 ± 0.2 kg/m², HbA_{1c} $7.7 \pm 0.1\%$ (61 ± 1.3 mmol/mol)) and 109 comparable healthy controls (57 men, 42.7 ± 1.3 yr, 24.5 ± 0.3 kg/m²). Fourteen (11.5%) patients had early-onset diabetes. Sixty-three patients (64.3%) had experienced one or more episodes of severe hypoglycemia with a mean of 8.0 ± 1.3 episodes per individual. The mean age at first episode was 25.5 ± 1.5 yr ($n=59$). Patients scored significantly higher on the HADS questionnaire (7.8 ± 0.5 vs. 5.3 ± 0.3 , $p < 0.001$). Microvascular complications were present in 35 patients (28 patients had a diabetic retinopathy, 21 patients had nephropathy, and 44 patients had peripheral polyneuropathy). Macrovascular complications were present in 12 (9.8 %) patients. Forty-three patients (35.2%) had hypertension; 56 (45.9%) patients had one or more other macrovascular risk factors.

Sleep questionnaires (data not shown)

Mean sleep duration in the PSQI did not differ between patients and controls (7.2 ± 0.1 vs. 7.1 ± 0.1 hr, $p=0.17$) nor did global PSQI-scores. However, a PSQI score >5 occurred in ~ 30% of the patients and in 18.3% of the controls, indicating poorer sleep quality in the patient group ($p=0.042$). More patients were at risk for obstructive sleep apnea than controls (BQ, 17.1 vs. 5.5%, $p=0.006$). There were no significant differences in ESS-score (patients 5.8 ± 0.4 vs. controls 5.3 ± 0.4 , $p=0.37$). Daytime sleepiness affected 19.0% of the patients vs. 11.9% of the controls ($p=0.14$).

Sustained attention to response task data (Table 2)

At the first SART test, error scores did not differ between groups, but the mean RT of patients was significantly higher than that of controls (331 ± 6 ms vs. 294 ± 4 ms, $p < 0.001$). In the second SART, the RT was still significantly longer in patients than in controls (323 ± 6 ms vs. 275 ± 4 ms, $p < 0.001$), but now patients made significantly fewer errors of commission than controls (8.1 ± 0.5 vs. 9.7 ± 0.5 , $p=0.005$), when compared unadjusted.

Table 1. Clinical variables of 122 adult patients with type 1 diabetes and 109 group-matched healthy controls.

	Patients with type 1 diabetes (n=122)	Controls (n=109)	P-value
Age (years)	43.4±1.1	42.7±1.3	0.751
Gender (n, % male)	74 (60.7)	57 (52.3)	0.200
BMI (kg/m ²)	24.5±0.2	24.5±0.3	0.782
HbA1c, % (mmol/mol)	7.7±0.1 (61±1.3)		NA
Insulin, n (%)			NA
Continuous s.c. insulin infusion	21 (17.2)		NA
Multiple insulin injections	101 (82.8)		
Mean insulin dose (units/kg/day)	0.69±0.0		NA
Medication, n (%)			
Antihypertensiva:			
Beta-blockers	8 (6.6)		
ACE-inhibitors	34 (27.9)		
Calcium antagonists	9 (7.4)		
Angiotensin II receptor antagonist	12 (9.7)		
Statins	43 (45.2)		
Type 1 diabetes duration (years)	25.4±1.0		NA
Age at diabetes onset (years)	18.2±1.0		
Early onset of type 1 diabetes (n, %)*	14(11.5)		NA
History of severe hypoglycemic events (n, %)	63 (64.3)		
Frequency severe hypoglycemic events**	8.0±1.3		NA
Age at first SH episode	25.5±1.5		NA
Hypertension (n, %)	43 (35.2)		NA
Retinopathy (n, %)	28 (23.0)		NA
None or background retinopathy	64 (74.4)		
(Pre)-proliferative diabetic retinopathy	1 (3.5)		
Not specified	4 (14.3)		
Laser treatment	23 (19.5)		
Nephropathy (n, %)	21 (17.2)		NA
Peripheral neuropathy (n, %)	44 (38.3)	1 (1.0)	<0.001
mTCNs-score	5.0±0.5	1.3±0.1	<0.001
Neurothesiometer (Volt)	11.1±0.8	6.4±0.4	<0.001
Macrovascular disease ***	12 (9.8)		NA
One or more other vascular risk factors	56 (45.9)		NA
HADS			
Anxiety score	4.8±0.3	3.6±0.3	0.010
Depression score	3.1±0.3	1.7±0.2	<0.001
Total score	7.8±0.5	5.3±0.3	<0.001
Score ≥ 13	15 (14.7)	6 (6.7)	0.067
Current cigarette smokers, n (%)	18 (14.8)	12 (11.2)	0.414
Fasting glycaemia > 7 mmol/l	71 (30.7)		NA

Data are mean ± SE for continuous variables and n (%) for categorical variables;

NA, not applicable; SH, severe hypoglycaemia; HADS, Hospital Anxiety and Depression score ; mTCNs, modified Toronto Clinical Neuropathy scale; BMI, body mass index; ACE, angiotensin-converting-enzyme.

*Early-onset of disease is defined as onset below 5 years of age

** Averaged amount of self-reported severe hypoglycemic events per individual.

***macrovascular complications without overt cerebrovascular disease.

Table 2. Unadjusted results of the two Sustained Attention To Response Tasks (SART) of 122 patients with type 1 diabetes compared to 109 group- matched healthy controls.

	Patients with type 1 diabetes (n=122)	Controls (n=109)	P-value
SART 1			
Errors of omission/200	1.9±0.4	1.0±0.2	0.117
Errors of commission/25	8.6±0.5	8.9±0.5	0.511
Total error score/225 *	10.4±0.7	10.0±0.5	0.914
Mean RT (ms)	330.6±6.1	294.4±4.1	<0.001
SART 2			
Errors of omission/200	1.3±0.4	0.8±0.2	0.097
Errors of commission/25	8.1±0.5	9.7±0.5	0.005
Total error score/225 *	9.4± 0.7	10.5±0.5	0.023
Mean RT (ms)	322.7±6.4	275.3±3.8	<0.001
Mean results **			
Errors of omission	1.6±0.3	0.9±0.1	0.092
Errors of commission	8.4±0.4	9.3±0.4	0.037
Total error score*	9.9±0.6	10.2±0.5	0.199
Mean RT (ms)	326.8±5.4	284.9±3.4	<0.001

Data are mean ± SEM;

RT, reaction time

* Total errors: sum of omission and commission errors.

** mean of the first (SART 1) second (SART 2) SART

Repeated measurement analysis of the combined effect of group and repetition (Table 3)

Reaction Time (RT), ms.

Comparison of the first and second SART test with repeated-measures analysis showed that diabetes *per se* was significantly associated with an increased RT (47.5, $p < 0.001$), *model 1*.

Model 3, including correction for possible confounders linked to the presence of diabetes, showed a significant association between diabetes mellitus and an increased RT (43.3, $p < 0.001$). In addition, older age (1.4, $p < 0.001$) and higher BMI (0.2, $p = 0.027$) were associated with increased RT. After correction for multiple comparisons, only older age still remained significantly associated with RT (*Data not shown*).

Errors of commission

There were significant differences in the rates of commission errors between the two groups. Diabetic patients made significantly fewer errors of commission (-0.19, $p = 0.015$). On repeating the SART the controls significantly made more errors of commission in contrast to patients (0.15, $p = 0.040$), *model 1*. When we included the RT in the analysis to control for its potential confounding effect on the error scores, *model 2*, the RT proved to have a significant negative association with the rate of errors of commission ($p < 0.001$). After correction for RT patients indeed tended to make *more* errors of commission, but this was not statically significant ($p = 0.079$). Corrected for the possible confounders that could possibly affect the commission errors, the diabetic patients

Table 3. Results of a repeated-measurement analysis to the influences of group, test and their interaction on SART RT and accuracy measures

	Reaction time (ms) Beta/S.E./ P	Errors of commission Beta/S.E./ P	Errors of omission Beta/S.E./ P
Model 1	Intercept:322.8/6.4/<0.001	Intercept:2.1/0.06/<0.001	Intercept:0.28/0.23/0.238
Group (0)	47.5/7.4/<0.001	-0.19/0.8/0.015	0.6/0.2/0.019
Test (0)	7.8/6.3/0.214	0.06/0.05/0.266	0.4/0.2/0.092
GroupxTest (0x0)	11.3/7.3/0.123	-0.15/0.07/0.040	N.I.
Model 2	N.A.	Intercept:4.5/0.1/<0.001	Intercept:0.28/0.23/0.238
Group (0)		0.10/0.6/0.079	0.6/0.2/0.019
Test (0)		0.13/0.4/<0.001	0.4/0.2/0.092
GroupxTest (0x0)		0.1/0.05/0.133	NI
Model 3	Intercept:285.3/60.8/<0.001	Intercept:3.8/0.3/<0.001	Intercept:-3.3/1.2/0.002
Group (0)	43.3/7.4/<0.001	0.12/0.04/0.010	0.5/0.3/0.081
Test (0)	7.8/6.3/0.220	0.10/0.03/<0.001	0.35/0.2/0.096
GroupxTest (0x0)	11.3/7.4/0.124	NI	NI

The model was created from a saturated model including all individual variables and the possible interaction, followed by removing non-significant parameters as long as the model fit was not significantly impaired.

Group 0= diabetes mellitus, Group 1= controls; Test 0 = SART1, Test 1= SART 2

Model 1: Unadjusted; Model 2: adjusted for RT; Model 3) model 2, plus total HADS scores, age, gender, BMI and the individual sleep characteristics.

S.E., standard error of the mean; P, P-value; NI, not included, i.e. no significant contribution to the final model; NA, not applicable

made significantly more commission errors ($p=0.010$) and all subjects (patients and controls together) made more errors in the first test than the second test (0.10 , $p=0.001$), *model 3*.

Errors of omission

Diabetes *per se* was significantly associated with a higher rate of errors of omission compared to controls (0.6 , $p=0.019$), *model 1*. After we included the possible confounders related to diabetes in the analysis (*model 3*), this association was no longer significant ($p=0.081$). In addition, male sex (0.65 , $p=0.006$) was significantly associated with more errors of omission (not shown).

Association of diabetic related risk factors with SART parameters in patients (Tables S4 and S5)

Reaction Time (ms)

Analysis of possible risk factors that could affect RT in patients with type 1 diabetes showed no significant associations with a disease-related risk factor after multiple corrections.

Errors of commission

Analyses for variables that could affect the commission errors in the patients showed significant associations between retinopathy (0.16 , $p=0.019$) and early onset of disease (0.29 , $p=0.021$). After multiple corrections, these associations were no longer significant.

Errors of Omission

Repeated measurement analysis within the patients showed no significant associations between the errors of omission and any of the disease-related factors.

Effect of glucose concentrations on SART outcome

Data of euglycemic versus hyperglycemic test in 10 type 1 diabetes patients showed that the individual parameters of sustained attention measured with the SART test were not different between the euglycemic (6.8 ± 0.6) and hyperglycaemic conditions (18.8 ± 0.7).

DISCUSSION

This study shows that type 1 diabetes *per se* is associated with impaired sustained attention, indicated by a significantly increased RT. These patients also made fewer commission errors, but previous studies showed that the number of commission errors was inversely correlated with RT.^(17;26-28) After correction for the RT, patients actually made *more* errors of commission. This could implicate that impaired accuracy in part was 'masked' by their increased RT. After correction of important confounders, more frequently found in diabetic patients, the RT and commission errors still remained significantly higher in patients. There was no relation between RT and impaired sleep characteristics or disease-related variables.

Sustained attention in patients with type 1 diabetes

Deficits in sustained attention have been reported in previous studies of type 1 diabetes⁽²⁹⁻³¹⁾, concerning a study in children⁽²⁹⁾, assessing the effect of acute hypo- or hyperglycemia⁽³¹⁾, using various tests.⁽²⁹⁻³¹⁾ The current study extends those observations in several aspects: (a) sustained attention was impaired in a large carefully characterized group of consecutive adult patients, after exclusion for most variables that could affect cognitive function; (b) we used the SART, an easily administrated tool allowing quick measurement of sustained attention, taking into account RT and accuracy, both important for everyday functioning; (c) and most importantly, we aimed to explore all possible risk factors for impaired sustained attention in the patients, including impaired sleep characteristics, frequently seen in these patients⁽¹⁴⁾ and associated with impaired sustained attention.^(15;16;32)

The main finding of our study was that diabetes itself was independently associated with impairments in RT. The finding that mean RT was significantly longer in patients than in controls is in accordance with previous literature.^(4;5;31;33) Evidence is increasing that cognitive slowing is the core feature of the diabetes-associated cognitive decline, sometimes inappropriately called a 'central neuropathy'.^(2;34) The complex multi-factorial pathophysiology of changes in the central nervous system in diabetic patients has not yet been fully elucidated. Different neuroradiological⁽³⁵⁾ and electrophysiological alterations, *i.e.* increased P300 latency⁽³⁶⁾, in brain were found in patients with type 1 diabetes, but data on the relation of imaging findings, risk factors and neurocognitive functions are scarce. Within the diabetic group HbA1c was significantly associated with more errors of commission, which could implicate more brain damage in poorly controlled diabetes.

There was no effect of current glycemic state on SART outcome in a small substudy, in line with previous studies showing minimal or no changes in cognitive function during hyperglycaemia.⁽²⁹⁾

Sleep characteristics and sustained attention

Although disturbed sleep characteristics are part of the complex syndrome of type 1 diabetes^(13;14), and the SART has been shown to be sensitive^(19;20), we did not find a relation between individual sleep characteristics and parameters of sustained attention in this study using *subjective* sleep questionnaires. The reasons are unclear; it is possible that the sleep disorders were not serious enough. We also cannot exclude that some of the missed glycemic control questionnaires included hypoglycemic episodes that influenced sleep. Additional studies with objective sleep characteristics are warranted.

CONCLUSIONS

The present explorative study demonstrates impaired sustained attention in patients with type 1 diabetes compared to healthy controls. Diabetes itself is an important risk factor for impaired sustained attention. However disturbed sleep characteristics were not associated with impaired sustained attention in our patients.

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Supplemental Table 4. Analysis of possible disease related risk factors determining reaction time (RT) in patients with type 1 diabetes

Potential factors	Reaction time (ms)
Basis (B/SE/P-value)	294.2/76.2/<0.001
Disease related variables	
Diabetes duration, years	1.1/0.8/0.168
HbA1c,%	-6.6/5.2/0.205
Early-onset of disease, <5 years	-0.7/19.1/0.969
SH-events in the past	-28.7/14.6/0.050
Presence of retinopathy	-10.7/15.6/0.518
Presence of nephropathy	39.9/22.7/0.078
Presence of peripheral neuropathy	23.7/14.3/ 0.097
Presence of macrovascular disease*	-23.2/22.7/0.306
Macrovascular risk factors, n (%)	
Hypertension	-11.8/13.3/0.374
Other vascular risk factor#	1.7/13.6/ 0.897

Data shown are the standardized B of independent predictive factors for impaired RT, ms. SH: severe hypoglycaemia; B:beta; SE: Standard error of the mean

. * Patients with cerebrovascular events were excluded, only patients with cardiovascular and peripheral vascular disease were included in the analyses.

defined as current smoker and/or presence of dyslipidaemia and/or obesity.

Model was created from a saturated model including all disease-related factors, Test (0/1), age, sex, BMI, and HADS scores followed by removing non-significant parameters as long as the model fit was not significantly impaired.

Supplemental Table 5. Analysis of disease related factors determining errors of commission and omission within patients with type 1 diabetes

Potential factors	Errors of commission	Errors of omission
Basis (B/SE/P-value)	4.0/0.40/ <0.001	1.52/1.8/0.400
Disease- related variables		
Diabetes duration ,years	NI	0.02/0.02/0.348
HbA1c, %	0.04/0.03/0.172	0.04/0.14/0.795
Early onset of disease, <5 years	0.29/0.12/0.021	-0.58/0.6/0.314
SH-events in the past	0.07/0.08/0.387	0.21/0.3/0.452
Presence of retinopathy	0.16/0.07/0.019	NI
Presence of nephropathy	-0.2/0.09/0.076	NI
Presence of peripheral neuropathy	-0.05/0.07/0.469	0.64/0.4/0.086
Presence of macrovascular disease*	NI	NI
Macrovascular risk factors, n (%)		
Hypertension	NI	-0.69/0.6/0.249
Other #	0.14/0.08/0.080	0.50/0.4/0.203

Data shown are the standardized B of independent variables predictive for the errors of commission and omission.

NI: not included, i.e. no significant contribution to the final model

SH: severe hypoglycaemia; B:beta; SE: Standard error of the mean

* Patients with cerebrovascular events were excluded, only patients with cardiovascular and peripheral vascular disease were included in the analyses.

defined as current smoker and/or presence of dyslipidaemia and/or obesity.

Model was created from a saturated model including all disease related factors, age, sex BMI, HADS scores, Test (0/1) and RT followed by removing non-significant parameters as long as the model fit was not significantly impaired.

CHAPTER

8

GENERAL DISCUSSION AND SUMMARY

CONTENTS

I. Introduction

II. Sleep characteristics and glucose regulation

1. Effect of sleep characteristics on glucose regulation
 2. Sleep duration
 3. Sleep quality
2. Sleep in patients with T1DM
3. Impact of hyperglycemic dysregulation on sleep

III. Sleep and sustained attention

IV. Summary and concluding remarks

I. INTRODUCTION

Intensive insulin therapy and frequent self-monitoring of blood glucose values (SMBG) are the main strategies for optimal replacement of endogenous insulin secretion and to attain normoglycemia in patients with T1DM.⁽¹⁾ Unfortunately, despite more physiological insulin replacement approaches and intensive treatment strategies, relatively few patients reach the glycemic and HbA_{1c} targets⁽²⁾, and in general large intra-individual variations in blood glucose persist.

Previous studies showed an important role for sleep as another physiological determinant of intra-individual variations in glucose metabolism in healthy controls.⁽³⁻⁷⁾ The impact of impaired sleep characteristics on glucose regulation had not been studied in patients with T1DM prior to the studies described in this thesis. We hypothesized that altered sleep characteristics might be one of the physiological factors contributing to impaired glucose regulation in patients with T1DM. In addition, we hypothesized that hyperglycemic dysregulation could impair sleep characteristics, which, in turn, could create a vicious circle by disturbing glucose metabolism.

In the present thesis we explored the interaction between sleep characteristics and glucose regulation in adult patients with T1DM and healthy controls. In addition, we assessed the impact of impaired sleep characteristics on sustained attention in patients with T1DM.

The major conclusions and implications of these studies are discussed in this chapter.

II. SLEEP AND GLUCOREGULATION

1. Effect of sleep characteristics on glucose regulation

a. Sleep restriction

Healthy subjects:

Previous studies showed a negative impact of (partial) sleep restriction during multiple nights on glucose tolerance in healthy controls.⁽³⁻⁵⁾ These studies were in some aspects limited as they did not assess insulin sensitivity by the euglycemic hyperinsulinemic clamp method, the gold standard for measurement of insulin sensitivity. In **Chapter 2** we aimed to compare the impact of one single night of reduced sleep duration (4 hr) with that of a night of normal sleep duration (8.5 hr) on insulin sensitivity in healthy controls. We assessed insulin sensitivity with the hyperinsulinemic euglycemic clamp technique using stable isotopes. The results of that study indicate that even a single night of partial sleep deprivation reduces insulin sensitivity by 19-25% in multiple metabolic pathways in healthy controls. In addition, our data also indicate that insulin sensitivity is not static in healthy controls, and can be modulated by sleep duration. This has also been observed to hold for other environmental factors, including exercise, and dietary macronutrient composition and –content.⁽⁸⁻¹⁰⁾

Clinical implications:

Sleep duration has been shortened considerably in Western societies during the past decades, together with a simultaneously increase in the prevalence of insulin resistance and T2DM. Our study confirmed that shortened sleep duration is another physiological determinant that contributes to insulin resistance and consequently to diabetes risk in healthy controls. Sleep

restriction, even of a single night, can induce such effects, indicating that they are most likely present on a daily basis in many people. Strategies to increase sleep duration might be considered as a potential intervention to prevent or delay the development of T2DM in high-risk populations

Patients with type 1 diabetes mellitus:

In most patients with T1DM glucose regulation cannot be completely normalized, despite intensive insulin treatment and large intra-individual variations in blood glucose levels persist. Subtle intra-individual variations in glucose regulation depend on variations in physiological determinants, such as dietary factors, stress and exercise.⁽¹¹⁻¹³⁾ In **Chapter 3** we aimed to explore whether sleep restriction was another physiological determinant of impaired glucose regulation in patients with T1DM. We evaluated the effects of a single night of partial sleep restriction (4 hr) with those of a night of normal sleep duration (8.5 hr) on *insulin sensitivity* in patients with T1DM. Our results showed that one night of partial sleep deprivation reduced insulin-mediated peripheral glucose uptake by 14-21%. The studies in **Chapter 2** and **Chapter 3** did not address the potential pathophysiological mechanisms involved in the negative effects of reduced sleep duration on insulin sensitivity. We speculate that these mechanisms involve alterations in the activity of the autonomous nervous system, in additions to the endocrine effects of shortened sleep duration.

Clinical implications:

This was the first study to assess the adverse effect of sleep restriction on insulin sensitivity in patients with T1DM. This study stresses that shortened sleep duration, even during only a single night, is another physiological determinant of insulin sensitivity in patients with T1DM and might become another therapeutic target to optimize glucoregulation in patients with T1DM. Endocrinologists perhaps ought to start explaining to their patients that not getting enough sleep may impair their insulin sensitivity, since it can be expected that sleep restriction increases postprandial glucose levels in the absence of concurrent adaptations of exogenous insulin dose. Further research of the underlying pathophysiologic mechanisms responsible for the effect of short sleep duration on insulin sensitivity in patients with T1DM is warranted.

b. Sleep composition

In addition to sleep duration, sleep composition is a determinant of glucose metabolism in healthy controls.⁽¹⁴⁾ The clear 'restorative' role of slow-wave-sleep (SWS) in the maintenance of glucose metabolism has been described before.⁽¹⁵⁻¹⁷⁾ The initiation of SWS coincides with neurophysiological, hormonal, and metabolic alterations, which can affect glucose regulation.⁽¹⁴⁾ Tasali et al.⁽¹⁴⁾ assessed the effect of multiple subsequent nights of SWS suppression on glucose tolerance, and documented that this intervention decreased glucose tolerance. However, these authors did not include the assessment of insulin sensitivity by the euglycemic hyperinsulinemic clamp method. The impact of one single night of selective on glucose regulation had not been studied in patients with T1DM previously. **Chapter 4** described the effects of a *single night* of SWS suppression compared to a night of normal sleep on insulin sensitivity in healthy controls.

We used acoustic stimuli of varying frequency and intensity to suppress the SWS. Our study showed that a single night of selective suppression of SWS, without a change in total sleep duration, did not affect insulin sensitivity in healthy controls.

Clinical implications:

Although we showed a negative impact of shortened sleep duration on insulin sensitivity in healthy controls and patients with T1DM in **Chapter 2** and **Chapter 3**, respectively, there was no effect of a single night of suppression of SWS during maintained sleep duration on insulin sensitivity in healthy controls. Therefore, sleep duration rather than sleep quality, is an important determinant of insulin sensitivity in healthy controls.

2. Sleep in patients with T1DM

Patients with T2DM report sleep disturbances more frequently than the general population does.^(18;19) This is relevant for glucose regulation, since previous publications have shown that reduced sleep duration and/or decreased sleep quality markedly reduce glucose tolerance and insulin sensitivity in healthy controls and patients with type 1 diabetes.^(3-5;7;14;20;21) Sleep characteristics, and their relation with glucoregulation, have not been studied in a large cohort of well-characterized adult patients with T1DM. In **Chapter 5** we describe a cross-sectional study in which we compared subjective sleep characteristics between a well-characterized large cohort of adult patients with T1DM and individually age, sex and BMI matched healthy controls. In addition, we related these sleep characteristics to glycemic control, i.e. HbA_{1c} - values, and assessed possible risk factors for impaired sleep. We used validated sleep questionnaires to assess different features of sleep. We reported significant disturbed sleep quality and a higher risk for obstructive sleep apnea (OSA) compared with matched healthy controls. Several aspects influenced the outcome parameters: the HADS depression score, presence of polyneuropathy, habitual snoring, and other sleep disturbances were independently associated with poor sleep quality. We found no significant association between individual sleep characteristics and impaired glucoregulation.

Clinical implications:

This explorative, cross-sectional, study is important, since disturbed subjective sleep characteristics are apparently part of the complex syndrome of long-standing T1DM. Although the presumed relationship between sleep characteristics and impaired glucose metabolism, assessed by HbA_{1c} values, was not observed in this study using questionnaires, additional studies with objective sleep measurements to assess the impact of impaired sleep characteristics on insulin sensitivity in patients with T1DM are described in **Chapter 2-4**.

3. Effect of metabolic dysregulation on sleep

Various aspects of diabetes could be linked to disturbed sleep characteristics in patients with T1DM, including complications of T1DM, psychological factors and high prevalence of sleep disturbances (see **Chapter 5**).

In **Chapter 6**, we aimed to investigate the effect of controlled metabolic dysregulation, frequently found in patients with T1DM, on sleep characteristics in well-controlled patients with T1DM on stable CSII. Hyperglycemic dysregulation was induced and maintained during the hyperglycemic night by 50% reduction of the basal and bolus insulin infusions compared with the euglycemic night. The results showed that hyperglycemia induced by reduction of insulin infusion did not affect objective sleep parameters. Therefore, a single night of hyperglycemic dysregulation does not constitute an important determinant of disturbed sleep duration and sleep quality in T1DM patients.

Clinical implications:

Although we showed that sleep duration is a determinant of glucose regulation in both healthy controls and T1DM patients in **Chapter 2** and **3**, hyperglycemic dysregulation did not turn out to be a determinant of impaired sleep in patients with T1DM. Since even well-controlled patients experience large daily fluctuations in glucose values, these fluctuations do not explain the potential disturbed sleep characteristics found in the patients.

Further objective research is warranted to elucidate the underlying mechanisms of the impaired sleep characteristics in patients with T1DM, since impaired sleep characteristics could impair glucoregulation in these patients.

IV. SLEEP AND SUSTAINED ATTENTION

Patients with T1DM perform less well on different neuropsychological tests, including sustained attention.^(22;23) Although different disease-related variables are associated with cognitive impairment⁽²⁴⁻²⁹⁾, the exact pathophysiology is not completely elucidated. The role of impaired sleep characteristics on sustained attention has been documented in healthy controls and patients with sleep disorders. The Sustained Attention to Response Task (SART)(32) has been proven to be sensitive to sleep deprivation in healthy controls and to excessive sleepiness in sleep-disorders.^(30;31) However, sleep characteristics and their relation with sustained attention had not been studied previously in patients with T1DM.

In **Chapter 7** we describe a cross-sectional study exploring the relation between subjective sleep characteristics, assessed by validated sleep questionnaires, and sustained attention in patients with T1DM and healthy controls. We used the SART to assess sustained attention. The results of this explorative study indicate the presence of impaired sustained attention in patients with T1DM compared to healthy controls. In addition, the presence of T1DM *per se* was an risk factor for impaired sustained attention. However, disturbed sleep characteristics were not associated with impaired sustained attention in our patients. Additional studies with objective sleep characteristics are warranted.

Clinical implications:

The results of this cross-sectional study are relevant, since even mild forms of cognitive dysfunction, as observed in our study, might influence everyday activities.

V. SUMMARY AND CONCLUDING REMARKS

The present thesis described the interaction between impaired sleep characteristics and glucose regulation in adult patients with T1DM and healthy controls. In addition, we described the impact of impaired sleep characteristics on sustained attention in patients with T1DM.

From the studies described in this thesis we can conclude that:

1. A single night of partial sleep restriction decreased insulin sensitivity of multiple metabolic pathways in healthy controls. **(Chapter 2)**
2. A single night of partial sleep deprivation reduced insulin-mediated peripheral glucose uptake in patients with T1DM. **(Chapter 3)**
3. A single night of selective SWS suppression did not induce insulin resistance in healthy controls; reduced sleep duration rather than altered sleep composition is therefore an important determinant of insulin sensitivity in healthy controls. **(Chapter 4)**
4. Disturbed sleep characteristics are part of the complex syndrome of patients with longstanding T1DM. **(Chapter 5)**
5. There is no effect of short-term controlled hyperglycemic dysregulation on objective sleep characteristics in patients with T1DM. **(Chapter 6)**
6. Diabetes *per se* is independently associated with impaired sustained attention in patients with T1DM. **(Chapter 7)**

Optimizing sleep duration could be a therapeutic target to optimize glucoregulation in these patients, since voluntary sleep curtailment is extremely common in this 24-hr modern society. Although we showed that reduced sleep duration has a negative impact on glucose regulation, short-term controlled hyperglycemia did not affect sleep characteristics, and, therefore, no vicious circle between sleep characteristics and dysregulation of glucose metabolism appears to be present.

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CHAPTER

9

NEDERLANDSE SAMENVATTING

INTRODUCTIE

Type 1 Diabetes Mellitus (T1DM) is een auto-immuun ziekte waarbij de insuline-producerende cellen in de alvleesklier verloren gaan. Hierdoor produceert het lichaam geen insuline meer, wat resulteert in een absoluut insuline-tekort. Het hormoon insuline is van groot belang voor een goede glucosehuishouding. De glucoseconcentratie in het bloed wordt normaliter strikt gereguleerd doordat het lichaam balanceert tussen het aanmaken van glucose (voornamelijk in de lever) en het opnemen van glucose uit het bloed in perifere weefsels (voornamelijk in skeletspieren en vetweefsels). Insuline remt normaliter de productie van glucose door de lever en zorgt dat het glucose opgenomen wordt in de perifere weefsels. Tevens remt het de afbraak van vetzuren, welke gebruikt worden als bouwsteen voor glucose in de lever. Zonder insuline wordt de bloedglucose-waarde te hoog (= *hyperglycemie*). De mate waarin de lever en perifere weefsels reageren op insuline bepaalt de *insuline-gevoeligheid*. Intensieve behandeling met insuline en frequente controle van de bloedglucose waarden zijn van groot belang voor een optimale glucoseregulatie voor deze patiënten, omdat hoge glucosewaarden zowel micro- (schade aan nieren, ogen, zenuwstelsel) als macrovasculaire complicaties (schade aan hart en bloedvaten) kunnen veroorzaken. Ondanks vele nieuwe ontwikkelingen in insuline therapieën de afgelopen 25 jaar, lukt het bij veel patiënten niet om een normale glucoseregulatie te bereiken. Dit is vooral te zien in de hoge bloedglucosewaarden en een relatief hoog HbA_{1c}, wat een maat is voor de gemiddelde bloedsuikerspiegel van de afgelopen 3 maanden. Ook zijn er veel intra-individuele variaties in de glucoseregulatie bij patiënten met T1DM. Dit wordt voor een deel verklaard door verschillende fysiologische factoren welke van invloed zijn op de glucoseregulatie, bijvoorbeeld voedsel, lichamelijke activiteit en stress.

De afgelopen jaren heeft onderzoek bij gezonde controlepersonen aangetoond dat slaap een belangrijke rol speelt bij de glucoseregulatie en een risico factor is voor het ontwikkelen van type 2 diabetes. Omdat een korte slaapduur en slaapproblemen veelvuldig voorkomen in onze 24-uurs maatschappij, wilden we onderzoeken of een gestoorde slaap (zowel slaapduur als kwaliteit) ook een rol speelt bij de glucoseregulatie in patiënten met T1DM. Ook is er is weinig bekend over hoe patiënten met T1DM slapen en of zij meer slaapproblemen hebben dan gezonde controlepersonen. Vervolgens werd gekeken of een gestoorde glucoseregulatie ook van invloed is op de slaapeigenschappen, zoals onder andere op de slaapduur, slaapopbouw en het hebben van slaapproblemen, en of zo een vicieuze cirkel aanwezig is bij patiënten met T1DM. Verder hebben wij gekeken of patiënten met T1DM een verminderde '*sustained attention*' (=vasthouden van aandacht) hebben dan gezonde controlepersonen en of dit gerelateerd was aan de slaapeigenschappen.

Dit proefschrift beschrijft de mogelijke interactie tussen slaap-kenmerken en glucoseregulatie bij volwassen patiënten met T1DM en gezonde controlepersonen. **Hoofdstuk 1** geeft een algemene inleiding over T1DM, slaap, de interactie tussen slaap en glucoseregulatie, en de relatie tussen slaap en cognitie, en met name de sustained attention (vastgehouden aandacht). In **Hoofdstuk 2-4** beschrijven we zowel het effect van een korte slaapduur als het effect van een verminderde slaapkwaliteit op de glucoseregulatie in patiënten met T1DM en gezonde controlepersonen. **Hoofdstuk 5** beschrijft subjectieve slaapkenmerken – met behulp van gevalideerde slaapvragenlijsten- in een grote groep patiënten met T1DM in vergelijking

met individueel overeenkomstige (op leeftijd, geslacht en body mass index (BMI)) gezonde controlepersonen. In **Hoofdstuk 6** onderzoeken we of een gestoorde glucoseregulatie de slaap in T1DM verstoort, en zo ja, of er een vicieuze cirkel aanwezig is met betrekking tot slaapkenmerken en glucoseregulatie bij deze patiënten. Daarnaast kijken we in **Hoofdstuk 7** of er een verschil is tussen de ‘sustained attention’ –gemeten met de ‘Sustained Attention to Response Task (SART)’- in patiënten met T1DM en gezonde controlepersonen. En zo ja, of deze gerelateerd is aan een kortere slaapduur of slechtere slaapkwaliteit in patiënten met een T1DM.

Hieronder worden de belangrijkste bevindingen van de studies die beschreven zijn in die hoofdstukken samengevat.

SLAAP EN GLUCOSEREGULATIE

Effect van slaap karakteristieken op de glucoseregulatie

Slaapduur

Uit eerder onderzoek blijkt dat een kortere slaapduur een ongunstig effect heeft op de glucoseregulatie bij gezonde controlepersonen. Deze onderzoeken hebben veelal het effect van *meerdere nachten* van een *totaal slaaptekort* of een *kortere slaapduur* onderzocht op de *glucosetolerantie* (= een test die meet hoe snel het lichaam het glucose vanuit bloed kan verwerken). Het effect van *één enkele nacht slaaptekort* op de *insulinegevoeligheid* is nog niet onderzocht bij gezonde controlepersonen. **Hoofdstuk 2** beschrijft het effect van *1 nacht* met een beperkte slaapduur op de *insulinegevoeligheid*, gemeten met de euglycemische hyperinsulinemische clamp methode met stabiele isotopen, bij gezonde controlepersonen. De ‘*euglycemische hyperinsulinemische clamp methode*’ is de gouden standaard voor meting van de insulinegevoeligheid. We vergeleken de insulinegevoeligheid na een nacht met een normale slaapduur (8.5 uur slaap: 23:00-7:30 uur) met de insulinegevoeligheid na een nacht met korte slaap (4 uur: 1:00-5:00 uur). Deze onderzoeken laten zien dat zelfs één nacht met korte slaapduur zowel de gevoeligheid van de lever als de perifere weefsels voor insuline vermindert met 19-25%. Ons onderzoek bevestigt dat een korte slaapduur een fysiologische factor is die bijdraagt tot insuline-resistentie bij gezonde controle-personen.

Het effect van een korte slaapduur op de insulinegevoeligheid was nog niet eerder onderzocht in patiënten met een T1DM. In **Hoofdstuk 3** vergeleken we het effect van 1 nacht korte slaapduur op de insulinegevoeligheid met de insulinegevoeligheid na een normale nacht slapen bij patiënten met T1DM. De insulinegevoeligheid werd gemeten met de hyperinsulinemische euglycemische clamp procedure met stabiele isotopen. Na één nacht kortere slaapduur was er een significante vermindering ~20% van de perifere insulinegevoeligheid in T1DM.

Deze onderzoeken bevestigen dat een kortere slaapduur een risicofactor is voor insulineresistentie bij zowel gezonde controlepersonen als bij patiënten met T1DM. Verder onderzoek is nodig om het onderliggende pathofysiologisch mechanisme waarom een kortere slaapduur de insulinegevoeligheid verminderd te onderzoeken.

Slaapopbouw

Ook de slaapopbouw lijkt een rol te spelen bij de glucoseregulatie. Normale slaap bestaat uit 'Rapid Eye Movement (REM)' slaap en 3 stadia van non-rapid eye movement (NREM)'' slaap: stadium I, II, en III. Stadium III wordt ook wel de slow-wave slaap (SWS) ('diepe slaap') genoemd. De SWS lijkt een belangrijke rol te spelen bij de glucosehouding. Een eerder onderzoek liet een belangrijk effect van 3 opeenvolgende nachten met volledige onderdrukking van SWS zien op de *glucosetolerantie* met de intraveneuze glucose tolerantie test (ivGTT) bij gezonde controlepersonen. **Hoofdstuk 4** beschrijft het effect van één enkele nacht met selectieve onderdrukking van de SWS op de insulinegevoeligheid in vergelijking met een nacht van normale slaap. Selectieve onderdrukking van de SWS werd bereikt door toediening van geluidsprikkelers via luidsprekers van verschillende frequentie en intensiteit. Indien de personen in SWS kwamen, werd er een stimulus gegeven zodat ze in een lichter slaapstadium kwamen, zonder dat ze hierbij wakker werden. De *slaapduur* was hetzelfde bij beide nachten. De insulinegevoeligheid werd gemeten met de hyperinsulinemische euglycemische clamp met stabiele isotopen. In dit onderzoek was er geen effect van een enkele nacht selectieve onderdrukking van de SWS op de insulinegevoeligheid in gezonde controlepersonen in vergelijking met de insulinegevoeligheid na een normale nacht slaap. Hieruit kunnen we concluderen dat *slaapduur* in plaats van de *slaapopbouw* een fysiologische rol speelt is bij de glucoseregulatie in zowel gezonde controlepersonen als patiënten met een T1DM.

Slaapkenmerken bij patiënten met type 1 diabetes

In eerdere onderzoeken hebben wij aangetoond dat een gestoorde slaapduur van invloed is op de glucoseregulatie (**Hoofdstuk 2 en 3**). Slaapkenmerken en hun relatie met glucoseregulatie waren nog niet goed onderzocht in een grote groep volwassen patiënten met T1DM. Twee kleinere onderzoeken lieten zien dat patiënten met T1DM vaker een lichtere slaap hadden. Een ander recente onderzoek aan 37 patiënten toonde dat het obstructieve slaap-apneu-syndroom (OSAS) vaker voorkwam bij patiënten met T1DM. **Hoofdstuk 5** beschrijft een onderzoek bij een grote groep volwassen patiënten met T1DM versus gezonde controlepersonen, van hun slaapkenmerken- en hun relatie met de glucoseregulatie. In dit onderzoek hebben wij in ruim 100 patiënten aangetoond dat een groot deel van de patiënten een verminderde slaapkwaliteit had en een hoger risico op OSAS in vergelijking met individueel gematchte gezonde controlepersonen. Verschillende ziekte- gerelateerde factoren bleken van invloed te zijn op deze uitkomst: de HADS depressiescore, het wel of niet hebben van polyneuropathie, snurken, andere slaapstoornissen. We vonden geen relatie tussen gestoorde slaap en gestoorde glucoseregulatie.

Effect van gestoorde glucoseregulatie op slaapeigenschappen

In **hoofdstuk 6** werd gekeken naar het effect van hoge bloedsuikers (hyperglycemie), vaak voorkomend bij patiënten met T1DM, op de slaapeigenschappen van goed-gereguleerde patiënten met T1DM met continue onderhuidse insuline-infusie (CSII) pomp. Hyperglycemische ontregeling werd bereikt door zowel de basale standen van de insulinepomp als de bolussen bij de maaltijden te halveren tijdens de hyperglycemische studiedag. Dit onderzoek toonde

geen effect van één enkele nacht met hyperglycemie op objectief gemeten slaapparameters in vergelijking met een euglycemische nacht (nacht met normale bloedsuikers). Alhoewel we hebben aangetoond, in **hoofdstukken 2 en 3**, dat de slaapduur een belangrijk effect heeft op de glucoseregulatie in patiënten met T1DM en gezonde controlepersonen, lijkt er andersom geen effect te zijn van een gestoorde glucoseregulatie op de slaapkenmerken bij patiënten met T1DM. Concluderend vormt hyperglycemische ontregeling geen belangrijke oorzaak van gestoorde slaap bij patiënten met T1DM.

SLAAP EN COGNITIE ('SUSTAINED ATTENTION')

Eerdere studies hebben aangetoond dat patiënten met T1DM verschillende neuropsychologische testen minder goed uitvoeren dan gezonde controlepersonen. Alhoewel verschillende aan de ziekte-gerelateerde oorzaken geassocieerd waren met de uitkomsten, is de exacte oorzaak (pathofysiologie) niet bekend. Eerdere studies hebben aangetoond dat 'sustained attention' (vastgehouden aandacht) beïnvloed wordt door slaaptekort en slaperigheid. De sustained attention en de eventuele rol van slaap hierop is nog niet eerder onderzocht in patiënten met een T1DM. **Hoofdstuk 7** beschrijft een onderzoek waarin het verschil in sustained attention, gemeten met de 'Sustained Attention to Response Task (SART)', tussen gezonde controlepersonen en patiënten met een T1DM onderzocht werd. Patiënten met diabetes hadden een gestoorde sustained attention in vergelijking met gezonde controles. We vonden geen relatie tussen slaapkenmerken en sustained attention in onze patiënten. Verder onderzoek met objectieve metingen van slaap zijn nodig.

SAMENVATTING EN CONCLUSIE

Dit proefschrift beschrijft de interactie tussen slaapeigenschappen en glucoseregulatie in volwassen patiënten met T1DM en gezonde controlepersonen. Daarnaast hebben we het effect van slaapkenmerken op de sustained attention (=vastgehouden aandacht) onderzocht.

We kunnen het volgende concluderen uit de studies in dit proefschrift:

1. Een enkele nacht met korte slaapduur vermindert zowel de gevoeligheid van de lever als perifere insuline-gevoeligheid in gezonde controlepersonen. **(hoofdstuk 2)**
2. Een enkele nacht met korte slaapduur vermindert de insuline-gemedieerde perifere glucoseopname in patiënten met T1DM. **(hoofdstuk 3)**
3. Eén enkele nacht met veranderde slaapopbouw – door selectieve onderdrukking van de slow-wave slaap (diepe slaap) – heeft geen effect op de insulinegevoeligheid in gezonde controlepersonen en is derhalve geen belangrijke determinant van insuline-gevoeligheid in gezonde controlepersonen. **(hoofdstuk 4)**
4. Gestoorde slaapkenmerken zijn onderdeel van het complexe syndroom van patiënten met een lang bestaande T1DM. **(hoofdstuk 5)**

5. Er is geen effect van een kortdurende hyperglycemische ontregeling op de slaapparameters gemeten met polysomnografie bij patiënten met T1DM. **(hoofdstuk 6)**
6. Patiënten met T1DM hebben een gestoorde 'sustained attention'. **(hoofdstuk 7)**

Het lukt heden ten dage nog steeds niet goed genoeg om de glucoseregulatie in patiënten met T1DM te perfectioneren ondanks nieuwe insuline-vervangende therapieën. Het is vrijwel onmogelijk voor patiënten met T1DM om normale glycemische en HbA_{1c} waarden te halen en er zijn relatieve grote intra-individuele schommelingen in de glucosewaarden. Het optimaliseren van de slaapduur kan een middel zijn om de glucoseregulatie te verbeteren, omdat een korte slaap zeer veel voorkomt in onze moderne 24-uurs samenleving. Alhoewel we hebben laten zien dat een verkorte slaapduur een negatief effect heeft op de glucoseregulatie, heeft een gestoorde glucoseregulatie gelukkig geen effect op de slaapeigenschappen. Concluderend is er geen vicieuze cirkel aanwezig tussen gestoorde slaap en glucoseregulatie bij patiënten met T1DM

CHAPTER

10

CURRICULUM VITAE

Marieke van Dijk werd geboren op 29 oktober 1982 te Almelo. Zij groeide op in Westerhaar-Vriezenveenschewijk, en behaalde in 2001 haar Gymnasium diploma aan het C.S.G. het Noordik, te Almelo.

Datzelfde jaar startte zij met de opleiding Psychologie aan de Universiteit van Groningen, welke zij het jaar erna beëindigde. In 2002 werd zij ingeloot voor de studie Geneeskunde aan de Universiteit Leiden. In 2008 startte verrichte zij haar wetenschapsstage op de afdeling Endocrinologie en Stofwisselingsziekten van het Leids Universitair Medisch Centrum. Onder begeleiding van Esther Donga deed zij onderzoek naar het effect van een korte slaapduur op de insulinegevoeligheid bij patiënten met een type 1 diabetes. Na het behalen van haar artsexamen in mei 2009 startte zij als promovenda op deze zelfde afdeling (in nauwe samenwerking met de afdeling neurologie) onder leiding van professor dr. J. A. Romijn, Dr. E.P.M. van der Kleij-Corssmit en professor dr. J.G. van Dijk. Zij heeft wetenschappelijk onderzoek verricht naar de mogelijke interactie tussen slaap op de glucoseregulatie bij patiënten met een type 1 diabetes. De resultaten van dit onderzoek staan beschreven in dit proefschrift. In juni 2012 was zij gestart met de opleiding Interne Geneeskunde in het Rijnland ziekenhuis te Leiderdorp (opleiders dr. M.F.J.M. Janssen, Dr. A.M. Schrander-van der Meer, drs. S. Anten en prof. dr. J.W. de Fijter). Per 1 sept 2014 is zij gestart met de opleiding tot cardiologe in het Rijnland ziekenhuis (opleiders Dr. C.J.H.J. Kirchhof en prof. dr. M.J. Schalij).

CHAPTER

11

LIST OF PUBLICATIONS

Esther Donga, **Marieke van Dijk**, J Gert van Dijk, Nienke Biermasz, Gert Jan Lammers, Klaas WK van Kralingen, Eleonora PM Corssmit, Johannes A Romijn. A single night of partial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects. *J Clin Endocrinol Metab* 2010; 95(6): 2963-2968.

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Marieke van Dijk, Esther Donga, J Gert van Dijk, Jitske Tiemensma, Gert Jan Lammers, Klaas WK van Kralingen, Petronella H. Geelhoed-Duijvestijn, Eleonora PM Corssmit, Johannes A Romijn. Controlled hyperglycemic dysregulation does not significantly affect sleep characteristics in patients with type 1 diabetes mellitus. *Submitted*

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Marieke van Dijk, Esther Donga, Mojca KM van Schie, Gert Jan Lammers, Erik W. van Zwet, Eleonora PM Corssmit, Johannes A Romijn, J Gert van Dijk. Impaired sustained attention in adult patients with type 1 diabetes related to diabetes per se. *Diabetes Metab Res Rev.* 2014;30 (2):132-9

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Accepted in Archives of Sexual behavior, 2015

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