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# **Glucose and fat metabolism in narcolepsy and the effect of sodium oxybate: A hyperinsulinemic-euglycemic clamp study**

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## ABSTRACT

**Introduction:** Narcolepsy is associated with obesity though it is uncertain whether this is caused by changes in glucose and fat metabolism. Therefore, we performed a detailed analysis of systemic energy homeostasis in narcolepsy patients, and additionally, investigated whether it was affected by three months of sodium oxybate (SXB) treatment.

**Methods:** Nine hypocretin deficient patients with narcolepsy-cataplexy, and nine healthy sex, age, and BMI matched controls were enrolled. A hyperinsulinemic-euglycemic clamp combined with stable isotopes ([6,6-<sup>2</sup>H<sub>2</sub>]-glucose and [<sup>2</sup>H<sub>3</sub>]- glycerol) was performed at baseline. In seven patients a second study was performed after three months of SXB treatment.

**Results:** Glucose disposal rate (GDR) per unit serum insulin was significantly higher in narcolepsy patients compared to matched controls ( $1.6 \pm 0.2$  vs.  $1.1 \pm 0.3$   $\mu\text{mol}/\text{kgFFM}/\text{min}/\text{mU}\times\text{L}$ ;  $P = 0.024$ ), whereas  $\beta$ -cell function was similar ( $P = 0.50$ ). Basal steady state glycerol appearance rate tended to be lower in narcolepsy patients ( $5.2 \pm 0.4$  vs.  $7.5 \pm 1.3$   $\mu\text{mol}/\text{kgFFM}/\text{min}$ ;  $P = 0.058$ ), suggesting a lower rate of lipolysis. SXB treatment induced a trend in reduction of the GDR ( $1.4 \pm 0.1$  vs.  $1.1 \pm 0.2$   $\mu\text{mol}/\text{kgFFM}/\text{min}/\text{mU}\times\text{L}$ ;  $P = 0.063$ ) and a reduction in endogenous glucose production ( $0.24 \pm 0.03$  vs.  $0.16 \pm 0.03$   $\mu\text{mol}/\text{kgFFM}/\text{min}/\text{mU}\times\text{L}$ ;  $P = 0.028$ ) per unit serum insulin. After SXB treatment lipolysis increased ( $4.9 \pm 0.4$  vs.  $6.5 \pm 0.6$   $\mu\text{mol}/\text{kgFFM}/\text{min}$ ;  $P = 0.018$ ), and body weight decreased in narcolepsy patients ( $99.2 \pm 6.0$  vs.  $94.0 \pm 5.4$  kg;  $P = 0.044$ ).

**Conclusion:** We show that narcolepsy patients are more insulin sensitive and may have a lower rate of lipolysis than matched controls. SXB stimulated lipolysis in narcolepsy patients, possibly accounting for the weight loss after treatment. While SXB tended to decrease systemic insulin sensitivity, it increased hepatic insulin sensitivity, suggesting tissue-specific effects.

## INTRODUCTION

Narcolepsy is a sleep disorder that is characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis, disturbed nocturnal sleep and obesity.<sup>1</sup> Both obesity and disturbed nocturnal sleep are important risk factors for the development of type 2 diabetes mellitus (T2DM).<sup>199</sup> Narcolepsy is caused by a loss of hypocretin (orexin) neurons in the hypothalamus.<sup>47;48</sup> Hypocretins are neuropeptides known to be involved in sleep-wake regulation, feeding behavior as well as body weight and temperature regulation.<sup>103;176</sup> Furthermore hypocretins are important hypothalamic regulators of glucose homeostasis. Disturbed activation of hypocretin neurons during sleep deprivation may lead to increased basal endogenous glucose production and a decreased insulin sensitivity in rats.<sup>200</sup> Moreover, hypocretin deficiency leads to an age-related defective glucose tolerance and insulin resistance.<sup>201</sup> Intracerebroventricular infusions of hypocretin-1 increase hepatic glucose production in rats,<sup>202;203</sup> but intracerebral injections of hypocretin-1 in the ventromedial hypothalamus increase glucose uptake and promote insulin induced glucose uptake and glycogen synthesis in skeletal muscle of mice.<sup>204</sup> Taken together, these data imply that the absence of hypocretin in human narcolepsy may disturb glucose homeostasis.

In the sixties and eighties of the previous century, some reports suggested a higher incidence of T2DM in narcolepsy patients.<sup>39;41;95</sup> Another study confirmed a higher risk of metabolic syndrome independent of body mass index (BMI) when comparing patients with narcolepsy to those with idiopathic hypersomnia.<sup>22</sup> However, in a recent study comparing narcolepsy patients with healthy controls matched for BMI, no differences in insulin resistance could be detected using the homeostatic model assessment (HOMA),<sup>42</sup> a method which estimates  $\beta$ -cell function and insulin resistance from a single pair of fasting glucose and insulin measurements.<sup>205</sup> Similarly, another more recent study could not find differences in glucose tolerance and  $\beta$ -cell function between 17 narcolepsy patients and healthy controls.<sup>96</sup> So far, there have been no studies using a hyperinsulinemic-euglycemic clamp to estimate insulin sensitivity in narcolepsy patients.

Metabolic syndrome and insulin resistance may lead to T2DM within several years if lifestyle is not adapted.<sup>206</sup> Under normal circumstances pancreatic islet  $\beta$ -cells compensate for insulin resistance by increasing insulin release. However, when these compensatory mechanisms fail T2DM will develop.<sup>207</sup> The most accurate method available for measuring insulin sensitivity is the hyperinsulinemic-euglycemic clamp technique.<sup>208</sup> During a clamp a fixed dose of insulin is continuously infused along with a variable amount of glucose so as to maintain

euglycemia, i.e., the plasma glucose levels are “clamped” at a predefined level. Thus, the amount of glucose infused necessary to keep plasma glucose levels constant can be used as a measure of peripheral insulin sensitivity since relatively lower amounts of glucose will be needed in case of insulin resistance.<sup>208</sup>

Sodium oxybate (SXB), also known as  $\gamma$ -hydroxybutyrate (GHB), is an effective treatment of narcolepsy. It reduces cataplexy, improves nocturnal sleep fragmentation and in higher doses it may also reduce excessive daytime sleepiness.<sup>73</sup> SXB activates dopaminergic circuits in the brain.<sup>77</sup> As diminished dopamine (D2) receptor mediated signal transduction appears to induce insulin resistance, the metabolic syndrome and T2DM,<sup>79</sup> it is conceivable that SXB might be protective against developing T2DM. Additionally, there are indications that SXB might reduce body weight,<sup>74</sup> which could also decrease the risk of T2DM.

The abovementioned discrepancies between the findings from earlier reports regarding potential disturbances in glucose metabolism in narcolepsy patients may be due to suboptimal assessment of insulin sensitivity. Moreover, to our knowledge, fat metabolism, which is obviously key to systemic energy homeostasis, has not been scrutinized before in narcolepsy patients. Therefore, in the present study we applied the gold standard for measuring insulin sensitivity, i.e., the hyperinsulinemic-euglycemic clamp, complemented with a stable isotope technique to assess both glucose and fat metabolism in a group of 9 hypocretin deficient narcolepsy with cataplexy patients and 9 individually matched healthy controls. In addition, we assessed the effect of three months of treatment with SXB on glucose and fat metabolism in narcolepsy patients.

## METHODS

### Subjects

Nine narcolepsy with cataplexy patients and nine, individually age, sex, BMI, fat percentage, and waist-to-hip ratio (WHR) matched healthy controls were enrolled. All patients fulfilled the ICSD-2 criteria for narcolepsy with cataplexy.<sup>118</sup> All were HLA DQB1\*06:02 positive, and CSF hypocretin-1 deficient. None of the controls used medication. Seven patients were drug naive, one discontinued methylphenidate 2 weeks prior to the study. The last patient was tapered off antidepressants and methylphenidate and did not take any drugs in the 2 weeks prior to the start of the study. None of the patients used any other medication. Subjects were eligible for study after exclusion of hypertension, any known (history of) pituitary,

psychiatric, or neurological disease (other than narcolepsy), alcohol or drug abuse, recent weight change (> 3 kg weight change within the last 3 months), or a sleep disorder history (controls). Routine laboratory tests were performed to rule out overt diabetes, thyroid disease, anemia, and hepatic and/ or renal disease. The study was approved by the ethics committee of the Leiden University Medical Centre. Written informed consent was obtained from all subjects.

### Clinical protocol

All studies started at 08:30 after an overnight fast. Alcohol consumption and excessive exercise on the day preceding the study was not allowed. Caffeinated drinks were also not allowed from 18:00 prior to and on the day of study, and smoking was prohibited until the end of the measurements. Height, weight, BMI, and waist-to-hip ratio were measured according to the WHO recommendations.<sup>209</sup> Bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK) was used to estimate lean body mass and fat percentage. Subjects were requested to lie down on a bed in a semi-supine position. A polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was inserted into a contralateral dorsal hand vein for blood sampling; this hand was kept in a heated box (60°C) throughout the test to obtain arterialized blood.<sup>210</sup> Samples were taken for measurement of basal levels of glucose, insulin, total cholesterol, LDL cholesterol, HDL-cholesterol, triglycerides, glycerol and background enrichment of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose and [<sup>2</sup>H<sub>5</sub>]-glycerol. At 08:30 (t = 0 min), an adjusted primed (17.6 μmol/kg × actual plasma glucose concentration (mmol/L)) continuous (0.33 μmol/kg/min) infusion of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose (enrichment 99.9%; Cambridge Isotopes, Cambridge, Massachusetts, USA) was started and continued throughout the study. After 60 min, a primed (1.6 μmol/kg) continuous (0.11 μmol/kg/min) infusion of [<sup>2</sup>H<sub>5</sub>]-glycerol (Cambridge Isotopes) was started and continued throughout the study. Subsequently, a primed continuous infusion of insulin (Actrapid, Novo Nordisk Pharma BV, Alphen aan de Rijn, The Netherlands; 40 mU/m<sup>2</sup>/min) was started (t = 120 min). Exogenous glucose 20% enriched with 3% [6,6-<sup>2</sup>H<sub>2</sub>]-glucose was infused at a variable rate to maintain the plasma glucose level at 5.0 mmol/ L. From t = 210 to 240 min, blood was drawn every 10 min for determination of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose and [<sup>2</sup>H<sub>5</sub>]-glycerol specific activities, glucose, insulin, and glycerol. While blood in the serum samples was allowed to clot, plasma samples were immediately put on ice. Within 60 min of sampling, all samples were centrifuged at 1610 g at 4°C for 20 min and then stored at -80°C until assay.

## Sodium oxybate

Narcolepsy patients started SXB treatment after completion of the first clamp session. SXB was given in a regular starting dose of 2 nighttime doses of 2.25 grams. Subsequently, the dose was titrated up to an optimum for each individual patient, although never exceeded the maximum dose of 9 grams per night. When there was satisfactory symptom control for 3 months, according to the patient and the treating neurologist, patients were allowed to embark on the second clamp session. The same neurologist (GJL) evaluated all cases. No other drugs were allowed during these 3 months.

## Assays

Serum insulin concentrations were measured by enzyme-labelled chemiluminescent immunometric assay (Immulite 2500; Siemens, Munich, Germany) with an intra-assay coefficient of variation (CV) of 4%. Enrichment of plasma [6,6-<sup>2</sup>H<sub>2</sub>]-glucose was determined in a single analytical run using gas chromatography coupled to mass spectrometry, as described previously.<sup>211</sup> All isotope enrichments were measured on a gas chromatograph mass spectrometer (model 6890/5973; Hewlett-Packard, Palo Alto, CA). Serum cholesterol, high-density lipoprotein (HDL) and triglycerides (TG) were measured with a fully automated P-800 module (Roche, Almere, The Netherlands). For both TG and total cholesterol (TC) the CV was less than 2%. For HDL the CV was less than 3%. Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald equation.<sup>212</sup>

## Calculations

In all clamp sessions a physiological steady state was achieved during the last 30 min of both the basal as well as the hyperinsulinemic period; therefore, the rates of appearance and disappearance for glucose and glycerol were calculated as the tracer infusion rate divided by the tracer to tracee ratio.<sup>213</sup> Under steady-state conditions the rate of appearance equals the rate of disappearance, or the disposal rate. Glucose flux rates were expressed per kg fat free mass (FFM), whereas glycerol flux rates were normalized per kg fat mass (FM). Endogenous glucose production (EGP) during the basal steady state is equal to the rate of appearance of glucose whereas EGP during the clamp was calculated as the difference between the rates of glucose appearance and infusion. Since the fasting plasma insulin concentration is a strong inhibitory stimulus for EGP, the basal hepatic insulin resistance index ( $\mu\text{mol}/\text{min}/\text{kgFFM}/\text{pmol}\times\text{L}$ ) was calculated as the product of fasting EGP and fasting

plasma insulin concentration.<sup>208</sup> The metabolic clearance rate of insulin was calculated as the constant infusion rate of insulin divided by the steady state serum insulin concentration corrected for endogenous insulin secretion.  $\beta$ -cell function was estimated using the HOMA as described previously.<sup>205</sup>

### Statistical analysis

Results are expressed as mean  $\pm$  standard error (SEM) unless otherwise specified. The nonparametric Mann-Whitney  $U$  test was used to assess differences in medians between patients and controls, whereas the Wilcoxon signed ranks test was applied to assess differences between baseline and SXB conditions. The nonparametric Spearman rank correlation coefficient was used to assess correlations between changes in weight and other metabolic parameters. A paired-samples t-test was used if applicable. All tests were two-tailed, and significance level was set at  $P < 0.05$ . Statistical analyses were performed using SPSS for Windows (release 18.0, SPSS, Inc.).

## RESULTS

### Subjects

Narcolepsy patients did not differ from controls with respect to sex, age, BMI, WHR, lean body mass, or fat percentage (all  $P \geq 0.33$ , Table 6.1). Moreover baseline glucose, insulin, total cholesterol ( $4.7 \pm 0.23$  vs.  $4.7 \pm 0.26$  mmol/L), LDL cholesterol ( $3.1 \pm 0.18$  vs.  $3.0 \pm 0.23$  mmol/L), HDL-cholesterol ( $0.98 \pm 0.06$  vs.  $1.2 \pm 0.11$  mmol/L), and triglycerides ( $1.4 \pm$

**Table 6.1** Demographics and body composition

	Narcolepsy patients	Controls	<i>P</i>
Male/Female	7/2	7/2	
Age (Y)	35.7 (0.9)	37.1 (1.5)	0.33
BMI	29.1 (1.3)	28.3 (1.1)	0.60
Fat (%)	25.0 (2.6)	24.4 (2.8)	0.95
Lean body mass (kg)	71.4 (3.6)	73.4 (3.6)	0.57
WHR	0.89 (0.2)	0.90 (0.2)	0.57

Data are means  $\pm$  SEM. Intergroup differences were assessed by the nonparametric Mann-Whitney  $U$  test. WHR, waist-to-hip ratio.



0.26 vs.  $1.0 \pm 0.14$  mmol/L) did not differ between the 2 groups (all  $P \geq 0.10$ ). SXB was well tolerated except in one patient who discontinued due to an unacceptable increase in sleep paralysis. Another patient with excessive daytime sleepiness as major complaint could not be assessed after treatment due to the fact that he insisted on taking stimulants. Thus 9 patients and controls were measured at baseline, and 7 patients were assessed for a second time. Narcolepsy patients lost an average amount of 5.2 kg of their body weight after 3 months of treatment with SXB ( $99.2 \pm 6.0$  vs.  $94.0 \pm 5.4$  kg;  $P = 0.044$ ).

### **Glucose metabolism in narcolepsy patients versus controls**

An overview of all metabolic parameters in narcolepsy patients and controls during both the basal steady state and the hyperinsulinemic steady state is presented in Table 6.2. During the basal steady state there were no significant differences in glucose disposal rate (Glucose Rd) between patients and controls. In addition, during the hyperinsulinemic steady state the glucose disposal rate was similar in narcolepsy patients and controls ( $55.9 \pm 7.9$  vs.  $40.2 \pm 5.3$   $\mu\text{mol}/\text{kgFFM}/\text{min}$ ,  $P = 0.122$ ), despite significantly lower steady state plasma insulin levels ( $33.9 \pm 2.9$  vs.  $42.6 \pm 3.5$  mU/L,  $P = 0.047$ ). When adjusted for the differences in plasma insulin levels, the glucose disposal rate ( $1.6 \pm 0.2$  vs.  $1.1 \pm 0.3$   $\mu\text{mol}/\text{kgFFM}/\text{min}/\text{mU} \times \text{L}$ ,  $P = 0.024$ ) was significantly higher in patients, whereas the rate of endogenous glucose production ( $0.26 \pm 0.03$  vs.  $0.24 \pm 0.07$   $\mu\text{mol}/\text{kgFFM}/\text{min}/\text{mU} \times \text{L}$ ,  $P = 0.085$ ) did not differ between both groups. In addition,  $\beta$ -cell function as assessed using the baseline glucose and insulin levels was equal in the two groups (8.7 vs. 10.3 Mann-Whitney  $U = 33$ ,  $Z = -0.66$ ,  $P = 0.50$ ).

### **Lipid metabolism in narcolepsy patients versus controls**

During the basal steady state plasma glycerol levels ( $46.7 \pm 2.8$  vs.  $67.2 \pm 11.3$   $\mu\text{mol}/\text{L}$ ,  $P = 0.070$ ), as well as the rate of glycerol appearance ( $5.2 \pm 0.4$  vs.  $7.5 \pm 1.3$   $\mu\text{mol}/\text{kgFM}/\text{min}$ ,  $P = 0.058$ ) tended to be lower in narcolepsy patients compared to controls, suggesting a lower rate of lipolysis in narcolepsy patients. However, these trends disappeared during the hyperinsulinemic steady state, indicating an intact insulin mediated suppression of lipolysis in narcolepsy patients. These findings remained unchanged when adjusted for plasma insulin levels (Table 6.2).

**Table 6.2** Metabolic parameters in nine patients with narcolepsy and nine matched controls during the basal steady state and during the hyperinsulinemic steady state

	Basal steady state			Hyperinsulinemic steady state		
	Narcolepsy	Controls	P	Narcolepsy	Controls	P
Glucose (mmol/L)	5.2 ± 0.1	5.4 ± 0.2	0.965	5.1 ± 0.1	4.8 ± 0.1	0.038*
Glucose $R_d$ ( $\mu\text{mol/kgFFM/min}$ )	19.0 ± 0.7	18.9 ± 0.4	0.757	55.9 ± 7.9	40.2 ± 5.3	0.122
M ( $\mu\text{mol/kgFFM/min}$ )	-	-	-	46.8 ± 7.6	31.4 ± 4.6	0.190
EGP ( $\mu\text{mol/kgFFM/min}$ )	19.0 ± 0.7	18.9 ± 0.4	0.757	8.3 ± 0.7	8.3 ± 0.9	0.627
Plasma insulin (mU/L)	6.2 ± 0.7	5.9 ± 1.0	0.796	33.9 ± 2.9	42.6 ± 3.5	0.047*
HIR ( $\mu\text{mol kgFFM/min/pmol} \times \text{L}$ )	806.5 ± 91.2	773.7 ± 127.4	0.566	1976.3 ± 242.1	2299.9 ± 104.9	0.310
MCR <sub>1</sub> (mL/m <sup>2</sup> /min)	-	-	-	0.11 ± 0.02	0.07 ± 0.01	0.085
Glucose $R_d$ per unit serum insulin ( $\mu\text{mol/kgFFM/min/mU} \times \text{L}$ )	3.6 ± 0.6	4.2 ± 0.9	0.825	1.6 ± 0.2	1.1 ± 0.3	0.024*
M per unit serum insulin ( $\mu\text{mol/kgFFM/min/mU} \times \text{L}$ )	-	-	-	1.4 ± 0.2	0.9 ± 0.3	0.031*
EGP per unit serum insulin ( $\mu\text{mol/kgFFM/min/mU} \times \text{L}$ )	3.6 ± 0.6	4.2 ± 0.9	0.825	0.26 ± 0.03	0.24 ± 0.07	0.085
Glycerol ( $\mu\text{mol/L}$ )	46.7 ± 2.8	67.2 ± 11.3	0.070	18.0 ± 1.4	20.8 ± 3.3	0.691
Glycerol $R_a$ ( $\mu\text{mol/kgFM/min}$ )	5.2 ± 0.4	7.5 ± 1.3	0.058	2.2 ± 0.2	2.0 ± 0.2	0.965
Glycerol $R_a$ per unit serum insulin ( $\mu\text{mol/kgFM/min}$ )	$9.7 \times 10^{-1} \pm 1.5 \times 10^{-1}$	$1.4 \pm 2.1 \times 10^{-1}$	0.102	$6.7 \times 10^{-2} \pm 8.1 \times 10^{-3}$	$4.9 \times 10^{-2} \pm 5.5 \times 10^{-3}$	0.102

Data are means ± SEM. Intergroup differences were assessed by the nonparametric Mann-Whitney U test, \*  $P < 0.05$ . Abbreviations: EGP = endogenous glucose production; FFM = fat free mass; HIR = hepatic insulin resistance; M = glucose metabolized; MCR<sub>1</sub> = metabolic clearance rate of insulin;  $R_d$  = rate of disappearance. During basal steady state glucose  $R_d$  equals EGP. During steady state conditions the rates of appearance ( $R_a$ ) of glucose and glycerol is equal to their respective rates of disappearance ( $R_d$ ).

### **Glucose metabolism in narcolepsy patients before and after SXB**

$\beta$ -cell function did not change after SXB treatment ( $P = 0.61$ ). SXB had no obvious effects on the basal steady state glucose metabolism (Table 6.3). After SXB treatment the steady state plasma insulin levels were significantly higher during the hyperinsulinemic conditions ( $33.8 \pm 3.8$  vs.  $48.9 \pm 4.8$  mU/L,  $P = 0.018$ ), perhaps due to a significantly lower metabolic clearance rate of insulin ( $0.11 \pm 0.02$  vs.  $0.06 \pm 0.01$  mL/m<sup>2</sup>/min,  $P = 0.018$ ). When adjusted for these differences in plasma insulin levels, SXB treatment induced a trend towards a reduced rate of glucose disposal ( $1.4 \pm 0.1$  vs.  $1.1 \pm 0.2$   $\mu\text{mol/kgFFM}/\text{min}/\text{mU} \times \text{L}$ ,  $P = 0.063$ ). In addition there was a significant reduction in endogenous glucose production ( $0.24 \pm 0.03$  vs.  $0.16 \pm 0.03$   $\mu\text{mol/kgFFM}/\text{min}/\text{mU} \times \text{L}$ ,  $P = 0.028$ ) during the hyperinsulinemic condition. Thus, SXB had a tendency to decrease peripheral (primarily muscle) insulin sensitivity, while it increased hepatic insulin sensitivity (Table 6.3).

### **Lipid metabolism in narcolepsy patients before and after SXB**

SXB treatment significantly increased both basal levels of glycerol ( $47.3 \pm 3.7$  vs.  $62.4 \pm 5.3$   $\mu\text{mol}/\text{L}$ ,  $P = 0.043$ ) and the rate of glycerol appearance ( $4.9 \pm 0.4$  vs.  $6.5 \pm 0.6$   $\mu\text{mol}/\text{kgFM}/\text{min}$ ,  $P = 0.018$ ), indicating that SXB treatment increases lipolysis in narcolepsy patients (Table 6.3). After SXB treatment the rate of lipolysis became lower during hyperinsulinemic conditions as evidenced by a trend towards lower plasma glycerol levels ( $17.8 \pm 1.6$  vs.  $14.6 \pm 1.9$   $\mu\text{mol}/\text{L}$ ,  $P = 0.063$ ) and a lower rate of glycerol appearance ( $1.9 \pm 0.2$  vs.  $1.4 \pm 0.2$   $\mu\text{mol}/\text{kgFM}/\text{min}$ ,  $P = 0.018$ ), suggesting that SXB increases the sensitivity of fat tissue to the inhibitory effects of insulin on lipolysis (Table 6.3). These findings remained largely unchanged when adjusted for plasma insulin levels (Table 6.3).

### **Metabolic parameters in relation to weight change**

The change in body weight after SXB treatment was strongly associated with alterations in glucose disposal rate per unit serum insulin ( $r = -0.93$ ,  $P = 0.003$ ; Figure 6.1), but not with changes in endogenous glucose production per unit serum insulin ( $r = 0.29$ ,  $P = 0.535$ ).

**Table 6.3** Metabolic parameters in seven patients with narcolepsy before and after sodium oxybate (SXB) treatment

	Basal steady state			Hyperinsulinemic steady state		
	Before SXB	After SXB	P	Before SXB	After SXB	P
Glucose (mmol/L)	5.2 ± 0.1	5.1 ± 0.1	0.063	5.1 ± 0.1	5.0 ± 0.1	0.028*
Glucose $R_d$ ( $\mu\text{mol/kgFFM}/\text{min}$ )	18.6 ± 0.7	18.0 ± 0.8	0.128	49.4 ± 7.8	47.5 ± 5.1	0.866
M ( $\mu\text{mol/kgFFM}/\text{min}$ )	-	-	-	41.0 ± 7.6	41.1 ± 5.1	1.000
EGP ( $\mu\text{mol/kgFFM}/\text{min}$ )	18.6 ± 0.7	18.0 ± 0.8	0.128	4.8 ± 0.47	7.2 ± 0.76	0.612
Plasma insulin (mU/L)	6.6 ± 0.9	7.0 ± 1.4	0.735	33.8 ± 3.8	48.9 ± 4.8	0.018*
HIR ( $\mu\text{mol/kgFFM}/\text{min}/\text{pmol} \times \text{L}$ )	847.9 ± 113.9	847.1 ± 151.1	0.499	1819.6 ± 277.4	2379.7 ± 246.5	0.063
MCR <sub>1</sub> (ml/m <sup>2</sup> /min)	-	-	-	0.11 ± 0.02	0.06 ± 0.01	0.018*
Glucose $R_d$ per unit serum insulin ( $\mu\text{mol/kgFFM}/\text{min}/\text{mU} \times \text{L}$ )	3.3 ± 0.7	3.7 ± 1.2	0.612	1.4 ± 0.1	1.1 ± 0.2	0.063
M per unit serum insulin ( $\mu\text{mol/kgFFM}/\text{min}/\text{mU} \times \text{L}$ )	-	-	-	1.2 ± 0.1	0.9 ± 0.2	0.091
EGP per unit serum insulin ( $\mu\text{mol/kgFFM}/\text{min}/\text{mU} \times \text{L}$ )	3.3 ± 0.7	3.7 ± 1.2	0.612	0.24 ± 0.03	0.16 ± 0.03	0.028*
Glycerol ( $\mu\text{mol/L}$ )	47.3 ± 3.7	62.4 ± 5.3	0.043*	17.8 ± 1.6	14.6 ± 1.9	0.063
Glycerol $R_a$ ( $\mu\text{mol/kgFM}/\text{min}$ )	4.9 ± 0.4	6.5 ± 0.6	0.018*	1.9 ± 0.2	1.4 ± 0.2	0.018*
Glycerol $R_a$ per unit serum insulin ( $\mu\text{mol/kgFM}/\text{min}$ )	$8.6 \times 10^{-1} \pm 1.6 \times 10^{-1}$	1.2 ± 0.3	0.063	$6.2 \times 10^{-2} \pm 8.8 \times 10^{-3}$	$2.9 \times 10^{-2} \pm 2.8 \times 10^{-3}$	0.018*

Data are means ± SEM. Intergroup differences were assessed by the nonparametric Wilcoxon signed ranks test, \*  $P < 0.05$ . Abbreviations: EGP = endogenous glucose production; FFM = fat free mass; FM = fat mass; HIR = hepatic insulin resistance; M = glucose metabolized; MCR<sub>1</sub> = metabolic clearance rate of insulin;  $R_d$  = rate of disappearance. During basal steady state, glucose  $R_d$  equals EGP. During steady state conditions the rates of appearance ( $R_a$ ) of glucose and glycerol is equal to their respective rates of disappearance ( $R_d$ ).

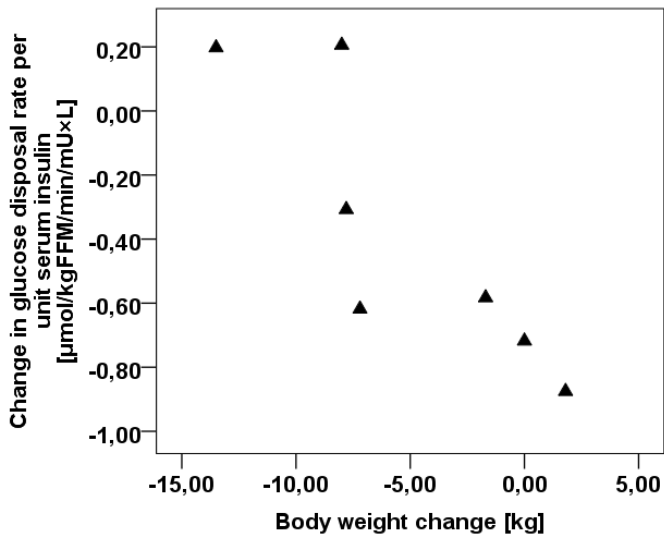


Figure 6.1 Correlations in body weight change and glucose disposal rate.

## DISCUSSION

The aim of this study was to accurately assess both glucose and fat metabolism in narcolepsy patients, as well as the metabolic effects of SXB treatment using the most accurate method available, i.e. a combination of a hyperinsulinemic-euglycemic clamp and stable isotope techniques. Surprisingly, we found increased peripheral insulin sensitivity in narcolepsy patients, whereas hepatic insulin sensitivity and  $\beta$ -cell function were not different from matched healthy controls. The higher insulin sensitivity in narcolepsy patients was reflected by a higher rate of insulin-mediated glucose uptake in peripheral tissues, of which skeletal muscle is the most important. Lipolysis tended to be lower in narcolepsy patients, which could be due to insulin sensitivity of adipose tissue. This finding may at least partly account for comorbid overweight in narcolepsy, which is seen in two thirds of the patients.<sup>18</sup> Also the higher insulin sensitivity itself might contribute.

Furthermore, we found that SXB treatment increased hepatic but tended to decrease whole body insulin sensitivity. Moreover, SXB stimulated lipolysis, which might be one of the reasons why patients lose weight while on this drug. In our study, patients lost on average 5.2 kg of weight after 3 months of SXB treatment which is in line with an earlier report that SXB treatment is associated with weight loss.<sup>74</sup> The mechanisms through which hypocretin-

deficiency and SXB treatment affect glucose and fat metabolism might involve modulation of autonomic nervous system activity, which recently has been demonstrated to be critically implicated in the regulation of energy homeostasis.<sup>214</sup> SXB appears to hamper insulin mediated glucose disposal in patients who maintain a stable body weight during treatment, but it simultaneously induces weight loss in other patients, which clearly compensates for the reduction in insulin sensitivity (Figure 6.1). On average, glucose disposal per unit of circulating insulin was reduced by SXB (Table 6.3). The impact of SXB on insulin action appears to be tissue specific: while glucose disposal is diminished, the capacity of insulin to suppress glucose production is reinforced by SXB (Table 6.3). The mechanism underlying the effects of SXB is unknown. As glucose disposal rate is primarily determined by glucose uptake in muscle tissue, and given the fact that the capacity of insulin to suppress lipolysis was not affected by SXB treatment, our findings suggest that the systemic effects of SXB on insulin sensitivity are chiefly mediated by changes in muscle, rather than fat, insulin sensitivity.

Since peripheral insulin sensitivity is increased and pancreatic  $\beta$ -cell function is normal in narcolepsy patients, our data do not support the notion that the risk of T2DM is increased in narcolepsy, which is in contrast to some earlier reports.<sup>22;39;41;95</sup> We found no differences in pancreatic  $\beta$ -cell function, which is in line with previous observations.<sup>42;96</sup> However, increased insulin sensitivity has not been previously reported in narcolepsy. Several explanations might account for these differences. Firstly, to our knowledge we are the first to apply the hyperinsulinemic-euglycemic clamp to assess insulin sensitivity, whereas previous studies used indirect and derived measures of insulin sensitivity such as the homeostasis model assessment and minimal model analysis.<sup>96</sup> Although the method used by Beitinger et al. highly correlates with the clamp technique, it is not the same and less accurate.<sup>215</sup> Secondly, in both earlier studies patients continued to be on their usual medication which might have confounded their results. For example, antidepressants, which are used as antiepileptic drugs, may induce insulin resistance.<sup>216</sup> Thirdly, all our patients were proven to be hypocretin deficient and HLA-DQB1\*06:02 positive which makes our group more homogeneous than the ones in earlier reports. There is a growing body of evidence that hypocretin plays a fundamental role in glucose metabolism.<sup>200;214;217</sup> However, the precise role of hypocretin in systemic glucose homeostasis is likely to be complex and seems even paradoxical since it can have both hyperglycemic<sup>202</sup> as well as hypoglycemic effects.<sup>214</sup> This dual effect of hypocretin in glucose homeostasis is probably related to its decisive role in the control of sleep/ wake and circadian rhythms, i.e., at awakening hypocretin not only stimulates arousal but also glucose production as well as glucose uptake.<sup>218</sup>

One limitation of this study is the relatively small number of subjects. However, this limitation is partly offset by the application of very sensitive techniques, which would have been unfeasible in larger groups of subjects due to the expensive and arduous nature of the experiments.

In conclusion, our findings show that narcolepsy patients are more insulin sensitive and tend to have a lower rate of lipolysis than weight matched controls. SXB stimulated lipolysis in narcolepsy patients, possibly accounting for the observed weight loss after treatment. While SXB had a tendency to decrease systemic insulin sensitivity, it increased hepatic insulin sensitivity, suggesting tissue-specific effects.

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