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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. As selective disruption of slow-wave sleep (SWS) during subsequent nights decrease glucose tolerance by ~23% in healthy subjects and previous literature showed the clear restorative role of SWS in glucose metabolism, 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. During SWS, changes occur in endocrine, metabolic and brain activity. The autonomic nervous system switches from sympathetic dominance during REM sleep to parasympathetic dominance in SWS sleep. 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. 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, ß-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. 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. 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. A primed [17.6 μmol.kg-1] continuous [0.22 μmol.kg-1.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 [6,6-2H2] 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. Briefly, this consisted of a primed (80 mU.m⁻².min⁻¹ for 5 minutes and subsequently 40 mU.m⁻².min⁻¹ for 5 min), followed by continuous (20 mU.m⁻².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% [6,6-2H2] 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 [6,6-2H2] 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 chemiluminescentimmunometric assay (Immulite 2500, Siemens, Germany) with an intra-assay coefficient of variation (CV) of 4%. Enrichment of plasma [6,6-2H2]glucose was determined in a single analytical run, using gas chromatography coupled to mass spectrometry, as described previously. 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 (Ra) and disappearance (Rd) 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 Ra of glucose, whereas EGP 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 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 from 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).

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

<table>
<thead>
<tr>
<th>Sleep stage</th>
<th>HR (beats/min)</th>
<th>LF/HF ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal sleep</td>
<td>SWS</td>
</tr>
<tr>
<td>Wake</td>
<td>62 ± 1.1</td>
<td>67 ± 1.2</td>
</tr>
<tr>
<td>Stage 1</td>
<td>58 ± 0.9</td>
<td>57± 0.9</td>
</tr>
<tr>
<td>Stage 2</td>
<td>55 ± 0.8</td>
<td>56±0.9</td>
</tr>
<tr>
<td>Stage 3(SWS)</td>
<td>55 ± 0.8</td>
<td>57± 0.9</td>
</tr>
<tr>
<td>REM</td>
<td>57 ±0.9</td>
<td>59±1.0</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SEM. HR, heart rate; LF, low frequency; HF, high frequency; SWS, slow wave sleep; NS, non-significant.
Table 2. Effects of SWS suppression on basal and clamp metabolic parameters in 10 healthy controls

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

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.\(^{(20)}\) 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.\(^{2,22}\) 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.
REFERENCE LIST


