Partial sleep restriction decreases insulin sensitivity in type 1 diabetes

Esther Donga¹, MD, Marieke van Dijk¹, MD, J Gert van Dijk², MD, PhD, Nienke R Biermasz¹, MD, PhD, Gert-Jan Lammers², MD, PhD, Klaas van Kralingen³, MD, PhD, Roel PLM Hoogma⁴, MD, PhD, Eleonora PM Corssmit¹, MD, PhD, Johannes A Romijn¹, MD, PhD

¹Depts of Endocrinology and Metabolic Diseases, ²Neurology and ³Pulmonology, Leiden University Medical Centre, Leiden, The Netherlands. and ⁴Groene Hart Ziekenhuis, Gouda, The Netherlands

Corresponding author:
E. Donga, MD
E-mail: e.donga@lumc.nl

Submitted 18 December 2009 and accepted 13 March 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org
Objective: Sleep restriction results in decreased insulin sensitivity and glucose tolerance in healthy subjects. We hypothesized that sleep duration is also a determinant of insulin sensitivity in patients with type 1 diabetes.

Research design and methods: We studied 7 patients (3 men, 4 women) with type 1 diabetes: mean age 44±7 yr, body mass index (BMI) 23.5±0.9 kg/m² and HbA1c 7.6±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 (NEFA) or endogenous glucose production. Sleep restriction did not alter endogenous glucose production during the hyperinsulinemic clamp compared to the unrestricted night (6.2±0.8 vs. 6.9±0.6 μmol.kgLBM⁻¹.min⁻¹, NS). In contrast, sleep restriction decreased the glucose disposal rate during the clamp (25.5±2.6 vs. 22.0±2.1 μmol.kgLBM⁻¹.min⁻¹, p=0.04), reflecting decreased peripheral insulin sensitivity. Accordingly, sleep restriction decreased the rate of glucose infusion by ~21% (p=0.04). Sleep restriction did not alter plasma NEFA levels during the clamp (143±29 vs. 133±29 μmol/L, NS).

Conclusions: Partial sleep deprivation during a single night induces peripheral insulin resistance in these 7 patients with type 1 diabetes. Therefore, sleep duration is a determinant of insulin sensitivity in patients with type 1 diabetes.
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, 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 are unable to compensate for these physiological variations, other than by adaptations of the dose of exogenous insulin. Normal glucose homeostasis shows a diurnal pattern with variations in glucose tolerance, in which sleep characteristics play a key role (4). In this respect, sleep duration may be particularly relevant, since sleep curtailment is a common aspect of our modern 24 hour 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 hypothesized 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, 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 9 patients (5 men and 4 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 glutamic acid decarboxylase 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. Their weight had been stable for at least three months prior to participation in this study. HbA1c levels had been below 8.5 % during the year prior to the start of the study. Exclusion criteria were a body mass index (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 rate was below 30 mg/24 h.

The 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. 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, UK) 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 hours in bed from 23:00 h
to 07:30 h on all 3 occasions. Subjects fasted throughout these nights from 22:00 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 01:00 h to 05:00 h AM. They were allowed to read or watch movies in an upward position, and their wakefulness was monitored and assured if necessary.

Polysomnography (PSG). Sleep was visually scored for each of the 3 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 PSG recorder (Titanium, Embla Systems, Inc, Broomfield, USA). Sleep and wake stages were visually scored in consecutive epochs of 30 seconds, resulting in a list of epochs spent in wake, stage I (drowsiness), stages II, III and rapid eye movement (REM) dream sleep. 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 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 [17.6μmol.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 hrs 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 and concentrations of glucose and plasma non-esterified fatty acids (NEFA). 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 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 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/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, 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 (Immuliite 2500, Siemens, Germany) with an intra-assay coefficient of variation (CV) of 4%. NEFA levels 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 (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 minutes of the basal period and the final 30 minutes 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. 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 between the effects of the night of normal sleep duration and the night of partial 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, USA). Significance was accepted at p<0.05.

**RESULTS**

**Clinical characteristics.** The results of 2 of the 9 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 7 patients (3 men) for analysis. Mean age of these 7 subjects was 44.3 ± 6.6 yr, mean weight 72.0 ± 4.0 kg, mean height 175 ± 3 cm and mean body mass index (BMI) 23.5 ± 0.9 kg/m². Mean HbA1c of the patients was 7.6 ± 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).

**The effects of partial sleep restriction on polysomnographic parameters (Table 1).** Sleep duration was considerably shorter in the night with partial sleep restriction, compared to the night with normal sleep duration (p=0.02). 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) than in the other night. The proportions of time spent in other stages did not differ between both conditions.

**The effects of partial sleep restriction on basal metabolic parameters (Table 1).** The mean overnight rate of subcutaneous infusion of insulin was 0.7 IE/h and was identical in both conditions. Compared to 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 endogenous
glucose production assessed by primed, continuous infusion of [6,6-^{2}H_{2} glucose]. 
The effects of partial sleep restriction on metabolic parameters during hyperinsulinemic euglycemic clamp conditions (Table 1 and Fig 1). Steady state glucose and insulin levels did not differ between the two clamp studies. Sleep restriction did not affect endogenous glucose production 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.

DISCUSSION
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 the data of only 7 patients with type 1 diabetes mellitus. The strictly controlled design of this pathophysiological study in combination with the fact that each subject served as his/her own control enabled 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 like gender, age, 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 a static but varies in 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 mellitus (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 a change in total sleep duration, decreased glucose tolerance in healthy subjects (23). The differential effects of altered sleep composition versus decreased total sleep duration on insulin sensitivity awaits further study.

Data on sleep physiology and sleep disturbances in patients with type 1 diabetes are rare. Jauch-Chara et al reported alterations in neuroendocrine sleep architecture and a trend towards less slow wave sleep in 14 patients with type 1 diabetes (24). 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.

ACKNOWLEDGMENTS
This study was supported by the European Foundation for the Study of Diabetes (EFSD) and the Dutch Diabetes Research Foundation. We thank all patients for participating in this study. We also thank Trea Streefland and all sleep technicians for their help. There are no conflicts of interest relevant to this article.
REFERENCES

15. Nedeltcheva AV, Kessler L, Imperial J, Penev PD. Exposure to recurrent sleep restriction in the setting of high caloric intake and physical inactivity results in increased insulin resistance and reduced glucose tolerance. J Clin Endocrinol Metab 2009;94:3242-3250
Table 1: The effects of a night of normal sleep duration *versus* a night of sleep duration 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.

<table>
<thead>
<tr>
<th>Sleep parameters</th>
<th>Normal sleep</th>
<th>Partial sleep deprivation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time (TST) (min)</td>
<td>469 ± 8.5</td>
<td>222 ± 7.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Stage 1 (% of TST)</td>
<td>10.6 ± 1.0</td>
<td>10.2 ± 2.1</td>
<td>0.49</td>
</tr>
<tr>
<td>Stage 2 (% of TST)</td>
<td>44.4 ± 2.8</td>
<td>40.4 ± 2.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Stage 3 (% of TST)</td>
<td>22.7 ± 3.4</td>
<td>35.3 ± 4.4</td>
<td>0.02</td>
</tr>
<tr>
<td>REM sleep (% of TST)</td>
<td>22.2 ± 1.7</td>
<td>13.7 ± 2.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Wake time during sleep (% of total sleep period)</td>
<td>4.2 ± 0.7</td>
<td>5.4 ± 2.3</td>
<td>0.87</td>
</tr>
</tbody>
</table>

**Basal values**

<table>
<thead>
<tr>
<th></th>
<th>Normal sleep</th>
<th>Partial sleep deprivation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>7.2 ± 1.0</td>
<td>6.7 ± 1.4</td>
<td>0.87</td>
</tr>
<tr>
<td>NEFA (umol/L)</td>
<td>673 ± 125</td>
<td>591 ± 99</td>
<td>0.35</td>
</tr>
<tr>
<td>Endogenous glucose production (μmol.kgLBM⁻¹.min⁻¹)</td>
<td>17.6 ± 0.9</td>
<td>16.3 ± 1.0</td>
<td>0.31</td>
</tr>
</tbody>
</table>

**Hyperinsulinemic euglycemic clamp study**

<table>
<thead>
<tr>
<th></th>
<th>Normal sleep</th>
<th>Partial sleep deprivation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1 ± 0.2</td>
<td>5.2 ± 0.1</td>
<td>0.23</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>24.0 ± 2.2</td>
<td>24.9 ± 1.5</td>
<td>0.40</td>
</tr>
<tr>
<td>NEFA (umol/L)</td>
<td>143 ± 29</td>
<td>133 ± 29</td>
<td>0.69</td>
</tr>
<tr>
<td>Endogenous glucose production (μmol.kgLBM⁻¹.min⁻¹)</td>
<td>6.2 ± 0.8</td>
<td>6.9 ± 0.6</td>
<td>0.74</td>
</tr>
<tr>
<td>Glucose Rd (μmol.kgLBM⁻¹.min⁻¹)</td>
<td>25.5 ± 2.6</td>
<td>22.0 ± 2.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose infusion rate (μmol.kgLBM⁻¹.min⁻¹)</td>
<td>19.0 ± 2.9</td>
<td>14.9 ± 2.1</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM.

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 patients with type 1 diabetes mellitus (n=7). Black horizontal lines represent the mean of the values of 7 subjects.
Partial sleep restriction in type 1 diabetes

A. P = 0.69

Endogenous glucose production (EGP) (μmol/kg LBM/min)

B. P = 0.74

NEFA (mmol/L)

C. P = 0.04

Glucose Disposal rate (GDR) (μmol/kg LBM/min)

D. P = 0.04

Glucose infusion rate (μmol/kg LBM/min)

Normal sleep Partial sleep deprivation