Timing Modulates the Effect of Sleep Loss on Glucose Homeostasis

Britta Wilms,[1,2,3] Rodrigo Chamorro,[1,2,4] Manfred Hallschmid,[3,5,6] Denisa Trost,[1] Nelli Forck,[1] Bernd Schultes,[7] Matthias Mölle,[2] Friedhelm Sayk,[8] Hendrik Lehnert,[1,2,3] and Sebastian M. Schmid[1,2,3]

[1]Department of Internal Medicine I, University of Lübeck, 23538 Lübeck, Germany; [2]Center of Brain, Behavior & Metabolism, University of Lübeck, 23562 Lübeck, Germany; [3]German Center for Diabetes Research, 85764 Munich-Neuherberg, Germany; [4]Department of Nutrition, University of Chile, 8380453 Santiago, Chile; [5]Department of Medical Psychology and Behavioral Neurobiology, University of Tübingen, 72076 Tübingen, Germany; [6]Institute for Diabetes Research and Metabolic Diseases, Helmholtz Centre Munich, University of Tübingen, 72076 Tübingen, Germany; [7]eSwiss Medical & Surgical Center, 9016 St. Gallen, Switzerland; and [8]Sana Hospital, 23560 Lübeck, Germany

ORCiD numbers: 0000-0003-3178-0567 (B. Wilms).

Context: Chronobiological factors may modulate the impact of sleep loss on glucose homeostasis. However, these interactions have not been systematically assessed in humans.

Objective: To assess the effect of sleep loss during the late vs early night on glucose homeostasis.

Design: Fifteen normal-weight men participated in three conditions of a randomized, balanced crossover study comprising two conditions with shortened sleep (*i.e.,* 4 hours of sleep during the first or the second half of the night) and a control condition with 8 hours of sleep. Glucose, insulin, cortisol, and glucagon were measured. Insulin sensitivity and secretion were assessed with a Botnia clamp.

Results: Compared with regular sleep duration, sleep loss reduced insulin sensitivity (M-value; $P = 0.031$) irrespective of early- or late-night timing ($P = 0.691$). The disposition index (*i.e.,* the β-cell response adjusted for insulin sensitivity) also tended to be impaired by short sleep ($P = 0.056$) but not by sleep timing ($P = 0.543$). In contrast, sleep loss in the second half but not the first half of the night induced reductions in morning glucagon and cortisol levels ($P < 0.031$) followed by a transient increase in cortisol ($P < 0.044$).

Conclusions: Although sleep deprivation acutely reduced insulin sensitivity irrespective of its nocturnal timing, sleep loss in the early morning compromised α-cell and hypothalamic-pituitary-adrenal axis activity to a greater extent than sleep loss in the first half of the night. This pattern suggests that the timing of sleep restriction can partly potentiate its deleterious metabolic effects. **(*J Clin Endocrinol Metab* 104: 2801–2808, 2019)**

Short sleep duration and disrupted sleep patterns have become common in modern societies (1). In parallel, obesity and metabolic comorbidities such as type 2 diabetes mellitus (T2DM) have risen on a worldwide scale (2). Large epidemiological studies show that impaired quantity and quality of sleep are associated with adverse metabolic conditions such as obesity and impaired glucose tolerance [for review see (3)]. A meta-analysis including more than 600,000 adults and 30,000 children clearly revealed a strong association between reduced

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Abbreviations: DI, disposition index; FPIR, first-phase insulin response; HEC, hyperinsulinemic-euglycemic clamp; HPA, hypothalamic-pituitary-adrenal; ivGTT, intravenous glucose tolerance test; REM, rapid eye movement; SWS, slow-wave sleep; T2DM, type 2 diabetes mellitus; TST, total sleep time.

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sleep duration (≤5 hours) and obesity (4). Longitudinal analyses of large population surveys further indicate that both long (~9 hours) and short (~5 hours) sleep durations elevate the risk for development of T2DM (5). However, laboratory studies on the impact of insufficient sleep on glucose metabolism are less unequivocal. Although there is solid evidence that sleep curtailment is associated with reduced peripheral insulin sensitivity of muscles and liver (6–8), results regarding islet function (*i.e.*, first-phase insulin response, basal insulin secretion, and α-cell function) are inconsistent. Some studies have shown impaired islet function (9), whereas others have reported unchanged (6) or even enhanced secretory function (10) after two nights of sleep restricted to 4 hours.

The mechanisms that might mediate detrimental effects of short sleep on energy balance and, in the long run, might favor obesity and T2DM are under intense investigation. Pathophysiological factors such as altered activity of neuroendocrine pathways [*e.g.*, the hypothalamic-pituitary-adrenal (HPA) axis (6, 9, 11)], elevated levels of free fatty acids (10), and increased sympathetic nerve activity (6, 9, 11) all appear to contribute to impaired glucose tolerance and positive energy balance after impaired sleep. First evidence exists that chronobiological aspects of sleep restriction affect metabolic conditions (12), but still little is known about whether the timing of sleep loss—in addition to sleep's shortened duration—affects glucose homeostasis. This question was addressed in the present experiment. We subjected healthy young men to three conditions: short sleep (4 hours) during the first half of the night, short sleep (4 hours) during the second half of the night, and regular sleep of 8 hours. We hypothesized that not only sleep curtailment *per se* but also timing of sleep loss and, in particular, waking up early after short sleep aggravates the adverse effects of sleep loss on glucose metabolism.

Material and Methods

Participants

Fifteen healthy normal-weight males aged 20 to 30 years (mean ± SEM: 24.6 ± 0.7 years) and with a body mass index between 20.0 and 24.9 kg/m^2 (23.3 ± 0.4 kg/m^2) were enrolled in this study. Exclusion criteria were chronic or acute illness, current medication of any kind, smoking, elevated alcohol (>50 g per day) and caffeine (>300 mg per day) consumption, use of prescription drugs, diabetes in first-degree relatives, shift work, travel across time zones during the past 4 weeks, and short habitual sleep duration (<6 hours per day). Furthermore, abnormal findings on physical examination or routine laboratory testing (complete blood counts, comprehensive metabolic panel, thyroid function, lipid composition) were also exclusion criteria. Physical activity, sleep behavior, nutrition, and subjective feelings during the day before experimental visits were assessed by a structured interview at each in-laboratory visit to ensure that participants adhered to the above-listed criteria. The study protocol was approved by the ethics committee on research involving humans at the University of Lübeck, and all participants gave written informed consent before participation.

Study design and procedure

Participants underwent an adaptation night in the laboratory that included a standard overnight polysomnographic recording to exclude sleep disorders and for familiarization with the experimental setting. Participants were tested in a randomized, counterbalanced, crossover design on three conditions spaced at least 3 weeks apart. In the week preceding the experimental nights, subjects were asked to maintain a regular sleep schedule with a minimum of 7 hours of sleep per night. Glucose homeostasis was assessed by the Botnia clamp technique (13) on the next morning after (i) one night with 4 hours of sleep during the first half of the night ("late-night sleep loss"; bedtime: 2230 to 0300 hours), (ii) one night with 4 hours of sleep during the second half of the night ("early-night sleep loss"; bedtime: 0215 to 0645 hours), and (iii) one night with regular 8 hours of sleep ("regular sleep"; bedtime: 2215 to 0645 hours).

Participants attended the research unit at 1915 hours for each laboratory night. An IV catheter was inserted into a vein of the participant's nondominant distal forearm. After a standardized light dinner (380 kcal) at 2015 hours, participants were prepared for polysomnographic recording. Thereafter, they were allowed to drink only water (max. 250 mL) until the next morning. Participants went to bed and lights were turned off at 2215 and 2230 hours, respectively, in the regular sleep and late-night sleep loss conditions, whereas they remained awake in a sitting position until 0300 hours in the early-night sleep loss condition. During sleep restriction conditions, participants were allowed to read and to watch nonarousing movies while being monitored constantly by the experimenters. Light intensity in the laboratory was 300 lux.

In the morning after each experimental condition, a second IV catheter was inserted into a vein of the subject's contralateral distal forearm, and a Botnia clamp was performed starting at 0800 hours (13). In brief, the Botnia clamp combines a frequently sampled IV glucose tolerance test (ivGTT) over 60 minutes (*i.e.*, 0800 to 0900 hours) with a hyperinsulinemic-euglycemic clamp (HEC) during the following 120 minutes (*i.e.*, 0900 to 1100 hours) to test for β-cell capacity and peripheral insulin sensitivity. For the ivGTT, a glucose bolus of 0.3 g/kg of body weight of a 20% glucose solution was injected IV directly before time point 0, and subsequent blood samples were collected at 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 minutes after the glucose load. At minute 60, the HEC was started with continuous fixed infusion rates (1.0 IU/kg of body weight) of short-acting human insulin (Insuman Rapid 40 I.E.; Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany) for 120 minutes. At the same time, plasma glucose concentration was kept stable at 5.5 mmol/L via adjustable infusion of 20% glucose solution. The glucose infusion rate was calculated from dynamics of measured (2- to 3-minute intervals throughout the clamp) plasma glucose by clamp software (EKF Diagnostics GmbH, Barleben, Germany). Approximately 60 minutes after starting the HEC, hepatic glucose production was halted and

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blood glucose reached a stable euglycemic plateau, which was maintained over an additional 60 minutes ("euglycemic steady state") until the end of the clamp.

Sleep recordings

For standard polysomnography, electrodes were attached to the scalp (C3, C4) for electroencephalographic recordings above, below, and beside the eyes for horizontal and vertical electrooculograms, and two electrodes were placed on the chin for electromyogram signals. Recordings were performed using a Nihon Kohden amplifier (EEG 4400 series; Nihon Kohden, Rosbach, Germany). Data were scored offline according to standard criteria (14). Because of technical issues, a full sleep data set was available in nine participants.

The following sleep parameters were determined: total sleep time (TST), time spent in non–rapid eye movement (REM) sleep stages 1, 2, 3, and 4 and in slow-wave sleep (SWS; *i.e.*, stages 3 and 4 combined), and REM sleep (all as absolute duration and as percentage of TST).

Blood samples and assays

Blood glucose level was determined immediately by EKF Diagnostics' Biosen C-Line (EKF Diagnostics GmbH, Barleben, Germany). The within-assay variation according to the manufacturer was below 1.5% (mean: 12 mmol/L). Concentrations of insulin and cortisol were assessed by immunoassays (Immulite 2000; Siemens Healthcare Diagnostics, UK) with detection limits and average within-assay coefficients of variation of 2.0 μUI/mL and ≤5.3% (insulin) and 5.5 nmol/L and ≤7.4% (cortisol). Glucagon concentrations were measured by RIAs (Glucagon RIA; IBL International, Hamburg, Germany) with a within-assay coefficient of variation of <2.5%. All values were determined from stored (−80°C) serum or plasma samples, respectively. Based on the results of the Botnia clamp data, the first-phase insulin response (FPIR) was calculated as the sum of insulin concentrations 2, 4, and 6 minutes after an IV glucose load of mIU/10 min (13). Insulin sensitivity was defined as the ratio of glucose infusion rate and steady-state mean insulin concentrations during the last 60 minutes of the clamp, and the M-value [in mg/(kg·min)] was calculated in addition. Higher M-values reflect better insulin sensitivity and *vice versa*. The disposition index (DI) as a measure of insulin secretion adjusted for insulin sensitivity was calculated as the product of the FPIR and the M-value. The homeostatic model assessment index was calculated with fasting values (15).

Statistical analyses

SPSS 22 (IBM Inc., Chicago, IL) for Mac was used for all analyses, and values are expressed as mean ± SEM. Analyses of sleep data were based on one-way ANOVA, including the factor "condition" (for late-night sleep loss vs early-night sleep loss vs regular sleep). Analyses of hormonal and neuropsychological data were based on ANOVA for repeated measures, including the factors condition (see preceding text) and "time" (for repeated measurements during the experiment). Helmert contrast tests were used to explore first- vs second-level differences (*e.g.*, "regular sleep" vs "short sleep"). For pairwise comparisons of single time points, the Student t test was applied. In all analyses, a P-value <0.05 was considered significant.

Results

Sleep

Participants slept 426 ± 43 minutes in the regular sleep condition, whereas TST was reduced to 249 ± 18 minutes and 263 ± 10 minutes in the late-night sleep loss and early-night sleep loss conditions, respectively ($P = 0.041$; Table 1). There was no statistical difference in TST between the two short sleep conditions ($P = 0.771$). The longer sleep duration in the regular sleep condition was primarily due to longer absolute and relative durations of REM sleep (both $P \leq 0.028$), whereas absolute and relative durations of SWS as well as shallow non-REM sleep stages 1 and 2 did not differ between all conditions (all $P \geq 0.134$).

Timing-independent effects

Morning fasting concentrations of glucose and insulin were not different between regular sleep, late-night sleep loss, and early-night sleep loss (glucose: 4.2 ± 0.1 vs

Table 1. Sleep Parameters

	Regular Sleep	Late-Night Sleep Loss	Early-Night Sleep Loss	*P* Value
TST, min	425.7 ± 43.6	249.4 ± 18.6	263.3 ± 10.4	0.041
Wake, min	3.9 ± 3.3	0.2 ± 0.2	0.3 ± 0.3	0.374
Wake, %	0.7 ± 0.6	0.08 ± 0.08	0.1 ± 0.1	0.374
S1 sleep, min	24.7 ± 8.3	12.2 ± 6.8	12.5 ± 3.4	0.470
S1 sleep, %	5.6 ± 2.1	4.9 ± 2.8	4.7 ± 1.2	0.717
S2 sleep, min	232.2 ± 44.0	114.0 ± 5.0	118.8 ± 7.1	0.134
S2 sleep, %	50.9 ± 18.1	46.6 ± 3.8	45.1 ± 2.1	0.782
SWS, min	78.1 ± 13.1	74.6 ± 16.2	88.6 ± 7.1	0.930
SWS, %	21.2 ± 6.0	35.4 ± 3.6	33.3 ± 1.9	0.390
REM sleep, min	80.6 ± 15.3	31.5 ± 8.2	38.9 ± 6.3	0.006
REM sleep, %	19.2 ± 3.1	11.9 ± 2.8	15.0 ± 2.4	0.028
MA, min	6.2 ± 1.1	2.1 ± 0.5	4.2 ± 1.0	0.043
MA, %	1.4 ± 0.1	0.8 ± 0.1	1.6 ± 0.4	0.370

Data are presented as mean ± SEM.

Abbreviations: MA, movement arousal; S1, stage 1; S2, stage 2; REM, rapid eye movement; TST, total sleep time; SWS, slow-wave sleep.

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4.2 ± 0.2 vs 4.2 ± 0.1 mmol/L, $P = 0.981$; insulin: 4.9 ± 0.7 vs 4.4 ± 0.6 vs 3.6 ± 0.6 μIU/mL, respectively, $P = 0.201$).

Glucose concentrations during the ivGTT were markedly affected by sleep restriction ($P = 0.016$ for the main effect of condition). Thus, blood glucose concentrations after the glucose load decreased later in the early-night sleep loss condition compared with the regular sleep condition (all $P < 0.01$ for pairwise comparisons from t = 30 minutes until t = 60 minutes after glucose load), whereas glucose concentrations after late-night sleep loss were intermediate (Fig. 1A). During the subsequent HEC procedure, glucose concentrations remained stable ($P = 0.126$ for the main effect of time) without any difference between conditions ($P = 0.809$ for the main effect of condition) as intended by study protocol.

Short sleep duration compared with regular sleep duration tended to dampen FPIR after glucose load ($P = 0.089$ for ANOVA Helmert contrast of regular sleep vs short sleep) (Fig. 1B). Of note, the reduction in FPIR seemed to be more pronounced after late-night sleep loss (*i.e.,* 18.5%; $P = 0.053$ for pairwise comparison with regular sleep) than after early-night sleep loss (*i.e.,* 9%; $P = 0.286$ for pairwise comparison). The amount of glucose needed to maintain euglycemia during the hyperinsulinemic clamp procedure was significantly decreased to the same extent after both short sleep conditions compared with the regular sleep condition ($P = 0.010$ for ANOVA Helmert contrast of regular sleep vs short sleep; $P = 0.580$ for pairwise comparison of early-night sleep loss vs late-night sleep loss) (Fig. 1C). In accordance, insulin sensitivity as displayed by M-values was markedly reduced—again to same extent—after both late-night sleep loss and early-night sleep loss compared with regular sleep (7.0 ± 0.7 mg/(kg·min) vs 6.8 ± 2.0 mg/(kg·min) vs 8.0 ± 0.9 mg/(kg·min), respectively; $P = 0.031$ for ANOVA Helmert contrast of

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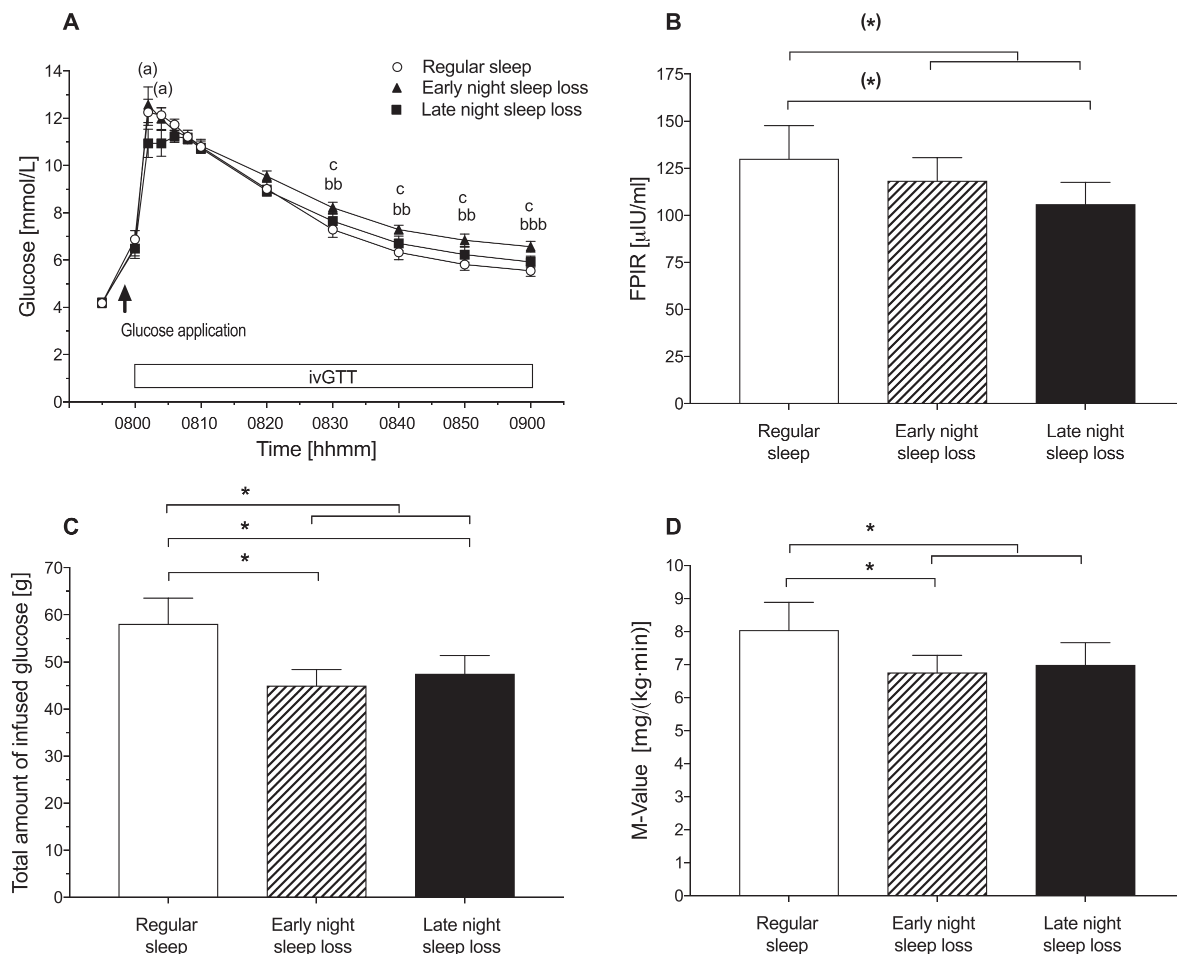

Figure 1. Glucose and insulin homeostasis after sleep restriction. (A) Glucose concentrations during the ivGTT are shown. [(a)]$0.05 < P < 0.01$ for regular vs late-night sleep loss; $^{bb}P < 0.01$; $^{bbb}P < 0.001$ for regular sleep vs early-night sleep loss; $^{c}P < 0.05$ for late-night sleep loss vs early-night sleep loss. (B) FPIR during the ivGTT is shown. $*0.05 < P < 0.1$. (C) Total amount of glucose infused during the hyperinsulinemic clamp to maintain euglycemia is shown. (D) M-values after regular sleep, early-night sleep loss, and late-night sleep loss is shown. $*P < 0.05$. Error bars, SEM.

regular sleep vs short sleep; $P = 0.691$ for pairwise comparison of early-night sleep loss vs late-night sleep loss) (Fig. 1D). Analysis of DI showed a trend for impaired secretory β-cell response after both late-night sleep loss and early-night sleep loss compared with regular sleep ($P = 0.056$ for ANOVA Helmert contrast of regular sleep vs short sleep). There was likewise no difference between both conditions of reduced sleep duration ($P = 0.543$ for pairwise comparison).

Timing-dependent effects

Morning fasting concentrations of glucagon were slightly different between conditions ($P = 0.072$ for the ANOVA main effect of condition). Although glucagon concentrations did not differ after short sleep compared with regular sleep ($P = 0.326$ for Helmert contrast of short sleep vs regular sleep), glucagon concentration was significantly lower after late-night sleep loss than after early-night sleep loss and regular sleep ($P = 0.030$ for Helmert contrast of late-night sleep loss vs early-night sleep loss and regular sleep) (Fig. 2A). A similar pattern was found for morning cortisol concentrations ($P = 0.015$ for Helmert contrast of late-night sleep loss vs early-night sleep loss and regular sleep) (Fig. 2B).

Although starting from different baseline levels, glucagon and cortisol decreased in all conditions (both $P < 0.001$ for ANOVA main effect of time) to reach comparable concentrations at the end of the Botnia clamp at 1100 hours ($P > 0.373$ for all respective pairwise comparisons). Because of lower morning concentrations, the decrease in glucagon after late-night sleep loss was less steep than after early-night sleep loss and regular sleep (both $P < 0.020$ for ANOVA condition $\times$ time interaction). Cortisol levels during the complete Botnia clamp between 0800 and 1100 hours differed between conditions ($P = 0.044$ for ANOVA of condition $\times$ time interaction). There was a slight increase in cortisol levels upon starting the experimental procedure at 0800 hours after late-night sleep loss, reaching levels at the end of the ivGTT at 0900 hours that are similar to those observed during baseline after regular sleep and early-night sleep loss ($P = 0.942$).

Discussion

In this balanced crossover trial, we examined the effects of the timing of acute sleep loss on glucose homeostasis. Irrespective of its timing, sleep curtailment to 4 hours reduced insulin sensitivity as measured by lower glucose infusion rates during an HEC (*i.e.*, lower M-values). Furthermore, β-cell function as reflected by the DI tended to be impaired by short sleep in general. In contrast, the impact of sleep loss on basal α-cell glucagon secretion depended on its timing, in that sleep loss during the second half but not the first half of the night reduced glucagon concentrations. Likewise, an impairing influence on cortisol concentrations emerged only after sleep deprivation in the second half of the night.

The dramatic decrease in insulin sensitivity by ~16% after both short-sleep conditions is in line with previous studies (8, 11), indicating that the detrimental effects of sleep restriction on insulin sensitivity are not modulated by timing. Although findings on the impairing effect of sleep restriction on insulin sensitivity are relatively consistent (8, 11), data on β-cell capacity have been more inconsistent (6, 9, 10). β-cell capacity has been unchanged after an oral glucose load (6) or even increased

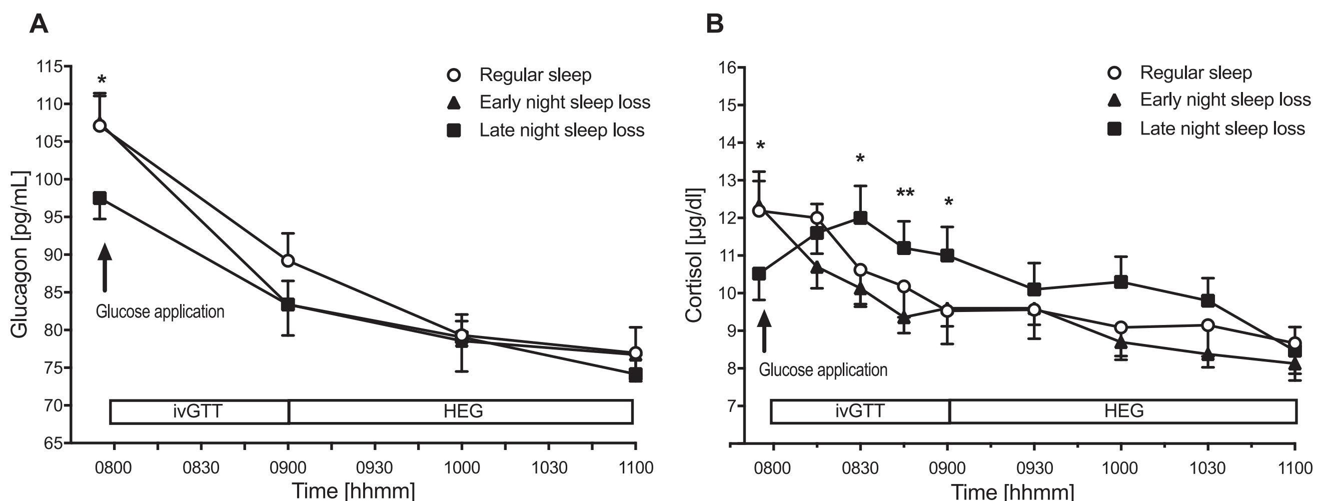

Figure 2. Glucagon and cortisol response after sleep restriction. (A) Glucagon (B) and cortisol concentration under fasting conditions (before glucose application) and during the ivGTT and hyperinsulinemic clamp after regular sleep (open circles), early-night sleep loss (black triangles), and late night sleep loss (black squares) are shown. *$P < 0.05$; **$P < 0.01$ for Helmert contrast of late-night sleep loss vs early-night sleep loss and regular sleep. HEC, hyperinsulinemic-euglycemic clamp.

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after moderate sleep restriction (10), whereas Spiegel *et al.* (9) reported on 30% lower β-cell capacity after total sleep deprivation. In the current study, β-cell capacity (*i.e.,* FPIR) was slightly lowered by short sleep, and there was a trend toward more pronounced effect after sleep deprivation in the late night. Therefore, an effect on β-cell capacity from prolonged wakefulness under fasting conditions cannot be completely ruled out.

As in our study, studies that reported no changes in β-cell capacity were designed with identical wake-up times in both control and short-sleep conditions (10, 16). However, a study showing impaired β-cell capacity included different wake-up times (0500 hours in the sleep-restriction condition vs 0900 hours in the recovery condition; *i.e.,* sleep restriction was accompanied by a prolonged awake wake time of 4 hours) (9). In contrast to our study, participants of the aforementioned study experienced sleep restriction over six consecutive nights, which may explain the more pronounced effect on β-cell capacity. However, our finding of unchanged fasting glucose and insulin concentrations is in line with previous data from our group (10) that likewise showed no changes after two nights with 4 hours of sleep each. In contrast, other studies showed that three nights with 4 hours of sleep caused an increase in insulin concentrations while glucose concentrations remained unchanged (16) and that 24 hours of total sleep deprivation impaired insulin secretion (17). Overall, a dose-response relationship between the "intensity" of sleep deprivation and impairment in β-cell activity can be assumed.

Of interest, we found no differences in peripheral insulin sensitivity after late-night sleep loss vs early-night sleep loss, indicating that sleep restriction *per se* impaired glucose tolerance independently of the timing of sleep loss. One explanation may be that the amount of SWS was identical in both conditions with restricted sleep duration. In a previous study (18), selective suppression of SWS during unchanged total sleep duration over three consecutive nights decreased insulin sensitivity. However, here we report comparable SWS in both short-sleep conditions as well as the regular sleep condition. Furthermore, it has been shown that two nights with only 4 hours of sleep and a comparable amount of SWS but reduced non–REM stage 2 and REM sleep dramatically impaired glucose tolerance and insulin sensitivity compared with regular sleep (10). Thus, not only SWS but also stage 2 sleep and total sleep duration may be essential for glucose homeostasis.

Reductions in α-cell activity, as measured by morning glucagon concentrations, depended to a greater extent on the timing of short sleep than on sleep duration *per se.* The morning plasma glucagon level was significantly lower when sleep restriction took place in the second half of the night than after early-night sleep loss or regular sleep. The impairing effect of sleep loss on glucagon concentration is in line with previous reports by our group (17, 19) and others (16). However, the respective interaction of sleep loss and timing identified in the present experiments allows the tempting assumption that impaired α-cell activity after sleep loss in the late night was due to prolonged preceding wakefulness rather than to the shortening of sleep time. Underlying mechanisms might involve specific inhibition of either α-cell functioning or innervation by the autonomic nervous system (20, 21), as autonomic nervous system activity is critically modulated by prolonged wakefulness.

Differential activity patterns of endogenous stress axes may have contributed to the observed results because increased stress axis activity induces distinct effects on β-cell activity, including reduced insulin secretion and decreased peripheral insulin sensitivity. Cortisol concentrations are highest after awakening and then decline continuously (22). In the current study, morning cortisol concentrations were markedly lower after late-night sleep loss than after early-night sleep loss and regular sleep, but they were relatively elevated during the ivGTT of the Botnia clamp. The sleep-wake cycle modulates the primarily circadian rhythm of cortisol release (23), so the observed reduction and subsequent increase in morning cortisol levels may be attributed to the timing of sleep restriction and moreover to differences in wake-up time. HPA axis activity physiologically peaks during awakening, presumably to prepare the organism for subsequent energy requirements of the waking state (24). The transient increase in cortisol during ivGTT after late-night sleep loss may reflect greater stress susceptibility to the experimental procedures, whereas stress axis activity after regular sleep and early-night sleep loss is already on a level to cope with respective stressors. However, during the subsequent HEC, cortisol concentrations were not different between conditions. Because insulin sensitivity was also decreased after early-night sleep loss, alterations in HPA axis activity are probably not a key mechanism. Although Spiegel *et al.* (9, 25) reported on dampened amplitude of 24-hour HPA axis rhythm (23) with increased cortisol in the evening after short sleep, impairment in glucose metabolism was already evident in the morning after sleep loss. As supported by earlier studies published by our group (10, 17, 19), this contradicts—at least in part—the hypothesis that alterations in glucose homoeostasis after short sleep are due to increased HPA axis activity.

GH, which was not measured in the current study, may be another factor linking sleep loss and impairments in glucose homeostasis. However, the impact of sleep deprivation on GH secretion appears to depend on the

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specific sleep restriction protocol. Prolonged GH pulses in the absence of effects on total secretion have been observed during four nights of sleep restriction to 0100 to 0530 hours (26), and acute sleep restriction to 2230 to 0300 hours did not alter morning GH concentrations (19). Mean 24-hour GH concentrations did not differ between bedtime schedules of 5.5 and 8.5 hours imposed over 14 days, although a subgroup of males displayed lower GH concentrations during the first 4 hours of bedtime when sleep restricted (6). Against this backdrop, the influence of (subtle) sleep loss–induced changes in GH secretion in response to the Botnia clamp should be clarified via further studies, which should also control for changes in melatonin arising from differences in the duration of light exposure (27, 28). In the current study, all subjects were exposed to light for at least 1 hour before the clamps so that differences in melatonin presumably could be abated before the start of the clamp protocol.

Several limitations need to be discussed. First, we assessed acute changes in glucose homeostasis after only one night of sleep restriction. Therefore, we cannot make conclusions about the effects of chronic sleep loss. However, 1 day of short sleep evoked detrimental effects on insulin sensitivity and glucose homeostasis, and it is tempting to speculate that short sleep for a longer period would result in even stronger effects. Second, in contrast to the early-night sleep loss condition, the late-night sleep loss condition implied 4 hours of additional wakefulness between awakening and the assessment of glucose homeostasis. Although this schedule allowed us to increase external validity by scheduling the glucoregulatory challenge in the morning hours in all conditions (rather than at night), future studies should control for any effects from additional preclamp wakefulness. Third, our study population comprised young, healthy, normal-weight men. Thus, results cannot be transferred to other groups (*e.g.*, women and subjects with obesity). However, epidemiological studies have shown similar associations between short sleep and glucose homeostasis in women and children over a wide range of body weights (29, 30).

Taken together, sleep loss *per se* impairs glucose homeostasis. However, the timing of sleep restriction can potentiate its deleterious endocrine effects, as α-cell and HPA axis activity are even more affected when waking up early after short sleep. These findings highlight the importance of sleep hygiene and appropriate recommendations in our 24-hour societies to prevent impairment of human glucose homeostasis.

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Correspondence and Reprint Requests: Britta Wilms, PhD, Department of Internal Medicine I, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: britta.wilms@uni-luebeck.de.

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