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A single night of sleep deprivation increases ghrelin levels and feelings of hunger in normal-weight healthy men

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SUMMARY Sleep loss is currently proposed to disturb endocrine regulation of energy homeostasis leading to weight gain and obesity. Supporting this view, a reduction of sleep duration to 4 h for two consecutive nights has recently been shown to decrease circulating leptin levels and to increase ghrelin levels, as well as self-reported hunger. We hypothesized that similar endocrine alterations occur even after a single night of sleep restriction. In a balanced order, nine healthy normal-weight men spent three nights in our sleep laboratory separated by at least 2 weeks: one night with a total sleep time of 7 h, one night with a total sleep time of 4.5 h and one night with total sleep deprivation (SD). On a standard symptom-rating scale, subjects rated markedly stronger feelings of hunger after total SD than after 7 h sleep (3.9 ± 0.7 versus 1.7 ± 0.3; $P = 0.020$) or 4.5 h sleep (2.2 ± 0.5; $P = 0.041$). Plasma ghrelin levels were 22 ± 10% higher after total SD than after 7 h sleep (0.85 ± 0.06 versus 0.72 ± 0.04 ng mL^{-1}; $P = 0.048$) with intermediate levels of the hormone after 4.5 h sleep (0.77 ± 0.04 ng mL^{-1}). Serum leptin levels did not differ between conditions. Feelings of hunger as well as plasma ghrelin levels are already elevated after one night of SD, whereas morning serum leptin concentrations remain unaffected. Thus, our results provide further evidence for a disturbing influence of sleep loss on endocrine regulation of energy homeostasis, which on the long run may result in weight gain and obesity.

KEYWORDS energy homeostasis, ghrelin, leptin, obesity, sleep loss

INTRODUCTION

Daily sleep duration has continuously decreased during the last decades (Knutson *et al.*, 2007). In parallel, the prevalence of overweight and obesity has dramatically increased in the western world (Ogden *et al.*, 2004, 2006). Recent epidemiological studies have pointed to a distinct relationship between sleep duration and body weight, suggesting a U-shaped association in that both shortened and extended sleep durations coincide with increases in body weight (Gangwisch *et al.*, 2005; Hasler *et al.*, 2004; Taheri *et al.*, 2004; Vorona *et al.*, 2005). The Quebec Family Study and the Wisconsin Sleep Cohort Study both indicated that short sleep duration is not only associated with an increased body mass index (BMI) but also with reduced circulating levels of leptin known to suppress appetite (Chaput *et al.*, 2007), as well as with an elevated level of ghrelin that promotes hunger (Taheri *et al.*, 2004). A recent experimental study in lean subjects showed that a restriction of time in bed to 4 h in comparison with an extended bedtime of 10 h for two consecutive nights markedly decreases leptin levels and increases ghrelin levels (Spiegel *et al.*, 2004b). Strikingly, these hormonal alterations were paralleled by increased feelings of hunger and appetite. Collectively, these data point to a distinct influence of sleep and sleep loss, respectively, on the endocrine regulation of energy homeostasis (Steiger, 2004). However, overall experimental evidence for a causal relationship between sleep loss and changes in the energy intake-regulating hormones is still scarce and, thus,

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strongly in need of further examination. In order to further elucidate the acute influence of short-term sleep curtailment on leptin and ghrelin secretion, we determined fasting morning levels of these hormones in nine normal-weight men after a night of 7 h sleep, a night of 4.5 h sleep and a night of total sleep deprivation (SD).

METHODS

Subjects were 10 healthy young men. Due to problems with blood sampling, data from one man were excluded. The remaining nine subjects' age was between 20 and 40 years (mean ± SEM: 24.2 ± 1.0 years). The BMI ranged between 20.7 and 25.0 kg m^{-2} (23.8 ± 0.6 kg m^{-2}). All subjects reported a stable body weight (±1 kg) within the last 3 months and did not follow a particular diet. Only subjects with a regular sleep–wake cycle were included. Shift workers were excluded from participation. All participants were instructed not to deviate from their regular sleep and eating habits during the study. To furthermore avoid any systematic influence of potential alterations in sleep habits during the study period, subjects were examined in randomized, balanced order in the different conditions. Self-reported habitual sleep time was 7–8 h (mean ± SEM: 459 ± 7 min) with time to bed ranging between 22:00–00:00 hours (23:16 hours ± 5 min) and wake-up time ranging between 06:00–08:00 hours (07:07 hours ± 10 min). The examinations reported here were part of larger study designed to test effects of sleep on hypoglycaemia counter-regulation (Schmid *et al.*, 2007). The study protocol was approved by the ethics committee of the University of Lübeck, and all participants gave written informed consent.

Subjects were examined on three different conditions spaced at least 2 weeks apart and performed in a balanced order. Conditions were as follows: (i) a night of total SD, (ii) a night of sleep time restriction to 4.5 h during the first part of the night and (iii) a night with 7 h of sleep.

Participants were instructed not to consume caffeine (for 10 h) and alcohol (for 24 h) on the day before the experiments, and to avoid extraordinarily intense physical activity. Compliance with these instructions was assessed by systematically interviewing the subjects before each experimental night. After arrival at 21:00 hours at the research unit, subjects were allowed only to drink water but to abstain from any intake of food until the end of the experiment. In the 7- and 4.5-h sleep conditions, subjects went to bed following the preparation of polysomnographical recordings. Until lights were turned off at 22:30 hours, they read and talked to the experimenter. Depending on the experimental condition, subjects were awakened either after 5 h or 7.5 h of bed time. In these conditions, subjects slept on average approximately 4.5 and 7.0 h, respectively. In the 4.5-h sleep condition, participants were not informed before that sleep ended after 4.5 h, so that early awakening took them by surprise. During the rest of the night, subjects read and watched a movie in a sitting position within the laboratory illuminated by standard office lighting (~500 lx). Brisk physical activities were not allowed. The same setting was used during total SD. Sleep was recorded polysomnographically in both sleep conditions. Sleep recordings were analysed offline according to standard criteria by Rechtschaffen and Kales (Rechtschaffen and Kales, 1968). During SD, subjects were monitored by the experimenter throughout the night.

Blood samples for determination of serum leptin and plasma ghrelin levels were drawn at 07:00 and 07:30 hours. Immediately thereafter, subjects rated their feelings of hunger from 0 (not at all) to 9 (severely) on a standard symptom questionnaire (Schultes *et al.*, 2005). Serum leptin concentrations (Human Leptin RIA KIT; Linco Research, St Charles, MO, USA) and total plasma ghrelin concentrations [Ghrelin (total) RIA KIT] were determined in duplicate by radio-immunoassay with the following intra-assay and inter-assay coefficients of variation (CV) and limit of sensitivity (LOS), respectively: leptin, CV < 8.3 and <6.2%, respectively, and LOS 0.5 ng mL^{-1}; ghrelin, CV < 10.0 and 14.7%, respectively, and LOS 93 pg mL^{-1}. For statistical analysis, ANOVA for repeated measures were performed including the factor 'sleep' (for the different sleep conditions) and 'time' (for measurements at 7:00 and 7:30 hours). Because there was neither a significant ($P < 0.05$) 'time' main effect nor a significant 'sleep' × 'time' interaction in any of the analyses, values obtained at 07.00 and 07.30 hours were averaged and thus reported here.

RESULTS

In the 7-h sleep condition, subjects slept on average 418 ± 11 min, while they slept 275 ± 9 min in the 4.5-h sleep condition. Sleep latency was 20 ± 4 min in the 7-h sleep condition and 26 ± 7 min in the 4.5-h sleep condition ($P = 0.20$). Hunger ratings indicated that subjects perceived stronger feelings of hunger after total SD than after 7 h sleep (3.9 ± 0.7 versus 1.7 ± 0.3; $P = 0.020$) or 4.5 h sleep (2.2 ± 0.5; $P = 0.041$; Fig. 1a). The difference in self-rated hunger between the 7- and 4.5-h sleep condition was not significant ($P = 0.34$).

Overall ANOVA pointed to an effect of the sleep condition on plasma ghrelin levels ($P = 0.075$; Fig. 1b). Pairwise comparisons revealed that mean plasma ghrelin levels were 22 ± 10% higher after total SD than after 7 h sleep (0.85 ± 0.06 versus 0.72 ± 0.04 ng mL^{-1}; $P = 0.048$). Compared with the 7-h sleep condition, mean plasma ghrelin levels were also increased by 11 ± 5% after 4.5 h sleep. However, this elevation was of small size only and failed to reach significance (0.77 ± 0.04 ng mL^{-1}; $P = 0.076$). In contrast to ghrelin levels, serum leptin levels remained completely unaffected by sleep restriction or deprivation ($P = 0.93$, for 'sleep' main effect; Fig. 1c).

Interestingly, correlation analyses (Pearson) showed a positive correlation ($r = 0.66$; $P = 0.05$) between the percent differences in mean ghrelin levels between the total SD and the 7-h sleep condition on the one hand and the difference in mean hunger ratings between the two conditions on the other hand.

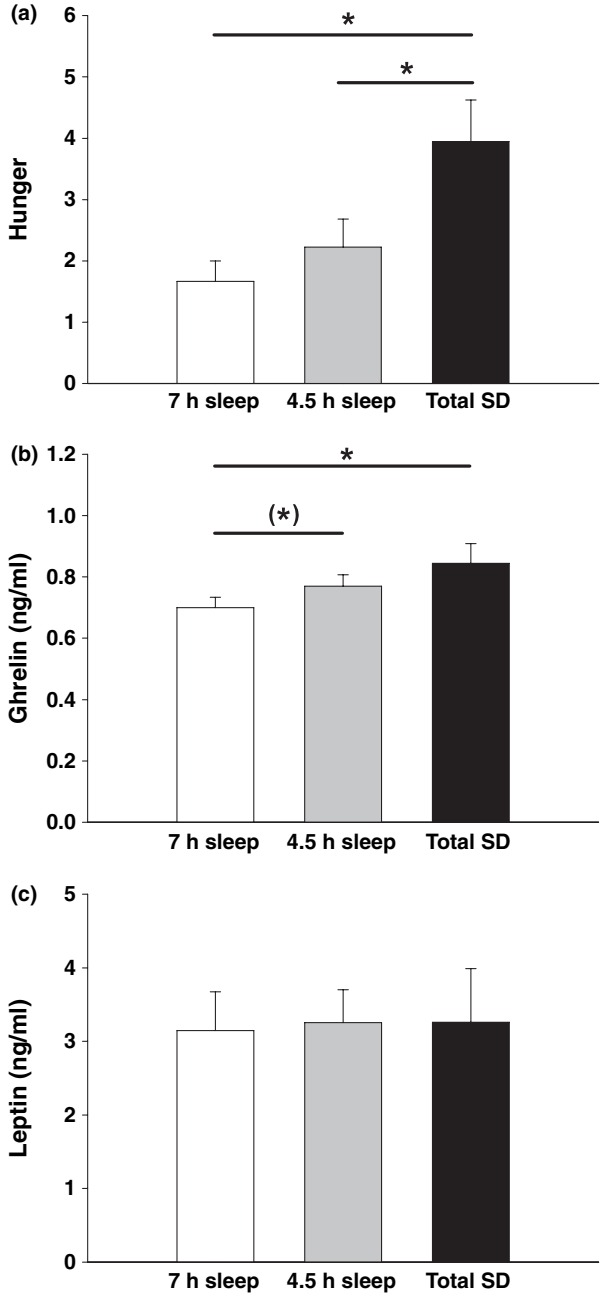

Figure 1. Mean ± SEM values of perceived feelings of hunger (a), plasma concentration of ghrelin (b) and serum concentration of leptin (c). Hunger was rated by and blood samples were taken from nine healthy men at 07:00 and 07:30 hours after a night with 7 h of sleep (white bars), after a night with only 4.5 h of sleep (grey bars), and after a night without sleep (sleep deprivation, black bars). Data shown represent averaged values assessed at the two points in time. $^{*}P < 0.05$, $(^{*})P = 0.08$.

DISCUSSION

We report that a single night of total SD acutely increases feelings of hunger and plasma levels of the hunger-promoting hormone ghrelin in normal-weight healthy men. A more subtle reduction of sleep duration by 2.5 h was revealed to induce smaller changes in the same direction, which, however, remained non-significant. While present results overall support a tight relationship between ghrelin levels, feelings of hunger and sleep duration, it must be emphasized that our results cannot be extrapolated to longer periods of sleep loss.

Our results on ghrelin levels are well in line with a previous report of elevated ghrelin levels after two consecutive days of sleep reduction to 4 h per night (Spiegel *et al.*, 2004b). However, in contrast to that study where sleep restriction also induced a clear-cut reduction of leptin levels, leptin levels in our study remained unchanged after sleep restriction or deprivation. Another study also by van Cauter's group revealed a distinct reduction of mean levels, maximal levels and rhythm amplitude in 24-h leptin profiles after six nights of ~4 h sleep when compared with profiles after six nights of ~9 h recovery sleep (Spiegel *et al.*, 2004a). An 88-h period of total SD reduced the diurnal amplitude of leptin (Mullington *et al.*, 2003). In combination, those and our data suggest a temporally graded response of hormones that regulate energy intake to increasing periods of SD with ghrelin already boosted by a single night of sleep loss and leptin levels not affected until after longer periods of SD. Alternatively, the lacking leptin response might reflect the inert nature of leptin release. Two measurements in the morning after SD may not have been sufficient to assess potential slower, gradual decreases in leptin levels, which might be an important limitation of our study. Considering that ghrelin is primarily associated with short-term regulation of food intake whereas leptin is rather regarded a tonic signal (Strubbe and van Dijk, 2002), the differential response of the two hormones to distinct patterns of sleep loss might serve to adjust eating behaviour to the actual state of sleep deficiency.

While increased feelings of hunger after sleep restriction in our study may have been triggered by elevated ghrelin levels, other mechanisms could likewise account for this observation. For instance, central nervous glucose utilization is known to be distinctly higher during wakefulness than during sleep, especially slow-wave sleep (Maquet, 1995; Maquet *et al.*, 1990). Thus, it may well be that a higher energy demand of the brain during prolonged wakefulness has evoked the increase in subjective feelings of hunger in our study.

Overall, our findings add to the as yet scarce experimental evidence that sleep affects the endocrine regulation of energy homeostasis. They corroborate the assumption of a tight connection between sleep–wake regulation and metabolic parameters (Knutson *et al.*, 2007). However, as the present as well as previous findings (Mullington *et al.*, 2003; Spiegel *et al.*, 2004a,b) were exclusively obtained in normal-weight healthy men, experimental studies now need to be extended to women and obese subjects.

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DISCLOSURE STATEMENT

None of the participating authors and institutions reported any conflict of interest regarding the study.

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