Sleep restriction is not associated with a positive energy balance in adolescent boys1–3

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ABSTRACT
Background: A short sleep (SS) duration has been linked to obesity in observational studies. However, experimental evidence of the potential mechanisms of sleep restriction on energy balance is conflicting and, to our knowledge, nonexistent in adolescents.
Objective: We investigated the effects of 3 consecutive nights of partial sleep deprivation on components of energy balance.
Design: In a randomized, crossover design, 21 healthy, normal-weight male adolescents (mean ± SD age: 16.8 ± 1.3 y) completed the following 2 experimental conditions, each for 3 consecutive nights: an SS (4 h/night) and a long sleep (LS; 9 h/night) duration. Endpoints were 24-h energy expenditure (EE), spontaneous physical activity (SPA), postintervention diet-induced thermogenesis (DIT), appetite sensations, ad libitum energy intake (EI), and profiles of plasma ghrelin and leptin.
Results: The 24-h EE on day 3 was 370 ± 496 kJ higher in the SS condition than in the LS condition (P = 0.003). This difference in EE was explained by prolonged wakefulness in the SS condition and a 19% higher SPA (P = 0.003). In a postintervention breakfast-meal challenge, there was a 0.19-kJ/min smaller incremental AUC in DIT over 4 h in the SS condition than in the LS condition (P = 0.012) with no time × condition effect (P = 0.29). Subjects consumed 13% less energy in the ad libitum meal in the SS condition (P = 0.031), with a concomitant decreased motivation to eat. Concentrations of ghrelin and leptin remained unchanged with sleep restriction.
Conclusion: Short-term sleep restriction in male adolescents is associated with a small negative energy balance driven by increased EE from prolonged wakefulness and a concomitant decreased EI and motivation to eat. This trial was registered at clinicaltrials.gov as NCT01198431.

INTRODUCTION
A mounting body of observational evidence has revealed that short sleep (SS)4 duration is associated with weight gain and increased incidence of obesity (1–3), especially in children (4–7). Intervention studies have contributed to our understanding of potential physiologic mechanisms that underpin this association. Spiegel et al (8) were the first to show experimentally that sleep restriction affects the regulation of key appetite hormones (ie, leptin and ghrelin) and appetite sensations in slightly hypocaloric subjects. However, in 3 similar intervention studies, albeit in eucaloric subjects, these observations were not reproduced (9–11), which left the question open as to whether the connection between SS and obesity can be explained by changes in homeostatic consumption behavior. In addition to these proposed physiologic mechanisms that favor an increased drive to eat, staying awake for a longer period of time exposes individuals to the obesogenic environment longer than normal sleepers are exposed, which leads to increased energy intake (EI) (12, 13) and, in particular, increased snacking behavior (10). In summary, an increased EI as a result of partial sleep restriction has not been consistently observed.

Evidence on the effects of sleep restriction on the other side of the energy-balance equation [ie, energy expenditure (EE)] is also conflicting. It has been shown that partial sleep deprivation does not influence 24-h EE (10, 11). This also seemed to be the case when the resting metabolic rate was investigated separately (14). However, divergent findings have also been reported, after both total sleep deprivation (15) and partial sleep deprivation (16). Conflicting results have also been observed regarding the effect of sleep restriction on physical activity levels and patterns (9, 11, 12, 14) that could influence total EE.

Because of these conflicting results and the fact that these investigations have, to our knowledge, been conducted only in adults, we aimed to investigate the effects of 3 consecutive nights of partial sleep deprivation on components of energy balance in a homogenous group of healthy teenage boys. This age group is particularly interesting because it has been observed that adolescents have experienced important declines in their sleeping time over the past decades (17), and they have an irregular sleep-wake cycle (18), which thereby constitutes a potential group at risk of developing
obesity. We hypothesized that 3 consecutive nights of only 4 h in bed would be accompanied by increased appetite sensations and an increased spontaneous food intake without affecting the total EE.

SUBJECTS AND METHODS

Subjects

Twenty-one healthy, normal-weight [age-adjusted BMI (in kg/m²) < 25 on the basis of Cole et al (19)] male adolescents between 15 and 19 y of age were recruited for the study through advertisements and by word of mouth. Volunteers were excluded from participation for any of the following reasons: smoking, unstable body weight (±3 kg) during the 6 mo before testing, regular physical exercise (>3 h/wk), excessive intake of alcohol (>7 drinks/wk), substance abuse, excessive intake of caffeine (>300 mg/d), metabolic disease (eg, thyroid disease, heart disease, or diabetes), medication use that could interfere with the outcome variables, eating disorder, highly restrained eating behavior (score ≥10 for cognitive dietary restraint on the Three-Factor Eating Questionnaire), irregular eating pattern (eg, skipping breakfast), self-reported sleep problems (score > 5 on the Pittsburgh Sleep Quality Index), transmeridian travel in the past month, and inability to comply with the protocol. All subjects (or parents of subjects aged <18 y) gave written informed consent to participate in this study, which had received approval from the ethical committee of the Capital Region of Denmark. Volunteers participated in the following 4 visits: an information meeting [ie, a preliminary visit aimed at informing subjects (and parents for subjects <18 y) about the procedures and protocol requirements], a screening visit to evaluate inclusion and exclusion criteria, and two 4-d experimental conditions.

Study design and procedure

A schematic overview of the study protocol is shown in Figure 1. With the use of a within-subject experimental design, each participant was engaged, in a random order, in each of the 2 following conditions: 1) SS (4 h/night from 0300 to 0700 for 3 consecutive nights) and 2) long sleep (LS; 9 h/night from 2200 to 0700 for 3 consecutive nights). These 2 experimental conditions were randomly assigned by using a computerized randomization scheme and separated by 3–4 wk. Between experimental conditions, participants were asked to maintain normal activities and, particularly, not to change sleeping, eating, or physical activity patterns. Vigorous physical activity was not allowed 24 h before testing, and a normal sleep schedule had to be respected for 3 d before testing. Subjects were required to arrive at the University of Copenhagen research laboratory in a fasting state, and all subjects were in good health on test days. Body weight was measured every morning on a calibrated scale (Lindell Tronic 8000; Samhall Lavi) with subjects in a fasting state with an empty bladder and wearing light clothes and without shoes. Likewise, subject temperature was measured in the ear canal with a thermometer (Braun ThermoScan; Braun GmbH) on awakening each morning.

The 3 nights were spent in a whole-body calorimetric chamber. During the 2 initial nights, the chamber door was open so that subjects could become accustomed to the environment. On the morning after the second night, the chamber door was sealed, and the subjects spent 24 h in the chamber for measurements of EE. Subjects had access to a computer with Internet, a telephone, and a television with a DVD player during the stay. The following time schedule was followed during the 24-h stay in the chamber: door closed at 0830, breakfast at 0900, 15 min of cycling at a constant resistance (75 W) and cadence (50 rpm) at 1000 and again at 1600, lunch at 1300, a short period of standardized light walking (25 rounds in the chamber, which corresponded to ~5 min) at 1430, and again at 1430, snack at 1530, dinner at 1800, snack at 2100, lights off at 2200 (for LS) or 0300 (for SS), awakened at 0700, basal metabolic rate (BMR) measurements at 0730, and chamber exit at 0830. Trained personnel regularly visually monitored subjects to ensure their safety and compliance to the protocol (eg, by making contact at any sign of sleeping during waking hours and BMR measurements).

Diet

The basal energy requirement was individually calculated by using the WHO formula (20) for this age group

\[
0.068 \times \text{weight (in kg)} + 0.57 \times \text{height (in m)} + 2.16 \times \text{physical activity factor (1.4 for chamber condition; 1.6 for free-living condition)}
\]

El was distributed as 25% from breakfast, 30% from lunch, 30% from dinner, and 15% from snacks. The macronutrient composition

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**Figure 1.** Study outline. Hatched areas represent time periods outside the study protocol. Participants spent both sleeping and wake time in the respiration chamber on days 1 and 2 to become accustomed to the chamber. Syringe symbols denote blood samplings (filled syringe denotes a fasting sample). Visual analog scales were provided at the same time points as blood sampling. Meals were given at the same time points in both conditions as follows: breakfast at 0900, lunch at 1300, snack at 1530, dinner at 1800, and snack at 2100. Meals outside of the laboratory were provided by our research kitchen staff. In free-living periods, participants were encouraged to engage in everyday activities but to abstain from scheduled physical activity. \textit{ad lib.}, ad libitum meal test; \textit{arrival}, arrival at the laboratory; depart., departure from the laboratory; laboratory, period during which participants stayed at the laboratory; LS, long-sleep condition (9 h from 2200 to 0700); meal, breakfast meal; SS, short-sleep condition (4 h from 0300 to 0700); ventilated hood, repeated ventilated hood measurements.
was, on average for the whole day, 10% of energy from protein, 30% of energy from fat, and 60% of energy from carbohydrates. Dietary calculations were made by using the food database from the Danish National Food Agency (version 6.0, 2005; Technical University of Denmark). Calculations were done with Dankost 3000 software (version 7.01; Dankost). All meals consisted of everyday products and were prepared in the research kitchen of the laboratory. Meals were given at the same time points in both conditions. Meals outside of the laboratory were provided to participants as ready-made meals with a written instruction on heating and handling. A text-message system was set up to remind participants to eat at the designated times. Participants were encouraged to eat the whole serving at each meal. Any leftovers were recorded, and the actual energy of that particular meal was recalculated. Water was offered ad libitum.

Sleep recordings and evaluation
Surface electrodes for electroencephalography, electrooculography, submental electromyography, and electrocardiography measurements were fitted on subjects by using a portable polysomnographic device (TrackitTM; Lifelines Neurodiagnostic Systems Inc) before they left the metabolic laboratory on the first day. Each morning, the electrodes were checked for signal strength and impedance. Post hoc analyses of sleep length were done by using visual scoring and were conducted by the same trained researcher for all recordings.

Each morning, at the same time point during both experimental conditions, subjective sleepiness was evaluated by using the Karolinska Sleepiness Scale (KSS) (21). The KSS consists of a question on subjective sleepiness with a 10-point scale with a phrase associated to each point that ranged from “1: extremely alert” to “10: extremely sleepy–can’t keep awake.” The KSS has been shown to be highly correlated with brain activity (α and θ activity) (21) and is a useful proxy behavioral indicator of sleepiness (22).

Measurement of EE
After a run-in period of 2 nights, the EE and respiratory quotient (RQ) were measured in one of 2 duplicate respiratory chamber calorimeters of the Department of Human Nutrition (23). The same chamber was used on both occasions for any subject. Gas exchange in the chamber was calculated from the measured airflow and concentrations of oxygen and carbon dioxide at the outlet of the chamber as well as in the fresh air going in. We performed a 24-h urine collection to determine nitrogen excretion. EE was calculated, including data on nitrogen excretion, on the basis of equations by Elia and Livesey (24). On the fourth day, subjects were awakened at 0700 but were instructed to remain lying still in bed, apart from getting up for voiding if needed. The registration of respiratory gas exchange between 0730 and 0830 was used for assessment of the BMR.

After subjects left the chamber, measurements of diet-induced thermogenesis (DIT) and RQ were performed before and for 3 h after a standardized breakfast that consisted of 25% of the individual daily energy requirement and with an energy distribution of 11% of energy protein, 27% of energy fat, and 62% of energy carbohydrate by using a ventilated hood system (Jaeger Oxycon Pro, Cardinal Health Care GmbH) as described in detail elsewhere (25). The duration of each measurement was 25 min where the first 5 min of each 25-min measurement was omitted. EE was calculated by using a formula assuming a fixed protein catabolism (26). The accuracy of the ventilated hood was validated by using an alcohol-burning test on a weekly basis with a CV of 1.5%.

Physical activity
Spontaneous physical activity (SPA) in the respiration chamber was assessed by using 2 microwave radar detectors (Sisor Mini-Radar; Statistic Input System SA). The radar detected when the subject was moving, and the generated signal was received by the transceiver and electronically stored for later analyses. SPA measurements indicated the percentage of time when the subject was moving.

Measurement of ad libitum EI
To measure spontaneous EI in an experimental context, participants were offered an ad libitum lunch at 1300 on day 4. The ad libitum meal was a semihomogenized spaghetti bolognese meal (961 kJ/100 g; 15% of energy protein, 30% of energy fat, and 55% of energy carbohydrate) and 200 mL tap water. Subjects were instructed to eat at a constant pace and to stop eating when they felt satiated. Subjects had a maximum of 30 min to consume the meal, and the serving of pasta was substantially larger than the expected intake (8 MJ offered). The meal was weighed before lunch, and the uneaten portion was weighed after lunch. Ad libitum EI was assessed by a food technician by using calculations performed on the amount of the meal consumed. The ad libitum test meal has been shown to be reproducible in our laboratory (27).

Measurement of appetite
Participants rated their sensations of hunger, satiety, prospective food consumption, fullness, and desire to eat meat, fish, something sweet, something salty, or something rich in fat on a visual analog scale (VAS) at different time points around the standardized breakfast on day 4. The VAS method has been described in detail and was shown to be both reproducible and valid for measuring appetite sensations in our laboratory (28). Assessments were completed before and immediately after the breakfast test meal and subsequently at every 30 min until the ad libitum lunch meal. In addition, the VAS was filled out every hour for a 4-h period after the ad libitum lunch.

Biochemical analyses
Blood samples were drawn through an indwelling superficial forearm venous catheter in the fasting state and every 30 min during the DIT measurement (a total of 8 time points). The sampling was done under standardized laboratory conditions. Samples were drawn into heparinized tubes, centrifuged, separated into aliquots, and frozen at −80°C until analyzed. Samples from each subject were analyzed continually in a single batch to eliminate assay variation. Blood samples were analyzed for total plasma ghrelin [determined by using an enzyme-linked immunosorbent assay (Millipore)], total plasma leptin [determined by using a radioimmunoassay (Millipore)], and free triiodothyronine, thyroxine, and thyroid-stimulating hormone [determined by using a solid-phase, enzyme-labeled chemiluminescent immunometric assay (Siemens Immulite 1000; Siemens Medical Solutions)].
Questionnaires

Three questionnaires were administered during the preliminary visit to better characterize participants. The 51-item Three-Factor Eating Questionnaire (29) was used to assess 3 factors related to cognition and eating behaviors: cognitive dietary restraint (intend to control food intake), disinhibition (overconsumption of food in response to cognitive or emotional cues), and susceptibility to hunger (food intake in response to feelings and perceptions of hunger). This questionnaire has been validated, and its 3 scales have been reported to show good test-retest reliability (29, 30). In addition, each participant completed the Pittsburgh Sleep Quality Index (31), which is a self-rated questionnaire that assesses sleep quality and disturbances over the preceding month. A total score >5 was associated with poor sleep. Finally, the Cohen’s Perceived Stress Scale (32) was completed to evaluate the level of stress in the everyday lives of participants. This questionnaire contained 10 questions, and a score of <10 indicated good management of stress.

Statistical analysis

The power-calculation analysis performed before the beginning of the study showed that data from 20 subjects gave a power of 90%, which was sufficient to detect a difference in 24-h EE between sleep protocols of 2% (SD: ±2%) with an α = 0.05. Before statistical analyses were conducted, all continuous variables were tested for normality and homogeneity of variance by using visual inspection of both quantile-quantile plots and plots of residuals against fitted values. A Box-Cox calculation was conducted, and the necessary transformations were done to ensure normality and homogeneity of variance. Differences in EE (respiration chamber), EI (ad libitum), AUC, and area over the curve (trapezoidal rule) and between SS and LS in fasting concentrations of hormones were analyzed by using a multiple linear regression model with robust estimation of variance (Huber-White estimation model) with the order of treatment as a fixed explanatory variable. Differences in repeated measurements of EE (hood), the VAS, and appetite-regulating hormones were tested by using a random coefficient model with maximum-likelihood estimation. Each model was tested for a correlation pattern, and the model was fitted as either unstructured or with an exponential correlation structure based on Akaike’s criteria. The time x condition effect in the random coefficient model was tested against a simpler model by using a likelihood ratio test. The order of intervention was a fixed explanatory variable. For statistical evaluation, Stata software (version 11; StataCorp) was used. Data are presented as means ± SDs unless otherwise specified. P < 0.05 was considered significant.

RESULTS

Participant characteristics and sleep data

Descriptive characteristics of subjects are shown in Table 1. None of the subjects were restrained eaters, and they all had low scores of disinhibition and susceptibility to hunger. In addition, subjects had a fairly good sleep quality in general as well as a normal perceived stress in their everyday lives.

Fasting morning weight did not change over the course of the 4-d intervention in either condition (random coefficient model), and there were not any differences in fasting weight on day 4 between conditions (paired sample t test).

Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Values (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>16.8 ± 1.3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>65.7 ± 5.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.0 ± 1.8</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>74.9 ± 4.5</td>
</tr>
<tr>
<td>Energy intake inside the chamber (MJ)</td>
<td>10.7 ± 0.6</td>
</tr>
<tr>
<td>Energy intake outside the chamber (MJ)</td>
<td>12.3 ± 0.7</td>
</tr>
<tr>
<td>Pittsburgh Sleep Quality Index score</td>
<td>3.86 ± 2.17</td>
</tr>
</tbody>
</table>

Cognitive dietary restraint | 4.73 ± 3.22 |
Disinhibition | 4.36 ± 2.13 |
Susceptibility to hunger | 4.55 ± 3.10 |
Cohen's Perceived Stress Scale score | 8.68 ± 4.20 |

1 All values are means ± SDs.

Data from polysomnography recordings showed that participants slept, on average, 506 ± 42 and 243 ± 15 min during the LS and SS conditions, respectively. Values for the KSS questionnaire went from 4.7 ± 1.7 (day 1) to 7.8 ± 0.9 (day 4) in the SS condition and from 5.2 ± 1.6 (day 1) to 4.1 ± 1.6 (day 4) in the LS condition. There was a significant time x condition effect (P = 0.012) with a significantly higher KSS score in SS compared with LS conditions on days 2, 3, and 4 (P < 0.001 for all days).

Measures of EE

Twenty-four-hour EEs in the respiration chamber were 10,469 ± 885 kJ and 10,099 ± 766 kJ for the SS and LS conditions, respectively (P = 0.003). These values corresponded to a 370 ± 496-kJ higher daily EE in the SS condition than in the LS condition (Figure 2). The profile of 24-h EE from the respiration chamber showed that the difference in 24-h EE originated from the difference in EE during the habitual nighttime period [6.8 ± 0.7 kJ/min (SS) compared with 5.3 ± 0.4 kJ/min (LS); P < 0.001 (Figure 3)]. There was no difference between conditions in either daytime EE [8.2 ± 0.7 kJ/min (SS) compared with 8.3 ± 0.7 kJ/min (LS); P = 0.42], sleeping EE [4.9 ± 0.5 kJ/min (SS) compared with 4.9 ± 0.4 kJ/min (LS); P = 0.54], or BMR [5.8 ± 0.6 kJ/min (SS) compared with 6.0 ± 0.7 kJ/min (LS); P = 0.082] (Figure 2). SPA followed the same pattern with a significantly higher SPA during habitual nighttime in the SS condition than in the LS condition (16 ± 7% (SS) compared with 3 ± 1% (LS); P < 0.001), which explained the 19% higher SPA over 24 h [16 ± 5% (SS) compared with 13 ± 5% (LS); P = 0.003; Figure 2]. The 24-h RQ value was significantly lower in the SS condition than in the LS condition [0.88 ± 0.02 (SS) compared with 0.89 ± 0.02 (LS); P = 0.022]. This difference in RQ was shown during sleep [0.88 ± 0.03 (SS) compared with 0.85 ± 0.03 (LS); P < 0.001] and BMR measurement [0.88 ± 0.04 (SS) compared with 0.85 ± 0.04 (LS); P = 0.049] (Figure 2).

DIT after the standardized breakfast meal challenge is shown in Figure 4. There was no significant time x condition effect for DIT (P-interaction = 0.29). However, there was a 0.19-kJ·min (29%) larger incremental AUC (baseline-subtracted AUC) in the LS condition than in the SS condition [0.67 ± 0.058 kJ·min (SS) compared with 0.86 ± 0.058 kJ·min (LS); P = 0.012]
temperature between days 1 and 4 were $-0.30^\circ$C and $0.00^\circ$C for SS and LS conditions, respectively ($P = 0.075$). There was no time × condition effect ($P$-interaction = 0.72). Measures of thyroid hormones showed a significant difference in free triiodothyronine between conditions on the morning of day 4 [$4.91$ pmol/L (SS) compared with $4.34$ pmol/L (LS); $P < 0.001$] but not in thyroxine [$1.12$ ng/dL (SS) compared with $1.10$ ng/dL (LS); $P = 0.26$] or thyroid-stimulating hormone [$1.76$ mIU/L (SS) compared with $1.85$ mIU/L (LS); $P = 0.34$].

Measures of appetite, EI, and appetite hormones

Profiles of subjective appetite (VAS scores for hunger, satiety, fullness, and prospective food intake) in response to the standardized breakfast on day 4 are presented in Figure 5. All of these VAS scores were affected toward a decreased motivation to eat in the SS condition. Thus, there was a significantly larger AUC or area over the curve for satiety, fullness, hunger, and prospective food intake ($P = 0.004$, $P = 0.003$, $P = 0.049$, and $P = 0.013$, respectively) (Figure 6). In the random coefficient model, there was a time × condition effect for prospective food intake ($P = 0.028$), whereas no effects were shown for fullness ($P = 0.20$), hunger ($P = 0.28$), and satiety ($P = 0.20$). Fasting values did not differ between conditions. There was a significantly larger AUC in the SS condition for the desire to eat meat and fish ($P = 0.048$) and something sweet ($P = 0.019$) but not for thirst ($P = 0.42$). There was a tendency toward an increased desire to eat something rich in fat ($P = 0.063$) and salty ($P = 0.064$). There was no time × condition effect in the random coefficient model for any of the scores ($P = 0.36$, $P = 0.25$, $P = 0.23$, $P = 0.39$, and $P = 0.64$ for fat, meat, salt, sweet, and thirst, respectively).

Data on the ad libitum EI assessment are presented in Table 2. Subjects consumed 13% less in the SS condition than in the LS condition ($P = 0.031$). The average eating time (min) and eating rate (kJ/min) were different between conditions.

Plasma concentrations of leptin and ghrelin are presented in Figure 7. Neither ghrelin nor leptin concentrations differed between conditions. In the leptin analyses, 3 subjects were excluded because of fasting concentrations >12 μg/L, which was considered above the normal range for this group of subjects. However, the significance of results did not change keeping these subjects in our analyses.

DISCUSSION

For the first time to our knowledge, the current study examined whether short-term sleep restriction affects components of energy balance in adolescents. Taken together, our data provided evidence that 3 consecutive nights of 4 compared with 9 h of sleep per night were associated with increased EE derived from increased wakefulness. Contrary to our hypothesis, we also observed a decreased EI after SS at a subsequent ad libitum test meal. This finding was accompanied by a decreased motivation to eat.

EE

The increased 24-h EE during the SS condition in our teenagers was not in line with our hypothesis and contradicted previous findings (10, 14, 16). However, in studies by Nedeltcheva et al (10, 16), the doubly labeled water technique was used to measure EE.
We used whole room calorimetry, which is able to detect even small changes in EE not picked up by the doubly labeled water technique (33). The increase in 24-h EE in our study was solely due to an increased EE during the time period in which subjects were awake in the SS condition and asleep in the LS condition. The extra energy spent during this period (from 2200 to 0300) constituted 98% of the total difference in 24-h EE. This effect has previously been reported by Jung et al (34), whose study supported our finding of a sleep-wake–related difference in EE. In addition, this observation was substantiated because BMR did not differ between conditions. An unchanged resting metabolic rate after sleep restriction has also been reported previously in different groups of subjects (10, 11, 14), whereas other studies have described a drop in resting metabolic rate after sleep restriction by using different methodologies (15, 16).

An increased thyroid activity has been reported previously after partial sleep restriction (11). Because we did not observe an increase in EE during either sleep or BMR, a possible effect of thyroxine 3 on EE in our teenagers was negligible. Previous studies have shown decreased body temperatures after total sleep restriction in both humans (35, 36) and rats (37), which could offer an explanation of increased thyroid activity. However, we showed a tendency toward a decreased body temperature only after SS in our study. Thus, the cause of elevated thyroxine 3 concentrations remains elusive.

Activity-related thermogenesis is probably a more obvious explanation of the increased EE. We showed that the increased nighttime SPA explained the increased 24-h SPA in the SS condition with no difference in either daytime or sleeping SPA between conditions (Figure 2). Previous studies have shown that both the amount and intensity of physical activity during free-living conditions after only one night of sleep curtailment can be suppressed (9), but opposite findings have also been reported by using both similar sleep-restriction protocols (12) as well as total sleep deprivation (14). In the current study, SPA in the respiration chamber was significantly increased in the SS condition because of the longer period of time spent awake. Although participants had access to recreational media, it is likely that the increased movement in the SS condition was a result of an increased effort to stay awake. However, physical activity in the respiration chamber was very limited because of the confinement and was by no means comparable to real-life conditions. Thus, caution must be exercised with regard to the clinical significance of these SPA data obtained in the respiration chamber.

![Energy-expenditure profile from the calorimetric chamber: profile of the 24-h energy expenditure between sleep conditions (n = 21). The cycle icon represents the time of standardized cycling at a constant resistance (75 W) and cadence (50 rpm) on an ergometer cycle.](image1)

![DIT data (±SEMs): DIT before and after the standardized meal challenge (A) and iAUC (trapezoid rule) calculations (B). Error bars represent SEMs. No statistically significant time × condition effect was shown in the random coefficient model (Pinteraction = 0.29; n = 19). *Significantly different between conditions, P < 0.05 (Huber-White estimation model with the order of intervention as an explanatory variable; n = 19). DIT, diet-induced thermogenesis; iAUC, incremental AUC.](image2)
DIT normally accounts for ~10% of 24-h EE when subjects are in energy balance, but changes in postprandial metabolism can give insight into a potential functional role of sleep in relation to long-term energy balance. After a standardized breakfast meal, we showed a small but significant difference in DIT calculated as incremental AUC over 4 h. On average, DIT was 40.1 ± 14.0 kJ lower in the SS condition than in the LS condition for the 4-h period. This result was contrary to results from recent studies (10, 16) in which no difference in DIT have been reported. However, another recent study showed a transient decrease (20%) in DIT after only one night of total sleep deprivation, which suggested a sleep-related change in metabolism (15). However, a comparison between the 2 studies was hampered because the 2 sleep protocols were very different, and a physiologically different response to total sleep deprivation and partial sleep deprivation has been proposed (38).

The observed decrease in RQ during both sleep and BMR suggested that sleep restriction was accompanied by a change in substrate use. However, we suggest that this difference was merely a consequence of the temporal difference in energy balance that stemmed from prolonged wakefulness in the SS condition and, thus, was not an intrinsic change in mobilization or breakdown of substrates after SS.

EI

In contrast to our hypothesis, we showed that the ad libitum food intake, which is a proxy for spontaneous EI, and subjective motivation to eat were reduced after 3 nights of sleep curtailment. To our knowledge, this is a new finding and is in contrast to previous findings in which both increased food intake (11–13) and unchanged food intake (9, 10) have been reported. However, there was a large interindividual variation in the difference in ad libitum food intake between conditions, and in addition, 7 of 21 subjects actually had an elevated EI in the SS condition.

**TABLE 2**

<table>
<thead>
<tr>
<th>Short sleep</th>
<th>Long sleep</th>
<th>P&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kJ)</td>
<td>3430.3 ± 1166.9</td>
<td>3883.6 ± 1341.2</td>
</tr>
<tr>
<td>Duration (min)</td>
<td>9.8 ± 3.3</td>
<td>10.6 ± 3.7</td>
</tr>
<tr>
<td>Eating rate (kJ/min)</td>
<td>354.9 ± 107.9</td>
<td>376.1 ± 92.5</td>
</tr>
</tbody>
</table>

<sup>1</sup> All values are means ± SDs. Energy intake, duration of the meal, and eating rate are from the ad libitum meal test.

<sup>2</sup> P values are for the difference between sleep conditions (Huber-White estimation model with the order of sleep condition as an explanatory variable; n = 21).
condition. To our knowledge, the use of an ad libitum setting has not been validated in teenagers, and on the basis of the current study, it seems that the group displays a considerable degree of heterogeneity.

Despite the large variation in food intake as a result of sleep restriction, the significant difference in ad libitum food intake was supported by data of appetite sensations. Indeed, the drive to eat was lower in the SS condition. However, there was no significant correlation between individual differences in food intakes and VAS scores (data not shown). This further raised the question as to whether the ad libitum meal settings and VAS scores are indeed a valid proxy for EI and appetite sensations in adolescents.

In the current study, we could not rule out the fact that the teenagers might have displayed compensatory eating behaviors after the study. Food intakes were highly controlled during the study until the ad libitum meal setting at lunch on day 4. It has previously been described that going to bed late is associated with late eating behavior, and this combination is associated with increased BMI (18). We continued to assess appetite sensations only until 1800 on day 4 and showed no difference between conditions during this period (data not shown). However, we did not assess compensatory eating behaviors in the afternoon or evening on day 4. This lack of assessment is a limitation of the study. A longer follow-up period is recommended in future studies to adequately capture potential compensations in energy balance after the intervention.

Increased ghrelin and decreased leptin concentrations have previously been associated with sleep restriction, and several studies have addressed this modulation of neuroendocrine regulation of appetite (8–11, 16, 39–44). We did not find support for an endocrine explanation to the decreased food intakes in our teenagers inasmuch as neither ghrelin nor leptin concentrations differed between conditions. However, the measurement of 24-h profiles of ghrelin and leptin could have provided a more robust conclusion in the current study. Although this lack of measurement was a methodologic limitation of the study, for ethical reasons, we were not able to collect blood during 24 h. The fact that we did not see any changes in leptin and ghrelin is in line with previous observations whereby the wakeup time was the same in both conditions (9, 10). This methodologic difference between studies (delay or difference between time of blood sampling and time of wake-up) made it difficult to compare results. Therefore, whether appetite regulation is acutely affected by restricted sleep can be questioned. Whether longer periods of sleep restriction have a more permanent influence on ghrelin and leptin concentrations as indicated in observational studies has, to our knowledge, not been investigated in intervention studies.

In conclusion, we showed that acute sleep restriction was associated with increased 24-h EE in adolescent boys as a consequence of prolonged wakefulness. Moreover, we observed that the motivation to eat and subsequent spontaneous EI, in contrast to what has previously been shown, decreased after SS. In this study, sleep restriction in male teenagers was associated with a small negative energy balance. Future studies should put efforts into better understanding the link between SS duration and energy balance in younger age groups over a longer period of time.

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