Effects of sleep fragmentation in healthy men on energy expenditure, substrate oxidation, physical activity, and exhaustion measured over 48 h in a respiratory chamber\textsuperscript{1–3}

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ABSTRACT
Background: Epidemiologic studies show an inverse or U-shaped relation between sleep duration and BMI. Decreases in total energy expenditure (TEE) and physical activity have been suggested to be contributing factors.

Objective: The objective was to assess the effect of sleep fragmentation on energy metabolism and energy balance in healthy men.

Design: Fifteen healthy male subjects [mean ± SD BMI (in kg/m\textsuperscript{2}): 24.1 ± 1.9; age: 23.7 ± 3.5 y] were included in a randomized crossover study in which energy expenditure, substrate oxidation, and physical activity (by radar) were measured twice for 48 h in a respiration chamber while subjects were monitored by electroencephalography to determine slow-wave sleep (SWS), rapid eye movement (REM) sleep, and total sleeping time (TST). During 2 nights of sleep fragmentation, sleep (2330–0730 h) was either fragmented or nonfragmented.

Results: Fragmented sleep led to reductions in TST, SWS, and REM sleep (\(P < 0.001\)). TEE did not differ (9.96 ± 0.17 compared with 9.83 ± 0.13 MJ/d, NS) between the sleep groups, nor did the components of energy expenditure, with the exception of activity-induced energy expenditure (AEE; 1.63 ± 0.15 compared with 1.42 ± 0.13 MJ/d for fragmented and nonfragmented sleep, respectively; \(P < 0.05\)). Physical activity, exhaustion, sleepiness, respiratory quotient (RQ), and carbohydrate oxidation were elevated in comparison with nonfragmented sleep [physical activity counts: 2371 ± 118 compared with 2204 ± 124 counts/d, \(P < 0.02\); exhaustion: 40.1 ± 3.8 compared with 21.8 ± 2.4 mm (by using a visual analog scale; VAS), \(P < 0.001\); sleepiness: 47.4 ± 4.2 compared with 33.9 ± 4.6 mm (VAS), \(P < 0.001\); RQ: 0.84 ± 0.04 compared with 0.91 ± 0.03, \(P < 0.05\); and carbohydrate oxidation: 346.3 ± 23.8 compared with 323.7 ± 22.5 g/d, \(P < 0.05\)], whereas fat oxidation was reduced (29.1 ± 9.1 compared with 61.0 ± 6.6 g/d, \(P < 0.01\)).

Conclusions: Fragmented compared with nonfragmented sleep contributed to weight gain. However, more research is needed to obtain a decrease in sleep duration (1–5). This has resulted in research on the effects of reductions in the different sleep stages—namely, non-REM\textsuperscript{4} sleep (which consists of stages 1–4), SWS (which is a part of non-REM sleep, namely stages 3–4), REM sleep, and TST—on energy balance and metabolic syndrome characteristics.

Experimental research focused initially on endocrine functions such as insulin sensitivity and glucose homeostasis. For instance, Stamatakis et al (6) found a decrease in insulin sensitivity after 2 nights of sleep fragmentation. Others (7–15) also described alterations in endocrine function after sleep restriction or SWS suppression without affecting TST, which may increase the risk of developing insulin sensitivity and type 2 diabetes. The designs of these studies used sleep restriction of ≤4 h/night and compared this with sleep recovery gained by “catching up” on sleep hours. Coinciding with these alterations in endocrine function, food intake appeared to increase, as shown by increased feelings of hunger and appetite (10, 11, 16–18), thus affecting energy balance. On the basis of observations made by Stamatakis et al (6), who showed that mild sleep deprivation might induce a positive energy balance, we decided to use mild sleep deprivation in the present study, which reflects a more realistic approach that resembles daily life.

The abovementioned studies point at insulin resistance and increased appetite, which would be present after mild fragmentation of sleep, as an explanation for the positive energy balance. Energy balance is maintained when energy intake equals energy expenditure. In addition to an increased food intake and alterations in glucose metabolism, energy expenditure and physical activity have been suggested to decrease after sleep restriction (11), thus contributing to a positive energy balance, which eventually will lead to weight gain. However, more research is needed to obtain

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\textsuperscript{4} Abbreviations used: AEE, activity-induced energy expenditure; EEG, electroencephalography; REM, rapid eye movement; RMR, resting metabolic rate; RQ, respiratory quotient; SMR, sleeping metabolic rate; SWS, slow-wave sleep; TEE, total energy expenditure; TST, total sleeping time; VAS, visual analog scale; \(\mathrm{VO}_2\), maximal oxygen uptake.

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evidence for the suggestion that sleep fragmentation may result in decreased energy expenditure and physical activity.

Therefore, the aim of the present study was to test the hypothesis that energy expenditure, especially physical activity, may be decreased because of disturbed sleep. We thus examined the effect of mild sleep fragmentation compared with nonfragmented sleep on energy expenditure, physical activity, and substrate oxidation in energy balance in a fully controlled situation in a respiratory chamber.

SUBJECTS AND METHODS

Subjects

Fifteen healthy male volunteers [mean ± SD BMI (in kg/m²): 24.1 ± 1.9; age: 23.7 ± 3.5 y] were recruited by advertisements on notice boards at Maastricht University. All volunteers participated in an initial screening that involved measurements of body weight and height and included completion of questionnaires related to eating behavior [Three-Factor Eating Questionnaire (19)], health, use of medication, physical activity, alcohol consumption, food allergies, and smoking and sleeping behavior. Selected subjects were in good health, had a BMI between 20–30, were nonsmokers, were not using any medication, were at most moderate alcohol consumers, were unrestrained eaters (as assessed by factor 1 of the Three-Factor Eating Questionnaire; cutoff ≤9) (19), and were sleeping 7–8 h/night. Baseline characteristics of the subjects are presented in Table 1. Subject recruitment started in June 2009, and the study was conducted between September 2009 and May 2010. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Medical Ethical Committee of Maastricht University Medical Centre. Written informed consent was obtained from all subjects. The study was registered as NTR1919 at www.who.int/isrctn and www.trialregister.nl.

Experimental design

The study had a randomized, single-blinded, crossover design. Subjects came to the university twice with ≥2 wk between sessions. Two days before their stay in the respiratory chamber, subjects were asked to abstain from strenuous exercise and to sleep for 8 h during the nights. During each visit, they stayed for 48 h in a respiration chamber in which energy expenditure, physical activity (radar), and substrate oxidation were measured during a condition of fragmented sleep compared with a condition of nonfragmented sleep. Subjects had fixed bedtimes, indicated by the lights that switched off automatically at 2330 h and switched on at 0730 h, resulting in 8 h sleeping time per night. During the daytime subjects were not allowed to sleep. To ensure this, the researchers addressed them on a regular basis, and polygraphic recordings were made continuously by using EEG (BrainRT Digital EEG System; OSG bvba). Furthermore, during the day, questionnaires concerning sleep and tiredness were completed. Artificial light intensity in the respiration chamber was always >400 lux (Energy Saver Tornado E27 900 lm; Philips Lighting). Fragmentation of sleep was accomplished with approximately hourly wake-up calls that varied in frequency of between 500–2000 Hz and in intensity of between 40–110 dB; subjects confirmed waking up by turning off their alarms after 2 min. Subjects were individually fed in energy balance 2 d before their stay in the respiration chamber. This diet had the same macronutrient composition (ratio of 12:55:33% energy for protein:carbohydrate:fat) as the diet they received during the subsequent stay in the respiration chamber and consisted of normal, everyday food products.

Sleep recordings

Before subjects entered the respiration chamber at 2000 h, electrodes for the EEG, electrocardiogram, electromyogram, and electrooculogram recordings were placed according to the appropriate standardized criteria (20). Polygraphic recordings were obtained throughout the entire 48 h (BrainRT Digital EEG System; OSG bvba). All records were visually scored in 30-s epochs according to the standardized criteria by a skilled researcher who was blinded to the conditions (20).

Energy intake

Calculations for both the diet at home and in the respiration chamber were based on individual average daily energy requirements. The daily energy requirement for the diet at home was estimated as 1.75 times the RMR (21). RMR was calculated with the formula of Harris and Benedict (22). The energy requirement in the respiration chamber was calculated as 1.35 times the measured SMR of the first night. Daily energy intake was divided over 3 meals: breakfast 20%, lunch 40%, and dinner 40%. Breakfast was given at 08:30 h, lunch at 13:30 h and dinner at 18:30 h.

Energy expenditure, physical activity, and substrate oxidation

Subjects stayed in the respiration chambers for 48 h, from 2000 h in the evening on day 1 until 2000 h in the evening of day 3. Energy expenditure, physical activity, and substrate oxidation were measured and calculated according to the similar protocol used in previous studies conducted at the department of Human Biology, Maastricht University (23–25). Body composition was determined between both sessions with the deuterium dilution (1H2O) technique (26, 27).

Questionnaires

The sleeping questionnaire consisted of VAS (in mm) questions on subjective feelings of napping, physical exhaustion, alertness,
sleepiness, and how satisfying the sleep was. Opposing extremes of each feeling were described at either end of a 100-mm horizontal line, and subjects marked the line to indicate how they felt at that moment.

Statistical analysis

Data from energy expenditure, physical activity, RQ, and substrate balances are presented as means ± SEs unless otherwise indicated. Data were analyzed by comparing the condition of fragmented sleep with the condition of nonfragmented sleep. This analysis was applied to SWS, REM, TST, TEE and its components, physical activity, sleeping questionnaire variables, and substrate oxidation. A 2-factor repeated-measures ANOVA was carried out for determination of possible differences between the conditions. The level for establishing significant differences was taken at $P < 0.05$. Data were analyzed by using SPSS version 11 (SPSS Inc.).

RESULTS

The duration of the sleep phases, namely the non-REM sleep stages 1–4, SWS and REM sleep, and TST were reduced during the nights with sleep fragmentation ($P < 0.001$) (Table 2). Between the 2 conditions, TEE and RMR did not differ significantly, whereas AEE significantly ($P < 0.05$) increased with fragmented sleep compared with nonfragmented sleep. In addition, physical activity increased as well with fragmented sleep ($P < 0.02$). SMR, as part of RMR, was not significantly affected by sleep fragmentation (Table 3).

After analyzing the data for measured energy expenditure and energy intake, subjects appeared to be slightly in positive energy balance. Energy balance did not differ significantly between both conditions, which implies that it did not affect the outcomes differently.

Fragmented sleep also coincided with an increase in RQ ($P < 0.05$), increased carbohydrate oxidation ($P < 0.05$), and reduced fat oxidation ($P < 0.01$).

Scores on the sleeping questionnaire differed significantly for exhaustion and sleepiness. Coinciding with fragmented sleep, subjects were more exhausted and sleepier ($P < 0.001$) (Table 3).

DISCUSSION

During fragmented compared with nonfragmented sleep, AEE, RQ, carbohydrate oxidation, physical activity counts, exhaustion, and sleepiness were elevated, whereas fat oxidation was reduced. TEE and its components RMR and SMR did not differ significantly between the 2 conditions. Subjects woke up ~7 h during the fragmented night, and consequently reductions in TST, SWS, and REM sleep were observed.

Our findings with respect to energy expenditure correspond to those reported by Nedeltcheva et al (28). Other studies (29–31) also found an increase in metabolic rate initiated after an (acute) disturbance of sleep. A study by Brevetti et al (32) reported that VO$_2$ was related to sleep stages, with the highest VO$_2$ during REM sleep and the lowest VO$_2$ during SWS. Fontvieille et al (33) concluded that sleep stages were associated with small differences in metabolic rate. Bonnet et al (34) have shown that even arousals during sleep, without waking up the subjects, caused elevations in VO$_2$ for another 3–9 min. The small differences in metabolic rate they measured over relatively short periods of time in partly controlled conditions may be transient in nature. Our study was fully controlled and corresponds with the results of Nedeltcheva et al (28) in which no effect on TEE was found. In addition, our results showed an increase in RQ and carbohydrate oxidation, whereas fat oxidation was reduced.

Nedeltcheva et al (28) reported no differences in TEE after measuring 10 subjects, who spent either 5.5 or 8.5 h time in bed, with doubly labeled water for 14 d during caloric restriction. They found decreased RMR after only 5.5 h in bed compared with 8.5 h, which may be an effect of a decrease in SMR because of fewer sleeping hours. In addition, they reported that subjects who spent 5.5 h in bed lost less fat mass and more fat-free mass than did subjects spending 8.5 h in bed. Comparable to our results, they also reported that RQ increased in the short sleepers, which implies less fat oxidation and more carbohydrate oxidation (28). These results were explained as the increased need for glucose by the brain in the wake state and other glucose-dependent tissues, thereby converting body protein into glucose (34). Despite our similar outcomes, we think that our results were primarily caused by mild sleep fragmentation and less because of a reduction in TST. TST was reduced.

### Table 2

<table>
<thead>
<tr>
<th>Sleep recordings</th>
<th>Nonfragmented sleep</th>
<th>Fragmented sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (min)</td>
<td>10.5 ± 0.9</td>
<td>4.0 ± 0.4*</td>
</tr>
<tr>
<td>S2 (min)</td>
<td>247.3 ± 7.7</td>
<td>214.0 ± 7.0*</td>
</tr>
<tr>
<td>SWS (min)</td>
<td>106.8 ± 7.3</td>
<td>88.3 ± 5.8*</td>
</tr>
<tr>
<td>REM (min)</td>
<td>111.9 ± 5.4</td>
<td>88.9 ± 4.4*</td>
</tr>
<tr>
<td>TST (min)</td>
<td>476.5 ± 5.9</td>
<td>395.3 ± 10.3*</td>
</tr>
<tr>
<td>Wake (min)</td>
<td>3.5 ± 8.3</td>
<td>84.7 ± 9.2*</td>
</tr>
</tbody>
</table>

*Significantly different from nonfragmented nights, $P < 0.001$.

### Table 3

| Outcome variables: energy expenditure, substrate oxidation, physical activity, and sleeping questionnaire
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Nonfragmented sleep</td>
<td>Fragmented sleep</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>TEE (MJ/d)</td>
<td>9.83 ± 0.13</td>
</tr>
<tr>
<td>RMR (MJ/d)</td>
<td>8.41 ± 0.05</td>
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<tr>
<td>SMR (MJ/d)</td>
<td>7.49 ± 0.10</td>
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<tr>
<td>AEE (MJ/d)</td>
<td>1.42 ± 0.13</td>
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<tr>
<td>CHOox (g/d)</td>
<td>323.7 ± 22.5</td>
</tr>
<tr>
<td>FATox (g/d)</td>
<td>61.0 ± 6.6</td>
</tr>
<tr>
<td>RQ</td>
<td>0.91 ± 0.03</td>
</tr>
<tr>
<td>EB (MJ/d)</td>
<td>+0.42 ± 0.13</td>
</tr>
<tr>
<td>PA (radar counts)</td>
<td>2204 ± 124</td>
</tr>
<tr>
<td>VAS (mm)</td>
<td>21.8 ± 2.4</td>
</tr>
<tr>
<td>Sleepiness</td>
<td>33.9 ± 4.6</td>
</tr>
</tbody>
</table>

*Significantly different from nonfragmented nights: $^*P < 0.05$, $^{**}P < 0.01$, $^*P < 0.001$. All values are means ± SEs.
by 1.0–1.5 h in our study, which is a minor reduction compared with the 3–4-h reduction in previous studies (14, 28). Despite the smaller reduction of TST, the mild sleep fragmentation caused a larger reduction in REM and SWS compared with the sleep restriction studies in which SWS often is preserved (14, 28). Mild sleep fragmentation mainly appears to affect REM sleep and SWS in comparison with reduced TST, which mostly affects REM.

Fontvieille et al. (33) reported a reduced RQ during the REM stage and an inverse correlation between REM-sleep duration and fat mass. Therefore, it is suggested that during REM-sleep fat oxidation takes place. Accordingly, when REM sleep was reduced, we observed reduced fat oxidation and increased carbohydrate oxidation. This may underscore diminished insulin sensitivity, which we observed in the same subjects during a 24-h test in which insulin concentrations appeared to be elevated without affecting glucose concentrations after a disturbed night (HKJ Gonnissen, R Hursel, F Rutters, EAP Martens, MS Westerterp-Plantenga, unpublished observations, 2010). Our findings again confirmed earlier observations (6, 7, 10, 14, 35). This shift in substrate oxidation, which implied increased carbohydrate oxidation, may also underscore previously reported increases in food intake by snack consumption (16, 18). Bosy-Westphal et al. (36) also showed an increase in carbohydrate oxidation, though they did not find a reduction in insulin sensitivity nor did they observe an effect of 4 nights of consecutive increasing sleep curtailment on TEE. Contrary to our observations, however, Katayose et al. (37) observed that REM sleep was associated with increased energy expenditure, RQ, and carbohydrate oxidation and decreased fat oxidation. In a different study, with total sleep deprivation compared with normal sleep duration, Benedict et al. (38) examined the effects on RMR and postprandial energy expenditure during the following day, with a ventilated hood system. They reported that fasting and postprandial energy expenditure decreased by 5% and 20%, respectively, after total sleep deprivation. The main difference in our study was that our study was conducted in a respiratory chamber in which subjects could move more freely and Benedict et al. (38) performed measurements with a ventilated hood system in which subjects had to lie calmly on a bed. It appears that different outcomes were observed depending on the design of the study and on the accuracy of the measurement methods. In our study, we aimed to approach daily life circumstances under controlled conditions. In daily life, sleep fragmentation often occurs, and this may have a small but cumulative effect on energy balance. We showed that after 2 fragmented nights, metabolic targets for a positive energy balance were affected.

Physical inactivity also may play a role in the etiology of obesity. Speculations about a decrease in physical activity after fragmented sleep were confirmed by Schmid et al. (39) who reported that short-term sleep loss decreased physical activity under free-living conditions. Because of our study design, we showed an initial increase in physical activity and AEE as an effect of sleep fragmentation, mainly because the subjects had to turn off their alarm clock 7 times during the night. However, the resulting increased exhaustion and sleepiness during the subsequent day might eventually counter-balance physical activity and AEE. Furthermore, the increased activity that we showed corresponds with the increased carbohydrate oxidation, thus depleting glycogen storage.

A possible limitation of the present study was the mild sleep disturbance, in comparison with the studies that used 4-h sleep deprivation. At the same time, our study shows that even after mild sleep disturbance, which often occurs naturally in today’s society with our modern lifestyle, there was a shift in substrate oxidation. Further limitations might be that the acute effects from this study cannot be extrapolated to chronic effects because adaptations might occur. Therefore, more research over the long term is necessary to establish the initial observations. In that respect, our observations correspond very well to those made over the long term by Nedeltcheva et al. (28). Also, the effect of sleep fragmentation needs to be studied in women because the present study was conducted in men only. Finally, our results may only partly resemble real life, because the space for physical activity in the respiration chamber is limited, and because we did not allow our subjects to sleep during the day.

In conclusion, sleep fragmentation while staying in a respiration chamber for 48 h induced reductions in the most important sleep phases, coinciding with elevated AEE, physical activity, exhaustion, sleepiness, RQ, and carbohydrate oxidation and reduced fat oxidation, which may predispose to overweight.

The authors’ responsibilities were as follows—RH, FR, HKJG and MSW-P: designed the study; RH, FR, HKJG, and EAPM: collected and analyzed the data; RH: wrote the manuscript; and MSW-P: contributed to the interpretation of the data and reviewed the manuscript. The study was executed under the supervision of MSW-P. None of the authors had a personal or financial conflict of interest.

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35. 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;94:342–50.


