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# Overnight energy expenditure determined by whole-body indirect calorimetry does not differ during different sleep stages<sup>1–3</sup>

Hanne KJ Gonnissen, Mathijs Drummen, Núria Rosique Esteban, Paul FM Schoffelen, and Margriet S Westerterp-Plantenga

## ABSTRACT

**Background:** Sleep has been associated with the regulation of energy balance, yet the relation between sleep stages and energy expenditure remains unclear.

**Objective:** The objective was to investigate the relation between sleep stages and energy expenditure, with sleep stage and overnight energy expenditure patterns taken into account.

**Design:** Thirteen subjects aged (mean  $\pm$  SD)  $24.3 \pm 2.5$  y with a BMI (in  $\text{kg}/\text{m}^2$ ) of  $23.6 \pm 1.7$  slept in a respiration chamber while sleep was polysomnographically recorded to determine wake after sleep onset (WASO), slow-wave sleep (SWS), and rapid eye movement (REM) sleep. Energy expenditure was calculated during each sleep stage for the whole night and separately for sleeping metabolic rate (SMR; ie, 3-h period during the night with the lowest mean energy expenditure) and non-SMR.

**Results:** Energy expenditure and sleep stages showed characteristic patterns during the night, independently of each other. Sleep stages exerted no effect on energy expenditure during the whole night, except for WASO compared with SWS ( $P < 0.05$ ) and WASO compared with REM sleep ( $P < 0.05$ ). During the SMR and non-SMR periods of the night, no overall effect of sleep stage on energy expenditure, except for WASO compared with SWS ( $P < 0.05$ ) and WASO compared with REM sleep ( $P < 0.01$ ) during the non-SMR period of the night, was found. Energy expenditure and activity counts during the night were positively correlated ( $r = 0.927$ ,  $P < 0.001$ ).

**Conclusions:** Energy expenditure does not vary according to sleep stage overnight, except for higher energy expenditure during wake episodes than during SWS and REM sleep. Coincidence of the sleep stage pattern and the overnight energy expenditure pattern may have caused accidental relations in previous observations. This trial was registered at <http://apps.who.int/trialsearch> as NTR2926. *Am J Clin Nutr* 2013;98:867–71.

## INTRODUCTION

The increased prevalence of obesity has developed over the same period as a reduction in sleep duration; therefore, recent research has attempted to unravel the role of sleep in the etiology of obesity (1). Experimental studies have shown that reduced time in bed (2) and changed sleep architecture (3, 4), including sleep fragmentation (5, 6), are related to a positive energy balance, ie, increased energy intake and/or decreased energy expenditure. Increased

energy intake may be directly caused by an increase in food intake (7) or indirectly by changes in appetite-regulating hormones (5, 8). Decreased energy expenditure may result from a decrease in physical activity (9). However, higher 24-h energy expenditure has been shown during short-term sleep restriction (10). Moreover, Hursel et al (6) showed higher physical activity during fragmented sleep. Important to note is the effect of the study design; in the study by Hursel et al, subjects had to turn off their alarm clock 7 times during the night, which led to higher activity-induced energy expenditure (6). This illustrates that it is difficult to draw conclusions about the effects of sleep disturbances on energy expenditure (11). Elucidating the relation between sleep and energy expenditure has raised the question of whether energy expenditure is influenced by sleep architecture. In previous work, we pointed out the importance of changes in sleep architecture and observed that energy balance variables are primarily related to the amount of slow-wave sleep (SWS)<sup>4</sup> and rapid eye movement (REM) sleep rather than to total sleeping time (3). Moreover, positive correlations have been found for total energy expenditure with quality sleep and SWS (12). Others have attempted to unravel relations between overnight energy expenditure and sleep stages, but have reported equivocal results (13–17). Therefore, it is still necessary to assess whether overnight energy expenditure is different according to different sleep stages, to reveal how sleep architecture affects energy expenditure and therefore energy balance.

The current study aimed to investigate the relation between sleep stages and energy expenditure. For this purpose, the effects of sleep stages on energy expenditure were determined by comparing energy expenditure during each sleep stage, with sleep stage distribution and the overnight energy expenditure pattern

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<sup>4</sup> Abbreviations used: REM, rapid eye movement; SMR, sleeping metabolic rate; SPT, sleep period time; SWS, slow-wave sleep; WASO, wake after sleep onset.

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taken into account. The study was performed in energy balance in a fully controlled condition in a respiration chamber.

## SUBJECTS AND METHODS

For the current study, a new analysis was performed from data previously collected by Gonnissen et al (18).

### Subjects

The subject group consisted of 13 healthy young adults (7 men and 6 women) with a mean ( $\pm$ SD) age of  $24.3 \pm 2.5$  y and a mean ( $\pm$ SD) BMI (in  $\text{kg}/\text{m}^2$ ) of  $23.6 \pm 1.7$ . The power calculation was based on previous research in the respiration chamber (19). Given  $\alpha = 0.05$  and  $\beta = 0.10$  (power  $1 - \beta = 0.90$ ),  $\geq 11$  subjects were needed. Subjects were recruited by advertising on notice boards at Maastricht University and underwent an initial screening to measure body weight and height and to complete questionnaires related to health, use of medications, smoking behavior, alcohol consumption, physical activity, eating behavior, food allergies, and sleeping behavior. Before enrollment of the subjects, screening questionnaires were evaluated on the basis of inclusion and exclusion criteria. All subjects were in good health, were nonsmokers, did not use medications, were moderate alcohol consumers, and did not have sleep disorders. In addition, subjects did not follow a diet and did not participate in an exercise program.

All participants provided written informed consent. The study was conducted in accordance with guidelines of the Declaration of Helsinki, and the Medical Ethical Committee of Maastricht University Medical Centre approved all procedures that involved human subjects. The study was registered in the International Clinical Trials Registry Platform (18).

### Study design

On the day of the experiment, subjects arrived at the university at 1800, and electrodes for the polysomnographic measurement were applied according to standardized criteria (18, 20). At 2000, subjects entered the respiration chamber. The respiration chamber provides a highly controlled situation in which the subjects can move freely. The subjects were instructed to sleep from 2330 until 0830, which provided a time in bed of 9 h to be able to achieve their habitual sleep duration. During the stay in the chambers, subjects were fed in energy balance and were not allowed to have daily naps or perform physical activity. Two days before the experiment, the subjects were asked to sleep according to their habitual sleep duration ( $490.55 \pm 16.42$  min) and were fed in energy balance according to individual energy requirements. A more detailed description of the study design can be found in the article by Gonnissen et al (18).

### Energy expenditure

The respiration chamber is a  $14\text{-m}^3$  room furnished with a bed, deep-freeze toilet, sink with hot and cold running water, chair, desk, telephone, television, and Internet connection. During the stay in the respiration chamber, oxygen consumption and carbon dioxide production were measured. The room was ventilated with fresh air at a rate of 70 to 80 L/min by using electronically modified dry gas meters (G6; Schlumberger). Air recirculation

flow was 1100 L/min and was measured by using electronically modified dry gas meters (G6; Schlumberger). Oxygen levels in the chamber were measured with magnetic oxygen analyzers (model 4100, Servomex; ABB/Hartman&Braun Magnos). Carbon dioxide levels were measured with infrared carbon dioxide analyzers (ABB/Hartman&Braun Uras). The gas samples to be measured were selected by a computer that stored and processed the data (18, 21). Physical activity was measured by using an analog ultrasound Doppler system (Advisor DU160; Aritech BV). The activity was calculated in total counts and provides information on small movements of the subjects during the night.

### Sleep monitoring

To measure the different sleep stages, polysomnographic recordings were obtained during the whole night (2330–0830) by using the BrainRT digital electroencephalogram system (OSG BVBA). Electrodes for electroencephalogram, 2 central (C3-A2 and C4-A1) and 2 occipital (O1-A2 and O2-A1), bilateral electrooculograms, and submental electromyograms were applied according to standardized criteria (18, 20). The different stages scored included wake after sleep onset (WASO), sleep stage 1, sleep stage 2, SWS, and REM sleep. Sleep period time (SPT) is defined as the time between sleep onset and awaking, including WASO. Total sleeping time is defined as the time between sleep onset and awaking, excluding WASO. The sleep latency is defined as the time to fall asleep in minutes. Hypnograms from each subject were plotted, including sleep stages between 2330 and 0830 by using GraphPad Prism software version 5.00 for Windows (GraphPad Software).

### Data analysis

The current study aimed at investigating the effects of sleep stages on overnight energy expenditure. For this purpose we first calculated mean overnight energy expenditure; second, we calculated energy expenditure for each sleep stage during the SPT (including wake episodes during the night) in all subjects. To investigate whether the general energy expenditure pattern could have an influence on our results, the night was divided into 2 periods: sleeping metabolic rate (SMR) and non-SMR periods. SMR is the 3-h period during the night with the lowest mean energy expenditure (22). Non-SMR is the remainder of the night, in which energy expenditure is more influenced by external factors. SMR was determined for each subject individually. In addition, the duration of sleep stages in SMR and non-SMR was compared to determine whether our results could be explained by the coincidence of the unequal distribution of sleep stages and the lower or higher mean energy expenditure during the night. Energy expenditure was calculated in kJ/min at 5-min intervals by using the formula of Weir (21). Sleep records were visually scored in 30s-epochs by an experienced researcher following the standard guidelines (18, 20). Only those sleep stages with a duration  $\geq 1$  min were included for analysis (17). Given that values of energy expenditure and sleep are obtained with different time intervals, the exact times of starting and ending a sleep stage were synchronized with energy expenditure data. All sleep recordings were delayed by 2 min to account for the response time of the analyzers to air changes in the respiration chamber.

## Statistical analysis

Data for energy expenditure, sleep stage duration, SPT, total sleeping time, SMR, non-SMR, sleep latency, and physical activity are presented as means  $\pm$  SEMs, unless otherwise indicated. Data were analyzed by comparing energy expenditure during the different sleep stages within the night and within each period (ie, SMR and non-SMR) of the night. Differences in energy expenditure between sleep stages were determined with repeated measures 1-factor ANOVA with Bonferroni post hoc for multiple comparisons. Repeated-measures 1-factor ANOVA was used to compare mean energy expenditure during each period of the night (SMR compared with non-SMR). To elucidate whether the observed changes in overnight energy expenditure are explained by variations in physical activity, Pearson correlation analysis was performed to analyze the relation between them. Data were analyzed by using SPSS 18 software, and figures were generated by using GraphPad Prism software version 5.00 for Windows (GraphPad Software).

## RESULTS

### Sleep stages and overnight energy expenditure

An example of the variations in magnitude of energy expenditure during the progress of the night, in combination with the sleep architecture of one subject, is shown in **Figure 1**. Sleep stage distribution during the night for all subjects is shown in **Table 1**. The average SPT of the subjects was  $510.31 \pm 5.78$  min with a sleep latency of  $31.34 \pm 5.71$  min. The average total sleeping time, which is the SPT without wake episodes during the night, was  $493.65 \pm 7.35$  min. Energy expenditure during WASO and the different sleep stages during SPT are shown in **Figure 2**. During SPT, there was no overall effect of stages on overnight energy expenditure, except for WASO ( $P = 0.01$ ). Average energy expenditure during WASO was significantly higher than during SWS ( $4.96 \pm 0.29$  kJ/min compared with  $4.37 \pm 0.12$  kJ/min;  $P < 0.05$ ) and during REM sleep ( $4.96 \pm 0.29$  kJ/min compared with  $4.44 \pm 0.15$  kJ/min;  $P < 0.05$ ).

**TABLE 1**

Absolute and relative duration of sleep-related variables during the whole night, the SMR period of the night, and the non-SMR period of the night<sup>1</sup>

Sleep variable	Whole night	SMR	Non-SMR
SPT (min)	$510.31 \pm 5.78$	180	$330.27 \pm 5.78$
WASO			
(min)	$16.65 \pm 4.86$	$5.69 \pm 2.44$	$10.96 \pm 4.29$
(%SPT)	$3.26 \pm 0.97$	$3.16 \pm 1.35$	$3.31 \pm 1.33$
Stage 1			
(min)	$5.50 \pm 1.82$	$0.62 \pm 0.50$	$4.88 \pm 1.69$
(%SPT)	$1.05 \pm 0.34$	$0.34 \pm 0.28$	$1.42 \pm 0.48$
Stage 2			
(min)	$246.88 \pm 10.88$	$86.85 \pm 7.47$	$160.04 \pm 9.34$
(%SPT)	$48.40 \pm 2.07$	$48.25 \pm 4.15$	$48.52 \pm 2.76$
SWS			
(min)	$130.92 \pm 14.11$	$39.69 \pm 8.49$	$91.23 \pm 12.38$
(%SPT)	$25.76 \pm 2.78$	$22.05 \pm 4.72$	$27.81 \pm 3.76$
REM sleep			
(min)	$110.31 \pm 7.88$	$47.15 \pm 5.94$	$63.15 \pm 7.48$
(%SPT)	$21.53 \pm 1.40$	$26.20 \pm 3.30$	$18.93 \pm 2.15$

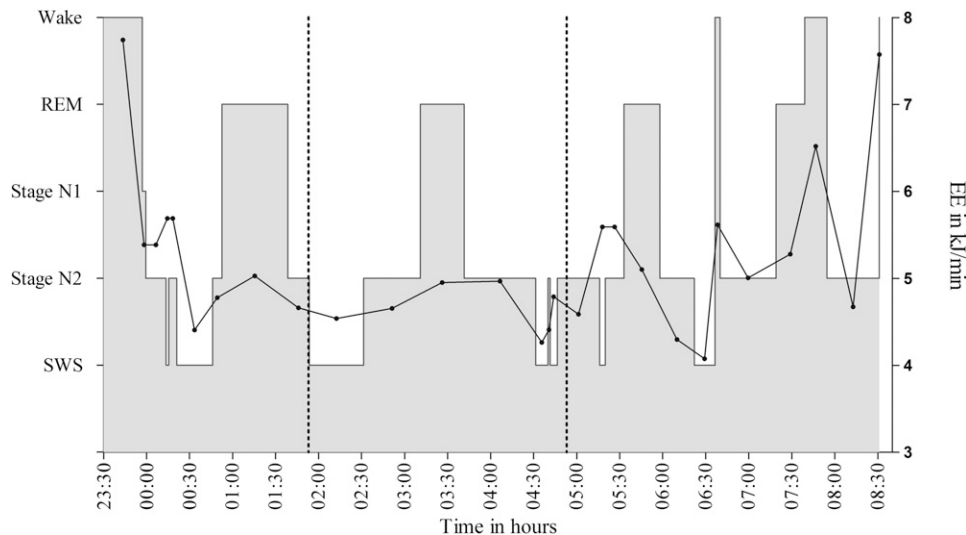
<sup>1</sup>All values are means  $\pm$  SEMs;  $n = 13$ . There were no significant differences between SMR and non-SMR (repeated-measures 1-factor ANOVA). REM, rapid eye movement; SMR, sleeping metabolic rate; SPT, sleep period time; SWS, slow-wave sleep; WASO, wake after sleep onset.

### Distribution of sleep stages between SMR and non-SMR

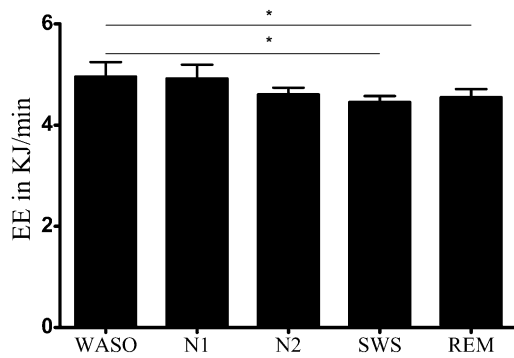
The night was divided for each subject individually into SMR and non-SMR. The SMR is the 3-h period (ie, 180 min) during the night with the lowest mean energy expenditure. The average non-SMR sleep period was  $330.31 \pm 5.78$  min. No significant differences in the relative durations of sleep stages were found between the SMR and the non-SMR periods of the night (Table 1).

### Sleep stages and energy expenditure during SMR and non-SMR

Mean energy expenditure of all subjects was significantly lower during SMR than during non-SMR ( $4.32 \pm 0.13$  kJ/min compared with  $4.73 \pm 0.14$  kJ/min;  $P < 0.001$ ). Energy expenditure during



**FIGURE 1.** Hypnogram of one representative subject together with changes in EE during one night. Sleep architecture during the night (from 2330 until 0830) is shown in gray. The black line indicates EE during the night. The dotted lines delimit the sleeping metabolic rate for this subject (0153 until 0453). EE, energy expenditure; N1, sleep stage 1; N2, sleep stage 2; REM, rapid eye movement; SWS, slow-wave sleep.



**FIGURE 2.** Mean ( $\pm$ SEM) changes in EE per sleep stage during sleep period time ( $n = 13$ ). \*EE during WASO significantly different from during SWS and REM sleep,  $P < 0.05$  (repeated-measures ANOVA with Bonferroni post hoc correction). EE, energy expenditure; N1, sleep stage 1; N2, sleep stage 2; REM, rapid eye movement; SWS, slow-wave sleep; WASO, wake after sleep onset.

WASO, SWS, and REM sleep within the SMR and non-SMR periods of the night is summarized in **Table 2**. No overall effect of stages on energy expenditure was found during the SMR. No overall effect of stages on energy expenditure was found during non-SMR, except for WASO ( $P = 0.02$ ). Within non-SMR, average energy expenditure during WASO was significantly higher than during SWS ( $5.19 \pm 0.28$  kJ/min compared with  $4.54 \pm 0.12$  kJ/min;  $P < 0.05$ ) and than during REM sleep ( $5.19 \pm 0.28$  kJ/min compared with  $4.60 \pm 0.19$  kJ/min;  $P < 0.01$ ).

### Overnight energy expenditure in relation to nocturnal physical activity

The average overnight energy expenditure was  $4.65 \pm 0.47$  kJ/min. A general pattern in overnight energy expenditure was shown for most of the subjects when energy expenditure values during the night were plotted (**Figure 3**; solid line). When radar counts were plotted during the night, the same pattern as for energy expenditure was shown (Figure 3; dotted line). Overnight energy expenditure was positively correlated with overnight radar counts ( $r = 0.927$ ,  $P < 0.001$ ).

### DISCUSSION

No overall effect of sleep stage on overnight energy expenditure, except for wake episodes during the night, by whole-body indirect calorimetry, was shown. Energy expenditure during WASO was significantly higher than during SWS and REM sleep over the whole night. However, overnight energy expenditure was not different between sleep stages 1 and 2, REM sleep, and SWS. Therefore, it may be concluded that there was no intrinsic effect of a particular sleep stage on energy expenditure. Because the only small increase in energy expenditure during the night took place during intermittent wake episodes, and because energy expenditure was positively related to the radar counts, we concluded that some movement during WASO may have caused the slight increase in energy expenditure. For closer inspection of the data, the night was divided into the SMR and non-SMR periods. No significant differences in the relative duration of the sleep stages between the SMR and the non-SMR periods of the night were observed. Sleep stage distribution was the same in the SMR and non-SMR periods

**TABLE 2**

Mean energy expenditure in SMR and non-SMR and during WASO, SWS, and REM sleep within SMR and non-SMR<sup>1</sup>

	Total	WASO	SWS	REM
SMR (kJ/min)	$4.32 \pm 0.13$	$4.19 \pm 0.27$	$3.84 \pm 0.15$	$4.12 \pm 0.23$
non-SMR (kJ/min)	$4.73 \pm 0.14^2$	$5.19 \pm 0.28$	$4.54 \pm 0.12^3$	$4.60 \pm 0.19^3$

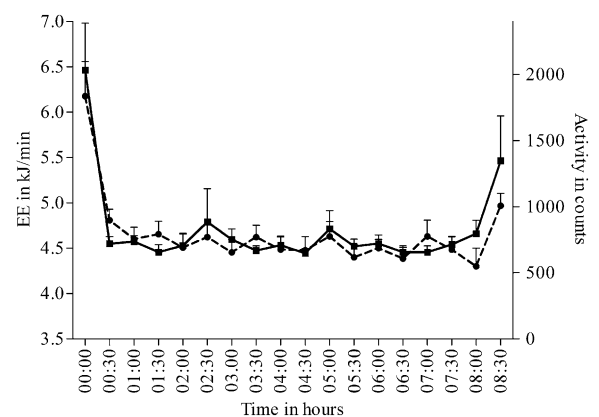
<sup>1</sup> All values are means  $\pm$  SEMs. REM, rapid eye movement; SMR, sleeping metabolic rate; SWS, slow-wave sleep; WASO, wake after sleep onset.

<sup>2</sup> Significantly different from SMR,  $P < 0.05$  (repeated-measures ANOVA with Bonferroni post hoc correction).

<sup>3</sup> Significantly different from WASO within the same period,  $P < 0.05$  (repeated-measures ANOVA with Bonferroni post hoc correction).

of the night. Sleep duration and wake-up time during the experiment were equal to habitual sleep duration and wake-up time.

In line with our results, Jung et al (17) found higher energy expenditure during WASO—the brief awakenings from sleep—than during sleep stages and no significant differences in energy expenditure between sleep stages. In addition, Ryan et al (13) also showed higher oxygen consumption during wakefulness than during other sleep stages by using a ventilated-hood method. Only Katayose et al (14) reported a higher energy expenditure during REM sleep than during SWS. In contrast with the current study, Katayose et al did not include wake episodes in their analyses (14). Fontvieille et al (16) suggested a minor role of sleep stages in the variance of SMR among subjects. However, our results showed the same sleep stage distribution during the SMR and non-SMR periods of the night. Therefore, previously shown associations between sleep stages and energy expenditure may be explained as accidental relationships because of a coincidence of the sleep stage pattern and the overnight energy expenditure pattern. During sleep, the human body cycles between stages of non-REM and REM sleep. The non-REM sleep consists of 4 stages of progressively deeper sleep. A sleep cycle begins with a short period of sleep stage 1 that progresses through sleep stage 2, followed by sleep stage 3 and sleep stage 4, and finally to REM sleep. Sleep stage 3 and sleep stage 4 are collectively referred to as SWS. REM sleep increases as the night progresses and is longest during the last one-third of the sleep cycle, whereas SWS occurs predominately during the first third of the night (23). Rather than remaining stable, overnight energy expenditure varies throughout the night. Immediately after sleep



**FIGURE 3.** Mean ( $\pm$ SEM) overnight EE (solid line) and physical activity counts (dotted line) ( $n = 13$ ) in 30-min intervals. Both EE and activity decreased after sleep onset and increased before awakening. EE, energy expenditure.

onset, overnight energy expenditure was at its highest value because of diet-induced thermogenesis of the dinner and the remaining effect of physical activity during the day (24–26). After approximately 1 h, overnight energy expenditure starts to decrease. At the end of the night before the subject wakes up, the overnight energy expenditure starts to rise again (16, 17, 22, 24) as a result of increased energy costs of arousal. Combined with the unequal distribution of the sleep stages, it is plausible that the differences in energy expenditure are attributable to a random coincidence of the pattern of energy expenditure and the pattern of sleep stages. Our finding of the same sleep stage distribution during SMR and non-SMR further confirms this.

In the current study, energy expenditure was determined by means of whole-body indirect calorimetry in 5-min intervals. The analysis of energy expenditure over an interval of 5 min may lead to partial overlapping of energy expenditure between sleep stages with a shorter duration, yet only sleep stages >1 min were included. The use of a whole-body calorimeter represents an advantage over other techniques, such as a ventilated hood, as used in previous studies (13, 16) because whole-body calorimetry facilitates the natural sleeping habit of the subject more and provides a 24-h continuous analysis of energy expenditure within a confined space. In addition, it allows determination of physical activity by radar, including small movements of the subjects during the sleep period. Our results showed a positive correlation between overnight energy expenditure and activity counts during the night, which explained the higher energy expenditure observed during non-SMR than during SMR. Furthermore, it may explain the higher energy expenditure previously found after sleep disturbance (6, 10). Sleep disturbance may result in higher energy expenditure via increased activity-induced energy expenditure because of more wakefulness resulting from a shorter time in bed (10) or fragmented sleep (6). However, sleep disturbance also results in higher energy intake (5, 7, 8), and the increased energy intake seems to overrule the increased energy expenditure, which eventually leads to a positive energy balance (12).

In conclusion, sleep stages do not differently affect overnight energy expenditure in healthy subjects. This was confirmed by comparing energy expenditure and duration of sleep stages within the SMR and non-SMR periods of the night. The differences in overnight energy expenditure between sleep stages previously described by other authors (13, 14, 16) may be accidental relations caused by coincidence of the sleep stage pattern and the overnight energy expenditure pattern.

The authors' responsibilities were as follows—HKJG and MSW-P: designed the study; HKJG: conducted the research; HKJG, MD, and NRE: wrote the manuscript; HKJG, MD, NRE, and PFMS: analyzed the data; and MSW-P: supervised the execution of the study and reviewed the manuscript. None of the authors had a personal or financial conflict of interest.

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