Nighttime snacking reduces whole body fat oxidation and increases LDL cholesterol in healthy young women

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1Health Care Food Research Laboratories, Kao Corporation, Tokyo, Japan; 2Department of Nutrition and Food Science, Ochanomizu University, Tokyo, Japan; 3Department of Home Economics, Tokyo Kasei University, Tokyo, Japan; and 4Department of Human Nutrition, SeiToku University, Chiba, Japan

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Hibi M, Masumoto A, Naito Y, Kiuchi K, Yoshimoto Y, Matsumoto M, Katashima M, Oka J, Ikemoto S. Nighttime snacking reduces whole body fat oxidation and increases LDL cholesterol in healthy young women. Am J Physiol Regul Integr Comp Physiol 304: R94–R101, 2013. First published November 28, 2012; doi:10.1152/ajpregu.00115.2012.—The increase in obesity and lipid disorders in industrialized countries may be due to irregular eating patterns. Few studies have investigated the effects of nighttime snacking on energy metabolism. We examined the effects of nighttime snacking for 13 days on energy metabolism. Eleven healthy women (means ± SD; age: 23 ± 1 y; body mass index: 20.6 ± 2.6 kg/m2) participated in this randomized crossover trial for a 13-day intervention period. Subjects consumed a specified snack (192.4 ± 18.3 kcal) either during the daytime (10:00) or the night time (23:00) for 13 days. On day 14, energy metabolism was measured in a respiratory chamber without snack consumption. An oral glucose tolerance test was performed on day 15. Relative to daytime snacking, nighttime snacking significantly decreased fat oxidation (daytime snacking: 520 ± 13.6 g/day; nighttime snacking: 45.8 ± 14.0 g/day; P = 0.02) and tended to increase the respiratory quotient (daytime snacking: 0.878 ± 0.022; nighttime snacking: 0.888 ± 0.021; P = 0.09). The frequency of snack intake and energy intake, body weight, and energy expenditure were not affected. Total and low-density lipoprotein (LDL) cholesterol significantly increased after nighttime snacking (152 ± 26 mg/dl and 161 ± 29 mg/dl; P = 0.03 and 76 ± 20 mg/dl and 83 ± 24 mg/dl; P = 0.01, respectively), but glucose and insulin levels after the glucose load were not affected. Nighttime snacking increased total and LDL cholesterol and reduced fat oxidation, suggesting that eating at night changes fat metabolism and increases the risk of obesity.

Obesity; dietary habit; night eating; circadian timing; energy expenditure; obesity

Night eating syndrome (NES), characterized by a delay in the circadian timing for food intake, is associated with obesity (29, 30). Recent studies reported that NES, diagnosed by nocturnal eating in which 25% or more of the total energy of the day is consumed after the evening meal or by waking up in the middle of the night to eat at least three times per week, is positively associated with a higher body mass index (BMI) (2, 7). Gluck et al. (13) reported that relative to non-nighttime eaters, nighttime eaters consumed more calories per day, especially during the night, and gained more weight over a 3-yr follow-up period. Night eating is more common among older women than in younger women, and it has been reported that 22% of 19-yr-old girls were defined as night eaters (25% or more calories eaten after dinner) (28). Moreover, participants with NES had higher glucose/insulin levels and lower ghrelin levels during the night relative to those without NES (1). Delayed circadian rhythm related to food intake associated with NES was accompanied by a similar delay in the circadian rhythm of food regulatory hormones (15). These studies argued that night eating may promote weight gain due to the positive energy balance created by evening hyperphagia and excessive energy intake during the night.

Although the positive energy balances associated with NES have been explained by weight gain, it is unclear whether altered daily eating pattern, per se, with NES affects the whole body energy metabolism. Recent animal studies have demonstrated a relationship between circadian rhythmicity and energy metabolism (9, 27, 35), and the timing of food intake itself reportedly influences weight gain (3). However, few intervention studies have examined the effects of nighttime eating on energy and glucose metabolism. Gluck et al. (14) demonstrated that individuals who exhibited habitual nighttime eating behavior had increased respiratory quotient (RQ) and decreased fat oxidation. Their study was conducted in healthy, but obese, Pima Indian and white subjects. In these subjects, energy metabolism was, at least in part, affected by energy intake (13). In a study examining how night eating patterns affect energy and glucose metabolism during a 24-h awake period, Holmback et al. (19) found no definitive effect on energy expenditure and substrate utilization when subjects followed the nighttime eating protocol. While this was certainly an important pioneering study, the intervention period may have been too short and/or the experimental protocol may not have represented the daily routine enough to effectively evaluate how nighttime eating affects energy metabolism in a nonobese population.

To test the hypothesis that nighttime eating habits, without changing meal frequency, lead to alterations in energy and glucose metabolism, the present study aimed to compare the effects of a 2-wk nighttime snacking intervention on energy metabolism and glucose/lipid metabolism with those of daytime snacking in lean young women. Energy expenditure and substrate utilization were measured using a respiratory chamber following both nighttime and daytime snacking interventions (13 days each). Glucose metabolism was assessed after the interventions by administering an oral glucose tolerance test (OGTT). Individual appetite and food intake during the intervention periods were measured using the visual analogue scale (VAS) questionnaire and food-weighing method.
SUBJECTS AND METHODS

Subjects. Thirteen healthy young women were recruited through poster advertisements and by word of mouth. Inclusion criteria were as follows: 18 to 40 yr of age, BMI between 18.5 and 25, usual eating pattern of 3 meals per day, regular menstrual cycles or taking oral contraceptive pills, and not pregnant or lactating. Exclusion criteria were as follows: eating disorders, smoking, excessive alcohol intake (over 30 g alcohol per day), shift-work, a history or required medication for cardiovascular disease, hypertension, diabetes, hypercholesterolemia, hyperglycemia, hyperlipidemia, allergies to ingredients in the test food, and a weight change of more than 2.0 kg during the 2 mo before the trial. Our study protocol was approved by the Ethics Committee of Ochanomizu University. All subjects provided written informed consent before study commencement.

Experimental design. The study was a randomized crossover design including two 13-day intervention periods comprising daytime snack intervention and nighttime snack intervention. A washout period of ~2 wk was inserted between interventions. Subjects attended a screening visit, a baseline visit, and two laboratory visits at the end of each intervention. Each laboratory visit was scheduled to coincide with the same point of the menstrual cycle during the trial period because all subjects were premenopausal. However, because self-reported and serum levels of progesterone were inconsistent in one subject, we were unable to schedule her visits to coincide with her menstrual cycle. For the daytime snack intervention, subjects were measured in a respiratory chamber in the follicular phase (n = 7) and in the luteal phase (n = 6). For the nighttime snack intervention, subjects were measured in a respiratory chamber in the follicular phase (n = 6) and in the luteal phase (n = 7).

At the baseline visit, subject baseline energy metabolism was determined during a 23-h period in a whole room respiratory chamber at the Kao Health Care Food Research Laboratory (Tokyo, Japan). After an ~2 wk adjustment to their menstrual period, subjects were instructed to consume the specified snack provided by the study coordinator at 10:00 during the 13-day daytime snack treatment period. Subjects then underwent the 23-h measurement period in the respiratory chamber from day 14 to day 15. A fasting blood sample was obtained from each subject on day 15, after which we performed a 75-g OGTT. After being allowed ~2-wk for a washout period to adjust to their menstrual period, subjects were instructed to consume the specified snack at 23:00 for the 13-day nighttime snack treatment. This treatment was also followed by a 23-h measurement period in the respiratory chamber, measurement of fasting blood glucose/insulin levels, and OGTT. The order of treatments (daytime or nighttime snacking) was randomly assigned to each subject. Subjects were allowed to choose their own meals aside from the assigned snacks but were encouraged to maintain their normal timing and location of meals. Subjects were also asked to avoid eating meals outside of breakfast, lunch, dinner, and the designated snack time but were free to drink noncaloric drinks and were instructed to maintain their normal level of physical activity. During the washout period, there were no restrictions or rules regarding food intake and exercise, and subjects were encouraged to maintain their normal lifestyle.

Snacks. Subjects were given commercially available snacks to cycle through a 7-day menu. Snacks comprised ~200 kcal [mean protein:fat:carbohydrate (CHO) ratio of 5:50:45], as shown in Table 1. According to the 2005 dietary intake reference for Japanese women (aged 18–29 yr) who maintain normal levels of physical activity, this caloric amount comprises 10% of the estimated mean energy requirement (1,950 kcal/day) (23).

Laboratory visits. Subjects were instructed to fast overnight for at least 12 h, refrain from exercise, and to avoid alcohol or caffeine-containing beverages for at least 24 h before the laboratory visit. The day before their laboratory visit, they were given a standard meal [738 kcal, 19% of energy (E%) protein, 21E% fat, and 60E% CHO] by 19:00 and required to fast thereafter. Subjects arrived at the laboratory by 07:00 and their height, weight, body temperature, and blood pressure measurements were obtained. They entered the respiratory chamber at 08:00, and energy metabolism was measured for 23 h. Subjects exited the chamber at 07:00 the next morning, and blood samples to measure fasting blood lipids, glucose, and hormone levels were collected at 08:30. For the OGTT, subjects ingested 75 g glucose solution (Trelan G75, Ajinomoto Pharma, Tokyo, Japan), and additional blood samples were obtained 30, 60, and 120 min thereafter.

Body composition measurements. Body weight was measured using a digital balance, accurate to 0.01 kg (CQ100 LW, Ohaus, Pine Brook, NJ) before the measurement of energy metabolism in the respiratory chamber. Body composition was measured using whole body dual-energy X-ray absorptiometry (QRD 4500 W, Hologic, Waltham, MA). Absolute fat mass and fat-free mass were determined using Hologic Systems Software, according to the procedures outlined in the Hologic QRD 4500 User’s Guide.

Food intake assessment. Subjects were interviewed and trained by a registered dietitian to record their previous food intake by the all food-weighing method. To record habitual food intake, subjects weighed and recorded the contents of the meals consumed during the 3 days before the baseline visit. Habitual snack intake and meal frequency were assessed by having each subject fill out a meal and snacking habits questionnaire. Subjects were also asked to provide food intake records for the last 3 days of each intervention (days 11–13 of each intervention). The nutritional value of the meals was calculated by the registered dietitian using nutrition calculation software compliant with the 5th edition of the Standard Tables of Food Composition in Japan (Healthy Maker Pro 501, Mushroon Software, Okayama, Japan).

Appetite questionnaire. Appetite profiles (hunger, fullness, prospective to eat, satiety) were measured with the 100-mm VAS questionnaire (12). The VAS was anchored at each end to...
express the most positive and negative words, which were translated into Japanese from English. Daily appetite profiles were recorded before consumption of breakfast, lunch, and dinner during the 13-day treatment periods. While subjects were in the respiratory chamber, hourly appetite profiles were obtained 16 times between the time they entered the chamber until sleeping.

Respiratory chamber measurements. Energy metabolism was measured in the respiratory chamber for 23 h at the end of each treatment period as well as at baseline. Breakfast, lunch, and dinner were all prepared by a dietician and provided at 09:00, 14:00, and 19:00. All ingredients were weighed in a metabolic kitchen, and energy intake requirements for each subject were determined according to subject age, height, and weight by multiplying the Harris-Benedict equation (16) by physical activity level of 1.4. Meal composition was 15%E protein, 25E% fat, and 60E% CHO, and meals of identical quantity and composition were provided while subjects underwent the three measurements in the respiratory chamber. Caloric distribution was 30%E breakfast, 30%E lunch, and 40%E dinner. Subjects exercised using a cycle ergometer (Aerobike 75XL-II, Combi Wellness, Tokyo, Japan) to raise their heart rate to 120 beats/min for 20 min at 13:00 and 18:00. They were instructed to be in bed at 23:30 and were awakened at 06:30.

Respiratory chamber measurements were performed by modifying previously described methods (18). Briefly, room temperature was set at 25°C, humidity was controlled at 50%, and fresh airflow rate was 70 l/min. Oxygen consumption (VO2) and carbon dioxide production (VCO2) were calculated and measured while subjects were in the respiratory chamber, hourly appetite profiles were recorded before consumption of breakfast, lunch, and dinner were all prepared by a dietician and provided at 09:00, 14:00, and 19:00. All ingredients were weighed in a metabolic kitchen, and energy intake requirements for each subject were determined according to subject age, height, and weight by multiplying the Harris-Benedict equation (16) by physical activity level of 1.4. Meal composition was 15%E protein, 25E% fat, and 60E% CHO, and meals of identical quantity and composition were provided while subjects underwent the three measurements in the respiratory chamber. Caloric distribution was 30%E breakfast, 30%E lunch, and 40%E dinner. Subjects exercised using a cycle ergometer (Aerobike 75XL-II, Combi Wellness, Tokyo, Japan) to raise their heart rate to 120 beats/min for 20 min at 13:00 and 18:00. They were instructed to be in bed at 23:30 and were awakened at 06:30.

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to the habitual snack intake form questionnaire completed before the trial, subjects consumed one snack between breakfast and lunch every 4.8 days, one snack between lunch and dinner every 2.3 days, and one snack between dinner and breakfast every 4.4 days. Corresponding snack intake values before the trial from the 3-day diet records according to the food-weighing method were one snack between breakfast and lunch every 3.0 days, one snack between lunch and dinner every 1.2 days, and one snack between dinner and breakfast every 4.7 days. According to dietary records, mean nutrient intake for the 3 days before the trial were as follows: total energy intake 1,932 kcal/day, protein intake 62.9 g/day, fat intake 61.2 g/day, and CHO intake 268.4 g/day.

Food intake. All subjects reported good adherence to snack interventions; mean compliance of specified snack intake was 100% adherence for the daytime snack intervention and 98.2% for the nighttime snack intervention. Dietary records revealed that frequencies of snack intake between breakfast and lunch and between dinner and breakfast were significantly higher during the daytime snack intervention than that during the baseline periods ($P < 0.01$). According to the food-weighing method, mean intake of energy, protein, fat, and CHO during the last 3 day before the interventions did not differ significantly by treatment (Table 3).

Appetite. Mean appetite values (hunger, fullness, prospective to eat, and satiety) before each meal (breakfast, lunch, and dinner) during each intervention are shown in Fig. 1. Mean hunger and prospective to eat values before lunch were significantly higher during the nighttime snack intervention than during the daytime snack intervention ($P = 0.02$ for hunger and $P = 0.04$ for prospective to eat). Mean fullness values before lunch were significantly lower in the nighttime snack intervention than in the daytime snack intervention ($P = 0.01$). No significant differences were noted in appetite values during intervention before breakfast or dinner. We observed no significant difference due to treatment in the four categories of appetite fluctuation over 16 h or in the AUC calculated from respiratory chamber measurements taken after the 13-day treatment (data not shown).

Energy expenditure and substrate utilization. Data for energy expenditure and substrate oxidation in the respiratory chamber are shown in Table 4. TEE and SMR values assessed after each intervention did not differ significantly by treatment.

### Table 3. Mean daily nutrient intake during the last 3 days of each intervention

<table>
<thead>
<tr>
<th></th>
<th>Daytime Snack</th>
<th>Nighttime Snack</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal/day</td>
<td>1,821 ± 316</td>
<td>1,930 ± 248</td>
</tr>
<tr>
<td>Protein, g/day</td>
<td>62.3 ± 8.5</td>
<td>64.5 ± 16.1</td>
</tr>
<tr>
<td>Fat, g/day</td>
<td>61.3 ± 9.5</td>
<td>68.6 ± 15.2</td>
</tr>
<tr>
<td>CHO, g/day</td>
<td>239.4 ± 55.6</td>
<td>246.2 ± 32.7</td>
</tr>
</tbody>
</table>

Data are means ± SD ($n = 11$). No significant differences between the interventions were detected.

Fig. 1. Mean appetite scores before meals during the 13-day interventions. Data are expressed as means ± SD ($n = 11$). *Significantly different from the daytime snack intervention ($P < 0.05$).
Table 4. Twenty-four hour energy expenditure and substrate utilization after the 13-day interventions

<table>
<thead>
<tr>
<th></th>
<th>Daytime Snack</th>
<th>Nighttime Snack</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEE, kcal/day</td>
<td>1,659 ± 129</td>
<td>1,674 ± 152</td>
</tr>
<tr>
<td>SMR, kcal/day</td>
<td>1,139 ± 85</td>
<td>1,149 ± 109</td>
</tr>
<tr>
<td>RQ</td>
<td>0.878 ± 0.022</td>
<td>0.888 ± 0.021</td>
</tr>
<tr>
<td>Fat oxidation, g/day</td>
<td>52.0 ± 13.6</td>
<td>45.8 ± 14.0*</td>
</tr>
<tr>
<td>CHO oxidation, g/day</td>
<td>303.0 ± 37.8</td>
<td>319.5 ± 42.8</td>
</tr>
<tr>
<td>Protein oxidation, g/day</td>
<td>59.7 ± 8.5</td>
<td>61.3 ± 16.0</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 11). SMR, sleeping metabolic rate. *Significantly different from the daytime snack intervention (*P < 0.05).

Energy balance measured during the respiratory chamber stay was slightly positive and did not differ between treatments (nighttime snack, 223.9 ± 78.7 kcal/day; daytime snack, 239.3 ± 78.7 kcal/day). Twenty-four hour fat oxidation was significantly lower after the nighttime snack treatment than after the daytime snack treatment (P = 0.02). Mean 24-h RQ tended to be higher following the nighttime snack treatment than following the daytime snack treatment (P = 0.09). The 24-h CHO oxidation and protein oxidation values taken at the laboratory did not differ significantly by treatment. TEE, fat oxidation, and RQ in each time zone throughout the day are shown in Fig. 2. We observed no significant effect of treatment or treatment-by-time in TEE (ANOVA). The RQ profiles tended to reflect the effect of treatment (P = 0.09). Fat oxidation showed a significantly different profile (P = 0.03, treatment effect). During the afternoon (14:00–19:00), RQ was significantly higher and fat oxidation was significantly lower after the nighttime snack treatment (P = 0.02 and P = 0.01, respectively). During other time periods, no significant differences were observed for RQ, fat oxidation, or CHO oxidation.

**Fasting and postprandial blood analysis.** Fasting blood lipid and hormone values following each treatment are shown in Table 5. Concentrations of total and LDL cholesterol were higher after the nighttime snack treatment than after the daytime snack treatment (P = 0.03 and P = 0.01, respectively). No significant differences were noted for levels of TSH, T3, T4, HDL cholesterol, triglycerides, NEFA, adiponectin, or leptin. In addition, no significant treatment differences were observed for glucose, insulin, and cortisol AUC values obtained from the OGTT after each intervention (Table 6).

**DISCUSSION**

The present study investigated the effects of a 13-day nighttime snack intervention on appetite, food intake, and metabolism in lean young women. Thirteen days of nighttime snacking did not alter body weight, energy expenditure, or glucose metabolism relative to 13 days of daytime snacking, but we did detect a decrease in fat oxidation and increases in total and LDL cholesterol levels. The strength of our study is that the effects of timing of snacks, without changing the total caloric intake and meal frequency, were evaluated by assessing 24-h energy metabolism in subjects who spent a whole day in the respiratory chambers. One limitation of this study is the small sample size, which contributed to insufficient statistical power. Another limitation was that while no significant difference was identified between crossover treatments, calorimeter measure-

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![Graphs showing energy expenditure, fat oxidation, CHO oxidation, and RQ over different time periods.](http://ajpregu.physiology.org/)
Table 5. Blood metabolites after interventions

<table>
<thead>
<tr>
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<th>Daytime Snack (mean ± SD)</th>
<th>Nighttime Snack (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH, µIU/ml</td>
<td>1.62 ± 0.20</td>
<td>1.62 ± 0.19</td>
</tr>
<tr>
<td>T₃, ng/ml</td>
<td>0.86 ± 0.05</td>
<td>0.88 ± 0.05</td>
</tr>
<tr>
<td>T₄, µg/dl</td>
<td>0.36 ± 0.34</td>
<td>0.30 ± 0.28</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>3.73 ± 5.93</td>
<td>4.88 ± 5.84</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>152 ± 8</td>
<td>161 ± 9*</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>57 ± 2</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>76 ± 6</td>
<td>83 ± 7*</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>77 ± 6</td>
<td>84 ± 7</td>
</tr>
<tr>
<td>NEFA, µEq/l</td>
<td>389 ± 50</td>
<td>354 ± 58</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>10.3 ± 1.0</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>8.79 ± 1.41</td>
<td>9.92 ± 1.79</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>90 ± 4</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>Insulin, µIU/ml</td>
<td>6.91 ± 2.99</td>
<td>6.86 ± 2.64</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 11). NEFA, nonesterified fatty acid; T₃, triiodothyronine; T₄, thyroxin; TSH, thyroid-stimulating hormone. *Significantly different from the daytime snack intervention (P < 0.05).

ments after intervention were made under a positive energy balance of over 200 kcal/day.

During the intervention period, daytime snacking compared with nighttime snacking, yielded a significant decrease in hunger and prospective to eat before lunch, as well as a significantly increased fullness (Fig. 1). This is likely due to snack intake before lunch, but no significant treatment difference was observed for mean meal consumption amount during each intervention period. Previous studies reported that eating a single high-CHO snack before a meal did not reduce subject-perceived appetite or energy intake. Moreover, whereas high-protein snacks suppressed appetite, they did not reduce meal intake (21). Our study results are consistent with those of others who examined between-meal interventions, in that they suggest that consumption of dense, high-fat and high-energy snacks has only a limited effect on subject appetite and the amount consumed during meals (22, 25).

Our study results were also consistent with those from previous studies reporting that circadian timing of energy intake affects the levels of LDL cholesterol and total cholesterol. Cell et al. (5) described a pattern of daily cholesterol synthesis that is strongly influenced by the timing of meals and is negatively correlated with cortisol secretion. Yamajuku et al. (34) found that restricting the timing of feeding without changing total energy intake affected the expression of the cholesterol-converting enzyme CYP7A1 in rat livers. They demonstrated that meal intake patterns influence circadian rhythms of cholesterol synthesis, potentially leading to increases in cholesterol levels during morning fasting. In the present study, 13 days of nighttime snacking might have changed the pattern of daily cholesterol synthesis, which may have led to the increased fasting LDL and total cholesterol levels. This increase in serum LDL cholesterol indicates that nighttime eating might increase the risk for cardiovascular disease.

The 24-h TEE and CHO oxidation after each 13-day intervention did not differ significantly, but we did not notice a significant (albeit small) decrease in 24-h fat oxidation following the nighttime snack intervention (Table 4). Fat oxidation was also significantly lower in the afternoon (14:00–19:00; Fig. 2). The nighttime snack intervention yielded a somewhat higher 24-h RQ as well as significantly higher RQ values in the afternoon (14:00–19:00; Table 4 and Fig. 2). The increase in fat oxidation during the 24-h period and the afternoon was consistent with decreasing, but nonsignificant, RQ during the 24-h period and significantly decreasing RQ in the afternoon. One possible reason for the lack of a significant difference for RQ during the 24-h period is small sample size. Accordingly, additional studies should be performed with a larger sample size. Moreover, energy balances in calorimeter measurements after both interventions were slightly positive because of the decreased physical activity in the limited space of the respiratory chamber, possibly affecting the differences in fat oxidation in this study. However, given the uniform lifestyle with no snack consumption maintained by all subjects while in the respiratory chamber, we speculate that changes in the 13-day meal intake patterns influenced fat metabolism. This change in fat oxidation is consistent with the reduced fat oxidation in NES patients reported by Gluck et al. (14), who evaluated the effects of long-term nighttime eating and suggested that increased RQ and decreased fat oxidation increases dietary intake and leads to obesity. Higher RQ values and lower fat oxidation are known risk factors for weight gain (27, 35). Lower fat oxidation and higher CHO oxidation in individuals predicted greater food intake throughout the lower glycogen store (8). Recent animal studies demonstrated that the relationship between biological circadian rhythms and energy regulation changes with the timing of food intake (20, 26), and that these circadian changes can affect weight gain (3). Another animal study on the rhythmic transcriptome and metabolome for tissues such as the liver in metabolic processes suggests a role for the Clock gene in metabolic regulation (9). Food intake was a potential zeitgeber for hepatic metabolites that control energy homeostasis (i.e., energy intake and energy metabolism). These studies suggest that nighttime meals lead to weight gain due to metabolic changes.

Shorter sleep durations such as those observed among NES patients alter the secretion of appetite-regulating hormones such as ghrelin and leptin (6, 31). In addition, sleep curtailment in healthy individuals also leads to increased caloric intake from snacks, especially at night (24). The present study was unable to identify significant differences due to intervention in the postprandial response of glucose, insulin, or cortisol during OGTT. Although very few studies have examined this issue, previous studies that compared irregular versus regular meals reported that insulin responses worsen with irregular meal intervention (10, 11). It is unclear why nighttime between-meal snacking in the present study did not influence glucose or insulin responses, and future studies with different participants or intervention periods are required to elucidate this matter.

In this study, food intake did not change during the 2 wk of nighttime snacking, and the 24-h appetite scores after each intervention were similar between treatments. Sleep duration and activity levels, however, were not measured during the study.

Table 6. Glucose metabolism values obtained from the OGTT after treatment

<table>
<thead>
<tr>
<th></th>
<th>Daytime Snack (mean ± SD)</th>
<th>Nighttime Snack (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg·dl⁻¹·h</td>
<td>284 ± 55</td>
<td>290 ± 66</td>
</tr>
<tr>
<td>Insulin, µIU·ml⁻¹·h</td>
<td>131.2 ± 50.6</td>
<td>127.2 ± 54.2</td>
</tr>
<tr>
<td>Cortisol, µg·dl⁻¹·h</td>
<td>35.9 ± 12.3</td>
<td>35.0 ± 10.3</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 11). No significant differences between treatments were detected.

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intervention; thus, it is unclear whether energy balance was altered during those periods.

In conclusion, we showed that a 2-wk intervention of nighttime snacking increases total and LDL cholesterol and reduces fat oxidation during the 24-h calorimeter measurement under the positive energy balances. Moreover, the intervention did not affect the 24-h total energy expenditure, RQ, appetite profiles, and glucose metabolism in healthy young women. These findings suggest that the timing of snack intake affects changes in fat metabolism and may increase the risk of obesity and cardiovascular disease.

**Perspectives and Significance**

Our findings that circadian timing of snack intake independent of meal frequency and daily energy intake affects serum cholesterol levels and whole body fat oxidation highlights the importance of the timing of nutrient intake with regard to whole body fat metabolism. The study sample size was small, however, so future studies with more subjects are required to determine how the timing of snack intake affects energy metabolism. A more complete appreciation of the contributions of glucose and insulin for 24-h rhythm dysfunction are needed to improve our understanding of pathological states such as metabolic diseases and diabetes, which are associated with an altered timing of energy intake.

**ACKNOWLEDGMENTS**

We thank the research volunteers for their outstanding dedication. We also thank Hiroko Yamaguchi, Tomomi Yamazaki, and Takae Nishizawa as the dietary staff in Kao Health Care Food Research Laboratories. We thank Hironobu Miyachi and Shigeru Nakajima of Fuji Medical Science Co., Ltd., for their technical expertise on respiratory chamber measurements.

**DISCLOSURES**

Masanobu Hibi, Yayoi Yoshimoto and Mitsuhiro Katashima are employed by Kao Corporation.

**AUTHOR CONTRIBUTIONS**

Author contributions: M.H., J.O., and S.I. interpreted results of experiments; M.H. drafted manuscript; K.J. interpreted results of the snacking intervention; thus, it is unclear whether energy balance was altered during those periods.

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