Metabolic Responses to Nocturnal Eating in Men Are Affected by Sources of Dietary Energy

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ABSTRACT Because night work is becoming more prevalent, we studied whether feeding at different times of a 24-h period would elicit different metabolic responses and whether dietary macronutrient composition would affect these responses. Seven men (26–43 y, 19.9–26.6 kg/m²) consumed two isocaloric diets, in a crossover design. The diets were a high carbohydrate (HC) diet [65 energy % (E%) carbohydrates, 20E% fat] and a high fat (HF) diet (40E% carbohydrates, 45E% fat). After a 6-d diet-adjustment period, the men were kept awake for 24 h and the food (continuation of respective diet) was provided as six isocaloric meals (i.e., every 4 h). Energy and substrate turnover, heart rate, mean arterial pressure (MAP), blood glucose, triacylglycerol (TAG), nonesterified fatty acid (NEFA) and glycerol were measured throughout the 24-h period. Significantly higher energy expenditure and NEFA concentration, and lower blood glucose and TAG concentrations were observed when the men consumed the HF diet than when they consumed the HC diet. Significant circadian patterns were seen in body and skin temperature (nadir, 0400–0500 h). When the men consumed the HF diet, significant circadian patterns were seen in fat oxidation (nadir, 0800–1200 h; plateau, 1200–0800 h), heat release (nadir, 0800–1200 h; plateau, 1600–0800 h), heart rate (nadir, 0000 h), blood glucose (nadir, 0800–1200 h; peak, 0000–0400 h), NEFA (nadir, 0800–1200 h; peak, 1200–2000 h) and TAG (nadir, 0800–1200 h; peak, 0400–0800 h) concentrations. Energy expenditure, carbohydrate oxidation, MAP and glycerol concentration did not display circadian patterns. Unequal variances eradicated most circadian effects in the HC-diet data. The increased TAG concentration in response to feeding at 0400 h might be involved in the higher TAG concentrations seen in shift workers. Distinct macronutrient/circadian-dependent postprandial responses were seen in most studied variables. J. Nutr. 132: 1892–1899, 2002.

KEY WORDS: • circadian • macronutrient • triacylglycerols • postprandial • meal • substrate utilization

The human body has an endogenous circadian rhythm for body temperature and endocrine environment (1). The circadian phase is set by external stimuli such as light, although dietary habits can affect this rhythm (2,3). During pharmacokinetic studies, a circadian variability has been shown in absorption of drugs (4). This is in part attributable to a decreasing rate of gastric emptying from morning to evening (5).

Although an increasing proportion of individuals in affluent societies have irregular work hours, a situation that affects their feeding cycle (6), there is still only limited knowledge on how the body reacts metabolically to this changed intake of food (7). Subjects respond differently when given identical test meals in the morning compared to the evening (7); glucose tolerance decreases from morning to midnight (8); and nocturnal eating increases the low density lipoprotein to high density lipoprotein ratio (6). The endocrine milieu may thus be less suitable for food intake during the night and the nocturnal hormonal pattern might be involved in the high incidence of obesity and cardiovascular diseases often seen in shift workers (9,10).

To further investigate the metabolic postprandial reaction when meals were consumed at different time periods throughout a 24-h period, we studied two different diets after a 6-d diet adaptation in a crossover design. The diets were a high carbohydrate (HC) diet and a high fat (HF) diet. The rationale for studying the HC diet was that it was previously shown that peripheral glucose tolerance varies during the 24-h period (8), and shift workers tend to nibble carbohydrate-containing food items at night (11). A high fat diet has been proposed by some to increase the risk of obesity (12), although others refute this connection (13). Dietary surveys indicate that shift workers tend to consume HF diets [i.e., about 40% of energy as fat (6)].

Because the body’s metabolic system is set for activity and meal intake during the day, and rest and fasting during the night, we anticipated a difference in metabolic response between day and night. Moreover, we expected the postprandial response to be different between the diets, with respect to circadian rhythm, and a more pronounced day/night difference when the high carbohydrate meals was consumed because

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carbohydrate metabolism is higher than fat metabolism in the oxidative hierarchy (14). The aim of this study was to examine the metabolic responses to nocturnal feeding as affected by sources of energy and possible effects on shift workers and other groups with irregular work hours.

MATERIALS AND METHODS

Subjects. Eight men were recruited for the study and seven of the eight subjects finished both experimental periods. Their (n = 7) mean age was 32 (range, 26–43) y; weight, 84.3 (69–95) kg; body mass index, 23.8 (19.9–26.6) kg/m²; body fat, 20.0 (11.4–31.2) g/100 g; and maximal oxygen uptake, 47 (36–60) mL/(min·kg⁻¹). All were in good health as determined by medical history and physical examination; none of the subjects was a smoker or had excessive alcohol consumption. They were screened for sleep disturbances, unusual sleep patterns and pathological blood lipid levels (one subject had slightly elevated plasma triacylglycerol (TAG)³ concentration, 2.67 mmol/L, at d1 of the study). All subjects gave their written informed consent, and the Ethical Committee of the Faculty of Medicine at Uppsala University approved the study.

Experimental design. The subjects participated in two 7-d experimental sessions, receiving two different diets in a crossover design with a 1-mo washout period between the two sessions. During the sessions they were followed on an outpatient basis at the metabolic unit from d1 to d6, and on d7 the 24-h metabolic study was performed. Approximative 1 wk before each session a test was performed using a high-precision scale (type KC120-1DI Multirange; Mettler Instruments, Greifensee, Switzerland), a skin caliper (John Bull; British Indicators, St Albans, UK) and bioimpedance spectroscopy (Hydra 4000B; Xitron Technical, San Diego, CA).

The same investigator assessed all subjects, and body composition was calculated using the three-compartment equation described by Forslund et al. (15). Maximal O₂ uptake (V˙O₂ max) was estimated by a submaximal (16) test on a bicycle ergometer (Monark 829E; Monark AB, Vansbro, Sweden). The body composition measurements were repeated on d1 of the second session. Weight and total body water were controlled on d3 to ensure that the subjects were in neutral energy balance. During these first 6 d the subjects also wore a wrist activity recorder (Activwatch, measures movements/min; Cambridge Neurotechnology, UK) and kept a diary on their daily activities and sleep patterns (results to be reported elsewhere). They were instructed to avoid any strenuous activity. In the evening of d6 they reported to the nutrition metabolic unit and electroencephalogram (EEG) electrodes were attached (results to be reported elsewhere). They received a snack at 2145 h and went to bed at 2300 h.

In the morning of d7, the basal metabolic rate (BMR) was measured for 30 min at 0600 h using an ergospirometer (SensorMedics 2900Z, Anaheim, CA), while the subjects were lying awake in bed. An intravenous catheter was inserted on the dorsal side of the left hand and the subjects were dressed in a direct calorimetric suit (17). At 0800 h the 24-h study began. During the subsequent 24 h the subjects remained awake. The 24-h study was divided into six identical 4-h periods. Each period started with a standardized meal, at 0800, 1200, 1600, 2000, 0000 and 0400 h, followed by computer-based mental-performance tests, another mental performance test, bioimpedance and blood pressure measurements (Vitalscan BP1000; Braun, Germany). These measurements were repeated every hour (results of the mental-performance tests will be reported elsewhere). Blood sampling occurred 30 min postprandially and at 1, 2, 3 and 4 h postprandially. At the end of each period, urine was collected and the subject was woken up to eat breakfast and to avoid skin temperature and body temperature were measured continuously throughout the 24-h period. As a pilot study, the activity of three of the subjects was monitored with the activity recorder. The subjects remained seated in a chair throughout the study and no physical activity was allowed.

³ Abbreviations used: BMR, basal metabolic rate; FQ, food quotient; HC, high carbohydrate; HF, high fat; MAP, mean arterial pressure; NEFA, nonesterified fatty acids; PUFA, polyunsaturated fatty acids; RMR, resting metabolic rate; RQ, respiratory quotient; TAG, triacylglycerol.

Diets. Two diets were compared: a high carbohydrate (HC) diet with a food quotient [FQ (18)] of 0.91, and a high fat (HF) diet (FQ 0.83). The HC diet consisted of 15% of the energy (E%) from protein, 65% from carbohydrates and 20% from fat. The HF diet consisted of 15% of protein, 40% from carbohydrates and 45% from fat. The fat composition in both diets was 40% saturated fatty acids, ~40% monounsaturated fatty acids and ~20% polyunsaturated fatty acids (PUFA). Both diets contained about the same amount of dietary fiber (~2.2 g/MJ).

A more complete description of the diets is given in the Appendix. Basal metabolic rate was calculated from height and weight according to the FAO/WHO/UNU equations (19). Energy requirements were estimated using a physical activity level of 1.55 × BMR during d1 to d6 and 1.4 × BMR during d7. The energy content of the diets was adjusted according to the previously determined energy requirement (d1 to d6, 12.5 ± 0.29 MJ/24 h; and d7, 11.3 ± 0.26 MJ/24 h), while keeping macronutrient proportions the same. All food items were provided in ready-to-eat containers and bottles. No other products were allowed during the 7-d period. The research kitchen at the Department of Public Health and Caring Sciences, Uppsala University, prepared the food.

In the evening of d6 at 2145 h, the subjects received a snack and then fasted until the start of the 24-h study.

The nutrient and energy content of the diets were estimated using computer software (Dietist version 1.1, Kost och Näringdata AB, Bromma, Sweden).

Procedures. The rates of O₂ consumption and CO₂ production during the 24-h study were assessed using an ergospirometer (SensorMedics 2900Z). Auto-calibration was performed every 30 min using two standard gases with known content of O₂ and CO₂ (16% O₂, 4% CO₂, and 26% O₂, 0% CO₂ in nitrogen, respectively). Inspiratory air was checked every 10 min. Interpolations of O₂ and CO₂ were carried out during short periods (~15 min), while the subject was eating, as well as every 8 h, when a manual recalibration of the instruments was performed. Energy expenditure and both fat and carbohydrate oxidation were calculated according to Simonson and DeFronzo (20). Data for BMR calculations were taken from the last 15 min of the 30-min BMR measurement. Protein oxidation was calculated from urinary nitrogen excretion (analyzed with the Kjeldahl technique), corrected for changes in the blood urea pool according to Jéquier et al. (21). Blood pressure was transformed into mean arterial pressure (MAP) with the formula MAP = [(systolic – diastolic)/3] + diastolic blood pressure. Blood samples were centrifuged and the supernatant was stored at –20°C until analyses. Plasma glucose was analyzed at the Clinical Chemistry routine laboratory at the University Hospital, Uppsala, Sweden. Triacylglycerol (TAG) concentration was analyzed in serum by enzymatic techniques (Instrumentation Laboratories, Lexington, MA) in a Monarch 2000 centrifugal analyzer. Serum nonesterified fatty acids (NEFA) and TAG concentrations were measured using an enzymatic colorimetric method (NEFA, Wako Chemical GmbH; glycerol, Boehringer Mannheim) applied for use in the Monarch 2000 centrifugal analyzer. Heat release and both skin temperature and body temperature were measured using the direct calorimetry suit (17).

Statistics. Because of a technical problem, valid indirect calorimetry was not obtained for subject 1 during one session. The data from that subject are omitted from all calculations based on indirect calorimetry. Subject 4 had high TAG concentrations and statistics on this subject are omitted.

The data were analyzed with a three-factor repeated-measures analysis of variance (RM-ANOVA) with Huyhn–Feldt correction for violations of the assumption of circularity. The independent factors were as follows: diet (difference between HC diet and HF diet), circadian (difference between the six 4-h time periods throughout the 24-h experiment) and meal (difference between the four (for blood samples) time points within each 4-h period). Within each
TABLE 1

Weight, basal metabolic rate (BMR) and blood variables in the morning of d7 before start of 24-h study in men who consumed high carbohydrate (HC) and high fat (HF) diets.

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>84.0 ± 3.5</td>
<td>84.2 ± 3.8</td>
</tr>
<tr>
<td>BMR, MJ/24 h²</td>
<td>7.4 ± 0.4</td>
<td>8.0 ± 0.5</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.3 ± 0.1</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>TAG, mmol/L</td>
<td>1.16 ± 0.04</td>
<td>0.71 ± 0.03*</td>
</tr>
<tr>
<td>NEFA, mmol/L</td>
<td>0.35 ± 0.06</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Glycerol, mmol/L</td>
<td>0.076 ± 0.010</td>
<td>0.073 ± 0.004</td>
</tr>
</tbody>
</table>

1 Means ± SEM; n = 7. * Different from HC diet, P < 0.001.
2 Abbreviations used: TAG, triacylglycerol; NEFA, nonesterified fatty acids.
3 Values without subject 1.
4 Values without subject 4 (with *4*: HC: 1.39 ± 0.23 mmol/L; HF: 0.84 ± 0.14 mmol/L).

RESULTS

Adjustment period (d1–d6). Body composition did not change significantly during or between the experimental periods (weight change ~ 1%). Activity measured with the activity recorder did not differ between the two experimental periods (HC 121.0 ± 6.5, HF 126.2 ± 7.0 mean activities/h). The BMR (measured in the morning of d7 before starting the 24-h study) tended to be greater (P = 0.064) after 6 d of consuming the HF diet (Table 1). Fasting blood glucose, NEFA and glycerol concentrations did not differ after consumption of the two diets, whereas the TAG concentration was greater after the HC diet (Table 1).

Metabolic 24-h study, d7. The results of the three-factor RM-ANOVA of combined 24-h data are shown in Table 2. Results from the two-factor RM-ANOVA (within each diet) are presented in the text. The results of the Day (1200–2000 h)/Night (0000–0800 h) comparisons are presented in the text.

Higher energy expenditure was observed when the men consumed the HF diet compared to the HC diet (Fig. 1, Table 3). Energy expenditure did not display any circadian pattern (tendency with the HC diet, P = 0.061), although the pattern within the 4-h periods differed between the periods, most likely because of an increased energy expenditure after the last meal (at 0400 h). No difference was found in energy expenditure between Day and Night.

The 24-h respiratory quotient (RQ) differed from FQ after consumption of both diets: HC diet, RQ 0.83 ± 0.01 vs. FQ 0.91; HF diet, RQ 0.77 ± 0.01 vs. FQ 0.83.

Carbohydrate oxidation was higher when the men consumed the HC diet than when they consumed the HF diet, and no circadian pattern was seen (Fig. 1, Table 3). Meal intake increased oxidation and there was an interaction with circadian pattern, probably the result of higher oxidation values 2 h postprandially in the 0800–1200 h and 0400–0800 h period compared to those of the other periods. No difference was found between Day and Night, although the patterns were different, probably the result of lower oxidation values at 1700 h compared to those at 0500 h (P < 0.028 for Day/Nighttime interaction).

Fat oxidation was lower when the men consumed the HC diet than when they consumed the HF diet; moreover, there

TABLE 2

Statistical summary of P-values from the 3-factor repeated-measurements (RM)-ANOVA, based on 24-h values from indirect calorimetry, the calorimeter suit, blood pressure measurements, and blood variables during d7 in men who consumed high carbohydrate (HC) and high fat (HF) diets.

<table>
<thead>
<tr>
<th></th>
<th>Diet (D)</th>
<th>Circadian (C)</th>
<th>Meal (M)</th>
<th>D-C</th>
<th>D-M</th>
<th>C-M</th>
<th>D-C-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy expenditure²</td>
<td>0.039</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>NS</td>
<td>NS</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate oxidation²</td>
<td>0.001</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>NS</td>
<td>NS</td>
<td>0.033</td>
<td>NS</td>
</tr>
<tr>
<td>Fat oxidation³</td>
<td>&lt; 0.001</td>
<td>0.054</td>
<td>&lt; 0.001</td>
<td>0.007</td>
<td>NS</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Protein oxidation³</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.004</td>
<td>NS</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature (rectal)²</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>NS</td>
<td>0.059</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature (skin)</td>
<td>NS</td>
<td>0.011</td>
<td>0.009</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heat release</td>
<td>NS</td>
<td>NS</td>
<td>0.005</td>
<td>0.009</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate²</td>
<td>NS</td>
<td>0.008</td>
<td>0.002</td>
<td>NS</td>
<td>0.047</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>NS</td>
<td>NS</td>
<td>0.015</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.036</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>NS</td>
<td>0.006</td>
<td>0.005</td>
<td>NS</td>
</tr>
<tr>
<td>TAG²</td>
<td>0.007</td>
<td>0.027</td>
<td>&lt; 0.001</td>
<td>0.008</td>
<td>0.002</td>
<td>&lt; 0.001</td>
<td>0.025</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.002</td>
<td>0.027</td>
<td>&lt; 0.001</td>
<td>NS</td>
<td>0.031</td>
<td>0.003</td>
<td>NS</td>
</tr>
<tr>
<td>Glycerol</td>
<td>NS</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>NS</td>
<td>0.017</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 P-values, n = 7. Abbreviations used: Diet, HC and HF diets; Circadian, comparison of the six different 4-h periods throughout the 24-h period; Meal, comparison of the time points within each 4-h period; D-C, the diet affects the circadian pattern; D-M, the diet affects the pattern within the 4-h periods; C-M, time of day affects the pattern within the 4-h periods; D-C-M, diet affects how time of day affects the pattern within the 4-h periods; Heat release, the heat dissipated from the subject measured with the calorimeter suit; Glucose, glucose concentration; TAG, triacylglycerol concentration; NEFA, nonesterified fatty acid concentration; Glycerol, glycerol concentration; NS, not significant (P-values > 0.07, the chosen threshold for tendency).
2 n = 6.
3 Protein oxidation is based on 4-h values.
was a tendency for a circadian pattern, possibly because of lower oxidation during the 0800–1200 h period compared to that of other periods (Fig. 1, Table 3). Fat oxidation did not differ between Day and Night, although the patterns were affected by diet, probably because of higher oxidation at 1700 h compared to that at 0500 h when consuming the HC diet (\(P = 0.023\) for diet \(\times\) Day/Night interaction).

Protein oxidation did not display any diet or circadian effects (data not shown).

Body and skin temperatures showed a circadian pattern, with a nadir in the early morning (0300–0500 h) (Fig. 2). Skin temperature transiently increased after meal consumption, although this was significant only when the HF diet was consumed (\(P = 0.014\)); body temperature, on the other hand, did not. The circadian rhythm in skin temperature was stronger with the HF diet than with the HC diet (HC diet, \(P = 0.063\); HF diet, \(P = 0.002\)). Higher body and skin temperatures were observed during Day than during Night (\(P < 0.001, P = 0.006\); body and skin, respectively). Body temperature decreased faster when the men consumed the HF diet than when they consumed the HC diet when comparing Day with Night (\(P = 0.041\) for diet \(\times\) Day/Night interaction).

Heat release was similar after consumption of both diets, but the pattern differed during the 24-h test period because heat release rapidly stabilized when the men consumed the HC diet; however, when the men consumed the HF diet, heat loss increased until the 1600–2000 h period (HF diet, \(P = 0.021\) for circadian pattern) (Fig. 2). Meal intake increased heat loss and this increase differed because of the circadian rhythm only after consuming the HC diet (\(P < 0.001\)). The pattern also differed when comparing Day with Night, in that heat loss increased with the HF diet and decreased with the HC diet from Day to Night (\(P = 0.023\) for diet \(\times\) Day/Night interaction).

The heart rate (pattern) differed after consumption of the diets during the 24-h test period, with lower heart rate 3 and 4 h postprandially after consumption of the HC diet (Fig. 3).

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy expenditure, MJ/24 h</td>
<td>10.2 ± 0.4</td>
<td>11.0 ± 0.6*</td>
</tr>
<tr>
<td>Carbohydrate oxidation, g/24 h</td>
<td>241 ± 9</td>
<td>150 ± 16*</td>
</tr>
<tr>
<td>Fat oxidation, g/24 h</td>
<td>115 ± 11</td>
<td>170 ± 13</td>
</tr>
<tr>
<td>Glucose, mmol/L (^{2,3})</td>
<td>5.85 ± 0.13</td>
<td>5.72 ± 0.12*</td>
</tr>
<tr>
<td>TAG, mmol/L (^{2,3})</td>
<td>1.24 ± 0.06</td>
<td>1.04 ± 0.05*</td>
</tr>
<tr>
<td>NEFA, mmol/L (^{2,3})</td>
<td>0.26 ± 0.02</td>
<td>0.32 ± 0.01*</td>
</tr>
</tbody>
</table>

1 Means ± SEM, \(n = 6\). * Different from HC diet, \(P < 0.05\).
2 \(n = 7\).
3 24-h average.
4 Values without subject 4. Abbreviations used: TAG, triacylglycerol; NEFA, nonesterified fatty acids.
The objective of this study was to describe the postprandial reaction relative to macronutrient composition when meals were consumed at different time points throughout a 24-h period. We defined circadian pattern as differences in postprandial response between different 4-h periods throughout a 24-h period. A more specific analysis was also done by comparing daytime values with nighttime values.

**Energy and heart parameters.** We did not see a decrease in energy expenditure or heat release in spite of a decrease in heart rate and both skin and body temperatures. On the other hand, energy intake increased heart rate. The decrease in heart rate from Day to Night was greater when the HC diet was consumed than when the HF diet was consumed ($P = 0.018$ for diet-Day/Night interaction).

Mean arterial pressure did not display any diet or circadian effects, except a diet-circadian interaction, seen as a difference in heart rate and both skin and body temperatures. On the other hand, energy intake increased heart rate. The decrease in heart rate from Day to Night was greater when the HC diet was consumed than when the HF diet was consumed ($P = 0.018$ for diet-Day/Night interaction).

Blood glucose concentration was higher and increased more at 0.5 h after consumption of the HC meals than after the HF meals (Fig. 4, Table 3). A circadian pattern was observed with higher concentration during the 2000–2400 h period than during the 0800–1200 h period, but this was mainly seen when the HF diet was consumed ($P = 0.041$). The 1-h postprandial response showed a distinct circadian pattern in both diets. We observed no differences in blood glucose concentration between Day and Night.

Triacylglycerol concentration was strongly affected by diet because higher concentrations were observed when the HC diet was consumed; furthermore, when the HF diet was consumed, higher amplitudes in response to meals were observed (Fig. 4, Table 3). This was also seen when comparing Day and Night ($P < 0.001$ for diettime interaction). Moreover, TAG concentration showed no circadian pattern with the HC diet, but a circadian pattern was seen with the HF diet ($P < 0.001$). However, after consumption of both diets, the postprandial response to the 0800 h meal was lower than that after the other meals. There was no absolute difference in TAG concentration between Day and Night, although the patterns were different, probably because of the higher TAG concentration at 0600 and 0700 h compared to that at 1800 and 1900 h ($P = 0.015$ for Day/Nighttime interaction).

Nonesterified fatty acid concentration was lower when the HC diet was consumed than when the HF diet was consumed, especially 2 h postprandially (Fig. 4, Table 3). We observed a circadian pattern, attributed to higher values seen during the 1600–2000 h period than during both the 0800–1200 h and the 2000–0400 h period when the HF diet was consumed ($P = 0.003$). We observed a circadian-meal interaction, probably from lower values 2 and 3 h postprandially in the 2000–0000 h period than in either the 1200–1600 h or 0400–0800 h periods (HC diet, $P = 0.064$; HF diet, $P < 0.001$ for circadian-meal interaction). We observed no differences in NEFA concentration between Day and Night.

Glycerol concentration was lower 2 h postprandially when the HC diet was consumed compared to when the HF diet was consumed; otherwise, we observed no diet or circadian effects (Fig. 4).

**DISCUSSION**

The 24-h curve and postprandial pattern of heart rate and mean arterial pressure (MAP) in men who consumed high carbohydrate (HC) and high fat (HF) diets. The vertical lines in the left figures separate the six different 4-h periods, each period starting with a meal. All values are means $\pm$ SEM, $n = 6$ in heart rate and $n = 7$ in MAP. The right figures serve to illuminate the postprandial responses within the six periods. Certain time points (means, chosen for reasons of clarity) are depicted with the start of each period on the x-axis (e.g., the 2-h line depicts the value 2 h after meal intake in all the six periods).

(P = 0.023). Energy intake increased heart rate. The decrease in heart rate from Day to Night was greater when the HC diet was consumed than when the HF diet was consumed ($P = 0.018$ for diet-Day/Night interaction).

Mean arterial pressure did not display any diet or circadian effects, except a diet-circadian interaction, seen as a difference in 1 and 2 h postprandially (Fig. 3).

Blood glucose concentration was higher and increased more at 0.5 h after consumption of the HC meals than after the HF meals (Fig. 4, Table 3). A circadian pattern was observed with higher concentration during the 2000–0400 h period than during the 0800–1200 h period, but this was mainly seen when the HF diet was consumed ($P = 0.041$). The 1-h postprandial response showed a distinct circadian pattern in both diets. We observed no differences in blood glucose concentration between Day and Night.

Triacylglycerol concentration was strongly affected by diet because higher concentrations were observed when the HC diet was consumed; furthermore, when the HF diet was consumed, higher amplitudes in response to meals were observed (Fig. 4, Table 3). This was also seen when comparing Day and Night ($P < 0.001$ for diettime interaction). Moreover, TAG concentration showed no circadian pattern with the HC diet, but a circadian pattern was seen with the HF diet ($P < 0.001$). However, after consumption of both diets, the postprandial response to the 0800 h meal was lower than that after the other meals. There was no absolute difference in TAG concentration between Day and Night, although the patterns were different, probably because of the higher TAG concentration at 0600 and 0700 h compared to that at 1800 and 1900 h ($P = 0.015$ for Day/Nighttime interaction).

Nonesterified fatty acid concentration was lower when the HC diet was consumed than when the HF diet was consumed, especially 2 h postprandially (Fig. 4, Table 3). We observed a circadian pattern, attributed to higher values seen during the 1600–2000 h period than during both the 0800–1200 h and the 2000–0400 h period when the HF diet was consumed ($P = 0.003$). We observed a circadian-meal interaction, probably from lower values 2 and 3 h postprandially in the 2000–0000 h period than in either the 1200–1600 h or 0400–0800 h periods (HC diet, $P = 0.064$; HF diet, $P < 0.001$ for circadian-meal interaction). We observed no differences in NEFA concentration between Day and Night.

Glycerol concentration was lower 2 h postprandially when the HC diet was consumed compared to when the HF diet was consumed; otherwise, we observed no diet or circadian effects (Fig. 4).
contrary, after consumption of both diets, higher energy expenditure in the late morning was recorded (seen as a difference in postprandial pattern in the 0400–0800 h period compared to that in the other periods). Because energy expenditure should follow body temperature, we would expect energy expenditure to decrease at night. We contend that the present finding may be an effect of increased fidgeting of the subjects as they were trying not to fall asleep, given that sleepiness was previously shown to increase rapidly at the nadir of body temperature (22). Fidgeting activities can consume substantial amounts of energy (23) and the activity pattern (derived from the activity recorder in three subjects; data not shown) was similar to the pattern of energy expenditure. That the thermogenic effect of food could be a factor is less likely because it was shown that the nighttime thermogenic effect of food is lower than either its morning or its afternoon thermogenic effect (24). The circadian rhythm seems to be stronger than the potential impact of fidgeting activities on body temperature and heart rate. Activities preceding the start of the study (i.e., application of catheter and calorimetry suit) probably explained why the body core temperature and heart rate did not display the characteristic cosine shape, with a peak in the late afternoon/early evening and a nadir in the early morning (22, 25). The shape of the skin temperature curve was most probably attributable to the calorimetry suit (17). However, we are confident that these factors did not affect the conclusions drawn from this study. We did not observe any circadian pattern in MAP, which was shown previously (26). This was most probably the result of a large variation in systolic blood pressure (data not shown). To our knowledge, however, the difference in pattern after consumption of HC and HF diets has not been previously reported. The physiological relevance of this finding remains to be elucidated.

We observed a higher energy expenditure after consumption of the HF diet than that after consumption of the HC diet, which is different from most other studies (27–29). There are a few reports that HF diets can affect energy expenditure (30). In a small subgroup of men, Cooling and Blundell (30) found that high fat eaters had a higher resting metabolic rate (RMR) than that of low fat eaters, when matched for body composition and energy intake. The finding is also interesting, considering the somewhat heterogeneous study group (age span 26 to 43 y, percentage body fat 11.4 to 31.2%); and the subject excluded from the indirect calorimetry calculations (see Statistics under Materials and Methods) also had values indicating higher energy expenditure after consumption of the HF diet. Further studies will be needed to confirm or refute this observation.

Substrate utilization. We found a tendency for a circadian meal interaction in carbohydrate oxidation after consumption of the HC diet and a clear circadian pattern in fat oxidation after consumption of the HF diet. Although no absolute difference was seen in carbohydrate and fat oxidation when comparing Day and Night, the pattern of carbohydrate and fat oxidation differed during daytime compared to that during nighttime when the men consumed the HC diet. After consumption of the HF diet, almost identical fat oxidation patterns were observed when Day and Night were compared. Plat et al. (31) showed a circadian rhythm in glucose metabolism and Frapé et al. (32) found an increased clearance of intralipid (10% lipid emulsion) during the afternoon compared with the morning values. The low fat oxidation and high carbohydrate oxidation (although not significantly higher) in the morning were most likely attributable to the morning gluconeogenesis, given that this circadian rhythm seems to override the possible effect of the nighttime fast. Several studies have shown that lean subjects adjust macronutrient oxidation to macronutrient composition during isoenergetic conditions (27, 28, 33). Although the subjects’ RQ came closer to the FQ, we found an unexpected discrepancy between FQ and RQ, especially with the HC diet, because in most other studies carbohydrate oxidation follows carbohydrate intake. However, it is important to state that the FQ is based on assumptions and estimated from nutritional tables. We contend that it is unlikely that possible errors in calculating dietary composition differed substantially between the diets, although it is more difficult to properly estimate the dietary content of carbohydrates than that of fat and protein (L. Hambraeus, personal observation, 1998).

Plasma glucose. Higher plasma glucose concentration was observed when the men consumed the HC diet and, like Raben et al. (34), we found that the plasma glucose increased more rapidly after a HC meal than after a HF meal. After consumption of both diets we observed a circadian pattern in plasma glucose concentration, although the circadian effect came from consumption of the HF diet. The largest postprandial response was observed 1 h after the 1600 h meal, but the 4-h average concentration reached its peak in the 0000–0400 h period, which is somewhat later than was observed by Van Cauter et al. (35). However, in their study a continuous glucose infusion was used, whereas our subjects were fed a meal at midnight. Perhaps another response would have been seen if the meals had been consumed earlier.

Serum lipids. We found a circadian pattern in TAG concentration when the men consumed the HF diet. The response to meals (as seen as concentration 1 h postprandially) showed a dramatic increase after HF meals were consumed, whereas a more blunted response was observed after HC meals, although the highest TAG value was seen 2 h after the 0400 h meal. Because our subjects were fed and kept awake throughout the 24-h period, comparisons with other studies are somewhat difficult. Nevertheless, in a recent study Sopowski et al. (36) studied the response to a premeal and test meal during the day compared to that during the night and found increased TAG levels at nighttime. Other studies have also found an increase in TAG concentration from morning to evening (37, 38). We observed higher TAG concentrations after consumption of the HC diet than after the HF diet, which is in accordance with several studies [reviewed by Parks and Hellerstein (39)]. In a recent review, it was concluded that elevated fasting TAG concentration is a strong and independent risk factor for ischemic heart disease (40). In one of the cited studies, the incidence rate of coronary heart disease was 7.7% in the middle tertile (1.10 to 1.59 mmol/L) (40). In our study, the fasting values after 6 d of HC-diet consumption was 1.1 ± 0.1 mmol TAG/L, just on the border of increased risk. Given that shift workers tend to have higher TAG concentration, caution has to be used before advocating low fat, high carbohydrate diets. After consuming the HF diet, increased TAG concentration was seen at 0500 and 0600 h, although these postprandial values (~1.6 mmol/L) were lower than what was previously reported to affect endothelial function (~2.2 mmol/L) (41). Still, this postprandial increase might be involved in the increased TAG concentrations seen in shift workers (42).

Nonesterified fatty acid concentration displayed a circadian rhythm when the HF diet was consumed, although this circadian rhythm was not related to glycerol concentration. If NEFA and glycerol are of the same origin, hydrolyzed TAG, these should appear as a NEFA/glycerol ratio close to 3:1. If reesterification is taking place, this ratio will then be lower if glycerol is not used during reesterification within the adipocyte.
Just after meal intake, NEFA decreased more than did glycerol in our study, indicating reesterification of NEFA into TAG. Then, NEFA increased more than did glycerol, indicating that glycerol must be metabolized intracellularly and/or reesterification of NEFA into TAG must be quite low. Most probably, lipolysis is inhibited after the meal and NEFA and glycerol respond somewhat differently to this. Higher NEFA concentrations were seen with the HF diet; however, although increased NEFA levels have been implicated in insulin resistance, this difference likely was not physiologically important (43).

**Circadian patterns.** Distinct circadian postprandial responses were seen in most studied variables, either as a main effect or through interaction effects. We would have expected a more pronounced difference between Day and Night, except for body and skin temperatures, no differences were seen. However, some effects were seen in the difference pattern, indicating that some Day vs. Night differences exist. However, some effects were seen in the difference pattern, indicating that some Day vs. Night differences existed. Perhaps the time periods we used for Day and Night were too long, but comparing 1600–2000 h with 0400–0800 h did not substantially alter the results (data not shown). Our hypothesis that HC meals would show a more pronounced daytime/nighttime difference was not supported by our results. Instead, the HF diet showed more circadian patterns. One reason for the lack of circadian effects seen with the HC diet was that the assumption of circularity (that the random error is in fact random) was violated in several of the measured variables, especially energy expenditure, carbohydrate oxidation and skin temperature. This was a consequence of unequal variance and was unexpected, so our estimation of sample size did not take this into account. Why later individual differences were observed with the HC diet is unclear; if anything, we would have expected the consumption of the HF diet to cause more variability.

The aim of this study was to gather more information about feeding at different time points, especially nocturnal eating, and to relate such patterns to possible health outcomes in shift workers. It should be noted that this study does not address the body clock "**rst night shift.** It seems to affect energy expenditure differently compared to day workers, particularly not on the first night shift.

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**LITERATURE CITED**


APPENDIX

Composition of high carbohydrate (HC) and high fat (HF) diets during d1 to d6 and d7

<table>
<thead>
<tr>
<th>d1 to d6</th>
<th>HC</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Sour milk, cereals, bread, margarine, cheese</td>
<td>Low fat yogurt, 0.5% fat</td>
</tr>
<tr>
<td></td>
<td>HC: orange juice; HF: milk (3% fat)</td>
<td>White bread</td>
</tr>
<tr>
<td>Lunch¹</td>
<td>Ham, pasta, white sauce, green peas or Chicken, rice, tomato sauce, green beans</td>
<td>Low fat margarine, 40% fat</td>
</tr>
<tr>
<td></td>
<td>HC: orange juice; HF: milk</td>
<td>Low fat cheese, 16% fat</td>
</tr>
<tr>
<td>Dinner¹</td>
<td>Meat sauce, rice, broccoli or Salmon, pasta, white sauce, carrots</td>
<td>Cucumber</td>
</tr>
<tr>
<td></td>
<td>HC: orange juice; HF: milk</td>
<td>Banana</td>
</tr>
<tr>
<td>Snacks</td>
<td>Bread, margarine, cheese, apple</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HC: orange juice; HF: milk</td>
<td></td>
</tr>
</tbody>
</table>

¹ The same dishes were used and carbohydrate and fat content were adjusted using water, milk, and rapeseed oil.