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Metabolic, endocrine and mood responses to nocturnal eating in men are affected by sources of dietary energy

Review of a thesis

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INTRODUCTION

Although the studies on **what** to eat are numerous, **when** to eat is an issue that has received less attention. About 20% of the work force in Sweden, about 800 000 people, have irregular working hours and of these approximately 200 000 are shift workers [2]. The same proportion of shift workers can be found in most of the industrialized world [66]. Shift-workers employed in two- and three-shift work seem to alter their timing of meals [60], with as yet not completely identified effects on metabolic and endocrine responses. Does it matter if some people eat at a different time point than the rest? Yes, there are most definitely reasons to worry about the health of shift workers and others with irregular work hours. Shift work has been shown to be associated with a higher frequency of gastro-intestinal problems [21] and with a higher prevalence of the "metabolic syndrome" [52], including a number of conditions such as high blood triacylglycerols (lipid) concentrations [88] and obesity [112], leading to an increased risk of myocardial infarction [56]. The "24-hour society" [77] demands that people work around the clock, so we must strive to decrease the negative health effects of shift work. It is therefore important to address what happens when people eat during irregular, especially nighttime, hours. In this thesis I have investigated some of the body's reactions to meal intake during the night. These processes are themselves extremely complex, just the conversion of glucose to energy requires some 25 steps and the aid of about as many enzymes and co-factors [93] and each step affect all others. Some of the variables in this thesis have been studied previously in models examining nocturnal eating, but this thesis differs from previous studies in the amount of variables that have been looked into and in that subjects underwent long and controlled preceding dietary period. We have also combined physiologic with psychologic variables as they might be influenced in different ways by meal patterns and composition. This article describes the results from one of the two studies where a high-carbohydrate diet was compared to a high-fat diet [46, 47, 65].

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Before going into the description of the meals and variables studied, here is a brief overview of the circadian rhythms that control most body functions. Most mammals display a clear circadian rhythm. The regulation of the ¨time keeper of the body clock¨ has attracted a lot of attention but the impact of rhythmicity on the human body has received less attention. The key regulator is believed to be located in the hypothalamus (more specific, in the suprachiasmatic nuclei) [57] where oscillations from ¨clock genes¨ are constantly corrected by the shift of light intensity [22]. The normal feeding cycle is divided into daytime intake of food and a long nocturnal fast. Endogenous circadian rhythms can be seen in body temperature and endocrine environment [40]. During pharmacokinetic studies, a circadian variability has been shown in absorption of several drugs [79]. This is partly due to a decreasing rate of gastric emptying from morning to evening [35]. How the body reacts to meals could thus depend on the time of day when the meal is provided. In this thesis, "circadian rhythms" is used as a more general term describing patterns and rhythms due to signals from the "body clock", and "time-of-day effect" as a more strict term meaning that we have found a statistical difference between different time periods.

Diet

Dietary surveys have indicated that shift workers tend to consume high-fat diets (i.e. about 40% of energy as fat [60]). A high-fat diet has been proposed by some to increase the risk of obesity [18] whereas others refute this connection [130]. Since shift workers have increased risk of becoming obese [112], the impact of their food preference certainly requires investigation. During short-term (one to seven days) food intervention studies, healthy subjects have been able to adjust their macronutrient oxidation to the composition of isocaloric diets in most studies [3, 15, 68, 91, 97, 99, 129], whereas other studies have not found that fat oxidation correlates with fat intake [92, 103]. The main reason for the divergent opinions regarding the high-fat diets role in the development of obesity is failure to take energy density into consideration. Fat content shows a strong correlation with energy density of the food but much stronger negative correlations are shown with water and fibre content [86]. It has been shown that peripheral glucose tolerance (~the capacity to transport blood sugar into the tissue) varies during the 24-h period [114]. Shift workers tend to nibble on carbohydrate (CHO) snacks to stay awake [78]. This nocturnal CHO intake could be physiologically unfavorable, although favorable for mental performance.

Metabolic variables

Energy expenditure would be expected to vary with *body temperature* and *heart rate*, which both show a clear circadian rhythm with minima around 04.00 in the morning [61, 95]. I have, however, not found any 24-h energy expenditure studies on shift workers or others during a 24-h wake. *Substrate utilization* has not often been addressed in a circadian perspective. A number of 24-h studies have been performed but none has measured fat, protein and CHO oxidation while the subjects were awake throughout the whole 24-h period. In subjects that are kept awake during a 26-h peri-

od, the *glucose* concentration has been shown to increase from morning to midnight and then decrease, although this study was performed with a continuous glucose infusion [113]. High blood *triacylglycerol* (lipid) concentrations are commonly found in shift workers [55, 88]. In studies comparing the blood lipids postprandial (after meal) response, higher concentrations are usually found in the evening compared to the morning [39, 66, 81]. The reason for this increase is so far unknown.

Endocrine variables

In this thesis 10 hormones have been studied as they represent different parts of the human metabolic system and I will just briefly describe their function.

Insulin plays a major role in the regulation of glucose metabolism, generally promoting the cellular utilization of glucose. It is also an important regulator of protein [49] and lipid [69] metabolism. Meal composition does not influence insulin concentration substantially [17, 43, 76] and insulin concentration is only weakly influenced by circadian rhythm [116].

In β-cells in the pancreas, proinsulin is enzymatically converted to insulin with the liberation of *C-peptide*. C-peptide has been viewed as biological inert index of insulin secretion, but some studies show a more active role for C-peptide [121].

Pancreatic polypeptide (PP) is a peptide, released from the pancreas in a biphasic manner in response to meals [102]; and it has been hypothesized to be a marker for vagal tone [83]. Large evening high fat (HF) or high carbohydrate (HC)-meals decrease the morning PP concentration [85] and a clear circadian rhythm has been shown in PP concentration[82].

Glucagon is a pancreatic hormone secreted by the alpha cells in the pancreas, and plays an important role in regulation of plasma glucose concentration, ketone metabolism, and several other physiologic processes.

The thyroid hormones [thyroid stimulating hormone (TSH), free thyroxin (fT4), and total triiodothyronine (tT3)] influence all major metabolic pathways. They increase basal energy expenditure by acting on protein, carbohydrate and lipid metabolism [75]. The thyroid hormones are not substantially affected by the dietary macronutrient composition [68], unless more extreme diets are used [120]. Thyroid hormones, especially TSH, show a clear circadian rhythm with lower values during the day and higher in the evening [34]. If subjects are kept awake, the TSH concentrations increase even more whereas T3 and T4 are less affected [34].

Cortisol affects energy expenditure [19], and protein [19], CHO [30] and fat metabolism [94], Cortisol displays a clear and steady circadian rhythm [6] but is not substantially affected by macronutrient composition [119].

Chromogranins (chromogranin A, CgA) are co-released when secretory granules from different neuroendocrine cells release their content (i.e. hormones); thus chromogranins might serve as a rough index of hormonal secretory activity [108]. No study has addressed the impact of dietary composition on chromogranin concentration. A circadian rhythm in CgA concentration has been found by some [31], but not others [108].

Leptin is a peptide hormone secreted from white fat cells and is implicated in the regulation of food intake and energy balance [41]. Leptin has been shown to display a circadian pattern but meal intake disrupts this pattern [96]. Moreover, meal composition has been shown to affect leptin concentration [42].

Psychological variables

Mood and mental performance varies throughout the 24-h period, sleepiness for example increases throughout the evening and night at reaches its maximum at about the same time as body temperature reaches its minimum [61]. Wells et al, have in several studies looked into the influence of macronutrient composition and diurnal effects on mood [125–127]. Subjects felt less vigorous and more dreamy and feeble after a HF-meal consumed at 10.30 than after a HC-meal [125]. These effects were not apparent if the meals were given at 12.30 instead [125]. This study has, however, been criticized for weak effects and questionable design [10]. The effects of macronutrient composition on mood thus seem to be modest and inconsistent [10]. The sum of the collected work seems to indicate that breakfast increases cognitive performance, lunch increases negative reports on mood and dinner might increase cognitive performance and improve mood ratings [51].

Tools

Of the tools used in this thesis, the principles behind indirect and direct calorimetry may require a brief explanation.

The basic principles behind *indirect* (respiratory) *calorimetry* are:

- Oxygen which is consumed during energy expenditure cannot be stored in the body and therefore a linear relationship exists between oxygen consumption and energy expenditure.
- With the knowledge of basic chemical stoichiometric constants, the oxidation of carbohydrate and fat can be estimated from oxygen consumption and carbon dioxide production.

Indirect calorimetry has been proven to be fairly reliable in estimating energy expenditure and not very sensitive to confounding metabolic processes [24;63]. Several 24-h studies have been performed showing good reproducibility [111]. The carbon dioxide production (CO_2)/ oxygen (O_2) consumption ratio is called the respiratory quotient or RQ. An RQ of 0.70 indicates almost exclusive fat oxidation whereas an RQ of 1.00 indicates CHO oxidation. As protein oxidation gives an average RQ of 0.85, protein oxidation's impact on the CO_2/O_2-ratio has to be subtracted (calculated from urinary nitrogen). The resulting carbon dioxide production/ oxygen consumption ratio is then called the non-protein RQ, which is used to calculate fat and CHO oxidation. A theoretical version of RQ is the food quotient or FQ. The food is analyzed and a theoretical value of the RQ the food should generate is calculated [14]. When using indirect calorimetry to estimate substrate utilization, there are a number of variables that have to be taken into consideration. When esti-

mating RQ, it is necessary to correct for changes in urea pool, ketogenesis and gluconeogenesis as this might otherwise lead to under- or overestimation of fat and CHO oxidation [63].

Direct calorimetry measures conductive, convective and radiant heat release in a ¨direct¨ manner, as all chemical processes produce heat, but does not measures evaporative heat loss [100]. One can estimated the evaporative heat release by converting the weight loss due to evaporation to energy spent. We, however, chose to "only" measure the heat released from the subjects by using a special insulated suit calorimeter [38]. Direct calorimetry differs from indirect calorimetry as direct calorimetry cannot measure the heat that is stored in the body. However, if the measurement continues for at least 24-h, one can assume that the heat will be released from the body.

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 (range) age was 32 (26–43) years; 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) %; and estimated maximal oxygen uptake 47 (36–60) mL/min/ kg. All were in good health as determined by medical history and physical examination; none of the subjects were smokers 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) concentration, 2.67 mmol/L, at day 1 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 seven-day experimental sessions, receiving two different diets in a crossover design with a one-month washout period between the two sessions. During the sessions they were followed on an outpatient basis at the metabolic unit from day 1 to day 6 and on day 7 the 24 h metabolic study was performed. Approximately one week before the first session body composition was assessed using a high-precision scale (Mettler, type KC120-ID1 Multirange; Mettler Instrumente, Greifensee, Switzerland), a skin caliper (John Bull, British Indicators, St Albans, UK) and bio-impedance 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. [28]. Maximal O_2 uptake (VO_{2max}) was estimated by a sub-maximal [8] test on a bicycle ergometer (Monark 829E; Monark Bodyguard, Vansbro, Sweden). The body composition measurements were repeated on day 1 of the second session. Weight and total body water was controlled on day three to ensure that the subjects were in

neutral energy balance. During these first six days the subjects also wore a wrist activity recorder (Actiwatch®, Cambridge Neurotechnology Ltd, UK; measures movements per minute) 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 day 6 they reported to the nutrition metabolic unit and electroencephalogram (EEG) electrodes were attached (results to be reported elsewhere). They received a snack at 21.45 and went to bed at 23.00. In the morning of day 7, basal metabolic rate (BMR) was measured for 30 minutes at 06.00 using an ergospirometer (SensorMedics® 2900Z, Anaheim, CA, USA) 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 [38]. At 08.00 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 08.00, 12.00, 16.00, 20.00, 00.00 and at 04.00. Then followed: computer based mental performance tests, another mental performance test, bio-impedance and blood pressure measurements (Vitalscan BP1000, Braun, Germany). These measurements were repeated every hour (the results of the mental performance test will be reported elsewhere). Blood sampling occurred 30 min postprandially and at 1, 2, 3 and 4 h postprandially (see table 1 for a detailed description of a 4-h period). At the end of each period, urine was collected and the subject was weighed. Heat release, skin and body temperature were measured continuously throughout the 24 h period. As a pilot study, three of the subjects' activity was monitored with the activity recorder. The subjects remained seated in a chair throughout the study and no physical activity was allowed.

Diets

Two diets were compared, a high carbohydrate diet (HC) with a Food Quotient [FQ; [14]] of 0.91, and a high fat diet (HF, FQ 0.83). The HC-diet consisted of 15 % of the energy (E%) from protein, 65 E% from carbohydrates (CHO) and 20 E% from fat. The HF-diet consisted of 15 E% from protein, 40 E% from CHO and 45 E%

Table 1. Description of the procedures in a 4-h period during the 24-h study.

08.01 Meal	10.15 Mental performance test I
08.15 Mental performance test I	10.45 BIA / Blood pressure measurement
08.30 Blood sample	10.55 Mental assessment
08.35 Mental performance test II	11.00 Blood sample
08.45 BIA[1] / Blood pressure measurement	11.15 Mental performance test I
08.55 Mental ("mood") assessment	11.25 Mental performance test II
09.00 Blood sample	11.35 BIA / Blood pressure measurement
09.15 Mental performance test I	11.40 Mental assessment
09.45 BIA / Blood pressure measurement	11.45 Urine collection
09.55 Mental assessment	11.55 Body weight
10.00 Blood sample	12.00 Blood sample

[1] Bioimpedence measurement (~body water measurement).

from fat. The fat composition in both diets was ~40% saturated fatty acids, ~40% monounsaturated fatty acids and ~20 % polyunsaturated fatty acids. Both diets contained about the same amount of dietary fiber (~2.2 g/MJ). A more complete description of the diets is given in table 2. Basal metabolic rate was calculated from height and weight according to the FAO/WHO/UNU equations [1]. Energy requirements were estimated using a physical activity level of 1.55 times BMR during day 1 to 6 and 1.4 times BMR during day 7. The energy content of the diets was adjusted according to the previously determined energy requirement: day 1 to 6: 12.5 ± 0.29 MJ/24 h and day 7: 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 seven-day period. The research kitchen at the Department of Public Health and Caring Sciences, Geriatric unit, Uppsala University, prepared the food. In the evening of day 6 at 21.45, 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 Näringsdata AB, Bromma, Sweden).

Procedures

The rates of O_2-consumption and CO_2-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 O_2 and CO_2

Table 2. Composition of high-carbohydrate (HC) and high-fat (HF) diets during day 1 to day 6 and day 7.

Day 1 to day 6	HC	Day 7	HF
Breakfast	Low-fat yogurt (0.5% fat)		Yogurt (3%)
Sour milk, cereals, bread, margarine, cheese	White bread		Grahams-bread
HC: orange juice HF: milk (3% fat)	Low fat margarine (40%)		Margarine (80%)
Lunch[1]	Low fat cheese (16%)		Low fat cheese (16%)
Ham, pasta, white sauce, green peas	Cucumber		Cucumber
or	Banana		Liquid margarine (40%)
Chicken, rice, tomato sauce, green beans			
HC: orange juice HF: milk			
Dinner[1]			
Meat sauce, rice, broccoli			
or			
Salmon, pasta, white sauce, carrots			
HC: orange juice HF: milk			
Snacks			
Bread, margarine, cheese, apple			
HC: orange juice HF: milk			

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

(16% O_2 + 4% CO_2, and 26% O_2 + 0% CO_2 in nitrogen, respectively). Inspiratory air was checked every 10 min. Interpolations of O_2 and CO_2 were carried out during short periods (~15 min) while the subject was eating, as well as every eight hours when a manual re-calibration of the instrument was performed. Energy expenditure, fat and CHO oxidation were calculated according to Simonson and DeFronzo [100]. Data for BMR calculations were taken from the last 15 minutes of the 30-minute 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. [50]. 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) in a Monarch 2000 centrifugal analyzer. Serum non-esterified fatty acids (NEFA) and glycerol concentrations were measured by an enzymatic colorimetric method (NEFA:Wako Chemical GmbH; Glycerol: Boehringer Mannheim) applied for use in the Monarch 2000 centrifugal analyzer. Insulin, C-peptide, thyroid stimulating hormone (thyrotropin; TSH), free thyroxin (fT4), total triiodothyronine (tT3), and cortisol concentrations were measured with an automated system for immunological analyses (Auto-Delfia, Wallac OY, Turku, Finland). Pancreatic polypeptide (PP) concentration was measured by a commercial RIA-kit (Euro-Diagnostica, Malmö, Sweden). Glucagon and leptin concentrations were measured by commercial RIA-kits (Linco Research Inc., St. Charles, MI, USA). Chromogranin A (CgA) concentration was measured with a competitive radioimmunoassay [107]. Heat release, skin and body temperature were measured using the direct calorimetry suit [38].

Mood

Subjective ratings covered four main areas. The first area related to gastrointestinal signals (3 ratings; hunger, thirst and "craving"). The ratings were given on a 9-point scale with verbal anchors (0=none, 2=weak, 4=moderate, 6=strong, 8=maximum). The next area was related to signs of sleepiness (results presented elsewhere) and the third area concerned the mental state (9 items; listless, irritated, uninterested, weary, finished, indifferent, emptied, exhausted, and interested). Most of these items used a 7-point scale with verbal anchors at the endpoints ranging from "not at all" to "very much", ("listless" and "irritated" used a similar 5-point scale). The fourth area concerned physical symptoms (not reported).

Statistics

Due to 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 TAG are computed without this subject (subject 4's 24 h average, day 7; HC-

diet: 3.11 mmol/L; HF-diet: 2.07 mmol/L). He was consistent with the other subjects in all other variables and was therefore included. The data were analyzed in two parts. First, data were analyzed using values from the whole 24 h period. Then, since shift work effects were of primary concern, we also compared daytime (*Day:* 12.00–20.00) values with nighttime (*Night:* 00.00–08.00) values. The data were analyzed with a three-factor repeated measurements analysis of variance (RM-ANOVA) with Huyhn-Feldt correction for violations of the assumption of circularity. The independent factors were: **diet** (difference between HC and HF-diet), **time-of-day** (difference between the six 4-h time periods throughout the 24-h experiment using combined data from both protocols) and **meal** (difference between the four (five for blood samples using combined data from both protocols) time points within each 4-h period). Within each diet, data were analyzed with a two-factor RM-ANOVA to pinpoint diet differences due to time-of-day. Time-of-day and meal were used as independent factors. For the DayNight analyses, the independent factors were diet, DayNight (difference between the two time periods) and time (difference between the eight (10 for blood samples) time points within each period). Partial correlations were obtained using longitudinal stepwise regression model, controlling for individual differences using adummy coding (forced into model). Statistical softwares (SuperANOVA, version 1.11, Abacus Concepts Inc, California, USA and StatView 5.0, SAS Institute Inc, NC, USA) were used for the analyses. All results are reported as mean ± SEM. Differences were considered significant at $P < 0.05$ and values of $P < 0.07$ are reported as tendencies.

RESULTS

Adjustment period (day 1–day 6) 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 per hour). Basal metabolic rate (measured in the morning of day 7 before commencing the 24 h study) tended to be greater ($P = 0.064$) after six days of consuming the HF-meals. The results of the three factors RM-ANOVA of combined 24 h data from day 7 are shown in Table 3. Results from the two factors RM-ANOVA (within each diet) are presented in the text. The results of the Day (12.00–20.00)/Night (00.00–08.00) comparisons are presented in the text.

Higher *energy expenditure* was observed when the men consumed the HF-meals compared to the HC-meals (Fig. 1, Table 3). Energy expenditure did not display any time-of-day pattern (tendency with HC-meals, $P = 0.061$) but the pattern within the 4-h periods differed between the periods, most likely due to an increased energy expenditure after the last meal (at 04.00). No difference was found in energy expenditure between *Day and Night*. Twenty-four h RQ differed from FQ after consumption of both diets: HC-meals, RQ 0.83 ± 0.01 vs. FQ 0.91; HF-meals, RQ 0.77 ± 0.01 vs. FQ 0.83.

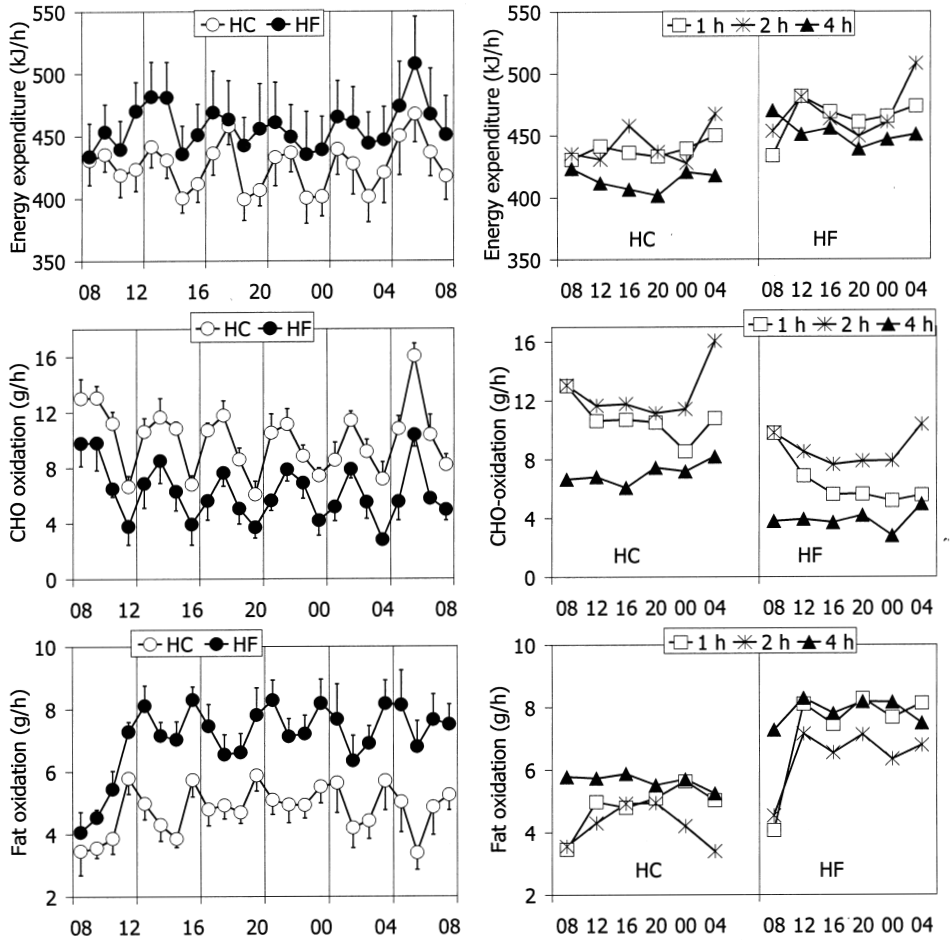

Fig 1. Twenty-four h curve and postprandial pattern of energy expenditure, CHO oxidation and fat oxidation in both diets from the 24 h study day 7. The vertical lines in the left figures separate the six different 4 h periods, each period starting with a meal. All values are means ± SEM, n=6. The right figures serve to illuminate thepostprandial responses within the 6 periods. Certain time points (chosen for reasons of clarity) are depicted with the start of each period on the x-axis. For example, the 2 h line depicts thevalue 2 h after meal intake in all the 6 periods, a.s.f.

Carbohydrate oxidation was higher with the HC-meals than with the HF-meals, and no time-of-day pattern was seen (Fig. 1, Table 3). Meal intake increased oxidation and there was an interaction with time-of-day pattern, probably due to higher oxidation values 2 h postprandially in the 08.00–12.00 and 04.00–08.00 period compared to the other periods. No difference was found between Day and Night although the patterns were different, probably due to lower oxidation values at 17.00 compared to 05.00 (P =0.028 for DayNight – time interaction).

Fat oxidation was lower with the HC-meals than with the HF-meals; and there

Table 3. Statistical summary of P-values from the 3-factor RM-ANOVA, based on 24-h values from indirect calorimetry, the calorimeter suit, blood pressure measurements and blood variables during day 7

	Diet (D)	Time-of-day (T)	Meal (M)	D•T	D•M	T•M	D•T•M
Energy expenditure[2]	.039[1]	•	<.001	•	•	.002	•
CHO oxidation[2]	.001	•	<.001	•	•	.033	•
Fat oxidation[2]	<.001	.054	<.001	.007	•	.002	•
Protein oxidation[3]	•	•		•			
Temp (rectal)[2]	•	<.001	•	.059	•	<.001	•
Temp (skin)	•	.011	.009	•	•	•	•
Heat release	•	•	.005	.009	•	•	•
Heart rate[2]	•	.008	.002	•	.047	•	•
Mean arterial pressure	•	•	•	.015	•	•	•
Glucose	.036	.001	<.001	•	.006	.005	•
TAG[2]	.007	.027	<.001	.008	.002	<.001	.025
NEFA	.002	.027	<.001	•	.031	.003	•
Glycerol	•	•	<.001	•	.017	•	•

Abbreviations used: Diet, HC and HF-diet; Time-of-day, comparison of the 6 different 4h periods throughout the 24 h period; Meal, comparison of the time points within each 4 h period; D • C, the diet affects the time-of-day pattern; D o 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, non-esterified fatty acid concentration; Glycerol, glycerol concentration; o, not significant (p-values > 0.07, the chosen threshold for tendency).

[1] P-values, n=7.

[2] n=6.

[3] Protein oxidation is based on 4 h values.

was a tendency for a time-of-day pattern, possibly due to lower oxidation during the 08.00–12.00 period compared to the other periods (Fig. 1, Table 3). Fat oxidation did not differ between *Day and Night* although the patterns were different depending on diet, probably due to higher oxidation at 17.00 compared to 05.00 when consuming the HC-meals (P = 0.023 for diet • DayNight • time interaction).

Protein oxidation did not display any diet or time-of-day effects (data not shown).

Body and skin temperature showed a time-of-day pattern with a nadir in the early morning (03.00 – 05.00) (Fig. 2, Table 3). Skin temperature transiently increased after meal consumption, although this was only significant with the HF-meals (HC-meals, *P* = NS; HF-meals, *P* = 0.014); whereas body temperature did not. The time-of-day rhythm in skin temperature was stronger with HF-meals compared to the HC-meals (HC-meals, *P* = 0.065; HF-meals, *P* = 0.002). Higher body and skin temperature were observed during *Day* than *Night* (*P* < 0.001, P = 0.006; body and skin, respectively). Body temperature decreased faster with the HF-meals than with

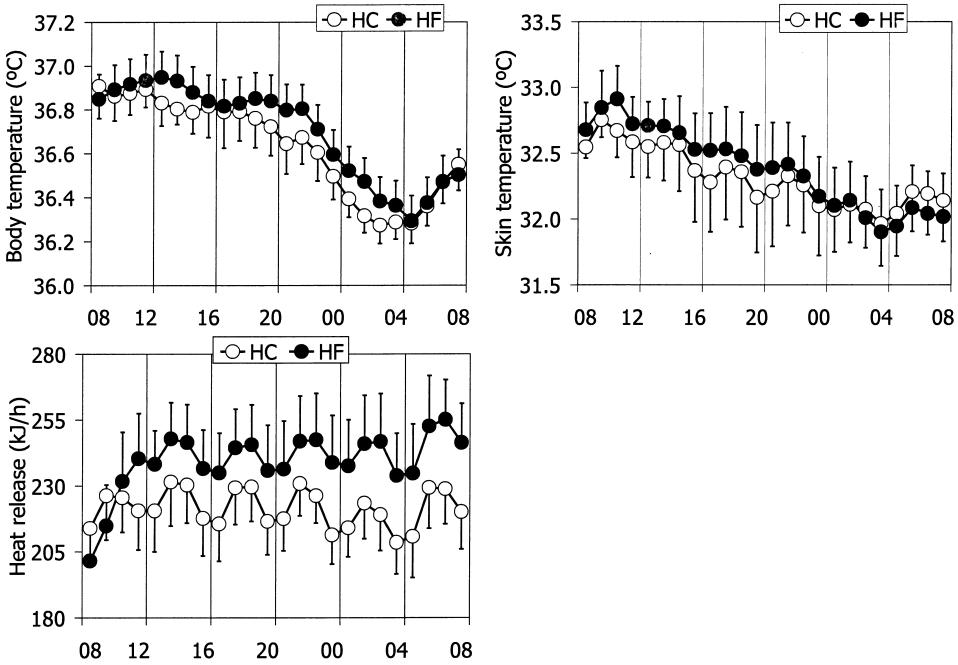

Fig. 2. Twenty-four h curve of body and skin temperature and heat release from the direct calorimetry suit in both diets from the 24 h study day 7. The vertical lines separate the 6 different 4 h periods, each period starting with a meal. All values are means ± SEM, n=7.

the HC-meals when comparing Day with Night (P = 0.041 for diet • DayNight interaction).

Heat release was similar with both diets, but the pattern differed during the 24 h period as heat release rapidly stabilized with the HC-meals; whereas with the HF-meals, heat release increased until the 16.00–20.00 period (HC-meals, P = NS; HF-meals, P = 0.021 for time-of-day pattern) (Fig. 2, Table 3). Meal intake increased heat release and this increase differed due to time-of-day rhythm only with the HC-meals (P <0.001). The pattern also differed when comparing *Day* with *Night* as heat loss increased with the HF-meals and decreased with the HC-meals from *Day* to *Night* (P = 0.023 for diet • DayNight interaction).

Heart rate (pattern) differed between the diets during the 24 h test period with lower heart rate 3 h and 4 h postprandially with the HC-meals (Fig. 3, Table 3). Heart rate showed a time-of-day pattern with a nadir in the 00.00–04.00 period, mainly seen with the HF-meals (HC-meals, P = NS; HF-meals, P = 0.023). Energy intake increased heart rate. The decrease in heart rate from *Day to Night* was larger with the HC-meals than the HF-meals (P = 0.018 for diet • DayNight interaction).

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

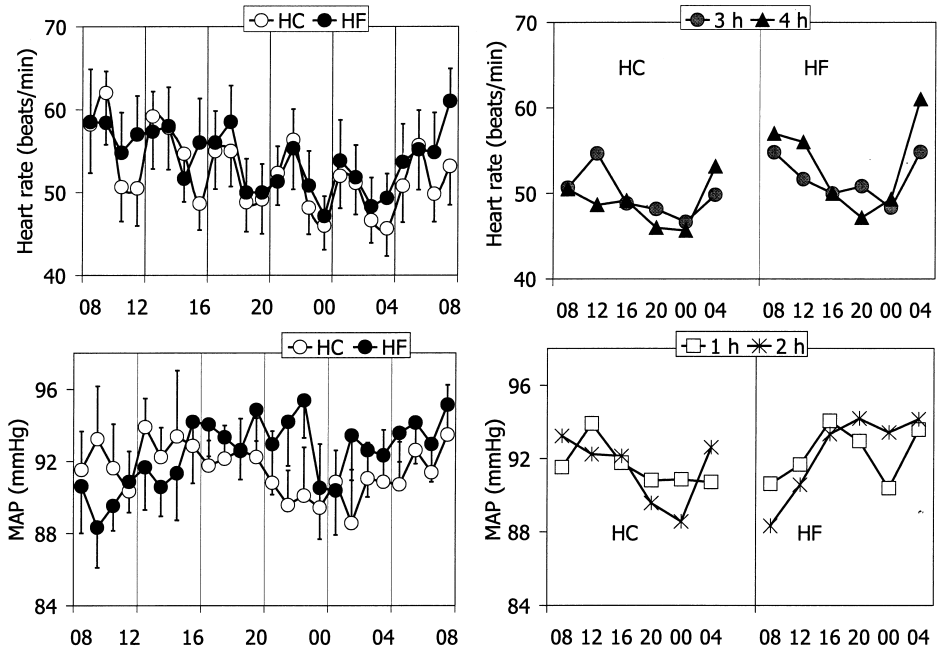

Fig 3. Twenty-four h curve and postprandial pattern of heart rate and mean arterial pressure (MAP) in both diets from the 24 h study day 7. The vertical lines in the left figures separate the six different 4 h periods, each period starting with a meal. All values are means ± SEM, n=6 in heart rate and n=7 in MAP. The right figures serve to illuminate the postprandial responses within the 6 periods.

Glucose concentration was higher and increased more at 0.5 h after meals with the HC-meals than with the HF-meals (Fig. 4, Table 3). A time-of-day pattern was observed with higher concentration during the 20.00–04.00 period compared to the 08.00–12.00 period, but this was mainly seen with the HF-meals (HC-meals, P = NS; HF-meals, P = 0.041 for time-of-day pattern). The 1 h postprandial response showed a distinct time-of-day pattern in both diets. No differences in glucose concentration were observed between *Day* and *Night* (P = NS).

Triacylglycerol concentration was strongly affected by diet because higher concentrations were observed with the HC-meals; and higher amplitudes in response to meals were observed with the HF-meals (Fig. 4, Table 3). This was also seen when comparing *Day* and *Night* ($P < 0.001$ for diet • time interaction). Moreover, TAG concentration showed no time-of-day pattern with the HC-meals (P = NS), but a time-of-day pattern was seen with HF-meals (P <0.001). However, with both diets, the postprandial response to the 08.00 h meal was lower than after the other meals. No absolute difference in TAG concentration between Day and Night, although the patterns were different, probably due to a higher TAG concentration at 06.00 and 07.00 compared to 18.00 and 19.00 (P = 0.015 for DayNight • time interaction).

Non-esterified fatty acid concentration was lower with the HC-meals compared

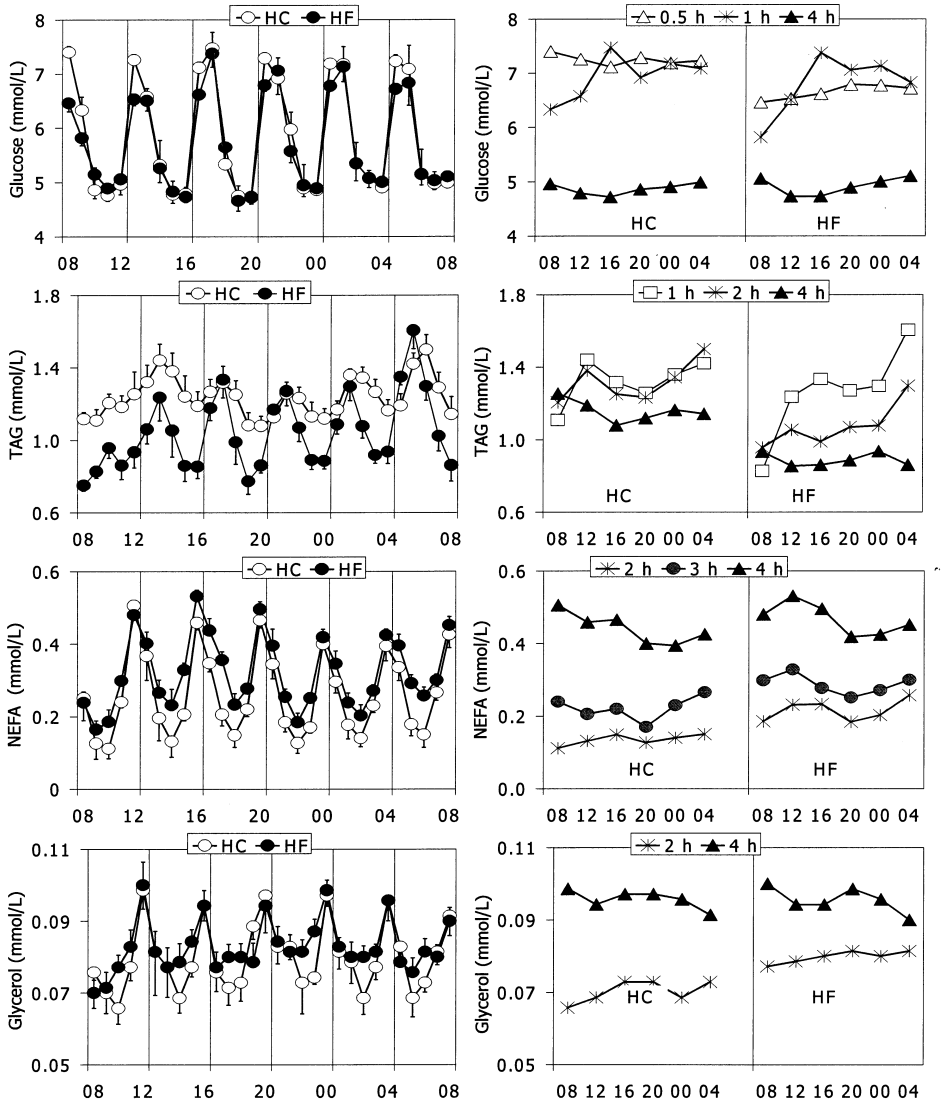

Fig 4. Twenty-four h curve and postprandial pattern of glucose, TAG, NEFA and glycerol concentrations in both diets from the 24 h study day 7. The vertical lines in the left figures separate the six different 4 h periods, each period starting with a meal. All values are means ± SEM, n=7. The right figures serve to illuminate the postprandial responses within the 6 periods.

to the HF-meals, especially 2 h postprandially (Fig. 4, Table 3). A time-of-day pattern was observed, due to higher values during the 16.00–20.00 period compared to the 08.00–12.00 and 20.00–04.00 period with the HF-meals (HC-meals, P = NS; HF-meals, P = 0.003). A time-of-day • meal interaction was observed, probably from lower values 2 h and 3 h postprandially in the 20.00–00.00 h period compared

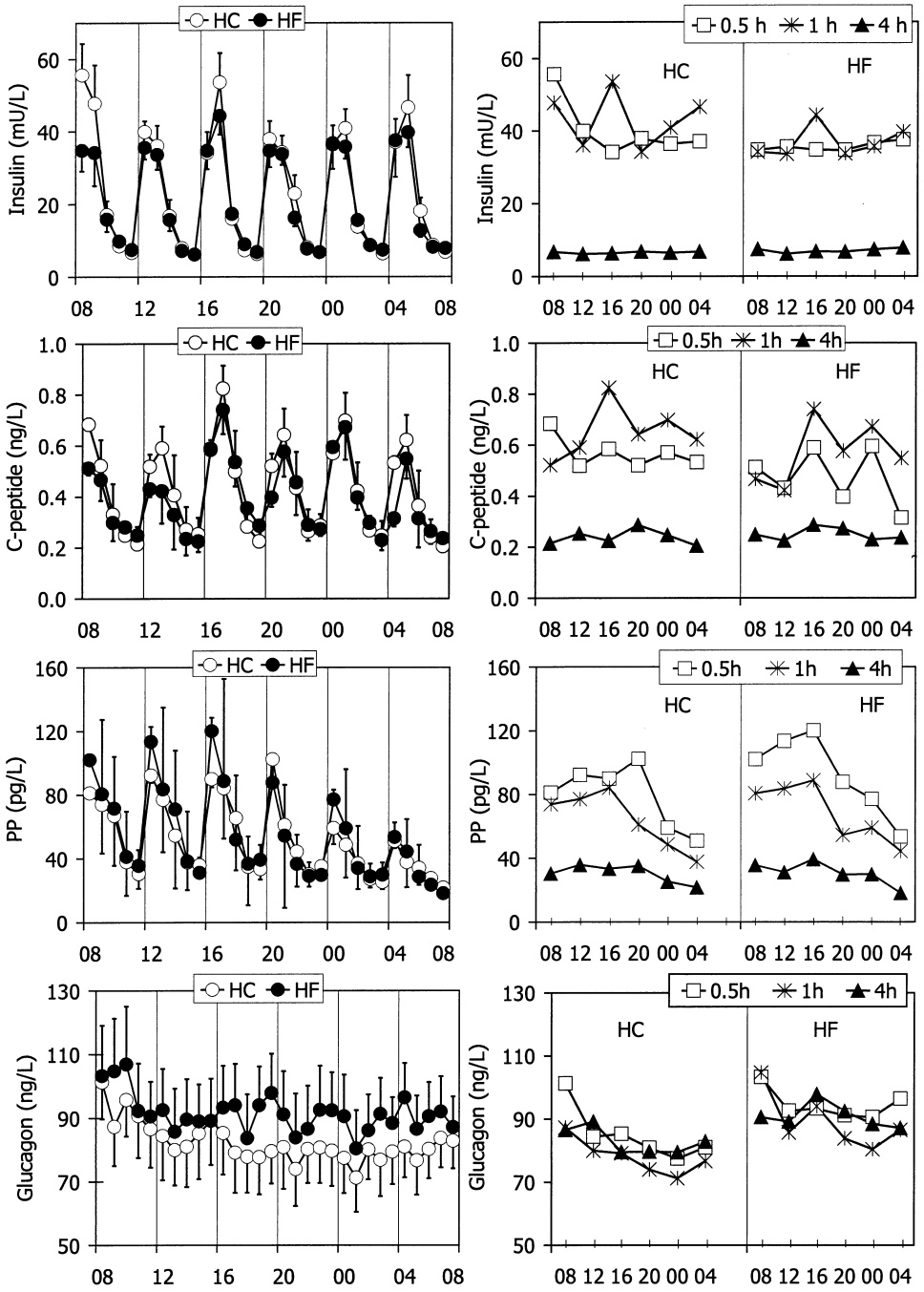

Fig. 5. Graphs depict the insulin, C-peptide, pancreatic polypeptide (PP) and glucagon concentrations during 24 h day seven. The vertical lines represents start of each time period (when the meal was provided), with the time in hours on the x-axis. The right figures serve to illuminate the postprandial responses within the 6 periods.

Table 4. Statistical summary of p-values from the three factor RM-ANOVA for hormone concentrations during day 7, based on 24-h values from HC- and HF-meals.

	Diet[1](D)	Time-of-Day [2](T)	Meal[3](M)	D•T[4]	D•M[5]	T•M[6]	D•T•M[7]
Insulin	ns	ns	<.001	.028	ns	ns	ns
C-peptide	ns	ns	<.001	ns	ns	ns	ns
PP	ns	.021	.032	ns	ns	.047	ns
Glucagon	.040	.011	ns	ns	ns	ns	ns
TSH	ns	<.001	.001	ns	ns	.002	ns
fT4	ns	<.001	ns	ns	ns	ns	.053
tT3	.053	.033	<.001	ns	ns	ns	ns
Cortisol	ns	<.001	.004	ns	ns	<.001	ns
CgA	.005	.034	ns	ns	ns	ns	ns
Leptin	ns	ns	ns	ns	ns	.007	ns

[1] Difference between HC and HF-diet.
[2] Difference between the 6 different 4-h periods throughout the 24-h period, using data from both diets.
[3] Difference between the time points within each 4-h period, using data from both diets.
[4] Diet o time-of-day interaction, the diets affect the pattern due to time of day differently.
[5] Diet o meal interaction, the diets affect the pattern within the 4-h periods differently.
[6] Time-of-day o meal interaction, time of day affects the pattern within the 4-h periods.
[7] Diet o time-of-day o meal interaction, diets affect how time of day affects the pattern within the 4-h periods.

to the 12.00–16.00 and 04.00–08.00 periods (HC-meals, $P = 0.064$; HF-meals, $P < 0.001$ for time-of-day • meal interaction). No differences in NEFA concentration were observed between *Day* and *Night*.

Glycerol concentration was lower 2 h postprandially with the HC-meals compared to the HF-meals, otherwise no diet or time-of-day effects were observed (Fig. 4, Table 3).

The insulin concentration was the same with both diets, although a diet o time-of-day interaction indicated a higher insulin concentration during the 08.00–12.00 h period with the HC-meals compared to the HF-meals (Fig. 5, Table 4). A tendency towards a time-of-day pattern was seen when the subjects consumed the HC-meals (P=0.057). Meal intake increased insulin concentration and this increase seemed to be especially high 0.5 h after the first meal and 1 h after the 16.00 meal (tendency for time-of-day • meal interaction with the HC-meals, P=0.051). No insulin concentration difference was observed between *Day* and *Night*.

The C-peptide concentration (Fig. 5, Table 4) showed a time-of-day pattern with the HF-meals (P=0.042), apparently due to lower concentration in the 12.00–16.00 period compared to 16.00–20.00 period. Meal intake increased C-peptide concentrations irrespective of the time of day with the HC-meals (P<0.001), whereas a tendency for a time-of-day • meal interaction was observed with the HF-meals (P=0.059). This was probably due to a difference in postprandial response between the 12.00–16.00 h period and the 16.00–20.00 h period. No C-peptide concentration difference was observed between *Day* and *Night*.

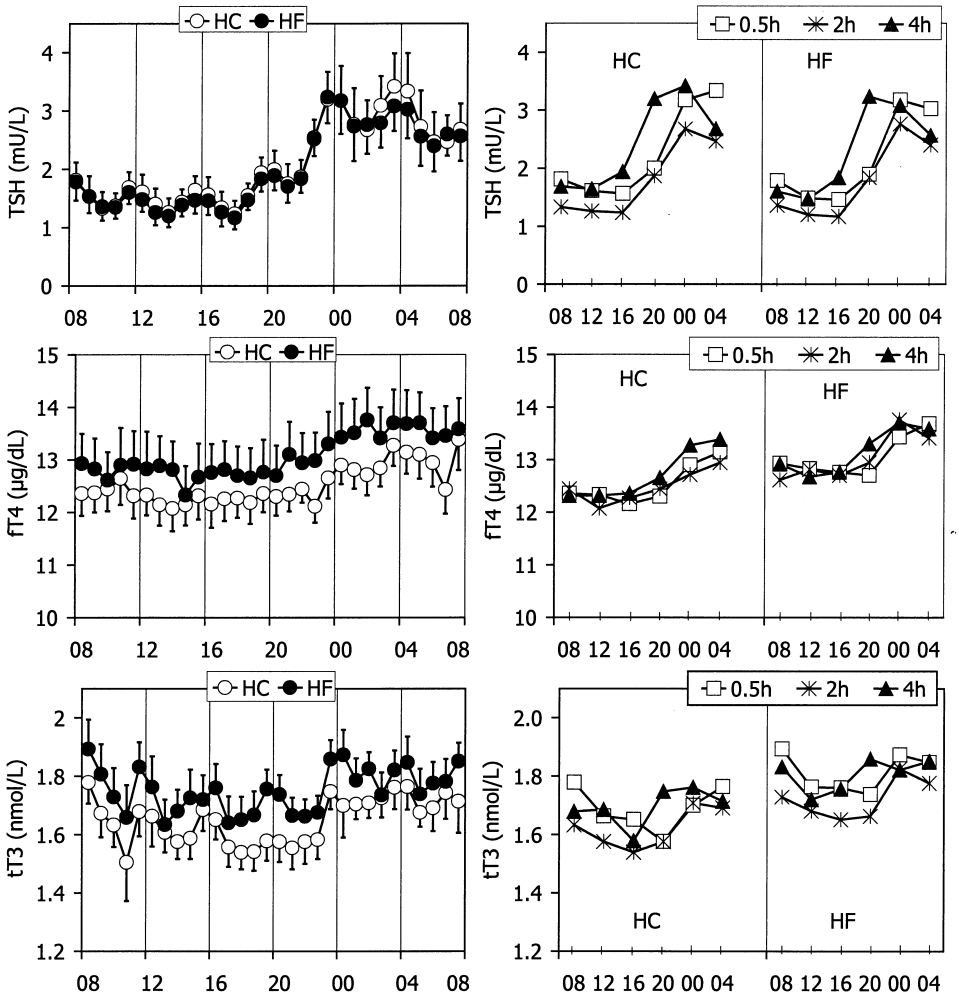

Fig 6. Twenty-four h curve and postprandial pattern of the thyroid stimulating hormone (TSH), free thyroxin (fT4) and total triiodothyronine (tT3) concentrations in both diets from the 24 h study day 7. The vertical lines in the left figures separate the six different 4 h periods, each period starting with a meal. All values are means ± SEM, n = 7. The right figures serve to illuminate the postprandial responses within the 6 periods.

The PP concentration showed a time-of-day pattern with a higher concentration in the 16.00–20.00 period compared to the 04.00–08.00 period (Fig. 5, Table 4). Meal intake increased the PP concentration but this increase diminished throughout the 24-h period (Fig. 6, Table 4). However, no significant PP concentration difference could be detected between Day and Night, due to large inter-individual variations (Fig. 5).

The glucagon concentration was higher with the HF-meals compared to the HC-

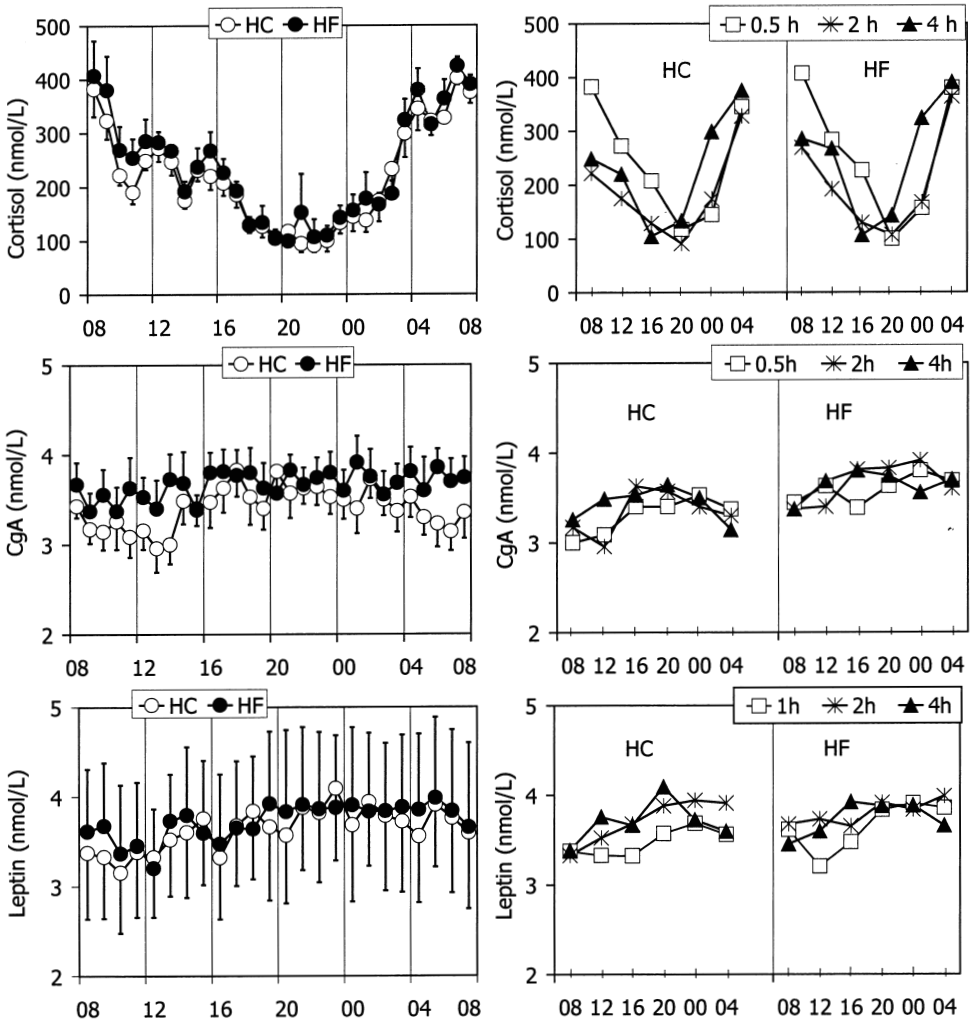

Fig. 7. Twenty-four h curve and postprandial pattern of cortisol, chromogranin-A (CgA) and leptin concentrations in both diets from the 24 h study day 7. The vertical lines in the left figures separate the six different 4 h periods, each period starting with a meal. All values are means ± SEM, n = 7. The right figures serve to illuminate the postprandial responses within the 6 periods.

meals and a time-of-day pattern was seen (Fig. 5, Table 4). A higher concentration was observed during the 08.00–12.00 period compared to the 20.00–04.00 periods with the HC-meals (P=0.002). Meal intake decreased glucagon concentration, irrespective of the time of day with the HC-meals (P=0.035). No distinct meal effect was seen for the HF-meals except for a tendency for time-of-day • meal interaction (P=0.062), probably due to a high postprandial response after the first meal. No glucagon concentration difference was observed between *Day* and *Night*, although

Table 5. Partial correlations (controlled for individual differences) between endocrine data from both diet periods during day 7 and metabolic variables.

	Insulin	PP	Glucagon	TSH	tT3	Cortisol	CgA
En-exp[1]	2.9%[4] (0.20)[5]	•	•	•	•	2.9% (0.20)	2.3% (0.16)
CHO-ox[2]	0.9% (–0.13)	5.5% (0.37)	4% (–0.26)	•	2.2% (–0.17)	5.7% (0.31)	•
Fat-ox[3]	2.6% (0.23)	2.2% (–0.33)	1.5% (0.06)	•	6.5% (0.20)	2.6% (-0.19)	2.9% (0.12)
Glucose	81.7% (0.93)	0.9% (–0.08)	0.5% (–0.08)	0.2% (0.05)	•	•	0.9% (0.11)
TAG	1.5% (0.14)	1.7% (0.25)	3.9% (–0.29)	2.2% (0.16)	•	1.4% (0.14)	•
NEFA	9.5% (–0.22)	1.3% (–0.15)	•	•	4.7% (0.21)	•	•
Glycerol	8.6% (-0.27)	•	•	•	3.6% (0.20)	•	•

Abbreviations used: PP, pancreatic polypeptide; TSH, thyroid stimulating hormone; tT3, total triiodothyronine; CgA, chromogranin A; •, not significant.
[1] Energy expenditure.
[2] Carbohydrate oxidation.
[3] Fat oxidation.
[4] Explained variance.
[5] Standard coefficient (b).

a diet • DayNight • time interaction was observed (P=0.034), most likely due to an increased glucagon concentration after the 04.00 meal with the HF-meals.

The TSH concentration did not differ between the diets and showed a time-of-day pattern with higher concentration during the 20.00–08.00 period compared to the 08.00–20.00 h period (Fig. 6, Table 4). Meal intake decreased TSH concentration irrespective of diet. A lower TSH concentration was observed during *Day* compared to *Night* (P=0.001) and there were smaller oscillations during *Day* than *Night* (DayNight • time interaction, P=0.002).

The fT4 concentration did not differ between the diets but displayed a time-of-day pattern with a nadir during the 12.00–20.00 periods and a peak in the 00.00–08.00 periods (Fig. 6, Table 4). With the HC-meals, the pattern within the periods differed depending on time of day (P=0.036). Especially during the 00.00–08.00 h periods the 3 h postprandial fT4 concentration seemed to be lower than the other time points during these periods (Fig. 6). A lower fT4 concentration was observed during *Day* compared to *Night* (P<0.001).

The tT3 concentration showed a tendency to be lower with HC-meals and a time-of-day pattern was observed with the HC-meals (P=0.004) with a nadir in the 16.00 –20.00 period and a peak in 00.00–04.00 h period (Fig. 6, Table 4). Meal intake decreased tT3 concentration irrespective of diet or time-of-day. Lower tT3 concentration was observed during *Day* than *Night* (P=0.006).

The cortisol concentration was unaffected by diet but displayed a time-of-day

Table 6. F-values and level of significance* for ratings of hunger and mood for the factors of Diet (HC/HF), Time-of-day (six 4 h blocks) and Meal (1–4 hours).

	Diet	Time of-day	Meal	D/T	D/M	T/M	D/T/M
Hunger	•	4.22*	19.8**	•	•	•	•
Thirst	•	•	11.5**	•	•	•	•
Irritated	7.27*	•	•	•	•	•	•
Indifferent	•	4.97*	•	•	•	•	•
Weary	•	11.4**	7.91**	•	•	2.40*	•

* p<0.05, ** p<0.01, • = n.s.

pattern with a peak around 0800 h and a nadir in the 20.00–00.00 h period (Fig. 7, Table 4). After the 08.00, 12.00 and 04.00 meals, a higher concentration was observed 0.5 h than 2 h postprandially, whereas no difference was seen in after the 16.00, 20.00 and 00.00 meals (time-of-day • meal interaction). Lower cortisol concentrations were observed during *Day* than *Night* ($P<0.001$).

The CgA concentration was higher with the HF-meals than the HC-meal (Fig. 7, Table 4). With the HC-meals, a time-of-day pattern was observed ($P<0.001$) with low concentrations in the 08.00–16.00 and 04.00–08.00 periods and a peak in the 20.00–00.00 period. Meal intake did not affect CgA concentration and no CgA concentration difference was seen between *Day* and *Night* (*P*=NS).

The leptin concentration did not display any main diet, time-of-day, meal or DayNight effects except a time-of-day • meal interaction after consumption of the HC-meals ($P=0.003$) (Fig. 7, Table 4). This was probably due to a different pattern within the 08.00–12.00 h period compared to the other periods.

In a forward stepwise linear regression analysis partial correlations were obtained between hormone concentrations and metabolic variables. Models were established for: energy expenditure (variance was to some degree explained by insulin, cortisol and CgA); carbohydrate (CHO) oxidation (PP, glucagon, tT3, cortisol, and insulin); fat oxidation (tT3, CgA, PP, glucagon, insulin and cortisol); glucose concentration (insulin, PP, CgA, glucagon and TSH); triacylglycerol (TAG) concentration (insulin, glucagon PP, TSH and cortisol); nonesterified fatty acid(NEFA) concentration (insulin, tT3, and PP); and glycerol concentration (insulin and tT3) (Table 5). C-peptide, fT4 and leptin concentrations did not fit into any model.

Table 7. Partial correlations between ratings and metabolic and endocrine variables, controlling for individual differences

	TAG	FFA	Insulin	Glucose	Leptin	Glucagon	Cortisol	Enexp
Hunger	–.39	.36	–.36	–.32	•	•	–.11	•
Thirst	–.16	.28	–.28	–.21	–.14	.14	•	•
Irritated	•	•	•	•	.14	•	•	•
Indifferent	•	•	•	•	•	•	.15	.15
Weary	•	•	•	•	.22	•	.31	•

• = n.s. (F-value to Enter<4.0).

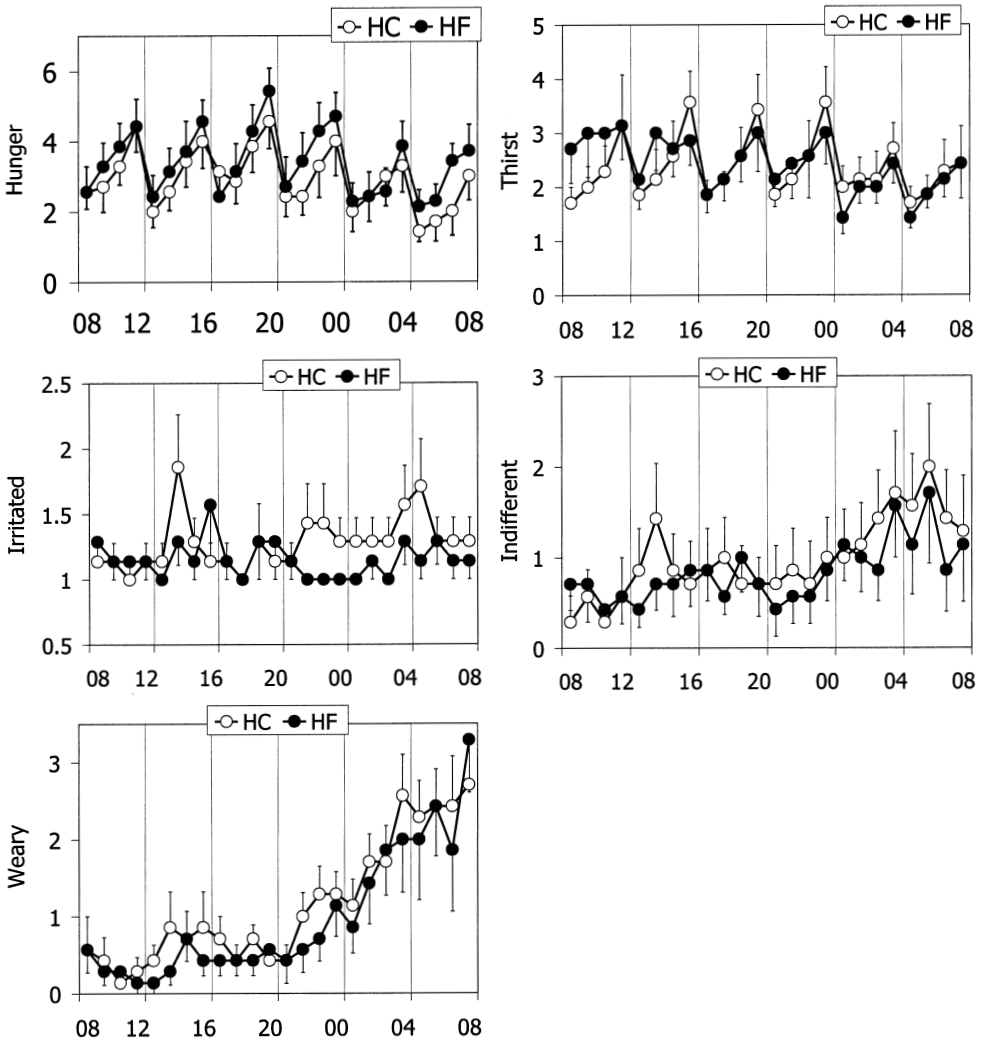

Fig. 8. Twenty-four h curve of ratings of Hunger, Thirst, Irritated, Indifferent and Weary in both diets from the 24 h study day 7. The vertical lines separate the 6 different 4 h periods, each period starting with a meal. All values are means ± SEM, n = 7. The scales for the ratings were 0 to 9 in Hunger and Thirst, 0 to 5 in Irritated and 0 to 7 in Indifferent and Weary.

Mood

Group mean levels across the 24 h are presented in Fig. 8 and the ANOVA statistics in Tables 6 & 7. Ratings of "Hunger" showed a clear time-of-day effect, mean levels being lower towards the end of the night (04.00–08.00) and the highest levels found in the evening (16.00–20.00). The strongest effect was that of meal, with a linear increase of hunger. "Thirst" only showed a significant effect of meal and did not show a time-of-day development. The feeling of being "Irritated" showed a significant

effect of diet with higher ratings for HC (mean HC=1.28±0.04; mean HF=1.15±0.03). But in general the levels of irritation were low throughout the 24-h period.

The item ”Weary” showed a strong time-of-day effect, with an increase from the lowest initial morning levels after awakening (08.00–12.00) and showed the highest levels at late night (04.00–07.00).

Partial correlations were calculated, using ratings as dependent variables and hormones and metabolites as independent, in a longitudinal regression model, controlling for individual differences.

Ratings of “Hunger“ correlated most strongly with serum TAG (negative correlation, Table 7), although the correlation was positive during the initial block of the experiment (08.00–12.00).

The first block differed from the other blocks since it was preceded by sleep and a nighttime fast. Also when including energy expenditure in the analysis, no significant correlations were found to hunger or thirst. The relation to FFA was significantly positive and significantly negative for insulin, glucose and cortisol. “Thirst” showed weaker relations to substances than did “hunger” but varied in a similar manner.

Thirst also varied negatively with leptin and positively with glucagon. The ratings of “irritated”, “indifferent”, “weary” or other ratings of mental states did not significantly correlate with substances with a few exceptions. “Irritated” was significantly correlated to leptin, “indifferent” correlated with cortisol and weary correlated with leptin and more strongly with cortisol.

DISCUSSION

This study does not address the situation in permanent night shift workers because they might show a partial circadian adaptation to night work. It does, however, address the situation of most shift workers, in rotating 2- or 3-shift schedules, who’s “body clock” is not different from day workers, particularly not on their first night shift [71]. Most shift workers (two thirds) do not take a nap before the first night shift (more between the first and the second) in rotating systems [7], which means that our model mimics a “real-life situation”for a majority of shift workers. Regarding meal composition, we chose two diets that may be bit extreme but all the same can be described as “normal”. Many studies that compare HF- and HC-diets have used macronutrient compositions that are far from what people usually eat, which makes it harder to apply the results. We chose to use meals instead of glucose infusion, as we think that meals are more physiologic. We also included a preceding strict 6-day dietary period to decrease the influence of individual dietary and physical activity habits. Standardization is always hard as you by restricting the protocol, also influence the end result.

Energy, heat, temperature and cardiac variables

There were no time-of-day effect in energy expenditure and heat release over the 24-h period, whereas a decrease in heart rate, and skin and rectal temperature was

found during the nighttime. A higher energy expenditure was found with the HF-meals compared to the HC meals.

Although a decrease in heart rate, and skin and rectal temperature was found during the nighttime, energy expenditure or heat release did not decrease with the HC-HF-meals. With both diets, a slightly higher energy expenditure was recorded (seen as a difference in postprandial pattern after the 04.00 meal compared to the postprandial pattern after the other meals). The discrepancy between decreased skin and rectal temperature, and constant energy expenditure and heat release indicates that heat may be released from the body without a change in skin temperature and that biochemical activity (thereby location of blood flow and heat) was shifted away from the core, probably to the periphery. Reduced gastric motility [35] and decreased hepatic blood flow [58] could be part of the decreased core activity. The increased peripheral activity could be increased fidgeting [62]. Fidgeting (~restless activity) may be a strategy not to fall asleep and sleepiness has been shown to increase rapidly at the nadir of rectal temperature (between 03.00 and 05.00) [61]. Fidgeting activities can consume substantial amounts of energy [62]. The physical activity patterns with HC- and HF-meals, and the pattern of energy expenditure were similar with increased fidgeting around the 04.00 meal (Fig. 11).

Much has been published on the thermogenic effect of food but in my opinion it is less probable that this effect could be a factor as it has been shown that the nighttime effect is lower than the morning and afternoon thermogenic effect of food [87].

We observed a higher energy expenditure with the HF-meals compared to the HC-meals, which is different from most other studies [15, 44, 91]. As seen in Fig 3, heat release, body and skin temperature were higher with the HF-meals (albeit not significantly), as well as a tendency for higher BMR with the HF-meals, adding weight to the difference in energy expenditure found with indirect calorimetry. There are a few reports, however, that have the same findings as in our studies, that HF-diets can affect energy expenditure [20, 67]. In a small sub-group matched for body composition and energy intake, Cooling and Blundell [20] found that high-fat eaters had higher resting metabolic rate (RMR) than low-fat eaters. Even in the larger group, which was not matched for energy intake, high-fat eaters had higher RMR and heart rate than low-fat eaters despite the same body composition. In this larger group, however, high fat eaters consumed significantly more calories [20].

One reason for the higher energy expenditure with the HF-meals could be a difference in the proportion of polyunsaturated fatty acids in the diets. A high proportion of polyunsaturated fatty acids in the diet can increase resting metabolic rate [67]. Although we used the same relative fatty acid distribution in both diets, the absolute amount of polyunsaturated fatty acids differed between HF-and HC-meals.

Furthermore, Else and Wu have shown that the level of unsaturation in the cell membrane greatly affects the activity of the Na+/K+- ATPase [27]. Dietary fat can change the skeletal fatty acid composition [4], but in my opinion six days is most probably too short a time to change cell membrane composition.

A further possible explanation for the diet effect could be that the HF-diet con-

tained more calcium from dairy products. Epidemiological data have shown that high calcium intake is associated with lower weight [23].

Although heart rate showed an effect of time-of-day, MAP did not with the HC-HF-meals. This is different to previous studies [104], and was most probably due to a large variation in systolic blood pressure (data not shown). The difference in MAP-pattern between the HC and HF-meals (Fig. 4) has, to our knowledge, not been reported previously. The physiological relevance of this finding remains to be elucidated.

Substrate utilization

We found a weak effect of time-of-day on CHO oxidation with the HC-meals and a clear effect of time-of-day in fat oxidation with the HF-meals. The RQ was lower than the FQ, especially with the HC-meals.

Although no absolute difference was seen when comparing *Day* (12.00–20.00) and *Night* (00.00–08.00) in CHO and fat oxidation, the HC-meals displayed a different pattern in substrate utilization during *Day* compared to *Night*. The HF-meals, on the other hand, had almost identical oxidation patterns when *Day* and *Night* were compared. Interestingly, with the HC-meals, the increase in energy expenditure after the 04.00 meal was mimicked by a sharp increase in CHO oxidation whereas fat oxidation was decreased. This might indicate a propensity to use CHO in the morning. This would agree with the oxidation patterns seen in the 08.00–12.00 period where both the HC-meal as well as HF-meal mainly stimulated CHO oxidation.

Plat et al. have shown a circadian rhythm in glucose metabolism [74] and Frapé et al. found an increased clearance of Intralipid (10 % lipid emulsion) during the afternoon compared with the morning [29]. Intuitively, one would expect fat oxidation to be high in the morning after a nighttime fast, and the body to give priority to glycogen storage, somewhat analogously to what happens after exercise when glycogen levels are depleted [54]. The problem is, however, to differentiate between circadian effects and the effects of the preceding nighttime fast [128]. The low fat oxidation and high CHO-oxidation (although not significantly higher than after the other meals) are most probably due to the morning gluconeogenesis as this circadian rhythm seems to override the possible effect of the nighttime fast. The morning gluconeogenesis could stem from the morning peak in cortisol secretion and increased glucagon concentration.

Although our subjects changed their RQ towards the FQ, we found an unexpected discrepancy between FQ and RQ, especially with the HC-meals , since in most other studies macronutrient oxidation follows dietary macronutrient intake [15, 44, 97]. It is, however, important to state that FQ is based on assumptions and estimated from nutritional tables. Roy et al. found a slight decrease in RQ after seven days on a HC-meals [92] and Hill et al. also observed that RQ was lower than the FQ using similar diets as we used [44]. Hill et al. speculated that seven days might be a too short a period to reach steady state (i.e. matching RQ with FQ) [44], although

Schrauwen et al. found a matching of RQ and FQ after seven days of a high fat diet [98]. In the latter study the subjects were in a negative energy balance for three days in the middle of the diet period, which the authors speculate could have facilitated the adjustment to HF-diet [98].

The discrepancy between RQ and FQ in our study could also come from weight changes during the dietary adjustment period [36], but only minor weight changes were found (~1%). Furthermore, the subjects were in a positive energy balance (data not shown) during day seven with the HC-meals, which should lead to higher, not lower RQs [36].

Although it is more difficult to estimate dietary content of carbohydrates than fat and protein (Hambraeus, personal observation), it is unlikely that calculation of dietary composition differed substantially between the diets. The food table values of the carbohydrate sources we used should be quite accurate [53].

In light of the difference between the meals in energy expenditure and the RQ-FQ difference, one would perhaps question the accuracy of the indirect calorimeter system. Although we have not performed a separate test apart from calibration (e.g. burning of alcohol), the calorimeter equipment was serviced before and after the studies with no malfunctions discovered. We have no explanation of why the high fat oxidation persisted, despite the positive energy balance, except for the possibility of small undetected changes in body composition.

Plasma glucose

The HC-meals caused higher plasma glucose concentration and both diets combined displayed an effect of time-of-day, although this effect came mainly from the HF-meals.

That HC-meals caused higher plasma glucose concentration and a faster postprandial increase compared to the HF-meals, agrees with the study by Raben et al [76]. The largest postprandial response was observed 1 h after the 16.00 meal but the 4-h average concentration reached its peak in the 00.00–04.00 period. This is somewhat later than was observed by Van Cauter et al [113]. They used a continuous glucose infusion in their study[113], whereas our subjects were given a meal at midnight. Perhaps another response would have been seen if this meal had been delivered earlier.

Peripheral glucose tolerance decreases from morning to evening whereas splanchnic glucose tolerance is the same [118]. When subjects were given intravenous glucose infusions and kept awake during the night, the glucose concentration increased during the evening to midnight and then decreased [113]. The glucose concentration then increased again, above “awake” concentration, when they were allowed to sleep in the morning [113] (somewhat analogous to the feeding-sleep patterns in shift workers). When they are allowed to sleep during the night a nocturnal increase in glucose concentration is seen with a peak around 04.00 [113], indicating that the circadian rhythm and sleep affect glucose tolerance separately [115]. Although the highest average glucose concentration was seen during the

00.00– 04.00 period, the largest increase was observed after the 16.00 meal with both diets, the physiological significance of this finding is unclear.

Serum triacylglycerols, non-esterified fatty acids and glycerol

We found an effect of time-of-day on TAG concentration with the HF-meals. Regardless of diet, the TAG concentration after the first meal was lower than the other periods. Total 24-h TAG concentration was higher after the HC-meals. Non-esterified fatty acid concentration was affected by time-of-day during the HF-meals and glycerol was not substantially affected by diet or time-of-day.

The postprandial TAG concentration (as seen as concentration 1 h postprandially) showed a dramatic increase from morning to night with the HF-meals whereas the HC-meals showed a more blunted response, although the highest TAG value was seen 2 h after the 04.00 meal. As our subjects were provided with food and kept awake throughout the 24-h period, comparisons with other studies are somewhat difficult. Nevertheless, in a recent study Sopowski et al. studied the response to a pre-meal and test meal during the day compared to night and found increased TAG levels at nighttime [105]. Other studies have also found an increase in TAG concentration from morning to evening [84, 122], although the risk with just comparing morning to evening is that one might see an effect from the preceding nighttime fast rather than an effect of circadian rhythm.

The high TAG concentration could perhaps be caused by increased residence time in the circulation due to decreased TAG clearance. A key enzyme in TAG metabolism is lipoprotein lipase, which has been shown to have a lower activity in the evening compared to the morning [5].

Triacylglycerol concentration was higher after the HC-meals, which is in accordance with several studies (reviewed by Parks and Hellerstein [73]). The HC-diet-induced hypertriacylglycerolemia has been shown to come mainly from increased secretion of very low-density lipoprotein triacylglycerols (VLDL-TAG) [70].

A recent review concluded that elevated fasting TAG concentration is a strong and independent risk factor for ischemic heart disease [32]. In one of the cited studies, the incidence rates of coronary heart disease was 4.6% in the lowest tertile and 7.7% in the middle tertile (1.10 to 1.59 mmol/L) [32]. In our study the fasting values after 6 days of HC-meals was 1.2 ± 0.1 mmol TAG/L, just above the border of increased risk, compared to HF-meals (0.7 ± 0.1 mmol/L). This would suggest that HF-meals are to be preferred if one wants to keep TAG concentrations low. With the HF-meals the postprandial TAG concentrations at 05.00 and 06.00 were ~1.6 mmol/L, which is lower than what has been reported to affect endothelial function (~2.2 mmol/L) [9]. Nevertheless, 1.5 mmol/L has been postulated to be the threshold for formation of large VLDL-particles [37]. These large VLDL-particles then cause the formation of artherogenic small dense low-density lipoprotein (LDL)-particles [37]. In a following study (Holmbäck et al, manuscript), we compared nocturnal eating (i.e. the HF-protocol) to nocturnal fasting (4 larger HF-meals during the day and fasting during the night). The highest postprandial TAG value during the

nocturnal fasting protocol was 1.83 mmol/L vs. 1.57 mmol/L for the nocturnal eating protocol. It therefore seems to be marginally better to eat smaller meals around the clock than larger meals during the day. However, it has been shown that those shift workers who redistributed most of their energy intake to the night shift, had the highest levels of total and low-density lipoprotein-cholesterol [59]. If and how the increased TAG response after the 04.00 meal relates to the increased TAG concentrations seen in shift workers[55, 88] remains to be elucidated. Apparently there is no clear advantage of any of the studied protocols and the last word is still to be spoken. Possibly, diet fatty acid composition might have more impact then the "when" and "how". Perhaps diets high in n-3 fatty acids should be recommended as they should decrease both fasting and postprandial TAG concentrations [131].

Non-esterified fatty acid concentration showed a bimodal shape with a maximum during the 12.00 to 20.00 period, and an additional peak after the 04.00 meal, but this time-of-day effect was not found in glycerol concentration. A similar afternoon peak was seen in a study by Van Gent et al. when subjects consumed three HF-meals between 09.00 and 17.00 or eight HF-meals between 09.00 and 23.00 [117]. However, when the subjects consumed eight HF-meals throughout the 24-period, the NEFA concentration curve was flattened [117].

Higher NEFA concentrations were seen with HF-meals but this difference was statistic albeit too small to be physiologic [16]. Increased NEFA concentrations have been implicated in insulin resistance and the circadian rhythm of insulin sensitivity [72], presumably via NEFA's role in the formation of intramuscular TAG [16]. In the study by Morgan et al., fasting NEFA concentration was higher in the evening than the morning [72]. However, the diurnal variations seen in the our and other studies when normal meals were provided throughout the 24-h period seem to be smaller (between 0.1 and 0.5 mmol NEFA/L) [72, 110, 117] than the 0.7 mmol NEFA/L above fasting levels needed to show a decreased insulin sensitivity [16].

Triacylglycerols, NEFA and glycerol displayed their lowest concentrations during the 08.00–12.00 period, at the same time fat oxidation was at its lowest. One interpretation is that during this period the body is set for CHO oxidation and fat storage is prioritized. Perhaps the intracellular stores of TAG are filled during the morning to be used later as fuel for the daily activities. However, to our knowledge, no study has determined the circadian patterns of intracellular fat metabolism. It is known that the cortisol peak in the early morning stimulates adipocyte hormone sensitive lipase and lipoprotein lipase activity [94]. The activity of these enzymes should lead to release of NEFA and glycerol from the adipocytes and increased clearance of TAG. Apparently, this is not the only mechanism involved as NEFA and glycerol concentrations both were low.

Insulin, c-peptide, glucagon and PP

Insulin did not show any substantial diet or time-of-day effects. Insulin concentration deviated from glucose concentration during the night with the HC-and HF-meals. A higher glucagon concentration was observed with the HF-meals compared

to HC-meals and the highest glucagon concentration was seen during the 08.00–12.00 period after which it remained relatively constant. The postprandial response of PP decreased from morning to evening-night.

After consumption of especially the HC-meals, a higher morning insulin peak was found, which might explain the lower glucagon peak seen with the HC-meals, since insulin suppresses glucagon secretion [26] and thereby decreases gluconeogenesis. The peak in plasma glucose 1 h after the 16.00 meal (Fig. 5) was mimicked by insulin and C-peptide concentrations, regardless of diet. In contrast, the steady increase in glucose concentration (4-h mean) with a peak in the 00.00–04.00 period was not reflected in insulin or C-peptide. The nocturnal disassociation between insulin and glucose concentration has also been shown in a similar experimental setting by Morgan et al [71]. A possible explanation for this nocturnal dissociation could for example be increased insulin clearance [116].

The highest glucagon concentration was seen during the 08.00–12.00 period, and higher glucagon concentrations were found with the HF-meals. Although it has been shown that the body seems to buffer differences in dietary CHO content with glycogenolysis rather than gluconeogenesis [12], the higher glucagon concentration with the HF-meals indicates that a slight increase in gluconeogenesis could perhaps have taken place all the same.

In our study, the postprandial concentration of PP decreased continuously during the evening and night after the 16.00 meal, indicating less gastrointestinal response to the meals. This is perhaps analogous to obese subjects, who respond with lower meal-induced increase in PP compared to normal subjects [33]. The PP concentration showed a large individual variation, so if the data were altered to proportions of the 24-h mean, the concentration difference between the daytime and nighttime became stronger (data not shown). The reduced amplitude of PP concentrations at night may be related to the changes in rectal temperature (used as an indicator of lower gastric activity) and appetite. As the postprandial PP response in this study was similar to the response seen in obese subjects [33], this could possibly indicate health implications of night eating

Thyroid hormones

The thyroid hormones increased from day to night, especially TSH, but did not show any substantial differences depending on diet.

As has been shown in other studies [34], the TSH concentration was higher at night than during the morning. This pattern was also seen in fT4 and tT3 but the nocturnal increase was, although significant, fairly small. Both TSH and fT4 concentrations showed larger postprandial variations during night time than day time, although the relative variation was the same (about 20%) in TSH concentration (Fig. 13). Goichot et al. did not see any nocturnal increase in neither fT4 nor free T3, using basically the same setting [34]. Hirschfeld et al. found a small nocturnal increase in free T3 but not in fT4 [45]. The reasons for these discrepancies could be that Goichot et al. [34] used continuous nasogastric enteral feeding whereas we pro-

vided meals at 4-h intervals. Furthermore, we measured total T3, whereas Goichot et al. [34] and Hirschfeld et al. [45] measured free T3. The thyroid hormones responded to meal intake by a postprandial concentration reduction, although the degree of reduction differed depending on time of day and diet. Sleep affects TSH strongly and the differences we found were much smaller than what has been shown when sleep is compared with no sleep [34]. Furthermore, thyroid hormones are involved in energy expenditure [75] and we observed a tendency for higher tT3 concentrations with the HF-meals. This could support the increased energy expenditure seen with the HF-meals as T3 has been linked to uncoupling [80].

Cortisol, chromogranin-A and leptin

Cortisol concentration showed a clear time-of-day effect, and the concentration was decreased by meal intake during the day but not during the night. Chromogranin-A concentration showed a time-of-day effect with the HC-meals, but the concentration was higher with the HF-meals. Leptin concentration showed no distinct diet or time-of-day effect.

Cortisol secretion was not affected by dietary changes in carbohydrate and fat, in accordance with Slag et al [101]. Meal intake during the morning hours of the day (after 08.00, 12.00 and 04.00 meals) suppressed cortisol concentration (seen as lower concentrations at 2 h compared to 0.5 h postprandially), but this effect was not seen after the 16.00, 20.00 and 00.00 meals. This lack of nocturnal meal feedback might mean that the central drive to increase cortisol concentration during the evening/night is stronger than the potential meal effect. This might have health effects as cortisol affects substrate utilization and lipid storage [89]. The morning meal-influenced decrease of cortisol concentration has also been seen in some other studies [42, 90, 116], whereas again other studies have seen a daytime cortisol increase after meals [48, 101]. In the latter studies, the meal-induced increase in cortisol concentration was only seen after a high protein meal.

Chromogranins are released when secretory granules from different neuroendocrine cells release their content (i.e. hormones); thus chromogranins might serve as an index of overall hormonal secretory activity [108]. The total 24-h CgA concentration was higher with the HF-meals than with the HC-meals. No time-of-day effect with the HF-meals was found in CgA concentration, which is similar to Takiyyuddin et al [108]. We found a time-of-day effect in CgA concentration with the HC-meals and Giampaolo et al. found a similar time-of day effect [31]. No information regarding meal composition before or during the study by Takiyyuddin et al. is provided so we can only speculate that the diet in their study was similar to our HF-meals and perhaps Giampaolo et al. used subjects who had consumed HC-meals before their study. Why HC-meals and not HF-meals would cause a time-of-day difference is not readily apparent, nor why HF-meals caused a higher 24-h CgA concentration than HC-meals. Chromogranin A has been shown to correlate with hypertension, and has therefore been suggested to be a useful marker for the sympato-adrenergic system [109]. It seems to respond to large-scale perturbations of the

sympathetic nervous system, but appears relatively insensitive to short-term behavioral challenge [25].

Leptin showed no distinct effect of time-of-day, except for a different postprandial pattern in the 08.00–12.00 period with the HC-meals. The large individual variation in leptin concentration may have hidden possible time-of-day effects and expressing leptin per kilo fat mass did not decrease this variation (data not shown). Leptin has been shown to display a circadian pattern but meal intake disrupts this pattern [96]. Moreover, meal composition has been shown to affect leptin concentration as high-fat/low CHO meals have been shown to result in lower 24 h area under the curve values compared to high CHO/low fat meals [42], although no diet adjustment period preceded that study. We found no difference in leptin concentration between the HC- and HF-meals. Leptin has been shown to be affected by energy balance [124] and glucocorticoids decrease leptin sensitivity [13]. Interpreting leptin concentrations is far from straightforward as leptin is influenced by so many factors.

Mood

"Hunger" decreased at night and increased linearly shortly after each meal. "Irritation" was higher with the HC-meals.

The decrease of "Hunger" at night is in line with previous observations in, for example, shift workers [123]. The measured metabolic and endocrine variables did not fully explain the reduction of "Hunger" at night. Other possible explanations could be decreased nocturnal gastric emptying rate [35] or the impact of sleepiness [61] on hunger perception. Hunger feelings were similar for both diets. This was surprising considering the greater palatability, according to verbal reports, and the higher energy density of the HF-diet (i.e. less volume than HC-meals)

The only diet effect on mood ratings was found for "Irritated", the HF-meals giving lower ratings. In an earlier study in which a "friendly-antagonistic" scale was used, a decrease of "friendliness" was found after an HC-meal [125].

The strongest overall reactions on the ratings "Indifferent" and "Weary" were related to time-of-day. There was no apparent breakfast-lunch-dinner difference in relation to meal intake and mood ratings, in contrast to Kanarek [51].

Correlations

The use of multiple regression analysis with only seven subjects might be questioned. We took the interindividual variation into account by using a dummy model in an attempt to make the results of the regression analysis more robust. Insulin's involvement in various metabolic pathways was evident in that it was part of all models explaining variance in metabolic parameters (Tables 5). Glucagon concentration correlated positively with CHO oxidation and negatively with fat oxidation. It has been shown that glucagon is an important gluconeogenic hormone [11], but its effect on lipolysis has been shown to be less pronounced [11]. The negative correlation between TSH and CHO oxidation most probably just reflects the inverse

response to meals of the two. In the experimental setting we used, other variables, than the concentration of endocrine variables we measured, explained the metabolic variables variance.

"Health" effects

Shift work has been shown to be associated with a number of conditions such as high TAG concentrations [88] and obesity [112], leading to an increased risk of myocardial infarction [56]. Could the type of macronutrient intake have a role in these metabolic disturbances? The differences we saw between the diets, higher TAG concentration and lower energy expenditure with the HC-diet, might be of concern in a shift work perspective. Is the nocturnal caloric intake also an issue? On the one hand, there was no clear advantage of any of the studied protocols regarding TAG concentrations, if anything, small meals around the clock would be preferred to keep the postprandial TAG concentrations low (Holmbäck et al, manuscript). Moreover, the subjects seemed to be more active if they ate at night (Holmbäck et al, manuscript). On the other hand, the increased TAG and glucose concentrations towards the night and the decreased responsiveness of cortisol and PP to nighttime meal intake compared to daytime may have health implications. Preliminary "mood" data indicate that nocturnal eating decreases ratings related to sleepiness more than nocturnal fasting [64]. However, it has been shown that redistributing most of the energy intake to the night shift increases total and LDL-cholesterol [59] Possibly, the physiologically sensible strategy differs from the psychologically sensible strategy.

Future perspectives

Long-term intervention studies are needed, in which different feeding regimens and dietary macronutrient compositions are tested. *Daytime sleep* decreases glucose tolerance [113] and its effects on TAG concentrations are unknown but have been studied recently (Holmbäck, data in process). *Sleep debt* has been shown to decrease insulin sensitivity [106] but its effects on TAG concentrations and macronutrient oxidation are so far unknown. *More health parameters* need to be determined before clear conclusions can be drawn. For example, immunological parameters (C-reactive protein, interleukin-6, monocyte count etc.) need to be included to obtain a more complete picture of health status.

CONCLUSIONS

The findings in this study can be summarized as:

- Postprandial responses differed depending on dietary macronutrient composition and the time of day.
- Nocturnal eating increased postprandial TAG concentration more than daytime eating.
- Energy expenditure was higher after the seven-day high fat-diet period than after the seven-day high carbohydrate diet period.

- Insulin, PP, TSH, fT4, cortisol and leptin concentrations after meal intake differed with respect to time of day.
- Cortisol and PP showed decreased responsiveness to nighttime meal intake compared to daytime meal intake.
- "Hunger" increased linearly shortly after each meal but "Hunger" in general was lower during the night, and "Irritation" was higher with the HC-meals.

I therefore conclude that:

- A well-balanced high fat diet seems to be better for health and mood than a high carbohydrate diet.
- Nocturnal eating seems to be good for mental energy (activity).
- Regarding blood lipid levels after meals, smaller evenly spaced meals throughout the 24-h period seem marginally better than larger meals during the day, although meals around 04.00 seems to be bad.
- Meal intake around midnight seems to be bad for plasma glucose concentrations.
- Nocturnal eating seems to be bad for gastrointestinal response and cortisol levels.

Further studies (especially long term) are needed before clear dietary guidelines can be given, especially regarding the impact of nocturnal eating on gastrointestinal response and cortisol. Nocturnal meals seem to be needed BUT the energy content should be low and aimed for maintaining mental energy.

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