Metabolic and Endocrine Responses to Nocturnal Eating

BY

ULF HOLMBÄCK
METABOLIC AND ENDOCRINE RESPONSES TO NOCTURNAL EATING

BY

ULF HOLMBÄCK

ACTA UNIVERSITATIS UPSALIENSIS
UPPSALA 2002
Dissertation for the Degree of Doctor of Philosophy (Faculty of Medicine) in Nutrition presented at Uppsala University

ABSTRACT


An increasing amount of people have their work hours displaced to the night and there are indications that shift work and other irregular working schedules are associated with an increased risk of developing the metabolic syndrome and other pathological conditions. It is therefore important to address the consequences of eating at irregular hours, especially nighttime. Papers I-III refer to a study in which 7 males were given a high-carbohydrate diet (HC) or a high-fat diet (HF), using a cross-over design. Subjects were kept awake for 24 h and food was provided as 6 equally spaced isocaloric meals. Higher energy expenditure and non-esterified fatty acids (NEFA) concentration, as well as lower glucose and triacylglycerol (TAG) concentrations were observed with the HF-diet, compared to the HC-diet. With the HF-diet, fat oxidation, heat release, heart rate, glucose, NEFA and TAG concentrations differed depending on time of day. The highest postprandial TAG concentrations were seen after the 04.00 meal with both diets. Insulin and leptin responses to meal intake differed with respect to diet and time of day. Time of day affected glucagon, thyroid stimulating hormone, free thyroxin, total triiodothyronine (tT3), cortisol, chromogranin A and pancreatic polypeptide (PP) concentrations. PP’s postprandial increase was greater during 08.00 – 16.00 compared to 20.00 – 08.00. Furthermore, the subjects felt less irritated when eating the HF-diet but hunger was not related to macronutrient composition. Hunger and thirst decreased throughout the 24 h period despite constant activity and energy intake; and were correlated with several endocrine and metabolic variables. In paper IV 7 males were studied twice during 24-h either given 6 isocaloric meals throughout the 24-h period, or 4 isocaloric meals from 08.00 to 20.00, followed by a nocturnal fast. Energy expenditure, glucose, TAG, insulin and glucagon concentrations were lower; and NEFA concentrations were higher during the nocturnal fast compared to nocturnal eating; although no 24 h differences between the protocols were apparent. The subjects were more passive during the fasting period compared to when food was given. Stepwise regression showed that correlations between metabolic variables and hormones differed between daytime and nighttime. The decreased evening/nocturnal responses of cortisol and PP to meal intake suggest that nocturnal eating might have health implications and that the body reacts unfavorably to nocturnal eating. Smaller meals around the clock, however, showed marginally better effects on postprandial TAG concentrations and mental energy compared to larger meals during daytime. Further studies (long term) are needed before dietary guidelines can be given to shift workers, especially regarding the impact of nocturnal eating on gastrointestinal response and cortisol.

Key words: postprandial, substrate utilization, circadian, hormones, mood.

Ulf Holmбёck, Department of Medical Sciences, Nutrition, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

© Ulf Holmбёck 2002

ISSN 0282-7476
ISBN 91-554-5456-9

Printed in Sweden by Akademitryck, Edsbruk, 2002
LIST OF ORIGINAL PAPERS

This thesis is based on the papers listed below, which are referred to in the text by their Roman numerals. Reprints were made with the permission of the publishers.


Paper II  **Ulf Holmbäck**, Anders Forslund, Arne Lowden, Jeanette Forslund, Torbjörn Åkerstedt, Maria Lennernäs, Leif Hambraeus, Mats Stridsberg. Endocrine responses to nocturnal eating - possible implications for night work. *European Journal of Nutrition, accepted for publication*

Paper III  Arne Lowden, **Ulf Holmbäck**, Torbjörn Åkerstedt, Anders Forslund, Jeanette Forslund, Maria Lennernäs. The expression of hunger and mental state for different macronutrient compositions and the relations to gastrointestinal hormones during a 24-hour wake. *Journal of Human Ergology, accepted for publication*

Paper IV  **Ulf Holmbäck**, Arne Lowden, Torbjörn Åkerfeldt, Maria Lennernäs, Leif Hambraeus, Jeanette Forslund, Torbjörn Åkerstedt, Anders Forslund, Mats Stridsberg. Metabolic and endocrine postprandial responses in men differ between nocturnal fasting and eating during a 24-h wake period. *Manuscript*
Table of Contents

Abbreviations......................................................................................................................6
INTRODUCTION..................................................................................................................7
Background.........................................................................................................................7
Diet .......................................................................................................................................8
Metabolic variables............................................................................................................9
Endocrine variables...........................................................................................................9
Psychological variables ...................................................................................................10
Tools ...................................................................................................................................11
Indirect calorimetry ........................................................................................................11
Direct calorimetry ............................................................................................................11
AIMS OF THE THESIS ....................................................................................................12
MATERIALS AND METHODS ........................................................................................13
Papers I- III ........................................................................................................................13
Subjects ............................................................................................................................13
Experimental Design .......................................................................................................13
Diets ..................................................................................................................................14
Procedures .......................................................................................................................15
Mood .................................................................................................................................16
Statistics ............................................................................................................................16
Paper IV ................................................................................................................................17
Subjects ............................................................................................................................17
Experimental design .......................................................................................................18
Diet ....................................................................................................................................18
Procedures .......................................................................................................................18
Statistics ............................................................................................................................18
RESULTS.........................................................................................................................19
Paper I ................................................................................................................................19
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMR</td>
<td>basal metabolic rate</td>
</tr>
<tr>
<td>CgA</td>
<td>chromogranin A</td>
</tr>
<tr>
<td>FQ</td>
<td>food quotient</td>
</tr>
<tr>
<td>fT4</td>
<td>free thyroxin</td>
</tr>
<tr>
<td>HC-meals</td>
<td>high-carbohydrate meals</td>
</tr>
<tr>
<td>HF-meals</td>
<td>high-fat meals</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>N-eat</td>
<td>nocturnal eating</td>
</tr>
<tr>
<td>NEFA</td>
<td>nonesterified fatty acids</td>
</tr>
<tr>
<td>N-fast</td>
<td>nocturnal fasting</td>
</tr>
<tr>
<td>RMR</td>
<td>resting metabolic rate</td>
</tr>
<tr>
<td>RQ</td>
<td>respiratory quotient</td>
</tr>
<tr>
<td>PP</td>
<td>pancreatic polypeptide</td>
</tr>
<tr>
<td>TAG</td>
<td>triacylglycerol</td>
</tr>
<tr>
<td>TSH</td>
<td>thyroid stimulating hormone</td>
</tr>
<tr>
<td>tT3</td>
<td>total triiodothyronine</td>
</tr>
<tr>
<td>VLDL</td>
<td>very-low-density lipoprotein</td>
</tr>
</tbody>
</table>
INTRODUCTION

“If something is so complicated that you cannot explain it in 10 seconds, then it’s probably not worth knowing anyway.”
Calvin

Background

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\(^1\). The same proportion of shift workers can be found in most of the industrialized world\(^2\). Shift-workers employed in two- and three-shift work seem to alter their timing of meals\(^3\), 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\(^4\) and with a higher prevalence of the “metabolic syndrome”\(^5\), including a number of conditions such as high blood triacylglycerols (lipid) concentrations\(^6\) and obesity\(^7\), leading to an increased risk of myocardial infarction\(^8\). The “24-hour society”\(^9\) 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\(^10\) 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.

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)\textsuperscript{11} where oscillations from “clock genes” are constantly corrected by the shift of light intensity\textsuperscript{12}. 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\textsuperscript{13}. During pharmacokinetic studies, a circadian variability has been shown in absorption of several drugs\textsuperscript{14}. This is partly due to a decreasing rate of gastric emptying from morning to evening\textsuperscript{15}. 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\textsuperscript{3}). A high-fat diet has been proposed by some to increase the risk of obesity\textsuperscript{16} whereas others refute this connection\textsuperscript{17}. Since shift workers have increased risk of becoming obese\textsuperscript{7}, 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\textsuperscript{18-24}, whereas other studies have not found that fat oxidation correlates with fat intake\textsuperscript{25,26}. 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\textsuperscript{27}.

It has been shown that peripheral glucose tolerance (the capacity to transport blood sugar into the tissue) varies during the 24-h period\textsuperscript{28}. Shift workers tend to nibble on carbohydrate (CHO) snacks to stay awake\textsuperscript{29}. This nocturnal CHO intake could be physiologically unfavorable, although favorable for mental performance. Regarding meal frequency, size and timing, the results from previous studies are quite disparate. Especially energy expenditure and blood lipids have been in focus in these studies. Some studies show that larger meals increase energy expenditure (or rather the dietary induced thermogenesis) more than small meals\textsuperscript{30,31}, whereas others do not find a difference due to meal size\textsuperscript{32}. In an extensive study, Murphy et al. compared two weeks of nibbling (12 meals per day) to gorging (three meals per day)\textsuperscript{33}. They found no difference between the protocols in blood lipids, glucose, insulin and other blood variables, except higher HDL-cholesterol after the gorging period. Previous more short term experiments had shown a reduction in cholesterol
concentration with a nibbling protocol. This might, however, be due to the short time period studied or unintentional changes in meal pattern. Regarding meal timing, no study has compared nocturnal eating with nocturnal fasting in neither metabolic nor endocrine or psychologic variables.

**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. 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 period, 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. High blood triacylglycerol (lipid) concentrations are commonly found in shift workers. In studies comparing the blood lipids postprandial (after meal) response, higher concentrations are usually found in the evening compared to the morning. 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 and lipid metabolism. Meal composition does not influence insulin concentration substantially and insulin concentration is only weakly influenced by circadian rhythm.

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.

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

*Glucagon* is a pancreatic hormone secreted by the alpha cells in the pancreas, and plays an important role in regulation of blood 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. The thyroid hormones are not substantially affected by the dietary macronutrient composition, unless more extreme diets are used. Thyroid hormones, especially TSH, show a clear circadian rhythm with lower values during the day and higher in the evening. If subjects are kept awake, the TSH concentrations increase even more whereas T3 and T4 are less affected.

Cortisol affects energy expenditure, and protein, CHO and fat metabolism. Cortisol displays a clear and steady circadian rhythm but is not substantially affected by macronutrient composition.

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. No study has addressed the impact of dietary composition on chromogranin concentration. A circadian rhythm in CgA concentration has been found by some, but not others.

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

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. Wells et al., have in several studies looked into the influence of macronutrient composition and diurnal effects on mood. Subjects felt less vigorous and more dreamy and feeble after a HF-meal consumed at 10.30 than after a HC-meal. These effects were not apparent if the meals were given at 12.30 instead. This study has, however, been criticized for weak effects and questionable design. The effects of macronutrient composition on mood thus seem to be modest and inconsistent. 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.
Tools
Of the tools used in this thesis, the principles behind indirect and direct calorimetry may require a brief explanation.

Indirect calorimetry
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. Several 24-h studies have been performed showing good reproducibility. The carbon dioxide production (CO₂)/oxygen (O₂) 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 CO₂/O₂-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. When using indirect calorimetry to estimate substrate utilization, there are a number of variables that have to be taken into consideration. When estimating 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.

Direct calorimetry
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. 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. 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, on can assume that the heat will be released from the body.
AIMS OF THE THESIS

- To study postprandial responses to meals eaten throughout the 24-h period and how macronutrient composition relates to these responses (I).
- To further examine the postprandial response with analyses of endocrine variables throughout the 24-h period (II).
- To study how macronutrient composition and time of day influence “mood” throughout the 24-h period (III).
- To study the effect of nocturnal fasting on metabolic and endocrine postprandial responses (IV).
MATERIALS AND METHODS

Papers I- III

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/m\(^2\); 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.\(^{76}\). Maximal \( \text{O}_2 \) uptake (\( \text{VO}_{2\text{max}} \)) was estimated by a sub-maximal \(^{77}\) 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. 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.

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

<table>
<thead>
<tr>
<th>Time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.01</td>
<td>Meal</td>
</tr>
<tr>
<td>08.15</td>
<td>Mental performance test I</td>
</tr>
<tr>
<td>08.30</td>
<td>Blood sample</td>
</tr>
<tr>
<td>08.35</td>
<td>Mental performance test II</td>
</tr>
<tr>
<td>08.45</td>
<td>BIA / Blood pressure measurement</td>
</tr>
<tr>
<td>08.55</td>
<td>Mental (“mood”) assessment</td>
</tr>
<tr>
<td>09.00</td>
<td>Blood sample</td>
</tr>
<tr>
<td>09.15</td>
<td>Mental performance test I</td>
</tr>
<tr>
<td>09.45</td>
<td>BIA / Blood pressure measurement</td>
</tr>
<tr>
<td>09.55</td>
<td>Mental assessment</td>
</tr>
<tr>
<td>10.00</td>
<td>Blood sample</td>
</tr>
<tr>
<td>10.15</td>
<td>Mental performance test I</td>
</tr>
<tr>
<td>10.45</td>
<td>BIA / Blood pressure measurement</td>
</tr>
<tr>
<td>10.55</td>
<td>Mental assessment</td>
</tr>
<tr>
<td>11.00</td>
<td>Blood sample</td>
</tr>
<tr>
<td>11.15</td>
<td>Mental performance test I</td>
</tr>
<tr>
<td>11.25</td>
<td>Mental performance test II</td>
</tr>
<tr>
<td>11.35</td>
<td>BIA / Blood pressure measurement</td>
</tr>
<tr>
<td>11.40</td>
<td>Mental assessment</td>
</tr>
<tr>
<td>11.45</td>
<td>Urine collection</td>
</tr>
<tr>
<td>11.55</td>
<td>Body weight</td>
</tr>
</tbody>
</table>

Diets
Two diets were compared, a high carbohydrate diet (HC) with a Food Quotient [FQ; 73] 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% 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 78. 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).

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

<table>
<thead>
<tr>
<th>Day 1 to day 6</th>
<th>Day 7</th>
<th>HC</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Low-fat yogurt, 0.5% fat</td>
<td>Yogurt, 3% fat</td>
<td></td>
</tr>
<tr>
<td>Sour milk, cereals, bread, margarine, cheese</td>
<td>White bread</td>
<td>Grahams-bread</td>
<td></td>
</tr>
<tr>
<td>HC: orange juice HF: milk (3% fat)</td>
<td>Low fat margarine, 40% fat</td>
<td>Margarine, 80% fat</td>
<td></td>
</tr>
<tr>
<td>Lunch¹</td>
<td>Low fat cheese, 16% fat</td>
<td>Cucumber, banana</td>
<td></td>
</tr>
<tr>
<td>Ham, pasta, white sauce, green peas or Chicken, rice, tomato sauce, green beans</td>
<td>Liquid margarine 40% fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC: orange juice HF: milk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner¹</td>
<td>Meat sauce, rice, broccoli or Salmon, pasta, white sauce, carrots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat cheese, 16% fat or Cucumber</td>
<td>Milk</td>
<td>Low fat cheese, 16% fat</td>
<td>Cucumber</td>
</tr>
<tr>
<td>Dinner¹</td>
<td>Bread, margarine, cheese, apple or HC: orange juice HF: milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snacks</td>
<td>Bread, margarine, cheese, apple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snacks</td>
<td>HC: orange juice HF: milk</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

**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 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 74. 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. 79. 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 \(^8\). Heat release, skin and body temperature were measured using the direct calorimetry suit \(^7\).

**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). Statistical software (SuperANOVA, version 1.11, SAS Institute Inc, Cary, NC, USA) was 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.

**Paper IV**

**Subjects**

Seven males were recruited for the study and their mean (range) age was 30 (22 – 43) y; weight 79 (63 – 92) kg; and body fat 22 (17 – 31)%.

Four subjects had participated in the previous study and their HF-data were included (Fig. 1). 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 TAG values [1.54 mmol/L, at 08.00 day 7 (mean of the two sessions)]. All subjects gave their written informed consent, and the Ethical Committee of the Faculty of Medicine at Uppsala University approved the study.

![Fig. 1 Description of the subjects (●) distribution in the different studies](image-url)
**Experimental design**

The subjects participated in two seven-day experimental sessions, receiving the same diet in two different eating protocols in a cross-over design with a one-month washout period between the two sessions. During the sessions they were followed on an outpatient basis during day 1 to day 6 and on day 7 the 24-h metabolic study was performed at the metabolic unit. 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 4-h periods. The two different eating protocols were: nocturnal eating (N-eat) where the subjects received six isocaloric meals; at 0800, 1200, 1600, 2000, 0000 and 0400 h; and nocturnal fasting (N-fast) where the same caloric content as N-eat was divided into four meals served at 0800, 1200, 1600 and 2000 h, and water (~200 ml) was given at 0000 and 0400 h. Mental performance tests were performed every hour (the results of the mental performance test will be reported elsewhere). Blood sampling occurred 30 minutes postprandially and at 1, 2, 3 and 4 h postprandially. At the end of each 4-h period, urine was collected. Activity, heat release and body temperature were measured continuously throughout the 24 h period. The subjects remained seated in a chair throughout the study and no physical activity was allowed.

**Diet**

The HF-diet described above was used.

**Procedures**

Similar to Paper I and II, except that energy expenditure was calculated according to Schutz⁸¹, and fat and CHO oxidation were calculated according to Jéquier et al⁷⁹.

**Statistics**

Data were analyzed as in Paper I and II, with the different factor protocol (24-h difference between N-eat and N-fast); A three-factor RM-ANOVA was also performed on the daytime (0800 – 0000 h) and nighttime period (0000 – 0800 h) separately. A multiple regression analysis was done on the whole 24-h period and the daytime (0800 – 0000 h) and nighttime period (0000 – 0800 h) separately. Statistics on TAG were computed without the subject with high TAG values. His results were consistent with the other subjects in all other variables and were therefore included. The sensitivity of the actimeters varied between the sessions, the data was then changed to proportions of the 24-h mean prior to statistical analyses.
RESULTS

“Under the most rigorously controlled conditions of pressure, temperature, volume, humidity, and other variables, the organism will do as it damn well pleases.”

Harvard’s Law

Paper I

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.

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

<table>
<thead>
<tr>
<th>Diet (D)</th>
<th>Time-of-day(T)</th>
<th>Meal (M)</th>
<th>D•T</th>
<th>D•M</th>
<th>T•M</th>
<th>D•T•M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy expenditure</td>
<td>.039$^1$</td>
<td>.001</td>
<td>&lt;.001</td>
<td>.002</td>
<td>.033</td>
<td>.002</td>
</tr>
<tr>
<td>CHO oxidation</td>
<td>.001</td>
<td>.002</td>
<td>&lt;.001</td>
<td>.002</td>
<td>.033</td>
<td>.002</td>
</tr>
<tr>
<td>Fat oxidation</td>
<td>&lt;.001</td>
<td>.054</td>
<td>&lt;.001</td>
<td>.007</td>
<td>.002</td>
<td>.002</td>
</tr>
<tr>
<td>Protein oxidation</td>
<td>.011</td>
<td>.009</td>
<td>.005</td>
<td>.009</td>
<td>.007</td>
<td>.007</td>
</tr>
<tr>
<td>Temp (rectal)</td>
<td>.008</td>
<td>.002</td>
<td>.047</td>
<td>.047</td>
<td>.047</td>
<td>.047</td>
</tr>
<tr>
<td>Temp (skin)</td>
<td>.008</td>
<td>.002</td>
<td>.047</td>
<td>.047</td>
<td>.047</td>
<td>.047</td>
</tr>
<tr>
<td>Heart rate</td>
<td>.008</td>
<td>.002</td>
<td>.047</td>
<td>.047</td>
<td>.047</td>
<td>.047</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>.008</td>
<td>.002</td>
<td>.047</td>
<td>.047</td>
<td>.047</td>
<td>.047</td>
</tr>
<tr>
<td>Glucose</td>
<td>.036</td>
<td>.001</td>
<td>&lt;.001</td>
<td>.006</td>
<td>.005</td>
<td>.005</td>
</tr>
<tr>
<td>TAG</td>
<td>.007</td>
<td>.027</td>
<td>&lt;.001</td>
<td>.002</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NEFA</td>
<td>.002</td>
<td>.027</td>
<td>&lt;.001</td>
<td>.031</td>
<td>.003</td>
<td>.003</td>
</tr>
<tr>
<td>Glycerol</td>
<td>.002</td>
<td>.027</td>
<td>&lt;.001</td>
<td>.031</td>
<td>.003</td>
<td>.003</td>
</tr>
</tbody>
</table>

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 • 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; •, 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.
Higher energy expenditure was observed when the men consumed the HF-meals compared to the HC-meals (Fig. 2, 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

**FIG. 2** 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 the postprandial 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 the value 2 h after meal intake in all the 6 periods, a.s.f.
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.

Carbohydrate oxidation was higher with the HC-meals than with the HF-meals, and no time-of-day pattern was seen (Fig. 2, 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 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. 2, 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. 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 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 test period as heat release rapidly stabilized with the HC-meals; whereas with the HF-meals, heat loss increased until the 16.00 – 20.00 period (HC-meals, $P = NS$; HF-meals, $P = 0.021$ for time-of-day pattern)(Fig. 3).
Meal intake increased heat loss 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

![Diagram showing glucose, TAG, NEFA, and glycerol concentrations over 24 hours for both diets.](image)

**FIG. 5** 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.
lower heart rate 3 h and 4 h postprandially with the HC-meals (Fig. 4). 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, $P = \text{NS}$; 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 • time-of-day interaction, seen as a difference 1 h and 2 h postprandially (Fig. 4).

Glucose concentration was higher and increased more at 0.5 h after meals with the HC-meals than with the HF-meals (Fig. 5, 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, $P = \text{NS}$; 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 ($P = \text{NS}$).

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. 5, 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 ($P = \text{NS}$), 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 to the HF-meals, especially 2 h postprandially (Fig. 5, 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, $P = \text{NS}$; 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 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. 5, Table 3).
Paper II

There were no differences in fasting hormone concentrations on the morning of day one, or day seven (before the 24-h study) between the diets (data not shown).

Twenty-four-hour-study data are presented in graphs and the results from the three-factor repeated measurements ANOVA are displayed in Table 4. The results of the two-factor repeated measurements ANOVA (within each diet and Day-Night comparisons) are presented in the following text.

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

<table>
<thead>
<tr>
<th></th>
<th>Diet1(D)</th>
<th>Time-of-Day2(T)</th>
<th>Meal3(M)</th>
<th>D•T</th>
<th>D•M</th>
<th>T•M</th>
<th>D•T•M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>ns</td>
<td>ns</td>
<td>&lt;.001</td>
<td>.028</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>C-peptide</td>
<td>ns</td>
<td>ns</td>
<td>&lt;.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>PP</td>
<td>&lt;.021</td>
<td>&lt;.032</td>
<td>ns</td>
<td>ns</td>
<td>.047</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Glucagon</td>
<td>.040</td>
<td>.011</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>TSH</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>.002</td>
<td>ns</td>
</tr>
<tr>
<td>fT4</td>
<td>&lt;.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>.053</td>
<td></td>
</tr>
<tr>
<td>tT3</td>
<td>.053</td>
<td>&lt;.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;.001</td>
<td>&lt;.004</td>
<td>ns</td>
<td>ns</td>
<td>&lt;.001</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CgA</td>
<td>.005</td>
<td>.034</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>.007</td>
</tr>
<tr>
<td>Leptin</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

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 • time-of-day interaction, the diets affect the pattern due to time of day differently.  5 Diet • meal interaction, the diets affect the pattern within the 4-h periods differently.  6 Time-of-day • meal interaction, time of day affects the pattern within the 4-h periods.  7 Diet • time-of-day • meal interaction, diets affect how time of day affects the pattern within the 4-h periods.

The insulin concentration was the same with both diets, although a diet • 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. 6, 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. 6, 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 1200 – 1600 h period and the 16.00 – 20.00 h period. No C-peptide concentration difference was observed between Day and Night.
Fig. 6  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 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. 6, Table 4). Meal intake increased the PP concentration but this increase diminished throughout the 24-h period (Fig. 1, Table 1). However, no significant PP concentration difference could be detected between Day and Night, due to large inter-individual variations (Fig. 1).

The glucagon concentration was higher with the HF-meals compared to the HC-meals and a time-of-day pattern was seen (Fig. 6, Table 4). A higher concentration was observed during the 0800 – 1200 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 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. 7, 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. 7, 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. 3). 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. 7, 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 pattern with a peak around 0800 h and a nadir in the 20.00 – 00.00 h period (Fig. 8, 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. 8, 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 \( (P=NS) \).

**Fig. 7** 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 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. 8, 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 variables from Paper I. 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 5 Partial correlations (controlled for individual differences) between endocrine data from both diet periods during day 7 and metabolic variables from Paper I

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insulin</th>
<th>PP</th>
<th>Glucagon</th>
<th>TSH</th>
<th>tT3</th>
<th>Cortisol</th>
<th>CgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>En-exp 1</td>
<td>2.9% (0.20)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.9% (0.20)</td>
<td>-</td>
</tr>
<tr>
<td>CHO-ox 2</td>
<td>0.9% (-0.13)</td>
<td>5.5% (0.37)</td>
<td>4% (-0.26)</td>
<td>-</td>
<td>-</td>
<td>2.2% (-0.17)</td>
<td>5.7% (0.31)</td>
</tr>
<tr>
<td>Fat-ox 3</td>
<td>2.6% (0.23)</td>
<td>2.2% (-0.33)</td>
<td>1.5% (0.06)</td>
<td>6.5% (0.20)</td>
<td>2.6% (-0.19)</td>
<td>2.9% (0.12)</td>
<td></td>
</tr>
<tr>
<td>Glucose 81.7%</td>
<td>0.9% (-0.08)</td>
<td>0.5% (-0.08)</td>
<td>0.2% (0.05)</td>
<td>-</td>
<td>-</td>
<td>6.5% (0.20)</td>
<td>-</td>
</tr>
<tr>
<td>TAG 1.5%</td>
<td>1.7% (0.25)</td>
<td>3.9% (-0.29)</td>
<td>2.2% (0.16)</td>
<td>-</td>
<td>-</td>
<td>1.4% (0.14)</td>
<td>-</td>
</tr>
<tr>
<td>NEFA 9.5%</td>
<td>1.3% (-0.15)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.7% (0.21)</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol 8.6%</td>
<td>3.6% (0.20)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 (E)

Paper III

Group mean levels across the 24 h are presented in Fig. 9 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 experimental 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).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Time-of-day Meal</th>
<th>D/T</th>
<th>D/M</th>
<th>T/M</th>
<th>D/T/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td>•</td>
<td>4.22*</td>
<td>19.8**</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Thirst</td>
<td>•</td>
<td>11.5**</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Irritated</td>
<td>7.27*</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Indifferent</td>
<td>4.97*</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Weary</td>
<td>11.4**</td>
<td>•</td>
<td>7.91**</td>
<td>•</td>
<td>2.40*</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01, • = n.s.
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 from Papers I and II 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.

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

<table>
<thead>
<tr>
<th></th>
<th>TAG</th>
<th>FFA</th>
<th>Insulin</th>
<th>Glucose</th>
<th>Leptin</th>
<th>Glucagon</th>
<th>Cortisol</th>
<th>Enexp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td>-.39</td>
<td>.36</td>
<td>-.36</td>
<td>-.32</td>
<td>•</td>
<td>•</td>
<td>-.11</td>
<td>•</td>
</tr>
<tr>
<td>Thirst</td>
<td>-.16</td>
<td>.28</td>
<td>-.28</td>
<td>-.21</td>
<td>-.14</td>
<td>.14</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Irritated</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>.14</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Indifferent</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>.15</td>
<td>.15</td>
<td>•</td>
</tr>
<tr>
<td>Weary</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>.22</td>
<td>•</td>
<td>.31</td>
<td>•</td>
</tr>
</tbody>
</table>

* = n.s. (F-value to Enter<4.0)

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.

Paper IV

None of the studied variables differed between the two protocols seen over the whole 24-h period. However, within the 24-period, distinct differences were observed.

Energy expenditure did not differ between the protocols during the eating period (08.00 – 00.00), but was lower during the fasting period (00.00 – 08.00) during the N-fast protocol then during the N-eat protocol (Fig. 10, Tables 8 & 9).

Carbohydrate oxidation did not differ between the protocols during the fasting or eating period (Fig. 10, Tables 8 & 9); although a protocol • time-of-day interaction was observed (Table 9). Further analyses showed a tendency for a protocol • time-of-day interaction during the nighttime period (Table 10). A time-of-day • meal interaction was observed during both protocols, probably from higher postprandial CHO oxidation after the 08.00 meal compared to the 16.00 meal (Fig. 10, Table 9). Fat oxidation did not differ between the protocols during the eating or fasting period (Fig. 10, Tables 8 & 9). A time-of-day • meal effect was seen during both protocols, probably from lower postprandial fat oxidation after the 08.00 meal compared to the 16.00 meal (Fig. 10, Tables 8 & 9).

Protein oxidation did not differ between the protocols or show any time-of-day pattern (Tables 8 & 9).
Activity (proportions of 24-h mean) showed no effect of time-of-day (P=NS), and only a tendency for a meal effect was observed (P=0.065; Fig. 11).

Heat release did not differ between the protocols and a time-of-day pattern was observed during both protocols (Fig. 11, Tables 8 & 9). During both protocols, a postprandial increase in heat release was observed (Table 9). A protocol • time-of-day • meal interaction was observed, probably due to a larger postprandial increase in heat release after the 16.00 and 20.00 meals during the N-fast protocol than during the N-eat protocol (trend for protocol • meal interaction); and a protocol • meal interaction during the nighttime (Tables 9 & 10).

Table 8  Energy expenditure, macronutrient oxidation, heat release, body temperature and blood variables during the feeding, fasting and total 24 h periods during the 24 h study day 7 during nocturnal eating protocol (N-eat) and nocturnal fasting protocol (N-fast)

<table>
<thead>
<tr>
<th></th>
<th>N-eat (D)</th>
<th>N-fast (D)</th>
<th>N-eat (N)</th>
<th>Nfast (N)</th>
<th>Neat (24 h)</th>
<th>Nfast (24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>En exp, MJ</td>
<td>6.0 ± 0.2</td>
<td>6.2 ± 0.3</td>
<td>3.8 ± 0.2</td>
<td>3.4 ± 0.1*</td>
<td>9.8 ± 0.4</td>
<td>9.6 ± 0.4</td>
</tr>
<tr>
<td>CHO ox, g</td>
<td>90 ± 6</td>
<td>117 ± 18</td>
<td>54 ± 3</td>
<td>38 ± 7</td>
<td>145 ± 9</td>
<td>155 ± 23</td>
</tr>
<tr>
<td>Fat ox, g</td>
<td>107 ± 5</td>
<td>98 ± 10</td>
<td>67 ± 5</td>
<td>65 ± 6</td>
<td>175 ± 9</td>
<td>163 ± 15</td>
</tr>
<tr>
<td>Pro ox, g</td>
<td>69 ± 5</td>
<td>78 ± 8</td>
<td>42 ± 3</td>
<td>42 ± 5</td>
<td>111 ± 6</td>
<td>120 ± 12</td>
</tr>
<tr>
<td>Heat rel, MJ</td>
<td>3.4 ± 0.3</td>
<td>3.5 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>5.3 ± 0.4</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Body temp, °C</td>
<td>36.8 ± 0.1</td>
<td>36.7 ± 0.1</td>
<td>36.4 ± 0.1</td>
<td>36.2 ± 0.2</td>
<td>36.6 ± 0.1</td>
<td>36.5 ± 0.1</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>5.54 ± 0.11</td>
<td>5.65 ± 0.10</td>
<td>5.72 ± 0.12</td>
<td>5.06 ± 0.12*</td>
<td>5.58 ± 0.10</td>
<td>5.48 ± 0.10</td>
</tr>
<tr>
<td>TAG, mM</td>
<td>0.98 ± 0.08</td>
<td>1.23 ± 0.11*</td>
<td>1.14 ± 0.07</td>
<td>0.80 ± 0.07*</td>
<td>1.02 ± 0.07</td>
<td>1.06 ± 0.09</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>0.30 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.29 ± 0.01</td>
<td>0.36 ± 0.03*</td>
<td>0.30 ± 0.01</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>17.7 ± 2.1</td>
<td>22.9 ± 2.7*</td>
<td>17.2 ± 2.0</td>
<td>5.3 ± 0.8*</td>
<td>16.7 ± 2.1</td>
<td>17.3 ± 2.2</td>
</tr>
<tr>
<td>PP, pmol/L</td>
<td>66.7 ± 19.0</td>
<td>115.1 ± 35.9†</td>
<td>48.3 ± 15.9</td>
<td>34.0 ± 7.9</td>
<td>42.6 ± 10.2</td>
<td>100.7 ± 7.65</td>
</tr>
<tr>
<td>Glucagon, mM</td>
<td>86.6 ± 14.2</td>
<td>96.7 ± 11.0</td>
<td>83.7 ± 10.8</td>
<td>78.9 ± 10.3*</td>
<td>87.1 ± 14.3</td>
<td>91.6 ± 10.7</td>
</tr>
<tr>
<td>TSH, μU/L</td>
<td>1.70 ± 0.25</td>
<td>1.58 ± 0.26</td>
<td>2.80 ± 0.44</td>
<td>2.83 ± 0.48</td>
<td>1.98 ± 0.37</td>
<td>1.88 ± 0.39</td>
</tr>
<tr>
<td>fT4, μg/dL</td>
<td>12.9 ± 0.6</td>
<td>12.5 ± 0.6</td>
<td>13.5 ± 0.6</td>
<td>13.3 ± 0.6</td>
<td>13.7 ± 0.4</td>
<td>12.7 ± 0.7</td>
</tr>
<tr>
<td>tT3, nmol/L</td>
<td>1.68 ± 0.08</td>
<td>1.64 ± 0.07</td>
<td>1.74 ± 0.09</td>
<td>1.72 ± 0.07</td>
<td>1.71 ± 0.09</td>
<td>1.67 ± 0.08</td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>190 ± 12</td>
<td>212 ± 22</td>
<td>276 ± 21</td>
<td>288 ± 22</td>
<td>224 ± 14</td>
<td>243 ± 20</td>
</tr>
</tbody>
</table>

Abbreviations used: En exp, energy expenditure; CHO ox, carbohydrate oxidation; Fat ox, fat oxidation; Prot ox, protein oxidation; Heat rel, Heat release; mM, mmol/L; TAG, triacylglycerol; NEFA, non-esterified fatty acids; PP, pancreatic polypeptide; TSH, thyroid stimulating hormone; fT4, free thyroxin , tT3, total triiodothyronine.  1 Means ± SEM; * Different from N-eat during the same time period (P<0.05). † Tendency for a difference from N-eat during the same time period (P=0.057).  2 Daytime, eating period in both protocols, 0800 to 0000, clock hours.  3 Nighttime, fasting period in the N-fast protocol, 0000 to 0800, clock hours.  4 time period sum  5 time period mean  6 n=6.

Body (rectal) temperature did not differ between the protocols and a time-of-day pattern was observed during both protocols (Fig. 11, Tables 8 & 9). During both protocols, a postprandial increase in body temperature was observed (Table 9).

Glucose concentration did not differ between the protocols during the eating period, but was lower during N-fast than during the N-eat protocol in the fasting period (Fig. 12, Tables 8 & 9). During both protocols, lower glucose concentrations were observed after the 08.00 meal compared to the 16.00 and 20.00 meals (Fig. 12).
A protocol • time-of-day• meal interaction was observed, most likely due to the plateau in the fasting period during the N-fast protocol, and a lower postprandial response after the 1600 and 2000 meals during the N-fast protocol, than during the N-eat protocol (Fig. 12, Table 9).

Triacylglycerol concentration was higher in the eating period and lower in the fasting period during the N-fast protocol than during the N-eat protocol (Fig. 12, Tables 8 & 9).
Insulin concentration was higher in the eating period and lower in the fasting period during the N-fast protocol than during the N-eat protocol (Fig. 13, Tables 8 & 9). Meal intake increased insulin concentration, and this increase was affected both by protocol and time-of-day (Fig. 13, Tables 8 & 9). A protocol • time-of-day • meal interaction was observed, most likely due to the plateau in the fasting period during the N-fast protocol, and a higher postprandial response after the 12.00, 16.00 and 20.00 meals during the N-fast protocol, than during the N-eat protocol (Fig. 13, Table 9).

A time-of-day pattern was seen during both protocols, and meal intake increased TAG concentration, except after the 08.00 meal (Fig. 11, Tables 8 & 9). A protocol • time-of-day • meal interaction was observed, most likely due to the plateau in the fasting period during the N-fast protocol, and a higher postprandial response after the 12.00, 16.00 and 20.00 meals during the N-fast protocol, than during the N-eat protocol (Fig. 11, Table 9).

Non-esterified fatty acid concentration did not differ between the protocols during the eating period, but was higher during the fasting period during the N-fast protocol than N-eat (Fig. 11, Tables 8 & 9). A time-of-day pattern was seen during both protocols and during both protocols, meal intake decreased NEFA concentration; although the response to meal intake seemed to be delayed after the 16.00 and 20.00 meal with the N-fast protocol (Fig. 11). A protocol • time-of-day • meal interaction was observed, most likely due to the increase in NEFA concentration during the first part of the fasting period during the N-fast protocol, and a lower postprandial response 4 h after the 12.00, 16.00 and 20.00 meals during the N-fast protocol, than during the N-eat protocol (Fig. 11, Table 9).

Insulin concentration was higher in the eating period and lower in the fasting period during the N-fast protocol than during the N-eat protocol (Fig. 13, Tables 8 & 9). Meal intake increased insulin concentration, and this increase was affected both by protocol and time-of-day (Fig. 13, Tables 8 & 9). A protocol • time-of-day • meal interaction was observed, most likely due to the plateau in the fasting period during the N-fast protocol (Fig. 13, Table 9).
Table 9  Statistical summary of P-values from the 3-factor repeated measurements (RM)-
ANOVA, based on 24 h values from indirect calorimetry, the calorimeter suit and blood variables
during day 7 during nocturnal eating protocol (N-eat) and nocturnal fasting protocol (N-fast)

<table>
<thead>
<tr>
<th></th>
<th>T</th>
<th>M</th>
<th>PeT</th>
<th>PeM</th>
<th>TeM</th>
<th>PeTeM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy expenditure</td>
<td>•</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.062</td>
<td>•</td>
<td>.057</td>
</tr>
<tr>
<td>Carbohydrate oxidation</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.008</td>
<td>•</td>
<td>.008</td>
<td>•</td>
</tr>
<tr>
<td>Fat oxidation</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
<td>•</td>
<td>.027</td>
<td>•</td>
</tr>
<tr>
<td>Protein oxidation 1</td>
<td>•</td>
<td>-</td>
<td>•</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heat release</td>
<td>.006</td>
<td>.003</td>
<td>•</td>
<td>•</td>
<td>.003</td>
<td>.005</td>
</tr>
<tr>
<td>Body temperature</td>
<td>.001</td>
<td>.038</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Glucose</td>
<td>.023</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.008</td>
<td>.001</td>
<td>.032</td>
</tr>
<tr>
<td>TAG 2</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NEFA</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.003</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insulin</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PP</td>
<td>.034</td>
<td>•</td>
<td>.042</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Glucagon</td>
<td>&lt;.001</td>
<td>.029</td>
<td>.024</td>
<td>.010</td>
<td>.003</td>
<td>•</td>
</tr>
<tr>
<td>TSH</td>
<td>&lt;.001</td>
<td>.001</td>
<td>•</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.032</td>
</tr>
<tr>
<td>tT4</td>
<td>&lt;.001</td>
<td>•</td>
<td>.062</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>tT3</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
<td>•</td>
<td>.012</td>
<td>•</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
<td>•</td>
<td>&lt;.001</td>
<td>•</td>
</tr>
</tbody>
</table>

Abbreviations used: T, Time of Day, comparison of the different 4-h periods throughout the 24-h period; M, Meal, comparison of the time points within each 4-h period; P • T, Protocol (N-eat and N-fast) affects the time of day pattern; P • M, the protocol affects the pattern within the 4-h periods; T • M, time of day affects the pattern within the 4-h periods; T • M, protocol 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, plasma concentration; TAG, serum triacylglycerol concentration; NEFA, serum non-esterified fatty acid concentration; PP, pancreatic polypeptide; TSH, thyroid stimulating hormone; fT4, free thyroxin , tT3, total triiodothyronine; •, not significant (P-values above 0.07, the chosen threshold for tendency).

1 Protein oxidation is based on 4-h values. 2 n=6.

Pancreatic polypeptide concentration showed a tendency to be higher in the eating period during the N-fast protocol than during the N-eat protocol (Fig. 13, Tables 8 & 9). Both protocols showed a time-of-day pattern with the highest postprandial responses after the 12.00 and 16.00 meals (Fig. 13, Table 9).

Glucagon concentration did not differ between the protocols during the eating period or the fasting period (Fig. 13, Tables 8 & 9). A protocol • time-of-day interaction was however observed, probably from lower concentration during the 04.00 - 08.00 period (Fig. 13, Tables 9 & 10).

Thyroid stimulating hormone concentration did not differ between the protocols during the eating period or the fasting period (Fig. 14, Tables 8 & 9). Both protocols showed a time-of-day pattern with the highest concentrations during the fasting period (Fig. 14, Table 9). A protocol • time-of-day• meal interaction was observed, most likely due to the difference in concentration pattern between the protocols during the fasting period (Fig. 14, Table 9).
Free T4 concentration did not differ between the protocols during the eating period or the fasting period (Fig. 14, Tables 8 & 9). Both protocols showed a time-of-day pattern with the highest concentrations during the fasting period (Fig. 14, Table 9). There was a tendency for a protocol • time-of-day interaction, probably due to lower concentrations during the 12.00 to 00.00 period in the N-fast protocol compared to the N-eat protocol (Fig. 14, Tables 9 & 10).

FIG. 12 Twenty-four h curve and postprandial pattern of glucose, TAG and NEFA concentrations in both protocols 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.
Total T3 concentration did not differ between the protocols during the eating period or the fasting period (Fig. 14, Tables 8 & 9). Both protocols showed a time-of-day pattern with the highest concentrations during the 00.00 to 04.00 h period (Fig. 14, Table 9).

**FIG. 13** Twenty-four h curve and postprandial pattern of glucose, TAG and NEFA concentrations in both protocols 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.
Table 10  Statistical summary of P-values from the 3-factor repeated measurements (RM)-ANOVA, based on daytime and nighttime values from indirect calorimetry, the calorimeter suit and blood variables during d7 during nocturnal eating protocol (N-eat) and nocturnal fasting protocol (N-fast)

<table>
<thead>
<tr>
<th></th>
<th>T M</th>
<th>PeT</th>
<th>PeM</th>
<th>TeM</th>
<th>PeTeM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day(D)</td>
<td>Night(N)</td>
<td>D</td>
<td>N</td>
<td>D</td>
</tr>
<tr>
<td>Enexp</td>
<td>•</td>
<td>•</td>
<td>&lt;.001</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>CHOox</td>
<td>.011</td>
<td>•</td>
<td>&lt;.001</td>
<td>.001</td>
<td>•</td>
</tr>
<tr>
<td>Fatox</td>
<td>•</td>
<td>•</td>
<td>&lt;.001</td>
<td>.069</td>
<td>•</td>
</tr>
<tr>
<td>Heatrel</td>
<td>.007</td>
<td>•</td>
<td>.009</td>
<td>.013</td>
<td>•</td>
</tr>
<tr>
<td>BodyT</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>.002</td>
<td>•</td>
</tr>
<tr>
<td>Glucose</td>
<td>.011</td>
<td>•</td>
<td>.001</td>
<td>&lt;.001</td>
<td>•</td>
</tr>
<tr>
<td>TAG ²</td>
<td>.013</td>
<td>•</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.037</td>
</tr>
<tr>
<td>NEFA</td>
<td>.004</td>
<td>•</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
</tr>
<tr>
<td>Insulin</td>
<td>•</td>
<td>•</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
</tr>
<tr>
<td>PP</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Glucagon</td>
<td>&lt;.001</td>
<td>.048</td>
<td>•</td>
<td>.065</td>
<td>•</td>
</tr>
<tr>
<td>TSH</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>fT4</td>
<td>.025</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>tT3</td>
<td>&lt;.001</td>
<td>.067</td>
<td>&lt;.001</td>
<td>.002</td>
<td>•</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>•</td>
</tr>
</tbody>
</table>

Abbreviations used: T, Time of Day, comparison of the different 4-h periods throughout the 24-h period; M, Meal, comparison of the time points within each 4-h period; P • T, Protocol (N-eat and N-fast) affects the time of day pattern; P • M, the protocol affects the pattern within the 4-h periods; T • M, time of day affects the pattern within the 4-h periods; P • T • M, protocol 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, plasma concentration; TAG, serum triacylglycerol concentration; NEFA, serum non-esterified fatty acid concentration; PP, pancreatic polypeptide; TSH, thyroid stimulating hormone; fT4, free thyroxin; tT3, total triiodothyronine; *, not significant (P-values above 0.07, the chosen threshold for tendency). ² Protein oxidation is based on 4-h values. ³ n=6.

Cortisol concentration did not differ between the protocols during the eating period or the fasting period (Fig. 14, Tables 8 & 9). Both protocols showed a time-of-day pattern with the highest concentrations during the 04.00 - 08.00 period (Fig. 14, Table 9).

Partial correlations from forward stepwise multiple regression analyses, based on 24 h, daytime and nighttime values from indirect calorimetry and blood variables are shown in Table 11. Insulin’s important role was clearly seen in that insulin correlated with all metabolic variables. The thyroid hormones as a group correlated with energy expenditure and substrate oxidation. Correlations differed between daytime and nighttime.
FIG. 14 Twenty-four h curve and postprandial pattern of TSH, fT4, tT3 and cortisol concentrations in both protocols 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.
Table 11  Partial correlations from forward stepwise multiple regression analyses, based on 24 h, daytime and nighttime values from indirect calorimetry and blood variables during day 7 during nocturnal eating protocol and nocturnal fasting protocol

<table>
<thead>
<tr>
<th></th>
<th>Subject</th>
<th>Insulin</th>
<th>PP</th>
<th>Glucagon</th>
<th>TSH</th>
<th>FT4</th>
<th>TT3</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enexp 24h 1</td>
<td>57.6 4</td>
<td>3.2 (0.19) 6</td>
<td>0.8 (0.14)</td>
<td>•</td>
<td>0.5 (-0.12)</td>
<td>0.5 (0.18)</td>
<td>0.5(0.21)</td>
<td>•</td>
</tr>
<tr>
<td>Day 2</td>
<td>59.7</td>
<td>1.9 (0.15)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Night 3</td>
<td>62.5</td>
<td>6.5 (0.26)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>1.0 (0.31)</td>
<td>•</td>
</tr>
<tr>
<td>CHOox 24h</td>
<td>13.5</td>
<td>2.3 (0.29)</td>
<td>3.7 (0.43)</td>
<td>7.5 (-0.38)</td>
<td>2.4 (0.40)</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>19.4</td>
<td>4.8 (0.48)</td>
<td>2.0 (-0.23)</td>
<td>1.9 (0.37)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Night 2</td>
<td>6.1</td>
<td>6.5 (0.26)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>1.0 (0.31)</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Fatox 24h</td>
<td>44.8</td>
<td>0.7 (0.10)</td>
<td>1.4 (-0.26)</td>
<td>2.3 (0.22)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>44.9</td>
<td>0.8 (0.11)</td>
<td>2.5 (-0.35)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Night 2</td>
<td>56.2</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Glucose 24h</td>
<td>7.0</td>
<td>46.8 (0.72)</td>
<td>5.5 (-0.34)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>7.6</td>
<td>43.3 (0.70)</td>
<td>5.2 (-0.35)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Night 3</td>
<td>6.0</td>
<td>73.3 (0.87)</td>
<td>0.8 (-0.14)</td>
<td>•</td>
<td>0.8 (-0.29)</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>TAG 24h</td>
<td>5.2</td>
<td>31.8 (0.59)</td>
<td>3.4 (0.21)</td>
<td>•</td>
<td>1.0 (0.21)</td>
<td>0 (-0.22)</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>37.6</td>
<td>7.8 (0.46)</td>
<td>0.3 (0.12)</td>
<td>•</td>
<td>1.4 (-0.41)</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Night 2</td>
<td>35.7</td>
<td>4.0 (0.41)</td>
<td>•</td>
<td>•</td>
<td>0.5 (0.42)</td>
<td>0.5(-0.17)</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>NEFA 24h</td>
<td>14.8</td>
<td>2.2 (0.16)</td>
<td>6.3 (-0.54)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>1.1 (0.12)</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>12.0</td>
<td>7.8 (0.30)</td>
<td>5.1 (-0.49)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Night 2</td>
<td>22.4</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>5.9 (0.28)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: Subject, variance explained by individual variation; PP, pancreatic polypeptide; TSH, thyroid stimulating hormone; FT4, free thyroxin , TT3, total triiodothyronine.; Enexp, energy expenditure; CHOox, carbohydrate oxidation; Fatox, fat oxidation; Glucose, plasma concentration; TAG, serum triacylglycerol concentration; NEFA, serum non-esterified fatty acid concentration; • , not significant P>0.05. 1 Data from both protocols during 24 h. 2 Data from both protocols from 08.00 to 00.00. 3 Data from both protocols from 00.00 to 08.00. 4 Individual variation 5 explained variance in % 6 β, standard coefficient 7 n=6
DISCUSSION

“If you cannot convince them, confuse them.”
Harry S. Truman

I have chosen to discuss the results in groups and not according to the order in the papers and thereby breaking an fundamental rule in publishing: “Keep the order!”. As nature does not always “produce” research results in a nice and orderly fashion, and everything is more or less connected, I hope you find this strategy agreeable.

The study designs in this thesis

This thesis 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. 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, which means that our model mimics a “real-life situation” for a majority of shift workers. Regarding meal composition in Papers I-III, 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. In Paper IV, the main difference between the N-eat and the N-fast protocol was that caloric intake in one case took place solely during the day and not only a mere difference in meal size. We realize that shift workers might not compensate for a nocturnal fast during work by eating more during the day, or for that matter eat less during the day to compensate for nocturnal eating. Nevertheless, we wanted our subjects to maintain energy balance over 24 h to facilitate data interpretation. If meals of the same size had been used in both protocols, effects of negative energy balance during the nighttime period would have made comparisons more difficult. 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, and no total 24-h difference between the N-eat and N-fast protocols, 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 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\textsuperscript{15} and decreased hepatic blood flow\textsuperscript{84} could be part of the decreased core activity. The increased peripheral activity could be increased fidgeting\textsuperscript{85}. 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)\textsuperscript{35}. Fidgeting activities can consume substantial amounts of energy\textsuperscript{85}. The physical activity patterns with HC- and HF-meals as well as during the N-eat-protocol, 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\textsuperscript{86}.

The larger meals during the N-fast protocol did not result in a larger postprandial increase in energy expenditure during the day although the meals contained 50% more energy than the N-eat meals. Some studies show that few larger meals lead to higher DIT than frequent smaller ones\textsuperscript{30,31}, whereas other studies do not find any difference\textsuperscript{87}. If anything, one would expect that nocturnal eating would lead to decreased 24-h energy expenditure as evening DIT is lower than morning DIT\textsuperscript{88}.

With the N-fast protocol we registered less physical activity, i.e. fidgeting. The subjects felt more tired\textsuperscript{89} and thus became less active when they did not receive food at night. The unexpected variance in the actimeters sensitivity and the large individual variation limits the conclusions that can be drawn from the activity data. I would still maintain that absence of food during the night decreased the activity of the subjects, thereby corroborating the energy expenditure data. These findings stand in contrast to the hypothesis that fidgeting is a strategy to stay awake, but
perhaps the food is required as a stimulus for activity. Levine et al. observed that a number of subjects responded to overfeeding with increased fidgeting\textsuperscript{90}, and thus it is possible that e.g. blood glucose concentration act as an activity stimulus.

The positive energy balance (about 15% of ingested energy) seen with both protocols might have hidden any smaller differences. The activity factor (PAL 1.4) we used previously was quite accurate in keeping subjects in energy balance in Paper I so the large positive energy balance was unexpected.

We found a larger postprandial increase in heat release after the 16.00 and 20.00 meals during the N-fast protocol than during the N-eat protocol. These two peaks were not seen in energy expenditure or rectal temperature, meaning that the body reacts to larger meals by radiating more heat. That rectal temperature should start to decline before the 20.00 meal during the N-fast protocol was not expected. It seems that the rectal temperature probe might have changed position in two subjects, thereby giving too low values. We would have expected the rectal temperature curves to be more or less the same between the protocols as the driving force for rectal temperature seems quite strong, as seen in Paper I.

We observed a higher energy expenditure with the HF-meals compared to the HC-meals, which is different from most other studies\textsuperscript{18,24,91}. 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\textsuperscript{92,93}. In a small sub-group matched for body composition and energy intake, Cooling and Blundell\textsuperscript{92} 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\textsuperscript{92}.

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\textsuperscript{93}. 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\textsuperscript{+}/K\textsuperscript{−} ATPase\textsuperscript{94}. Dietary fat can change the fatty acid composition (Andersson, submitted), 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 contained more calcium from dairy products. Epidemiological data have shown that high calcium intake is associated with lower weight.

Although heart rate showed an effect of time-of-day, MAP did not with the HC-HF-meals. This is different to previous studies, 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 N-fast protocol lead to a higher carbohydrate oxidation during the feeding period and lower oxidation during the nighttime period whereas fat oxidation was not affected by protocol. 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 and Frapé et al. found an increased clearance of Intralipid (10 % lipid emulsion) during the afternoon compared with the morning. 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. The problem is, however, to differentiate between circadian effects and the effects of the preceding nighttime fast. 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 seen in Paper II.

The N-fast protocol lead to a higher carbohydrate oxidation during the feeding period and lower oxidation during the nighttime period (seen as a protocol • time-
of-day interaction); whereas fat oxidation was not affected by protocol. As carbohydrate oxidation precedes fat oxidation in the oxidative hierarchy\textsuperscript{101}, we expected that the larger meals in the N-fast protocol (i.e. more CHO) would lead to transiently higher carbohydrate oxidation during the eating period, followed by higher fat oxidation during the fasting period. One reason why fat oxidation did not increase during the fasting period could be the positive energy balance.

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\textsuperscript{18,20,91}. 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\textsuperscript{25} and Hill et al. also observed that RQ was lower than the FQ using similar diets as we used\textsuperscript{91}. Hill et al. speculated that seven days might be a too short a period to reach steady state (i.e. matching RQ with FQ)\textsuperscript{91}, although Schrauwen et al. found a matching of RQ and FQ after seven days of a high fat diet\textsuperscript{102}. 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\textsuperscript{102}.

The discrepancy between RQ and FQ in our study could also come from weight changes during the dietary adjustment period\textsuperscript{103}, 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\textsuperscript{103}.

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\textsuperscript{104}.

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. Glucose concentration was lower in the fasting period during the N-fast protocol.
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. 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. They used a continuous glucose infusion in their study, whereas our subjects were given a meal at midnight. Perhaps another response would have been seen if this meal had been delivered earlier.

As would be expected, glucose concentration was lower in the fasting period during the N-fast protocol. Perhaps more surprising was the smaller oscillations seen in glucose concentration with the larger meals during the N-fast protocol as carbohydrate oxidation was higher at the same time. The even secretory pattern observed might come from the slower gastric emptying rate. It can be hypothesized that the larger meals would lead to slower gastric emptying rate since energy content and meal volume have been shown to correlate inversely with gastric emptying rate.

Peripheral glucose tolerance decreases from morning to evening whereas splanchnic glucose tolerance is the same. 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. The glucose concentration then increased again, above “awake” concentration, when they were allowed to sleep in the morning (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, indicating that the circadian rhythm and sleep affect glucose tolerance separately. 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. During the N-fast protocol, the highest postprandial TAG levels were observed after the 16.00 meal, whereas the N-eat protocol showed the highest postprandial TAG levels after the 04.00 meal. Regardless of diet or protocol, 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 oscillated less during the eating period in the N-fast protocol. 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\textsuperscript{108}. Other studies have also found an increase in TAG concentration from morning to evening\textsuperscript{109,110}, 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 postprandial TAG concentrations were higher during the eating period in the N-fast protocol than in the N-eat protocol in the same period. During the N-fast protocol, the highest postprandial TAG concentrations were observed at 17.00, which is similar to other studies\textsuperscript{110,111}, although Rivera-Coll et al. also found a large postprandial peak at 03.25\textsuperscript{110}. The postprandial TAG concentration could be described as a simple dose-response and seen over 24 h, it does not matter how the meals are taken. For the postprandial periods after the 08.00, 12.00, 16.00 and 20.00 meals, the dose-response scheme seems to be correct with TAG levels about 25\% higher in the N-fast protocol than in the N-eat protocol. However, the postprandial response after the 04.00 meal during the N-eat protocol was almost as high as after the meals during the N-fast protocol, despite a much smaller meal (Fig. 12).

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\textsuperscript{112}.

Triacylglycerol concentration was higher after the HC-meals, which is in accordance with several studies (reviewed by Parks and Hellerstein\textsuperscript{113}). The HC-diet-induced hypertriacylglycerolemia has been shown to come mainly from increased secretion of very low-density lipoprotein triacylglycerols (VLDL-TAG)\textsuperscript{114}.

A recent review concluded that elevated fasting TAG concentration is a strong and independent risk factor for ischemic heart disease\textsuperscript{115}. 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)\textsuperscript{115}. 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)\textsuperscript{116}. Nevertheless, 1.5 mmol/L has been postulated to be the threshold
for formation of large VLDL-particles\textsuperscript{117}. These large VLDL-particles then cause the formation of artherogenic small dense low-density lipoprotein (LDL)-particles\textsuperscript{117}. When comparing N-fast to N-eat, the highest postprandial TAG values during the N-fast protocol was 1.83 mmol/L vs. 1.57 mmol/L for N-eat. 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\textsuperscript{118}. If and how the increased TAG response after the 04.00 meal relates to the increased TAG concentrations seen in shift workers\textsuperscript{6,38} 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”. My personal bias would lead me to recommend diets high in \textit{n}-3 fatty acids as this should decrease both fasting and postprandial TAG concentrations\textsuperscript{119}.

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\textsuperscript{120}. However, when the subjects consumed eight HF-meals throughout the 24-period, the NEFA concentration curve was flattened \textsuperscript{120}. Non-esterified fatty acid concentration oscillated less during the N-fast protocol, perhaps as an effect of the larger insulin oscillations observed during the N-fast protocol, as insulin decreases NEFA concentrations\textsuperscript{121}.

Higher NEFA concentrations were seen with HF-meals but this difference was statistic albeit too small to be physiologic\textsuperscript{121}. Increased NEFA concentrations have been implicated in insulin resistance and the circadian rhythm of insulin sensitivity\textsuperscript{122}, presumably via NEFA’s role in the formation of intramuscular TAG\textsuperscript{121}. In the study by Morgan et al., fasting NEFA concentration was higher in the evening than the morning\textsuperscript{122}. 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)\textsuperscript{120,122,123} than the 0.7 mmol NEFA/L above fasting levels needed to show a decreased insulin sensitivity\textsuperscript{121}.

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. 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, and during the daytime period in the N-fast protocol. 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. Glucagon concentration was lower during the fasting period in the N-fast protocol. 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 and thereby decreases gluconeogenesis. The peak in blood 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. Moreover, higher postprandial insulin peaks were found in the daytime period during the N-fast protocol compared to the N-eat protocol whereas no difference in glucose concentration between the protocols was found during the same time period. The nocturnal disassociation between insulin and glucose concentration has also been shown in a similar experimental setting by Morgan et al. A possible explanation for this nocturnal dissociation could for example be increased insulin clearance. The increased insulin concentration during the eating period in the N-fast protocol was most likely a result of the larger meals, with more insulin needed to maintain glucose concentration.

The highest glucagon concentration was seen during the 08.00 - 12.00 period, and higher glucagon concentrations were found with the HF-meals. Glucagon concentrations were lower during the fasting period in the N-fast protocol and a tendency for a protocol • meal interaction was found in the daytime period (Table 10). Although it has been shown that the body seems to buffer differences in dietary CHO content with glycogenolysis rather than gluconeogenesis, the higher glucagon concentration with the HF-meals indicates that a slight increase in gluconeogenesis could perhaps have taken place all the same. This higher glucagon concentration together with its concentration patterns during the N-fast protocol indicates that glucagon is stimulated by meal size and dietary fat content, and
decreased in the absence of food. As glucagon is major gluconeogenic hormone, one would expect glucagon to be decreased of large meals and increased by fasting. Possibly the glycogen stores were sufficient after the larger meals to maintain glucose concentrations above the threshold for gluconeogenesis.

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. The PP concentration showed a large individual variation, so the data were altered to proportions of the 24-h mean, after which the concentration difference between the daytime and nighttime became significant (data not shown). In both Paper II and IV, the postprandial PP concentration decreased from morning to evening, and decreased even further during the night. Despite that there was no food intake during the N-fast protocol, there was no difference between the N-eat and the N-fast protocol in PP concentration during the night. 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 seen in Papers I and III. As the postprandial PP response in this study was similar to the response seen in obese subjects, 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 or meal size/timing.

As has been shown in other studies, 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. Hirschfeld et al. found a small nocturnal increase in free T3 but not in fT4. The reasons for these discrepancies could be that Goichot et al. used continuous nasogastric enteral feeding whereas we provided meals at 4-h intervals. Furthermore, we measured total T3, whereas Goichot et al. and Hirschfeld et al. 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. Furthermore, thyroid hormones are involved in energy expenditure 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 in Paper I as T3 has been linked to uncoupling. We found some differences between the N-eat and the N-
fast protocol in TSH levels during the fasting period. Sleep, however, affects TSH strongly and the differences we found between the protocols, although statistically significant, were much smaller than what has been shown when sleep is compared with no sleep\textsuperscript{54}. The fT4 and tT3 concentrations were not affected by differences in meal intake, except a tendency for fT4 concentration to be slightly lower during the day during the N-fast protocol.

**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\textsuperscript{130}. 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of 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\textsuperscript{131}. Cortisol concentration did not differ statistically between N-eat and N-fast; although the graphs suggest some small differences. The concentration pattern differed slightly between the protocols around 04.00, indicating that the meal intake affects cortisol concentration. The morning meal-influenced decrease of cortisol concentration during the evening/night is stronger than the potential meal effect. This might have health effects as cortisol affects substrate utiliza...
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\textsuperscript{134}. It seems to respond to large-scale perturbations of the sympathetic nervous system, but appears relatively insensitive to short-term behavioural challenge\textsuperscript{135}.

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\textsuperscript{63}. 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\textsuperscript{64}, 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\textsuperscript{136} and glucocorticoids decrease leptin sensitivity\textsuperscript{137}. 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\textsuperscript{138}. 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\textsuperscript{15} or the impact of sleepiness\textsuperscript{35} 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\textsuperscript{65}.

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\textsuperscript{69}.

**Correlations**

The use of multiple regression analysis with only seven subjects might be questioned. We took the individual 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 & 11). Glucagon concentration correlated positively with CHO oxidation and negatively with fat oxidation. It has been shown that glucagon is an important gluconeogenic hormone\textsuperscript{139}, but its effect on lipolysis has been shown to be less pronounced\textsuperscript{139}. 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\textsuperscript{6} and obesity\textsuperscript{7}, leading to an increased risk of myocardial infarction\textsuperscript{8}. 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. Moreover, the subjects seemed to be more active if they ate at night. 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\textsuperscript{89}. However, it has been shown that redistributing most of the energy intake to the night shift increases total and LDL-cholesterol\textsuperscript{118} 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\textsuperscript{32} and its effects on TAG concentrations are unknown but have been studied recently (Holm bäck, personal communication, data in process). *Sleep debt* has been shown to decrease insulin sensitivity\textsuperscript{141} 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

“No matter where you go, there you are.”
Buckaroo Banzai

The findings in this thesis 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 a seven-day high fat-diet than after a seven-day high carbohydrate diet.
• 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.
• Distributing energy intake to take place solely during the day did not affect total 24-h values of the metabolic and endocrine variables measured compared to meals given throughout the 24-h period.

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 blood 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. If, however, I combine the data from my thesis with my personal bias, I would not “ban” nocturnal meals BUT the energy content should be low and aimed for maintaining mental energy.
Svensk sammanfattning av publikationerna och avhandlingen


Publikation III är en följdstudie där samma personer som i I även fick sinnestämningen kvantifierad genom att de under dygnet fick skatta följande sinnestämningar: Hunger, törst, irritation, likgiltighet och känsla av att vara utarbetad. De kände sig mindre irriterade på HF-kost. Kostens sammansättning hade mindre betydelse för känslan av
hunger, som minskade under nattimmarna. Hunger och törst korrelerade med flera hormonella och metabola variabler, för hunger var den starkaste korrelationen (negativ) med TAG-koncentrationen. Om man tar hänsyn till försökspersonernas aptitkänsla verkar det som om mindre måltider krävs på natten trots bibehållen aktivitet.

I publikation IV beskrivs en studie där sju män under 24-timmars vakenhet antingen åt 6 måltider under hela 24-timmarsperioden (nattätning), eller 4 något större måltider under dagen och fastade under natten (nattfasta). Under dessa 24 timmar mättes samma variabler som i publikation I och II. Energisättningen, glukos-, TG-, insulin- och glukagonkoncentrationen var lägre; och fria fettsyraaktiviteten högre under nattfasta jämfört med nattätning; även om det inte syntes någon skillnad mellan protokollen sett på 24 timmar. Nattfasta påverkade inte kortisol och tyroideahormonerna nämnvärt. Korrelationsanalyser visade att korrelationen mellan metabolab och hormonella variabler varierade mellan dag och natt. TG-koncentrationen efter måltid klockan 0400 under nattätning var jämförbar med TG-koncentrationen under nattfasteprotokollets dagmåltider, trots att måltiderna var 50% större under nattfasteprotokollet. Vi fann inga klara belägg för att det skulle vara bättre att äta 4 större måltider under dagen jämfört med 6 mindre måltider utspridda under hela 24-timmarsperioden, kroppen verkar kunna buffra mindre skillnader i måltidsstorlek och måltidstdin.

Sammanfattning av avhandlingen

Acknowledgements

These studies were carried out at the Department of Medical Sciences, Nutrition. I would like to express my gratitude and appreciation to all who have in one way or another contributed to this thesis. I especially would like to thank:

The subjects for their willingness and determination in performing the very tough 24-studies.

Mats Stridsberg, my supervisor, for your support and providing me with an “of-course-you-can-do-it”-feeling, for providing the means for me to finish this thesis, and for your not-too-bad taste in music.

Anders Forslund, my co-supervisor, for your endless enthusiasm, happy spirit, and willingness to perform monstrous 24-studies en masse.

Leif Hambraeus, my supervisor emeritus, for providing me with a rewarding atmosphere, and “forcing” me to go to Galveston.

Maria Lennernäs, the true creator of this thesis, without your help, knowledge and connections, this thesis would not be.

Torbjörn Åkerstedt, for your interest and profound knowledge of everything.

Arne Lowden, for your countless hours of calculations with never-ending optimism and for helping me understand statistics.

Jeanette Forslund, for being the chief organizer during the first 24-h studies and in command of the diets.

The “Näringslära”-crew: Roger Olsson for helping out with the studies and being The Organizer, Torbjörn Åkerfeldt for helping out with the studies and your truly immense nutritional knowledge, Stefan Branth, for being everywhere and doing everything, Gunilla Hjort and Maivor Liedén for helping out with the studies and being excellent coffee break partners, Inger Winkler for being a first-rate proof-reader and superb coffee break partner, Linley Karltun and Salaam Elnour for being/having been terrific coffee break partners. Anders Sjödin for conferences in the past and in the future.
Agneta, Annika, Anette and Erica at the Department of Public Health and Caring Sciences; and Jenny, Katarina, Hanna, Inger, Ylva, Helen, Anette, Åsa and all others at the Department of Domestic Sciences for games, conference activities, barbeque parties and interesting lunch discussions.

To all my splendid coffee break partners at Clinical Chemistry

The Thursday Lunch Gang: Johanna, Helena, Elisabet, Cilla and Johanna for lunch discussions big and small.

Semper and Findus for making the ideal “pre-hand-in-your-thesis-food”

The members of RA, for non-scientific intellectual gymnastics, by the way, Whisky is better than Cognac.

All my friends for activities and festivities.

My mother, father, sister, brother and sister-in-law for encouragement and your confidence in me.

Aili, for your love, support and for being the best proof-reader ever – stjärnstopp!

This work was financially supported by Swedish Dairy Association, The Swedish National Defense Research Institute, and Swedish Council for Forestry and Agricultural Research.

\[1\] gruel (välling) (Holmbäck, study in progress)
References

7. van Amelsvoort LG, Schouten EG, Kok FJ. Duration of shiftwork related to body mass index and waist to hip ratio. Int.J.Obes.Relat Metab Disord. 1999;23(9):973-8.


Errata

“Proofreading is more effective after publication”
Barker

Page
11 Last sentence: “on” should be changed to “one”.
17 Line 12: The following sentence should be inserted: “Partial correlations were obtained using a longitudinal stepwise regression model controlling for individual differences using dummy coding (forced into model).
Line 12 “Statistical software…… was used” should be changed to “Statistical softwares (SuperANOVA, version 1.11, Abacus Concepts Inc, California, USA and StatView 5.0, SAS Institute Inc, North Carolina, USA) were used for the analyses.”
22 Line 1 and 16: “(Fig. 3)” should be “(Fig. 3, Table 3)”
Line 14: “test” should be deleted.
24 Line 1 and 7: “(Fig. 4)” should be “(Fig. 4, Table 3)”
26 The following text should be inserted in the figure text: “The right figures serve to illuminate the postprandial responses within the 6 periods”.
27 Line 4: “(Fig. 1, Table 1)” should be “(Fig. 6, Table 4)”
Line 6: “(Fig. 1)” should be “(Fig. 6)”
Line 6 in the fT4 concentration paragraph: “(Fig. 3)” should be “(Fig. 7)”
30 Last sentence before table 6: “experimental day” should be changed into “24-h period”.
33 In table 8 explanations, after “(P=0.057)”, the following sentence should be added: “Students T-test for dependent samples.”
35 Line 4, 11, 17, 24 and 32: “(Fig. 11)” should be “(Fig. 12)”
39 Table 10, title: “during d7” should be “during day 7”.
43 Line 11, “pattern” should be inserted after “postprandial”.
Line 8 from bottom, reference 88 should be reference 86.
46 Line 18, reference 102 is the same as reference 19
47 Line 14 to 17, should be “…slower gastric emptying rate, but energy content and meal volume has been shown to correlate with gastric emptying rate 105.”