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ORIGINAL CONTRIBUTION

Endocrine responses to nocturnal eating – possible implications for night work

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Summary *Background* Night work is becoming more common and shift workers display several metabolic disturbances. *Aim* To study the endocrine responses in relation to time of day during a 24-h period and how dietary macronutrient composition affects these responses. *Design* Seven males (26–43 y and 19.9–26.6 kg · m^{-2}) were studied in a crossover design. Isocaloric diets described as high-carbohydrates (HC; 65 energy percent (E%) carbohydrates and 20E% fat) or high-fat (HF; 40E% carbohydrates and 45E% fat) were given. After a 6-day diet adjustment period, the subjects were kept awake for 24 h in a metabolic unit and were served an isocaloric meal (continuation of respective diet) every 4-h. Blood samples were taken throughout the 24-h period. *Results* Insulin and leptin responses to meal intake differed with respect to time of day ($p < 0.05$). Time of day affected glucagon, thyroid stimulating hormone (TSH), free thyroxin (fT4), total triiodothyronine (tT3), cortisol, chromogranin A (CgA) and pancreatic polypeptide (PP) concentrations ($p < 0.05$). Meal intake decreased cortisol concentration after meals at 0800, 1200 and 0400 but not at 1600, 2000 and 0000 h. The PP's postprandial increase was greater during 0800–1600 h compared to 2000–0800 h. With the HC meals, lower glucagon and CgA concentrations ($p < 0.05$), and a tendency for lower tT3 concentrations ($p = 0.053$) were observed compared to the HF meals. *Conclusion* Insulin, PP, TSH, fT4, cortisol and leptin responses to meal intake differed with respect to time of day. The decreased evening/nocturnal responses of cortisol and PP to meal intake indicate that nocturnal eating and night work might have health implications.

Key words circadian – thyroid – cortisol – pancreatic polypeptide – postprandial – meal

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Introduction

Around twenty percent of individuals in the industrialised countries have irregular work hours [1], a situation that causes changes in their feeding cycle [2]. There is only limited knowledge on the metabolic and endocrine responses to these changes [3]. From a circadian point of view, the human body does not expect a nocturnal load of energy and nutrients, as a circadian variation has been seen in gastric emptying rate, intestinal blood flow, kidney and liver activity [4]. It has been shown that subjects respond differently when given identical test meals in the morning compared to the evening [5]; glucose tolerance decreases from morning to midnight [6]; and nocturnal eating increases the low-density lipoprotein to high-density lipoprotein ratio [2]. The endocrine milieu may therefore be less suitable for food intake during the night and the nocturnal hormonal pattern might be involved in the high incidence of obesity and cardiovascular diseases often seen in shift workers [7, 8].

The aim of this study was to examine how high-carbohydrate (HC) and high-fat (HF) meals affect en-

docrine variables when meals were given during a 24-h period of continuous wakefulness. We chose 10 different hormones to obtain an overview of the endocrine system: insulin (and C-peptide), glucagon and cortisol as they are involved in different pathways of the metabolic system; the thyroid hormones as they have an impact on energy and lipid turnover; Chromogranin A (CgA) as an indicator of overall catecholamine secretion [9] and thereby stress [10]; pancreatic polypeptide (PP) to assess the activity of the gastro-intestinal tract [11]; and leptin as an indicator of energy status [12]. To obtain a more complete picture of the metabolism throughout a 24-h period, the endocrine responses were related to energy expenditure, substrate utilisation; and blood glucose, triacylglycerol and non-esterified fatty acid concentrations presented in a previous publication [13].

Subjects and methods

Subjects

Eight males were recruited for the study and seven of the eight subjects completed both experimental periods. Their (n = 7) mean age was 32 (range, 26–43) y; weight 84.3 [69–95] kg; body mass index 23.8 (19.9–26.6) kg/m^2; body fat 20.0 (11.4–31.2) %; and 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) values, 2.67 mmol/L, at day one of the studies). All subjects gave their written informed consent, and the Ethical Committee of the Faculty of Medicine at Uppsala University approved the study.

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 each session they were followed on an outpatient basis during days one to six and on day seven the 24-h metabolic study was performed at the metabolic unit. In the evening of day six they reported to the metabolic unit where they received a snack at 2145 h and went to bed at 2300 h. At 0800 h the study began and during the subsequent 24 h the subjects remained awake. The 24-h study was divided into six identical 4-h periods with a standardised meal at 0800, 1200, 1600, 2000, 0000 and at 0400 h. Blood sampling occurred 0.5, 1, 2, 3, and 4 h postprandially. The subjects remained seated in a chair throughout the study and no physical activity was allowed. A more detailed explanation of the experimental design has been presented in Holmbäck et al. [13].

Diets

Two isocaloric diets, calculated to maintain energy balance, were compared, a high carbohydrate diet (HC) and a high fat diet (HF). The HC diet consisted of 15 energy percent (E%) from protein, 65 E% carbohydrates (CHO), and 20 E% fat. The HF diet consisted of 15 E% protein, 40 E% CHO, and 45 E% fat. The fat composition and amount of dietary fibre were similar between the diets. The nutrient and energy content of the diets were estimated using computer software (Dietist© version 1.1, Kost och Näringsdata AB, Bromma, Sweden).

Assays

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 [14].

Statistics

Data were analysed using values from the whole 24-h period with a three-factor repeated measurements analysis of variance (RM-ANOVA) with Huyhn-Feldt correction for the violation of assumption of sphericity. The three 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 diets); and "meal" (difference between the five (four for leptin) time points within each 4 h period, using combined data from both diets).

To further pinpoint diet differences in the circadian pattern within each diet, data were also analysed with a two-factor RM-ANOVA with "time-of-day" and "meal" as factors.

Since shift work effects were in focus, values from a daytime period (Day: 1600–2000 h) were compared to those of a nighttime period (Night: 0400–0800 h) values. For the Day-Night analyses the independent factors

were "diet", "DayNight" (difference between the two time periods), and "meal".

A forward multiple regression model was used when analysing the covariance between hormones in the present publication and energy expenditure, substrate utilisation, plasma glucose, and serum lipid concentration in a previous publication [13]. The hormone concentrations were z-transformed (controlling for variances originating from differences between individuals) before analyses. Two statistical software packages (SuperANOVA, version 1.11, Abacus Concepts Inc, California, USA and StatView 5.0, SAS Institute Inc, North Carolina, USA) were used for the analyses. All results are reported as mean ± SEM. Significance was accepted at $p < 0.05$ and p-values < 0.07 are reported as tendencies.

Results

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 1. The results of the two-factor repeated measurements ANOVA (within each diet and Day-Night comparisons) are presented in the following text.

The *insulin* concentration was the same with both diets, although a diet · time-of-day interaction indicated a higher insulin concentration during the 0800–1200 h period when the subjects consumed the HC meals compared to the HF meals (Fig. 1, Table 1). 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 1600 h 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. 1, Table 1) showed a time-of-day pattern with the HF meals ($p = 0.042$), apparently due to lower concentration in the 1200–1600 h period compared to 1600–2000 h 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 1600–2000 h period. No C-peptide concentration difference was observed between Day and Night.

The *PP* concentration showed a time-of-day pattern with a higher concentration in the 1600–2000 h period compared to the 0400–0800 h period (Fig. 1, Table 1). 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. 1, Table 1). A higher concentration was observed during the 0800–1200 h period compared to the 2000–0400 h 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 inter-

Table 1 Statistical summary of p-values from the three factor RM-ANOVA for hormone concentrations during day 7, based on 24-h values from both diets

	Diet[1] (D)	Time-of-Day[2] (ToD)	Meal[3] (M)	D · ToD[4]	D · M[5]	ToD · M[6]	D · ToD · M[7]
Insulin	ns	ns	< 0.001	0.028	ns	ns	ns
C-peptide	ns	ns	< 0.001	ns	ns	ns	ns
PP	ns	0.021	0.032	ns	ns	0.047	ns
Glucagon	0.040	0.011	ns	ns	ns	ns	ns
TSH	ns	< 0.001	0.001	ns	ns	0.002	ns
fT4	ns	< 0.001	ns	ns	ns	ns	0.053
tT3	0.053	0.033	< 0.001	ns	ns	ns	ns
Cortisol	ns	< 0.001	0.004	ns	ns	< 0.001	ns
CgA	0.005	0.034	ns	ns	ns	ns	ns
Leptin	ns	ns	ns	ns	ns	0.007	ns

[1] Difference between HC and HF diet. [2] Difference between the 6 different 4-h periods throughout the 24-h period, using data from both diets. [3] Difference between the time points within each 4-h period, using data from both diets. [4] Diet · 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

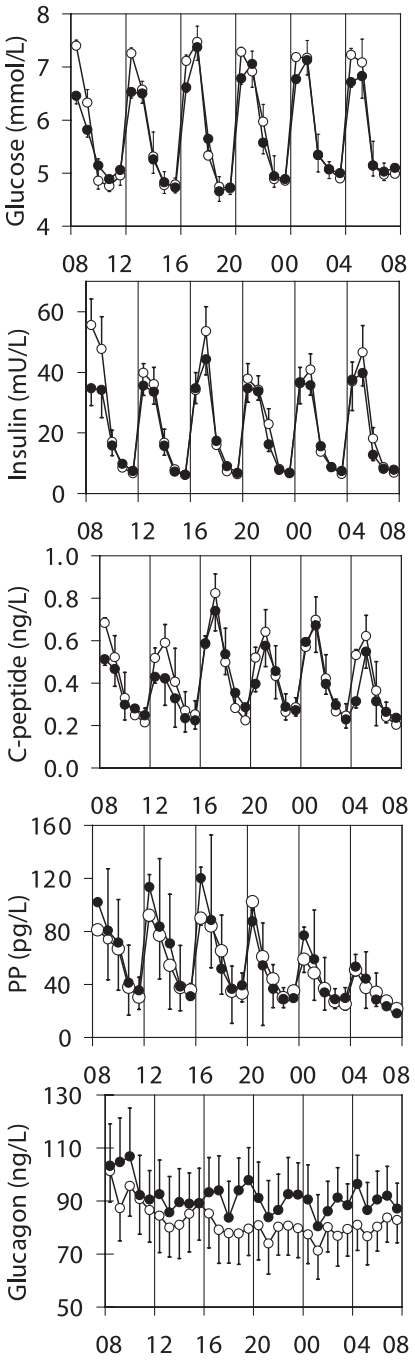

Fig. 1 Graphs depict the glucose, insulin, C-peptide, pancreatic polypeptide (PP) and glucagon concentrations during 24 h on day seven. The vertical lines represent start of each time period (when the meal was provided), with the time in hours on the x-axis. ○ high-carbohydrate meals, ● high-fat meals. Glucose data are taken from [13] with permission

action ($p = 0.062$), 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 0400 h 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 2000–0800 h period compared to the 0800–2000 h period (Fig. 2, Table 1). 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 1200–2000 h periods and a peak in the 0000–0800 h periods (Fig. 2, Table 1). With the HC meals, the pattern within the periods differed depending on time of day ($p = 0.036$). Especially during the 0000–0800 h periods the 3 h postprandial fT4 concentration seemed to be lower than the other time points during these periods (Fig. 2). 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 1600–2000 h period and a peak in 0000–0400 h period (Fig. 2, Table 1). 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 2000–0000 h period (Fig. 2, Table 1). After the 0800, 1200 and 0400 meals, a higher concentration was observed 0.5 h rather than 2 h postprandially, whereas no difference was seen after the 1600, 2000 and 0000 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. 2, Table 1). With the HC meals, a time-of-day pattern was observed ($p < 0.001$) with low concentrations in the 0800–1600 h and 0400–0800 h periods and a peak in the 2000–0000 h period. Meal intake did not affect CgA concentration and no CgA concentration difference was seen between Day and Night.

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-diet ($p = 0.003$)(Fig. 2, Table 1). This was probably due to a different pattern within the 0800–1200 h period compared to the other periods.

In a forward stepwise linear regression analysis partial correlations were obtained between hormone concentrations and variables from previously presented data [13]. Models were established for *energy expenditure* (variance was to some degree explained by insulin, cortisol and CgA), *carbohydrate (CHO) oxidation* (PP,

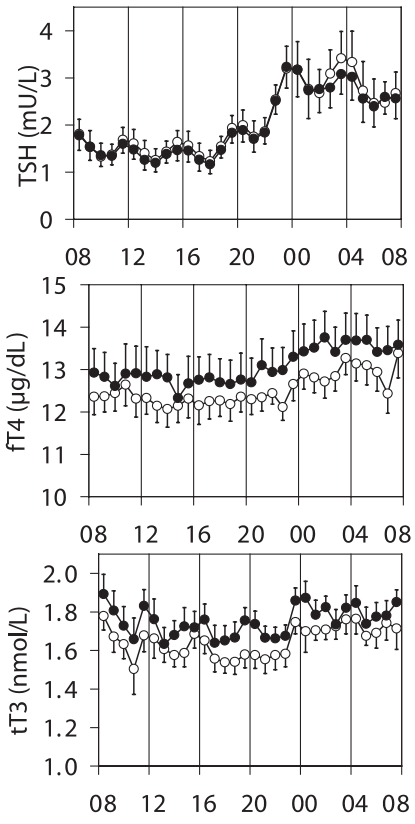

Fig. 2 Graphs depict the thyroid stimulating hormone (TSH), free thyroxin (fT4) and total triiodothyronine (tT3) concentrations during 24 h on day seven. The vertical lines represent start of each time period (when the meal was provided), with the time in hours on the x-axis. ○ high-carbohydrate meals, ● high-fat meals

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 2). C-peptide, fT4 and leptin concentrations did not fit into any model.

Discussion

In this study the postprandial endocrine response at different times of day and after HC and HF meals was examined. The results were related to previously published data on metabolic variables from the same study to obtain a more complete picture of the metabolic and endocrine situation encountered during night work. It should be noted that this study does not address the situation in permanent night shift workers because they might show a partial circadian adaptation to night work. However, most shift workers work in rotating 2- or 3-shifts, so their "body clock" is not different from day workers, particularly not on the first night shift [15]. Furthermore, 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 [16], which means that our model mimics a "real-life situation" for many shift workers.

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 diet, since insulin suppresses glucagon secretion [17] and thereby decreases gluconeogenesis. Furthermore, the peak in blood glucose 1 h after the 1600 h meal seen in the previous study [13] 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 0000–0400 h period was not reflected in insulin or C-peptide. This nocturnal disassociation between insulin and glucose concentration has also been shown in a similar experimental setting by Morgan et al. [15]. A possible explanation for this nocturnal disassociation between insulin and glucose concentration could for example be increased insulin clearance [18]. Insulin concentration correlated with energy expenditure, possibly as an indication of its involvement in dietary induced thermogenesis [19], although other studies have shown that it is cellular carbohydrate metabolism per se rather than insulin concentration that affects dietary induced thermogenesis [20]. An ef-

Table 2 Partial correlations (controlled for individual differences) between endocrine data from both diet periods during day 7 and metabolic variables [13]

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

[1] Energy expenditure; [2] Carbohydrate oxidation; [3] Fat oxidation; [4] Explained variance; [5] Standard coefficient (β)
PP pancreatic polypeptide; *TSH* thyroid stimulating hormone; *tT3* total triiodothyronine; *CgA* chromogranin A; *ns* not significant

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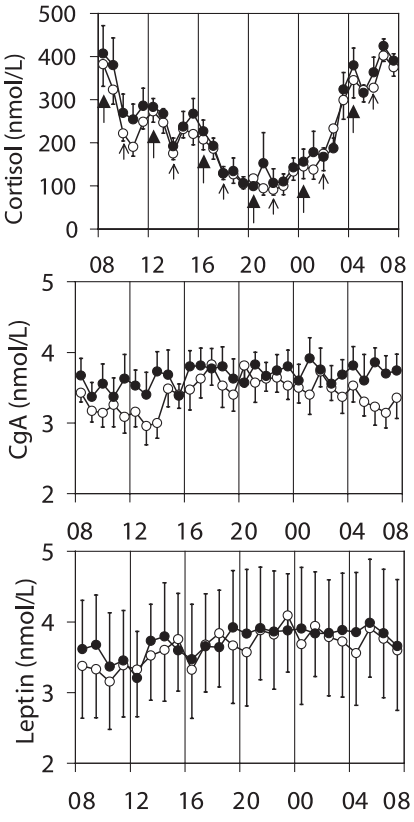

Fig. 3 Graphs depict the cortisol, chromogranin A (CgA) and leptin concentrations during 24 h on day seven. The vertical lines represent start of each time period (when the meal was provided), with the time in hours on the x-axis. ○ high-carbohydrate meals, ● high-fat meals. In the cortisol graph, the postprandial time points 0.5 h and 2 h are indicated with ↑ and ↑, respectively

fect on lipolysis was seen with insulin concentration correlating negatively with NEFA and glycerol concentrations. Insulin concentration partly explained the TAG variance, and could thus have been involved in the nocturnal postprandial TAG increase via lipoprotein lipase [15]. In this study, the concentration pattern for insulin looks more or less the same for the two diets. Triacylglycerol concentration, however, differed substantially between the diets in that TAG concentration was higher after the HC meals and a more pronounced effect of time-of-day was seen with the HF meals [13]. Any effect on TAG from insulin therefore most probably does not stem from the plasma insulin concentration.

Pancreatic polypeptide is a polypeptide, released from the pancreas in a biphasic manner in response to meals [21]; and it has been hypothesised to be a marker for vagal tone [11]. Pancreatic polypeptide affects hepatic insulin sensitivity, perhaps by affecting insulin receptor availability, and PP deficiency can contribute to persistent hyperglycaemia [21]. Obese subjects with normal glucose tolerance respond with lower meal-induced increase in PP compared to normal subjects [22]. In our study, the postprandial response of PP decreased from the meal at 1600 h indicating less gastrointestinal response to the meals. PP showed a large individual variation, which eradicated any statistical difference between Day and Night. However, if the data were altered to proportions of the 24-h mean, the concentration difference between Day and Night became significant (data not shown). The reduced amplitude of PP concentrations at night may be related to the changes in metabolism and appetite seen in night wake [23]. As the postprandial PP response in this study was similar to the response seen in obese subjects [22], this could possibly indicate health implications.

The highest glucagon concentration was seen during the 0800–1200 h period after which it remained relatively constant. A higher glucagon concentration was observed with the HF meals compared to HC meals. Glucagon is an important gluconeogenic hormone but its effect on lipolysis seems to be less pronounced [24]. Although the body seems to buffer differences in dietary CHO content with glycogenolysis rather than gluconeogenesis [25], the higher glucagon concentration indicates that a slight increase in gluconeogenesis with the HF diet could perhaps still have taken place. Only a negative correlation was seen between glucagon and TAG concentration and no correlation with NEFA or glycerol concentrations. The nocturnal increase in TAG concentration observed in our previous study [13], however, does not seem to be influenced by glucagon since the nocturnal concentration patterns differ substantially between glucagon (Fig. 1) and TAG [13]. It is therefore more probable that other hormones, e. g. adrenaline [26], affected lipolysis more than glucagon.

As has been shown in other studies [27], 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. 2). Goichot et al. [27] did not see any nocturnal increase in either fT4 or free T3, using basically the same setting. Hirschfeld et al. [28] found a small nocturnal increase in free T3 but not in fT4. The reasons for these discrepancies could be that Goichot et al. [27] used continuous nasogastric enteral feeding whereas we provided meals at 4-h intervals. Furthermore, we measured total T3, whereas Goichot et al. [27] and Hirschfeld et al. [28] 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. Thyroid hormones affect lipid metabolism (reviewed in [29]), and we observed correlations between tT3 and NEFA and glycerol concentrations, whereas TSH correlated with TAG concentration.

Thyroid stimulating hormone's effect on TAG seems small (only 2.2 % of the variance could be explained by TSH), but TSH might still be involved in the nocturnal postprandial increase in TAG concentration, as TSH has been shown to decrease lipoprotein lipase activity [30]. 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 diet [13] as tT3 has been linked to uncoupling [31]. However, tT3 concentration did not explain any variance in energy expenditure so the possible effect might come from the preceding 6-day diet period. All the same, the fasting tT3 concentration (at 0800 h on day 7) was not higher after the HF diet.

As expected, the circadian rhythm of cortisol was pronounced. Cortisol secretion was not affected by dietary changes in carbohydrate and fat, in accordance with Slag et al. [32]. Cortisol secretion is stable and not affected by short time changes in the sleep-wake cycle [33]. Meal intake during the early hours of the day (after 0800, 1200 and 0400 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 1600, 2000 and 0000 meals. The morning meal-influenced decrease has also been seen in some other studies [18, 34, 35], whereas other studies have seen a daytime cortisol increase after meals [32, 36]. In the latter studies, the meal-induced increase in cortisol concentration was only seen after a high protein meal. 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 utilisation and lipid storage [37].

Chromogranins are 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 [9]. We found a circadian rhythm in CgA with the HC meals, in contrast to Takiyyuddin et al. [9] who found no evidence of circadian rhythms. The 24-h pattern of CgA concentration seen with the HF meals, however, was similar to Takiyyuddin et al. [9]. No information regarding meal composition before or during the study by Takiyyuddin et al. is provided so we can only speculate that the diet in their study was similar to our HF diet. Why HC meals and not HF meals would cause a circadian pattern is not readily apparent, nor is it clear why HF meals cause a higher 24-h CgA concentration than HC meals. Chromogranin A concentration correlated with energy expenditure and fat oxidation. Chromogranin A can be processed peripherally to pancreastatin, which has a metabolic function counter-regulatory to insulin action [38]. This might explain CgA's involvement in energy expenditure and fat oxidation, apart from being co-released with catecholamines [9]. Chromogranin A seems to respond to large-scale perturbations of the sympathetic nervous system, but appears relatively insensitive to short-term behavioural challenge [39]. Nevertheless, CgA has been shown to correlate with hypertension, which suggests that it is a useful marker for the sympato-adrenergic system [40].

Leptin showed no distinct effect of time-of-day, except for a different postprandial pattern in the 0800–1200 h period with the HC meals. The large individual variation in leptin concentration hid possible circadian patterns 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 [41]. Moreover, meal composition has been shown to affect leptin concentration as high-fat/low CHO meals result in lower 24 h area under the curve values compared to high CHO/low fat meals [34], 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 [42] and glucocorticoids decrease leptin sensitivity [43]. In other words, leptin is influenced by so many factors that interpreting leptin concentrations is far from straightforward.

One of the main issues in this study was whether the response to a meal would differ depending on time of day. This issue was studied for all variables and for some such an effect was seen: insulin, PP, TSH, fT4, cortisol and leptin responses to meal intake differed with respect to time-of-day. This could be of significance in relation to the health of night workers. Insulin concentration did not show an effect of time of day despite a nocturnal increase in glucose concentration, which is in accordance with other studies that indicate decreased insulin sensitivity [44]. The postprandial PP concentration decrease during the night and the decreased nocturnal influence of meal intake on cortisol concentration also indicates that the body is not prepared for caloric intake. The nocturnal increase in TSH might be involved in the increased nocturnal postprandial TAG response, by decreasing lipoprotein lipase activity [30].

People who work during night time have been shown to display a number of metabolic disturbances. Obesity is more prevalent in shift workers [8, 45], although to our knowledge it has not been shown whether day-shift workers are less susceptible to becoming overweight than night-shift workers. Moreover, shift workers commonly display increased blood lipid concentrations [46, 47] as well as higher low-density lipoprotein levels [2]. Could the type of macronutrient intake have a role in these metabolic disturbances, or is it just the nocturnal caloric intake that is the culprit? The present results support the notion that the endocrine system reacts unfavourably to nocturnal eating. Even if the

results from the present study suggest negative effects of night-time food intake, more information is needed before giving advice regarding dietary macronutrient composition.

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