Neuroendocrine perturbations in human obesity

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Chapter 10

Summary and Discussion

The evolutionary advantage of several animal species to conserve energy in the form of adipose issue in order to survive long periods of food shortage in the past, turned into a major health problem in current times of plenty. Excess accumulation of body fat, or "obesity", is associated with severely increased co-morbidity and mortality risks and is a global epidemical medical condition which is difficult to manage. The exact pathophysiologic mechanism of obesity remains elusive and various factors such as genetic, social, behavioural and physiological cues are involved in its development. From a biological point of view, obesity might be explained by differences in the regulation of energy intake, expenditure and storage (energy homeostasis) between obese and lean individuals. The neuroendocrine system provides a source of humoral messengers, which can modulate energy homeostasis. This thesis will focus on changes of the neuroendocrine environment of obese women. First of all, spontaneous diurnal plasma hormone concentrations and secretion of different hormonal systems were studied (the results of these studies will be discussed and summarized in **Paragraph 1**). Secondly, the effect of weight loss on neuroendocrine perturbations of some of these hormonal axes was evaluated (the results of these studies will be discussed and summarized in **Paragraph 2**). Finally, the impact of modulation of potential physiological cues which might be involved in the neuroendocrine changes and metabolic alterations (increased circulating FFAs and deficit dopaminergic signalling), was investigated (the results of these studies will be discussed and summarized in **Paragraph 3 and 4** respectively).

1. Changes of spontaneous diurnal plasma hormone concentration patterns and secretion in obese premenopausal women

The first aim of this thesis was to delineate differences of diurnal spontaneous hormonal concentrations and secretion of the lactotroph, thyrotroph and corticotroph axis in obese vs. lean premenopausal women. Therefore, blood samples were taken during 24 h with a sampling interval of 10 min for the assessment of plasma hormone concentrations in obese premenopausal women and lean premenopausal female controls of similar age. All subjects were studied in the early follicular stage of their menstrual cycle. 24 h Plasma hormone concentration rhythms were mathematically analysed as described in Appendix B. Hormonal secretion rates were estimated by (multi parameter or waveform-independent) deconvolution analysis. Sizes of regional body fat mass were measured using MRI, whereas total body fat mass was calculated using DEXA.

Lactotroph axis in obese vs. lean premenopausal women (Chapter 2)

The release of PRL by the pituitary is tonically inhibited by dopamine through activation of the dopamine D2 receptor (D2R) on lactotroph cells (1). Obese humans appear to have reduced D2R binding sites in their brain (2). Therefore, it is hypothesized that spontaneous PRL release is enhanced in obese humans. Results of this study showed that PRL secretion was significantly enhanced in obese women (total daily release 137 ± 8 vs. lean controls 92 ± 8 µg/L/24 h, $P = 0.001$) in proportion to their BMI ($R^2 = 0.55$, $P < 0.001$) and in particular the size of their visceral fat depot (total PRL secretion vs. visceral fat area $R^2 = 0.64$, $P = 0.006$). These findings are in conflict with previous studies reporting that basal (single measured) PRL levels were similar and exogenously stimulated PRL concentrations were blunted in obese individuals (3-11). These differences might either be explained by the methods used (spontaneous PRL secretion has not been estimated

in obese humans before) or subjects enrolled in the present and previous studies. The observation that PRL was enhanced in obese women in proportion to the size of their visceral fat mass is in line with previous studies showing that PRL has lipogenic effects (12-18) and knock-out of the PRL receptor gene in mice causes loss of body fat, primarily from the visceral depot(19). Since PRL is inhibited by D2R activation, the elevated PRL secretion may reflect reduced D2R availability in the brain in obese premenopausal women. Diminished dopaminergic neuronal activity promotes body fat accumulation in (seasonally) obese animal models and in humans. Furhtermore, anti psychotic drugs, blocking D2R, promote body weight gain (20-22). Thus, this study implicates that PRL may be one of the endocrine messengers that relay reduced D2R mediated dopaminergic neural signals to peripheral tissues to promote (visceral) fat storage.

Thyrotroph axis in obese vs. lean premenopausal women (Chapter 4)

The hypothalamic pituitary thyroid (HPT) hormonal ensemble regulates energy balance (23-25). Recent evidence implicates leptin as an important modulator of thyroid axis activity (23;26-30). As obesity might be considered as a phenotypic expression of energy imbalance (31) and obese humans are hyperleptinemic, it is hypothesized that obese individuals have altered HPT axis activity. Results of this study showed that mean TSH concentration (obese 1.9 ± 0.2 vs. lean 1.1 ± 0.1 mU/L, P = 0.009) and secretion rate (obese 43.4 ± 5.5 vs. in lean 26.1 ± 2.2 mU/V_{dl} x 24 h, P = 0.011) were significantly enhanced in obese women, whereas the fasting free thyroxine concentrations were similar compared to normal controls (free T_4 in obese 15.4 ± 1.5 vs. in lean 16.4 ± 1.5 pmol/L, P = 0.147). Furthermore, TSH secretion was positively related to 24 h leptin concentrations (R^2 = 0.31, P = 0.007). Previous studies documented that basal (single measured) serum TSH concentrations are normal in obese humans (32;33), whereas the stimulated TSH response to TRH is enhanced, normal or impaired in obese subjects compared to normal weight controls (7;9;33-39). However, spontaneous TSH concentration profiles over 24 hours have not been measured in obese humans before. Different physiological cues, such as the stage of the menstrual cycle in which the women were studied or sex differences, might explain the differences between results of this study and those of previous investigators. As several studies provide strong evidence that leptin stimulates TSH production in rodents and humans, the finding that 24 h TSH secretion was positively related to mean 24 h leptin concentrations in the present study and may be interpreted as circumstantial evidence of a stimulatory impact of hyperleptinemia on TSH release in obese individuals. Alternatively, dopamine inhibits TSH synthesis and release through D2R activation in thyrotrophs of the pituitary gland, whereby it appears to specifically reduce the amplitude of pulsatile TSH release (40). As the increased TSH secretion rates of the obese subjects were primarily attributable to enhanced TSH pulse amplitude and the availability of D2R binding sites is considerably reduced in human obesity (2), reduced dopamine D2 receptor (D2R) mediated neurotransmission may also be involved in the enhanced TSH release in the obese humans enrolled in the present study.

Although a few studies demonstrated that serum T_3 concentrations were elevated in obese subjects (32;44;45), the majority of data suggests that there is no change in basal thyroid hormone concentrations in obese humans (33;41-43), which is in line with the results of the present study. However, the finding that TSH levels are elevated in the face of normal free T_4 in our obese subjects has never been described before. This phenomenon might be explained by impaired biological activity of TSH (through reduced dopaminergic signalling (46-48)) or unresponsiveness to exogenous TSH through increased sympathetic activity, as autonomic nervous system regulates the sensitivity of the thyroid gland to TSH (49-51).

Corticotroph axis in obese vs. lean premenopausal women (Chapter 7)

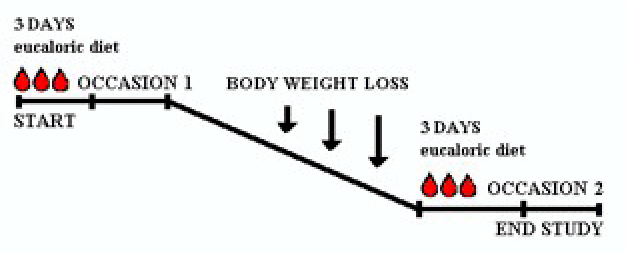
Based on several animal and clinical studies which document that obesity is associated with an exceedingly active hypothalamo-pituitary-adrenal (HPA) axis (52-63), it was hypothesized that the secretion rates of pituitary-adrenal hormones are enhanced in obesity. Daily ACTH secretion was substantially higher in obese than in lean women (7950 ± 1212 vs. 2808 ± 329 µg/24 h, P = 0.002), whereas cortisol was not altered (obese 36 362 ± 5639 vs. lean 37 187 ± 4239 nmol/24 h, P = 0.912). ACTH release rates correlate strongly with BMI, whereas the sizes of various fat areas (including visceral and subcutaneous fat depots) do not appear to be independently associated with ACTH production. Furthermore, the ACTH release process is less regular (as evidenced by ApEn statistics) in obese than in lean women. Regularity of hormonal secretion patterns mirrors the net result of feed forward signalling and feedback restraint. As cortisol secretion was not altered, it is stated that CRH, which is one of the strongest feed forward drives activating the HPA axis, may be increased

in obese humans. The occurrence of relatively low cortisol levels in face of elevated ACTH in the obese women enrolled in the present study, has been found in a few previous studies (64;65). Various mechanistic explanations for this neuroendocrine anomaly can be proposed, such as increased urinary cortisol excretion, insensitive adrenals (through increased sympathetic activity or leptin mediated peripheral inhibition of adrenal glucocorticoid production), reduced 21-hydroxylase activity (which would direct cortisol precursors towards androgen synthesis), increased 5- reductase activity (which converts cortisol to inactive cortisone) or increased 11βHSD type 1 (which catalyses the conversion of cortisone into active cortisol at tissue level). The exact pathophysiological implications of high plasma ACTH concentrations in the face of normal cortisol levels remain to be established.

2. Impact of weight loss on neuroendrocrine perturbations in obese premenopausal women

The second aim was to investigate the impact of body weight loss on the altered hormonal secretion of the lactotroph and thyrotroph axis in obese women. Therefore,
24 h plasma PRL and TSH concentrations were measured at 10 min intervals before and after weight loss (50% reduction of overweight, using a very low calorie diet) in eleven obese premenopausal women (BMI before weight loss 33.3 ± 0.7 kg/m^2) in the follicular phase of their menstrual cycle. Mathematical analysis of the hormone concentration patterns was performed (Appendix B). 24 h Hormone secretion rates were calculated using waveform-independent deconvolution technique (Pulse). Figure 1 is a schematic overview of the study.

Figure 1.



Lactotroph axis before and after weight loss in obese women (Chapter 3)
PRL release is inhibited by dopamine 2 receptor (D2R) and dietary restriction/weight loss are associated with increased dopaminergic signalling in animals(66). Therefore, it was hypothesized that enhanced PRL release in obese humans would be reversed by weight loss. Results of this study show indeed that elevated spontaneous 24 h PRL secretion was significantly reduced after weight loss in obese women (mean daily release before 128 ± 24 vs. after weight loss 110 ± 17 µg/V$_{dl}$ x 24 h, P = 0.05). Body weight loss particularly blunted PRL secretory burst mass (Pulse area before 230 ± 28 vs. after weight loss 221 ± 31 µg/V$_{dl}$ x min, P = 0.03), whereas burst frequency was unaffected (Number of pulses before 11 ± 1 vs. after weight loss 12 ± 1 n/24 h, P = 0.69). So far, variable results of studies evaluating the effects of caloric restriction and body weight loss on plasma PRL concentrations in humans have been described (67-71) and this is the first study to evaluate the effect of body weight loss on diurnal spontaneous PRL secretion rates in obese humans. Amelioration of deficit dopamine D2 receptor mediated neurotransmission can be involved in the physiology of this phenomenon, however dopaminergic neuronal activity was not directly assessed in the present study. The reduction of 24 h PRL secretion in response to weight loss in the present study was closely associated with the mean decrease of plasma leptin concentrations. Furthermore, findings of previous studies suggest that leptin plays a role in the control of PRL release (72-77). Thus, changes of leptin might be involved in the physiology of altered PRL secretion in response to body weight loss in the present study. In a variety of animal species PRL exerts potent lipogenic and diabetogenic effects and caloric restriction and weight loss tend to restore the metabolic profile to normal in obese individuals (78). Based on the data of this study it is postulated that the beneficial effect of long term caloric restriction on metabolic parameters in obese individuals may be brought about by amelioration of

deficit D2R mediated dopaminergic transmission in hypothalamic nuclei and that PRL serves as a messenger mediating the favourable effects of dopamine on glucose and lipid metabolism in peripheral tissues.

Thyrotroph axis before and after weight loss in obese women (Chapter 5)

Changes in body weight are accompanied by compensatory changes in energy expenditure (79), which may be brought about in part by adaptations of HPT axis activity (23-25). Studies in animals and humans show that leptin appears to be a regulator of the HPT axis. Therefore, it was hypothesized that weight loss induces adaptations of HPT axis activity in obese humans and that putative changes in leptin correlate with alterations of HPT axis activity. Results of this study show that weight loss significantly lowers TSH release (before 43.4 ± 6.4 vs. after weight loss 34.4 ± 5.9 mU/Lx24 h, P = 0.02) and circulating free triiodothyronine levels (from 4.3 ± 0.19 to 3.8 ± 0.14 pmol/L (P = 0.04). Differences in 24 h TSH release correlated positively with the decline of circulating leptin concentrations ($P < 0.01$, $R^2 = 0.62$). Most of the previous clinical studies evaluating the impact of body weight loss on the HPT axis showed that weight loss lowers single measurement of TSH and the TSH release in response to TRH, whereas others report unchanged thyroid hormones, plasma TSH or TRH induced TSH responses in obese individuals after weight loss (80-87). As the reduction of 24 h TSH secretion correlated with the decline of mean 24 h leptin concentrations in response to weight loss, this might implicate that leptin plays a possible role in the control of pituitary TSH release in (obese) humans.

Alternatively, other factors might modulate TSH production so as to decrease in response to weight loss in obese women. As TSH release is inhibited by D2R activity (40) and calorie restriction and weight loss are accompanied by increased D2R signalling in animals and probably also in humans (11;66), up-regulation of D2R tone in response to weight loss may reduce TSH secretion. As exogenous estrogens raise TSH concentrations (88) and estrogen levels significantly dropped after weight loss, estrogen might be involved in the modulation of HPT axis activity. Whatever the underlying mechanism, changes of HPT activity in response to body weight loss in obese humans may be of clinical and physiological relevance. Since thyroid hormones are among the regulatory cues involved in stimulating energy expenditure and basal metabolic rate (40), this neuroendocrine adaptation potentially frustrates obese humans in their attempts to lose weight.

3. Effect of Acipimox on neuroendocrine perturbations in obese premenopausal women

The **third aim** of this thesis was to study the impact of Acipimox, known as a lipid lowering drug which reduces circulating FFA levels, on the somatotroph and the corticotroph hormonal ensemble in obese premenopausal women. Therefore, plasma hormone concentrations of healthy obese premenopausal women were studied twice in the follicular phase of their menstrual cycle, with a time interval of at least 8 weeks where body weight remained stable. Obese women were randomly assigned to treatment with either Acipimox (an inhibitor of lipolysis, 250 mg orally four times daily) or placebo in a double blind cross-over design, starting one day prior to admission until the end of the blood sampling period. At each study occasion, blood samples were taken during 24 h with a sampling interval of 10 min for assessment of plasma hormone concentrations and hormone secretion was estimated by deconvolution analysis (**Appendix B**). Figure 2 is a schematic overview of the study.

Figure 2.

Somatotroph axis before and after Acipimox treatment in obese women (Chapter 6)

Both clinical as well as experimental animal studies have shown that Free Fatty Acids (FFAs) reduce hormonal secretion of the somatotroph axis (89-92). Obesity is associated with high circulating free fatty acid (FFA) concentrations (93;94) and hyposomatotropism (95). Therefore it hypothesized that reduction of circulating FFA levels with Acipimox, a powerful anti-lipolytic drug, enhances GH secretion in obese humans.

Results of this study showed that Acipimox unleashes spontaneous GH secretion in obese (Acipimox 113 ± 50 vs. Placebo 66 ± 10 mU/V_{dl}/24 h, P = 0.02). Diurnal GH secretion rates remained lower compared to lean controls (controls 201 ± 23 mU/V_{dl}/24 h, P = 0.005 vs. obese during Acipimox). Neuroendocrine alterations of the GH axis particularly occur in viscerally obese patients (96). Visceral fat is morphologically and functionally distinct from subcutaneous fat, in that cellularity and FFA turnover are higher per unit adipose tissue (97;98). Furthermore, venous output of visceral fat drains directly into the portal system of the liver, while FFAs from subcutaneous fat enter the systemic circulation. FFA infusion specifically into the portal vein enhances pituitary-adrenal axis and sympathetic nervous system activity, whereas systemic FFA infusion does not exert appreciable effects on these neuroendocrine systems (99-101). Thus, a high portal FFA flux, brought about by excess visceral fat, may particularly inhibit GH release. Therefore, we sought to determine the relationship between the effects of Acipimox and the size of various adipose depots. However, further analysis did not show any relationship between the effects of Acipimox on GH secretion and regional body fat distribution. This might be due to the limited size of our study population. The mechanism through which Acipimox stimulates GH secretion in obese individuals might be due to lowering circulating FFA, however, other potential mechanistic explanations for the profound impact of Acipimox on GH secretion may relate to its impact on plasma insulin levels or neural pathways such as dopamine. Finally, a direct effect of Acipimox on GH cannot be excluded. Findings of this study are in line with previous studies evaluating the effect of Acipimox on GH plasma levels in response to various exogenous secretagogues in obese humans (102-106). Thus, present and previous studies show that specifically enhances both exogenously as well as endogenously driven GH secretory burst mass. Therefore it is postulated that Acipimox may enhance somatotroph sensitivity to GHRH feed forward inputs, which appears to be a critical determinant involved in obesity related hyposomatotropism.

Corticotroph axis before and after Acipimox treatment in obese women (Chapter 7)
Experimental studies show that circulating free fatty acids (FFAs) promote the secretory activity of the HPA axis (99-101;107). Human obesity is associated with high circulating FFAs (93;94) and an exceedingly active hypothalamo-pituitary-adrenal (HPA) axis (52;54-60;62). Therefore, it is hypothesized that lowering of circulating FFAs by Acipimox would reduce HPA axis activity in obese humans. Results of this study showed that Acipimox significantly reduced ACTH secretion in the obese subjects (Acipimox 5850 ± 769 µg/24 h, P = 0.039 vs. placebo), while cortisol release did not change (Acipimox 33 542 ± 3436 nmol/24 h, P = 0.484 vs. placebo). This is the first study to evaluate the impact of Acipimox on secretion rates of pituitary-adrenal hormones in obese humans by deconvolution analysis. Findings of this study are in line with data from experimental studies, showing that elevation of circulating FFA by intralipid infusion raises plasma levels of ACTH (and corticosterone) (99-101;107). Although the exact mechanistic pathway through which Acipimox blunts ACTH secretion in obese individuals remains elusive, present results implicate that FFAs are indeed involved in the pathophysiology of this neuroendocrine anomaly.

4. Effect of Bromocriptine on neuroendocrine perturbations and food metabolism in obese premenopausal women

The **fourth aim** of this thesis was to study the impact of Bromocriptine, a dopamine D2 receptor (D2R) agonist which ameliorates dopaminergic neurotransmission, on food metabolism and leptin in obese premenopausal women. Therefore, eighteen healthy obese women were studied twice in the follicular phase of their menstrual cycle with a time interval of four weeks where body weight remained stable. Obese women were assigned to treatment with Bromocriptine or placebo in a single blind parallel design, starting eight days prior to admission until the end of the blood sampling period. At each study occasion, blood samples were taken during 24 h with a sampling interval of 10 min for the assessment of blood glucose and plasma insulin concentrations and with a 20 min sampling interval for the measurement of circadian plasma leptin concentrations. Plasma free fatty acids (FFA) and triglyceride (TG) plasma levels were measured hourly during 24 hours. Standardized eucaloric meals were served one day prior to admission until the end of the blood sampling period and caloric intake was identical at both study occasions. During each blood sampling period 24 h urine was collected. Fuel oxidation was determined by indirect calorimetry (ventilated hood) while subjects were fasting. Percentage total body fat was measured using DEXA. Figure 3 is a schematic overview of the study.

Figure 3.

Diurnal metabolic profiles and energy expenditure before and after Bromocriptine treatment in obese women (Chapter 8)

Diminished dopaminergic neuronal activity severely impairs insulin sensitivity and promotes body fat accumulation in (seasonally) obese animal models (108). In humans, anti psychotic drugs, blocking D2R, promote body weight gain and the development of type 2 Diabetes and hyperlipidemia (20-22). Obese humans appear to have reduced D2R binding sites in their brain (2). Therefore, it is hypothesized that short term amelioration of deficit D2R dopaminergic transmission by Bromocriptine would favourably affect diurnal metabolic profiles and energy balance in obese individuals.

Results of this study show that mean 24 h blood glucose (Bromocriptine 4.9 ± 0.1 vs. Placebo 5.4 ± 0.1 mmol/L, $P < 0.01$) and insulin (Bromocriptine 10.9 ± 0.8 vs. Placebo 13.3 ± 1.4 mU/L $P < 0.01$) were significantly reduced by Bromocriptine, whereas mean 24 h FFA and TG were increased (FFA Bromocriptine 0.57 ± 0.05 vs. Placebo 0.44 ± 0.03 mmol/L $P <0.01$ and TG Bromocriptine 1.34 ± 0.101 vs. Placebo 1.24 ± 0.1 mmol/L, $P = 0.14$). Bromocriptine increased oxygen consumption (Bromocriptine 243.6 ± 8.2 vs. Placebo 232.2 ± 5.7ml/min, $P = 0.03$) and resting energy expenditure by 50 kCal/day, $P = 0.03$). Finally, systolic blood pressure was significantly reduced by Bromocriptine (Bromocriptine 112 ± 3 vs. 122 ± 4 mmHg, $P = 0.04$). Previous studies have shown long-term Bromocriptine treatment effectively reduces fasting insulin and glucose levels in rodents and improves glucose tolerance in healthy and diabetic obese humans (109-115). However, chronic Bromocriptine administration consistently reduces body fat and food intake might have been altered in these studies, which could explain these metabolic corollaries of treatment. Data of this study strongly suggest that stimulation of D2R facilitates glucose metabolism in obese humans independent of body adiposity or food intake. The rise of circulating FFA levels induced by Bromocriptine may mirror the lipolytic properties of the drug, shifting energy balance away from lipogenesis in obesity. Although the exact mechanisms through which D2R dopaminergic neurotransmission impacts energy balance and fuel metabolism remain to be established, these findings support the notion that reduced D2R availability in the brain of obese humans directly contributes to their altered energy homeostasis and their metabolic anomalies.

Leptin before and after Bromocriptine treatment in obese women (Chapter 9)

Obese humans are hyperleptinemic and it has been postulated that obese individuals are leptin resistant (116). However, the mechanism involved with this neuroendocrine perturbation remains elusive and very little is know about the regulation of leptin secretion in vivo. Dopamine is among the neurotransmitters involved in the central adjustment of food intake, metabolism and hormonal secretion. A few previous studies provide evidence for an inhibitory effect of dopaminergic system activity on leptin secretion (117;118). As D2R binding capacity in the brain of obese humans is reduced, one might postulate that impaired dopaminergic signalling might be involved in the occurrence of hyperleptinemia in obese humans. Furthermore, short term treatment with the D2R agonist Bromocriptine profoundly alters metabolic profiles in obese women (P. Kok et al unpublished data) and previous studies have shown that changes of circulating metabolic parameters such as glucose, insulin and lipids are related to altered leptin secretion (119-130). Therefore, it is hypothesized that short term treatment with Bromocriptine reduces leptin concentrations in obese humans. Results of this study show that Bromocriptine significantly lowered diurnal leptin concentrations in obese premenopausal women (Mean 24 h concentration Bromocriptine 30.5 ± 2.5 vs. Placebo 33.6 ± 2.5 µg/L, $P = 0.03$). Furthermore, the decline of circadian leptin plasma levels is associated with the increase of FFA levels in response to Bromocriptine treatment in the obese subjects ($R^2 = 0.46$, $P = 0.03$). These results are in line with data obtained in these previous studies observing the effect of modulation of the dopaminergic activity on plasma leptin levels (117;118). Although the observed effect of Bromocriptine on leptin may also be mediated through other indirect mechanistic pathways, e.g. the effect of Bromocriptine on metabolic or hormonal

parameters, these findings implicate that leptin signalling/secretion is centrally regulated by neuronal dopaminergic systems in the brain. Thus, deficit dopaminergic signalling might be involved in the hyperleptinemic/leptin resistant state associated with obesity.

General Discussion and Future Perspectives

The studies of this thesis provide new insight of hormonal aberrations in obese women. In most of the previous studies investigating hormonal systems in obesity, single plasma hormone measurements were performed or exogenously stimulated hormone response peaks were studied. As the majority of plasma hormone concentrations fluctuate over the day and these circadian variations of serum hormone concentrations appear to be important for their biological function (17;131), proper appreciation of spontaneous hormonal concentrations requires analysis of circadian hormonal concentration patterns. Furthermore, circulating hormone concentrations result from combined influences of prior and ongoing hormone secretion, distribution and elimination. In the studies of this thesis different mathematical techniques were used to calculate these hormonal secretory and kinetic parameters from the hormone concentration time series data. It seems important to emphasize, that the design of the studies in this thesis also has some limitations. First of all, the studies were performed in a clinical setting under standardized physiological conditions which might be different from normal life outside the research center. For example, no changes of cortisol levels were found in obese and lean women when they remained recumbent in a clinical set-up. However, their cortisol levels in response to anticipatory stress or stressful experiences during daily activities could be different. Secondly, because of practical reasons, electro encephalogram (EEG) sleep recording was not performed during the studies. Therefore, great care was taken not to disturb and touch subjects during withdrawal of blood samples while they were sleeping. Lights were switched off/on and subjects went to sleep/were awakened at fixed time points. Periods of wakefulness and toilet visits during the night were recorded by the personnel performing nocturnal blood sampling. However, quantified data about sleep stages and sleep/wake cycles was not collected. In most of the studies standardized eucaloric meals were consumed at fixed time points at each study occasion, to limit nutritional confounding. It is important to note, that it is unclear from the literature how long a wash out period is needed exactly to "wash out" any potential confounding effect of calorie restriction per se on hormonal secretion. As, the secretion rate and/or plasma concentration of some hormones responds rather quickly (i.e. within hours to days) to changes in nutrient availability (132;133), we prescribed all obese subjects a standard liquid, eucalorie diet for 3 days prior to each study occasion to limit the putative impact of calorie restriction on hormonal release. However, we can not completely rule out the possibility of a persistent effect of the VLCD on the changes of hormonal secretion induced by weight loss. Finally, all subjects enrolled in the studies were premenopausal females. Whether the data of the studies in this thesis is extensible to obese men requires further investigation. Furthermore, all subjects were studied in the early follicular phase of their menstrual cycle. In this context, one might wonder whether the observed differences of hormonal secretion as measured in the follicular phase of lean and obese premenopausal women are a peculiarity of this phase of their menstrual cycle. Some clinical studies found altered basal hormone concentrations the peri-ovulatory and the luteal phase of the menstrual cycle (134-137), whereas others reported that no changes throughout different stages of the menstrual cycle (138-140). Thus, there is no conclusive evidence that the observed differences of hormonal secretion between obese and lean women are invariable during different stages of the menstrual cycle.

This thesis describes new observations and elucidated several facets of the altered hormonal milieu in obesity. Practising science is a never ending story; several fascinating questions remain to be answered. This paragraph will discuss some general conclusions and future perspectives. First of all, the changes of diurnal plasma PRL and TSH hormone levels and secretion in obese premenopausal women, provide indirect evidence for reduced dopaminergic signalling as a potential cue involved in the pathophysiology of hormonal alterations in obesity. Weight loss partly restores these neuroendocrine anomalies. Future studies are needed to directly assess the impact of weight loss on dopaminergic neuronal activity. For example, imaging studies assessing D2R availability in the brain of obese humans before and after weight loss could be performed. Alternatively, it is postulated that prolactin may be one of the endocrine messengers that relay reduced D2R mediated dopaminergic neural signals to peripheral tissues to promote (visceral) fat storage. Thus, PRL itself might have impact on peripheral glucose metabolism and adipogenesis in humans, which remains to be investigated.

Thirdly, TSH and ACTH were enhanced in face of normal thyroid hormones and cortisol levels in obese premenopausal women. Although we did not measure peripheral hormone metabolism and we therefore can not exclude the possibility that hormonal signalling at the level of the peripheral target organs was altered, these data implicate that peripheral sensitivity of the thyroid and adrenal gland towards the feed forward drive of the pituitary hormones (TSH and ACTH) is somehow hampered. Evidence from experimental animal studies suggests that the sensitivity of the adrenal cortex and thyroid gland to ACTH and TSH is centrally regulated by the suprachiasmatic nuclei via the autonomic nervous system. Since obesity appears to be associated with increased sympathetic activity, this might explain these endocrine phenomena. Present and previous studies show that Acipimox specifically enhances both exogenously as well as endogenously driven GH secretory burst mass. Furthermore, Acipimox specifically reduced the enhanced ACTH secretion by the pituitary gland. Collectively, these data suggest that FFA enhance HPA output and blunt GH secretion through effects on neuronal control systems in brain centres at the supra pituitary level. Although we cannot exclude that Acipimox itself directly impacts hormonal secretion, these findings implicate that circulating FFA are involved in the pathophysiology of pituitary-adrenal hyperactivity and hyposomatropism in obese humans.

Energy homeostasis is achieved by variable effects on energy intake, expenditure and storage, coordinated through the central nervous system (141;142). Signals related to either short term nutrient availability (e.g. nutrients and gastro intestinal peptides) or the amount of energy consumed over a more prolonged time period and proportion of body adiposity (the so called “long term” signals) emanate from adipose, endocrine, gastro-intestinal and neuronal systems. These efferent signals are received and integrated in the hypothalamus. On its turn, this specific brain area exerts homeostatic control over neuroendocrine secretion and energy homeostasis. Dopaminergic neurotransmission of this brain area is involved in the regulation of hormonal secretion and energy homeostasis. Hormonal changes described in chapter 2, 3, 4 and 5 provide indirect evidence that reduced dopaminergic signalling is involved in the pathophysiology of hormonal alterations in obesity and the studies in chapter 8 and 9 showed indeed that modulation of the dopaminergic system improves energy metabolism and blunts leptin levels in obese humans. Thus, next to its role regulating peripheral sensitivity of endocrine organs, the brain appears to be a central factor involved in the development and/or maintenance of the obese state and its associated metabolic perturbations.

Finally, one might wonder whether modulation of dopamine 2 Receptor signalling is a potential target for restoration altered neuroendocrine ensemble in human obesity. A few studies have shown long-term bromocriptine treatment consistently reduces body fat, fasting glucose and improves glucose tolerance in healthy and diabetic obese humans (109-115). However, chronic bromocriptine administration might have side effects and such corollaries of treatment have not been investigated in these studies. Long term follow up studies should be performed to evaluate long term effects and safety of chronic bromocriptine treatment.

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