Neuroendocrine perturbations in human obesity

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Chapter 1

General Introduction

Most individuals match energy intake, expenditure and storage with great precision (1-3). This phenomenon reflects an active regulatory process, which is termed energy homeostasis. Energy homeostasis promotes stability in body weight and in the amount of body energy stored in the form of fat. The ability of animals to conserve energy in the form of adipose tissue and the timed process of body fattening can be considered as an evolutionary advantage to survive periods of food shortage (4). For example, body fat stores provide energy for hibernation, migration or pregnancy. However, nowadays the abundance of highly palatable energy dense foods combined with minimal requirement for physical activity (increased industrialization, urbanization and mechanization) strongly promotes the expansion of adipose tissue mass towards levels at which the risk of morbidities and mortality are severely increased.

Obesity - Definition and Classification

In medical terms, the excessive accumulation of body fat is called "obesity". "Obesity" originates from the Latin word "Obesus" that means fat, plump or swollen and its past principle "Obedere" means to eat upon or to eat away. A rough measurement for the diagnosis and the classification of obesity is the body mass index (BMI), which is calculated as follows: weight (kg)/(length (m)) 2. A BMI of 25-30 kg/m^2 is considered as overweight and a BMI > 30 kg/m^2 indicates obesity (5). The classification of obesity according to the WHO guidelines, using the BMI is given in Table 1.

Table 1.

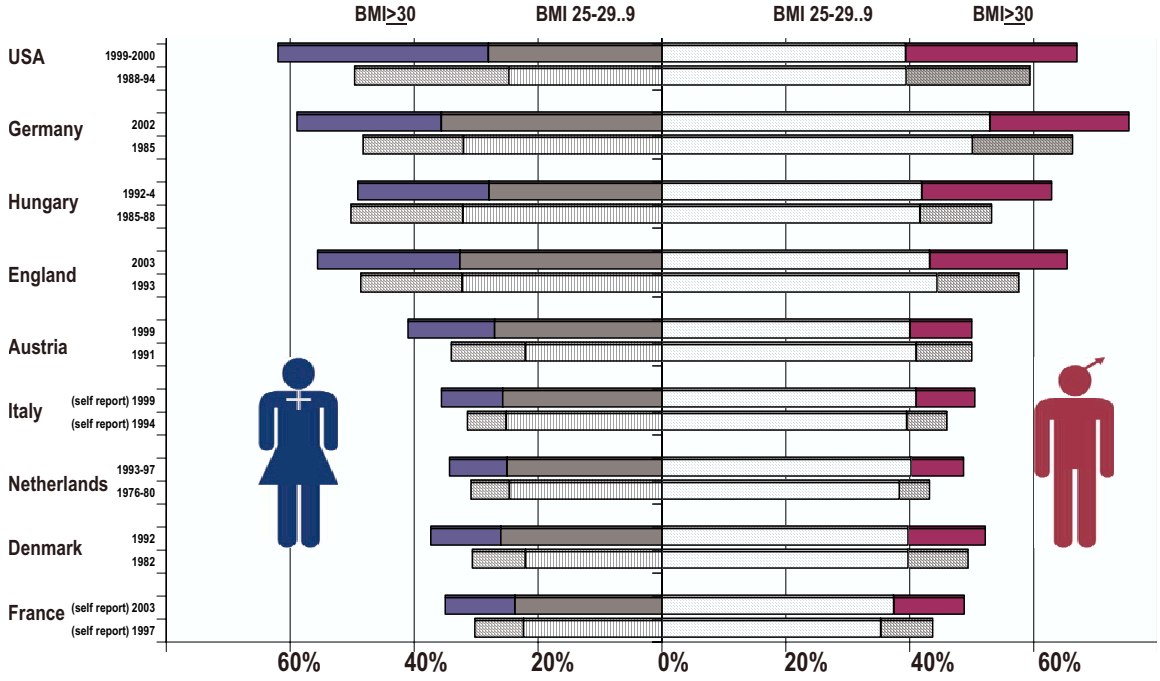
Classification	BMI (kg/m^2)	Risk co-morbidity	Action level & Consequences
Normal	18.5-24.9	Medium	
Overweight	25-29.9	Slightly increased	1: Prevention weight gain
Obesity	≥ 30		2: Weight reduction (10-15%)
Level I	30-34.9	Increased	and stabilisation body with
Level II	35-39.9	Severely increased	professional care
Level III	≥ 40	Highly increased	

Derived from: Meinders AE, Fogteloo J. NTvG 2003 Sep 20; 147(38):1847-51 and Guidelines WHO Tech Rep Ser 894, 2000

Obesity - Epidemiology

The overall prevalence of obesity has risen dramatically over time. Globally there are more than 1 milliard overweight adults and at least 300 million of them are obese (World Health Organization). The obesity epidemic is not restricted to industrialized societies; obesity often co-exists with under-nutrition in developing countries and the increasing prevalence of obesity in these countries is often faster than in the developed world (5). Furthermore, obese adults of developing countries, who were undernourished in early life, tend to develop hypertension, cardiovascular disease and diabetes at earlier age and in more severe form than those who were never undernourished. Figure 1 shows the increasing prevalence of adult overweight and obesity in the USA and Europe.

Figure 1.



Derived from: European Obesity Task Force EU Platform Briefing Paper March 2005

In the Netherlands the prevalence of adult obesity has risen from 4.9 to 8.5% in men and from 6.2 to 9.3% in women between the late 1970s and mid- 1990s. Table 2 represents prevalence of overweight and obesity among adults in the Netherlands based on data collected from studies between 1998 and 2002.

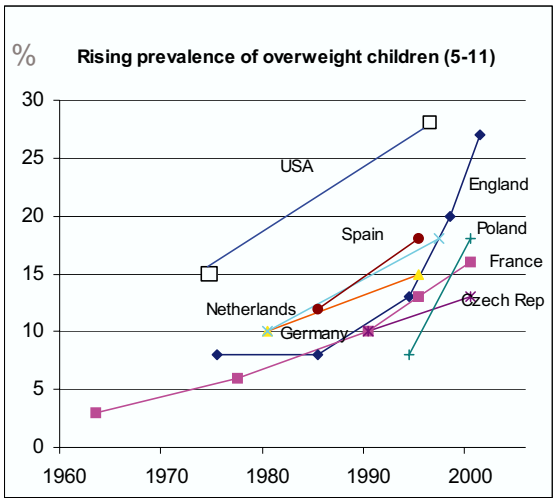
Table 2.

Prevalence in the Netherlands	% BMI 25-29.9	% BMI ≥ 30	% BMI ≥25
Male	43.5	10.4	53.9
Female	28.5	10.1	38.6

Derived from: European Obesity Task Force EU Platform Briefing Paper March 2005

The global obesity epidemic affects individuals of all ages and the rising prevalence and incidence of overweight and obesity among youngsters is a rapidly growing problem in many countries. This rapid increase is especially worrisome because of the well described association between childhood obesity and increased cardiovascular risk and mortality in adulthood (6;7). Furthermore, persistent obesity in adulthood after childhood obesity is associated to a higher degree of adverse health consequences compared to the pattern of adversity of obesity confined to adult life (8). The cut off point for excess fatness of overweight or obesity in children and adolescents is based on the sex-specific "percentile of BMI for age". Overweight is defined as ≥ 85th percentile of BMI for age and obesity ≥ 95th percentile of BMI for age (9). According to global estimates of the WHO, world wide about 22 million children under the age of five are overweight. Figures of the International Obesity Task Force show that one in 10 school-age children (age 5-17 years old) is overweight, which includes a total of 155 million children world wide. 30-45 Million children within that figure are classified as obese. Although the rates of increase vary among different countries, a rapidly rise of childhood overweight and obesity has been observed in the USA and Europe (Figure 2).

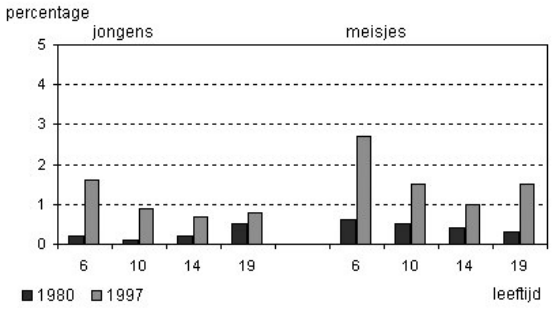
Figure 2.



Derived from: European Obesity Task Force EU Platform Briefing Paper March 2005

Figure 3 shows the severely increased prevalence of overweight and obesity among boys and girls in the Netherlands between 1980 and 1997.

Figure 3.



Derived from: Hirasing RA, et al. NTvG 2001; 145(20);1303-4)

Finally, a cynical remark could be made that the wide spread occurrence of obesity not only affects human beings but also the domestic animals they take care of. This is well illustrated by the increasing incidence and prevalence of obesity among cats and dogs and treating co-morbidities such as diabetes mellitus type 2 of obese pets is one of the growing common activities for veterinary clinicians (10;11). Thus, the abundance of highly palatable energy dense foods in the civilized society even badly affects man's best friend.

Obesity-Health Consequences

Obesity is associated with numerous metabolic disturbances, such as insulin resistance, diabetes mellitus type 2, dyslipidemia and hypertension (12-15). The development of type 2 diabetes is not only confined to older adults with increased body fatness but also affects obese children even before puberty. Conversely, 85% of people with type 2 diabetes are overweight (16). Furthermore, obesity leads to several other health problems including disturbances of the respiratory and musculoskeletal system (for example sleep apnoea and osteoarthritis), gallbladder disease, skin difficulties and infertility (17;18). Obesity is also associated with increased risks of cancer, in particular cancer of the breast, colon, prostate, endometrium, kidney and gallbladder (19-22). Epidemiologic studies in primarily white populations have shown a strong linkage between obesity and increased mortality rates. This increase begins to rise slowly at a BMI > 25 kg/m^2 and steeply increases at a BMI>30 kg/m^2, towards a 1.5-2.0 fold excess independent risk of mortality compared to individuals with a BMI < 25 kg/m^2 (23).

Obesity - Visceral Adiposity

Excess of fat deposition within the abdomen, or so called visceral adiposity, confers an independent risk for metabolic (diabetes mellitus type 2) and cardiovascular complications than does adipose accumulation elsewhere (24-27). This close relation between excess body fat in the visceral depot and metabolic disturbances or cardiovascular disease might be explained by the specific endocrine features of the visceral fat depot and/or its unique anatomical relation to the hepatic portal circulation (thereby releasing adipokines and free fatty acids directly into the portal venous system in stead of the peripheral systemic circulation).

Waist circumference appears to be a reliable index of intra-abdominal fat mass (27). Changes in waist circumference reflect changes in risk factors for cardiovascular disease and other co-morbidities associated with visceral obesity (28). Therefore, waist circumference can be used as a simple additional tool to assess health risks associated with visceral obesity. Table 3 shows the cut off point of sex-specific waist circumferences that denote increased risk for metabolic complications associated with obesity in the Caucasian population (27).

Table 3.

	Risk metabolic complications	
	Increased	Substantially increased
Male	≥ 94 cm	≥ 102 cm
Female	≥ 80 cm	≥ 88 cm

Obesity-Treatment

The fundamental approach to reverse the obesity epidemic is effective weight management for obese individuals and groups at risk for developing obesity (5;29). Most of the putative strategies in order to achieve weight reduction include life style management focused on dietary intervention and increased physical activity, with the use of a variety of pharmacological agents such as orlistat and sibutramine. In the most severe cases invasive techniques (bariatric surgery) might be used in order to achieve weight loss. However, obesity remains a medical condition which is difficult to manage and weight regain is among the greatest challenges related to a weight loss intervention (30).

Obesity - Pathophysiology

The pathophysiologic mechanism of obesity remains elusive and explanations are still lacking why some individuals are more likely to suffer from obesity than others in the same environment. Various factors such as genetic, social, behavioural and physiological cues are responsible for the development of obesity. As obesity runs in families it has been recognized and appreciated that a genetic component is involved its development (31). So far, a number of families with rare pleiotropic obesity syndromes have been studied by linkage analysis and some chromosomal loci for obesity syndromes have been recently described (see Online Mendelian Inheritance in Man (OMIM) http://www.ncbi.nlm.nih.gov/omim/). The Prader-

Willi syndrome is the most common syndromal cause of human obesity (estimated prevalence of about 1 in 25.000) caused by deletion or disruption of the paternal segment 15q11.2-q12. Other syndromes in which obesity is a recognized part of the phenotype are for example Albright hereditary osteodystrophy, Fragile X syndrome or the Bardet Biedl Syndrome. Single gene mutations, such as either the dominant (yellow, Ay/a) or recessive (ob/ob, db/db, fa/fa, tb/tb) gene defects, are known to cause genetic and experimental syndromes of obesity in rodents (32-35). Based on these studies it has been hypothesized that mono genetic defects can lead to disorders of energy balance and obesity in humans. Indeed, these candidate gene mutations have also been identified in obese humans. For example, mutations of the leptin gene (leading to congenital leptin deficiency) and leptin receptor genes are linked to early onset, severe obesity (36;37). Additionally, mutations of the gene encoding pro-opiomelanocortin (POMC), which is known as one of the anorexigenic hypothalamic neuropeptides, are associated with early onset childhood obesity (38). Also, loss of function mutations of other signalling molecules (Prohormone Convertase 1 deficiency) or receptors (Melanocortin 4 Receptor deficiency) of the melanocortin system which is involved in the regulation of body weight in humans, lead to severe childhood obesity (39). Finally, de novo mutations in the neurotrophin receptor TrkB and missense mutations in the cocaine- and amphetamine-regulated transcript (CART) induce severe obese phenotypes through physiological disturbances in the regulation appetite and energy intake (40). Although a strong linkage has been described between genetics and obesity, in general, the development of obesity is not simply due to single gene mutations (41). A comprehensive and updated reference for all association studies in obesity genetics is available in the form of the obesity gene map established by Bouchard, Chagnon, Perusse and colleagues at The Pennington Biomedical Research Centre (link to http://www.obesite.chaire.ulaval.ca/genemap.html).

Obesity-Neuroendocrinology

From a biological point of view, obesity might be explained by differences in the regulation of energy homeostasis between obese and lean individuals. Energy homeostasis is achieved by variable effects on energy intake, expenditure and storage, coordinated through the central nervous system (42;43). 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 energy intake, expenditure and storage through modulation of various processes, including food intake, physical activity and neuroendocrine secretion.

The neuroendocrine system provides a source of humoral messengers many of which can modulate energy homeostasis in target cells of different organ systems. As neuroendocrine factors are involved in the regulation of energy homeostasis, alterations of the endocrine environment might contribute to the development or maintenance of excess adipose tissue mass and the obese phenotype. This thesis will focus on changes of the neuroendocrine environment in obese women. The hormonal systems studied in the obese women, will be shortly introduced in the next paragraph.

Growth Hormone

Growth Hormone (GH) is an anabolic hormone which has several effects on glucose, lipid and protein metabolism. GH increases plasma glucose concentrations through stimulation of endogenous glucose production of the liver and GH reduces peripheral glucose uptake (diabetogenic hormone)(44). Furthermore, GH stimulates protein synthesis, whereas GH inhibits protein breakdown and amino acid oxidation (45-47). Finally, GH has a profound impact on fat storage; it enhances adipose tissue lipolysis through stimulation of lipolytic enzymes and the inhibition of lipogenic enzymes (48-50) and GH facilitates lipolytic actions of epinephrine (51). Although most of the GH deficient patients are not clinically obese, they show an increased amount of body fat, with a predominant visceral adiposity (52;53). GH replacement reduces their body fat with the largest decrease in visceral fat mass independently of changes in body weight (54;55). However, it is not known whether these changes of body fat deposition observed in GH deficient patients are primarily due to the consequential loss of the lipolytic and anabolic GH actions per se. Nevertheless, it has been invariably observed that both spontaneous pulsatile GH secretion as well as the GH response to various provocative exogenous stimuli are markedly blunted in obese individuals (56). Thus, obesity and in particular visceral obesity (57), is associated with hyposomatotropism. As it has been found that

hyposomatotropism is not compensated by increased adipose tissue responsiveness to GH (58), the reduced circulating plasma GH might contribute to enlarged adipose tissue mass in obese humans.

Corticotroph Axis

The hypothalamic-pituitary-adrenal (HPA) axis is essential for the response to stress and survival. However, the HPA hormonal ensemble also regulates lipid metabolism and body fat distribution. Changes in circulating glucocorticoid levels are associated with alterations of energy homeostasis. For example, it has been shown that removal of the adrenals reduces energy intake and adipose tissue weights in rodents, which is reversed by glucocorticoid replacement (29;59-65). Furthermore, glucocorticoid administration promotes body weight gain in rodents and humans (66-68). Hypercortisolism in patients with Cushing's syndrome leads to excess of fat in the visceral depot. Lowering of plasma cortisol levels in these patients returns body fat accumulation back to normal (69;70). Although cortisol is considered to be the main messenger conveying HPA signals to target tissues, adipocytes express ACTH receptors and ACTH poses lipolytic actions in some animal species (71). Corticotrophin releasing hormone (CRH), which stimulates ACTH secretion in the pituitary gland, reduces both food intake (acting as a satiety factor at hypothalamic level) and body weight. Furthermore, CRH simultaneously increases energy expenditure in normal weight and obese rodents (72;73). Several studies suggest that the hypothalamo-pituitary-adrenal (HPA) axis is hyperactive in obese animals and humans. Experimental studies in genetically obese rodents show that these animals have high levels of glucocorticoids (74-79). Clinical studies report that both plasma ACTH and cortisol concentrations rise to higher levels in response to CRH administration alone or in combination with arginine vasopressin (AVP) in obese humans compared to normal weight controls (80-82). Moreover, the cortisol response to ACTH is exaggerated in obese volunteers (83-85) and it has been reported that stress induced cortisol secretion is increased in abdominally obese women (86). A few previous papers reported that diurnal plasma ACTH concentrations are higher in obese individuals, while circulating cortisol levels are similar to those in lean controls (87;88). Furthermore, urinary free cortisol excretion appears to be elevated in abdominally obese humans (83;84), while suppression of plasma cortisol levels by dexamethasone (89) or hydrocortisone is blunted (89-91). Recently it has been published that tissue specific changes in cortisol metabolism are associated with obesity. At tissue level, the conversion of cortisone into active cortisol is catalysed by the enzyme 11βHSD type 1, which in turn stimulates adipocyte differentiation of stromal cells to mature adipocytes. Both experimental animal studies as well as clinical studies have shown that 11βHSD type 1 is increased in the liver and visceral adipose tissue in obesity (92). Furthermore, urine analysis of cortisol and cortisone metabolites show that cortisol/cortisone ratios are significantly lower in patients with obesity, which might indicate enhanced 11βHSD type1 activity in obese individuals (93). Finally, transgenic 11βHSD type 1 over expressing mice are characterized by visceral obesity while its circulating corticosterone levels are normal (94). These genetically mutated mice were also hyperglycaemic, hyperinsulinemic and glucose intolerant. These findings suggest that increased production of active glucocorticoids in adipose tissue through 11βHSD type 1 over expression, leads to visceral obesity and its associated metabolic perturbations. It has been suggested that this phenomenon possibly reflects a tissue specific (visceral) Cushing's syndrome in obese humans (95). Taken together, previous data implicate that changes of the HPA axis might be involved the development or maintenance of the (upper body) obese phenotype.

Prolactin

Prolactin (PRL) is a versatile hormone that, among many other biological actions, affects energy balance and food metabolism. Exogenous PRL administration increases fat storage in animals when injected at certain times of the day (96;97). PRL influences body fattening directly or indirectly through stimulation of food intake and multiple metabolic routes (98-100). PRL augments activity of the key enzyme for lipid accumulation (lipoprotein lipase) in bird adipocyte tissue and in the liver of rats (101;102). Furthermore, PRL also modulates adipocyte differentiation (103). Indeed, PRL receptor gene knockout mice have considerably reduced fat mass and primarily visceral fat is diminished (104). PRL has been reported to influence carbohydrate metabolism through its direct effects on pancreatic functioning. For example, PRL increases pancreatic insulin secretion and decreases the glucose threshold for insulin secretion through increasing glucokinase and glucose transporter 2 (factors involved in organ specific glucose disposal) (60;105-108). However, insufficient data exists about the direct impact of PRL on peripheral glucose metabolism in humans. Increased body weight or a recent history of weight gain is

frequently observed in hyperprolactinemic men and women (109), whereas these patients lose weight once treated effectively with dopaminergic agents (dopamine 2 receptor agonists), which decrease PRL secretion. Variable abnormalities of plasma PRL concentrations have been observed in obese humans. Several papers report that both basal (single measurement) as well as 24 h (hourly measured) integrated plasma PRL levels are similar in obese and normal weight humans, whereas the PRL release in response to a number of secretagogues was blunted in obese individuals (110-118). Thus, PRL can be considered as another humoral messenger being causally involved in or maintaining the obese state.

Thyrotroph Axis

The hypothalamic pituitary thyroid (HPT) hormonal ensemble orchestrates a variety of metabolic processes, including thermogenesis and energy expenditure, thereby affecting energy balance (119-121). Hypothyroidism is associated with a moderate increase in body weight and decreased appetite, whereas weight loss with normal or increased food intake is a hallmark of thyrotoxicosis. Numerous studies have evaluated the HPT axis status in obese humans even when they were clinically and biochemically euthyroid and the results were conflicting. The majority of these studies suggests that there is no substantial change in basal thyroid hormone concentrations, although a few papers document serum triiodothyronine (T_3) elevation in obese subjects (122-125). The basal serum TSH concentration in a single plasma sample was similar in obese and non-obese subjects in some studies (126;127), whereas others documented higher basal TSH concentrations in obese humans (123;126-129). Also, a larger rise of plasma TSH in response to TRH stimulation is found in obese subjects, while other studies revealed normal or reduced TSH responses (111;113;123;126-134). Synthetic thyroid hormones as well as various other thyroid hormone preparations have been and are still used as adjunctive measures to induce or facilitate weight loss. However, as triiodothyronine treatment enhances mostly body protein loss and only to a small extent loss of body fat (135), thyroid hormone supplements are not recommended in the treatment of obesity.

Leptin

The adipocyte is well recognized as a bona fide endocrine cell and several adipocyte derived hormones, or adipokines have been recently discovered (136;137). Leptin is among these adipocyte derived hormones and is one of the afferent signals informing the brain of adipose tissue energy reserves (fat stores). There exists a positive correlation between the amount of fat cell mass and leptin secretion. The effects of leptin are achieved by its interaction with specific leptin receptors, which are both located in peripheral tissues and within the central nervous system. Leptin is transported across the blood-brain barrier and it binds to specific receptors on appetite modulating neurons, most notably but not exclusively in the hypothalamic arcuate nucleus. Leptin promotes negative energy balance (inhibition food intake and stimulation energy expenditure) in order to maintain body weight homeostasis (138-141). Next to its effect on energy balance and food intake, many other activities of leptin have been described. For example, leptin affects bone formation, functioning of the immune system, the gonadal system and modulates fertility (142). Leptin deficient animals and humans are hyperphagic, obese and infertile (36;143), whereas exogenous leptin administration reverses obesity in leptin deficiency. However, leptin deficiency and leptin receptor deficiency is an extremely rare cause of human obesity. In fact, the majority of obese humans have high circulating leptin concentrations and this hyperleptinemic state is accompanied by a relatively low ratio of leptin CSF to serum levels compared to lean individuals (144). Therefore, it has been proposed that obese humans are leptin resistant. This leptin resistance might result from defects in transport across the blood-brain barrier or might be due to impaired leptin signalling. Thus, changes in leptin are associated with obesity and this neuroendocrine perturbation might be involved in the generation or the persistence of the obese state.

Effect of weight loss on neuroendrocrine perturbations associated with obesity

Caloric restriction and weight loss ameliorates the metabolic profile and affects energy expenditure in obese individuals. Also, changes of different hormonal systems after weight loss have been described in literature. For example, it has been invariably observed that reduced GH secretion and secretion reversed to nearly normal levels after substantial weight loss in obese humans (145;146). Furthermore, weight loss is also associated with a profound decrease in circulating leptin levels in obese humans (147). Variable effects of weight reduction on the HPA hormonal ensemble in obese humans has been

described literature. Some studies reported that weight loss reduced single measurements of cortisol concentrations (148;149), whereas others found increased (150) or unchanged (151) plasma cortisol levels after weight loss in obese individuals. Furthermore, some authors reported that plasma ACTH concentrations in response to CRH administration increased towards similar levels before and after weight loss in obese humans, whereas the cortisol response to CRH was either blunted or unaltered (81;82;152;153). Weight loss appeared to have no effect on suppression of plasma cortisol levels by dexamethasone (152;153) and 24 h urinary cortisol concentrations were decreased or unaltered after weight loss (152;154). Previous clinical studies concerning the impact of body weight loss on the thyrotroph and lactotroph endocrine systems have shown variable results. Some studies reported that the serum PRL response to TRH injection is blunted after a four week period of caloric restriction (320 kCal/day) or a 36 hour fast in obese subjects (155;156), whereas others found no impact of a 3-9 week period of total fasting on TRH induced PRL release in obese males (157). Prolonged fasting (no caloric intake) during twelve days significantly increased hourly integrated (spontaneous) PRL concentrations in six obese women compared to normal controls (six women, one man)(158), whereas others found no changes in basal serum PRL levels during caloric restriction in obese females (155). Furthermore, most studies have shown that weight loss reduces TSH concentrations and the TSH response to TRH, whereas others report unchanged plasma TSH or TRH induced TSH responses in obese individuals after weight loss (157;159-162). As plasma PRL and TSH concentrations are characterized by circadian fluctuations, adequate appreciation of the impact of body weight loss on PRL/TSH release requires frequent measurement of these hormones over time. However, the impact of weight loss on diurnal PRL/TSH concentration patterns and secretion rates has not been studied before. Therefore, the impact of body weight loss on spontaneous diurnal concentrations/ secretion rates of the thyrotroph and lactotroph hormonal systems will be studied in this thesis.

Factors involved in neuroendrocrine perturbations associated with obesity

The cause of the neuroendocrine perturbations associated with obesity remains elusive and numerous physiological cues may be involved in the altered hormonal milieu in obese humans. The impact of two different factors are studied in this thesis:

1. Free Fatty Acids

Free Fatty Acids (FFAs) are released from the fat cell into the blood. Obesity is associated with high circulating FFA concentrations (163;164). Previous studies in animals have shown that circulating FFAs inhibit GH secretion (165-168). Therefore, it has been hypothesized that the increased amount of circulating FFAs might be among the physiological factors involved with the hyposomatotropism.

Excess fat can be stored in various adipose depots and it appears that neuroendocrine alterations particularly occur in viscerally obese patients (145;169-171). Visceral fat is morphologically and functionally distinct from subcutaneous fat (171;172). Venous output of visceral fat drains directly into the portal system of the liver, while FFAs from subcutaneous fat enter the systemic circulation. Moreover, cellularity and FFA turnover are higher per unit adipose tissue. FFA infusion 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 (173-175). Therefore, it has been hypothesized that the high portal FFA flux, brought about by excess visceral fat, may particularly modulate hormonal secretion of the exceedingly active hypothalamo-pituitary-adrenal (HPA) axis of obese individuals.

2. Dopamine

Dopamine is among the neurotransmitters involved in the central adjustment of food metabolism and hormonal secretion (176-178). Dopamine exerts its effect through activation of the dopamine D2 receptor (D2R), which is located on the cell membrane of its target cells. A myriad of experimental and clinical studies suggests that reduced dopamine 2 receptor (D2R) mediated neurotransmission is associated with the metabolic syndrome, the cluster of clinical features including insulin resistance, hyper insulinemia, dyslipidemia, visceral obesity and hypertension (179). It has been reported that central dopamine 2 receptor expression is reduced in obese individuals (180). Based on previous studies, one might postulate that deficit D2R dopaminergic transmission might be involved in the metabolic and neuroendocrine perturbations in obese humans.

Methods for investigating neuroendocrine changes in obesity

In most of the previous studies investigating hormonal systems in obesity, only single plasma hormone measurements were performed or exogenously stimulated hormone response peaks were studied. However, the majority of plasma hormone concentrations fluctuate over the day. These circadian variations of serum hormone concentrations appear to be important for their biological function (4;181). Furthermore, hormonal secretion into the blood often occurs in a pulsatile fashion. Frequent blood sampling at short time intervals is required to adequately detect these high frequency variations.

Evaluating hormone secretion is different from primarily inspecting plasma or serum hormone concentrations over time. Circulating hormone concentrations result from combined influences of prior and ongoing hormone secretion, distribution and elimination. Hormone distribution and elimination kinetics associated with metabolism and/or removal of intact hormone from the circulation and calculation of regularity and circadian rhythmicity of hormone concentration time series data provides insight of hormonal release. Various validated computer techniques have been developed to appraise information about hormonal kinetics, secretory parameters, regularity and nyctohemeral rhythmicity, calculated from in vivo measured hormone concentrations (for review see (182)). In the studies of this thesis different mathematical techniques were used to calculate these parameters from the hormone concentration time series data, which is further explained in Appendix B.

Thus, proper appreciation of spontaneous hormonal concentrations requires frequently measured hormone concentrations over 24 hours. During all experiments described in this thesis, blood was sampled for 24 hours at 10 min time intervals, while physiological conditions were standardized and kept constant (sleep-wake cycles, activities, meal schedules).

Aims of the thesis

The spontaneous diurnal plasma concentration patterns and the secretion of the thyrotroph, lactotroph and corticotroph axis have not been studied in obese women before and variable changes have been found in previous studies evaluating these endocrine systems in obesity. Thus, the **first aim** of this thesis is to delineate differences of diurnal spontaneous hormonal concentrations and secretion of the thyrotroph, lactotroph and corticotroph axis in obese and lean premenopausal women.

Both PRL as well as TSH synthesis and secretion is inhibited by dopamine (DA) through dopamine 2 receptor (D2R) activation at the lactotoroph/thyrotroph cell membrane. Dietary restriction/weight loss is associated with increased dopaminergic signalling in animals. This might implicate that weight loss affects diurnal secretion rates of thyrotroph and lactotroph endocrine systems. As the thyrotroph axis regulates energy expenditure, oxygen consumption and fuel metabolism and changes in body weight are accompanied by compensatory changes in energy expenditure, this might also implicate that weight loss is associated with adaptations of the spontaneous diurnal activity of these endocrine systems. Therefore, 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.

Free Fatty Acids (FFAs) modulate hormonal secretion of the somatotroph and corticotroph axis. It has been postulated that the increased amount of circulating FFAs and in particular the FFAs released from visceral adipose tissue into the portal circulation might be among the pathophysiological cues causing the altered hormonal secretion of somatotroph and corticotroph endocrine systems in obese humans. Therefore, the **third aim** 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.

Hormonal secretion and food metabolism is centrally regulated by the dopaminergic system. Hormonal release by the pituitary is regulated by dopamine through activation of the dopamine D2 receptor (D2R) of its target cells. Obese humans appear to have reduced D2R binding sites in their brain. Therefore, altered central regulation of hormonal secretion by the dopaminergic system might be involved in the neuroendocrine and metabolic perturbations in obese humans. Thus, the **final aim** of this thesis was to study the impact of enhanced dopaminergic signalling on neuroendocrine perturbations and metabolic profiles in obese premenopausal women.

Outline of the thesis

Chapter 1 is the general introduction of the thesis. In **Chapter 2** spontaneous 24 h PRL secretion in obese premenopausal women is compared to PRL release in a control group of similar age and sex and **Chapter 3** evaluates the impact of body weight loss (induced by a very low calorie diet) on PRL release in obese premenopausal women. **Chapter 4** delineates differences between spontaneous 24 h TSH secretion in obese premenopausal women and lean controls and in **Chapter 5** the effect of body weight loss induced by long term caloric restriction on diurnal TSH levels of obese females is studied.
In **Chapter 6** the impact of lowering circulating FFAs by Acipimox, a powerful anti-lipolytic drug, on spontaneous GH release in obese individuals is investigated. **Chapter 7** represents differences of spontaneous diurnal ACTH and cortisol secretion in obese and lean premenopausal women and the effect of Acipimox on the HPA hormonal ensemble in obese individuals. In Chapter 8 the effect of short term treatment with bromocriptine (D2R agonist) on spontaneous diurnal insulin, glucose and lipid plasma concentration time series and resting energy expenditure in obese premenopausal women is shown. In Chapter 9 the effect of short term bromocriptine treatment on spontaneous diurnal leptin concentrations in obese premenopausal women is described. Results of all studies published in Chapter 2 to 9, are discussed and summarized in **Chapter 10**. A Dutch summary of the thesis is given in **Chapter 11**.
Appenix A is the list of abbreviations used in the thesis. **Appendix B** briefly explains the different mathematical methods used in this thesis to analyse diurnal hormonal rhythms.

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