Chapter 7

Summary and General discussion
GENERAL ASPECTS

In the last decades, at least in the western world, obesity has become a major public health problem. It is very complicated and affects all age categories and socioeconomic groups. Individually it is known that substantial weight loss will have beneficial effects. Still, the knowledge on the exact pathophysiological processes and interactions in obesity remains scarce as it involves so many issues such as genetic, environmental and behavioural factors (Figure 1). Another important fact is that the homeostatic systems in the human body are more capable of defending against weight loss than against weight gain (1). Therefore it remains challenging to understand the processes involved and consequently why it is so difficult to lose weight and even more difficult to maintain the weight loss. A subgroup of obese subjects have been identified as a “metabolically healthy” phenotype referring to obese subjects without metabolic derangements such as dyslipidaemia, insulin resistance and hypertension. Although it seems that even subjects in this group have a higher risk of cardiovascular events compared to non-obese, further investigations on this specific phenotype might lead to some more answers (2,3). The discovery of many small signalling proteins such as adipokines the last decades has led to new insights into the regulation of the energy balance. Also, lately, hepatokines and myokines have been suggested to be possible biomarkers for insulin sensitivity (4). A recent exciting discovery of the effect of gut microbiomes on the regulation of body weight (5), has opened a wide field of possible research, and perhaps in the future, more effective therapeutical options.

Another interesting category are the elderly. The homeostasis during aging is complex involving both insulin resistance and anorexia (6,7).
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**Figure 1.** Multifactorial involvements of obesity

**THIS THESIS**

The objective of this thesis was to study various processes involved in the regulation of body composition and energy balance both in healthy lean and obese subjects as well as in the elderly.

To begin with, in **chapter 2** we reviewed two of the most essential hormones involved in the regulation of both short- and long-term energy balance, Leptin (identified in 1994) and Ghreline (identified in 1999). Leptin in combination with insulin and serotonin, which share a common signalling pathway, is involved in regulating body weight and energy homeostasis. Its receptors are found widely in the brain, mainly in the hypothalamus and cerebellum, but also peripherally such as in the stomach and vessels. It mainly signals the levels of fat stores...
to the brain and has an appetite lowering (anorexogenic) effect. Its levels are increased in obesity, but in a way there is leptin resistance in the brain, leading to “abnormal” processing of this peripheral information. Also, leptin seems to be involved in adaptation to energy deprivation with a significant decline in leptin levels after a few days of fasting, much greater than the change in adipose mass, as a neuroendocrine response to starvation but the consequences of this are not clear. The last few years more literature has been published on some of the diverse other effects of leptin, such as positive effect on immune response, neural plasticity and regulation of neuronal damage (8,9). The effect of leptin on the vascular system is contradictory with reports on pro- and anti-inflammatory effects (10, 42). Leptin treatment in subjects with undetectable levels of leptin due to mutations in the leptin gene have improved metabolic parameters such as insulin resistance and lipid profile (11). Improving leptin sensitivity is potentially a useful strategy to treat obesity and its complications, but targeting the leptin resistance either with drugs or with food compound modulation have not yet shown safe or efficient results (12,13). More clarification of the interaction of leptin with insulin and serotonin is likely to give some more insights.

Ghrelin is mostly involved in the short-term energy balance. It has been identified in the hypothalamus, the gastrointestinal tract and adrenal cortex. The secretion of ghrelin depends mainly on the nutritional state and is the only peripheral hormone that activates receptors in the hypothalamic and pituitary appetite centers. Recently a collaborative mechanism with the endocannabinoid system in modulating the non-homeostatic feeding has been suggested (15). There is a pre-prandial rise and postprandial decrease in ghrelin levels suggesting a role in meal initiation. Ghrelin also seems to be involved in decreased energy expenditure and promotes the storage of fatty acids in adipocytes therefore contributing to a positive energy balance (14). Antagonizing ghrelin function has been shown to attenuate body weight gain and glucose intolerance induced by a high caloric diet. In contrary, an orally active
ghrelin receptor agonist has been introduced as treatment for cancer related anorexia-cachexia (16).

In obesity the circulating levels of ghrelin are decreased probably as a compensatory mechanism and recently a novel concept of obesity induced ghrelin resistance in neural circuits has been introduced, as a system to maximize energy reserves during a time of food scarcity (17). When obese subjects lose weight the low ghrelin levels increase or “normalize” again.

Besides leptin and ghrelin, there are 2 essential neuroendocrine regulators of the body composition, the hypothalamic-pituitary-adrenal axis and the growth hormone (GH)/IGF-I axis. Glucose and insulin can influence the function of these regulators in diabetes, insulin resistance and obesity (18,19). In normal weight persons there is a very strong reaction in the pituitary to low blood glucose levels and high blood glucose levels suppress growth hormone secretion. In patients with diabetes GH levels are abnormally high, suggesting a central derangement caused by glucose, FFA, IGF-I and/or insulin levels. Less is known about the effect of these metabolic parameters in a situation of normoglycemia. We studied the hormonal reaction of the pituitary gland to physiological levels of glucose as shown in chapter 3. Under stable clamp conditions the blood glucose levels were stabilized at fixed levels of 4, 8 and 12 mmol/l, and in these conditions stimulation tests were performed. There were no significant differences in the reaction of cortisol, ACTH and growth hormone at these different levels of blood glucose (4, 8 and 12 mmol/l). In obesity, especially in subjects with great abdominal fat tissue, there is evidence of a greater responsivity of the HPA axis as well as an upregulation of cortisol output in adipocytes (20). There is an increased neuroendocrine stress response involving other factors and leading to for example changes in sleep rhythm and reactions to stress (21).
Growth hormone is a well-known essential hormone involved in body composition. The main effects of growth hormone is exerted via IGF-1. IGF-1 is not only involved in tissue growth but has a wide range of other physiologic effects such as in glucose and lipid metabolism (22). With aging various changes in body composition occur. Body weight increases until the age of about 60-65 years due to an increase in fat mass, but simultaneously there is a decrease of muscle mass and bone mineral density. It has been suggested that some of these changes are related to the physiological age-related decline in the GH/IGF-1 axis. This decline might make older adults vulnerable and more susceptible to falls due to loss of muscle tissue (sarcopenia) (23). The appetite signalling also differs with aging, although studies in this area are scarce. It is known that in this phase levels of pro-inflammatory cytokines are higher. During aging there is possibly leptin and insulin resistance stimulating the production of these pro-inflammatory cytokines (24). This is sometimes called the “paradox of insulin resistance in aging”. At this stage hunger signals seem to prevail satiety signals. After the age of about 70-75 years, there is an increase in satiety signals, such as GLP-1, due to relatively large amounts of fat and less ghrelin, leading to less energy intake. This has been referred to as the “anorexia of aging” (7). In conclusion although the mechanisms involved in the regulation of energy homeostasis in the elderly are complex and not clear, there seems to be a very delicate balance between over- and undernourishment with aging.

In older people information is scarce regarding the “normal reference range” of IGF-1 levels. As this hormone has widespread effects, the relation between these levels and measurements of body composition are of great interest, especially in the elderly. We were able to investigate serum IGF-1 levels in older people in a large cohort, the Longitudinal Aging Study Amsterdam (LASA), as shown in chapter 4. The reference range was in the highest age category of 80-90 years was between 10-15 nmol/l, significantly lower when
compared to the age category of 65-70 years. After correction for various possible confounders such as nutritional status, we were able to show an association between IGF-1 levels and some measurements of body composition. For example, there was a significant association between low IGFI-I levels, low BMI and biceps skinfold measurements in men aged above 75 years. It is possible that lower IGF-I levels are favourable with aging. The relationship between IGF-I and fat distribution remains unclear. The findings of a large British birth cohort study of people aged between 53 and 64 years suggests some role of IGF-I in regulating fat mass but not lean mass (25). On the other hand, there are still speculations on the relationship between IGF-I levels and lean mass as it is known that there is a high prevalence of sarcopenia in the elderly (26). Although we did not find an association between IGF-I and muscle mass, in our study men with a low physical activity score had lower BMI and lower IGF-I levels.

Measurements of IGF-I and physical activity in the elderly can be variable. After 12 weeks resistance training, these levels either decrease or are not affected (27,28).

Further studies on body composition and frailty are essential to be able to preserve, or at least delay the functional decline associated with aging. More studies are necessary especially evaluating exercise and nutritional interventions (29).

In the last decades various techniques such as Functional MRI (fMRI) and PET scans have been evolved and widely used in brain research inducing possibilities to examine the connection of peripheral metabolic measurements to the central processing systems. Areas such as the amygdala and orbitofrontal cortex are involved in receiving information on visual food stimuli followed by further processing of this information into rewarding or motivational values, by means of an integrated neural system. As would be expected, this processing is different in the hungry and the sated state. Furthermore, various areas involved in reward perceptions have been shown in subjects with craving and obesity (30,31,32).
We performed an fMRI study on healthy young males, with the aim to visualize the areas involved in the processing of food stimuli both in sated and hungry state as well as to connect the measurements of leptin, ghrelin, insulin, FFA and glucose with these areas as shown in chapter 5. During the fMRI scans the subjects were shown pictures of food, landscape, animals and some things and were asked to perform some assignments. We observed, during fasting, an increased activity in the fusiform gyrus and hippocampus areas which are known to be involved in visual perception and memory, most likely due to increased salience to food pictures in this state. During the fasting state there seemed to be a correlation between the areas involved in appetite and ghrelin levels but this did not reach significance in our study. On the other hand, there was a strong negative correlation between the higher leptin levels during satiation and activation in the hippocampus and insula areas, emphasizing the fact that in the sated state, the reward reaction is blunted in normal weight subjects.

In obese subjects, the normal function of the neural reward system is different (33,34). Therefore it is interesting to investigate whether, and to what extent, this changes with weight loss. In chapter 6 the results of the effects of caloric restriction in overweight subjects are shown. We were especially interested in the early metabolic and hormonal effects of caloric restriction and the correlation of these parameters with the brain areas involved in satiation and hunger. After only 1 week of caloric restriction there were various favourable changes such as lower systolic blood pressure, lower leptin, resistin and triglyceride levels.

Before caloric restriction food stimuli resulted in an increased activation of the right amygdala, which is known to be involved in memory, decision making and emotional reactions. Also, there were correlations between FFA and glucose with various areas involved in food reward processing. Remarkably, when fMRI scans were repeated 1-2 weeks
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after the caloric restriction, the activation of the amygdala was no more present, and the correlations of these areas with leptin and ghrelin, had shifted to the more “normal” situation.

The “reversal” of brain reaction is not new. For example after gastric bypass (bariatric) surgery, a partial reversal of hypothalamic dysfunction has been shown (35). Yet, it is interesting to see these changes even after a modest short term intervention in overweight subjects.

Recently a study on the effect of diet induced weight loss on memory and brain activity was published, showing improvement of memory function and increased activity in the hippocampus and this was linked to decreased plasma levels of FFA (36).

The reasons for the changes in the reward system are still not known. The dopaminergic system seems to be an important factor, as there is substantial evidence for a decrease in dopamine signalling in obese subjects (37). Dopamine signalling appears to be restored after caloric restriction or gastric bypass surgery (34,38). Eric Stice et al have done much research on the reward circuit, and have published data on genetic propensity (TaqIA A2/A2 allele) for higher dopamine signalling suggesting that the risk of overeating is either related to too much or too little dopamine signalling. This could also be used to predict future weight gain (39).

In summary, with the evolving brain research techniques, such as fMRI and PET scans, brain activation can be assessed in various conditions such as before and after weight loss treatment and, most interestingly, it will possibly be useful in identifying which subjects could benefit from a specific treatment and which don’t (40). Research in this area is ongoing, for example on the changes in the reward circuit in binge eating, anorexia and the changes in the “healthy obese”. The question is how do the regulatory hormones as drug targets come in, such as recombinant human leptin and ghrelin antagonists. Recombinant human leptin
has been approved for the treatment of congenital and acquired generalized lipodystrophy, and is further being evaluated in other conditions such as diabetes, obesity, depression (11).

Figure 2. Summary of some of the changes found in obesity. HPA: Hypothalamus-pituitary-adrenal axis
PERSPECTIVES

Obesity is being defined as a social and environmental disease (WHO), and has to be tackled at all levels. Of course, preferentially the main emphasis should be on prevention and not only on the treatment of the obesity epidemic. It begins with a global awareness of the issues involved such as the importance of a healthy diet and increased physical activity. The WHO/FAO report (41) : physical activity and food intake are both specific and mutually interactive behaviours that are and can be influenced partly by the same measures and policies. Therefore this should be together with the other risk factors for chronic disease such
as alcohol and tobacco, the most important subjects in public health policies. Healthier food should be affordable and the fast food which is richly available should be higher taxed and perhaps less easier to get. The influence of the food industry is massive, but the governments nationwide should be able to take more responsibility on this very essential issue. On an individual basis the acknowledgement of the importance of food choice and meal size has to be more emphasized.

Physical activity should also be stimulated, as sedentary lifestyle is one of the main factors involved in the progression of obesity. More research is needed on these interfering factors in all age categories, leading to a healthier population.

With more knowledge on the pathophysiology, environmental and genetical factors involved in obesity, it is most likely that the treatment for obesity will become more individualized. Probably predictions can be made, whether specific treatments, such as cognitive behavioural therapy, specific training programs or medication targeting for example the insulin or leptin resistance would be likely to be effective.

Also the acknowledgement of the delicate balance between over and undernourishment in the elderly will assumingly lead to an improved quality of life, but more research on this is needed.
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