General Introduction
In the last 40 years the world has changed from an era in which underweight was twice as common as obesity, to a time when more people are obese than underweight. Currently, more than 600 million people are obese worldwide, and numbers are still growing. Whereas previously, obesity was predominantly a problem in Western high-income countries, its prevalence is now emerging in developing countries. In the Netherlands, 12 percent of men and 16 percent of women are obese (i.e. body mass index (BMI) ≥30 kg/m²), while 54 percent and 46 percent are overweight (i.e. BMI ≥25 kg/m²), respectively. Adiposity increases the risk of type 2 diabetes, cardiovascular disease and several forms of cancer. Furthermore, obese people are more likely to suffer from joint problems, sleep apnoea and depression. Due to these co-morbidities, adiposity strongly increases the risk of mortality.

Despite the abundance of diet books and heavily promoted schemes for rapid weight loss, lifestyle interventions have been largely unsuccessful in maintaining healthy body weights. More long-term success on weight loss is obtained with bariatric surgery, such as gastric banding and bypass. However, surgical treatments are expensive and not without risks, given the association with peri-operative complications and long-term nutritional deficiencies. Also the search of anti-obesity medication has been rather disappointing, mostly because of observed, sometimes severe, side effects. Therefore, it is important to further elucidate risk factors that contribute to overeating and weight gain, since this might add in the development of new prevention and treatment strategies against obesity.

**FOOD INTAKE REGULATION BY THE BRAIN**

Obesity results if there is an imbalance between energy intake and energy expenditure. Energy expenditure can be divided into resting metabolic rate, which is the amount of energy burnt while being at complete rest, and physical activity, which comprises all additional activity such as standing, walking around and the performance of exercise. If energy intake exceeds energy expenditure in the long run, the surplus of energy is stored as body mass, of which 60-80 percent as body fat. To maintain the body in a state of energy balance (i.e. homeostasis), intake and expenditure of energy are controlled by the central nervous system through complex interactions with key informative sites of the body. For the control of food intake, the brain receives information about short-term meal-related energy intake mainly from the gustatory system and the gastro-intestinal tract. These signals are mediated either by neural connections provided by the autonomic nervous system, or by hormones and metabolites. Whereas some of the peptides produced in the gut stimulate feeding and the initiation of a meal, such as ghrelin, others mediate satiety and the termination of a meal, such as cholecystokinin and glucagon-like peptide-1. For the regulation of food intake in the long-term, the brain receives information about
the amount of fat stored in the body by the hormone leptin, which is secreted by adipocytes in proportion to the amount of body fat mass. Increased leptin signalling limits food intake and supports energy expenditure through negative feedback in the brain. Key brain sites that regulate this short-term and long-term homeostatic feeding are the brainstem and the hypothalamus (Figure 1)

In addition to this homeostatic feeding, palatable food is also consumed for its hedonic properties independent of energy status. Such reward-related eating is highly influenced by external cues that signal the availability of palatable foods, such as its sight, smell or taste. These reward signals can override the homeostatic signals of the body, which may result in energy intake exceeding energy requirements. Numerous brain areas are involved in the processing of food reward, including cortico-limbic and midbrain areas such as the insula, amygdala, striatum, nucleus accumbens and orbitofrontal cortex (Figure 1).

Neurotransmitters that are responsible for these neural processes are mainly dopamine and opioids. Signalling by dopamine is thought to contribute to the ‘wanting’ of food (e.g. motivational aspects and craving), whereas opioids are involved in the ‘liking’ of food (e.g. hedonic value or palatability). Brain circuits involved in food reward are highly integrated with those regulating homeostatic feeding, for example energy depletion increases the rewarding value of food. Since the obesity epidemic is characterized by energy intakes that go beyond metabolic needs, reward-related hedonic feeding is likely to be an important contributor to weight gain and the development of obesity.

FIGURE 1
Sagittal image of the brain with schematic representation of regions involved in homeostatic feeding (hypothalamus and ventral tegmental area), hedonic feeding (striatum, amygdala, orbitofrontal cortex, nucleus accumbens) and inhibitory control (prefrontal cortex).
ALTERED BRAIN REWARD FUNCTION IN OBESITY

Studies in animals and humans have documented that chronic overeating leads to neuroadaptations of reward circuits that are comparable to changes observed in individuals with drug addiction\(^\text{17}\). Animal experiments demonstrated that overfeeding results in lower dopamine signalling and reduced responsivity of reward regions to food intake\(^\text{18}\). This is consistent with evidence from human studies using neuroimaging techniques, such as positron emission tomography and functional magnetic resonance imaging (fMRI)\(^\text{19}\). These studies reported lower dopamine receptor availability\(^\text{20}\) and decreased striatal responses to palatable food intake\(^\text{21}\) in obese compared to lean individuals. This hypo-function of the reward system is suggested to result from overeating and weight gain\(^\text{22}\) and could, in turn, lead to more overeating by means of compensation for a lack of reward during eating.

In contrast to the lower reward response to actual food consumption, the reward response to cues of palatable food availability has repeatedly shown to be higher in obese compared to lean individuals. FMRI studies repeatedly observed higher brain activation in reward regions when comparing obese and lean participants who were presented highly palatable food pictures\(^\text{23}\). Such increased reward responsiveness to food cues may result in higher craving for food until the food is obtained and consumed. Interestingly, this hyper-reward responsiveness has been demonstrated to predict future weight gain\(^\text{24}\), which suggests that it reflects an initial vulnerability factor to the development of overeating and obesity.

In addition to these alterations in the reward system, obesity has been associated with lower inhibitory control over food intake, which may result in overeating due to greater impulsivity. Brain regions that are responsible for this inhibition deficit are prefrontal regions implicated in behavioural control, such as the dorsolateral and ventral lateral prefrontal cortex\(^\text{25}\).

Except of the evidence coming from prospective studies\(^\text{22,24}\), most insights for these etiological hypotheses come from cross-sectional studies, which makes it difficult to determine the nature and direction of causality within the observations. Therefore, one could argue that none of the observed alterations in reward system function cause overeating and weight gain but, rather, that they all develop secondary to overweight. This would disqualify them as meaningful targets for intervention.

ENVIRONMENTAL AND GENETIC FACTORS

Environmental factors Obesity is a complex disease, arising from a multitude of genetic and environmental factors, and their interactions. The increase in obesity prevalence has occurred very rapidly during the last 40 years, and is seen in many parts of the world. Since our genes have not substantially changed during this period, the obesity epidemic
is largely explained by environmental factors. Changes in the environment include the increased availability of highly palatable foods and increased meal sizes. Between 1971 and 2004 the mean energy intake per individual in the US is suggested to have increased with 314 kcal per day. Due to the industrialization of food processing, the cost of food has fallen drastically, especially of energy-dense foods high in fat and sugar. Importantly, these palatable energy-dense foods have rewarding properties which, through positive reinforcement, increases its consumption. On the energy expenditure side, simultaneous advances in technology (e.g. development of computers and television) and transportation reduced the need of physical activity during work and leisure time.

**Genetic factors** However, in any given environment not all individuals become obese, which suggests that individual susceptibility factors determine how people respond to certain environments.

These individual differences in body weight regulation reflect differences in genetic make-up as well as the exposure to a host of environmental factors that influence body weight throughout the life course. The evidence supporting the importance of both genetic and environmental factors contributing to obesity can be structured based on their level of influence, i.e. 1) the population at large, 2) individual human beings, or 3) specific organ systems, in this case the brain reward system (Figure 2).

Twin and adoption studies confirmed that variation in body mass index (BMI) has a strong genetic component. The correlation of BMI between genetically identical (i.e. monozygotic) twins has consistently shown to be higher than BMI correlations between same-sex non-identical (i.e. dizygotic) twins. Furthermore, adoption studies demonstrated that, with respect to BMI, children were more similar to their biological parents than to their adoptive parents. In addition, overfeeding studies showed higher intra-pair similarity that between-pair similarity in the amount of weight gain in response to a positive energy balance. During the years, the contribution of genetic effects to BMI variation has been estimated to be 40-70%. Interestingly, high heritability estimates have also been documented for food-related functioning of the brain reward system, by studies showing high intra-pair correlations in multiple aspects of appetite and eating behaviour, including the rewarding value of food.

Recent advances in DNA analysis and computational techniques have allowed to study the effects of specific genetic loci on a variety of traits, including adiposity. In the candidate-gene approach, alleles of a pre-specified gene that may be involved in a disease are associated with that specific disease itself. In obesity, several candidate-genes have been reported, however, due to limited study sizes many have never been replicated. Another disadvantage of this approach is that the selection of candidate genes relies on a priori knowledge of...
pathophysiology. Since obesity is a complex disease, it involves many biological pathways which makes the identification of candidate genes a difficult task. In the last decade, progress has been made due to the rise of large population-based cohorts and high throughput techniques that facilitate the genotyping of thousands of genetic variants. Genome-wide association studies (GWAS) investigate the association between genetic variants and a disease in a hypothesis-free exploratory approach, which allows the discovery of novel variants without a priori assumptions about their function. To date, GWAS have identified 97 genetic loci associated with BMI and body fat distribution. These common genetic variants have modest effect sizes and, together, explain a small proportion (less than 5%) of BMI variation. Nevertheless, the discovery of genetic variants can gain insight in the biological pathways that underlie the aetiology of obesity. For instance, variants in or nearby the fat mass and obesity associated (FTO) gene, which have the strongest effects on BMI, have shown to impact on food intake and resting energy expenditure.

Interestingly, many recently discovered variants associated with BMI are suggested to act through the central nervous system, specifically in regions implicated in homeostatic and hedonic feeding such as the hypothalamus and cortico-limbic areas, as observed by gene enrichment analyses. These findings align with studies in patients with rare, monogenic forms of obesity, which demonstrated that genetic mutations, such as in the melanocortin 4 receptor (MC4R) and leptin receptor (LEPR), can cause severe obesity by disrupting pathways involved in food intake regulation by the brain. In line with this, recent neuroimaging studies observed altered brain responses to food cues in regions mediating reward in humans with rare genetic mutations or common obesity-associated variants.

With heritability of BMI estimated between 40% and 70%, a large amount of the individual differences in BMI (30%-60%) must arise from exposure to environmental factors that influence body weight regulation. Although an ‘obesogenic’ lifestyle can be a characteristic of an entire population, compared to other populations globally or that population’s past, large variation can exist within the population in the degree of exposure to palatable foods, a physical inactive lifestyle, and factors mediating these deviant behaviours. In contrast to the influence of genetic factors, the contribution of environmental factors to obesity-related alterations in brain reward responsiveness to food in humans remains largely unexplored.
THE INTRAUTERINE ENVIRONMENT AND FOOD INTAKE

In addition to environmental factors that exert their effect during life after birth, risk to disease in later life is influenced by environmental conditions during foetal development in utero. In 1990 Barker and colleagues observed that reduced foetal growth was associated with increased risk of cardiovascular disease, type 2 diabetes and associated mortality 43. The ‘foetal origins of disease’ hypothesis postulates that during critical periods in foetal development, environmental factors may induce persisting changes in the body structure and function, which influences the risk of disease in later life, especially when the prenatal period is followed by an adverse environment in adulthood 44. This foetal programming may occur through altering the expression of genes in response to environmental conditions. Since these alterations in gene regulation relate to modification without changing the DNA sequence, this phenomenon is known as ‘epigenetic’ mechanisms 45.

In the last decade, studies have investigated which tissues and organs mediate the deleterious effects of foetal programming. Several studies observed that individuals with poor intrauterine conditions before birth not only ate more food in general, but also had specific food preferences for palatable high-calorie foods in later life, which suggests involvement of mechanisms underlying the brain’s regulation of food intake 46.

The main criticism on the foetal origins hypothesis, which was
primarily based on epidemiological studies, was its vulnerability to confounding by factors, such as social class, that influence both the intrauterine as the adult environment. Part of this confounding was reduced by pseudo-experiments of intrauterine malnutrition in which humans born during the Dutch hunger winter of 1944-1945 were compared with unexposed time-controls or siblings. In these studies, it was again observed that the intrauterine malnourished individuals had a preference for high-fat foods in adult life. Despite these more convincing results, however, the possibility of confounding remains, in particular by genetic factors. Hence, genes that influence the way the foetus responds to unfavourable prenatal conditions could also explain the way it develops feeding preferences in later life. Clarification of the relation between intrauterine conditions and food preferences in later life is needed, since possible interventions that could modify the intrauterine environment would only reduce disease risk in later life if the association between intrauterine growth and adult dietary preferences resulted from a true causal relationship.

**AIMS AND OUTLINE OF THESIS**

Although the understanding of the complex regulation of food intake and the development of obesity has expanded greatly in the last decade, there are still many unresolved issues that should be addressed. The overarching aim of this thesis was to investigate the contribution of genetic and environmental factors to food intake, physical activity, and brain reward responsiveness to food. Further, we aimed to disentangle whether the altered reward system functioning in individuals with obesity precedes overeating and weight gain, or develops secondary to overweight itself.

This thesis is subdivided into three parts which consecutively describe the contribution of 1) the intrauterine environment, 2) the environment in general and 3) genetic factors to food intake, physical activity and the regulation of food reward by the brain. Since for each of these parts a different study design was used, this thesis starts with a detailed description of why and how the data were collected in each of these study designs (Chapter 2). Then, in the first part of the thesis, the influence of intrauterine environmental factors is investigated (Chapter 3). This chapter investigates whether the previously observed association between intrauterine growth restriction and unfavourable feeding preferences in later life is indeed a result of intrauterine environmental factors, independent of genetic confounding. To this end, birth weight was associated with dietary intake of adolescent dizygotic and monozygotic twin pairs. The second part of this thesis deals with the role of unique environmental influences. In Chapter 4 and 5 rare monozygotic twin pairs with an intra-pair difference in BMI were investigated to study the contribution of unique environmental factors to food intake and physical activity (Chapter 4) and brain reward responsiveness to food (Chapter 5).
food was examined using fMRI measurements during the presentation of appealing food pictures and the anticipation and receipt of a palatable food stimulus. Chapter 6 investigates the nature of the correlation between environmentally-induced overweight and altered functional connectivity of so-called resting state brain networks involved in food intake and motivation. To this end, brain activation was measured during the resting state using fMRI within BMI discordant monozygotic twins. In the third and final part of this thesis, the influence of genetic factors is studied. Chapter 7 and 8 investigate whether genetic susceptibility to obesity is related to differences in food intake and physical activity (Chapter 7) and brain reward responsiveness to food (Chapter 8). To this end, a genetic risk score based on previously identified obesity-related genetic variants was used to identify individuals with either a low or high genetic risk for obesity. Further classification of this study sample into individuals with either a low or high current BMI allowed to investigate whether altered food intake, physical activity and brain reward responsiveness to food are a cause or, rather, a consequence of obesity. Chapter 9 is the closing chapter, which summarizes the main findings of this thesis, discusses the relevance and clinical implications, reviews the possible study limitations and recommends suggestions for future research.
REFERENCES


8 Artz DE, Courcoulas AP. Bariatric surgery for obesity and metabolic conditions in adults. BMJ. 2014;349:g3961.


47 Stein AD, Rundle A, Wada N, Goldbohm RA, Lumey LH.