Chapter 1

General introduction: Genetics of the link between obesity and its metabolic complications
Chapter 1

In our contemporary society, obesity is a major health problem with pandemic proportions. The number of obese individuals has been rapidly increasing over the last few decades, and latest estimates of the World Health Organization (WHO) show that around the globe currently 500 million people are obese. As yet, the growth of the obesity pandemic did not come to a stop and the numbers of obese individuals is expected to continue rising.

The obesity pandemic is the main cause of a rise in the prevalence of various serious diseases such as Type 2 Diabetes and Non-Alcoholic SteatoHepatitis (NASH), leading to cardiovascular complications and irreversible liver damage. In line with this, obesity has a severe contribution to mortality. A Body Mass Index (BMI) of 30 to 35 kg/m$^2$ decreases average life expectancy by 2 to 4 years, and a BMI over 40 kg/m$^2$ decreases average life expectancy by 10 years (1). To perform proper clinical management of obesity and obesity-related diseases, understanding the link between obesity and such diseases is essential.

**Definition and prevalence of obesity**

Obesity is a state of increased body weight due to an excessive accumulation of body fat. The most commonly used measure to quantify obesity is the Body Mass Index (BMI), which is the individual’s mass in kilograms divided by the square of its length in meters. The WHO defined obesity as a BMI above or equal to 30 kg/m$^2$. As shown in Figure 1, a large percentage of the population throughout the world, and especially in Western countries, is obese (not only overweight); In the United States about one third of the adult population meets the WHO criteria for obesity.

Other useful measures for obesity are the waist circumference or the waist-hip ratio. These measures for obesity take into account the distribution of fat across the body to distinguish between visceral (“apple-shaped”) and other types (“pear-shaped”) of obesity. Since in particular visceral obesity increases the risk to develop obesity related morbidity, waist related obesity measures are better disease predictors (2-4).

**Major causes of the obesity pandemic**

As shown in Figure 2, the energy consumption per individual has risen with about 25% over the last 40 years. The coincidence of this rise in individual daily energy
consumption with the obesity pandemic makes it highly assumptive that the recent obesity pandemic is mainly due to the rise in daily individual energy consumption. Besides this, lack of physical exercise as a result of an increase in office-bound work, is also likely to contribute to the obesity pandemic. Other causes for the rise in the prevalence of obesity have been proposed as well, but increased food intake and diminished physical exercise are the primary contributors to the obesity pandemic.
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Figure 2: Map of energy consumption per country in 1961 (upper map) and 2001–2003 (lower map).
Colours represent the caloric intake (kcal/person/day) as shown in the legend. The world average energy intake per individual was 2254 and 2800 kcal/person/day in 1961 and 2001–2003 respectively. Data are derived from (20) and maps are published on (21).

Regulation of energy balance

Intake and expenditure of energy is carefully balanced (Figure 3). This is illustrated by the fact that even in case of weight gain of 20 kilogram over 20 years, which amounts to a positive energy balance of 140 000 kilocalories, the average excess of daily energy uptake is only 1% (5).

The main organ controlling energy balance is the hypothalamus in the brain. This organ acts as a control centre that receives and integrates signals from other organs reflecting energy intake and expenditure. Consequently, the hypothalamus controls
the decision to eat and modulates the function of metabolic tissues by secreting hormones or by nerve signalling. As an example, in an obese state adipose tissue secretes large amounts of the hormone leptin, which leads to raised leptin levels in the blood (6). The hypothalamus can sense and interpret these increased leptin levels in the blood, and will then signal to other parts of the brain that food intake should be reduced, and to the gut that less energy is needed to be taken up (7).

Although this concept is straightforward, the actual regulation of long-term energy balance is tremendously complex. First, many factors can modulate hypothalamic output, including seasonal variations (day length/ light intensities), temperature, sickness (immune system activation), duration and quality of sleep, medication, hedonic input, and psychological factors (8, 9). Second, the number of signalling hormones that are secreted to reflect the metabolic state of an individual or organ is very large. Finally, signalling molecules that can signal to the hypothalamus, for example insulin, often also have a direct effect on other organs (Figure 3).

This complexity makes that the regulation of long-term energy balance is poorly understood. The various metabolic organs (liver, pancreas, adipose tissue, muscle, stomach, gut) release a variety of hormones that reflect their physiological state. The hypothalamus is a main control centre that receives, integrates and interprets all these hormonal signals in order to modulate the peripheral metabolic organs in such a way that energy uptake and expenditure are carefully balanced.

**Complications of obesity**

Obesity increases the risk to develop a great variety of serious diseases. As compared to lean individuals, the risk for obese individuals to develop hypertension is up to 5 times higher. For coronary artery disease and stroke this risk is 3–6 times higher, and for diabetes the risk is even greater with half of all Japanese 70-year old individuals with a BMI over 28 kg/m² having diabetes. The prevalence of Non-Alcoholic Steato-Hepatitis (NASH) is also rising as a result of the obesity pandemic. Other diseases that show an increased risk in obese individuals are arthritis, respiratory diseases, cancers, and reproductive abnormalities (5). This thesis will confine itself to obesity complications involving the liver and the pancreas (type 2 diabetes).

The mechanisms that link obesity to increased risk on other diseases, are complex and poorly understood. Often, the risk to develop a complication of obesity also increases the risk to develop other complications, making it hard to distinguish which mechanisms are causally related to the development of different obesity-related dis-
Figure 3: Setting the stage for obesity.

Tissues release various hormones that reflect their physiological state. The hypothalamus is the main control centre that receives, integrates, and interprets all these hormonal signals, in order to balance energy intake and expenditure. Taken from (10).

...eases. However, two primary obesity-related mechanisms seem to account for most of the obesity complications (Figure 4). First, accumulation of fat in adipose tissue often goes together with a diminished capacity of adipose tissue to store additional fat. This aberrant fat storage could lead to a “spillover” of nutrients into the circulation and consequent hyperlipidemia, characterized by increased plasma triglyceride and cholesterol levels, due to increased low density lipoprotein (LDL), increased very low density lipoprotein (VLDL), and decreased high density lipoproteins (HDL). On its turn, hyperlipidemia is related to lipotoxicity in various organs including the liver and pancreas (11). Second, obesity is related to deregulation of the hormonal secretion of adipose tissue, which is involved in maintenance of proper energy homeostasis. Besides their role in signalling to the hypothalamus, adipose tissue hormones, can
be involved in autocrine, paracrine, or endocrine signalling that might affect the proper functioning of other organs. Examples of such adipokines are adiponectin (ADIPOQ), leptin (LEP), resistin (RETN), and visfatin (NAMPT) (12-15).

In the liver, obesity and consequent hyperlipidemia can lead to increased hepatic fat accumulation which is the benign form of Non-Alcoholic Fatty Liver Disease (NAFLD) (17), which occurs in 90% of all obese individuals. A subset of individuals with NAFLD will develop liver inflammation (Non-Alcoholic SteatoHepatitis; NASH), scarring (fibrosis), cirrhosis, and hepatocellular carcinoma. The exact mechanisms underlying the progression of NAFLD are poorly understood, although it has been proposed that adipokine release from adipose plays an important role in the
transition of benign steatosis to NASH (12-15).

Obesity is also tightly linked to failure of the insulin secreting pancreatic β-cells and consequent type 2 diabetes. This might be related to lipotoxicity (16) and to insulin resistance of adipose tissue, i.e. diminished capacity of adipose tissue to react properly to insulin. Insulin resistance in adipose tissue puts a demand on pancreatic β-cells to produce more insulin, which induces stress on β-cells that could eventually lead to their failure. In addition to this cellular stress, insulin resistance in adipose tissue might lead to a diminished clearance of glucose from the circulation, contributing directly to hyperglycaemia (11). To conclude, obesity induces hyperlipidemia, chronic inflammation, disturbed adipokine secretion, and insulin resistance. In liver this can induce NAFLD, NASH and subsequent progressive liver disease, and in the pancreas this can lead to β-cell failure and consequent type 2 diabetes.

Genetics of obesity and type 2 diabetes

Both environmental changes and genetic factors are involved in the onset of obesity. Changes in the genetic make-up of individuals did not likely induce the obesity pandemic, but are important factors in an individual’s response to environmental changes. In other words: Some individuals are predestined to become obese with the current environmental changes due to their genetic make-up. Moreover, while research into environmental factors has remained complicated, genetic research has been revolutionized over the last few years because of technological advances. Initially, genetic linkage studies were largely ineffective to discover genetic variation associated with complex diseases. However, more recently, Genome-Wide Association Studies (GWAS) became possible (18), and led to the discovery of over 5000 genetic variations associated to various complex diseases. At this moment genetic variation at 50 loci has been reproducibly associated to type 2 diabetes. Chapter 2 discusses the functional role of the genes located in the vicinity of genetic variation associated with type 2 diabetes. The genetics of NASH have not been investigated on a large scale since diagnostic challenges make it hard to collect large numbers of patients. For NAFLD only genetic variation on one locus, near the *PNPLA3* gene, was found to be associated (19).

The main advantage of identification of genetic variants associated to disease is that those variants can lead us to the genes causing the disease, and thereby a reliable starting point to perform functional research. However it is hard to make a direct translation from a genetic variant to disease aetiology, because it is often unclear
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which gene is actually affected by the genetic variant. A reason for this is that through the non-random inheritance of genetic variation, or linkage disequilibrium often several genetic variants in a single locus are associated to a disease. A further reason is that a genetic variation can be located in a regulatory element that affects the function of a gene which is located over a million base pairs away. Therefore the first step that needs to be taken in order to make full advantage of GWAS results, is the identification of the genes that are actually affected by the genetic variants associated to disease.

**Aim and outline of the thesis**

Although obesity induces several mechanisms that can lead to disease, not all obese individuals will develop the entire spectrum of obesity complications. However, it is unclear why some obese individuals develop specific complications, whereas other obese individuals remain relatively healthy. Our aim was to identify differences between obese individuals with and without the metabolic complications of obesity, particularly type 2 diabetes and NASH. To detect such differences we combined both genetic information, gene expression data, extensive phenotypic information, and bio-informatics in order to find out which genes are involved in type 2 diabetes and NASH. Our study contributed to the understanding of the aetiology of obesity-induced NASH and type 2 diabetes, and revealed promising targets for research into new therapeutic applications, which eventually may lead to improved clinical management of obesity complications and metabolic diseases. Chapter 2 discusses the functional role of the genes located in the vicinity of genetic variation associated with type 2 diabetes. Chapter 3 describes a study in which we sought for genes that have expression levels in adipose tissue associated to hyperlipidemia and diabetes. In this chapter we show that expression in adipose tissue of genes involved in the immune response are associated to glucose and HDL plasma levels.

In chapter 4 we examined correlations between the expression of genes encoding adipokines in subcutaneous (SAT) and visceral adipose tissue (VAT) of obese individuals and liver damage such as seen in NAFLD and NASH. We identified expression of the adipokines leptin (LEP), retinoic acid receptor responder (tazarotene induced) 2 (RARRES2; alias chemerin), and angiopoietin 2 (ANGPT2) in VAT to be strongly correlated to lobular inflammation of the liver. Leptin expression in VAT was also correlated to steatosis and hepatocellular ballooning (liver apoptosis). These adipokines are important players in the link between obesity and liver disease.
Chapter 5 describes a genome-wide search for the influence of genetic variation on gene expression levels in 5 different tissues. In this study we identified several genetic variants that have an effect on the expression of genes. We also unveiled that the same genetic variation in different tissues can have different, in some cases even opposite, effects on gene expression levels. This is an important step in the elucidation of tissue-dependent gene expression and is important for proper interpretation of the results obtained through GWAS.

In chapter 6 we investigated genes that are located in the vicinity of genetic variants associated to type 2 diabetes through GWAS. We used different methods to identify the genes that are affected by the genetic variants associated to type 2 diabetes. We found that \textit{THADA}, \textit{PTPLB}, \textit{MEST}, \textit{CCNE2}, \textit{NUCB2}, and \textit{ATP2B3} are very likely candidate genes to be involved in the aetiology of type 2 diabetes, and we prioritized these genes for further research.

A conclusion and a general discussion of this thesis is provided in chapter 7.

References

22. OECD Health Data 2012 - Frequently Requested Data. © OECD.