Summary
Worldwide the number of obese individuals is increasing at an alarming rate, with currently more than half a billion people being obese. This obesity epidemic coincides with an increase in the incidence of diseases which can be initiated by obesity, including type 2 diabetes and Non-Alcoholic SteatoHepatitis (NASH; fatty degeneration and inflammation of the liver, and progressive liver damage such as fibrosis and cirrhosis). The main driver of the obesity epidemic is a change in environmental factors to which the general population is exposed, in particular changes in food intake and a decreased physical activity. However, besides these environmental factors, also genetic factors contribute to obesity and diseases that can be initiated by obesity. Such genetic factors often do not cause disease by themselves, but they determine whether an individual under the changed environmental circumstances, develops or does not develop a disease.

In this thesis the link between obesity and diseases that can be initiated by obesity, in particular type 2 diabetes and NASH, was studied. To this end, 90 individuals suffering severe obesity were collected. Some of these individuals also suffered type 2 diabetes and/or NASH, whereas others were relatively healthy. In these individuals genome-wide gene expression levels were determined in four different tissues, which are known to play a role in the disease mechanism of type 2 diabetes and/or NASH: liver, skeletal muscle, subcutaneous adipose tissue, and visceral adipose tissue. Next, differences in gene expression levels in healthy as compared to unhealthy severely obese individuals, were investigated. Genes that were found to have changed expression levels in these individuals, probably play a role in the aetiology of type 2 diabetes and/or NASH.

Furthermore, in the same 90 severely obese individuals, genetic variation was assessed on a genome-wide scale. These data were used to investigate relationships between genetic variation and gene expression levels. In particular, the effects of approximately 50 genetic variants, which were previously found to be associated to type 2 diabetes, on the expression of genes in their vicinity were studied. The major strength of this approach was that genetic variation associated to disease represents causal factors involved in the mechanisms underlying the disease. Therefore, studying the effects of such genetic variation provides a direct route to unveil causal disease mechanisms.

Chapter 1 gives an overview of current knowledge about the obesity epidemic, and consequences of obesity in relation to type 2 diabetes and NASH. Also this chapter briefly discusses current knowledge regarding the genetics of obesity and type 2 diabetes. Chapter 2 gives a more detailed overview of genetic factors associated to
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type 2 diabetes, and functions of genes that possibly play a role in the disease process of type 2 diabetes.

In chapter 3, correlations were determined between expression levels of genes in subcutaneous and visceral adipose tissue and several plasma parameters reflecting an individuals’ metabolic health, in particular diabetes and hyperlipidemia. This was done by reducing the genome-wide data—consisting of data about approximately 20,000 genes—to approximately 70 groups of genes that showed strongly correlated expression levels between the individuals. A few of those 70 groups of genes were associated to plasma levels of glucose and HDL-cholesterol. Most of the genes that were part of these groups are involved in immunity-related processes. Thus, it can be concluded that genes that show differential expression in adipose tissue of unhealthy as compared to healthy severely obese individuals, are mainly involved in immunity-related processes. Changes in immune-related processes in adipose tissue thus seem to be related to differences between healthy and unhealthy severely obese individuals.

Chapter 4 describes a study into the relationships between microscopic features of NASH in the liver and adipose tissue expression of genes that encode hormones that can be secreted by adipose tissue, termed adipokines. Expression values in visceral adipose tissue of three such adipokines—Leptin (LEP), Chemerin (RARRES2), and Angiopoietin 2 (ANGPT2)—were associated to features of NASH in the liver, in particular lobular inflammation. These results show that these three adipokines play a role in the origin of liver disease in severely obese individuals.

In chapter 5, correlations were determined between genetic variants on a genome-wide scale and expression levels of genes in the vicinity of such variants in 5 tissues: liver, skeletal muscle, subcutaneous adipose tissue, visceral adipose tissue, and also blood. These correlations are important for interpretation of data derived from genetic association studies; results of these analysis can be used as a catalogue to find genes that have expression levels correlated to genetic variants that were found to be associated with a disease. Moreover, this analysis unveiled that the same genetic variant can have a different—sometimes even opposite—effect on the expression of a gene in different tissues. This demonstrates that it is pivotal to choose the proper tissue to perform research into the role of genetic variation in the molecular mechanisms of a disease.

Chapter 6 describes a tripartite study to find out which genes in the vicinity of genetic variation associated to type 2 diabetes, are most likely involved in the mechanism of this disease. First, genes were identified that showed different expression in any tissue relevant to diabetes of diabetic as compared to non-diabetic mice. Second,
relations were investigated between the expression of genes in tissues relevant to diabetes of severely obese individuals, and plasma parameters related to diabetes: glucose, insulin, HOMA-IR, and HOMA-B. Finally, relations were assessed between genetic variants associated to type 2 diabetes, and expression of genes located nearby these genetic variants. The latter analyses also were performed in 4 different tissues of severely obese individuals. The results of these three approaches were combined, and revealed that the genes *THADA*, *PTPLB*, *MEST*, *CCNE2*, *NUCB2* and *ATP2B3* are very likely be causally involved in the disease mechanism underlying type 2 diabetes.

Chapter 7 provides a general discussion, and discusses future possibilities of genetic research to contribute to enhanced knowledge about the mechanisms underlying complex diseases, including type 2 diabetes and NASH.

**The major conclusions of this thesis are:**

- Genes with expression levels in subcutaneous and visceral adipose tissue that are correlated with plasma levels of glucose and HDL-cholesterol, are mainly involved in immune processes.

- Expression levels of *LEP*, *RARRES2* en *ANGPT2* in visceral adipose tissue of severely obese individuals are correlated to microscopic features of NASH in the liver.

- For a proper interpretation of results from genetic association studies, it is very important to investigate correlations between the associated variants and expression levels of genes in the vicinity of such variants. We have shown that it is pivotal to investigate these correlations within the proper tissue, in order to find correlations that are relevant for the disease mechanism.

- It is very likely that the genes *THADA*, *PTPLB*, *MEST*, *CCNE2*, *NUCB2* en *ATP2B3* play a role in the disease mechanism of type 2 diabetes.