# CHAPTER 1

## General introduction and outline of the thesis

## OBESITY AND TYPE 2 DIABETES

### Epidemiology

Currently, one of the major worldwide health issues is the increasing number of people with obesity. This is primarily caused by changes in dietary habits, such as the increased intake of high-calorie foods, and a decrease in physical activity. Obesity is defined as a body mass index (BMI; calculated as bodyweight (kg)/height (m)$^2$) of 30 kg/m$^2$ or more. According to the World Health Organization (WHO) the prevalence of obesity has more than doubled since 1980 and it is estimated that almost 500 million adults worldwide are obese.[1] In the Netherlands 13% of men and 14% of women are currently obese.[2] Furthermore, obesity is already present in 2% of children and adolescents aged 2 to 21 years.[3] Since obesity is one of the major risk factors for type 2 diabetes mellitus (T2DM), a steep increase in T2DM prevalence is seen as well. In 2012, more than 371 million people worldwide had diabetes (~90% of them having type 2 diabetes) and it is estimated that by 2030 this will have risen to 552 million.[4]

### Morbidity, mortality and costs

The rise in obesity and T2DM is of great concern, as both conditions are associated with increased morbidity and mortality. Aside from the risk on T2DM, obesity is associated with a higher incidence of hypertension, cardiovascular disease (CVD), gallstones, liver disease, infertility, sleep apnea, muscoskeletal complaints and certain types of cancer.[5,6] Furthermore, obese subjects have a higher all-cause mortality rate.[7]

Diabetes is also associated with several serious complications such as neuropathy, microvascular complications (retinopathy and nephropathy) and macrovascular complications (CVD). In most high-income countries, diabetes is a leading cause of blindness, end-stage renal disease, lower limb amputation and CVD. These complications are associated with increased mortality; it is estimated that worldwide every seven seconds a person dies from the consequences of diabetes.[4,8] In patients with T2DM, up to 70% of deaths are attributed to CVD.[8,9] For unclear reasons there is a greater excess risk of mortality from coronary artery disease in diabetic women than in diabetic men. Compared with their non-diabetic counterparts, the mortality rate from CVD is more than twice as high in men with diabetes and more than four times as high in diabetic women.[10,11]

Treatment of T2DM and its complications also puts a high burden on healthcare costs. The average medical expenditure is 2.3 times higher in diabetes patients compared to persons without diabetes. In 2011 diabetes healthcare expenditures accounted for 11% of total healthcare costs in the world.[4,8,12]

Apart from the impact on morbidity and health care costs, T2DM has also been shown to result in a decreased quality of life (QoL) as compared to healthy controls.[13,14] A reduction in QoL not only influences individual well-being and that of their direct en-

vironment, but may also have an impact on participation in the working process, social functioning, compliance to therapy and therefore on socioeconomic costs, making it a very important aspect of the disease.

## PATHOPHYSIOLOGY OF T2DM

T2DM is a chronic disease characterized by disturbances in glucose homeostasis, eventually leading to hyperglycemia. Normally, blood glucose concentrations are maintained within a narrow range since low as well as elevated blood glucose levels are hazardous. Brain function depends on glucose, and severe hypoglycemia can lead to seizures, coma and death. Hyperglycemia on the other hand can lead to acute complications such as dehydration, and in the long term can result in the aforementioned micro- and macrovascular complications. Blood glucose levels are tightly regulated by balancing endogenous glucose production (EGP), which predominantly occurs in the liver (± 90%), and the uptake of glucose by peripheral tissues. Insulin is the most important hormone involved in glucose homeostasis. It is secreted by pancreatic ß-cells mainly in response to increased blood glucose levels, and it reduces plasma glucose by stimulating glucose disposal in peripheral tissues and inhibiting EGP.[15] In patients with T2DM, these processes are disturbed.

The underlying cause of T2DM is both complex and multifactorial. There seems to be a strong genetic component of the disease; the risk for T2DM is 3.5-fold higher among offspring with a single diabetic parent and even 6-fold higher if both parents have T2DM.[16] Furthermore, there is a high concordance rate of T2DM in monozygotic twins.[17,18] Around 40 genes have currently been identified that are associated with T2DM. However, variants in these genes have a modest effect size. For example, the variants with the greatest effects result in approximately twofold the lifetime risk of T2DM in persons carrying two copies of the risk allele as compared to persons with none. Most of the currently identified genes are related to insulin secretion.[19,20]

Obesity is also one of the major factors in the pathophysiology of T2DM, as over 80% of T2DM patients are overweight or obese.[21,22] Especially the increase in truncal (visceral and abdominal subcutaneous) adipose tissue ('male-type adiposity'), as reflected by an increase in waist circumference, seems to be detrimental for T2DM development. In addition, physical inactivity has also been recognized as a risk factor for T2DM.[23]

Ultimately, T2DM develops when a combination of these genetic and environmental factors result in insulin resistance of target tissues and diminished insulin secretion by pancreatic ß-cells. Insulin resistance is present early in the course of T2DM development. Glucose tolerance, however, remains near-normal for over a long period of time because the pancreatic ß-cells are able to compensate by increasing insulin output. Eventually,

insulin secretion capacity is unable to overcome the insulin resistance of peripheral tissues and glucose tolerance deteriorates, ultimately resulting in overt T2DM.[15,24,25] As this thesis mainly focuses on insulin resistance, the pathophysiology of insulin resistance will be discussed in more detail.

## INSULIN RESISTANCE

Insulin resistance is a condition characterized by the reduced responsiveness of tissues to a given concentration of insulin, also referred to as a state of decreased insulin sensitivity.[25-27] Insulin sensitivity declines with increasing age and varies widely within the general population; within an age-group the most insulin-sensitive person can be four times more sensitive than the most resistant individual.[24]

Insulin resistance may not necessarily be pathologic and can even be considered an evolutionary conserved adaptive response. For example, insulin resistance is seen after extreme diet-induced obesity in animals preparing for hibernation. This short-term insulin resistant state, which disappears after the winter fast, is thought to direct tissues to switch from glucose to fatty-acid metabolism to prevent starvation and does not have pathological consequences.[28-30] A decrease in insulin sensitivity after a period of fasting is also seen in humans.[31,32] Furthermore, in humans insulin resistance develops during pregnancy[33] and infection[34], resulting in a decreased nutrient uptake by non-priority tissues and thereby reserving glucose for important processes such as brain function, immune system activation and fetal development. The capacity to develop insulin resistance can thus be considered a beneficial adaptive response, which is conserved after millions of years of evolution, aiding in the survival of infections and famine, and supporting reproduction. However, in our current environment, where food is abundant and physical activity is no longer strictly necessary, insulin resistance can become chronic and have many detrimental consequences.[30,35]

Insulin resistance is currently very prevalent in the general population and it often goes unnoticed since it will not result in T2DM as long as ß-cells are able to compensate by increasing their insulin secretion capacity. It can be acquired, as is the case in subjects with obesity, the most important risk factor for insulin resistance. It is however also present in virtually all T2DM patients, obese as well as lean, and in these cases it seems to have a strong genetic component as well.[24,26] Insulin resistance is an independent predictor of not only future T2DM development, but also other serious diseases such as CVD, hypertension, stroke and cancer.[36,37] The exact pathophysiology of insulin resistance is currently not completely understood, but several tissues are known to be involved (Figure 1).

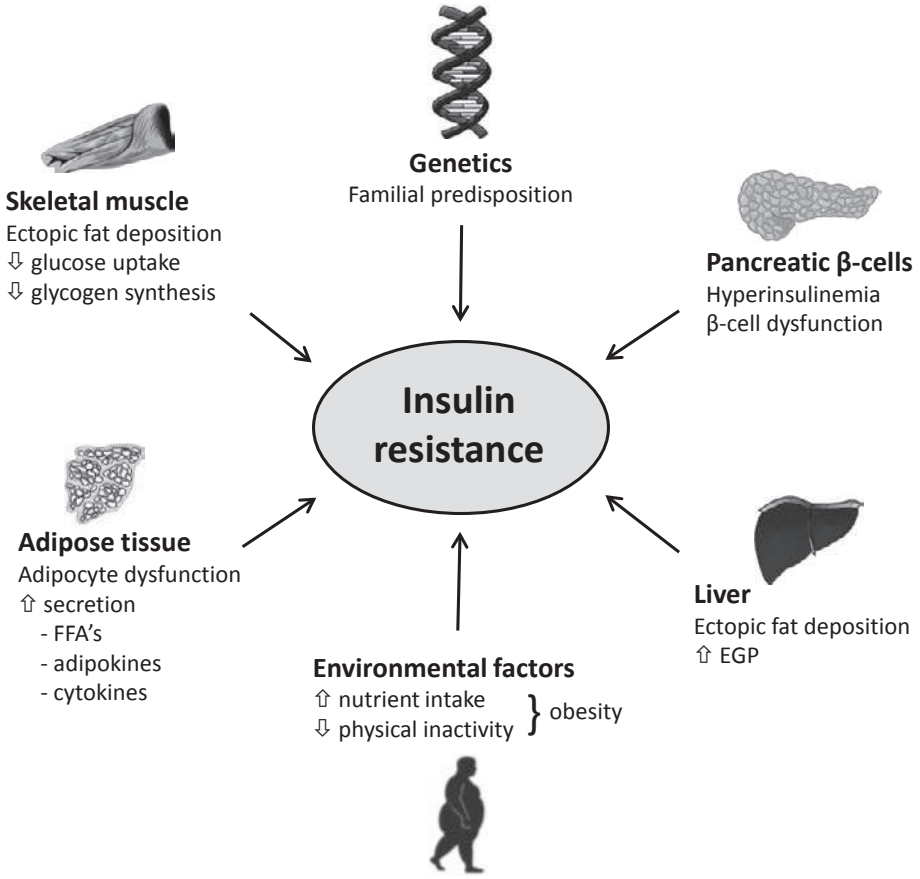


**Figure 1.** Multifactorial pathophysiology of insulin resistance

### Skeletal muscle

Skeletal muscle is the main site of insulin-stimulated glucose uptake and is therefore considered one of the most important tissues involved in whole-body insulin resistance. During hyperinsulinemia, it is estimated that around 80% of glucose disposal occurs in skeletal muscle.[38] Insulin activates a complex cascade of phosphorylation/dephosphorylation reactions, also called the insulin signaling cascade, eventually resulting in the uptake of glucose by the cell.[39] When glucose is taken up by the cell, it can either be oxidized or stored in the form of glycogen, also referred to as non-oxidative glucose disposal (NOGD). It seems that an impairment in NOGD is the primary defect responsible for skeletal muscle insulin resistance. Glycogen synthesis is approximately 60% lower in T2DM patients as compared to healthy lean controls.[40] Furthermore, a decrease in glycogen synthesis is seen early in T2DM development and is already present in normal glucose tolerant offspring of T2DM patients.[41]

Currently, a number of defects in the insulin signaling cascade that can ultimately result in impaired glucose uptake and glycogen synthesis have been described in insulin resistant subjects. Even so, much remains to be elucidated because of the complexity of the cascade.[39] One of the mechanisms that is known to disturb processes within the signaling cascade is lipotoxicity by ectopic fat storage in myocytes (discussed in detail under 'Adipose tissue'). With the exception of athletes, intramyocellular lipid (IMCL) accumulation is inversely related to skeletal muscle insulin sensitivity and is increased in obesity and T2DM.[42]

Because of the aforementioned fact that skeletal muscle accounts for the major part of glucose uptake under hyperinsulinemic conditions, its role in whole-body insulin resistance is widely acknowledged. However, whether it is the primary defect in the pathophysiology of insulin resistance is still under debate, as other tissues are currently also thought to be involved.

### Adipose tissue

Adipose tissue accounts for only ~10-20% of whole-body glucose uptake.[43] Still, accumulating evidence suggests an important role for adipose tissue in the pathophysiology of insulin resistance.

Humans have two major adipose tissue compartments, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). SAT is present directly under the skin and it accounts for the major part of adipose tissue in the body. VAT is found intra-abdominally and comprises only 7–18% of total body fat.[44-46] Studies suggested that an increase in VAT is associated with insulin resistance and that SAT may even be protective against obesity-related insulin resistance.[44,47-50] This led to the hypothesis that VAT accumulation is of importance in insulin resistance development, possibly explained by the fact that VAT has a higher lipolytic activity and directly secretes free fatty acids (FFAs) into the portal system, resulting in hepatic insulin resistance.[51] Yet, VAT is responsible for only 10-15% of the total FFA flux. Also, other studies showed that truncal SAT is inversely related with insulin sensitivity.[46,52] Moreover, although obesity is the most important risk factor for the development of insulin resistance and T2DM, approximately 20% of obese subjects remain metabolically healthy, whereas ~18% of lean individuals develop metabolic abnormalities.[53] Therefore it is hypothesized that adipose tissue dysfunction, independent of bodyweight or fat mass, plays a crucial role in the pathogenesis of insulin resistance.[43,54] Enlargement (hypertrophy) of adipocytes, a sign of adipocyte dysfunction, is frequently seen in obesity, but also in pre-diabetic subjects and T2DM patients. It is associated with insulin resistance independent of BMI and an independent predictor for the development of T2DM.[54-56]

One of the most important functions of adipocytes is to serve as a buffer for the daily influx of fat by storing FFAs as triglycerides. When adipocytes are overloaded, they

become hypertrophic and are thought of as dysfunctional as they are no longer able to store lipids. This leads to overexposure of other tissues to FFAs and results in the storage of triglycerides in non-adipose tissues (liver, skeletal muscle, heart), also called ectopic fat deposition.[42,54] This ectopic storage of fat disturbs cellular function, also called 'lipotoxicity'. Lipotoxicity can impair the insulin signaling cascade and thereby result in hepatic and skeletal muscle insulin resistance.[42]

The idea that this impaired adipocyte buffer capacity can lead to decreased insulin sensitivity of target tissues is further supported by the fact that patients suffering from lipodystrophy, a group of rare diseases characterized by the partial or total lack of SAT, display severe insulin resistance and hepatic steatosis.[57] Lipoatrophic mice also display severe insulin resistance and increased lipid content of the liver and skeletal muscle. Transplantation of fat tissue into these mice restores insulin sensitivity and reverses ectopic fat storage.[58,59]

Apart from its function in the storage of lipids, adipose tissue also actively secretes cytokines and proteins called adipokines. Adipocyte size seems to be an important determinant of adipokine secretion. Enlarged adipocytes are characterized by increased secretion of leptin, resistin, ASP and other proteins, and a decreased production of adiponectin, which are all linked to insulin resistance.[54,60] Furthermore, pro-inflammatory cytokines that are associated with insulin resistance, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, are also secreted in excess. It is thought that macrophages that infiltrate the adipose tissue are the major source of these inflammatory factors. Macrophage infiltration of adipose tissue is increased in obesity and is positively associated with adipocyte size.[61,62] Obesity as well as T2DM are well known to be associated with a chronic low-grade inflammatory state.[63] Pro-inflammatory cytokines can induce insulin resistance in different tissues by inhibiting the insulin signaling cascade.[64]

Thus, adipose tissue dysfunction, caused by either obesity or other (environmental or genetic) factors, results in ectopic fat depositions, disturbed adipokine production and increased pro-inflammatory cytokine secretion and this may induce, or at least maintain, whole-body insulin resistance (Figure 2).

### Liver

The liver is the major source of EGP. Glucose is produced by converting stored glycogen into glucose (glycogenolysis) and by the generation of glucose from other substrates, such as amino acids, glycerol and lactate (gluconeogenesis). EGP is particularly important in the post-absorptive state when it provides the brain with glucose, as it is not capable of storing glucose itself. The liver also stores and consumes glucose and lipids and is involved in lipid synthesis.

In the liver, as well as in skeletal muscle, lipotoxicity by ectopic fat depositions results in insulin resistance by impairing the insulin signaling cascade.[42] Hepatic insulin

resistance leads to decreased glucose uptake and impaired insulin-mediated inhibition of hepatic EGP. In the fasting state, the increase in EGP is the primary determinant of elevations in fasting blood glucose levels in T2DM patients. Furthermore, it also contributes to postprandial hyperglycemia.[65,66] The increased delivery of FFAs to the liver not only leads to ectopic fat deposition, but also results in increased glucose production because of higher substrate delivery, higher very low-density lipoprotein (VLDL) output and reduced insulin clearence.[54]

In several studies hepatic fat content was not only associated with hepatic but also peripheral (skeletal muscle and adipose tissue) insulin resistance.[47-50,67-69] Hepatic fat accumulation can lead to chronic inflammation of the liver, and this could possibly induce or aggravate peripheral insulin resistance via the secretion of pro-inflammatory cytokines.[66]

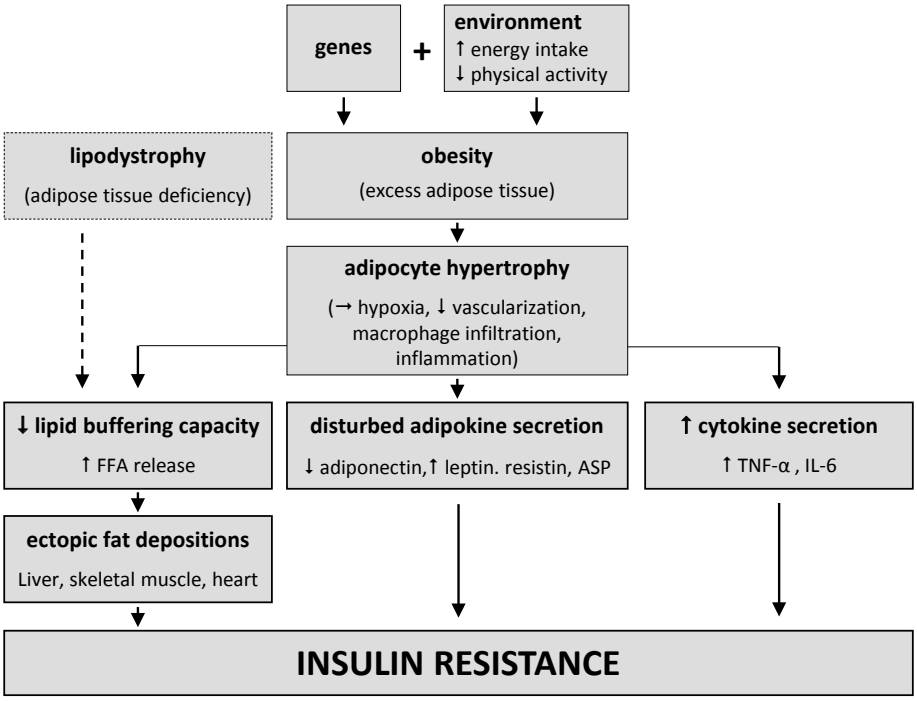


**Figure 2.** Adipocyte dysfunction

Adipose tissue dysfunction may play a crucial role in the pathogenesis of insulin resistance. Adipocyte hypertrophy can be considered a pathophysiologic response to genetic, environmental, and behavioral factors such as excess energy intake and decreased physical activity. When adipocytes become too large, hypoxia and endoplasmatic reticulum (ER) stress occur and this may trigger the activation of stress and inflammatory pathways. Dysfunctional adipocytes produce excess inflammatory cytokines and adipokines and attract immune cells, leading to a state of chronic inflammation. Furthermore, stressed and hypertrophic adipocytes are less capable of taking up free fatty acids (FFAs), leading to ectopic fat depositions and thereby insulin resistance.

### Pancreatic ß-cells

There is some evidence that the compensatory hyperinsulinemia, which is considered an adaptive response of the ß-cell to overcome insulin resistance, can aggravate insulin resistance itself.[27,70] Normally, insulin is secreted in a pulsatile fashion; it is estimated that as much as 70% of total insulin delivery comes from this pulsed output.[71] However, in T2DM patients these pulses are markedly attenuated and total insulin secretion is higher.[72] Interestingly, abnormal oscillatory insulin secretion is already seen in offspring of T2DM patients.[73] It is well known that hormones that are normally secreted intermittently, can desensitize target cells when hormone levels are persistently high (for example gonadotropin-releasing hormone (GnRH)).[27,70] Hyperinsulinemia has been shown to result in insulin resistance in mice[74] as well as in humans.[75] The idea that hyperinsulinemia can lead to insulin resistance is supported by the fact that patients with an insulin producing tumor (insulinoma), display signs of insulin resistance which resolve after removal of the tumor.[27,70]

In conclusion, although the exact primary defect in insulin resistance remains to be elucidated, several mechanisms in different tissues are currently known to induce and/or aggravate the insulin resistant state in obesity and T2DM.

## INSULIN RESISTANCE IN SOUTH ASIANS

The risk of developing T2DM is exceptionally high among native South Asians, as well as in migrants of South Asian descent (originating from the Indian subcontinent; India, Pakistan, Bangladesh, Nepal and Sri Lanka).[76,77] In the Netherlands, most South Asians are Hindustani Surinamese who migrated from the former Dutch colony Surinam, and whose ancestors came from the Indian subcontinent. Of all ethnic minorities living in the Netherlands, Hindustani Surinamese have the highest prevalence rate of T2DM, which is estimated to be almost 5-fold higher than in ethnic Dutch.[78,79] In addition to the increased prevalence, South Asians develop diabetes at a much younger age and at a lower BMI than Caucasians.[76,77,80,81] It seems that the mechanism responsible for the increased incidence of T2DM in South Asians is insulin resistance and not impaired insulin secretion.[82-86] Since South Asians represent over 20% of the world's population, the extremely high prevalence of T2DM is becoming a major health and socioeconomic burden. The cause of this excess risk is still not completely understood. In **Chapter 2** the underlying mechanisms involved in the higher prevalence of insulin resistance in South Asians will be reviewed extensively.

## THERAPEUTIC STRATEGIES IN T2DM AND INSULIN RESISTANCE

Treatment of T2DM can consist of improving insulin sensitivity or increasing the available insulin concentration. The latter can be achieved by medical therapy using insulin secretagogues or exogenous insulin.[87] Insulin sensitivity can be enhanced by caloric restriction[88], weight loss[89,90], exercise[91], or with medication.[87]

### Pharmacological interventions

According to the guidelines of the Dutch general practitioners association (Nederlands Huisartsen Genootschap), the first drug of choice for the treatment of T2DM is metformin, a biguanide.[92] Metformin lowers blood glucose levels by reducing hepatic EGP and improving insulin sensitivity without increasing the risk of hypoglycemia, and is considered weight-neutral. When metformin is no longer sufficient in controlling blood glucose levels, usually a sulfonylurea (an insulin secretagogue) is added. Sulfonylureas stimulate insulin release from pancreatic ß-cells. They increase the risk of hypoglycemia and are associated with modest weight gain. Alternatively, another class of oral glucose-lowering medications, the thiazolidinediones (TZDs), can be considered. TZDs activate the peroxisome proliferator–activated receptor (PPAR) γ, which results in an improvement of insulin sensitivity in skeletal muscle and a reduction in hepatic EGP. TZDs are not associated with increased hypoglycemia risk, but have other side effects such as weight gain, fluid retention leading to edema and an increased risk of fractures.[87] TZDs are therefore currently not often prescribed.

In time, oral therapy frequently fails to effectively reduce hyperglycemia because of the progressive β-cell dysfunction and insulin replacement therapy is therefore often required. At first, only intermediate or long-acting ('basal') insulin once daily is added. However, this is seldom adequate to reach treatment goals. Therefore insulin doses are often increased, or multiple daily injections with short-acting ('prandial') insulin are added.[93] Unfortunately, insulin therapy is associated with risk of hypoglycemia and significant weight gain. Severely obese (BMI > 35 kg/m$^2$) T2DM patients often require high doses of insulin due to grave insulin resistance and this will eventually result in a vicious cycle of increasing insulin requirement and persistent weight gain.[94,95]

Because of the influence of body weight on insulin sensitivity and the fact that T2DM is also associated with hypertension, dyslipidemia and CVD, there is much interest in pharmacological interventions that not only lower blood glucose, but also have beneficial effects on weight loss and cardiovascular risk factors. Over the past decade, drugs that influence the incretin system have been introduced.[96] Incretins are gut hormones that stimulate insulin secretion after oral glucose administration. One of the most important incretins is glucagon like peptide (GLP)-1. GLP-1 is made in the enteroendocrine L cells in the distal ileum and colon and increases within minutes after eating. GLP-1 low-

ers blood glucose levels by increasing glucose-dependent insulin secretion, suppressing glucagon secretion, inhibiting gastric emptying, and reducing appetite and food intake.[97] Injectable GLP-1 receptor agonists, for example exenatide and liraglutide, have been developed and are currently used as add-on therapy in obese patients with T2DM on oral hypoglycemic drugs. They reduce HbA1c and result in a significant amount of weight loss.[98-101] Furthermore, there is some evidence that GLP-1 receptor agonists lower blood pressure and lipid levels, and are cardioprotective.[102]

The search for new pharmacologic interventions still continues. A possible new option in the treatment of T2DM may be topiramate. Topiramate is a neuro-therapeutic agent which is currently used for selected seizure disorders and migraine prophylaxis. In healthy obese subjects, topiramate treatment leads to a dose-dependent reduction in body-weight.[103-106] In animals it has an insulin-sensitizing effect and improves ß-cell dysfunction, independently of a decrease in food intake and weight loss.[107-110] In obese T2DM patients it lowers HbA1c and 2-h plasma glucose levels.[111-113] However, whether these effects on glucose metabolism are also independent of body weight loss in humans is currently unknown. Also, phytosphingosine, one of the sphingolipids and a constituent of plants, fruits and yeasts, might be of interest. In mice dietary supplementation with 1% phytosphingosine decreased plasma cholesterol and TG levels and seemed to positively affect insulin sensitivity.[114] It is not known whether addition of phytosphingosine to the diet is also beneficial for humans.

Currently, medical therapy is only initiated in patients with overt T2DM. It remains the question however, whether patients with insulin resistance or impaired glucose tolerance (IGT) should be treated to prevent the onset of T2DM. In several randomized controlled trials in individuals at high risk of developing T2DM lifestyle intervention (i.e. diet and/or exercise) as well as metformin treatment resulted in a significant reduction in the incidence of T2DM.[115-118] The fact that lifestyle modification was as effective as metformin treatment, and in one study even more effective[117], highlights the importance of weight loss as therapeutic intervention in insulin resistance and T2DM.

### Weight loss interventions

Weight reduction is one of the hallmarks of therapy in T2DM. Hypocaloric diets (< 1200 kcal/day) generally lead to moderate weight loss (5-10% loss of body weight), but this already improves insulin action, dyslipidemia and hypertension.[119-122] Still, in T2DM patients, substantial weight loss is needed to improve peripheral insulin sensitivity.[123] Very low calorie diets (VLCD), containing ≤ 800 kcal/day, can be used to achieve such a substantial amount of weight loss. On average, in obese subjects a VLCD results in a reduction in body weight of 20 kg after 12 to 16 weeks. In T2DM, treatment with a VLCD leads to an improvement of hepatic as well as peripheral insulin sensitivity[85,86] and the

mobilization of ectopic fat depositions.[89,90,124] Unfortunately, long-term maintenance of weight loss is difficult.

One of the interventions that is known to result in more sustained weight loss (up to 20 years) is bariatric surgery.[125] The most widely applied methods of bariatric surgery are laparoscopic adjustable gastric banding (LAGB), a purely restrictive procedure, and Roux-en-Y gastric bypass (RYGB), a combined restrictive and malabsorptive procedure. The Swedish Obese Subjects (SOS) study showed that surgically-induced weight loss in obese subjects was associated with a long-term reduction in overall mortality[126] and a decreased incidence of diabetes[127] and cardiovascular events.[128,129] Furthermore, bariatric surgery in T2DM patients improves glycemic control and even leads to diabetes remission in ~70-80% of these patients after 2 years-follow up.[130,131] Bariatric surgery has been shown to improve liver, adipose tissue and skeletal muscle insulin sensitivity in obese non-diabetic as well as obese T2DM patients.[132,133]

## OUTLINE OF THE THESIS

### Part I Insulin resistance: pathophysiology in South Asians

The first part of this thesis focuses on the excess risk of T2DM development in individuals of South Asian descent. In **Chapter 2** the possible mechanisms leading to the increased incidence of insulin resistance in this ethnic group will be reviewed. In **Chapter 3** we describe a study in which we performed a prolonged oral glucose tolerance test (OGTT) in healthy, young Caucasian and South Asian men. South Asians have higher insulin levels during an OGTT than Caucasians, and these differences are still present 120 minutes after the glucose ingestion when the OGTT normally ends. Therefore we performed a prolonged OGTT (up to 360 minutes post glucose ingestion) to see if these higher levels of insulin persist for longer periods of time and if this will lead to reactive hypoglycemia later on. Reactive hypoglycemia is a condition characterized by a drop in glucose levels 4-6 hours after a glucose load and is considered a sign of early latent diabetes.[134-136] Further, during the OGTT GLP-1 levels were measured to uncover a possible explanation for the increased insulin levels consistently found in healthy subjects of South Asian descent, since GLP-1 is known to stimulate insulin secretion from pancreatic β-cells in response to glucose.[96]

### Part II Insulin resistance: therapeutic strategies

The second part of this thesis focuses on the effects of pharmacological and weight loss interventions on insulin resistance and associated parameters.

In **Chapter 4** we assessed the effect of topiramate treatment on insulin sensitivity and secretion in obese, insulin-resistant women in a randomized double-blind cross-

over placebo-controlled study. To assess the effects of topiramate independent of weight loss, short-term (4 weeks) treatment was used. Insulin sensitivity and secretion were measured using a two-step hyperinsulinemic euglycemic clamp and a hyperglycemic clamp. In **Chapter 5** we describe a retrospective study on the effect of adding a GLP-1 receptor agonist to existing insulin therapy in obese, highly insulin resistant T2DM patients. Main outcome parameters were changes in HbA1c, body weight and insulin dose during combination therapy. In **Chapter 6**, the effect of dietary supplementation with phytosphingosine on blood cholesterol levels and insulin sensitivity was assessed. Male subjects with the metabolic syndrome were included in a randomized double-blind cross-over placebo-controlled study. Insulin sensitivity was measured with an intravenous glucose tolerance test (IVGTT).

In **Chapter 7** and **Chapter 8** results of a study assessing the effect of a 16-week VLCD, with or without exercise, in obese T2DM patients are described. Earlier we showed that this intervention resulted in an increase in liver, adipose tissue and skeletal muscle insulin sensitivity. The addition of exercise to the 16-week VLCD induced more fat loss and increased maximum aerobic capacity, but did not result in a higher insulin-stimulated glucose disposal rate.[137] Over the past few years wide-scale proteome analysis ('proteomics') has become available and has been applied in studies on obesity and T2DM.[138,139] However, the plasma proteome of T2DM patients before and after a VLCD has not been studied. Changes in this profile after a diet, with or without exercise, could potentially help to further elucidate the physiology of weight loss and physical activity. Therefore proteomic (**Chapter 7**) analysis was performed in the VLCD-study. In addition, the plasma proteome profiles of the T2DM patients were compared to those of matched obese and lean controls to uncover proteins differentially expressed in T2DM patients as compared to the controls, searching for possible new biomarkers to identify patients at risk.

It is known that T2DM is associated with a decreased QoL. Some[140-142], but not all[143] studies reported that weight loss results in an improved QoL. The effect of exercise on QoL has also not been fully elucidated.[144,145] Therefore, in **Chapter 8** we assessed whether the addition of exercise to the 16-week VLCD had a greater impact on QoL than the VLCD alone, both immediately and 18 months after the intervention.

Diet-induced weight loss can result in ectopic fat mobilization and improvements in diastolic heart function.[42,146] In **Chapter 9** we studied the effect of surgically-induced weight loss on ectopic fat depositions and cardiac function, using magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI). Insulin-dependent T2DM patients were studied before and 16 weeks after a RYGB.

In **Chapter 10** the results of the studies described in this thesis are summarized and discussed.

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