The effects of a very low calorie diet and exercise in obese type 2 diabetes mellitus patients

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CHAPTER 1

Introduction and outline of the thesis

INTRODUCTION

Incidence of obesity and type 2 diabetes mellitus
Pathophysiology type 2 diabetes mellitus
- Skeletal muscle
- Adipose tissue
- Liver
- Pancreatic β-cells

Role of ectopic fat in the pathogenesis and organ dysfunction associated with type 2 diabetes mellitus
- Skeletal muscle (IMCL)
- Liver (hepatic steatosis)
- Heart (myocardial triglyceride content; pericardial fat mass)

Quality of Life (QoL)
Diet-induced weight loss (using very low calorie diets)
Exercise
Outline thesis

INCIDENCE OF OBESITY AND TYPE 2 DIABETES MELLITUS (T2DM)

Global increases in overweight and obesity are attributable to a number of factors including a shift in diet towards increased intake of energy-dense food and a trend towards decreased physical activity due to the increasingly sedentary nature of many forms of work, changing modes of transportation and less physical exercise. Once, the obesity epidemic was considered to be a problem in high-income countries only, however, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings.

The World Health Organization (WHO) defines "overweight" as a body mass index (BMI) (calculated as the bodyweight in kilograms divided by the square of the height in meters (kg/m^2)) equal to or more than 25 kg/m^2, and "obesity" as a BMI equal to or more than 30 kg/m^2. The WHO estimates that in 2005 worldwide approximately 1.6 billion adults had overweight and at least 400 million adults were obese. They further calculated that by 2015 approximately 2.3 billion adults will have overweight and more than 700 million will be obese (http://www.who.int; obesity and overweight fact sheet).

Obesity presents a risk to health and this risk increases progressively as BMI increases. Obesity per se is associated with an increased mortality rate (Table 1). Furthermore, obesity is tightly associated with insulin resistance, hyperlipidemia and hypertension, and thus with diseases such as type 2 diabetes mellitus (T2DM), stroke and ischemic heart disease. But also other diseases have a higher incidence in overweight or obese people, such as gallstones, disruption of the menstrual cycle, infertility and arthrosis (Table 1) (1-4).

It is therefore not surprising that the prevalence of T2DM is rising steadily along with the obesity epidemic. The WHO estimates that there will be at least 366 million people worldwide suffering from T2DM in 2030, which is more than 5% of the adult population (5). Diagnostic

Table 1. The estimated relative 10-year risk for mortality and disease in overweight (BMI 25-30 kg/m^2) and obese (BMI ≥ 30 kg/m^2) men and women.

	Overweight		Obesity	
	Men	Women	Men	Women
Mortality rate	1.1	1.1	1.3 - 2.2	1.4 - 1.6
Type 2 Diabetes Mellitus	3.5	4.6	11.2 - 23.4	10.0 - 17.0
Cardiovascular disease	1.5	1.4	2.0 - 2.2	1.5 - 1.6
Stroke	1.2	1.2	2.0 - 2.3	1.0 - 1.1
Hypertension	1.7	1.7	2.7 - 3.0	2.1 - 2.3
Gallstones	1.4	1.9	2.3 - 2.9	2.5 - 3.0
Colon carcinoma	1.2	1.2	1.7 - 1.8	1.3 - 1.8

The relative risks for the women are derived from the follow-up study of the Nurses' Health Study and for the man from the Health Professionals Study (2). There is a range since relative range varies within age and the amount of obesity.

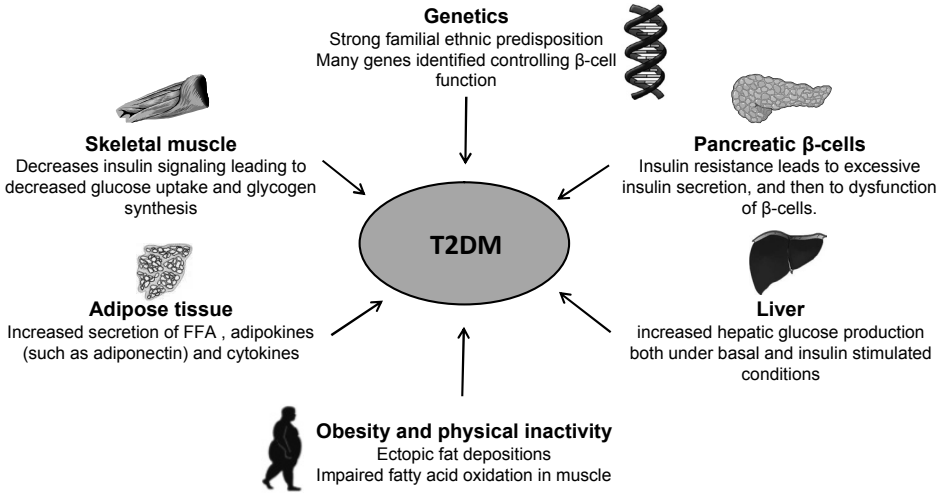
criteria for T2DM set by the WHO and the American Diabetes Association (ADA) are HbA1c ≥ 6.5% OR fasting (defined as no caloric intake for at least 8 hours) plasma glucose (FPG) ≥ 7.0 mmol/L OR 2-hour glucose ≥ 11.1 mmol/L during a 75 grams oral glucose tolerance test (OGTT), confirmed by repeat testing on a different day in the absence of unequivocal hyperglycemia OR when classical symptoms of hyperglycemia are present a random plasma glucose level ≥ 11.1 mmol/L (6).

The cut-off points of overweight and obesity (25 and 30 kg/m^2) provide a benchmark for individual assessment, but there is evidence that the risk of chronic disease in the population increases progressively above a BMI of 21 kg/m^2 (7). The BMI provides the most useful measure of overweight and obesity at the population level as it is the same for both sexes and for all ages. However, the BMI should be interpreted with caution at the individual level, because it does not predict body composition let alone regional body fat distribution. Individuals, especially the groups of elderly (8), children (9) and people from a different ethnicity (10), with equal BMI can be highly variable in terms of body fat mass and regional body fat distributions (visceral and subcutaneous fat mass) (11,12). This is of note since visceral fat accumulation is associated with a greater risk to develop T2DM and cardiovascular disease. Waist circumference is a valid index for visceral fat mass and can therefore be used as an indicator of health risk associated with excessive visceral fat mass (13).

PATHOPHYSIOLOGY T2DM

T2DM is a multifactorial, chronic disease characterized by hyperglycemia (Figure 1). The complex nature of T2DM is reflected in the multifaceted genetic background and the varied environmental interaction. There is cross-sectional evidence which suggests a strong genetic component of the disease. Positive family history confers a 2.4 fold increased risk for T2DM. The lifetime risk of T2DM is 7% in a general population, 38% in offspring of one parent with T2DM and about 70% if both parents have T2DM (14,15). Furthermore, it is well established that about 80% of T2DM is associated with obesity especially visceral fat accumulation and sedentary life styles (16). The pathophysiology of T2DM also comprises a combination of insulin resistance of target tissues (liver, adipose tissue, skeletal muscle) and impaired insulin secretion in the pancreatic β-cell. This leads to a combination of defects in insulin-mediated glucose uptake (predominantly muscle tissue), dysregulation of the adipocyte as a storage and secretory organ, dysregulation of the endogenous glucose production (predominantly in the liver), and a progressive decline in beta-cell function and mass in the pancreas leading to impaired insulin secretion, as will be discussed in detail in the following sections.

Figure 1. Type 2 Diabetes Mellitus (T2DM) is a multifactorial disease.

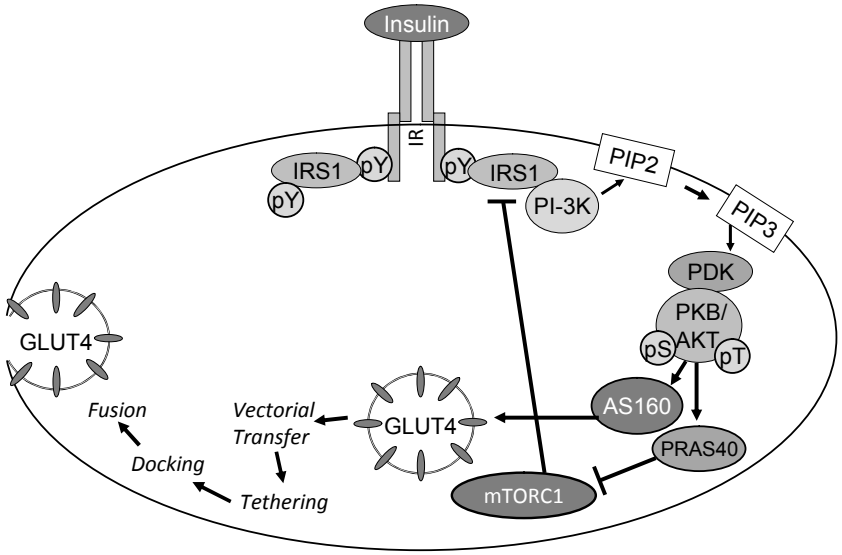


Skeletal muscle

Muscle glucose uptake accounts for 75-80% of insulin stimulated glucose uptake by the body (17). Therefore in T2DM patients the largest part of the impairment in insulin-mediated glucose uptake is explained by the defect in skeletal muscle. This involves both impaired glucose uptake as well as impaired glucose disposal. The major pathway for overall glucose metabolism is glycogen synthesis or non-oxidative glucose disposal (NOGD). In patients with T2DM glycogen synthesis is only 60% of that of healthy lean control subjects.

The cellular events through which insulin initiates its stimulatory effect on glucose uptake start with the binding of insulin to the α-subunit of the insulin receptor (IR) leading to a conformational change that induces a process of autophosphorylation; the intracellular kinase domain of one half of the receptor phosphorylates the tyrosine residues of the other half of the receptor (18). The phosphorylated tyrosines on the IR can now serve as docking sites for other proteins such as the insulin receptor substrate 1 (IRS1) (19). Phosphorylated IRS1 binds to phosphatidylinositol 3-kinase (PI3K) (20), which is recruited to the plasma membrane and converts phosphatidylinositols-4,5-bisphophate (PIP2) to phosphatidylinositols-3,4,5-trisphophate (PIP3). PIP3 subsequently attracts phosphatidylinositol-dependent protein kinase (PDK) and protein kinase B (PKB)/Akt to the plasma membrane where Akt is activated by PDK-mediated phosphorylation (21,22). Activated Akt thereupon dissociates from the cellular membrane to affect several metabolic processes, such as glycogen synthesis and glucose transport into the cell (23). Activated Akt inactivates glycogen synthase kinase 3 (GSK3), hereby abrogating the inhibitory action of GSK3 on glycogen synthase, and thus stimulating glycogen synthesis (24). Activated Akt also leads to phosphorylation of Akt substrate 160 (AS160) that allows glucose transporter 4 (GLUT4) storage vesicles to move to, dock, and

Figure 2. Insulin signaling cascade in the skeletal muscle cell.



IR: Insulin receptor; IRS: insulin receptor substrate; PI3K: phosphatidylinositol 3-kinase; PIP2: phosphatidylinositols-4,5-bisphophate; PIP3: phosphatidylinositols-3,4,5-trisphophate; PDK: phosphatidylinositol dependent protein kinase; PKB/AKT: Protein kinase B/AKT; AS160: Akt substrate 160; GLUT4: glucose transporter protein 4; PRAS40: Proline rich Akt substrate 40 kDa; mTORC1: mammalian target of rapamycin complex 1.

fuse with the plasma membrane. GLUT4 translocation consists of 4 stages: vectorial transfer: GLUT4 vesicles are transported to the cell periphery; tethering: GLUT4 vesicles are retained near the cell periphery; docking: GLUT4 vesicles bind to plasma membrane; fusion: irreversible incorporation of GLUT4 vesicles in the plasma membrane (25-27). Activated Akt also phosphorylates the nuclear protein Proline-rich Akt Substrate of 40 kDa (PRAS40). The exact function of PRAS40 is still under debate. Possibly phosphorylation of PRAS40 disrupts the interaction between mammalian target of rapamycin complex 1 (mTORC1) and PRAS40, which may relieve an inhibitory constraint on mTORC1 activity. The mTORC1 signaling pathway abrogates insulin-mediated activation of the PI3K-PKB/Akt pathway by inducing inhibitory serine phosphorylation on the insulin receptor and IRS1/2 (28) (Figure 2).

In T2DM patients a number of defects in the insulin signaling cascade have been described as compared to lean insulin sensitive control subjects, however it has been difficult to replicate the results in different studies both *in vitro* and *in vivo*. The complexity of the insulin signaling pathway grows, new studies lead to the discovery of new proteins, protein isoforms and new regulatory sites and defects in insulin resistant subjects or T2DM patients. In Table 2 a summary of the defects in the insulin signaling cascade in T2DM patients are shown compared to findings in lean healthy controls (29-40). One of the mechanisms by which insulin signal transduction is disturbed is excessive ectopic triglyceride storage in the skeletal muscle cell (as will be discussed in the following sections).

Table 2. Defects in insulin signaling pathway in the skeletal muscle in type 2 diabetes mellitus (T2DM) patients compared to healthy controls.

	T2DM vs. healthy controls	reference
IR activity or autophosphorylation	unchanged	30, 31, 32
IRS1 tyrosine phosphorylation	impaired	31, 33, 34, 35
IRS1 association with PI3K	impaired	30, 32, 36
PKB/AKT phosphorylation	impaired or unchanged	32, 34, 37, 38, 39, 40
GS activity	impaired	30-40
Glucose disposal rate	impaired	30-40

IR: Insulin receptor; IRS: insulin receptor substrate; PI3K: phosphatidylinositol 3-kinase; PKB/AKT: Protein kinase B/AKT; GS: glycogen synthase.

Adipose tissue

In lean healthy subjects approximately 10% of insulin-stimulated glucose uptake occurs in adipose tissue. This suggests a minor role of adipose tissue in the pathophysiology of insulin resistance. However, in the adipose tissue of patients with T2DM the expression of GLUT4 is down-regulated, hereby leading to a diminished uptake of glucose in this organ. Also, adipocyte-selective GLUT4 knockout mice show a systemic insulin resistance (41), suggesting that adipocytes secrete proteins that are responsible for cross-organ communication. Factors secreted by adipocytes that may alter insulin action and hepatic glucose production include adipokines (like adiponectin, resistin, leptin) (as reviewed in (42)), pro-inflammatory cytokines and free fatty acids (FFAs) (see in section ectopic fat depositions). In obesity, the adipose tissue is characterized by adipocyte hypertrophy and increased lipolysis leading to elevated production of FFAs. Furthermore, macrophages are present in much higher numbers in adipose tissue of obese subjects.

Cross-sectional studies have shown that insulin resistant states such as obesity and T2DM are associated with chronic low-grade inflammation (43,44). Macrophages, in the adipose tissue appear to be major sources of inflammatory mediators that are linked to insulin resistance such as pro-inflammatory cytokines (interleukin 6 (IL6) and tumor necrosis factor α (TNFα)) and elevated levels of highly sensitive C-Reactive Protein (hsCRP) (45,46). These cytokines can inhibit insulin signaling downstream of the IR, this might be the primary mechanism through which the chronic low-grade inflammatory status causes insulin resistance. TNFα and IL6 stimulate phosphorylation of serine residues of the IRS1/2. This phosphorylation reduces tyrosine phosphorylation of IRS1/2 in response to insulin which prevents further downstream signaling and thus GLUT4 translocation to the cellular membrane (47).

Visceral fat has a higher lipolytic activity and is less responsive to the anti-lipolytic activity of insulin as compared to subcutaneous adipose tissue (48,49). In addition, the adipokines, FFAs and (pro-inflammatory) cytokines produced by the visceral adipose tissue will be secreted directly into the portal vein and will have direct detrimental effects in the liver (50-52). However the visceral adipose tissue contributes only 10-15% of the total systemic free fatty acid flux, thus the impact of excess visceral adipose tissue on peripheral insulin sensitivity is

questioned. It seems that the combination of excessive subcutaneous adipose tissue with excessive visceral adipose tissue is important in insulin resistance.

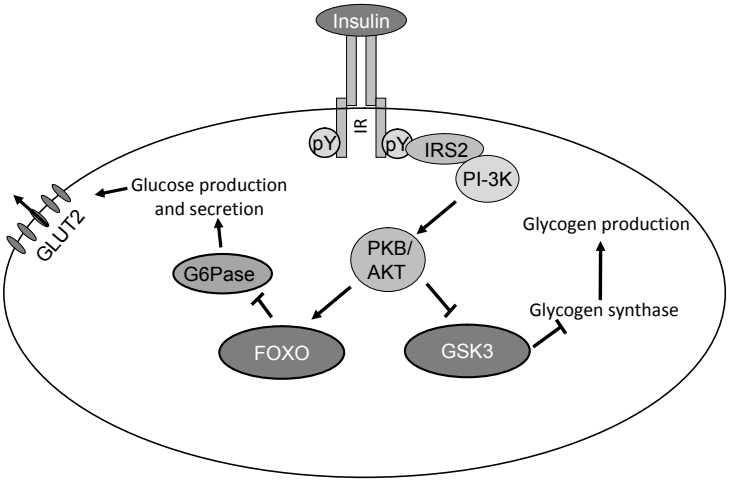
Liver

The liver has the ability to both consume, store as well as produce glucose and lipids. The liver is the major source of endogenous glucose production (EGP) but with prolonged fasting the contribution of the kidney increases (to 20% or even higher). EGP comprises 2 pathways: glycogenolysis (the conversion of glycogen to glucose) and gluconeogenesis (the generation of glucose from non-sugar carbon substrates (such as amino acids, mainly alanine, glycerol and lactate)).

In the post-absorptive state, the liver of healthy subject produces glucose at a rate of 2.0 mg/kg/min. This glucose efflux is essential to meet the need of the brain and other neural tissue, since these tissues lack the ability to store glucose (53,54). In the post-absorptive state, hepatic insulin resistance of T2DM is manifested by overproduction of glucose despite fasting hyperinsulinemia. Indeed the increased rate of EGP by the liver is the primary determinant of the elevated FPG concentration in T2DM individuals. In the non-fasting state hepatic insulin resistance leads to an impaired suppression of the EGP by the liver which contributes to the postprandial hyperglycemia (54).

The first steps of insulin signaling in hepatocytes is quite similar to that in skeletal muscle cells; binding of insulin to its receptor leads to phosphorylation of the tyrosine-kinase on the IR. This is followed by ligand-receptor interaction. In the liver, as opposed to skeletal muscle, the PI3K/Akt pathway is not only controlled by IRS1 but also by IRS2 (55,56). In addition,

Figure 3. Insulin signaling cascade in the liver cell.



IR: Insulin receptor; IRS: insulin receptor substrate; PI3K: phosphatidylinositol 3-kinase; PKB/AKT: Protein kinase B/AKT; FOXO: forkhead box protein O; G6Pase: glucose-6-phosphatase catalytic subunit; GSK3: glycogen synthase kinase 3; GLUT2: glucose transporter protein 2.

Akt in the liver regulates the expression of numerous genes important in controlling lipid synthesis and gluconeogenesis (57). For example Akt can regulate the phosphorylation of the forkhead box protein O (FOXO) family of transcription factors, which in turn inhibit the expression of the glucose-6-phosphatase catalytic subunit (G6Pase), leading to a suppression of glucose production (58). Also, insulin promotes glycogen synthesis by inactivating the enzyme glycogen synthase kinase 3 (GSK3) through the PI3K/AKT pathway. In the absence of insulin GSK3 phosphorylates glycogen synthase, which becomes inactive and thus glycogen synthesis will be inhibited (Figure 3).

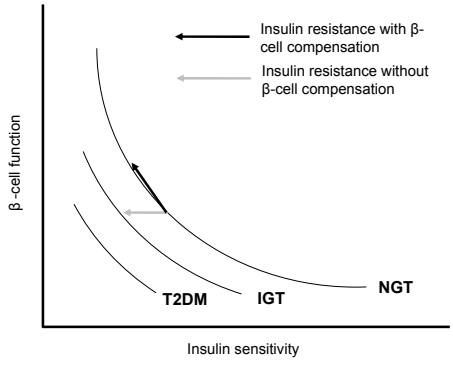
Due to ethical considerations liver biopsies in human studies with T2DM patients are rare. Animal studies confirm impaired insulin signaling from IRS1/2 to PI3K/Akt leading to increased gluconeogenesis (54). In, addition glycogen synthesis is inhibited (59). One of the mechanisms by which insulin signal transduction is disturbed is excessive ectopic triglyceride storage in the liver (as will be discussed in the following sections).

Pancreatic β-cells

Early in the development of T2DM, insulin resistance is well established but glucose tolerance remains normal because of a compensatory increase in insulin secretion. There is a dynamic interaction between insulin secretion and overall insulin resistance within the early stages of T2DM. The progression from impaired glucose tolerance to T2DM is characterized by an inability of the beta cell to maintain the previously elevated rate of insulin secretion in response to a glucose challenge. Tissue sensitivity to insulin deteriorates only minimally in this stage (unless of course the patient is able to lose weight) (Figure 4) (60,61).

Insulin secretion is biphasic with an early burst of insulin release within the first 10 minutes followed by a progressive increase in insulin secretion that persists as long as the hyperglycemic stimulus is present (62). Loss of the first phase insulin secretion is a characteristic and an early abnormality in patients developing T2DM. Loss of the first phase insulin secretion has

Figure 4. Hyperbolic relation between β-cell function and insulin sensitivity.



NGT: normal glucose tolerant; IGT: impaired glucose tolerant; T2DM: type 2 diabetes mellitus

important pathogenic consequences, because this early burst of insulin primes insulin target tissues, especially the liver (63,64). The second phase insulin secretion is important to prevent hyperglycemia by stimulating the uptake of glucose by the different target tissues.

A number of genetic and acquired factors have been implicated in the progressive impairment in both first and second phase insulin secretion (65-67), including chronic hyperglycemia (glucotoxicity) (68), chronic hyperlipidemia (lipotoxicity) (69,70) and pro-inflammatory cytokines (IL6 and TNFα) (71). However, the exact pathogenesis has not been elucidated yet.

ROLE OF ECTOPIC FAT IN THE PATHOGENESIS AND ORGAN DYSFUNCTION ASSOCIATED WITH T2DM

Adipocytes have a unique capacity to store large amounts of excess FFAs in cytosolic lipid droplets. Under healthy conditions, most triglycerides are stored in adipocytes. Cells of non-adipose tissues (such as the liver, the skeletal muscle, myocardium and the pancreas) have a limited capacity for storage of lipids and this is very tightly regulated. When the capacity of the adipose tissue to store triglycerides is exceeded, lipids accumulate in non-adipose tissues, termed ectopic fat deposition. Ectopic fat disturbs cellular function and may even lead to cell death, called lipotoxicity (72,73). The reason this ectopic deposition occurs is not elucidated. Bluher (74) recently proposed a model in which genetic and environmental factors lead to adipocyte hypertrophy, hypoxia and endoplasmatic reticulum stress causing inflammation within adipose tissue (via attraction of macrophages) and a different adipokine secretion profile. This leads to impaired adipocyte differentiation, reduced lipid accumulation and increased lipolysis in adipocytes, altogether culminating in a redirection of lipids towards non-adipose tissues.

Obesity and especially T2DM is associated with elevated plasma FFA concentrations postprandially. The ability of insulin to inhibit the elevated basal rate of lipolysis and hence to reduce the plasma FFA concentration is markedly impaired (75,76). The surplus of FFA in the circulation will lead to ectopic fat depositions in several organs including the skeletal muscle (intramyocellular lipid accumulation (IMCL)); the liver (steatosis hepatis); and the heart (pericardial fat and intramyocardial triglyceride (TG) content) and may result in lipotoxicity. The surplus fatty acids enter non-oxidative pathways leading to re-esterification into triglycerides within the non-adipose cell. Triglycerides per se are not harmful, however it is the availability of fatty acid derivatives like diacylglycerol (DAG), ceramide and long chain fatty acid-CoA (LC-CoA), which can negatively influence cellular processes (as described in the following sections).

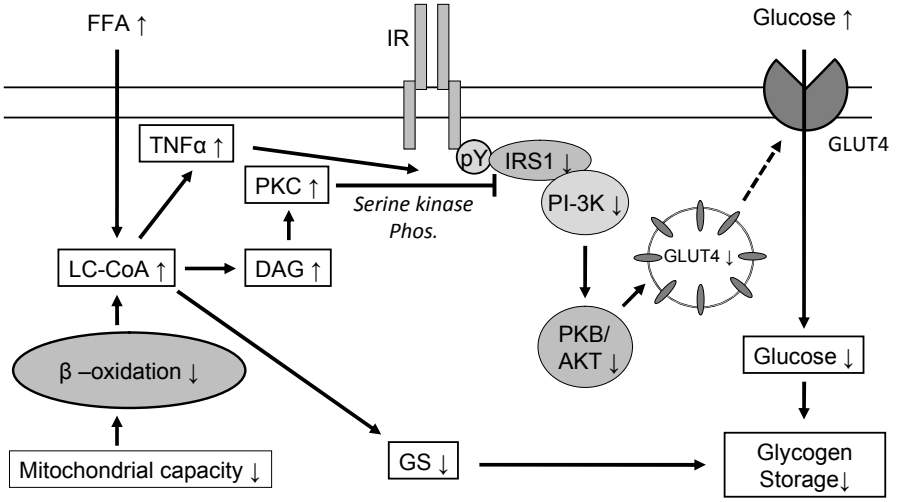
Skeletal muscle (IMCL)

Cross-sectional studies have demonstrated that intramyocellular lipid (IMCL) accumulation is increased in obesity and T2DM (77-80). IMCL positively correlates with insulin resistance both in obese and non-obese subjects with or without T2DM (77,78,81).

Triglyceride derivatives, such as DAG, ceramide and LC-CoA are known to activate protein kinase C (PKC) that, in turn, phosphorylates the serine residues of IRS1. Serine-phosphorylated IRS1 is unable to associate with and activate PI3K, leading to disruption early in the insulin-signaling cascade and hence diminished trafficking of GLUT4 to the cell membrane (as reviewed by Morino *et all* (82)). Furthermore LC-CoA upregulates the de novo synthesis of TNFα, which is also associated with diminished insulin signaling, through the same pathway (83). In addition, an increase in the cytosolic pool of LC-CoA could directly inhibit glycogen synthase activity which leads to lower glycogen storage (80,81,84). Via these mechanisms, lipotoxicity can disturb cellular processes leading to insulin resistance in the skeletal muscle cell (Figure 5).

A decreased metabolic flexibility in T2DM patients is part of the explanation how lipids can accumulate in the skeletal muscle cell. The switch in fuel oxidation is normally dependent on the amount of nutrients (glucose, FFA or amino acids) available for oxidation. After a meal, in the insulin-stimulated state, glucose oxidation is high while lipid oxidation is suppressed. In the fasting/postabsoptive state the situation is just the opposite. However, in T2DM patients the switch in fuel oxidation is impaired, termed metabolic inflexibility (as reviewed in (85)). This leads to decreased oxidation of FFA and FFA derivatives. The reduction in metabolic

Figure 5. Cellular processes leading to insulin resistance in the skeletal muscle cell.



FFA: free fatty acid; LC-CoA: long chain fatty acid-CoA; TNFα: tumor necrosis factor α; DAG: diacylglycerol; PKC: protein kinase C; IR: insulin receptor; IRS: insulin receptor substrate; PI3K: phosphatidylinositol 3-kinase PKB/AKT: Protein kinase B/AKT; GLUT4: glucose transporter protein 4; GS: glycogen synthase.

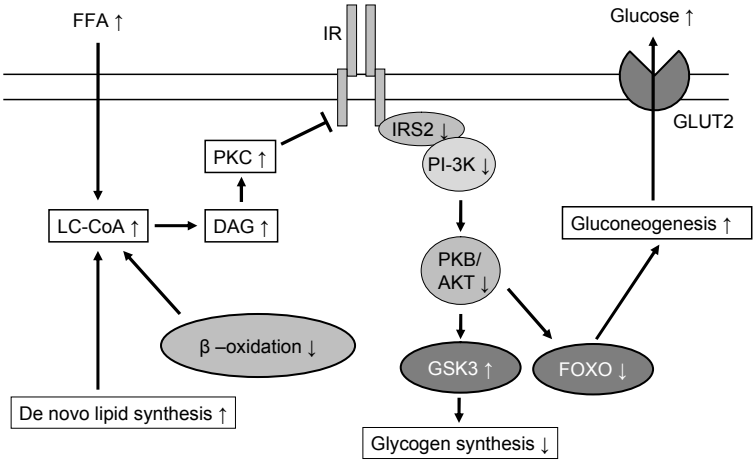
flexibility can partly be explained by reduced mitochondrial function and capacity. Indeed studies show reduced mitochondrial density and function in skeletal muscle cells of T2DM patients (86-88).

Liver (hepatic steatosis)

Cross-sectional studies show a positive correlation between hepatic steatosis (high hepatic TG content) and hepatic insulin resistance, both in T2DM patients and non-diabetic subjects (89,90).

The exact underlying pathophysiological mechanism by which hepatic triglyceride accumulation leads to hepatic insulin resistance is unknown. However, it is very likely that similarly as in the skeletal muscle lipid intermediates (such as DAG) are important. In the liver as well as in the skeletal muscle, DAG activates PKC which in turn binds and inactivates the IR resulting in reduced IRS1/2 and hence PI3K/AKT phosphorylation. Subsequently, this leads to an increase in GSK3 and decrease in FOXO phosphorylation, and thus respectively reduced liver glycogen synthesis and impaired suppression of hepatic gluconeogenesis. Thus there is augmented glucose release into the circulation (Figure 6) (as reviewed by Morino *et all* (82)).

Figure 6. Cellular processes leading to insulin resistance in the liver cell.



FFA: free fatty acid; LC-CoA: long chain fatty acid-CoA; DAG: diacylglycerol; PKC: protein kinase C; IR: insulin receptor; IRS: insulin receptor substrate; PI3K: phosphatidylinositol 3-kinase PKB/AKT: Protein kinase B/AKT; GSK3: glycogen synthase kinase 3; FOXO: forkhead box protein O; GLUT2: glucose transporter protein 2.

Heart (Myocardial triglyceride content; pericardial fat mass)

Cross-sectional studies report that stores of myocardial triglyceride are positively related to FFA exposure and are increased in obese and T2DM subjects (91,92). Ectopic fat depositions in the heart lead to diminished heart function. Triglyceride intermediates, such as DAG, ceramide and LC-CoA activate apoptotic processes, which ultimately alters the structure and

thus function of the heart. In cross-sectional studies, the increase in myocardial triglyceride stores in obese or T2DM subjects is associated with impaired systolic and diastolic function (92).

Pericardial fat is the adipose tissue surrounding the heart. The physiological function of this fat depot is still under debate. It may serve as protection for the coronary arteries and/or energy supply for the myocardium. On the other hand, it may be a metabolically active organ and secrete pro-inflammatory cytokines (93,94). Several cross-sectional studies have suggested a positive relation between an increased pericardial fat volume and coronary artery disease and insulin resistance in obese patients with or without T2DM (95-97).

QUALITY OF LIFE (QOL)

Several studies have shown that patients with T2DM have a worse Quality of Life (QoL) as compared to healthy controls. Lower QoL scores were associated with the use of insulin, the presence of diabetic complications or co-morbidities, physical inactivity and poor glycemic control. As in the normal population, socioeconomic status, demographic location and age are also of influence (98-101).

Obesity per se is also associated with a diminished quality of life. This is due to symptoms of obesity-related diseases, a negative general health perception, restricted physical activity, decreased self-image and a decline in social functioning. An improvement in QoL can increase patients' compliance with their diabetes treatment and enhances their commitment to self-management, resulting in positive adjustments in lifestyle and diabetes care (101).

DIET-INDUCED WEIGHT LOSS (USING VERY LOW CALORIE DIETS)

Weight reduction with diet and exercise is one of the cornerstones in the treatment of obese and T2DM patients. Weight loss improves morbidity associated with obesity such as insulin resistance, dyslipidemia and hypertension (77,102-104). In obese patients a substantial energy restriction for a longer period of time is necessary to achieve weight loss. Moreover, in obese T2DM patients substantial weight loss is needed to improve peripheral insulin sensitivity, the mainstay of glucose disposal. Eight percent weight loss improved hepatic but not skeletal muscle insulin resistance (105) while 9-11% weight loss slightly (106) and 20% weight loss greatly improved peripheral insulin sensitivity (107). To achieve such energy restriction and weight loss very low calorie diets (VLCD) can be used. VLCDs contain 800 kcal/day or less. Usual food intake is completely replaced by specific foods or liquid formulas. Weight loss on VLCDs averages 1.5 to 2.5 kg/week; total loss after 12 to 16 weeks averages 20 kg in obese patients. These results are superior to standard low-calorie diets of 1200 kcal/

day, which lead to weight losses of 0.4 to 0.5 kg/week and an average total loss of only 6 to 8 kg in 12 to 16 weeks.

Studies show that VLCDs can be used safely in obese insulin-dependent T2DM patients even up to a year (107). Already after 2 days of a VLCD, basal EGP declines (108). VLCD-induced loss of 50% of the excess weight significantly improves hepatic and peripheral insulin sensitivity. The more than 100% increase in insulin-stimulated glucose disposal was accompanied by an improvement in insulin signaling at the cellular level. Both basal and insulin-stimulated phosphorylation of AS160 improved after the loss of 50% of the excess weight by the VLCD (107).

Some (101,109,110) but not (111) all investigators have found an improvement in QoL after diet-induced weight loss. This was mainly due to a reduction in symptoms of the diseases associated with excess weight such as low self-image and joint pain. Long-term studies on the effect of diet-induced weight loss on QoL in obese T2DM patients are lacking.

Diet-induced weight loss induces a decline in low-grade inflammation (as expressed in hsCRP levels), both in obese non-diabetic subjects as well as in obese T2DM patients (112-114). No data is available on the specific effect of long-term VLCDs on low-grade inflammation in T2DM.

Diet-induced weight loss might decrease ectopic fat depositions and hereby decrease the harmful effects of these excess lipids in non-adipose tissues. Indeed, a decrease in IMCL accumulation following weight loss has been shown in obese subjects and obese T2DM patients by some but not all investigators (107,115-117). Even a relatively small drop in BMI considerably reduces hepatic triglyceride content as measured by proton magnetic resonance spectroscopy (^{1}H-MRS). The main reduction in hepatic TG content already occurs in the first two weeks of the diet (116,118). This is associated with improved hepatic insulin resistance as measured by the hyperinsulinaemic euglycaemic clamp technique (78,105,119,120). The effect on myocardial TG stores following weight loss in obese T2DM patients has not yet been studied.

Long-term maintenance of weight loss with VLCDs is not very satisfactory and is no better than with other forms of weight reducing treatment with the exception of bariatric surgery.

EXERCISE

Physical activity has long been recognized as an effective interventional strategy in the treatment of T2DM. The current guidelines for the treatment of diabetes from the ADA, The European Association for the Study of Diabetes (EASD) or the American College of Physicians (ACP) all firmly recognize the therapeutic strength of exercise interventions. The ADA states that "to improve glycemic control, assist with weight maintenance, and reduce risk of CVD, at least 150 min/week of moderate-intense aerobic physical activity is recommended distributed over at least 3 days/week" (121,122).

Prolonged application of either endurance or the combination of resistance- and endurance-type exercise training has been shown to increase whole body insulin sensitivity and improve cardiovascular risk profile in obese T2DM and non-diabetic subjects. This is attributed to the concomitant induction of modest weight loss, the up-regulation of GLUT4 via non-insulin mediated pathways (i.e. adenosine monophosphate-activated kinase (AMPK)), improved nitric oxide-mediated skeletal muscle blood flow, and the normalization of blood lipid profiles (123-127). However, studies assessing the effect of exercise training in long-standing, insulin-dependent T2DM patients are lacking since these patients are usually unable to perform a reasonably intensive exercise program. Literature regarding the effect of exercise on QoL in patients with T2DM is conflicting. Exercise can either improve QoL because it increases physical fitness and is associated with increased social activity or it can decrease QoL due to an increase in body or joint pain, or the negative perception of high psychological demands and pressure of participating in an exercise program (128-131).

The effects of acute and chronic exercise are different with respect to the effect on low-grade inflammation (132,133). Acute exercise can elicit a pro-inflammatory response whereas chronic exercise is thought to mediate an anti-inflammatory effect (134). However, in several long-term exercise studies the effects on low-grade inflammation were less clear as they showed an improvement of hsCRP and IL6 without effects on TNFα levels.

OUTLINE THESIS

In previous studies we showed that 50% reduction of excess body weight in obese insulin-dependent T2DM patients using a VLCD without an exercise program significantly improved, but not normalized hepatic and peripheral insulin resistance (107). In these studies ectopic fat depositions, mitochondrial capacity, QoL and low-grade inflammation were not studied. Therefore in this thesis, we studied both short and long-term effects of addition of exercise to a 16-week VLCD on insulin sensitivity, ectopic fat depositions, QoL and low-grade inflammation. Our study population consisted of obese insulin-dependent T2DM patients, who still had endogenous insulin secretion as measured by a 1 mg glucagon stimulation test.

Our **first aim** was to systematically review the literature to look at the effect of diet-induced weight reduction and exercise on ectopic fat depositions in the liver, skeletal muscle and heart and the function of these organs (hepatic and peripheral insulin sensitivity and cardiac function) (**Chapter 2**).

The **second aim** was to evaluate whether the addition of exercise had extra beneficial effects on insulin sensitivity. Our a priori hypothesis was that addition of exercise would further improve and might even normalize insulin sensitivity in T2DM patients. We therefore studied both hepatic and peripheral insulin sensitivity before and after the 16-week intervention using a hyperinsulinaemic euglycaemic clamp with stable isotopes ([$^2H_5$]-glycerol

and [6,6-$^{2}H_{2}$]-glucose). In addition, muscle biopsies were taken to evaluate the (differential) effects of the two interventions on insulin signaling at the myocellular level. Importantly, we also evaluated the possible additional effects of an exercise program on mitochondrial copy number (muscle biopsy), maximum aerobic capacity (incremental cyclo-ergometer exercise test) and substrate (lipid and glucose) oxidation (indirect calorimetry with a ventilated hood) (**Chapter 3**).

The **third aim** was to evaluate long-term effects (18 months) on weight en glycemic control of a 16-week VLCD with or without exercise, and to evaluate the (differential) effects of the two interventions (**Chapter 4**).

Improvement of QoL in T2DM patients is an important treatment goal. Interventions aimed at improving the perception of patients of their physical and mental health can enhance their commitment to self-management and adherence to therapy that will lead to positive lifestyle changes and better diabetes control. Therefore, the **fourth aim** was to evaluate whether QoL could be improved or even normalized using a 16-week VLCD with or without exercise in obese T2DM patients. Both short- and long-term (18 months) results of a 16-week VLCD with or without exercise on QoL are described in **Chapter 4**. QoL of the patients was compared to that of a healthy lean and healthy obese control population.

Chronic low-grade inflammation is a pathogenetic factor in the development of insulin resistance and T2DM. Diet and exercise have been recognized to control T2DM and to ameliorate the classic CVD risk factors, such as hyperlipidemia and hypertension (7,135). Reduction in bodyweight in obese subjects is associated with a decline in hsCRP levels, and hence low-grade chronic inflammation. However, it is unclear whether exercise has additional beneficial effects, besides the weight loss effect, on chronic low-grade inflammation. Most physical/fitness studies have been cross-sectional in nature. Therefore, the **fifth aim** was to study both the short- and long-term effect of a 16-week VLCD with or without exercise in obese insulin-dependent T2DM patients on low-grade inflammation and cardiovascular risk factors (**Chapter 5**).

Our **sixth aim** was to evaluate both short (**Chapter 6**) and long-term (**Chapter 7**) effects of a 16-week VLCD with or without the addition of exercise on quantity and functional effects of ectopic fat depositions in the heart. To this end a subpopulation of the study patients was studied before, directly after and 18 months after the intervention. Ectopic fat deposition in the heart (intramyocardial TG content) was measured using ^{1}H-MRS and was related to function of the heart.

Our last and **seventh aim** was to examine the short (**Chapter 6**) and long-term (**Chapter 8**) effects of a 16-week VLCD with or without the addition of exercise on quantity of visceral and subcutaneous fat mass and ectopic fat depositions (in the liver and the pericardium). To this end magnetic resonance imaging (MRI) was used to measure pericardial fat, visceral and subcutaneous fat mass and ^{1}H-MRS for hepatic TG content.

In the last chapter (**Chapter 9**) the results are summarized and discussed.

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