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

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ISBN: 978-94-6169-102-6

Cover Design: Els Kok, Meeple, Den Haag.
Lay-out and print: Optima Grafische Communicatie, Rotterdam.

This thesis was partly financially supported by Roba Metals B.V., IJsselstein and Nutrition and Santé, Antwerpen, Belgium.
Publication of this thesis was financially supported by the research fund of the Bronovo hospital, Den Haag.

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Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op
donderdag 1 september 2011
klokke 13.45 uur

door

Marieke Snel
geboren te Delft
in 1979

PROMOTIECOMMISSIE

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Overige Leden:	Dr. E.J.P. de Koning
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CONTENTS

LIST OF ABBREVIATIONS

ACP	American college of Physicians
ADA	American Diabetes Association
AGE	advanced glycation end products
ALT	alanine aminotransferase
AMPK	adenosine monophosphate-activated kinase
AST	aspartate aminotransferase
AS160	Akt substrate 160
ATM	adipose tissue macrophage
au	arbitrary units
BMI	body mass index
CPT1	carnitine-palmitoyl transferase 1
CR	caloric restriction
CT	computertomografie
CVD	cardiovascular disease
DAG	diacylglycerol
E	exercise
E/A ratio	early and atrial diastolic filling phase ratio
EASD	European association for the study of diabetes
ECG	electrocardiogram
E/Ea	estimated left ventricular filling pressure
EDV	end-diastolic volume
EGP	endogenous glucose production
ELISA	enzyme-linked immunosorbent assay
ESV	end-systolic volume
FAT/CD36	fatty acid transporter CD36
FATP	fatty acid transport protein
FABP	fatty acid binding protein
FFA	free fatty acid
FM	fat mass
FOXO	forkhead box protein O
FPG	fating plasma glucose
GLM	general linear model
γ-gt	gamma-glutamyl transferase
GLUT2	glucose transporter protein 2
GLUT4	glucose transporter protein 4
GS	glycogen synthase
GSK3	glycogen synthase kinase 3

1.	G6Pase	glucose-6-phosphatase catalytic subunit
2.	HADS	hospital anxiety and depression score
3.	HbA1c	hemoglobin A1c (glycosylated hemoglobin)
4.	HDL	high-density lipoprotein
5.	HIR	hepatic insulin resistance
6.	^{1}H-MRS	proton magnetic resonance spectroscopy
7.	HOMA-IR	homeostatic model assessment of insulin resistance
8.	HPLC	high-performance liquid chromatography
9.	hsCRP	high sensitive c-reactive protein
10.	IGT	impaired glucose tolerant
11.	IHL	intrahepatic lipids
12.	IL	interleukin
13.	IMCL	intramyocellular lipid
14.	IFNγ	interferon gamma
15.	IR	insulin receptor
16.	IRS	insulin receptor substrate
17.	LBM	lean body mass
18.	LC-CoA	long chain fatty acid-CoA
19.	LDL	low density lipoprotein
20.	LUMC	Leiden university medical center
21.	LV	left ventricle
22.	LVEDVI	left ventricle end-diastolic volume index
23.	LVEF	left ventricular ejection fraction
24.	MCR_I	metabolic clearance rate of insulin
25.	MFI20	multidimensional fatigue index 20
26.	MRI	magnetic resonance imaging
27.	mtDNA	mitochondrial DNA
28.	mTORC1	mammalian target of rapamycin complex 1
29.	NAFLD	non-alcoholic fatty liver disease
30.	NASH	non-alcoholic steatohepatitis
31.	NGT	normal glucose tolerant
32.	NHP	Nottingham health profile
33.	NOGD	non-oxidative glucose disposal
34.	NS	non significant
35.	OGTT	oral glucose tolerance test
36.	ORO	oil red o
37.	PCR	polymerase chain reaction
38.	PI3K	phosphatidylinositol 3-kinase
39.	PIP2	phosphatidylinositols-4,5-bisphophate

1.	PIP3	phosphatidylinositols-3,4,5-trisphophate
2.	PDK	phosphatidylinositol dependent protein kinase
3.	PKB/AKT	Protein kinase B/AKT
4.	PKC	protein kinase C
5.	ppm	parts per million
6.	PRAS40	Proline rich Akt substrate 40 kDa
7.	QoL	quality of life
8.	R_a	rate of appearance
9.	R_d	rate of disappearance
10.	SEM	standard error of the mean
11.	SF36	short form 36
12.	SU	sulfonylureum
13.	TC	total cholesterol
14.	TE	echo time
15.	TG	triglyceride
16.	TNFα	tumor necrosis factor α
17.	TR	repetition time
18.	T2DM	type 2 Diabetes Mellitus
19.	UKPDS	United Kingdom Prospective Diabetes Study
20.	VLCD	very low calorie diet
21.	VLDL	very low density lipoprotein
22.	VO_{2max}	maximum aerobic capacity
23.	WHO	World Health Organization
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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.

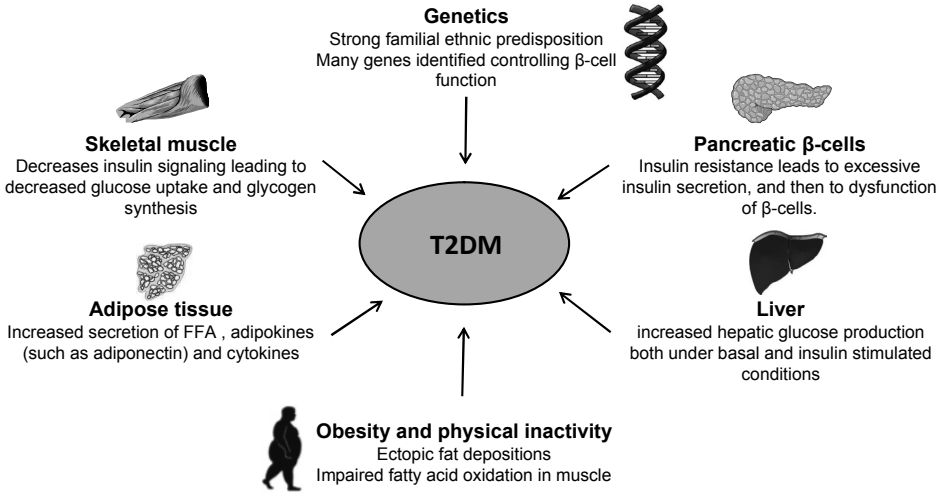
criteria for T2DM set by the WHO and the American Diabetes Association (ADA) are HbA1c ≥ 6.5% OR fasting (defined as no caloric intake for at least 8 hours) plasma glucose (FPG) ≥ 7.0 mmol/L OR 2-hour glucose ≥ 11.1 mmol/L during a 75 grams oral glucose tolerance test (OGTT), confirmed by repeat testing 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.

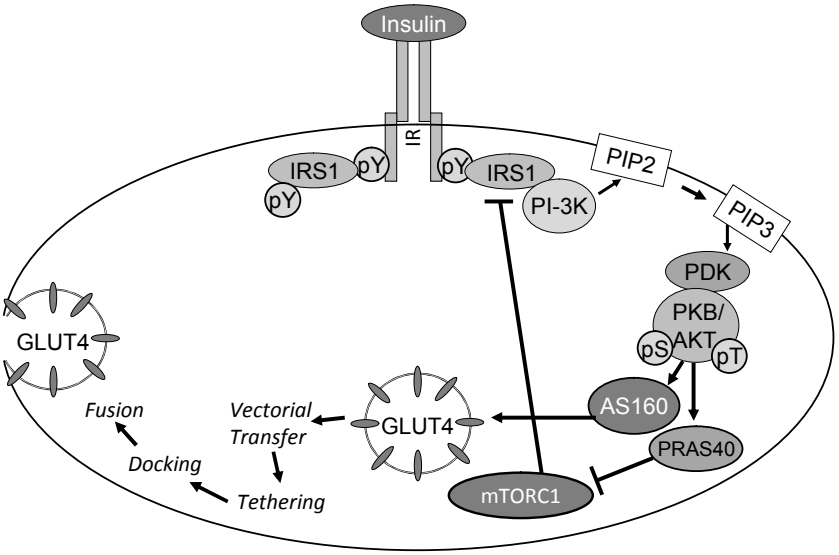


Skeletal muscle

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)), 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 pro-inflammatory cytokines vesicles 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.

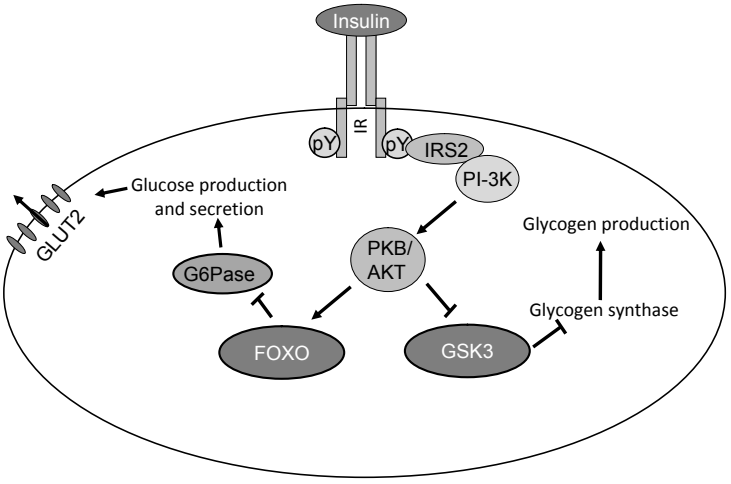
Liver

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,

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.

the PI3K/Akt pathway is not only controlled by IRS1 but also by IRS2 (55,56). In addition, 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).

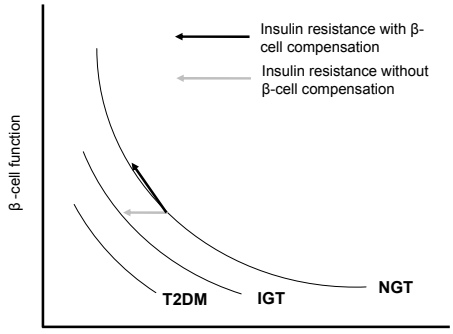
Due to ethical considerations liver biopsies in human studies with T2DM patients are rare. Animal studies confirm impaired insulin signaling form IRS1/2 to PI3K/Akt leading to increased gluconeogenesis (54). In, addition glycogen synthesis is inhibited (59). One of the mechanisms by which insulin signal 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

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



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

early abnormality in patients developing T2DM. Loss of the first phase insulin secretion has 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).

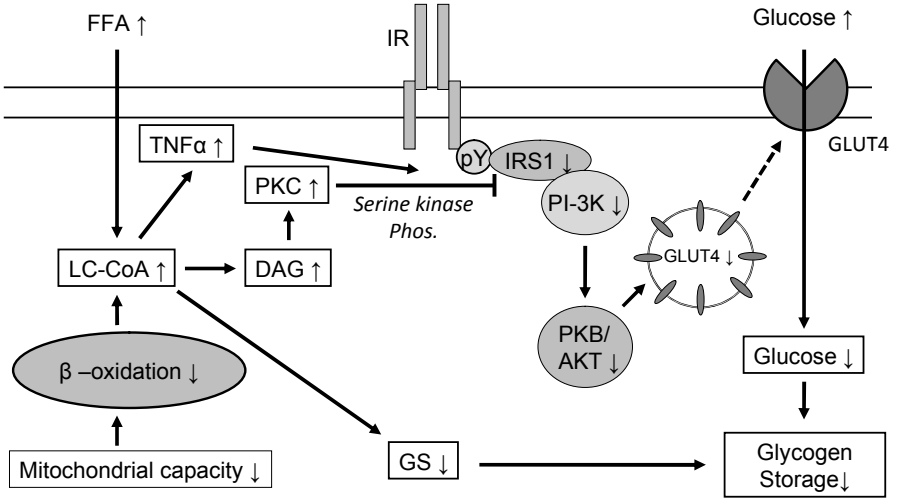
Skeletal muscle (IMCL)

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.

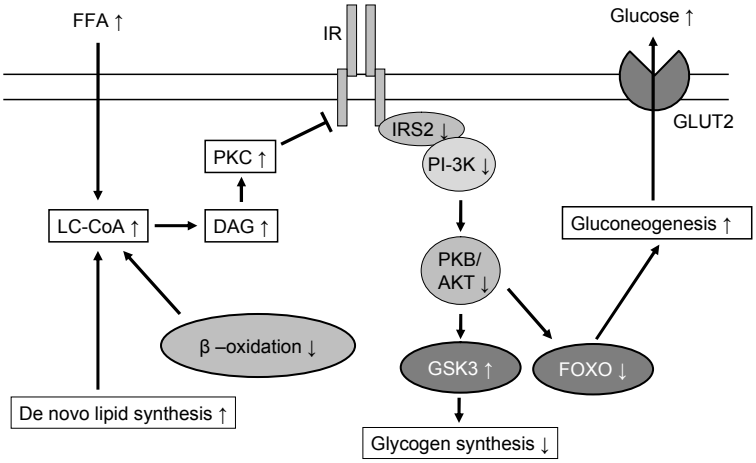
flexibility can partly be explained by reduced mitochondrial function and capacity. Indeed studies show reduced mitochondrial density and function in skeletal muscle cells of T2DM patients (86-88).

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. The 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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CHAPTER 2

Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions

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Submitted

ABSTRACT

Obesity predisposes to the development of insulin resistance, type 2 diabetes mellitus (T2DM) and cardiovascular disease. In obese subjects that develop insulin resistance, adipose tissue dysfunction plays a role, causing redirection of triglycerides (TG) towards non-adipose tissues. If in these tissues TG supply exceeds oxidative capacity intracellular lipid accumulation occurs. Ectopic fat is defined as storage of TG in tissues other than adipose tissue, that normally contain only small amounts of fat, such as the liver, skeletal muscle, heart and pancreas. The consequences of ectopic fat accumulation depend on the specific cell type and organ in which TG are deposited. Ectopic fat in the liver and muscle are positively correlated with insulin resistance and T2DM. Myocardial steatosis refers to TG accumulation in the myocardiocytes and is associated with impaired diastolic function. In this review we will discuss the consequences of ectopic fat accumulation in the liver, muscle and heart and, if known, the underlying pathophysiological pathways. In addition, we will discuss the effect of diets with or without exercise on these ectopic fat localizations and the effect of diminishing ectopic fat on insulin resistance in obesity and T2DM.

INTRODUCTION

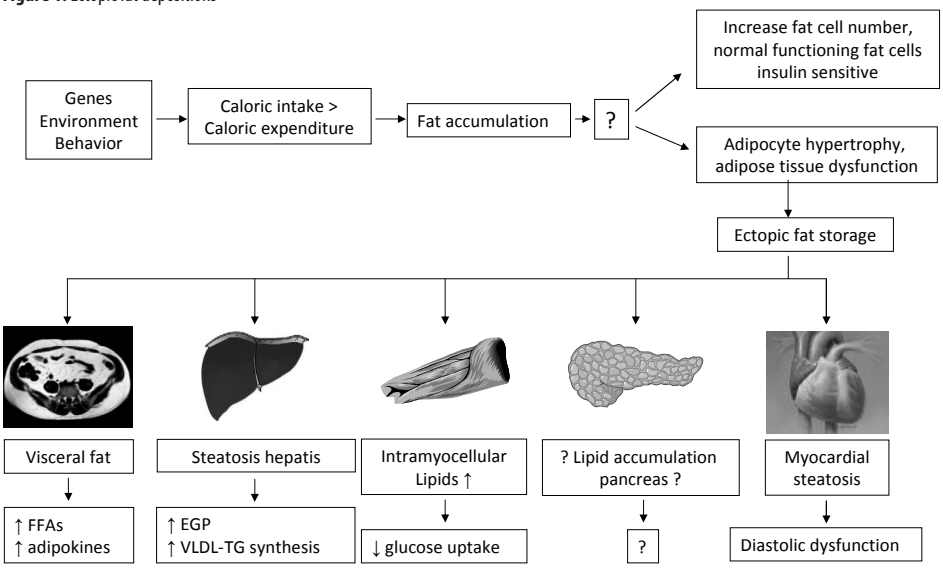
The amount of people with obesity has increased dramatically over the past decades to an estimated number of 400 million adults worldwide with a projected 700 million in 2015 (http://www.who.int/mediacentre/factsheets/fs311/en/index.html). Obesity predisposes to the development of insulin resistance, type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (1-6). However, about 30% of obese men and women are metabolically healthy (7), that is, do not have hypertension, dyslipidemia or disturbances in glucose metabolism. Vice versa, these metabolic abnormalities occur in 20-30% of normal weight people.

Adipose tissue consists of adipocytes and the so-called stromal-vascular fraction that encompasses blood vessels and stroma with macrophages. Adipose tissue has the unique capacity to store large amounts of energy in the form of triglycerides (TG). Before long, it has been presumed that this was the only function of adipose tissue. However, adipose tissue acts as an endocrine organ by secreting various hormones and cytokines (also referred to as adipokines) with effects on glucose and lipid metabolism and energy homeostasis (8). It now appears that in those obese subjects that develop insulin resistance, adipose tissue dysfunction plays a role as reviewed by Bluher (9). Adipose tissue dysfunction is characterized among others by large adipocytes, secretion of adipokines with a pro-inflammatory profile and ectopic fat deposition. Ectopic fat is defined as storage of TG in tissues other than adipose tissue, that normally contain only small amounts of fat, such as the liver, skeletal muscle, heart and pancreas.

The cause for adipose tissue dysfunction and ectopic fat storage is largely unknown. Bluher 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 (9). For example, tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) impair adipocyte differentiation, reduce lipid accumulation and increase lipolysis in adipocytes. Hence, lipids are redirected towards non-adipose tissues. If in these tissues lipid supply exceeds oxidative capacity intracellular lipid accumulation occurs.

The consequences of ectopic fat accumulation depend on the specific cell type and organ in which TG are deposited. Ectopic fat in the liver (10) and muscle (11) are positively correlated with insulin resistance and T2DM. Myocardial steatosis refers to TG accumulation in the myocardiocytes and is associated with impaired diastolic function (12). Recently, increasing interest has focused on fat deposition around the heart: epicardial and pericardial fat. In cross-sectional studies pericardial fat accumulation is associated with coronary artery disease (13) and related to whole body insulin resistance (14). As to the existence and clinical consequences of lipid accumulation in the pancreas controversy exists as elegantly reviewed recently (15). Ectopic fat depositions can be measured with several techniques. Traditionally, biopsies were used to quantify lipid content in liver and skeletal muscle. Nowadays most

Figure 1. Ectopic fat depositions



FFA: free fatty acids; EGP; endogenous glucose production; VLDL-TG: very low density lipoprotein-triglyceride

studies use non-invasive methods such as computertomografie (CT), ultrasound and proton magnetic resonance spectroscopy (^{1}H-MRS) (Figure 1).

In this review we will discuss the consequences of fat accumulation in the liver, muscle and heart and, if known, the underlying pathofysiological pathways. In addition, we will discuss the effect of diet with or without exercise on these ectopic fat localizations and the effect of diminishing ectopic fat on insulin resistance in obesity and T2DM.

METHODS

The following databases were searched: PubMed (1949 to November 2010), EMBASE (OVID-version, 1980 to November 2010), Web of Science (1945 to November 2010), and Cochrane Library (1990 to November 2010). The search strategy consisted of the AND combination of three main concepts:

1. Type 2 Diabetes Mellitus, Obesity, or Insulin Resistance;
2. Weight Loss, Diet or Exercise;
3. Ectopic Fat.

For these three concepts, all relevant keyword variations were used. References were limited to human studies, adults, written in English or Dutch. In addition, only studies that used techniques that can quantify the amount of lipid accumulation and measured insulin sensitivity were included. Studies using surrogate markers for lipid accumulation (e.g. alanine

aminotransferase (ALT) or aspartate aminotransferase (AST) as a proxy for hepatic steatosis) were excluded. Hypocaloric diets are defined as containing less calories than required for energy demands and usually contain 1000-1200 kcal/day. Very low calorie diets (VLCD) typically contain less than 800 kcal/day.

SKELETAL MUSCLE

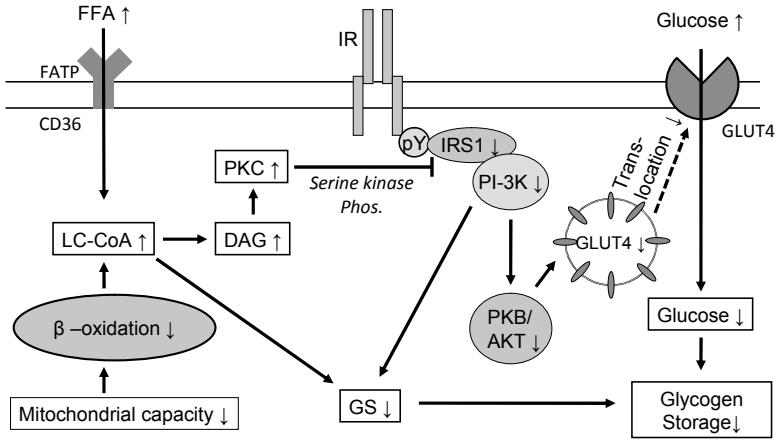
Intramyocellular lipids and peripheral insulin resistance

Several factors are involved in skeletal muscle insulin resistance: muscle fibre type (less type I, oxidative fibres), impaired capillary recruitment, diminished free fatty acid (FFA) oxidative capacity and an increased plasma FFA concentration (leading to perturbations in insulin signaling). The latter 2 conditions are involved in accumulation of intramyocellular lipid (IMCL). FFAs are taken up by the cell mainly by protein-mediated membrane transport (CD36, fatty acid transport protein (FATP)), along with passive diffusional uptake (16). Inside the cell fatty acid binding protein (FABPc) is the most important cytosolic protein for guiding long-chain fatty acids in the cell to places of oxidation or esterification. Long-chain fatty acyl-CoA is taken up by the mitochondria via carnitine-palmitoyl transferase 1 (CPT1). Inside the mitochondria β-oxidation and further degradation in the tricarboxylic acid cycle takes place. Therefore, IMCL accumulation occurs as a consequence of continuous oversupply of FFAs (caused by enhanced lipolysis, adipocyte dysfunction) together with an impairment in FFA oxidation in the mitochondria.

Accumulation of IMCL is associated with insulin resistance (17-20) and T2DM (11). However, it is not synonymous with the condition given the fact that endurance-trained athletes, who are highly insulin sensitive, also have a high IMCL content (11). Rather, the capacity to oxidize IMCL determines whether they represent a physiological or a pathological role as reviewed by van Loon and Goodpaster (21). In endurance-trained athletes IMCL serve as a readily available energy source. The close proximity of lipid droplets to the mitochondria supports this hypothesis. In these athletes, IMCL are not deleterious because of the increased capacity to oxidize lipids.

T2DM is characterized by a low oxidative capacity which leads to accumulation of lipids and intermediates of fatty acid metabolism such as long chain acyl-CoA (LC-CoA), diacylglycerol (DAG) and ceramides. These fatty acid metabolites induce a sustained activation of serine/threonine kinases such as protein kinase C (PKC) isoforms, IKB-kinase-β and Jun N-terminal kinase, which phosphorylate insulin-receptor substrate (IRS) 1 on serine residues (22). Serine-phosphorylated forms of IRS1 cannot associate with and activate phosphatidylinositol-3-kinase (PI3K), resulting in a decreased glucose transporter 4 (GLUT4) regulated glucose transport over the cell membrane (Figure 2).

Figure 2. Cellular processes leading to insulin resistance in the skeletal muscle cell.



FATP: fatty acid transport protein; 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; GLUT4: glucose transporter protein 4; GS: glycogen synthase.

The fact that impaired oxidation is involved led to the speculation that mitochondrial dysfunction is the cause of IMCL accumulation and the ensuing insulin resistance. Indeed, a decreased mitochondrial density and/or function has been reported in insulin-resistant offspring of T2DM patients (23-25) and T2DM patients (26-29). However, three of these studies reported decreased mitochondrial function at normal IMCL levels (27-29) suggesting that impaired mitochondrial function is not a prerequisite for IMCL accumulation. Rather, it might be that mitochondrial dysfunction is the consequence of the increased amount of fatty acid metabolites, for example via the formation of lipid peroxides (30). In that case it might be that the lipid-induced mitochondrial dysfunction induces progressive deterioration of oxidative capacity and further accumulation of lipid intermediates in the skeletal muscle cell. Further investigations are warranted to elucidate which one is cause or consequence: IMCL or mitochondrial dysfunction. In this review we focus on IMCL and will not elaborate on mitochondrial function.

In summary, an imbalance between fatty acid supply and oxidation (the contribution of esterification is very low) leads to accumulation of IMCL and lipid intermediates that interfere with insulin signaling and hence reduce insulin-stimulated glucose uptake in the skeletal muscle cell. Therefore, IMCL are associated with insulin resistance with the exception of endurance-trained athletes.

Effect of diets on IMCL accumulation

In a 6-month study in normal glucose tolerant (NGT) obese subjects, 25% calorie restriction (amount of calories not mentioned) leading to 10% weight loss (8 kg) had no effect on insulin sensitivity or IMCL. A parallel group that received a low calorie diet (890 kcal/day) but also

had moderate weight loss (14%,11 kg) showed a significant improvement in insulin sensitivity as measured by the insulin-modified frequently sampled intravenous glucose tolerance test and a tendency to decreased IMCL (measured by ^{1}H-MRS) that was not significant (31). In another 6-month study in morbidly obese NGT subjects, 10% weight loss (14 kg) by hypocaloric diet (1200 kcal/day) also had no significant effect on insulin sensitivity as measured with the hyperinsulinaemic euglycaemic clamp technique nor on IMCL as measured by skeletal muscle biopsies (32). In obese T2DM patients, a similar percentage of weight loss (10%, 8 kg) with a 1200 kcal/day very low fat (3%) diet during an average of 7 weeks, also had no effect on peripheral insulin sensitivity as measured with the hyperinsulinaemic euglycaemic clamp technique nor on IMCL (measured by ^{1}H-MRS) (33).

Moderate weight loss of 10-11% using a VLCD (600-800 kcal/day) for around 8 weeks in obese NGT persons improved insulin sensitivity as measured by homeostasis model assessment insulin resistance index (HOMA-IR) but had no effect on IMCL as measured by skeletal muscle biopsies (34). On the contrary, one study (35) showed a significant decrease in IMCL accumulation but no significant effect on peripheral insulin sensitivity after a 6-days VLCD (700 kcal/day) with a weight loss of 2.5% (in T2DM patients) and 5% (in obese subjects) compared to baseline. It should be noted however IMCL were measured by ^{1}H-MRS in the soleus muscle which has different characteristics than the vastus lateralis muscle. All other studies measured IMCL by muscle biopsy or ^{1}H-MRS of the vastus lateralis muscle. In addition in this study, a very high insulin infusion rate (200 mU/m^{2}/min) was used during the hyperinsulinaemic euglycaemic clamp, leading to supraphysiological insulin concentrations and hence higher glucose uptake rates as compared to other studies.

Moderate weight loss with hypocaloric diets seems to have no effect on IMCL or on insulin sensitivity in either obese or obese T2DM patients (31-33,36-39). More pronounced weight loss using a VLCD leads to a decrease in IMCL. A prolonged VLCD (450 kcal/day, on average 17 weeks duration) leading to 50% reduction of excess weight (mean 22 kg) in obese insulin-treated T2DM patients led to a significant improvement of both peripheral insulin sensitivity as well as IMCL in skeletal muscle biopsies (40) (Table 1).

Effect of diet and exercise on IMCL accumulation

Several studies investigated the effect of the addition of exercise to a hypocaloric or VLCD in obese NGT or T2DM patients. One group investigated the effect of a hypocaloric diet (goal weight loss 7 kg) with or without exercise (3 to 5 times weekly at 60-70% of maximal heart rate) in obese NGT (41) and obese T2DM patients (42), respectively. The obese NGT groups (diet-only and diet with exercise) both lost ~ 10% of body weight (9-10 kg) and had equal increases in glucose disposal rates. IMCL decreased in the diet-only group, not in the diet with exercise group. In the other study of the same group, 10 obese T2DM patients lost 7% of body weight (7 kg) and had an 54% increase in insulin-stimulated glucose disposal and a slight but significant increase in IMCL as measured by CT (42). Other studies in obese NGT

Table 1. Effect of diet and exercise on insulin sensitivity and intramyocellular lipid (IMCL) content.

Ref	patients	n	BMI start kg/m2	age yrs	intervention	duration	body weight loss kg	effect on skeletal muscle insulin sensitivity	Effect on IMCL
30	obese NGT	12	31±2	unknown	25% caloric restriction	6 months	- 8 kg	Si, no change	no change
		12	33±2		12,5% caloric restriction + 12,5% exercise		- 8 kg	Si 37±18%, p<0.01	no change
		11	33±2		15% weight loss hypocaloric diet 1200 kcal/ day		- 11 kg	Si 70±34%, P<0.04	no change
		11	31±2		controls		0 kg	Si, no change	no change
31	morbid obese NGT	9	48±9	39±12	diet 1200 kcal/day	6 months	-14±12 kg	M-value, no change	no change
		8	51±8	39±12	biliopancreatic diversion		-33±10 kg	M value 23±3 to 52±11 µmol/ kgFFM/min, p<0.05	1.6±1.1 to 0.2±0.4, p<0.05
	controls	7	27±1	35±11	controls		unknown	M-value, no change; baseline 53±13 µmol/kgFFM/min	no change; baseline 0.1±0.2
32	obese NGT	20	34±1	42±2	-700 kcal compared to normal diet	15 weeks	- 11 kg	OGTT, no change	no change
					followed bij energy restriction+exercise	21±2 weeks	- 5 kg	OGTT, no change	no change
33	obese T2DM	8	30±1	47±3	1200 kcal/day 3% fat diet (untill normoglycemia)	3-12 weeks (mean 7)			
					studies after weight stabilisation period	4 weeks	- 8±1kg	GDR, no change	no change
34	obese NGT	13	33±2	unknown	-522 kcal compared to normal diet	3 months	- 6 kg	M-value, no change	no change
35	overweight T2DM	7	27± 3	55±5	-25-30 kcal/kgLBM	2 weeks	BMI -1.5±0.0%	M-value, no change	no change
		7	27±3	46±3	- 25-30 kcal/kgLBM + advice to walk 2-3 td 5-6days/week		BMI - 2.3±0.1%	M-value 5.3±0.3 to 8.2±0.5 mg/ kg/min; P< 0.001	3.8±0.4 to 3.1±0.4, p<0.03
36	morbid obese NGT	7	44±6	unknown	diet 1200 kcal/day	6 months	- 5±4 kg	M value, no change	no change
38	obese T2DM	13	36±1	50±3	VLCD 600-800 kcal/day	8 weeks	- 9±1 kg	HOMA-IR -0.9 is -44±7%, p<0.001	no change
37	obese NGT	5	36±5	38±12	VLCD 700 kcal/day	6 days	- 2.3 kg	GDR, no change	- 56%, p=0.006 (^{1}H-MRS)

Table 1. (continued)

Ref	patients	n	BMI start kg/m2	age yrs	intervention	duration	body weight loss kg	effect on skeletal muscle insulin sensitivity	Effect on IMCL
	obese T2DM	7	37±7	43±6	VLCD 700 kcal/day	6 days	- 3.7 kg	GDR, no change	- 40%, p=0.04 (^{1}H-MRS)
40	obese T2DM	10	40±2	55±3	VLCD 500 kcal/day until 50% excess weight was lost	mean 17 weeks	- 22 kg	GDR 18.8±2.0 to 39.1±2.8 umol/ kgLBM/min, P<0.001	7±14 to 4±1, p<0.002
41	obese NGT	7	33±1	46±2	25% caloric restriction, goal 7% weight loss	18.6±0.7 weeks	-11±2% , ca. - 8 kg	M value increased 29±7% p<0.05	decreased, p<0.05
		9	35±1	42±3	25% caloric restriction + 3/5 days/wk exercise at 60-70% MHR	19.2±0.4 weeks	- 9±1%, ca - 9 kg	M value increased 38±9% p<0.05	no change
42	obese T2DM	10	34±1	44±3	25% caloric restriction + 3/5 days/wk exercise at 60-70% MHR	16-20 weeks	-7.1±0.1% = ca 7 kg	GDR 4.1±0.6 to 6.3±0.9 mg/ kgLBM/min, p<0.05	48±1 to 50±1, p<0.01 (CT)
43	obese NGT	21	33	40	- 500-1000 kcal compared to normal diet with 4-6x/wk exercise at 65-75% MHR	16 weeks	-10 kg	GDR 6.5 naar 9.7 mg/kgLBM/ min p<0.05	no change
44	obese IGT	11	34±1	67±1	-600kcal/day compared to normal diet and aerobic exercise 5days/wk 60 min at 75% VO_{2max}	12 weeks	ca -8 kg	M value 2.9±0.3 to 4.7±0.6 mg/ kgLBM/min, P<0.01	3.9±0.6 to 2.5±0.3, p<0.05
		12	35±2	66±1	aerobic exercise 5days/wk 60 min at 75% VO_{2max}	12 weeks	ca -3 kg	M value 3.0±0.4 to 4.2±0.7 mg/ kgLBM/min, P<0.05	3.9±0.6 to 3.0±0.4, p<0.05
45	obese NGT	25	30±1	66±1	4-5days/wk supervised aerobic exercise at 75% MHR	16 weeks	- 1.3 kg	M-value, no change	21% increase, p<0.01
46	obese NGT	20	30±1	59±1	2x/week 30 min aerobic + 1x/week resistance exercise both at 55% VO_{2max}	12 weeks	no change	GDR, no change	no change
	obese T2DM	18	30±1	59±1			no change	GDR 18.4±1.4 to 21.0±1.4 umol/ kg/min, p<0.05	no change

NGT: normal glucose tolerant; GDR: glucose disposal rate; HOMA-IR: homeostatic model assessment of insulin resistance; IGT: impaired glucose tolerant; LBM: lean body mass; T2DM: Type 2 Diabetes Mellitus.

(31,43), obese impaired glucose tolerant (IGT) (44) or obese T2DM patients (38) consistently showed increased insulin sensitivity with no differences between diet-only or diet with exercise group (with the exception of (31) all measured with the hyperinsulinaemic euglycaemic clamps) The effect on IMCL in these studies was less consistent showing either no effect (31,43) or a decrease (38,44) in IMCL accumulation (Table 1).

Effect of exercise on IMCL accumulation

Few studies have addressed the effect of exercise-only on IMCL and insulin sensitivity. A 4-month exercise program (4-5 times weekly 45 minutes at 75% of maximal heart rate) in obese NGT subjects increased insulin-stimulated glucose disposal as well as IMCL (by 21%) as measured by muscle biopsy (45). Interestingly intramuscular ceramide and DAG levels decreased. No control group participated in the study. A study in obese subjects with IGT investigated the effect of exercise-only (5 days/week 60 min at 75% maximum aerobic capacity (Vo_{2max})) vs. diet (circa 1300 kcal/day) combined with exercise for 12-week. The diet with exercise group lost more body weight but the improvement in peripheral insulin sensitivity and the decline in IMCL were similar in the two intervention groups (44). Recently the effect of a 12-week combined aerobic and resistance exercise program was investigated in 18 T2DM (body mass index (BMI) 30 kg/m^2, HbA1c 7.2%, age 59 years) and 20 healthy controls matched for age, body weight and BMI. The intervention had no effect on body weight (46). Exercise increased insulin-stimulated glucose disposal only in the T2DM patients but not to levels comparable to that in the control group. In addition, exercise had no effect on IMCL in the controls and increased IMCL in the T2DM patients along with an increase in insulin-stimulated glucose oxidation and suppression of fat oxidation (Table 1).

Conclusion

Moderate weight loss has no effect on IMCL but can improve peripheral insulin sensitivity if a VLCD is used. Larger weight losses are needed to improve both peripheral insulin sensitivity and deplete IMCL stores. With respect to the effect of exercise on IMCL the story is more complex. Athletes have increased IMCL stores that serve as a readily available energy supply and training further increases IMCL in healthy subjects. On the other hand, sedentary insulin-resistant subjects also have increased IMCL, caused by diminished oxidative capacity. These are associated with increased intramyocellular lipid intermediates that interfere with insulin signaling and other cellular processes. Exercise training in these subjects either increases, decreases or has no effect on IMCL accumulation. This is probably associated with the stage of disease (can fat oxidation and/or mitochondrial function be restored) as well as with the intensity of the exercise program and sort (aerobic/resistance) exercise. Overall, studies show that exercise improves peripheral insulin sensitivity. Factors involved are a reduction in lipid intermediates and hence improved insulin signaling and mitochondrial function, increased oxidative capacity and increased capillary blood flow. In addition, contraction and

hypoxia activate adenosine monophosphate-activated protein kinase (AMPK) which leads to increased GLUT4 translocation independent of insulin.

LIVER

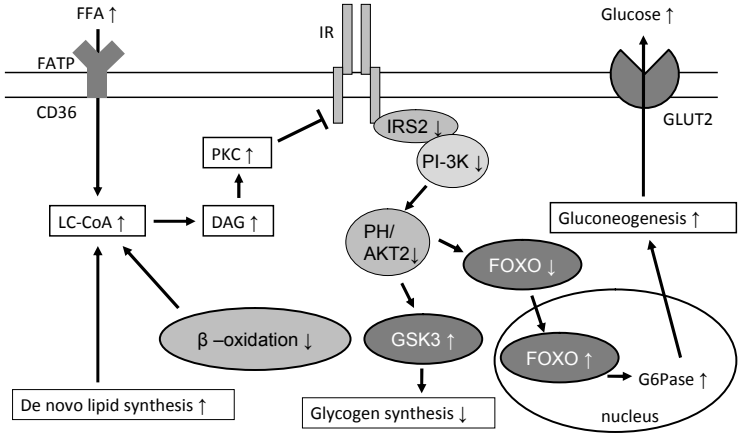
Intrahepatic lipids and insulin resistance of the liver

Accumulation of fat in the liver in the absence of excessive alcohol ingestion is referred to as non-alcoholic fatty liver disease (NAFLD). The spectrum of liver abnormalities within this entity ranges from hepatic steatosis with or without mild increases in serum AST/ALT to non-alcoholic steatohepatitis (NASH) with or without fibrosis, cirrhosis and incidental hepatocellular carcinoma. The world-wide estimated prevalence in the general population is about 20%, with large differences across countries (47). There is a strong association with obesity. A cross-sectional study in 1515 severely obese NGT subjects showed abnormal liver biopsies in 90% of people (48). The majority had hepatic steatosis, but one third had portal inflammation and fibrosis. A prospective study demonstrated a 4-times increased risk to develop hepatic steatosis in obese persons as compared to controls (49).

Non-invasive methods for measuring hepatic TG content such as ultrasound, CT and ^{1}H-MRS cannot distinguish NAFLD from NASH and fibrosis. A definite diagnosis can only be made by liver biopsy with histologic examination. The cut-off value for abnormal lipid accumulation in the liver has been defined as more than 5% of liver volume or when more than 5% of hepatocytes contain visible intracellular lipids (50). Two recent studies in respectively a NGT mixed (Hispanic, non-Hispanic, African American) population (51) and a lean Caucasian population (52) found that the 95th percentile for hepatic TG content was 5.6% and 3% respectively, using ^{1}H-MRS. The Pathology Committee of the NASH Clinical Research Network designed and validated scoring system of 14 histological features examining liver biopsy findings detailing steatosis, fibrosis, inflammation, and liver cell injury (53). An NAFLD activity score > 5 was universally associated with NASH.

Fat accumulation in the liver is associated with hepatic insulin resistance as well as with peripheral insulin resistance in skeletal muscle and adipose tissue (10,54-56). In a large European cohort of 1307 middle-aged NGT subjects, patients with a high fatty liver index (an estimate for hepatic steatosis based on an algorithm including BMI, waist circumference, TG, and gamma-glutamyltransferase) had a lower glucose disposal rate as measured by a euglycaemic hyperinsulinaemic clamp as well as higher FFA levels at the end of the insulin infusion (57). The latter is suggestive for decreased insulin sensitivity of adipose tissue. Korenblat et al. (54) found a negative correlation between hepatic insulin sensitivity measured by the hyperinsulinaemic euglycaemic clamp and hepatic TG content (measured by ^{1}H-MRS) in 42 nondiabetic obese subjects. Indeed, a multivariate linear regression analysis found that hepatic TG content was the best predictor of insulin sensitivity in liver, skeletal muscle and

Figure 3. Cellular processes leading to insulin resistance in the liver cell.



FATP: fatty acid transport protein; 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; GSK3: glycogen synthase kinase 3; FOXO: forkhead box protein O; GLUT2: glucose transporter protein 2.

adipose tissue, independent of BMI and percent body fat. Some claim that the amount of hepatic TG content is directly correlated with the severity of insulin resistance but this cannot be confirmed by others.

The mechanism behind hepatic TG accumulation and the development of hepatic insulin resistance is similar to that described for skeletal muscle (22,58). An increase in DAG in hepatic cells leads to activation of PKC, leading to decreased insulin receptor kinase activity and subsequently lower insulin-stimulated IRS2 tyrosine phosphorylation and lower IRS2–associated PI3K-activity which results in reduced insulin stimulation of glycogen synthase activity. This ultimately leads to decreased insulin-stimulated hepatic glucose uptake and reduced insulin suppressibility of hepatic glucose production. Furthermore, reduced activity of AKT2, a protein kinase downstream of IRS and PI3K, results in decreased phosphorylation of the forkhead box O (FOXO) transcription factor, allowing it to enter the nucleus and activate the transcription of the rate-controlling enzymes of gluconeogenesis. The result is increased hepatic glucose production and decreased hepatic glucose uptake, which both contribute to increased plasma glucose levels (Figure 3).

Effect of diet on intrahepatic lipids

Non-invasive techniques like CT and ^{1}H-MRS have shown that weight loss by nutritional interventions can result in a large decrease in hepatic TG content in obese and T2DM subjects (33,37,38,59-63). Because of the different populations and differences in baseline hepatic TG content, studies are not well comparable with respect to the individual effect of level of caloric restriction and/or amount of weight reduction on loss of hepatic TG content. Nonetheless, it has been shown that even a relatively small drop in BMI of 3-6% is associated with a

considerable reduction in hepatic TG content of 34-40% (37,60,61). The main reduction in hepatic TG content already occurs in the first two weeks of dietary restriction (38,59). The percentual decline in hepatic TG content positively correlates with the hepatic TG content at baseline, as patients with a high hepatic TG content at start of the diet lose relatively more TG than patients with low hepatic TG content, with the same amount of weight loss (59,60,62). Two studies measured both hepatic TG content and hepatic insulin sensitivity with a hyperinsulinaemic euglycaemic clamp (33,63). In obese (BMI 30.1 kg/m^2) T2DM patients, 7 weeks of a liquid formula diet (1200 kcal/day) led to a weight reduction of 8%. Hepatic TG content decreased by 81% and insulin mediated suppression of the endogenous glucose production (EGP) improved substantially (33). Viljanen et al. (64) found similar results in obese NGT subjects (BMI 33.7 kg/m^2). In this 6-week study a VLCD (550 kcal/day) led to a weight reduction of around 11 kg, a decrease in hepatic TG content of 60% together with a 40% decrease in basal EGP and hepatic insulin resistance index as well as a diminished hepatic FFA uptake.

Although the abovementioned studies clearly show that diet-induced weight loss leads to a decrease in hepatic TG content, the effect of this decrease in hepatic TG content on liver histology (i.e with liver biopsies) has only been scarcely studied. In obese patients with NASH moderate weight loss over a 12-month period, obtained with dietary advices only, led to an improved steatosis score in 9 of the 15 subjects. The improvement was associated with greater weight loss as compared to the patients that showed no change in steatosis (64). Another study in 15 patients with obesity and NASH combined a hypocaloric diet with exercise during 12 weeks while 10 obese subjects with NASH served as controls. The BMI decreased by 3 kg/m^2 in the intervention group and the steatosis score decreased from 2.3 to 1.3 (30-50% steatosis to less than 30%) while no changes were observed in the control group (65). In contrast, severe caloric restriction during 8 months in 41 morbidly obese (BMI 43.3 kg/m^2) subjects leading to an impressive median weight loss of 34 kg (-27% of BMI) showed normalization of liver architecture in 19 patients (66). However, they also found an increase in hepatic inflammation and fibrosis in some patients, this was associated with a greater weight loss and elevated FFAs.

Effect of diet and exercise on intrahepatic lipids

Studies on the effect of exercise on hepatic steatosis are scarce (review (67)). Most of these combine an exercise program with a hypocaloric diet. Both in obese as well as in T2DM patients these combined interventions led to a reduction of hepatic steatosis. Even a 2-week intervention with minimal weight reduction led to a significant 20% reduction in hepatic TG content in T2DM patients (38). However, since moderate weight loss alone already reduces hepatic steatosis the role of exercise is uncertain. Larson-Meyer studied a diet-only (25% calorie restriction (CR)) vs. a diet (12.5% CR) combined with exercise (12.5% CR) obese NGT group. In this 6-month study no additional effect of exercise upon the diet was found at an equal total amount of caloric restriction (31).

Effect of exercise on intrahepatic lipids

Only one study has investigated the influence of exercise alone on hepatic TG content in overweight (BMI 27.7 ± 0.5 kg/m^2) sedentary men (68). After a 6-week aerobic exercise programme (60-85% of VO_{2max} for a minimum of 20 min. at least three times per week) without significant effect on body weight no changes were found in hepatic TG content as measured by ^{1}H-MRS, although both peripheral and hepatic insulin sensitivity (measured by the hyperinsulinaemic euglycaemic clamp) improved. In this study, the hepatic TG content was already low at the start of the intervention and the amount of exercise was modest. Nevertheless, this study suggests that exercise alone (without weight loss and/or dietary caloric restriction) has beneficial effects on hepatic insulin resistance. The mechanism by which exercise improves hepatic insulin resistance is probably different than that in muscle. Indirect signals via factors mediated by the muscle and adipose tissue and a decrease in circulating FFAs are thought to play a role.

EPICARDIAL AND PERICARDIAL FAT

Pericardial fat is the adipose tissue surrounding the heart. It consists of two layers: epicardial fat (visceral fat) originating from mesothelial cells and paracardial or mediastinal fat, originating from mesenchymal cells (69). For more in depth information on the anatomic and pathophysiologic role of pericardial fat we refer to two excellent reviews on this subject (69,70).

Several functions have been proposed for epicardial fat tissue (70). Scientific proof is however rather difficult to obtain since most animal species have very little epicardial fat (71). In guinea pigs, rates of lipolysis and lipogenesis were 2-fold higher in epicardial fat than in other fat depots. This led to the assumption that epicardial fat might act as a buffer to protect the myocardium from highly toxic fatty acid levels and to provide fatty acids as a direct energy source in times of energy demand (71,72). Coronary arteries are embedded in epicardial fat so that another putative function might be to protect the coronary arteries from the tension and torsion induced by the arterial pulse wave and provide an environment in which the coronary arteries can easily expand. This fat compartment also acts as a metabolically active organ, secreting cytokines (73,74).

Several cross-sectional studies have suggested a positive relation between an increased epicardial fat volume and coronary artery disease (14,75-77). Furthermore, an increased epicardial fat volume has been associated with insulin resistance in non-diabetic obese patients (13) and with the presence of T2DM in a Han Chinese population (78,79).

Effect of diet on epicardial fat

Two studies have examined the effect of diet-induced weight loss on epicardial fat. Kim et al. (80) studied 27 moderately obese NGT subjects, who lost 11% (9.5 kg) of weight during

a 12-week weight loss intervention study. Epicardial fat thickness measured over the right ventricle wall by echocardiography decreased by 17% from baseline. Iacobellis et al. (81) studied 20 severely obese (BMI 45 ± 5 kg/m^2) subjects (probably including patients with IGT or T2DM) who followed a 6-month low calorie diet (900 kcal/day) and lost 20% (25 ±10 kg) of bodyweight. Epicardial fat decreased by 32% from baseline. This was accompanied by an improvement in left ventricular mass and diastolic cardiac function. The change in diastolic function was also positively correlated with the change in epicardial fat thickness. Moreover, in 15 obese patients with T2DM, we found a significant decrease in pericardial fat after weight loss with a 16-week VLCD, measured with MRI (unpublished data) (82).

Effect of exercise on epicardial fat

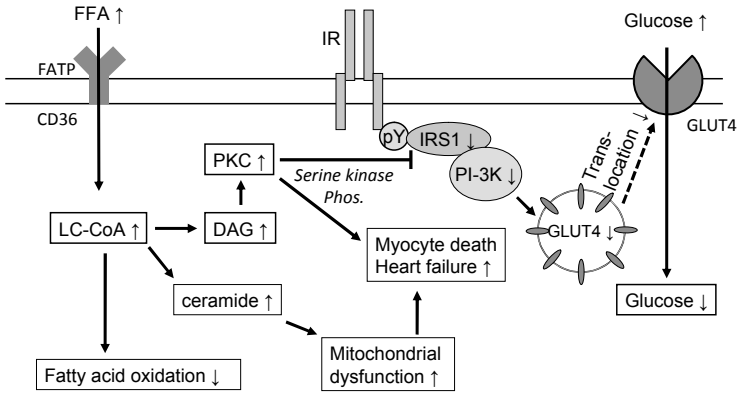
To date, only two studies examined the effect of (aerobic) exercise on epicardial fat (83). In one study, 24 obese NGT (BMI 30.7 ± 3.3 kg/m^2) middle-aged Japanese men followed a supervised exercise program for 3 months. The exercise intensity was gradually increased in 4 weeks from 50-60 to 60-70% of the maximum heart rate 3 days/week 60 minutes, which was continued for the remainder of the study. Following the intervention the BMI decreased by 4.3 ± 3.0% (circa -1 kg/m^2) and VO_{2max} increased by 20%. Epicardial fat thickness measured by echocardiography over the free wall of the right ventricle decreased significantly. The change in visceral adipose tissue (-15%) was significantly correlated with the change in epicardial adipose tissue (-8.6%).

In the other study, 32 obese postmenopausal women were randomized to diet-only or diet combined with moderate or intensive exercise for 20 weeks. All three groups had a similar 15% reduction in bodyweight and a 17% reduction in pericardial fat. However, no differences were observed between the diet-only or diet with exercise group (84).

MYOCARDIAL TRIGLYCERIDE CONTENT

In addition to the epicardial/pericardial fat depositions, TG can also be stored within the cardiomyocytes. This is referred to as myocardial TG content or myocardial steatosis. Myocardial TG accumulation can be measured with great sensitivity by ^{1}H-MRS (85). Patients with IGT and T2DM have an increased myocardial TG content compared to obese and lean controls (12,86,87). This accumulation of myocardial TG is a result of excessive fatty acid uptake relative to the oxidation. If fatty acids are converted to myocardial TG, several intermediates are released (e.g. ceramide) which in animal models caused cardiac dysfunction (88,89). In T2DM patients, myocardial steatosis was associated with impaired left ventricular diastolic function (12). Indeed, an increased fatty acid uptake in the myocardium has been found in healthy obese and T2DM patients compared to healthy lean subjects (90,91). But in the one study that investigated the fate of the intracellular fatty acids in the resting state in insulin-naïve

Figure 4. Cellular processes in the cardiomyocyte leading to myocardial dysfunction



FATP: fatty acid transport protein; 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; GLUT4: glucose transporter protein 4.

T2DM patients as compared to controls an increase, not a decrease in fatty acid oxidation was found. Fatty acid re-esterification was negligible but lower in T2DM patients as compared to the controls (91). Interestingly, neither plasma FFA levels nor myocardial blood flow was increased both in subjects with obesity (90) and T2DM (91) suggesting another mechanism, for example at the (cellular) level of the FAT/ CD36, to account for the increased fatty acid uptake (Figure 4).

The myocardial TG content is not static. Three days of severe caloric restriction (450 kcal/day to complete starvation) in healthy volunteers and patients with T2DM increases myocardial TG content, which is associated with a decrease in left ventricular diastolic function (92,93).

Effect of diet on myocardial triglyceride content

Thirty-four obese (BMI 33.7 ±0.7 kg/m^2) healthy persons underwent a 6-week VLCD (550 kcal/day). Before and after the intervention intramyocardial TG were measured, a hyperinsulinaemic euglycaemic clamp was performed and either glucose uptake or fatty acid uptake was measured by positron emission tomography. The intervention led to a weight loss of 11.2± 0.6 kg and a non-significant decrease in myocardial TG content of 31% (n=8, p=0.076). Myocardial fatty acid uptake decreased significantly. Myocardial mass and work decreased significantly by 7 and 26% respectively (94).

Our group investigated the effect of a 16-week VLCD (450 kcal/day) on myocardial TG content in obese patients with T2DM (95). We showed that a decrease in BMI (from 35.6 ± 1.2 to 27.5 ± 1.3 kg/m^2) was associated with a significant decrease in myocardial TG content and an improvement in left ventricular diastolic function.

Effect of exercise on myocardial triglyceride content

No other studies regarding the effect of diet-induced weight loss and/or exercise are available. It would be interesting to study endurance trained athletes to see whether they have, like in skeletal muscle, higher levels of intramyocardial TG than healthy controls and whether this can be accounted for by impaired myocardial fatty acid oxidation, and is related with cardiac function.

DISCUSSION

T2DM is a multifactorial disease in which genetic, environmental and lifestyle factors induce insulin resistance and impaired insulin secretion, ultimately leading to chronic hyperglycemia and its complications. Given the association between T2DM and obesity the recent focus of research has been the link between them. Vague already described a link between visceral adipose tissue, insulin resistance and T2DM in 1947 (96). But it has not been until the start of the obesity and diabetic epidemic that further elaboration on his work started. This research revealed that adipose tissue is not merely a storage depot for TG but actively secretes a vast array of factors such as cytokines, metalloproteinases and adipokines that can induce inflammation and insulin resistance (8).

In addition, with the advancement of radiological techniques it has become apparent that patients with insulin resistance and T2DM not only have a higher visceral to subcutaneous fat ratio as compared to healthy subjects but that TG are also stored in other organs called ectopic fat depositions for example in the liver, skeletal muscle, heart, and perhaps the pancreas (97,98). TG in the cells of these organs disrupts normal metabolic processes leading to increased hepatic glucose production, decreased insulin-stimulated glucose disposal and impaired cardiac function respectively. The consequences of elevated fatty acids and/or lipid accumulation in the pancreas seem to be only in order when elevated glucose levels are already present (15). Whether these ectopic fat storage is cause or consequence of insulin resistance and T2DM is currently under investigation. If ectopic fat is the cause of insulin resistance than it should be present already before the onset of insulin resistance (primary steatosis). On the other hand, when ectopic fat is the consequence of insulin resistance than insulin resistance should always be present. To date only cross-sectional studies have been performed.

Several large lifestyle intervention studies such as the Diabetes Prevention Study (99), the Da Qing study (100) and the Diabetes Prevention Program (101) all showed that lifestyle changes aimed at weight loss decrease the risk of developing T2DM by 31-58%. In patients already affected by T2DM, weight loss also decreased insulin resistance and improved glucoregulation (40). The data in this review show that substantial weight loss mobilizes ectopic fat stores in all organs and that this was associated with an improvement of the function

of that organ. Thus, a reduction in hepatic TG was accompanied by a decline in fasting EGP (33,63) and an improvement in the insulin-suppressibility of EGP. A decrease in myocardial TG (95) and epicardial fat (81) were both associated with improved diastolic cardiac function. Finally a decline in IMCLs leads to an improved insulin-stimulated glucose disposal (40). It should be noted however that the amount of weight loss and/or the severity of caloric restriction are of influence on the effect and that there seems to be a tissue-specific reaction. For example, around eight kilograms weight loss following a 1200 kcal/day diet for 7 weeks led to a decrease in hepatic TG and improved insulin sensitivity of the liver but had no effect on insulin-stimulated glucose disposal or IMCL in obese T2DM patients. When obese women with a history of gestational diabetes were subdivided in groups with high and low liver fat, a similar weight loss led to greater loss of hepatic fat in the high liver fat group while both groups lost an equal amount of visceral and subcutaneous fat (62). A prolonged VLCD in more severely obese insulin-dependent T2DM patients leading to around 22 kg of weight reduction also decreased IMCL and improved insulin-stimulated glucose disposal (40). We recently corroborated these findings in a similar group of patients who underwent a 16-week VLCD. The largest reduction occurred in hepatic TG content (-85%) whereas IMCL accumulation in the skeletal muscle decreased by 38%. The relative reduction in visceral fat was larger than the reduction in subcutaneous abdominal fat (-60%, -45% resp.) (82). The above studies suggest that hepatic TG content is the most easily mobilized, followed by visceral fat. The tissue-specific reaction to dietary interventions is also present when, vice versa, patients are subjected to high-fat feeding. Three days of a high-fat high-energy diet in health young males greatly increased hepatic TG stores but had no effect on myocardial TG (102).

Few studies have investigated the effect of exercise per se, that is exercise without weight loss and/or caloric restriction. The effect of exercise varies in the different organs with respect to TG accumulation. In muscle, exercise can even increase IMCL. However, when this is accompanied with increased fatty acid oxidation this is positive and in accordance with the athlete's paradox. The latter refers to the fact that endurance-trained athletes have increased IMCL but are very insulin sensitive. In these athletes the IMCL are a substrate source during exercise and the high turnover rate prevents accumulation of lipid intermediates that have a negative effect on insulin signaling and can form lipid peroxides. In the sedentary state, when metabolic flexibility is low, IMCL accumulate with the aforementioned deleterious effect on cellular processes. Exercise can either, increase, decrease or have no effect on IMCL but does improve insulin sensitivity. Apart from a decrease in lipid intermediates/increased fatty acid oxidation, an increase in capillary density and activation of AMPK with subsequently enhanced GLUT4 translocation are also involved. Exercise in combination with diet also depletes hepatic TG content and improves hepatic insulin sensitivity whereas the one study that investigated the effect of exercise alone found no effect on hepatic TG content but an increase in hepatic insulin sensitivity. The underlying mechanism is probably not direct but via a decrease in factors produced by adipose (8) and skeletal muscle tissue. Only one study

investigated the effect of exercise on epicardial fat: this was reduced but cardiac function was not measured. No studies on the effect of exercise on myocardial TG content have been published.

The clinical significance of measuring ectopic fat depositions after weight-loss interventions is limited. Performing an MRI/^{1}H-MRS in every patient is not practical from a logistical and cost perspective, especially since it has no implications for treatment at this moment.

From a scientific point of view the most intriguing question is how and why ectopic fat storage begins and whether this process is genetically determined or can be modified by measures other than the always so difficult to obtain weight loss. Therefore, it is interesting that exercise leads to improvements in insulin sensitivity, whereas it does not lead to decreases in ectopic fat. For that matter it is interesting that Europeans are relatively protected from (diet-induced) obesity and insulin resistance. On the other hand, aboriginals adapting a Western lifestyle rapidly obtain obesity and diabetes (103). Whether this is associated with the deposition of ectopic fat is unknown although reasonable to assume. Also the sequence of affected organs is unknown. The fact that people adapting a Western lifestyle develop obesity and T2DM suggests however that food might induce an inflammatory process. This of course upon a genetic background that, from an evolutionary standpoint, is set to store calories. Dietary intervention studies in which radiologic techniques, metabolic studies and tissue biopsies are combined hopefully will shed more light upon the question what determines why people store fat in non-fat compartments. And more importantly, what is the most effective treatment in order to halt the increasing diabetes epidemic.

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CHAPTER 3

Adding exercise to a 16-week very low calorie diet in obese, insulin-dependent type 2 diabetes mellitus patients improves metabolic flexibility, VO_{2max} and mitochondrial copy number in skeletal muscle

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Submitted

ABSTRACT

Objective. Loss of 50% overweight using a very low calorie diet (VLCD, 450kcal/day) improves insulin sensitivity in obese type 2 diabetes mellitus patients. This study investigates whether adding exercise to the VLCD has additional benefits.

Methods. Twenty-seven obese (BMI 37.2±0.9kg/m^2 (mean±SEM)) insulin-treated type 2 diabetes mellitus patients followed a 16-week VLCD. Thirteen of them simultaneously participated in an exercise program (E) consisting of one-hour in-hospital training and four 30-minute training sessions on a cyclo-ergometer weekly. Oral glucose-lowering agents and insulin were discontinued 3 weeks prior to and at the start of the VLCD respectively. Anthropometric measurements, hyperinsulinaemic euglycaemic clamp with skeletal muscle biopsies and peak oxygen consumption testing (VO_{2max}) were performed before and after the intervention.

Results. Baseline characteristics were identical in both groups. Substantial weight loss occurred (-23.7±1.7kg VLCD-only vs. -27.2±1.9kg VLCD+E, p=NS). The exercise-group lost more fat mass. Glycemic control improved considerably. Insulin-stimulated glucose disposal increased similarly in both study groups (15.0±0.9 to 39.2±4.7µmol.min^{-1}.$kglbm^{-1}$ VLCD-only vs. 17.0±1.0 to 37.5±3.5 µmol.min^{-1}.$kglbm^{-1}$ in VLCD+E), as did phosphorylation of PI3K-PKB/AKT insulin signaling pathway. In contrast, skeletal muscle mitochondrial DNA (mtDNA) content increased only in the VLCD+E group (1211±185 to 2288±358, arbitrary units p=0.016 vs. 1397±240 to 1196±179, p=NS VLCD-only group). VO_{2max} also only increased significantly in the VLCD+E group (+6.6±1.7 ml.min^{-1}.$kglbm^{-1}$ vs. +0.7 ±1.5 ml.min^{-1}.$kglbm^{-1}$ VLCD-only, p=0.017).

Conclusions. Addition of exercise to a 16-week VLCD induces more fat loss. Exercise augments VO_{2max} and skeletal muscle mtDNA content. These changes are, however, not reflected in a higher insulin-stimulated glucose-disposal rate.

INTRODUCTION

In the obese type 2 diabetes mellitus patients (T2DM) insulin resistance is of pivotal importance. Caloric restriction, increasing physical activity and cognitive restructuring are the mainstays of the treatment of obesity especially in case T2DM is present (1). Diet-induced weight loss in obese T2DM improves insulin resistance and phosphatidylinositol 3-kinase-protein kinase B/AKT (PI3K-PKB/AKT) insulin signaling in the skeletal muscle (2).

Moderate exercise (70% of maximum aerobic capacity) does not play a major role in losing body weight but helps to maintain diet-induced weight loss. Moderate exercise can enhance peripheral insulin sensitivity even without weight loss (3). Exercise plays an important role in improving mitochondrial capacity and aerobic fitness in healthy individuals (4).

The current study compares the effect of a very low calorie diet (VLCD) with and without an exercise program in obese insulin-dependent T2DM patients to elucidate whether the addition of exercise to a VLCD has incremental benefits in terms of weight reduction, glucoregulation, insulin sensitivity, myocyte morphology and mitochondrial capacity.

PATIENTS AND METHODS

Patients

Twenty-seven sedentary (14 males, 13 females) T2DM patients were enrolled in the study. Clinical details are summarized in Table 1. All patients were obese (BMI >30 kg/m^2) and used at least 20 units of insulin per day, with or without oral glucose-lowering medication. In addition, patients had to have residual beta-cell capacity, defined as a fasting plasma C-peptide level greater than 0.8 ng/mL and a 2-fold increase of the basal C-peptide level in response to administration of 1 mg glucagon intravenously (5). The residual beta-cell capacity is necessary to safely stop all glucose-lowering medication.

Exclusion criteria were smoking, recent weight change, any other chronic (endocrine) conditions and silent cardiac ischemia. Written informed consent was obtained from all patients. The study was approved by the local ethics committee.

Study design

Throughout the 16-week intervention period no blood glucose-lowering medication (both oral and insulin therapy), was utilized by the patients. All oral blood glucose and lipid lowering medication was discontinued 3 weeks prior to the study (because of the longer half-life of some of the oral glucose-lowering medication). Insulin therapy was intensive during these three weeks to prevent hyperglycemia. One day before the start of the intervention only short-acting insulin was prescribed, and long-acting insulin was omitted to prevent the presence of exogenous insulin during the hyperglycaemic euglycaemic clamp the next day.

After a baseline visit (outlined below), all patients started a 16-week VLCD (Modifast®, Nutrition & Santé, Antwerpen, Belgium). Modifast® provides a total of ~450 kilocalories per day and all necessary vitamins and micronutrients, divided over 3 meals of liquid shakes. Modifast provides about 50 g protein, 50 to 60 g carbohydrate, 7 to 9 g lipid, and 10 g of dietary fibre.

Thirteen of the 27 subjects were randomized to follow an exercise program simultaneously. This exercise program consisted of at least four training sessions per week at home and an one-hour in-hospital training. For the home-training sessions patients had to exercise at least 4 times a week for 30 minutes at 70% of their maximum aerobic capacity on a cyclo-ergometer. The one-hour in-hospital training entailed primarily aerobic exercise, under supervision of a physiotherapist. Patients in the VLCD-only group were instructed not to alter their pattern of physical activity.

During the 16-week intervention period patients visited the outpatient clinic weekly to confirm compliance by questionnaires, providing sachets of Modifast® and reading the heart rate monitor (Polar S610 $_{i}^{tm}$, Polar Electro Oy, Finland), which recorded the duration, heart rate and intensity of every training session. Patients were instructed not to perform physical activity in the last 48 hours before the hyperinsulinaemic euglycaemic clamps.

Hyperinsulinaemic euglycaemic clamp

All studies started after an overnight fast. Height, weight and waist circumference were measured. Lean body mass was assessed by bioelectrical impedance analysis (Bodystat® 1500, Bodystat Ltd., Douglas, Isle of Man, UK).

Metabolic studies were performed as described previously (6). In short, first samples were taken for the measurement of basal levels of glucose, insulin, and background enrichment of [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol. Basal rates of glucose and glycerol turnover were assessed after 3 hours of continuous infusion of [6,6-$^2H_2$]-glucose and 1.5 hours of continuous infusion of [$^2H_5$]-glycerol (Cambridge Isotopes, Cambridge, USA). Subsequently, insulin-stimulated rates of glucose and glycerol turnover were measured after 4.5 hours of a hyperinsulinaemic euglycaemic clamp (Actrapid®, Novo Nordisk Pharma, Alphen aan de Rijn, The Netherlands; rate 40 mU/m^2/min). Glucose values were clamped at 5.5 mmol/L via the infusion of a variable rate of 20 % glucose enriched with 3 % [6,6-$^2H_2$]-glucose.

A physiological and isotopic steady-state was achieved during the last 30 min of both the basal as well as the hyperinsulinaemic period, therefore, the rates of appearance (R_a) and disappearance (R_d) for glucose and glycerol were calculated as the tracer infusion rate divided by the tracer-to-tracee ratio (7). Endogenous glucose production (EGP) during the basal steady-state is similar to the R_a of glucose, whereas EGP during the clamp was calculated as the difference between the rates of glucose appearance and infusion. The hepatic insulin resistance index (HIR) (µmol/min/kg$_{LBM}$/pmol*L) was calculated as the product of EGP and plasma insulin concentration (8). The metabolic clearance rate of insulin (MCR_I) was calculated as the constant infusion rate of insulin divided by the steady-state insulin concentra-

tion corrected for endogenous insulin secretion (basal insulin concentration x [steady state c-peptide/basal c-peptide concentration) (9).

Aerobic fitness

Each subject performed an incremental cyclo-ergometer exercise test to determine their maximum oxygen consumption ($VO_{2\ max}$) both before and directly after the intervention period. Exercise intensity was progressively increased while measuring ventilation, oxygen and carbon dioxide concentration of the inhaled and exhaled air. $VO_{2\ max}$ was reached when oxygen consumption remained constant despite an increase in workload.

Indirect calorimetry

Both under basal and hyperinsulinaemic conditions, indirect calorimetry with a ventilated hood (Oxycon Beta, Mijnhardt Jaegher, Breda, The Netherlands) was performed for 30-min. The molar ratio of oxygen consumed to carbon dioxide produced was used to calculate total glucose and lipid oxidation rates as described previously by Simonson and DeFronzo (10). Non-oxidative glucose disposal (NOGD), as a measurement for glycogen storage was calculated by subtracting the glucose oxidation rate from R_d of glucose.

Muscle biopsy (Mitochondria, Insulin Signalling, Oil Red O staining)

Under localised anaesthesia, with 1% lidocaine, muscle biopsies were taken from the vastus lateralis muscle under basal conditions and 30 minutes after the start of the insulin infusion (6) using a modified Bergström needle. Muscle samples were divided into two parts: one frozen in liquid nitrogen for subsequent determination of insulin signaling, whereas the other part was snap-frozen in liquid nitrogen-cooled isopentane and stored at -80°C for determination of intramyocellular lipid accumulation (IMCL).

mtDNA content was assessed using a modification of the quantitative real-time PCR-based method as we described previously (11). Insulin signaling was measured as described previously (12). Tissue sections of basal biopsies were stained with Oil Red O (ORO) combined with a double-immunofluorescence assay (anti-laminin and a monoclonal antibody raised against adult human slow myosin heavy chain) to allow quantification of IMCL as described previously (13).

ASSAYS

Serum insulin was measured with an immunoradiometric assay (Biosource, Nivelles, Belgium). Serum C-peptide levels were measured with a radioimmuno assay (Linco Research, St. Charles MO, USA). HbA1c was measured with a semi automated HPLC machine Primus Ultra 2 (Kordia, Leiden, the Netherlands)

Plasma free fatty acids (FFAs) concentrations were measured by a commercial kit (Wako Chemicals, Neuss, Germany). [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol were measured in a single analytical run using gas chromatography-mass spectrometry as described previously (14).

STATISTICAL ANALYSIS

Results are expressed as mean ± standard error (SEM). Paired *t* tests were applied to assess mean differences before and after the intervention within groups, whereas unpaired *t* tests were used to assess differences in means or deltas between groups. Non-parametric tests (Wilcoxon signed-rank test for paired samples, Mann-Whitney for unpaired samples resp.) were performed, when appropriate. Significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS for Windows (release 16.0, SPSS, Inc., Chicago, IL).

RESULTS

Anthropometric measurements

As shown in Table 1, the baseline (pre-intervention) characteristics of the two patient groups (VLCD with exercise (VLCD+E) and VLCD-only) did not differ with respect to both clinical and metabolic parameters. After the 16-week intervention both groups (VLCD+E and VLCD-only) showed significant improvements in clinical and metabolic characteristics.

Similar weight loss was achieved in both patient groups (-27.2±1.9 kg VLCD+E; -23.7±1.6 kg VLCD-only). The VLCD+E group lost significantly more fat mass (-21.8±2.2 kg VLCD+E; -16.6±1.7 kg VLCD-only) and also waist circumference decreased more (-25±1 kg VLCD+E; -19±2 kg VLCD-only) compared to the VLCD-only group (Table 1).

Glucose and lipid metabolism

After the intervention fasting plasma glucose, insulin and HbA_{1C} levels improved substantially and similarly in both intervention groups. During the clamp pre- and post-intervention steady state plasma glucose concentrations were similar in the two intervention groups (VLCD+E 5.4±0.4 vs. 5.4±0.5 mmol/L; VLCD-only 5.5±0.7 vs. 5.6±0.6 mmol/L, pre- and post-intervention resp.; both NS) Steady state plasma insulin during the clamp was significantly lower after the intervention but similar in both groups, as a result of an increase in clearance of exogenous insulin after weight loss (Table 2).

There was a similar reduction in basal EGP in both patient groups. The HIR index diminished both under basal and hyperinsulinaemic conditions after the intervention to a similar extent in both patient groups. Also peripheral insulin sensitivity improved considerably; glucose R_d increased with ~150% in both patient groups. The R_a of glycerol as a measure of the rate

Table 1. Clinical characteristics, body composition and fasting plasma levels before and after a 16-week VLCD +/- exercise in obese insulin-dependent type 2 diabetes mellitus patients.

	VLCD only			VLCD + exercise		
	baseline	after 16 wks		baseline	after 16 wks	
sex (M/F)	6/8			8/5		
age (years)	56.1 ± 2.4			53.0 ± 2.5		
weight (kg)	112.7 ± 5.6	89.0 ± 4.3	*	113.5 ± 5.1	86.3 ± 4.2	*
BMI (kg/m²)	37.9 ± 1.4	30.0 ± 1.1	*	36.4 ± 1.1	27.7 ± 1.0	*
waist (cm)	122 ± 3	103 ± 3	*	123 ± 3	98 ± 3	* $
fat mass (kg)	49.9 ± 3.6	33.2 ± 2.8	*	45.4 ± 3.2	23.5 ± 2.2	* $
systolic bloodpressure (mmHg)	161 ± 4	140 ± 4	*	145 ± 5	132 ± 5	*
diastolic bloodpressure (mmHg)	87 ± 3	78 ± 2	*	81 ± 3	75 ± 3	
HbA1c (%)	7.8 ± 0.3	6.7 ± 0.3	*	7.8 ± 0.4	6.3 ± 0.4	*
fasting plasma glucose (mmol/L)	12.1 ± 0.5	7.7 ± 0.6	*	10.9 ± 0.7	6.6 ± 0.8	*
fasting insulin (mU/L)	24.4 ± 4.3	12.6 ± 2.0	*	25.1 ± 2.2	8.8 ± 0.8	*
fasting C-peptide (nmol/L)	2.9 ± 0.3	2.2 ± 0.2		3.5 ± 0.3	2.0 ± 0.1	*
metformin (number of patients)	9	0		10	0	
SU-derivative (number of patients)	1	0		3	0	
average insulin (IU/day)	86.2	0		77.2	0	

Data are presented as mean±SEM. * $p<0.001$ within the group; $ $p< 0.05$ between the groups.
M: male F: female; kg: kilogram; cm: centimeter; IU: international units.

of lipolysis in basal conditions was somewhat higher after VLCD+E (whereas VLCD-only did not affect this measure), but the capacity of insulin to suppress lipolysis improved only by VLCD+E (Table 2).

Insulin signaling

Both patient groups had a higher insulin receptor (IR) expression after the intervention. IR expression increased further in the VLCD+E compared to the VLCD-only. Phosphorylation of proline rich substrate 40 (PRAS40) was similarly increased in both groups after the intervention, in basal as well as in hyperinsulinaemic conditions. AKT substrate 160 (AS160) phosphorylation showed a similar trend, but this increase was not statistically significant (Figure 1).

Glucose and lipid oxidation rates

Basal lipid oxidation and NOGD increased significantly post-intervention only in the VLCD+E group (Table 2). Before the intervention the switch between glucose and lipid oxidation was lost, post-intervention this improved in both groups. The basal lipid oxidation and insulin-mediated suppression of lipid oxidation improved more in the VLCD+E group.

Table 2. Metabolic parameters before and after a 16-week VLCD +/- exercise in obese insulin-dependent type 2 diabetes mellitus patients.

	VLCD only		VLCD+ exercise	
	baseline	after 16 wks	baseline	after 16 wks
basal EGP (µmol/kg_{LBM}/min)	17.7 ± 0.7	15.1 ± 0.6 *	17.1 ± 0.7	14.1 ± 0.4 *
clamp EGP (µmol/kg_{LBM}/min)	4.8 ± 0.7	1.2 ± 0.6 *	3.1 ± 0.8	1.8 ± 0.6
suppression EGP (%)	-73.7 ± 3.4	-93.1 ± 3.2 *	-82.0 ± 4.5	-87.6 ± 4.1
basal HIR (µmol/kg_{LBM}/min/pmol*L)	3009 ± 419	1242 ± 187 *	2644 ± 267	983 ± 136 *
clamp HIR (µmol/kg_{LBM}/min/pmol*L)	3492 ± 558	737 ± 335 *	2135 ± 572	1134 ± 392 *
glucose R_d (µmol/kg_{LBM}/min)	15.5 ± 1.2	38.6 ± 4.6 *	16.6 ± 1.2	41.8 ± 3.6 *
clamp insulin (mU/L)	102.4 ± 9.0	86.6 ± 7.3 *	102.0 ± 5.6	79.4 ± 5.5 *
metabolic clearance rate insulin (ml/m^2/min)	8.9 ± 1.6	17.9 ± 4.2 *	7.3 ± 1.3	16.2 ± 2.0 *
basal glucose ox. (µmol/kg_{LBM}/min)	10.9 ± 1.5	6.1 ± 1.4 *	15.8 ± 1.8	3.6 ± 1.1 * $
clamp glucose ox. (µmol/kg_{LBM}/min)	16.2 ± 2.1	17.4 ± 1.8	16.9 ± 2.2	12.9 ± 1.6
increase glucose ox. (µmol/kg_{LBM}/min)	5.2 ± 1.6	11.3 ± 1.9 *	1.0 ± 1.3	9.3 ± 1.2 *
basal NOGD (µmol/kg_{LBM}/min)	6.8 ± 1.4	9.1 ± 1.4	1.4 ± 1.7	10.5 ± 1.2 * $
clamp NOGD (µmol/kg_{LBM}/min)	0.0 ± 2.4	21.3 ± 4.2 *	0.0 ± 2.1	29.0 ± 2.7 *
basal R_a glycerol (µmol/kg_{FM}/min)	11.3 ± 1.2	11.3 ± 1.3	12.9 ± 1.0	15.9 ± 1.1 *
clamp R_a glycerol (µmol/kg_{FM}/min)	5.9 ± 0.9	5.8 ± 1.0	7.2 ± 1.3	7.9 ± 1.3
suppression R_a glycerol (µmol/kg_{FM}/min)	-5.4 ± 0.7	-5.5 ± 0.8	-5.6 ± 0.8	-7.9 ± 0.9 *
basal FFA levels (mmol/L)	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0
clamp FFA levels (mmol/L)	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.1 ± 0.0
basal lipid ox. (µmol/kg_{LBM}/min)	7.0 ± 0.4	6.6 ± 0.3	4.9 ± 0.4	6.9 ± 0.3 * $
clamp lipid ox. (µmol/kg_{LBM}/min)	4.6 ± 0.6	3.6 ± 0.5	4.7 ± 0.5	4.6 ± 0.5
suppression lipid ox. (µmol/kg_{LBM}/min)	-2.4 ± 0.6	-2.9 ± 0.6	-0.2 ± 0.4	-2.4 ± 0.4 *

Data are presented as mean±SEM.* $p<0.001$ within the group; $ $p< 0.05$ between the groups.
EGP: endogenous glucose production; LBM: lean body mass; HIR: hepatic insulin resistance index; Rd: rate of disappearance; Ra: rate of appearance, FM: fat mass; FFA: free fatty acid; ox: oxidation; NOGD: non-oxidatieve glucose disposal.

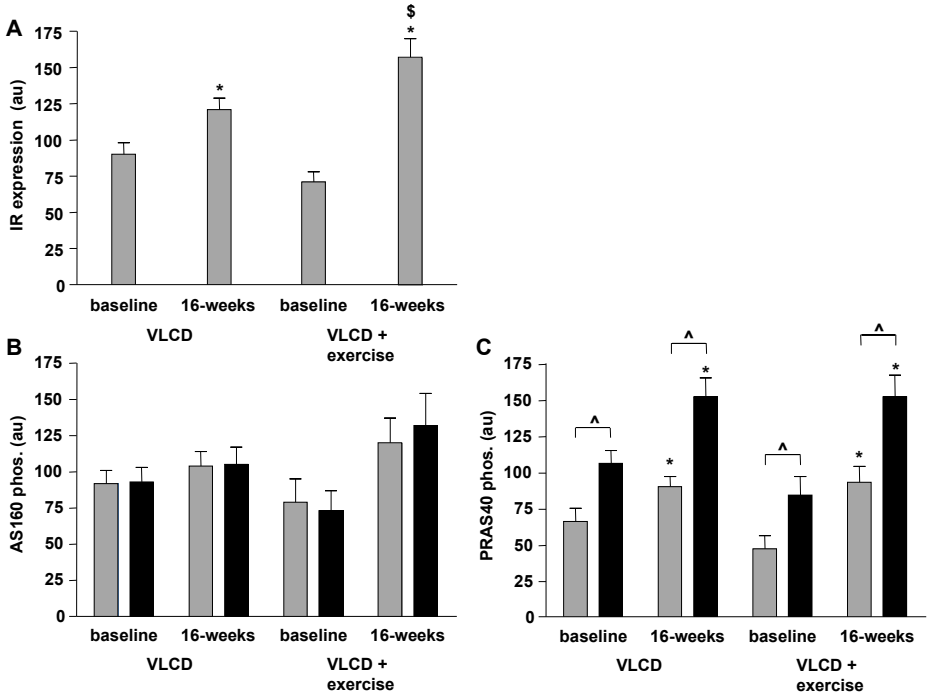
Maximal aerobic capacity

At baseline, VO_{2max} was similar in both groups. Participants in the VLCD-only group had a non-significant change (0.7±1.5 mg/kg_{LBM}/min difference from baseline) in $VO2_{max}$. In contrast there was a significant increase in VO_{2max} in the VLCD+E group (6.6±1.7 mg/kg_{LBM}/min difference from baseline) (Figure 2A).

Mitochondria

Muscle mtDNA content was not affected following a VLCD-only (1398±240 vs. 1127±180 au, pre- and post-intervention resp.). In contrast, the VLCD+E group showed a significant increase following the intervention (1211±185 vs. 2288±359 au, pre- and post-intervention resp.; $p<0.05$) (Figure 2B).

Figure 1. A. Insulin receptor (IR) expression at baseline and after 16 weeks of intervention; B. basal (grey bars) and insulin stimulated (black bars) Akt substrate 160 (AS160) phosphorylation (phos); C. basal (grey bars) and insulin stimulated (black bars) Proline rich Akt substrate 40 (PRAS40) phosphorylation.



* p<0.05 within the group compared to baseline; $ p< 0.05 between the groups; ^ p<0.05 basal vs. insulin stimulated.
au: arbitrary units.

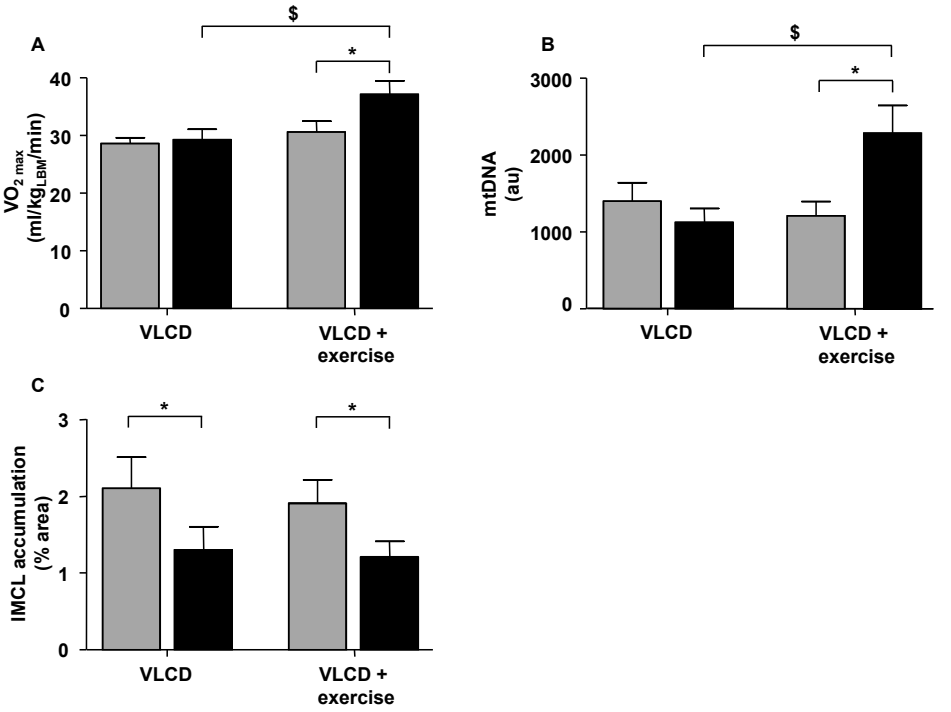
Muscle morphology and IMCL

In both intervention groups there was a similar decline in IMCL accumulation in the skeletal muscle fibers after the 16-week intervention (Figure 2C). The percentage of type 1 fibers in the skeletal muscle increased, whereas the percentage of type 2 fibers decreased significantly and similarly in both groups (VLCD+E type 1 and 2: 52.4±3.0% and 47.6±3.0% vs. 58.4±3.2% and 41.6±3.2% pre- and post-intervention resp.; both p<0.05; VLCD-only type 1 and 2: 51.8±3.0% and 48.2±3.0% vs. 60.7±4.1% and 39.3±4.1%, pre- and post-intervention resp.; both p<0.05).

DISCUSSION

A 16-week VLCD in obese insulin-dependent T2DM patients with or without moderate intense exercise resulted in substantial weight reduction and decrease of waist circumference. Exercise did not result in extra weight loss. However, it induced a greater loss of fat mass

Figure 2. A: Maximum aerobic capacity (VO_{2max}); B: mitochondrial DNA (mtDNA) content of the skeletal muscle; C: intramyocellular lipid accumulation (IMCL) as relative fraction of cell area containing lipid droplets at baseline (grey bars) and after (black bars) a 16-week VLCD +/- exercise in obese insulin-dependent type 2 diabetes mellitus patients.



* p<0.05 within the group; $ p< 0.05 between the groups.
LBM: lean body mass, au: arbitrary units.

and thus conservation of lean body mass. Glucoregulation improved to the same extent in both groups despite the cessation of all glucose-lowering agents including insulin. Insulin sensitivity of the liver, adipose tissue and skeletal muscle improved similarly in both groups, which is in accordance with the observed similar improvement in insulin signaling and decrease in IMCL in skeletal muscle. Also a significant increase in type 1 oxidative muscle fibers was observed in both groups. Maximal aerobic capacity and mitochondrial copy number increased only in the exercise group while these parameters remained unchanged in the VLCD-only group.

The current study confirms that diet-induced weight reduction improves glucoregulation in obese patients with T2DM by ameliorating both hepatic and peripheral insulin resistance. The high dosage used during insulin infusion almost completely suppressed EGP in both groups. Since plasma insulin clearance was increased by weight loss, insulin infusion resulted in lower circulating insulin concentrations during clamp steady state after the intervention. Thus, for a more accurate comparison of hepatic insulin sensitivity before and after the intervention the HIR was used which showed improved hepatic insulin resistance in both groups.

The current study failed to show additional effects of 16-weeks moderate intense exercise on both peripheral and hepatic insulin sensitivity. One possible explanation for this lack of an additional effect of exercise might be that the magnitude of caloric restriction and the achieved weight loss masked the potential additional effect of exercise.

The substantial increase in peripheral insulin sensitivity is in accordance with the increased level of insulin receptor expression and improvement in the PI3K-PKB/AKT insulin signaling pathway in skeletal muscle cells as reflected by increased PRAS40 phosphorylation. These data confirm the results of other studies showing that diet-induced weight loss (2) or the combination with exercise improve peripheral insulin sensitivity (15,16) and the insulin signaling pathway (17) in obese patients with or without T2DM. In our study, insulin receptor expression increased even further with the addition of exercise. However, this was not accompanied by further improvement of glucoregulation, as evidenced by similar baseline and insulin induced levels of PRAS40 and AS160 phosphorylation in both groups.

A low oxidative capacity leads to accumulation of lipids in the skeletal muscle. IMCL content is elevated in the skeletal muscle in obese type 2 diabetes mellitus patients and associated with insulin resistance. Not IMCLs per se, but IMCL derivatives, such as diacylglycerol (DAG) and long chain fatty acid-CoA are known to activate protein kinase C, which in turn, phosphorylates the serine residue of insulin receptor substrate 1(IRS 1). Serine-phosphorylated IRS 1 is unable to activate PI3K and leads to disruption in the PI3K-PKB/AKT insulin signaling cascade (18). The improvement in insulin signaling found in our study might partially be explained by the observed decrease in IMCL content in both intervention groups. IMCL content in the skeletal muscle is not only increased in obese and insulin resistant subjects, it is also high in endurance trained athletes; here IMCLs represent a physiological role as readily available energy source. In literature, exercise in obese non-diabetic and obese T2DM subjects, either increased (19), decreased (15,20) or led to unchanged (21,22) IMCL accumulation. In our group of patients a possible exercise-induced increase in IMCL could be hidden by the strong effect of caloric restriction and weight loss.

Prospective studies have shown that a low ability to oxidize fat is a risk factor for weight gain, obesity and insulin resistance (23). Impaired muscle fatty acid oxidation, reflected by reduced number of mitochondria, could be considered as a primary defect causing IMCL accumulation in T2DM patients (24). T2DM patients are characterized by low basal fat oxidation and increased lipogenesis rates (25). The current study showed that only a VLCD with exercise increased the reliance on lipid oxidation and lipolysis during fasting after the intervention.

It has been shown that the reduced muscle mitochondrial content and functional capacity in obese subjects are reversible with moderate weight loss (10%) combined with moderate-intensity regular physical activity (16,21). This suggests that sedentary behavior might be responsible for the reduction in mitochondrial capacity in obese T2DM patients. Indeed, here we showed that only combining VLCD with exercise increases mitochondrial copy number. However, the increase in mitochondrial copy number in the VLCD with exercise group was

not associated with a further decrease in IMCL and a greater increase in glucose disposal rate as compared to VLCD-only group.

We found an identical and significant increase in type 1, and decrease in type 2 muscle fibers in both intervention groups. A low capacity to oxidize fat due to a low percentage of type 1 (oxidative) muscle fibers might lead to obesity and T2DM, although a causal relation has not been established. To the best of our knowledge this is the first study which shows a significant increase in type 1 oxidative muscle fibers in T2DM patients after weight loss. Type 2 muscle fibers are responsible for generating strength and power, the decrease in type 2 muscle fibers could well be a reflection of reduced weight-bearing, since both groups lost similar but excessive amounts of weight after the intervention.

Even moderately-intense regular exercise leads to improved fitness in obese T2DM patients as shown in the present and previous studies (20,21,26), whereas aerobic capacity did not improve in the group receiving dietary advice without exercise program.

In conclusion, diet-induced weight loss improves insulin sensitivity and glucoregulation, decreases intramyocellular lipids, and improves insulin signaling in skeletal muscle. Adding exercise to the diet leads to additional loss of fat mass and conservation of lean body mass, an increased number of intramyocellular mitochondria and an improvement of maximum aerobic capacity. However, despite all these beneficial metabolic effects, exercise does not reinforce insulin action in this setting, perhaps because a VLCD per se ameliorates insulin resistance to a maximal extent in obese patients with T2DM.

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CHAPTER 4

Quality of life is improved for at least 18 months by the addition of exercise to a 16-week very low calorie diet in obese patients with type 2 diabetes mellitus

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Accepted in European Journal of Internal Medicine

ABSTRACT

Objective. To evaluate whether the addition of exercise to a very low calorie diet (VLCD) has beneficial short- and long-term effects on health-related Quality of Life (QoL) in obese patients with type 2 diabetes mellitus (T2DM).

Methods. We included 27 obese, insulin-dependent T2DM patients in a 16-week VLCD study, of whom 13 participated simultaneously in an exercise program (VLCD+E). Before, immediately after and 18 months after the intervention anthropometric measurements, glucoregulation and QoL (SF-36, HADS, NHP and MFI-20) were assessed. Patients were compared to healthy lean and obese (matched for body mass index) controls matched for gender and age.

Results. At baseline, T2DM patients had significantly worse QoL scores in 18 and 14 of the 22 subscales of the QoL questionnaires, compared to lean and obese controls, resp. The 16-week VLCD (n=27) decreased bodyweight (-25.4±1.3kg, $p<0.0001$, $p=0.179$ between groups), and improved glucoregulation (HbA1c -1.3±0.3%, $p<0.0001$, $p=0.488$ between groups) and 9 (VLCD-only) and 11 (VLCD+E) of the 22 subscales of QoL. After 18 months, in the VLCD+E group the QoL subscales did not differ from those in obese controls and only 4 of the 22 subscales were significantly worse compared to lean controls. However, in the VLCD-only group 17 and 13 of the 22 QoL subscales were significantly worse compared to the lean and obese controls, resp.

Conclusion. A 16-week VLCD induces considerable weight loss, metabolic amelioration, and major improvements in QoL in obese T2DM patients. The addition of exercise is of paramount importance for the maintenance of better QoL.

INTRODUCTION

The number of patients with type 2 diabetes (T2DM) is steadily increasing. Almost 90% of T2DM patients are overweight or obese. Medical attention is focused primarily on improving metabolic control to diminish long-term complications. However, patients with chronic diseases such as T2DM also have a poorer health-related quality of life (QoL) compared to healthy control subjects (1). Reduced QoL not only affects individual happiness but may also have impact on participation in the working process, social functioning, compliance to therapy and hence socioeconomic costs.

Improvement of QoL in T2DM patients is associated with an increase in self-management, adherence to therapy and positive changes in lifestyle (2). The magnitude of the effects on QoL is dependent on the type of intervention. Behavioral interventions have the smallest effect, but are nevertheless able to improve QoL, reduce the number of hospitalizations and use of medication (2,3). Bariatric surgery has the largest effect on QoL, mainly through the loss of excess weight and the waning of obesity-associated symptoms (2,4). However, surgery is expensive, invasive, associated with substantial morbidity. Furthermore it is logistically impossible to operate all obese patients with T2DM. Therefore, diet and lifestyle interventions remain the mainstay of therapy for most obese T2DM patients.

Diet-induced weight loss improves QoL in the short-term but not in the long-term, mostly because of regain of bodyweight (3). The effect of exercise on QoL in T2DM patients is less clear (5,6).

Therefore, the aim of this study was to assess whether addition of exercise to a 16-week very low calorie diet (VLCD) in obese, insulin-dependent T2DM patients has greater effects on QoL than a VLCD-only, both immediately after the 16-week intervention and 18 months after the intervention. Secondary aims were to compare QoL in our T2DM patients with lean and obese healthy controls.

PATIENTS AND METHODS

Patients

Twenty-seven obese (body mass index (BMI) 37.2±0.9 kg/m^2), insulin-dependent T2DM patients (age 58.0±1.6 years, duration of T2DM 8.9±0.8 years, 58±8 months on insulin therapy) participated in the study. Inclusion criteria were insulin-dependent T2DM patients (who used at least 20 EH of insulin per day) with or without oral glucose-lowering medication; at baseline a BMI above 30 kg/m^2; remaining endogenous insulin secretion defined as a fasting plasma C-peptide level greater than 0.8 ng/mL and a 2-fold increase of the basal C-peptide level in response to administration of 1 mg glucagon intravenously. Exclusion criteria were recent weight changes; smoking; any other chronic (endocrine) conditions; signs of depres-

sion or antidepressant medication and silent cardiac ischemia. Patients were recruited via advertisements in local news papers and from the endocrinology and internal medicine out-patient clinics of our hospital.

For each T2DM patient, 2 lean and 2 obese control subjects, matched for age, gender, race and in the obese group for BMI, were included. Control subjects were recruited via advertisements in local news papers.

The Medical Ethics Committee of the Leiden University Medical Center approved the study protocol. Written informed consent was obtained from each subject.

Study design

In all T2DM patients oral blood glucose-lowering medication was discontinued 3 weeks prior to the start of the intervention. One day before the start of the intervention, insulin therapy was stopped as well. The patients did not use any anti-diabetic medication during the whole 16-week intervention period.

At day 0 baseline observations were obtained in all patients (outlined below), followed by a 16-week intervention period. The intervention period consisted of a 16-week VLCD with or without the addition of an exercise program. The VLCD contained a total of ~450 kilocalories per day, divided into 3 sachets of Modifast® (Nutrition & Santé, Antwerpen, Belgium). Modifast® provides all necessary vitamins and micronutrients.

Thirteen of the 27 subjects were randomized to follow an exercise program simultaneously. This exercise program entailed one-hour in-hospital training per week (primarily aerobic exercise, supervised by a physiotherapist) and at least four training sessions at home on a cyclo-ergometer at 70% of their maximum aerobic capacity (VO_{2max}).

At the start, immediately after and 18 months after the 16-weeks intervention period, patients visited the research center after an overnight fast. Height, weight and waist circumference were measured. Fat mass was determined by bioelectrical impedance analysis (Bodystat® 1500 MDD, Bodystat Ltd., Douglas, Isle of Man, UK). Blood samples were obtained for fasting plasma glucose (FPG), insulin and HbA1c levels.

To assess QoL, patients were asked to fill in 4 different questionnaires. Each patient completed the questionnaires before, directly after, and 18 months after the intervention, resulting in a 100% response rate. The healthy (lean and obese) controls were asked to complete the QoL questionnaires only once.

During the 16-week intervention the subjects visited the outpatient clinic weekly, for measurement of weight and to check glucoregulation. Furthermore, compliance with the diet and exercise was established by counting sachets of Modifast that were supplied weekly and reading the heart rate monitor, that was worn during the exercise sessions (Polar S610 $_{i}^{tm}$, Polar Electro Oy, Finland). After the 16-week intervention period patients were treated according to current guidelines either in the primary care setting or at the out-patient clinic of our hospital. A follow-up visit for further investigation was scheduled 18 month later. All

patients completed the whole study period of 18 months, there were no dropouts from the study.

Questionnaires

Short Form-36 (SF-36)

The 36 items of the SF-36 record general well-being during the previous thirty days. The items are formulated as questions or statements and are subdivided into nine subscales: (1) physical functioning, (2) social functioning, (3) limitations in usual role activities due to physical problems, (4) limitations in usual role activities due to emotional problems, (5) bodily pain, (6) general health perception and change in health, (7) general mental health and (8) vitality (energy and fatigue) (9) health change. Because the HADS and the MFI-20 (see below) are more specific questionnaires for mental health, vitality and general mental health, these items were left out in this evaluation. Scores vary between 0 and 100. Higher scores are associated with a higher quality of life. (7).

Multidimensional Fatigue Index (MFI-20)

The MFI-20 contains 20 statements to assess fatigue. Five different dimensions of fatigue (four items each) are calculated from these statements: general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue. Scores fluctuate between 0 and 20; higher scores indicate more fatigue (8).

Hospital Anxiety and Depression Scale (HADS)

The HADS consists of fourteen items pertaining to the three subscales anxiety, depression and total score. Every item is scored on a four-point scale. Scores for the anxiety and depression subscale range from 0 to 21 and the total score from 0 to 42. A higher score indicates more severe anxiety or depression (9).

Nottingham Health Profile (NHP)

The NHP consists of 38 yes/no questions, subdivided in six scales: pain, energy, sleep, emotional reactions, social isolation and disability/ functioning, i.e. physical ability. The scores of the subscales are calculated as a weighted mean of the associated items. The scores are expressed as a value between 0 and 100 and the total score is the mean of the six subscales. A higher score is related to a worse quality of life (10).

Total QoL score

For an integral comparison of the QoL parameters addressed in the 4 questionnaires, our research-group developed a total QoL score which is the sum of all different QoL questionnaires. As described previously (11), all subscales were converted to a 100-point score. The

SF-36 scores were inverted so that a higher score is a worse QoL. Subsequently all subscale scores were added and a mean was calculated, generating a total QoL score (minimum value 0, maximum value 100). A higher score indicates a greater impairment of QoL

Assays

FPG were measured with a fully automated P-800 module (Roche, Almere, the Netherlands). Serum insulin was measured with an immunoradiometric assay (IRMA, Biosource, Nivelles, Belgium). HbA1c was measured with a semi automated HPLC machine Primus Ultra 2 (Kordia, Leiden, the Netherlands)

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR, normal values approach 1) was calculated from FPG and insulin levels according to the updated computed version of the formulae of Wallace et al (12).

Statistical analyses

Data are presented as mean ± SEM. Data analyses were performed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Differences between all groups (the two intervention groups and the two healthy controls) were analyzed using a one-way ANOVA. Differences within the group between the three different time points (baseline; directly after the intervention and 18 months after the intervention) were analyzed with a general linear model (GLM) for repeated measures, with time as within-subject factor. LSD post-hoc tests were used in case of a significant F-ratio. Differences in effect of the intervention between both intervention groups were analyzed by calculating a delta between two time points. The delta values were subsequently compared by non-parametric tests for independent samples or, when appropriate, by a two tailed Student's *t*-test for unpaired data. A p-value of < 0.05 was considered to be statistically significant.

RESULTS

Baseline characteristics

Clinical characteristics of the patients and healthy controls are shown in Table 1. Baseline characteristics of the two patient groups (VLCD with exercise (VLCD+E) and VLCD-only) were not different. The lean and obese control groups had significantly lower levels of FPG, insulin and HbA1c compared to the patients. The obese controls were well matched with respect to weight, BMI and waist circumference to the T2DM patients.

Short- and long-term effect of a 16-week VLCD on bodyweight and glucoregulation

After the 16-week intervention both groups (VLCD+E and VLCD-only) showed a significant improvement in clinical and metabolic characteristics. There was an impressive loss of weight in

Table 1: Clinical and metabolic characteristics at baseline, directly after and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients and comparisons with (obese and lean) healthy controls.

	VLCD + exercise			VLCD only			controls	
	baseline	16 weeks	18 months	baseline	16 weeks	18 months	obese	lean
sex (M/F)	8/5			6/8			28/26	28/26
age (years)	53±3			56±2			56±1	59±2
weight (kg)	114±5 *	86±4 !	98±5 !	113±6 *	89±4 !	103±5 !	112±3 *	73±2
BMI (kg/m²)	36.4±1.1*	27.7±1.0!	31.6±1.2 !	37.9±1.4*	30.0±1.1!	34.7±1.3 !	37.6±0.7*	23.8±0.3
waist (cm)	123±3 *	98±3 !	107±4 !	122±3 *	103±3 !	114±3 !	118±2 *	87±1
fat mass (kg)	45.4±3.2*	23.5±2.2!	35.4±2.6 !	49.9±3.6*	33.2±2.8!	44.2±3.0 !	39.2±2.4*	33.4±2.2
HbA1c (%)	7.8±0.4*#	6.3±0.4!	7.5±0.6	7.8±0.3*#	6.7±0.3!	8.2±0.5	5.5±0.1*	5.2±0.0
fasting glucose (mmol/L)	10.9±0.7*#	6.6±0.8!	9.2±1.0	12.1±0.5*#	7.7±0.6!	12.2±1.1	5.5±0.1*	4.9±0.1
fasting insulin (mU/L)	25±2 *#	9±1 !	13±2 !	24±4 *#	13±2 !	17±6	14±1 *	5±0
HOMA-IR	12.3±1.3*#	2.5±0.2!	4.7±0.8 !	12.9±2.3*#	4.3±0.8!	9.0±3.1	3.4±0.3*	1.1±0.1

Data are presented as mean ± SEM. ! significant difference vs. baseline values within the group; * significant difference vs. lean controls; # significant difference vs. obese controls

M: male; F: female; BMI: body mass index; HOMA-IR: homeostatic model assessment insulin resistance

all subjects (-27.2±1.9 kg VLCD+E; -23.7±1.6 kg VLCD-only), which consisted mostly of fat mass (-21.8±2.2 kg VLCD+E; -16.6±1.7 kg VLCD-only). There was significantly more loss of fat mass and waist circumference in the VLCD with exercise group. FPG and HbA1C levels improved substantially (respectively p=0.910 and p=0.488 between groups), despite the cessation of all glucose-lowering medication throughout the 16-week intervention period (Table 1).

After 18 months both groups had regained some weight. However, weight was still significantly decreased compared to baseline observations (Table 1). The beneficial effects of the 16-week intervention on HbA1c and FPG levels were also decreased. Nonetheless, the patients still used substantially less glucose-lowering medication than before the intervention (Table 2). Fasting insulin and HOMA-IR levels were only significantly better after 18 months compared to baseline in the VLCD with exercise group.

Table 2: Overview of medication used at baseline, directly after and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients.

	VLCD + exercise			VLCD only			controls	
	baseline	16 weeks	18 months	baseline	16 weeks	18 months	obese	lean
insulin (number of pts)	13	0	0	14	0	6	0	0
insulin (EH/day)	77	0	0	86	0	36	0	0
metformin (number of pts)	10	13	12	9	14	12	0	0
SU derivative (number of pts)	3	0	4	1	0	4	0	0
exercise (minutes/week)	34	180	192	24	0	45	82	167

pts: patients; SU: sulfonylureum

Short- and long-term effect of VLCD with or without exercise on QoL

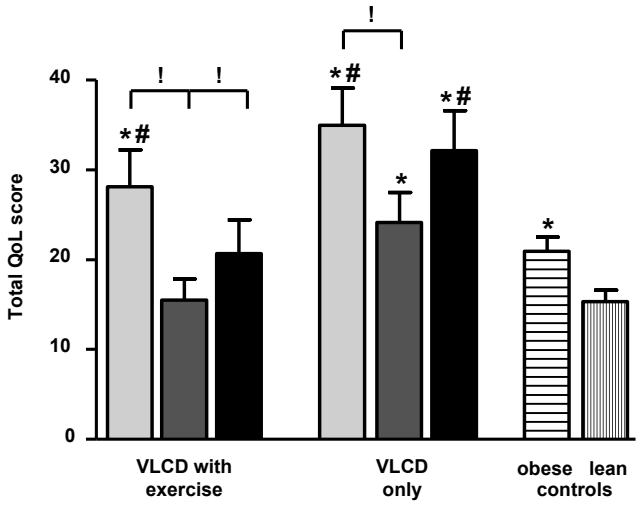
The results of the QoL questionnaires before, immediately after and 18 months after the intervention period are shown in Table 3. At baseline there were no differences in QoL between

Table 3: Quality of life (QoL) at baseline, directly after and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients and comparisons with (obese and lean) healthy controls. Short form-36 (SF-36): score 1-100 higher scores are associated with better QoL. Multidimensional fatigue index-20 (MFI-20): score 1-20 higher scores indicate higher experienced fatigue. Hospital Anxiety and depression scale (HADS): scores 0-21, higher score indicated more severe anxiety or depression. Nottingham Health Profile (NHP): score 0-100, higher score is related to a worse QoL.

	VLCD + exercise			VLCD only			controls	
	baseline	16 weeks	18 months	baseline	16 weeks	18 months	obese	lean
SF-36								
Physical functioning	72±6 *#	82±6 ! *	83±5 !* ?	63±5 *#	78±6 !*	68±7 *#	81±3 *	94±1
Social functioning	81±6 *#	88±3	87±8	79±5 *#	88±4	80±4 *#	91±2	93±2
Limits due to physical problems	65±11 *#	90±5 !	75±9 *	68±10 *#	71±10 *	59±11 *#	85±4	94±2
Limits due to emotional problems	95±5	100±0	100±0	74±9 *#	86±8	86±8	90±4	94±2
Pain	79±8 *	85±6	82±8	70±6 *	72±6 *	70±6 *	80±3 *	91±2
General health perception	52±6 *#	76±6 !	68±5 !* ?	51±5 *#	58±5 *#	51±6 *#	74±2 *	80±2
Health change	50±7	92±3 ! *#	67±7 *#	55±7 *#	88±6 !*#	66±7 #	51±2	55±2
MFI-20								
General fatigue	12±1 *#	7±1 !	9±1 ! ?	14±1 *#	10±1 !*	12±1 *#	9±1 *	7±1
Physical fatigue	12±1 *#	7±1 ! #	8±1 ! ?	13±1 *#	9±1 !*	12±1 *#	9±1 *	6±0
Reduction in activity	10±1 *	8±1	7±1 ! ?	12±1 *#	8±1 !	10±1 !*	8±1	7±1
Reduction in motivation	8±1 *	6±1 !	7±1	10±1 *#	8±1	10±1 *#	7±1	6±0
Mental fatigue	9±1	7±1	8±1	8±1	7±1	8±1	8±1	8±1
HADS								
Anxiety	4±1	3±1 !	4±1	5±1 *#	4±1 !	4±1	3±0	3±0
Depression	3±1	1±0	2±1 ?	5±1 *#	3±1 !	5±1 *#	3±0 *	2±0
Total	7±1	4±1 !	6±1 ?	10±1 *#	7±1 !	9±1 *#	6±1	5±1
NHP								
Energy	18±7 *	2±2 !	11±8 ?	36±11 *#	9±5 !	31±12 *#	7±3	4±2
Pain	13±8 *	9±7	15±9	20±6 *#	21±7 *#	18±6 *	8±2	4±2
Emotional reaction	4±2	5±2	3±2	9±3 *	7±3	9±4 *	4±1	2±1
Sleep	10±6	11±6	15±5 ?	21±7 *#	30±10 *#	30±9 *#	9±2	5±2
Physical ability	14±5 *#	4±2 !	12±5 *	15±5 *#	5±3	15±5 *#	7±2 *	3±1
Social isolation	0±0	0±0	0±0	2±2	0±0	0±0	3±1	1±1
NHP total score	10±3 *	5±2	9±4 ?	17±5 *#	12±3 *#	17±4 *#	6±1 *	3±1

Data are presented as mean ± SEM. ! significant difference vs. baseline values within the group; * significant difference vs. lean controls; # significant difference vs. obese controls; ? significant difference between the VLCD + exercise and VLCD only.

Figure 1. Total Quality of life (QoL) score before (grey bars), directly after (dark grey bars) and 18 months (black bars) after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients and comparisons with (obese and lean) healthy controls (white bars).



Data are presented as mean ± SEM. ! significant difference vs. baseline values within the group; * significant difference vs. lean controls; # significant difference vs. obese controls.

the two intervention groups. All patients scored significantly better directly after the 16-week intervention compared to baseline measurements on several subscales but mostly those concerning fatigue and physical ability. The VLCD with exercise group scored significantly better on 11 of the 22 subscales (i.e. the total of subscales of all four questionnaires) after the 16-weeks intervention. These results were partly lasting, since after 18 months still 5 of the 22 subscales remained improved. The VLCD-only group had a similar improvement in 9 of the 22 subscales directly after the 16-week intervention. However, in this group all but one subscale of QoL had returned to baseline levels at 18 months.

The total QoL score (Figure 1) improves equally in both groups directly after the 16-week intervention. However only in the VLCD with exercise group this effect is lasting.

QoL in healthy controls vs. patients

At baseline, T2DM patients scored significantly worse on subscales concerning mostly physical functioning and fatigue compared to both lean and obese healthy controls of the same gender, age and ethnicity (Table 3). Also the total QoL score is significantly worse in both groups compared to lean and obese controls

Immediately after the 16-week intervention program, most QoL scores improved, even to the level of the lean control subjects. In the VLCD with exercise group patients performed worse only at 1 of the 22 subscales compared to lean or obese controls. The VLCD-only group performed worse at 9 and 4 of the 22 subscales compared to healthy lean and obese controls, resp. The total QoL score in the VLCD with exercise group improved to the level of the healthy

lean controls. The patients in the VLCD-only group also improved but not beyond the total QoL score of the obese control group.

The difference between the two intervention groups became more apparent after 18 months. In the VLCD with exercise group the improvements in QoL persisted, none of the subscales differed from obese controls and only 4 of the 22 subscales were significantly worse compared to healthy lean controls after 18 months. The total QoL score also showed a persistent improvement; after 18 months there were still no significant differences in total QoL scores between patients in the VLCD with exercise group and healthy lean and obese controls. However, the QoL scores of the VLCD-only group returned almost back to their original levels; 17 and 13 of the 22 items were significantly worse compared to the lean and obese healthy controls, resp. In addition, the total QoL score of the VLCD-only group after 18 months follow-up was significantly worse than that of healthy lean and obese controls.

In one of the subscales of the SF-36 namely health change both patients groups scored significantly better directly after the intervention compared to both lean and obese control subjects. In the VLCD with exercise group this was a lasting effect up to 18 months after start of the intervention. In the VLCD-only group, patients only scored significantly better compared to the obese control subjects.

DISCUSSION

This study demonstrates that QoL parameters are considerably impaired in obese, insulin-treated T2DM patients. Treatment with a 16-week VLCD with or without an exercise regimen considerably improved QoL, associated with major improvements in anthropometric characteristics and metabolic regulation. However, long-term follow-up shows that exercise is vital in maintaining the achieved anthropometric, biochemical and QoL improvements. Ultimately, in the VLCD with exercise group the total QoL score did not differ and only 4 of the 22 QoL subscales negatively differed from the values obtained in healthy lean controls. In contrast, the achieved improvements in QoL and glucoregulation were completely abolished in patients in the VLCD-only group after 18 months.

Our patients scored slightly better in the HADS items (anxiety and depression) at baseline compared to the T2DM patients described by Pouwer et. al (13). This might be due to the exclusion of patients with psychiatric problems and the use of antidepressant drugs in the present study. In addition, our patients were slightly younger, which is relevant since increasing age is associated with decreased QoL (14). As far as we know, the MFI-20 questionnaire (containing items concerning fatique) has not been performed previously in T2DM patients. The NHP questionnaire (containing item regarding physical ability, social isolation energy levels) has been used sporadically in T2DM patients (15), whereas the SF-36 is the most used health-related QoL questionnaire (recording general well–being during the previous thirty

days) regardless of the population that is tested. Our patients and T2DM patients from previous other studies had comparable scores for the NHP and SF-36 questionnaire at baseline (4,15,16).

Our finding that diet-induced weight loss can lead to improvements in QoL directly after the intervention is in agreement with other studies (2,16). For example, Kaukua et al. (16) reported improvement in physical functioning and perception of health change related to weight loss after a low caloric diet in combination with Sibutramine or placebo measured by SF-36 in T2DM patients.

Several factors might have contributed to the improvement in QoL in our patients. Firstly, all patients lost a considerable amount of weight. Other studies have already shown that weight loss *per se* (e.g. after bariatric surgery) improves QoL (2). Secondly, insulin therapy was discontinued during the entire 16-week intervention period in our T2DM patients. After the intervention none of the patients in the VLCD with exercise group and almost 2/3 of the patients in the VLCD-only group still did not use insulin at 18 months follow-up. The use of insulin is associated with a lower QoL because of the burden of injections and self-control (1). Thirdly, the oral glucose-lowering medication was stopped as well, which may have decreased potentially present side-effects. However, since these medications were reinstituted after the intervention they cannot contribute to the lasting effect on QoL found in the VLCD with exercise group. The same applies to the fourth issue: improved glycemic control. This can ameliorate QoL via a decrease in metabolic oscillations as well as via reduced fear for the occurrence of long-term diabetic complications (1). However, improved metabolic control was only present at 16 weeks, not at 18 months. Fifthly, the undoubtedly better physical condition, due to more minutes of exercise per week at 18 months (Table 2) in the exercise group, might have contributed to the lasting effect on QoL. Lastly, there may also be the confounding effect of participation in a study, because intensive counseling and education has been shown to improve QoL (17). However, this did not apply at 18 months of follow-up. In our study none of the above ascribed factors could independently predict the improvements in QoL (data not shown).

In contrast to what we hypothesized, the addition of an exercise program to a VLCD had no immediate additional positive effect on QoL as compared to a VLCD-only. This has also been reported by some (6,18), but not all previous publications (5,19). One possible explanation for this lack of a direct additional effect of exercise on QoL is that the magnitude of the achieved weight loss masked the effect of exercise. Moreover, it is possible that exercise also had some negative effects. For example, it may have increased muscle or joint pain or interfered with social life (19). However, this study clearly shows that exercise is of paramount importance in maintaining the diet-induced improvements in QoL, weight loss and glucoregulation.

After the intervention patients were referred to their own health care provider. We were not able to influence treatment strategies, neither with respect to weight loss nor on metabolic regulation. This may be the reason that glycemic control was not as tight as required

by guidelines. Reluctance to reinstitute insulin therapy might have played a role. This makes it difficult to compare the two intervention groups regarding glucoregulation. The improvement in HOMA-IR and insulin levels after the 16-week intervention only lasted in the VLCD with exercise group. Moreover, in none of the patients in this group insulin therapy was restarted. Together these observations are suggestive of a better glucoregulation and less insulin resistance after 18 months in the VLCD with exercise group. We observed a lasting improvement in body weight in both groups. The long-term effect of a VLCD on bodyweight and glucoregulation (20-24) in obese diabetic patients has been addressed before. Some investigators found lasting improvements in glucoregulation but not on body weight (21,24) whereas others found a deterioration of both body weight and glucoregulation (22,23). Our group found improvements in both glucoregulation and body weight (20) 18 months after following a 30-day VLCD .

A limitation of the present study is the relatively small sample size. Nonetheless, our results are in line with the existing literature regarding the effects of bariatric surgery which also causes a significant amount of weight loss, on QoL.

The improvement of QoL in T2DM patients is an important treatment goal. Interventions aimed at improving the perceptions of patients of their physical and mental health can enhance their commitment to self-management and adherence to therapy which will lead to positive lifestyle changes and better diabetes control. Therefore, our finding of an improved QoL after a 16-week VLCD with or without exercise in obese, insulin-dependent T2DM patients is very relevant. The positive effect of weight loss on QoL, in addition to its beneficial effects on glycemic control, insulin resistance and cardiovascular risk factors, should stimulate T2DM patients and health care givers to make a serious effort in achieving and maintaining weight loss. For that matter, we emphasize the paramount importance of exercise in maintaining the positive effects achieved by diet-induced weight loss.

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CHAPTER 5

Immediate and long-term effects of addition of exercise to a 16-week very low calorie diet on low-grade inflammation in obese, insulin-dependent type 2 diabetes mellitus patients

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Submitted

ABSTRACT

Objective. To assess the short- and long-term effects of addition of exercise to a very low calorie diet (VLCD) on low-grade inflammation in obese patients with type 2 diabetes mellitus (T2DM).

Methods. 27 obese, insulin-dependent T2DM patients followed a 16-week VLCD with (n=13) or without (n=14) exercise (E) and were followed up to 18 months. Anthropometric measurements, metabolic and inflammatory parameters were assessed before, directly after the intervention and at 6 and 18 months follow-up. The same measurements were performed only once in 56 healthy lean and 56 healthy obese controls.

Results. At baseline hsCRP, IL10 and IL8 were significantly elevated in obese T2DM compared to lean healthy controls. After 16 weeks, despite substantial weight loss (-25.4±1.3kg), neither the VLCD nor VLCD+E had an effect on plasma cytokines. At 6 months, in the weight-stabilizing period, measures of low-grade inflammation had decreased substantially and equally in both intervention groups. Despite subsequent weight regain, beneficial effect was sustained upto 18 months in both groups, except for IL1 and hsCRP which had returned to baseline in the VLCD-only group.

Conclusion. Our findings suggest that severe caloric restriction increases cytokine production by adipose tissue macrophages and that the beneficial effects of weight loss become apparent only in the eucaloric state.

INTRODUCTION

Insulin resistant states such as obesity and type 2 diabetes mellitus (T2DM) are associated with chronic low-grade inflammation, as indicated by higher circulating levels of C-reactive protein (CRP), interleukin 6 (IL6) and tumor necrosis factor alpha (TNFα) (1). These pro-inflammatory proteins and cytokines can intervene in intracellular signaling pathways involved in glucose uptake and insulin secretion (2,3). However, the temporal and causal relationship between insulin resistance and elevated markers of inflammation is as of yet unclear.

Both obesity and T2DM are independent risk factors for the development of premature atherosclerosis and ischemic heart disease (1,4). Patients with T2DM have a 2-4 times increased cardiovascular disease (CVD) risk compared to healthy controls (5). This increased cardiovascular risk appears to be associated not only with traditional cardiovascular risk factors such as smoking, gender, hypertension and dyslipidemia but also with the abovementioned markers of inflammation (6,7).

Caloric restriction (CR), weight loss and exercise improve glucoregulation in patients with T2DM and ameliorate the classic CVD risk factors hypertension and dyslipidemia (8). CR and weight loss induce a decline in CRP levels, both in obese, non-diabetic subjects as well as in obese diabetic subjects (9). In addition, weight loss and lifestyle interventions decrease plasma IL6 and TNFα levels in obese non-diabetic subjects (10-13). The effect of exercise on these two markers of inflammation is controversial (14,15). Moreover, the effects of a very low calorie diet (VLCD) on IL6, TNFα and CRP has only been studied after a 3-week intervention (16) in obese patients with T2DM and no long-term follow-up data are available. Therefore, the aim of this study was to assess the effects of a 16-week VLCD (Modifast, 450 kcal/day) on classic cardiovascular risk factors and low-grade inflammation in obese, insulin-dependent T2DM patients. We also assessed whether adding exercise to the VLCD had additional beneficial effects on these outcomes. Patients were re-examined at 6 and 18 months after the start of the 16-week intervention, to evaluate the durability of the effects. In addition, the levels of these inflammatory markers were compared with those obtained in lean and obese healthy controls.

PATIENTS AND METHODS

Patients

Twenty-seven (14 males, 13 females) obese, insulin-dependent T2DM patients (disease duration 9±0.8 years, mean±standard error of the mean (SEM)) were enrolled in the study between June 2006 and June 2007. Clinical characteristics are summarized in Table 1. Exclusion criteria were smoking, known history of CVD and/or other chronic or endocrine disease, weight change within three months prior to the study and silent myocardial ischemia (as

Table 1. Clinical characteristics.

	VLCD + exercise	VLCD only	controls obese	controls lean
sex (M/F)	6/8	8/5	28/26	28/26
age (years)	56 ± 2	59 ± 2	56 ± 1	59 ± 2
weight (kg)	114 ± 5 *	113 ± 6 *	112 ± 3 *	73 ± 2
BMI (kg/m^2)	36.4 ± 1.1 *	37.9 ± 1.4 *	37.6 ± 0.7 *	23.8 ± 0.3
waist (cm)	123 ± 3 *	122 ± 3 *	118 ± 2 *	87 ± 1
fat mass (kg)	45.4 ± 3.2 *	49.9 ± 3.6 *	39.2 ± 2.4 *	33.4 ± 2.2
HbA1c (%)	7.8 ± 0.4 * #	7.8 ± 0.3 * #	5.5 ± 0.1 *	5.2 ± 0.0
insulin (IU/day)	77	86	0	0
metformin (number of pts)	10	9	0	0
SU-derivatives (number of pts)	3	1	0	0

Data are presented as mean ± SEM. * significant difference vs. lean controls; # significant difference vs. obese controls.
M: Male; F: female; BMI: body mass index; IU: international units; pts: patients; SU: sulfonylureum.

measured by an incremental cyclo-ergometer cardiac stress test). Patients had to use a minimum dosage of 20 IU insulin/day.

For each T2DM patient, 2 lean control subjects and 2 obese control subjects, matched for age, gender, race, geographical area and in the obese group for BMI were included in the study. In Table 1 baseline characteristics of the lean and obese control groups are summarized.

Patients and healthy controls were recruited via advertisements in local newspapers, and patients also via the outpatient clinics of the departments of Endocrinology/Internal Medicine in the Leiden University Medical Center. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of our center.

Study design

All T2DM patients discontinued their oral glucose-lowering medication three weeks prior to the start of the study. Two days before the start of the study the last dose of long-acting insulin was given and the day before the start of the study the last dose of short-acting insulin was given at the evening meal. The glucose-lowering medication remained discontinued during the 16-week intervention. After the baseline study day (as outlined below) T2DM patients started a 16-week VLCD (450 kcal/day) consisting of three sachets of Modifast® (Nutrition & Santé, Antwerp, Belgium) per day. Modifast® contains all necessary vitamins and micronutrients. In addition, 13 of the 27 patients simultaneously followed an exercise program. This weekly exercise program comprised a minimum of 4 days training at home for 30 minutes at 70% of maximum aerobic capacity on a cyclo-ergometer and a one-hour in hospital training under supervision of a physiotherapist (the attendance rate was 97%).

Patients visited the outpatient clinic weekly, for support, to keep up with the diet, measurement of bodyweight and to check glucoregulation. Compliance with the diet and exercise was confirmed by questioning, counting sachets of Modifast that were supplied weekly and reading the heart rate monitor which was worn during exercise sessions both at home and in the hospital (Polar S610 $_{i}^{tm}$, Polar Electro Oy, Finland). After the intervention a regular diet was slowly reintroduced with the aim of weight maintenance. Thereafter, patients were referred back to their original doctors and treated with routine care. The researchers performed a follow-up visit at 6 and 18 months after the start of the intervention.

At day 0 (start of the study), 16 weeks (end of the intervention), 6 and 18 months (follow-up) patients were studied in the morning after an overnight fast and after 2 days without any exercise. All patients performed all the follow-up visits, so no patients were lost to follow-up. The lean and obese healthy controls were studied only once. Length, weight, BMI and waist circumference were measured according to the World Health Organization recommendations. Blood pressure was measured with an Omron 705IT blood pressure device (Omron Matsusaka Co., Ltd., Japan) and recorded within the limits of 1 mmHg. Fat mass was assessed by bioelectrical impedance analysis (Bodystat® 1500 MDD, Bodystat Ltd., Douglas, Isle of Man, United Kingdom). Fasting blood samples were drawn for the measurement of plasma glucose, insulin, hemoglobin A1c (HbA1c), high-sensitive C-reactive protein (hsCRP), IL1, IL2, IL6, IL8, IL10, TNFα and interferon gamma (INFγ), total cholesterol (TC), high density lipoprotein (HDL)-cholesterol, triglyceride (TG) levels and free fatty acids (FFA). Low density lipoprotein (LDL)-cholesterol was calculated with the Friedewald formula. Ten-year CVD risk was calculated with the United Kingdom Prospective Diabetes Study (UKPDS) risk engine (17). Only at baseline and after the 16-week intervention rate of appearance (Ra) of glycerol and free fatty acid levels were measured. Basal rates of glycerol appearance were measured after a 1.5 hours of continuous infusion of [$^{2}H_{5}$]-glycerol (Cambridge Isotopes, Cambridge, USA) as described previously. A physiological and isotopic steady-state was achieved during the last 30 min therefore, the Ra of glycerol were calculated as the tracer infusion rate divided by the tracer-to-tracee ratio.

Assays

Plasma glucose, TC, HDL-cholesterol and TG concentrations were measured with a fully automated P-800 module (Roche, Almere, the Netherlands). Insulin was detected with an immunometric assay on an automated Immulite 2500 (Siemens, Breda, the Netherlands). Glycosylated hemoglobin (HbA1c) levels were measured with a high-performance liquid chromatography (HPLC) system (Variant, Biomed, Hercules, California, USA). hsCRP levels were determined with an enzyme-linked immonosorbent assay (DSL, Webster, Texas, USA). Plasma free fatty acids (FFAs) concentrations were measured by a commercial kit (Wako Chemicals, Neuss, Germany). Plasma levels of the various cytokines were assessed using precoated 96-well multispot plates from Meso Scale Discovery (MSD; Gaithersburg, Maryland, USA), an

enzyme linked immunosorbent assay (ELISA) based electrochemiluminiscence assay. Briefly, plates were incubated with plasma or calibrator (25 µl/well) for 2 hours. After washing (3x, PBS with 2% Tween 20), detection antibody labeled with MSD SULFO-TAG (1 µg/mL) was added and incubated for another 2 hours. Wells were washed and reading buffer was added. Plates were immediately read using the SECTOR Imager 2400 and cytokine concentrations were determined by a non-linear standard curve fit.

Statistical analyses

A general linear model for repeated measurements analyzed differences within the patient groups at the various time points. Differences between both intervention groups were analyzed by calculating a delta between two time points. The deltas were subsequently compared using the two-tailed Student's *t*-test for unpaired data or, when appropriate, by non-parametric tests for independent samples. Differences between all groups (the two intervention groups and the two healthy controls) were analyzed using a one-way ANOVA, LSD post-hoc tests were used in case of a significant F-ratio. Data are presented as mean±SEM. Data analyses were performed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

RESULTS

Effect on bodyweight and glucoregulation

The VLCD with exercise (VLCD+E) and VLCD-only groups did not differ with respect to clinical and metabolic parameters at baseline (Table 1). Moreover, gender, age, weight, BMI and waist circumference of the obese control group were well matched with the T2DM patients. The lean and obese control groups had significantly lower levels of fasting glucose and HbA1c as compared to the two patient groups. Medication use of the patients can be found in Table 1.

Bodyweight and glucoregulation improved significantly (baseline vs. after the 16 week intervention bodyweight: VLCD+exercise 114±5 vs. 86±4 p<0.0001; VLCD only 113±6 vs. 89±4 p<0.0001 HbA1c: VLCD+exercise 7.8±0.4 vs. 6.3±0.4 p<0.0001; VLCD only 7.8±0.3 vs. 6.7±0.3 p<0.0001) and to an equal extent in both intervention groups directly after the 16-week VLCD despite the cessation of all glucose-lowering medication. The VLCD+E group lost significantly more fat mass and waist circumference compared to the VLCD-only group (delta VLCD+exercise vs. delta VLCD fat mass -21.8±2.7 vs. -16.6±2.7 p=0.020; waist -24.8±1.5 vs. -19.3±1.7 p=0.049). At 6 and 18 months, both groups had gained weight. However, weight, fat mass and waist circumference were still significantly lower compared to baseline values in both groups (Table 2). Nevertheless at 18 months, glucoregulation had deteriorated in both intervention groups, mostly because patients were not restarted on insulin therapy (Table 3).

Table 2. Clinical, metabolic characteristics and cholesterol levels before, directly after 6, and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients and comparisons with (obese and lean) healthy controls.

		VLCD + exercise	VLCD only	controls	
				obese	lean
weight (kg)	baseline	114 ± 5 *	113 ± 6 *	112 ± 3 *	73 ± 2
	16 weeks	86 ± 4 !	89 ± 4 !		
	6 months	90 ± 5 ! ?	93 ± 5 ! ?		
	18 months	98 ± 5 ! ?	103 ± 5 ! ?		
BMI (kg/m^2)	baseline	36.4 ± 1.1 *	37.9 ± 1.4 *	37.6 ± 0.7 *	23.8 ± 0.3
	16 weeks	27.7 ± 1.0 !	30.0 ± 1.1 !		
	6 months	28.9 ± 1.3 ! ?	31.1 ± 1.2 ! ?		
	18 months	31.6 ± 1.2 ! ?	34.7 ± 1.3 ! ?		
waist (cm)	baseline	123 ± 3 *	122 ± 3 *	118 ± 2 *	87 ± 1
	16 weeks	98 ± 3 !	103 ± 3 !		
	6 months	101 ± 3 !	105 ± 3 !		
	18 months	107 ± 4 ! ?	114 ± 3 ! ?		
fat mass (kg)	baseline	45.4 ± 3.2 *	49.9 ± 3.6 *	39.2 ± 2.4 *	33.4 ± 2.2
	16 weeks	23.5 ± 2.2 !	33.2 ± 2.8 !		
	6 months	29.5 ± 2.8 ! ?	35.2 ± 2.9 ! ?		
	18 months	35.4 ± 2.6 ! ?	44.2 ± 3.0 ! ?		
systolic bloodpressure (mmHg)	baseline	145 ± 5	161 ± 4	149 ± 3	141 ± 3
	16 weeks	132 ± 5 !	140 ± 4 !		
	6 months	136 ± 4 !	142 ± 4 !		
	18 months	146 ± 5 ?	157 ± 4 ?		
diastolic bloodpressure (mmHg)	baseline	81 ± 3	87 ± 3	87 ± 1	82 ± 1
	16 weeks	75 ± 2	78 ± 2 !		
	6 months	77 ± 3	83 ± 2 ?		
	18 months	82 ± 2 ?	89 ± 3 ?		
heart rate (beats/min)	baseline	79 ± 2 *	82 ± 3 *	70 ± 2	67 ± 2
	16 weeks	64 ± 3 !	69 ± 3 !		
	6 months	65 ± 3 !	70 ± 3 !		
	18 months	70 ± 4	76 ± 3		
HbA1c (%)	baseline	7.8 ± 0.4 * #	7.8 ± 0.3 * #	5.5 ± 0.1 *	5.2 ± 0.0
	16 weeks	6.3 ± 0.4 !	6.7 ± 0.3 !		
	6 months	6.1 ± 0.4 !	6.7 ± 0.3 !		
	18 months	7.5 ± 0.6	8.2 ± 0.5 ?		
fasting glucose (mmol/L)	baseline	10.9 ± 0.7 * #	12.1 ± 0.5 * #	5.5 ± 0.1 *	4.9 ± 0.1
	16 weeks	6.6 ± 0.8 !	7.7 ± 0.6 !		
	6 months	7.4 ± 0.7 !	8.4 ± 0.8 !		
	18 months	9.2 ± 1.0	12.2 ± 1.1 ?		

Table 2. (continued)

		VLCD + exercise	VLCD only	controls obese	controls lean
fasting insulin (mU/L)	baseline	25 ± 2 * #	24 ± 4 * #	14 ± 1 *	5 ± 0
	16 weeks	9 ± 1 !	13 ± 2 !		
	6 months	9 ± 2 !	11 ± 2 ! ?		
	18 months	13 ± 2 !	17 ± 6		
HOMA-IR	baseline	12.3 ± 1.3 * #	12.9 ± 2.3 * #	3.4 ± 0.3 *	1.1 ± 0.1
	16 weeks	2.5 ± 0.2 !	4.3 ± 0.8 !		
	6 months	2.7 ± 0.5 !	4.3 ± 1.0 !		
	18 months	4.7 ± 0.8 ! ?	9.0 ± 3.1		
TC (mmol/L)	baseline	5.4 ± 0.4	6.1 ± 0.4	6.0 ± 0.1	6.0 ± 0.1
	16 weeks	4.5 ± 0.3 !	5.5 ± 0.3		
	6 months	4.4 ± 0.4 !	4.7 ± 0.3 ?		
	18 months	4.3 ± 0.3 !	5.5 ± 0.4		
TG (mmol/L)	baseline	2.5 ± 0.5 * #	2.3 ± 0.2 * #	1.6 ± 0.1 *	1.1 ± 0.1
	16 weeks	1.2 ± 0.1 !	1.5 ± 0.2 !		
	6 months	1.2 ± 0.2 !	1.5 ± 0.2 !		
	18 months	1.9 ± 0.3 ?	2.8 ± 0.6 ?		
HDL (mmol/L)	baseline	1.1 ± 0.0 * #	1.2 ± 0.1 * #	1.4 ± 0.0 *	1.8 ± 0.1
	16 weeks	1.2 ± 0.1	1.2 ± 0.1		
	6 months	1.4 ± 0.1 ! ?	1.5 ± 0.1 ?		
	18 months	1.2 ± 0.1	1.4 ± 0.1 !		
Chol:HDL ratio	baseline	4.9 ± 0.4 *	5.3 ± 0.4	4.2 ± 1.4 *	3.5 ± 1.4
	16 weeks	4.0 ± 0.3 !	4.8 ± 0.4 !		
	6 months	3.0 ± 0.2 !	3.1 ± 0.2 !		
	18 months	3.7 ± 0.3 !	4.1 ± 0.3 !		
LDL (mmol/L)	baseline	3.6 ± 0.3	4.4 ± 0.4	3.8 ± 0.1	3.7 ± 0.1
	16 weeks	3.0 ± 0.3 !	3.7 ± 0.3 !		
	6 months	2.4 ± 0.2 ! ?	2.8 ± 0.2 ! ?		
	18 months	2.5 ± 0.7 !	3.5 ± 1.0		
FFA (mmol/L)	baseline	0.9 ± 0.1	1.0 ± 0.1		
	16 weeks	0.9 ± 0.0	1.0 ± 0.1		
Ra glycerol (µmol/kgFFM/min)	baseline	12.9 ± 1.0	11.3 ± 1.2		
	16 weeks	15.9 ± 1.1 !	11.3 ± 1.3		

Data are presented as mean ± SEM. ! significant difference vs. baseline values within the group; ? significant difference vs. 16 weeks values within the group; * significant difference vs. lean controls; # significant difference vs. obese controls.

BMI: Body mass index; HOMA-IR: homeostatic model assessment of insulin resistance; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; FFA: free fatty acids; Ra: rate of appearance; FFM: fat free mass.

Table 3. Use of medication before, directly after 6 and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients.

	VLCD + exercise				VLCD only			
	before start of the study	16 weeks	6 months	18 months	before start of the study	16 weeks	6 months	18 months
insulin (IU/day)	77	0	0	0	86	0	2	36
metformin (*)	10	13	13	12	9	14	13	12
SU-derivatives (*)	3	0	1	4	1	0	5	4
statine (*)	9	13	13	12	9	14	13	10
0/1 antihypertensiva (*)	6	8	8	6	3	6	7	7
2 antihypertensiva (*)	3	3	3	4	6	4	3	1
3 of more antihypertensiva (*)	4	2	2	3	5	4	4	6
exercise (min/week)	34	180	132	192	24	0	75	45

IE: international units; SU: sulfonylureum; * number of patients.

Effect on lipids and blood pressure

After the 16-week VLCD ± exercise intervention, systolic blood pressure, TG, cholesterol /HDL-cholesterol ratio and LDL-cholesterol decreased significantly (Table 2), whereas TC improved only in the VLCD+E group. At 18 months the effects on blood pressure and TG were completely abolished in both intervention groups while the beneficial effect on the cholesterol/HDL-cholesterol ratio was maintained. The decline in LDL-cholesterol was sustained only in the VLCD+E group.

Ten year coronary heart disease risk estimates (UKPDS risk score) showed a non-significant improvement in both intervention groups (baseline vs. 18 months follow up: 17.4±2.9% to 11.0±1.8% VLCD+E group and 24.7±4.3% to 19.2±3.5% VLCD-only group).

Effect on low-grade inflammation

Directly after the 16-week intervention hsCRP levels declined only in the VLCD+E group (p=0.011) (Table 4). All plasma cytokines in both intervention groups were equal or even higher compared to baseline values immediately after the intervention. On the contrary, at 6 months hsCRP was lower in both the VLCD-only group (p=0.005) and the VLCD+E group (p=0.005). Moreover, all pro-inflammatory cytokines (including TNFα and IL6) were significantly lowered in both groups. At 18 months, hsCRP and all measured pro-inflammatory cytokines were still reduced compared to baseline in the VLCD+E group (p=0.038). In the VLCD-only group, hsCRP (p=0.065) and IL1 (p=0.410) returned to baseline whereas other plasma cytokine levels remained lowered.

Table 4. Low-grade inflammation before, directly after 6 and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients and comparisons with (obese and lean) healthy controls.

		VLCD + exercise	VLCD only	controls	
				obese	lean
hsCRP	baseline	5.1 ± 0.9 *	5.5 ± 1.1 *	4.5 ± 0.7 *	1.7 ± 0.5
	16 weeks	2.8 ± 0.7 !	4.9 ± 2.0 *		
	6 months	1.6 ± 0.4 ! #	2.2 ± 0.5 !		
	18 months	2.2 ± 0.7 !	3.3 ± 0.5		
IFNγ	baseline	2.6 ± 0.9	2.3 ± 0.2	2.7 ± 0.5	1.8 ± 0.4
	16 weeks	2.5 ± 0.4	2.0 ± 0.1		
	6 months	1.0 ± 0.3 ! ?	1.6 ± 0.3 !		
	18 months	2.0 ± 0.9 !	1.6 ± 0.3 !		
IL10	baseline	10.2 ± 2.9 * #	6.7 ± 1.8 *	4.2 ± 0.6	3.5 ± 0.7
	16 weeks	15.2 ± 6.9 * #	6.7 ± 2.0		
	6 months	7.4 ± 3.7 * ?	3.6 ± 1.9 ! ?		
	18 months	5.4 ± 2.2 !	3.8 ± 1.5 ! ?		
IL1	baseline	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.4 ± 0.1
	16 weeks	0.4 ± 0.1	0.8 ± 0.2 * #		
	6 months	0.2 ± 0.1 !	0.3 ± 0.1 ! ?		
	18 months	0.1 ± 0.0 ! ?	0.4 ± 0.1		
IL2	baseline	0.6 ± 0.1	1.0 ± 0.2 *	0.6 ± 0.1	0.3 ± 0.1
	16 weeks	0.7 ± 0.1	1.2 ± 0.2 * #		
	6 months	0.1 ± 0.0 ! ?	0.3 ± 0.1 ! ?		
	18 months	0.2 ± 0.1 ! ?	0.3 ± 0.1 ! ?		
IL6	baseline	2.0 ± 0.3	2.4 ± 0.4	3.1 ± 0.6	1.6 ± 0.4
	16 weeks	1.9 ± 0.2	2.2 ± 0.4		
	6 months	1.0 ± 0.1 ! ?	1.1 ± 0.2 ! ?		
	18 months	1.1 ± 0.2 ! ?	1.2 ± 0.2 ! ?		
IL8	baseline	6.3 ± 0.7 * #	7.5 ± 1.3 * #	2.6 ± 0.2 *	2.1 ± 0.1
	16 weeks	5.9 ± 0.5 * #	7.0 ± 0.9 * #		
	6 months	2.8 ± 0.4 ! ?	2.2 ± 0.3 ! ?		
	18 months	2.8 ± 0.3 ! ?	2.9 ± 0.4 ! ?		
TNFα	baseline	7.6 ± 1.3	6.6 ± 0.5	6.2 ± 0.6	5.8 ± 0.5
	16 weeks	7.2 ± 0.5 * #	7.0 ± 0.7		
	6 months	4.2 ± 0.5 ! ?	4.5 ± 0.6 ! ?		
	18 months	4.4 ± 0.5 ! ?	4.5 ± 0.6 ! ?		

Data are presented as mean ± SEM. ! significant difference vs. baseline values within the group; ? significant difference vs. 16 weeks values within the group; * significant difference vs. lean controls; # significant difference vs. obese controls.
hsCRP: high sensitive c-reactive protein; IFNγ: interferon gamma; IL: interleukin; TNFα: tumor necrosis factor alpha

Parameters of low-grade inflammation in healthy controls vs. patients

At baseline, both patient groups and the healthy obese controls had significantly higher hsCRP and IL8 levels compared to healthy lean controls, also patients had higher levels of the presumed anti-inflammatory cytokine IL10. Directly after the 16-week intervention hsCRP declined only in the VLCD+E group and not to the level of healthy lean controls. It was only 2 months after the cessation of both interventions (i.e. at 6 months), when patients were on a eucaloric diet, that parameters of chronic inflammation improved to the levels of healthy lean controls, with the exception of IL8 in the VLCD+E group (Table 4). At 18 months pro-inflammatory markers were still reduced and comparable to healthy lean controls in both patient groups.

DISCUSSION

This study shows that a 16-week VLCD or a 16-week VLCD+E have equal effects on lowering body weight and improving glucoregulation and dyslipidemia. Remarkably, this led not to an immediate lowering of markers of low-grade inflammation with the exception of a decrease in hsCRP in the VLCD+E group. Surprisingly, at 6 months all measured cytokines and hsCRP were significantly lower and comparable to the values in healthy lean controls. These effects were sustained after 18 months of follow-up. The direct effect of the 16-week intervention on the cardiovascular risk factors hypertension and dyslipidemia was not lasting; only cholesterol-HDL ratio and LDL-cholesterol were still lower compared to baseline at 18 months in the VLCD+E group.

The effect of dietary interventions on hsCRP levels has mostly been studied in obese non-diabetic subjects. Weight loss was clearly associated with a decrease in hsCRP in these subjects and is related to the amount of weight loss (9). Exercise had no, or only a minimal effect on hsCRP levels in either obese non-diabetic or obese diabetic patients (14,15,18). We showed that hsCRP levels decreased immediately after the intervention in the VLCD+E group. In the VLCD-only group, hsCRP levels declined just at six months when energy intake was eucaloric and weight loss still preserved. Interestingly, another study in obese T2DM patients, using a diet of ~1600 kcal for 8 weeks leading to 5-6 kg weight loss, also showed no improvement in hsCRP directly after the intervention but did so at 12 months follow-up (19). These findings could suggest that a VLCD combined with exercise is lowering activation of the acute phase response by the liver, while VLCD-only does not. The exact mechanism by which diets and exercise lower hsCRP levels needs further investigation.

In obese non-diabetic patients, diet, exercise or combined interventions have controversial effects on plasma IL6 and TNFα levels (10-13,20-22). Studies performed in obese T2DM patients are scarce. In one study, 7 obese T2DM women received a VLCD for 3 weeks which decreased plasma IL6 levels but had no effect on TNFα levels (16). We also observed no direct

effect of a VLCD of longer duration (16 weeks) on IL6 and TNFα levels but at 6 and 18 months both were improved compared to baseline in the two intervention groups alike. It should be noted that the effects of acute and chronic exercise are different (23). Acute exercise can elicit a pro-inflammatory response whereas chronic exercise is thought to mediate an anti-inflammatory effect. To purely study the chronic effects of exercise we performed our measurements after 2 days of abstinence of exercise. At 16 weeks no effects of a VLCD+E on plasma TNFα and IL6 levels were observed but at 6 and 18 months TNFα and IL6 levels were decreased compared to baseline. In several other exercise studies the effects on IL6 were equivocal and there were no effects on TNFα (15,22).

The effects on the presumed anti-inflammatory cytokine IL10 are paradoxical. We found elevated levels in both patient groups compared to healthy lean controls at baseline and a slow decrease in IL10 levels during the intervention with near-normalization at 18 months. On the contrary, a 4-week aerobic exercise training (3 times/week 60 minutes, level of intensity not specified) had no effect on IL10 levels in twenty obese T2DM patients (14) whereas a 6-month aerobic exercise intervention (4 times/week 45-60 minutes) significantly increased IL10 levels in 60 obese T2DM patients (24). Perhaps patient characteristics (obese vs. obese diabetics on insulin therapy), differences in intervention and/or assays used can explain the discrepancy found.

We could not find articles discussing the effects of a VLCD±exercise on the other cytokines measured (IL1, IL2 and INFγ). All these cytokines are pro-inflammatory cytokines. We hypothesize that this is the reason why these cytokines show the same trend as IL6 and TNFα.

The mechanisms by which CR, weight loss and exercise decrease plasma pro-inflammatory cytokines and increase anti-inflammatory cytokines are largely unknown. One putative mechanism is the decrease in visceral fat mass. Visceral adipose tissue (which consists of fat cells, adipose tissue macrophages (ATMs) and stromal vascular fraction) releases several so-called adipocytokines among which IL1, IL6 and TNFα that in turn stimulate CRP-production by the liver (25). Indeed, waist circumference, an indirect measure of visceral fat mass was decreased significantly as compared to baseline at all measured time points. Furthermore, the decrease in waist circumference was larger in the VLCD+E group, which indeed exhibited the greatest although non-significant beneficial effects on plasma cytokines. However, we could not find a correlation between the change in waist circumference and the change in plasma cytokines. The lack of an immediate effect of a VLCD+/-E on low-grade inflammation has been observed by others. In 22 obese women undergoing a 4 week VLCD (800 kcal/day) followed by a 2-month hypocaloric diet (-600 kcal of estimated energy requirements) a euglycaemic hyperinsulinaemic clamp with adipose tissue biopsies for adipocyte and macrophage gene expression and markers were performed (26). It appeared that adipocyte genes involved in metabolism were downregulated during the VLCD and upregulated during weight stabilization (hypocaloric diet) whereas macrophage genes involved in inflammatory pathways were upregulated during the VLCD (with increased plasma inflammatory markers) and downregulated during the

hypocaloric diet (with decreased plasma inflammatory markers). This is compatible with our findings of lower levels of inflammation after the 16 week-intervention during the weight stabilization period, and has recently been further investigated in mice (27). CR led to a gradual decrease in body weight and fat mass in previously high-fat fed mice due to a decrease in adipocyte size not number. However, ATM content increased during the first 3 days of the diet (and these were lipid-loaded macrophages) to gradually decrease thereafter. This rise and fall of ATM content was closely correlated with plasma free fatty acid levels and adipose tissue lipolysis. Total lipolysis is the sum of basal lipolysis (which is elevated in adiposity and greater in visceral vs. subcutaneous adipose tissue) and demand lipolysis (which is driven by hormones in response to nutritional demands). The authors speculate that in early weight loss, adipocyte size is the same and therefore basal lipolysis remains high. However, demand lipolysis increases with an increase in local free fatty acids that subsequently attract ATMs. These ATMs phagocytose excess lipids and might even secrete antilipolytic factors followed by a decrease of the free fatty acid-driven cytokine production. We only measured rate of appearance of glycerol and plasma free fatty acids in the patients before and after 16 weeks of a VLCD+/-E. Indeed, at 16 weeks free fatty acids levels were equal as compared to the start of the diet and rate of appearance of glycerol, which was high at baseline, had not changed in the VLCD-only group whereas it was further increased in the VLCD+E group. This suggests that our patients were still having a high (demand) lipolytic rate at 16 weeks (while still on the VLCD) with probably still elevated levels of ATMs, explaining the lack of decrease in plasma cytokines at 16 weeks. We presume that when a eucaloric diet was reinstituted the demand lipolysis decreased with lower local free fatty acid levels, a decrease in ATMs and subsequent decrease in plasma cytokines. This hypothesis, already tested in mice (27), should be further investigated in obese humans during various stages of CR with adipose tissue biopsies. The set-up will be limited however, by the fact that it is difficult to obtain visceral fat biopsies.

A limitation of the present study is the relatively small sample size. Nonetheless, our results are in line with the existing literature. Furthermore we measured the control population once only. The reason for this is that the lean control subjects were not able to perform a 16 week VLCD. Another limitation is the lack of fat biopsies. Fat biopsies could have provided more information for example with regard to the amount of macrophages in the fat mass. A possible confounder in the data could be that all patients in who insulin therapy was reinstated at 18 months came from the VLCD-only group, this could mean higher advanced glycated end products in this group. However fasting glucose levels and HbA1c levels were similar in both groups at 18 months, other advanced glycated end products have not been measured.

In conclusion, a 16-week VLCD with or without exercise does not directly decrease pro-inflammatory plasma cytokine levels despite a large decrease in body weight and waist circumference and an improvement in dyslipidemia and glucoregulation. The beneficial effects on chronic inflammation became apparent only when patients were on a eucaloric regular diet and were sustained up till 18 months after the start of the diet despite bodyweight re-

gain and deterioration of glucoregulation. Our findings are compatible with the hypothesis that demand lipolysis activation during CR leads to local free fatty acid accumulation with attraction of ATMs and hence an increased production of cytokines (27). When nutritional status is eucaloric and demand lipolysis decreases the amount of ATMs will decrease along with a reduction in cytokine production. This hypothesis should be confirmed in humans with adipose tissue biopsies during various amounts of calorie restriction. The initial question was whether exercise has additional beneficial effects on low-grade inflammation. This appears to be true only for hsCRP. Nonetheless, since chronic inflammation is associated with cardiovascular risk this study again shows the importance of weight reduction and exercise for the modification of these risk factors.

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CHAPTER 6

Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function

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Journal American College of Cardiology 2008;52:1006–12

ABSTRACT

Objectives. Myocardial triglyceride (TG) content is increased in patients with type 2 diabetes mellitus (T2DM) and may reflect altered myocardial function. It is unknown whether myocardial TG content is influenced during a therapeutic intervention. This study sought to assess the effects of prolonged caloric restriction in obese patients with T2DM on myocardial TG content and myocardial function.

Methods. Myocardial TG content (magnetic resonance [MR] spectroscopy), myocardial function (MR imaging), plasma hemoglobin A1c, and body mass index (BMI) were measured in 12 obese, insulin-treated T2DM patients before and after a 16-week very low calorie diet (VLCD) (450 kcal/day) to achieve substantial weight loss. Insulin was stopped during the VLCD.

Results. The BMI decreased from 35.6 ± 1.2 kg/m2 (baseline, mean ± SEM) to 27.5 ± 1.3 kg/m^2 (after the VLCD, $p < 0.001$) and was associated with an improvement in hemoglobin A1c from 7.9 ± 0.4% (baseline) to 6.3 ± 0.3% (after the VLCD, $p = 0.006$). Myocardial TG content decreased from 0.88 ± 0.12% to 0.64 ± 0.14%, respectively ($p = 0.019$), and was associated with improved diastolic function (reflected by the ratio between the early and atrial filling phase) from 1.02 ± 0.08 to 1.18 ± 0.06, respectively ($p = 0.019$).

Conclusions. Prolonged caloric restriction in obese T2DM patients decreases BMI and improves glucoregulation associated with decreased myocardial TG content and improved diastolic heart function. Therefore, myocardial TG stores in obese patients with T2DM are flexible and amendable to therapeutic intervention by caloric restriction.

INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are associated with increased deposition of triglycerides (TG) in nonadipose tissue, such as the heart, liver, pancreas, and skeletal muscle (1-4). There are indications from animal experiments and human observations that the increase in myocardial TG content is associated with altered myocardial function. In animal experiments, increased myocardial TG content is associated with impaired myocardial function (5,6) via complex routes involving free fatty acid (FFA) derivatives, such as FFA acyl-coenzyme A and diacylglycerol (7-9).

In humans, myocardial TG content can be measured noninvasively *in vivo* by proton magnetic resonance spectroscopy (^{1}H-MRS) (10-14). Studies have documented that increased myocardial TG stores in obese subjects are accompanied by increased left ventricular (LV) mass (13) and changes in LV diastolic function (2).

In healthy subjects, myocardial TG stores are not fixed, but vary depending on nutritional conditions. For instance, short-term caloric restriction dose-dependently increases myocardial TG content (15), whereas a single high-fat meal does not affect myocardial TG stores (12). Recently, we reported that the increase in myocardial TG content induced by short-term caloric restriction is associated with impaired diastolic function in healthy normal-weight subjects (15,16).

Caloric restriction is an important lifestyle factor in the treatment of obese patients with T2DM. However, the effects of caloric restriction on myocardial TG content have not been studied in these patients. Therefore, the primary goal of the present study was to evaluate the effects of prolonged caloric restriction in obese patients with T2DM on myocardial TG content and LV myocardial function in relation to metabolic regulation. In addition, T2DM is associated with ectopic deposition of TG in the liver (17,18). To assess the tissue-specific effects of caloric restriction, we also assessed liver TG content in these obese T2DM patients.

PATIENTS AND METHODS

Patients

We studied 12 obese (body mass index (BMI), mean ± SEM: 35.6 ± 1.2 kg/m^2) T2DM patients (7 men, 5 women). The mean duration of T2DM was 9.6 ± 1.4 years. The patients' age was 48.3 ± 2.8 years. Patients were recruited from the outpatient clinic. All subjects used insulin treatment (mean dosage 93 ± 21 IU/day) with or without concomitant use of oral blood glucose-lowering agents. Exclusion criteria were smoking; an abnormal stress electrocardiogram (ECG); the use of other medication known to influence lipolysis and/or glucose metabolism; and renal, hepatic, or other endocrine disease. Furthermore, subjects were excluded if the remaining insulin secretory capacity was insufficient, defined by fasting C-peptide levels <0.8

ng/l and/or <2-fold increase after glucagon stimulation (1.0 mg intravenously). This criterion was included because we documented in a previous study that preservation of the capacity of beta cells to secrete insulin predicts a favorable metabolic response to a very low calorie diet (VLCD) in obese T2DM patients (19,20). Body weight was stable for at least 3 months, and subjects were instructed not to change lifestyle habits (eating, drinking, and exercise) from screening until the start of the study. The protocol was approved by the institutional ethical committee, and all subjects provided written informed consent before participation.

Study design

The study consisted of 2 study occasions separated by a 16-week intervention period, during which the subjects used a VLCD to induce substantial weight loss. The VLCD consisted of 3 sachets of Modifast per day (450 kcal/day, Nutrition & Santé, Antwerp, Belgium), providing about 50 g protein, 50 to 60 g carbohydrates, and 6 g lipids daily. Three weeks before start of the intervention period, all oral blood glucose–lowering drugs were discontinued and the insulin therapy was intensified. Baseline magnetic resonance (MR) measurements were obtained in the postprandial state (4 h after the last meal) within 1 week before the start of the VLCD. Baseline blood samples were obtained after an overnight fast. At the start of the VLCD and during the whole intervention period, all glucose-lowering medication, including insulin, was discontinued. Six of the 12 subjects followed an exercise program in addition to the VLCD, but were not different with respect to outcome parameters. After 16 weeks, MR measurements (4 h after the last meal) were repeated. Blood samples were taken after an overnight fast.

^{1}H-MRS of the heart and the liver

All measurements were performed on a 1.5-T Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, the Netherlands) in the supine position. For ^{1}H-MRS measurements, a body coil for radiofrequency transmission and a surface coil (diameter of 17 cm) for signal receiving were used. A point-resolved spatially localized spectroscopic pulse sequence was used to acquire single-voxel (8 ml) spectra. For the heart, the voxel was placed in the myocardial septum on 4-chamber and short-axis images at end systole, avoiding contamination with epicardial fat. Data acquisition was double-triggered using ECG triggering and navigator echoes to minimize breathing artifacts (14). For the liver, voxel sites were matched at the study occasions (by using the 12th thoracic vertebra as an anatomical landmark), carefully avoiding blood vessels and bile ducts. Water-suppressed spectra with 128 averages were collected to detect lipid signals from the heart, and suppressed spectra with 64 averages were acquired from the liver. Spectral parameters included: repetition time of at least 3,000 ms, echo time 26 ms, and 1,024 data points over 1,000-kHz spectral width. Furthermore, unsuppressed spectra with 4 averages were acquired in the same voxel, using the same parameters except for a repetition time of 10 s. Spectra were analyzed in the time domain, using the

advanced MR algorithm in the Java-based MR user interface software (jMRUI version 2.2, A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium) (21), as described earlier (14). Peak estimates of lipid resonances of myocardial and hepatic TG at 1.3 and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (%TG, TG/water × 100) and used in further analysis.

Left ventricular function

Imaging was performed at 1.5 T in a single session together with spectroscopy measurements, using a body coil for radiofrequency transmission and a 5-element synergy coil for signal receiving. The heart was imaged in the short-axis orientation using an ECG-triggered, sensitivity-encoding balanced steady-state free procession sequence to assess systolic function. Imaging parameters were: field-of-view = 400 × 320 mm, matrix size = 256 × 256, slice thickness = 10 mm, slice gap = 0 mm, flip angle = 35°, echo time = 1.67 ms, and repetition time = 3.34 ms. Temporal resolution was 25 to 39 ms (depending on the heart rate). End-diastolic and –systolic images were identified on all slices, and dedicated postprocessing software (MASS, V2007-EXP, Leiden University Medical Center, Medis, Leiden, the Netherlands) was used to quantify LV ejection fraction, LV mass, cardiac output, stroke volume, and end-diastolic and -systolic volume as described previously (22). Furthermore, we calculated cardiac index, LV mass index, stroke volume index, end-diastolic index, and end-systolic index by dividing the parameter by body surface area. To asses LV diastolic function, an ECG-gated gradient echo sequence with velocity encoding was performed to measure blood flow across the mitral valve (23). Imaging parameters were: echo time = 5 ms, repetition time = 14 ms, flip-angle = 20°, slice thickness = 8 mm, field of view = 350 mm, matrix size = 256 × 256, velocity encoding = 100 cm/s, and scan percentage = 80%. Flow velocities in early diastole (E) and at atrial contraction (A) were measured and their peak flow ratio was calculated (E/A ratio) using the FLOW analytical software package (V2006-EXP, Leiden University Medical Center, Medis). Furthermore, the downslope of the early filling phase (E deceleration) and an estimation of LV filling pressures (E/Ea) (24) were calculated. During MR imaging, blood pressure and heart rate were measured with an automatic device (Dinamap DPC100X, Freiburg, Germany).

Assays

Plasma glucose, total cholesterol, and TG concentrations were measured on a fully automated P800 analyzer (Roche, Almere, the Netherlands). Insulin was measured with an immunoradiometric assay (Biosource, Nivelles, Belgium). Coefficients of variation were <2% for glucose and <5% for insulin. Plasma levels of free fatty acids (FFA) were measured using a commercial kit (FFA-C, Wako Chemicals, Neuss, Germany). The hemoglobin A1c levels were measured with an HPLC system (Variant, Biomed, Hercules, California). Leptin and adiponectin were measured with a radioimmunoassay from Linco Research (St.Charles, Missouri), with coefficients of variation ranging from 3.0% to 5.1% for leptin and 7% to 9% for adiponectin, and a

sensitivity of 0.5 µg/l. The high-sensitivity C-reactive protein enzyme-linked immunosorbent assay came from DSL (Webster, Texas). The sensitivity was 0.03 mg/l, and the coefficient of variation was between 3% and 6%.

Statistical analyses

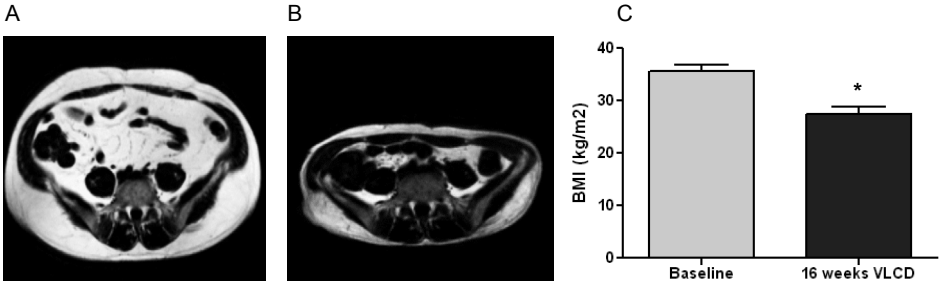
All statistical analyses were performed with SPSS version 14.0 (SPSS Inc., Chicago, Illinois). Statistical comparisons between baseline measurements and measurements after prolonged caloric restriction were made by paired *t* test. Data are shown as mean ± SEM. A value of $p < 0.05$ was considered to reflect significant differences.

RESULTS

Metabolic parameters

Caloric restriction reduced BMI from 35.6 ± 1.2 kg/m^2 at baseline to 27.5 ± 1.3 kg/m^2 after the intervention period ($p < 0.001$) (Figure 1). Metabolic parameters before and after prolonged caloric restriction are shown in Table 1 and Figure 2. After a 16-week VLCD, glycemic control was significantly improved; fasting plasma glucose levels decreased from 11.4 ± 0.6 mmol/l at baseline (despite glucose-lowering therapy by high-dose insulin) to 6.7 ± 0.6 mmol/l after prolonged caloric restriction (only on a VLCD without any glucose-lowering therapy for 16 weeks, $p < 0.001$). Furthermore, HbA1c levels decreased from 7.9 ± 0.4% to 6.3 ± 0.3% at baseline and after prolonged caloric restriction, respectively, $p = 0.006$). Plasma FFA levels were 0.92 ± 0.07 mmol/l at baseline and decreased to 0.67 ± 0.05 mmol/l after prolonged caloric restriction ($p < 0.001$) (Figure 2A). Furthermore, plasma levels of liver enzymes, total cholesterol, TGs, leptin, and C-reactive protein were significantly decreased after the VLCD compared with baseline, whereas plasma adiponectin levels were increased (Table 1, Figure 2).

Figure 1. Fat stores and body mass index. Example of a transverse slice at the level of the 5th lumbar vertebrae showing visceral and subcutaneous fat depots, illustrating the effects of a 16-week very low calorie diet (VLCD) in the same patient (A and B). Body mass index (BMI) is decreased after prolonged caloric restriction (C).



Data are presented as mean ± SEM. * $p < 0.001$.

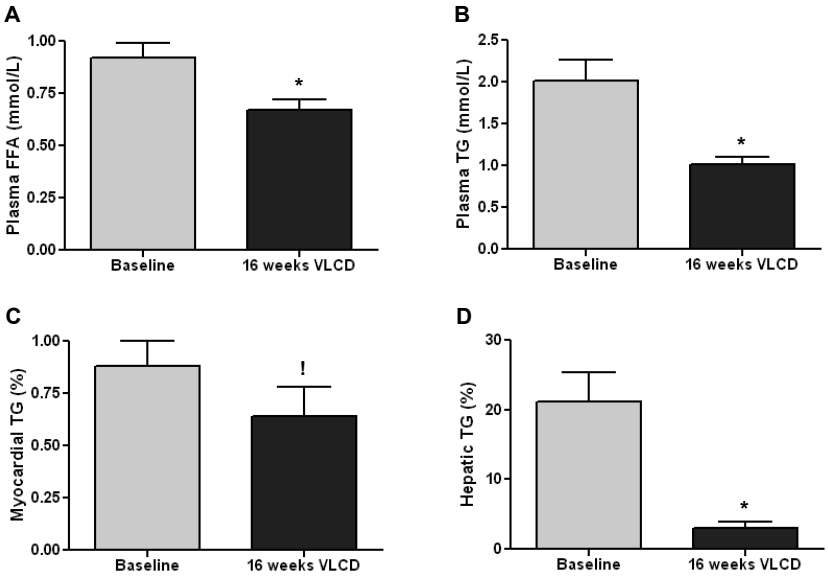
Table 1. Metabolic response to 16 weeks of caloric restriction in obese patients with type 2 diabetes mellitus.

	baseline	after 16 wks	
Glucose (mmol/l)	11.4 ± 0.6	6.7 ± 0.6	*
HbA1c (%)	7.9 ± 0.4	6.7 ± 0.6	!
Insulin (mU/l)	39 ± 9	10 ± 3	!
AST (mmol/l)	44 ± 5	27 ± 3	!
ALT (mmol/l)	52 ± 12	23 ± 3	!
γGT (mmol/l)	38 ± 5	18 ± 2	!
Total cholesterol (mmol/l)	5.7 ± 0.5	4.8 ± 0.2	!
Free fatty acids (mmol/l)	0.92 ± 0.07	0.67 ± 0.05	*
TG (mmol/l)	2.1 ± 0.3	1.1 ± 0.1	*
Leptin (µg/l)	21.5 ± 4.3	7.6 ± 3.4	*
Adiponectin (µg/l)	5.2 ± 0.7	7.8 ± 1.1	!
hsCRP (mg/l)	18.5 ± 4.2	7.5 ± 2.0	!

Data are mean ± SEM. * $p < 0.001$; ! $p < 0.01$ versus baseline.

γGT: gamma-glutamyl transferase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; hsCRP: high sensitive c-reactive protein; TG: triglyceride.

Figure 2. Metabolic changes at baseline and after a 16-week very low calorie diet (VLCD). Changes in plasma free fatty acids (FFA) (A), plasma triglyceride (TG) levels (B), and myocardial (C), and hepatic (D) TG content on prolonged caloric restriction.

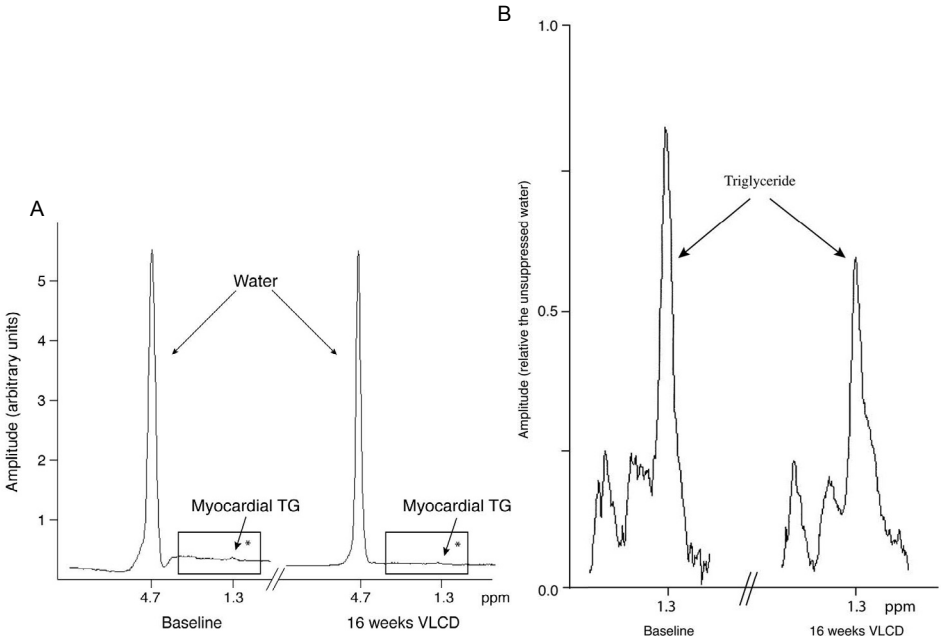


Data are mean ± SEM.* $p < 0.001$; ! $p < 0.05$.

Myocardial and hepatic TG content

Typical myocardial proton spectra of a patient at baseline and after caloric restriction are shown in Figure 3. Myocardial TG content decreased from 0.88 ± 0.12% (baseline) to 0.64 ± 0.14% (after the VLCD, p = 0.019, based on n = 11 successful myocardial spectral measurements) (Figure 2C). Concomitantly, hepatic TG content decreased from 21.2 ± 4.2% to 3.0 ± 0.9%, respectively (p < 0.001) (Figure 2D).

Figure 3. Myocardial Proton Spectra. Typical unsuppressed proton spectra of the same patient at baseline and after a 16-week very low calorie diet (VLCD) (A). The starred boxes indicate the part of spectrum where the myocardial lipids resonate, of which the suppressed spectra are shown in (B).



ppm: parts per million; TG: triglyceride.

Myocardial systolic and diastolic function

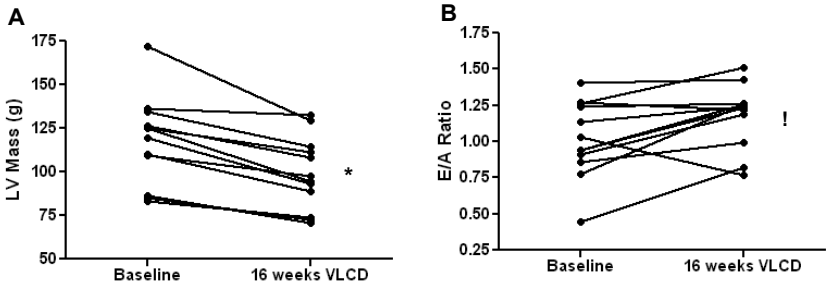
Systolic blood pressure decreased from 144 ± 8 mm Hg to 118 ± 6 mm Hg at baseline and after substantial weight loss, respectively (p < 0.001). Diastolic blood pressure decreased from 81 ±2 mm Hg at baseline to 71 ± 2 mm Hg after weight loss (p < 0.001). Heart rate was significantly decreased after substantial weight loss (Table 2). During caloric restriction, myocardial function improved. Cardiac output decreased significantly from 7,971 ± 601 ml/min at baseline to 6,508 ± 401 ml/min after prolonged caloric restriction (p = 0.001). Furthermore, LV mass was significantly decreased as well (from 118 ± 7 g to 99 ± 6 g, respectively, p < 0.001) (Figure 4A). The E/A ratio increased from 1.02 ± 0.08 at baseline to 1.18 ± 0.06 after the VLCD (p = 0.019), reflecting improved diastolic function (Figure 4B).

Table 2. Effects of 16 weeks of caloric restriction on systolic and diastolic function in obese patients with type 2 diabetes mellitus.

	baseline	after 16 wks	
Systolic blood pressure (mm Hg)	144 ± 8	118 ± 6	*
Diastolic blood pressure (mm Hg)	81 ± 2	71 ± 2	*
Heart rate (beats/min)	78 ± 3	61 ± 2	*
LVEF (%)	57 ± 2	58 ± 2	
Stroke volume (ml)	102 ± 6	103 ± 8	
Stroke volume index (ml/m^2)	45 ± 2	51 ± 3	!
Cardiac output (ml/min)	7971 ± 601	6508 ± 401	!
Cardiac Index (l/min/m^2)	3.5 ± 0.2	3.2 ± 0.2	
LV Mass (g)	118 ± 7	99 ± 6	*
LV mass index (g/m^2)	53 ± 3	49 ± 3	!
ED volume (ml)	177 ± 8	177 ± 11	
ED index (ml/m^2)	79 ± 3	88 ± 4	!
ES volume (ml)	76 ± 4	74 ± 5	
ES index (ml/m^2)	34 ± 2	37 ± 2	
E deceleration (ml/s^2 x 10^{-3})	4.04 ± 0.50	4.30 ± 0.42	
E/A peak ratio	1.02 ± 0.08	1.18 ± 0.06	!
E/Ea	11.9 ± 1.2	11.4 ± 1.5	

Data are mean ± SEM. * $p < 0.001$; ! $p < 0.05$ versus baseline.
A: atrial filling phase; E: early filling phase; E/Ea: estimated left ventricular filling pressure;
ED: end-diastolic; ES: end-systolic; LV: left ventricular; LVEF: left ventricular ejection fraction.

Figure 4. Changes in myocardial function. Intraindividual changes in left ventricular (LV) mass (A) and the ratio between the early filling phase and the atrial filling phase (E/A ratio) after a 16-week very low calorie diet (VLCD) (B).



* $p < 0.001$; ! $p < 0.05$.

DISCUSSION

This study shows that prolonged caloric restriction decreases BMI and considerably improves glucoregulation, associated with decreased myocardial TG content and beneficial effects on blood pressure and myocardial function in insulin-treated obese patients with T2DM. The data prove that myocardial TG stores in obese patients with T2DM are flexible and amendable to therapeutic intervention by caloric restriction.

Myocardial TG accumulation is the net result of excessive FFA uptake in relation to oxidative FFA requirements. In animal experiments, this increased myocardial TG pool is associated with impaired myocardial function (5,6). In human studies, myocardial TG accumulation is also associated with impaired myocardial function. For instance, a post-mortem study in obese patients with severe metabolic dysregulation and heart failure documented myocardial lipid accumulation that was higher in subjects suffering from obesity and T2DM (25). Recently, McGavock et al. (2) documented that in patients with T2DM myocardial TG content is increased, and suggested that myocardial TG accumulation precedes overt changes in systolic function. Therefore, myocardial TG content may be an interesting marker for the risk of nonischemic heart disease, and a potential surrogate marker for assessing the effects of metabolic interventions on the heart. In rodents, the restoration of myocardial TG metabolism is associated with improvements in cardiac function (6,26), in accordance with our findings. Nonetheless, the improvement in myocardial function on caloric restriction in the present study cannot merely be ascribed to the decreased myocardial TG stores, because there were also major alterations in other factors that affect cardiac mass and function, such as BMI and blood pressure.

Others reported beneficial effects of weight loss on cardiac function after bariatric surgery (27) or VLCD (28). Moreover, we found a decrease in heart rate, which is beneficial because heart rate is independently associated with increased mortality (29). In addition to this decreased heart rate, we observed a decrease in cardiac output and LV mass, in line with previously reported data (30). The LV ejection fraction was normal and did not change after the intervention period, in accordance with previous data showing that normal LV ejection fraction was unchanged 3 months after weight loss in obese subjects (31). The LV mass is predictive of cardiovascular morbidity and mortality and can be decreased by improvements in blood pressure (32). In addition, the decrease we found in LV mass is influenced by the substantial weight loss (33) and possibly by the improvements in insulin sensitivity (34). Because of the dramatic changes in body size, some of the indexed values for LV dimensions were changed after the intervention period. The LV mass index decreased, whereas the end-diastolic index was increased. The decrease in LV mass can directly influence LV filling pressures, and consequently, parameters of LV diastolic function (35). However, the presently used estimation of LV filling pressures (E/Ea) showed no changes after prolonged caloric restriction. Therefore, an alternative explanation for the increase in the E/A ratio may

be improved elastic properties of the LV, in line with results from animal models, documenting the relationship between myocardial TG accumulation and myocardial function (5,6). One of the alternative mechanisms may be that changes in plasma FFAs change the calcium homeostasis in the myocardium (36), which influences LV diastolic function (37). Furthermore, the present improvements in the inflammatory parameter C-reactive protein may influence myocardial function as well (38).

In addition to the decrease in myocardial TG content, the VLCD dramatically decreased hepatic TG content, associated with improvements in plasma lipid profile and liver enzymes. Moreover, insulin sensitivity was markedly increased after substantial weight loss, in accordance with previous studies (19,20,39,40). The improvement in hepatic TG content indicates that there is a general reduction in ectopic deposition of TG in nonadipose tissues, including the liver and heart.

There are limitations to this study. First, the study is descriptive and does not establish a causal relationship between myocardial TG accumulation and myocardial function, although the results are in accordance with data obtained in different animal models of obesity and, additionally, show the metabolic flexibility of the diabetic heart. Second, the sample size is relatively small. However, the patients are their own controls, and the magnitude of the metabolic and functional changes is illustrative because it indicates dynamic features of myocardial TGs and diastolic function.

In conclusion, prolonged caloric restriction in obese T2DM patients decreases BMI and improves glucoregulation associated with decreased myocardial TG content and improved diastolic heart function. Therefore, myocardial TG stores in obese patients with T2DM are flexible and amendable to therapeutic intervention by caloric restriction.

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CHAPTER 7

Sustained cardiac remodeling after a 16-week very low calorie diet in type 2 diabetes mellitus patients

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Submitted

ABSTRACT

Objective. A 16-week very low calorie diet (VLCD) in type 2 diabetes mellitus (T2DM) patients results in cardiac remodeling and improved diastolic function. It is unknown how long these effects sustain after reintroduction of a regular diet. Therefore, we aimed to assess the long-term effects of initial weight loss by a VLCD on cardiac dimensions and function in patients with T2DM.

Methods. Fourteen patients with insulin-dependent T2DM (mean ± SEM: age 53 ± 2 years; body mass index (BMI) 35 ± 1 kg/m^2) were treated by a VLCD (450 kcal/day) during 16 weeks. Cardiac function was measured by magnetic resonance imaging before and after the 16-week VLCD and again after 14 months of follow-up on a regular diet.

Results. BMI decreased from 35 ± 1 kg/m^2 to 28 ± 1 kg/m^2 after the VLCD and increased again to 32 ± 1 kg/m^2 at 18 months (both $p<0.05$ vs. baseline). Left ventricular (LV) end-diastolic volume index increased after the 16-week VLCD (80 ± 3 to 89 ± 4 ml/m^2, $p<0.05$) and remained increased after 14 months of follow-up (90 ± 3 ml/m^2; $p<0.05$ vs. baseline) at comparable filling pressures. The improvement in LV diastolic function after the 16-week VLCD, was sustained after 14 months of follow-up (early (E) / atrial (A) diastolic filling phase ratio: 0.96 ± 0.07 (baseline); 1.12 ± 0.06 (after VLCD); 1.06 ± 0.07 (18 months, $p<0.05$ vs. baseline)).

Conclusion. Weight reduction by a 16-week VLCD in T2DM patients results in sustained cardiac remodeling and improved diastolic function after 14 months of follow-up, despite weight regain on a regular diet.

INTRODUCTION

Diastolic dysfunction in type 2 diabetes mellitus (T2DM) is associated with an increased risk of development of heart failure and mortality, independent of coronary disease and hypertension (1). Moreover, even well-controlled patients with uncomplicated T2DM have significantly lower left ventricular (LV) end-diastolic volume indices compared to age-matched healthy controls (2). Accordingly, cross-sectional data from the Framingham Offspring cohort have indicated an association between increased insulin resistance and concentric remodeling of the LV (3). Several mechanisms underlying diastolic dysfunction in T2DM have been proposed including: hypertension, fibrosis and deposition of advanced glycation end products (AGEs), altered calcium handling and myocardial lipotoxicity (4,5).

Weight loss and lifestyle alteration are important cornerstones in the treatment of T2DM. In obesity, diet-induced weight loss not only improves insulin resistance, but also diastolic cardiac function (6-8). In obese patients with T2DM, we showed that a 16-week VLCD improves diastolic function, associated with a decrease in myocardial triglyceride (TG) content (9). There are no data available on long-term effects of diet-induced initial weight loss on cardiac function and myocardial TG content, particularly in T2DM. Therefore, we performed a 14 month follow-up study after completing a 16-week very low calorie diet (VLCD) in obese patients with insulin-dependent T2DM. We assessed cardiac function by cardiac MR imaging and myocardial triglyceride (TG) content by MR spectroscopy.

PATIENTS AND METHODS

Patients

We included 14 insulin-dependent T2DM patients (8 men, mean age: 53 ± 2 years, diabetes duration: 9±1 years). Patients were eligible for inclusion if they: had stable weight at baseline, were non-smoking, had a normal stress electrocardiogram, did not use medication which is known to influence lipolysis and/or glucose metabolism, had no other endocrine disease and had sufficient residual insulin secretory capacity. The details of the initial VLCD study have been previously described (9). To increase the power we included 2 extra patients who underwent the VLCD and were studied after 14 months of follow-up, therefore the exact numbers at baseline and at 16 weeks in this follow-up study, are slightly different from our previous publication (9).

Patients were studied on three occasions: at baseline (within 1 week before the start of the VLCD), directly after the 16-week VLCD and at 18 months (78.9 ± 1.6 weeks) from baseline. The VLCD consisted of 3 meals per day containing 450 kcal/day and all essential micro- and macronutrients (Modifast, Nutrition & Santé, and Antwerpen, Belgium). During the 16-week VLCD all glucose-lowering medication, including insulin therapy was discontinued. After the

VLCD, patients were reintroduced to a regular diet. Six months after the start of the intervention all patients were referred back to their own specialist, for regular medical care including if necessary reintroduction of glucose-lowering medication.

This study was approved by the local ethics committee. Written informed consent was obtained from all patients and the study was performed in accordance with the Declaration of Helsinki.

Cardiac function

All MR measurements were performed in the postprandial state (4 hours after the last meal) and in supine position. During MR imaging, blood pressure and heart rate were measured, using an automatic device (Dinamap, DPC100X, Freiburg, Germany).

We used a 1.5 Tesla Gyroscan ACS-NT MR imaging scanner (Philips, Medical Systems, Best, The Netherlands), with a body coil for radiofrequency transmission and 5-element synergy coil for signal receiving. A sensitivity encoding balanced steady-state free processing sequence, with ECG-gating and breath holding, was used with the following parameters: echo time (TE) = 1.7ms, repetition time (TR) =3.4 ms, slice gap = 0 mm, flip angle = 35°, field of view = 400 * 320 mm, reconstructed matrix size = 256*256, slice thickness = 10 mm, to image the heart in short-axis orientation from apex to base with 12 slices. All images were quantitively analyzed using dedicated software (MASS, Medis, Leiden, the Netherlands) to assess left ventricular (LV) end-diastolic volume, LV end-systolic volume, stroke volume, cardiac output, LV ejection fraction and LV mass (10). We calculated cardiac index, LV end-diastolic volume index, LV end-systolic volume index, LV stroke volume index and LV mass index, by dividing the parameters by the body surface area. Flow dynamics across the mitral valve were assessed using an ECG-gated gradient-echo sequence with velocity encoding with the following scan parameters: TE = 4.8 ms, TR = 14 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350*350 mm, matrix size = 256*256, velocity encoding = 100 cm/s and scan percentage = 80%. Flow encoded MRI data were analyzed using the FLOW software package (Medis, Leiden, the Netherlands). The early filling phase (E) and atrial contraction (A) were analyzed and the maximum flow rate of E and A were calculated to obtain an E/A peak flow ratio. Furthermore we quantified an estimation of filling pressure (E/Ea) (11).

Proton magnetic resonance spectroscopy

The body coil was used for radiofrequency transmission and a surface coil (17 cm) for signal receiving. A point resolved spectroscopy sequence was used to acquire single-voxel spectra (12). The voxel (8 ml) was placed in the interventricular spectrum and data were acquired at end-systole. Spectral parameters were: TR of at least 3000 ms, TE = 26 ms. A total of 1024 data points was collected over a 1000-Hz spectral width. Data acquisition was ECG-triggered and with respiratory echoes, to minimize breathing influences. Water-suppressed spectra with 128 averages and unsuppressed spectra with 4 averages (TR = 10000) were acquired, using

the same voxel location. Spectra were analyzed using Java-based MR user interface software (jMRUI version 2.2) as previously described (12). Myocardial triglycerides (peak at 1.3 parts per million (ppm) and 0.9 ppm were summed) were calculated as percentage of the unsuppressed water signal (TG/water*100).

Statistical analyses

All statistical analyses were performed using SPSS 17.0 (SPSS Inc.Chicago, Illinois, USA). A 2-tailed probability value of 0.05 or less was considered statistically significant. Data are expressed as mean values ± standard error of the mean (SEM) or median (interquartile range). Non-normally distributed data were log-transformed and checked for normality after transformation. A general linear model for repeated measures, with time as within-subject factor was used for comparison between the three assessments. LSD post-hoc tests were used in case of a significant F-ratio. GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, USA) was used for creation of the figures.

RESULTS

Body mass index (BMI) decreased from 35 ± 1 kg/m^2 to 28 ± 1kg/m^2 ($p<0.05$) after the VLCD. Patients regained weight during the 14 months of follow-up to a BMI of 32 ± 1 kg/m^2 ($p<0.05$ vs. baseline, Table 1). At baseline, all patients used insulin, 8 patients used metformin and 3 patients a sulfonylureum (SU)-derivate. During the 16 week-VLCD all patients had stopped insulin therapy and oral glucose-lowering medication. Directly after the VLCD 14 patients used metformin and 3 patients a SU-derivate. At 18 months, four patients had restarted insulin therapy, 12 patients used metformin and 4 patients used a SU-derivate.

Table 1. Changes in metabolic parameters at baseline, after a 16-week very low calorie diet and after 14 months of follow-up on a regular diet.

	baseline	16 week VLCD		18 months		
Body mass index (kg/m^2)	35.3 ± 1.1	27.5 ± 1.1	*	31.7 ± 1.1	*	!
HbA1c (%)	8.4 ± 0.3	7.0 ± 0.4		7.7 ± 0.5		
fasting glucose (mmol/L)	11.9 ± 0.6	7.8 ± 0.8	*	10.4 ± 1.0		!
fasting insulin (mU/L)	23.5(14.3-29.3)	10.0(6.8-12.0)	*	6.5(5.0-14.3)		
Free fatty acids (mmol/L)	0.97 ± 0.07	0.76 ± 0.08	*	0.68 ± 0.06	*	
Triglycerides (mmol/L)	2.3 ± 0.2	1.2 ± 0.1	*	2.8 ± 0.6		!

Data are expressed as mean ± SEM. * $p<0.05$ vs. baseline, ! $p<0.05$: 18 months vs.16 weeks

Hemodynamics

Systolic and diastolic blood pressures decreased from 155 ± 5 mmHg and 89 ± 3 mmHg respectively at baseline to 136 ± 4mmHg and 80 ± 2 mmHg directly after the 16-week VLCD ($p<0.05$, Table 2). At 18 months both systolic and diastolic blood pressures returned to baseline values (149 ± 5 mmHg and 88 ± 3 mmHg resp.). Heart rate decreased from 85 ± 2 beats/min at baseline to 69 ± 3 beats/min after the VLCD ($p<0.05$) and remained decreased at 18 months (71 ±3 beats/min, $p<0.05$ vs. baseline). This resulted in a decreased rate pressure product after the VLCD and at 18 months compared to baseline (baseline: 13218 ± 636 beats/min·mmHg vs. 16 weeks: 9426 ± 550 beats/min·mmHg, $p<0.05$; 18 months: 10592 ± 576 beats/min·mmHg, $p<0.05$ vs. baseline).

Prescription of antihypertensives did not significantly change during the study (number of classes of antihypertensives prescribed: at baseline: 2.4 ± 0.3; after VLCD: 1.9 ± 0.3; 18 months: 2.1 ± 0.3 ($p>0.05$). Statin use did not significantly change during the study either.

Table 2. Changes in hemodynamic parameters and cardiac function at baseline, after a 16-week very low calorie diet, after 14 months of follow-up on a regular diet.

	baseline	16 week VLCD		18 months	
Hemodynamics					
Systolic blood pressure (mmHg)	155 ± 5	136 ± 4	*	149 ± 5	
Diastolic blood pressure (mmHg)	89 ± 3	80 ± 2	*	88 ± 3	
Heart rate (beats/min)	85 ± 2	69 ± 3	*	71 ± 3	*
Rate pressure product (beats/min.mmHg)	13218 ± 636	9426 ± 550	*	10592 ± 576	*
Cardiac function and dimensions					
LVEDV (ml)	177 ± 9	177 ± 11		190 ± 9	
LVEDV index (ml/m^2)	80 ± 3	89 ± 4	*	90 ± 3	*
LVESV (ml)	78 ± 5	75 ± 5		80 ± 4	
LVESV index (ml/m^2)	35 ± 2	38 ± 2		38 ± 2	
LV stroke volume (ml)	99 ± 6	102 ± 7		110 ± 7	*
LV stroke volume index (ml/m^2)	45 ± 2	51 ± 3	*	52 ± 2	*
Cardiac index (L/min/m^2)	4 ± 0.2	3.3 ± 0.2		3.6 ± 0.2	
Ejection fraction (%)	56 ± 2	57 ± 2		57 ± 1	
LV mass (g)	119 ± 8	102 ± 7	*	109 ± 9	*
LV mass index (g/m^2)	54 ± 3	51 ± 3		51 ± 3	
LV mass/LVEDV	0.67 ± 0.03	0.59 ± 0.03	*	0.56 ± 0.03	*
E/A ratio	0.96 ± 0.07	1.12 ± 0.06	*	1.06 ± 0.07	*
E/Ea	10.0 ± 1.4	10.2 ± 1.3		9.9 ± 0.8	

Data is expressed as mean ± SEM. * $p<0.05$ vs. baseline, ! $p<0.05$: 18 months vs. 16 weeks.
LV: left ventricular; EDV: end-diastolic volume; ESV: end-systolic volume; E: early diastolic filling phase; A: diastolic atrial contraction; E/Ea: estimate of left ventricular filling pressure.

Cardiac dimensions and function

LV end-diastolic volume, end-systolic volume and end-systolic volume index did not change significantly after the 16-week VLCD and at 18 months (Table 2). End-diastolic volume index showed a sustained increase (baseline: 80 ± 3 ml/m^2, 16 weeks: 89 ± 4 ml/m^2, 18 months: 90 ± 3 ml/m^2 (both p<0.05 vs. baseline)). This increase occurred with unchanged filling pressures (Figure 1). Simultaneously, LV stroke volume index increased from 45 ± 2 ml/m^2 to 51 ± 3 ml/m^2 after the VLCD and 52 ± 2 ml/m^2 at 18 months (both p<0.05 vs. baseline).

Cardiac index and ejection fraction did not significantly change directly after the VLCD and at 18 months compared to baseline (Table 2). LV mass showed a sustained decline from 119 ± 8 grams to 102 ± 7 grams at 16 weeks and 109 ± 9 grams at 18 months (both p<0.05 vs. baseline). LV mass index was unchanged between the occasions (Table 2). A reduction in LV mass / LV end-diastolic volume was observed (0.67 ± 0.03 at baseline to 0.59 ± 0.03 at 16 weeks and 0.56 ± 0.03 at 18 months resp. (both p<0.05 vs. baseline, Figure 1)). Diastolic E/A

Figure 1. Relation of LV end-diastolic volume index and LV filling pressure. Relation of LV end-diastolic volume index (LVEDVI) and E/Ea ratio (estimate of LV filling pressure) at baseline (●), after a 16-week very low calorie diet (VLCD) (■) and after an additional 14 months of follow-up on a regular diet (▲) in 14 patients with T2DM. Bars represent means ± SEM. Note the increase in LVEDVI from 80±3 ml/m^2 at baseline to 89±4 ml/m^2 after a 16-week VLCD and 90±3 ml/m^2 after an additional 14 months of follow-up on a regular diet.

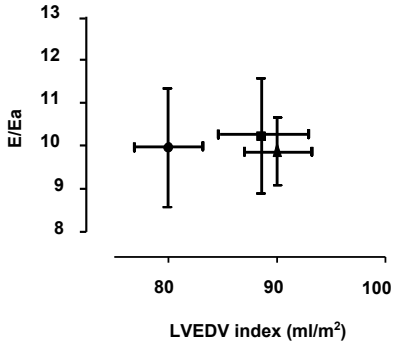
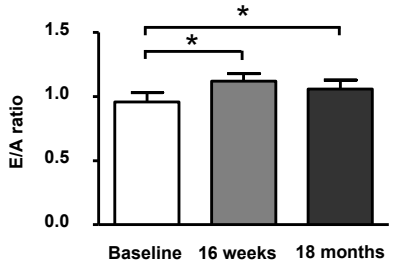


Figure 2. Change in left ventricular diastolic function. Changes in E/A ratio at baseline (white bar), after a 16-week VLCD (grey bar) and after an additional 14 months of follow-up on a regular diet (black bar) in 14 patients with T2DM. Note the sustainment of improved diastolic heart function at 18 months as compared to baseline.



Data are expressed as mean ± SEM. * p<0.05

VLCD: very low calorie diet; E/A ratio: ratio between early filling phase and atrial filling phase.

ratio increased after the VLCD and remained increased compared to baseline at 18 months (baseline: 0.96 ± 0.07; after VLCD: 1.12 ± 0.06, 18 months: 1.06 ± 0.07, both $p<0.05$ vs. baseline, Figure 2). E/Ea did not significantly change compared to baseline.

Myocardial TG content

Myocardial TG content decreased from 0.74 (0.41-1.10)% at baseline to 0.45 (0.31-0.54)% after the VLCD ($p<0.05$ vs. baseline, based on n = 11 successful ^{1}H-MRS measurements on all three occasions), but had returned to baseline values at 18 months (0.76 (0.65-1.32)%, $p>0.05$ vs. baseline).

DISCUSSION

This study aimed to assess the long-term effects of an initial 16-week VLCD on cardiac measures and function in obese patients with insulin dependent T2DM. Our previous study demonstrated that a 16-week VLCD decreased LV mass and improved diastolic function in insulin-dependent, obese T2DM patients (9). The present study extends these observations by showing that cardiac remodeling and diastolic function remains improved even after 14 months of follow-up on a regular diet, despite some weight regain.

Systolic and diastolic blood pressures decreased upon the use of the VLCD, but returned to baseline values after 14 months of follow-up. Nonetheless, the sustained decreased heart rate resulted in a decreased rate pressure product at 16 weeks and 18 months compared to baseline. The rate-pressure product is an estimate of myocardial oxygen consumption (13,14). In diabetic mice, cardiac efficiency is decreased, which is related to increased myocardial oxygen consumption (15). Therefore, the sustained decreased rate pressure product in our patients may reflect improved cardiac efficiency. Accordingly, short-term clinical studies found that weight loss induced a decrement in blood pressure (6,8,16) and rate pressure product (8) in obese, non-diabetic subjects.

Previously, it was shown that T2DM patients have decreased LV end-diastolic volume indices compared to healthy controls, associated with diminished compliance (2). In this study we show that weight loss can reverse this process up to 14 months of follow-up, by an increase in LVEDV index at comparable estimated LV filling pressure (E/Ea, Figure 1). The E/A ratio, a load-dependent parameter, also remained improved during long-term follow-up. However since the E/Ea ratio did not change, this change in E/A ratio most likely reflects an improvement in diastolic function. In accordance with our observation in T2DM patients, a 2-year caloric restriction study (maximum weight loss at 6 months) performed in obese, non-diabetic subjects found an improvement in diastolic function, which lasted up to 2 years after the start (6). Rider et al. (17) accordingly found an improvement in LV diastolic function after 1 year of weight loss in obese non-diabetic subjects, however they did not find improvement

in LV remodeling. Our study is the first to show that weight reduction also leads to sustained improvement in diastolic function and an increase in compliance of the left ventricle in T2DM patients.

Little is known about the underlying pathophysiological mechanisms of the improved diastolic function after weight loss. Van Heerebeek et al. (18) demonstrated that heart failure in patients with normal ejection fraction is associated with an increase in cardiomyocyte diameter and a higher resting tension of the cardiomyocytes, whereas AGEs and collagen deposition were more important in patients with restricted ejection fraction. In obese diabetic mice, diet-induced weight loss also resulted in improved diastolic function, which could be partly attributed to normalization in SERCA2 activity, which determines the Ca2+ removal from the myofilaments

We aimed to assess changes in myocardial triglyceride content, because previous studies showed an inverse correlation between myocardial triglyceride content and cardiac function (9,20,21). This association was explained by the concept of lipotoxicity. Accordingly, we found a decrease in myocardial TG content directly after the VLCD. However, after 14 months of follow-up, myocardial TG content returned to baseline values, even though there was sustained improvement in cardiac function. Viljanen et al. (8) studied the effect of a 6-week VLCD in obese subjects, without diabetes and similarly found a decrease in myocardial TG content. Apparently the regain in myocardial TG content does not immediately correspond with deterioration in diastolic function, which argues against a simple relation between cardiac triglyceride content and cardiac dysfunction. In previous short-term nutritional interventions (20,21) we showed a relation between increases in myocardial TG content and deteriorated diastolic function in healthy subjects and subjects with T2DM. It could be hypothesized that the regain in myocardial TG content precedes deterioration in diastolic function. Otherwise, the contribution of the positive effects on cardiac remodeling may be more substantial to diastolic function than the regain in myocardial TG content.

In accordance with our results, other studies have demonstrated a decrease in LV mass (6-8,16,17,22) after diet-induced weight loss in obesity, however no change in LV mass index (16,17,22). As insulin acts as a growth factor and can induce cardiac hypertrophy, the persistently decreased plasma insulin levels could contribute to the decrease in LV mass in our study. Accordingly, in obese rats a VLCD decreased myocardial mass, due to a decrease in myocardial cell size during weight loss and a strong association between the relative decrease in heart weight and the decrease in body weight was found (23).

A limitation of the present study is the relatively small sample size. The extensive dietary intervention, long-term follow-up and imaging protocol did not allow to include more patients. However, patients served as their own controls. Moreover, we used the highly reproducible and sensitive techniques of MRI and ^{1}H-MRS in controlled metabolic conditions. Using this study design, we observed major short-term effects on hemodynamic and cardiac parameters, which are in line with the existing literature.

In conclusion, weight reduction by a 16-week VLCD in T2DM patients results in sustained cardiac remodeling and improved diastolic function after 14 months of follow-up, despite weight regain on a regular diet.

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CHAPTER 8

Long-term beneficial effect of a 16-week very low calorie diet on pericardial fat in obese type 2 diabetes mellitus patients

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Submitted

ABSTRACT

Objective. Pericardial fat accumulation has been associated with an increased cardiovascular risk. A very low calorie diet (VLCD) improves the cardiovascular risk profile in patients with type 2 diabetes mellitus (T2DM), by improving the metabolic profile, heart function and triglyceride (TG) stores in (non)adipose tissues. However, long-term effects of a VLCD on pericardial fat volume and tissue-specific TG accumulation have not been documented. The aim of this study was therefore to assess the effects of a 16-week VLCD and of subsequent 14 months follow-up on a regular diet on pericardial fat in relation to other TG stores in obese T2DM patients.

Methods. We included 14 obese patients with insulin-treated T2DM (mean±SEM: age 53±2 years; BMI 35±1 kg/m^2). Pericardial fat and other (non)adipose TG stores were measured using magnetic resonance (MR) imaging and proton spectroscopy before and after a 16-week VLCD and after a 14-month follow-up without dietary interventions.

Results. A 16-week VLCD reduced bodyweight, pericardial fat, hepatic TG content, visceral and subcutaneous abdominal fat volumes to 78, 83, 16, 40 and 53% of baseline values respectively, (all $p<0.05$). After an additional 14 months of follow-up on a regular diet, the reduction in pericardial fat volume sustained, despite a substantial regain in body weight, visceral abdominal fat and hepatic TG content (resp. 90, 83 and 73% of baseline values).

Conclusion. VLCD-induced weight loss in obese T2DM patients is accompanied by a substantial decrease in pericardial fat volume, which is sustained even after subsequent weight regain.

INTRODUCTION

One of the cornerstones in the treatment in type 2 diabetes mellitus (T2DM) is lifestyle intervention with diet-induced weight reduction and exercise. A very low calorie diet (VLCD) is safe and effective to induce considerable weight loss and to improve insulin resistance, even in patients with insulin-dependent T2DM (1-3). During short-term weight loss, there is a close relationship between tissue-specific decreases in triglyceride (TG) stores and improvement in insulin sensitivity in obese nondiabetic and diabetic patients. Even a small reduction in weight can induce major reductions in hepatic TG content already within the first two weeks of a diet (4,5) and visceral abdominal fat even within 3 days of a VLCD in patients with T2DM (6). Reductions in hepatic TG content and visceral fat are associated with improvements in hepatic and peripheral insulin sensitivity (7,8). Studies on the long-term effects of a period of VLCD in T2DM have only focused on weight reduction and metabolic effects. The long-term effects of a VLCD on TG accumulation in pericardial fat and other (non)adipose tissues have not been studied.

Pericardial fat is adipose tissue surrounding the heart, which consists of two layers: epicardial and paracardial fat (9). An increased pericardial fat volume has been associated with insulin resistance in non-diabetic obese subjects (10) and T2DM patients (11). Moreover it has been associated with an increased cardiovascular risk (9,12-17). Recently, it has been documented by ultrasound studies that weight loss is associated with a decrease in pericardial fat stores in non-diabetic obese subjects (18,19). These studies also reported a larger decrease in visceral fat volume compared to pericardial fat volume (19). Dietary effects on pericardial fat have been studied only to a limited extent in patients with T2DM.

Therefore, the aim of this study was to assess the effects of a 16-week VLCD and subsequent 14-month follow-up on a regular diet on pericardial fat in relation to other TG stores in obese insulin-dependent patients with T2DM, using magnetic resonance imaging and spectroscopy.

PATIENTS AND METHODS

Patients

Fourteen obese patients (8 men/6 women) with T2DM were studied (mean ± standard error of mean (SEM): age: 53 ± 2 years). Patients were diagnosed with T2DM since 9 ± 1 years and used 81 ± 16 units of insulin per day. Selection criteria were: body mass index (BMI) > 30 kg/m^2, use of at least 20 units of insulin per day with or without oral blood glucose-lowering agents, remaining endogenous insulin secretory capacity (defined as fasting C-peptide levels > 0.8 ng/liter and/or a twofold increase in C-peptide levels upon infusion of 1 mg glucagon) and stable bodyweight during 3 months before inclusion. Exclusion criteria were: smoking, abnormal stress-electrocardiogram (ECG), renal, hepatic or other endocrine disease and contra-indications for magnetic resonance imaging (MRI).

This study was approved by the local ethics committee. Written informed consent was obtained from all patients and the study was performed in accordance with the Declaration of Helsinki.

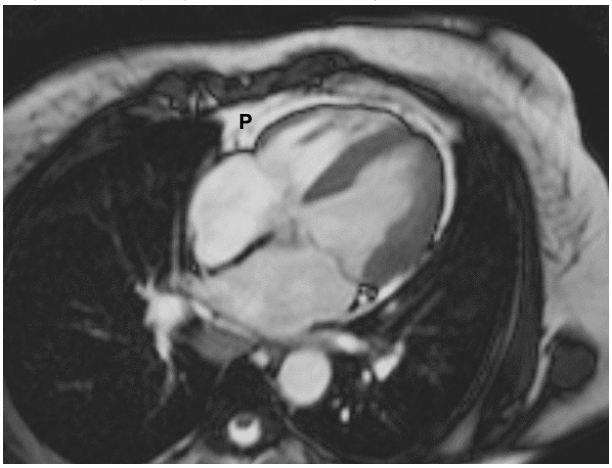
Study procedure

The study procedure during the 16-week VLCD has been described in detail before, but will be summarized here (2). After screening, the patients entered a three week run-in period, in which all oral blood glucose-lowering medication was omitted and insulin therapy was intensified. Within one week before the start of the intervention a baseline magnetic resonance (MR) scan was performed to assess the different fat compartments (see below). The intervention consisted of a very low calorie diet (VLCD), prescribed during 16 weeks, to achieve considerable weight loss. During the VLCD, patients consumed 3 sachets of Modifast Intensive per day (containing 450 kcal, 50 g protein, 50-60 g carbohydrates and 6 g lipids per day, Nutrition & Santé, Antwerpen, Belgium). At the start and, subsequently, during the whole period of the VLCD all glucose-lowering medication including insulin was discontinued. Five of the 14 patients additionally followed an exercise program during the VLCD, however this had no effect on the outcome parameters. At the end of the 16-week VLCD a second MRI-scan was performed. Subsequently, patients received dietary advice and were slowly reintroduced to their diet of choice. Twenty weeks after the start of the study, patients were referred back to their own specialists for regular medical care either in primary care or at the out-patient clinic of our department. Fourteen months after the end of the VLCD a third MRI-scan was performed. The average caloric intake was estimated on basis of the food intake three days before the study days. Blood was drawn on each occasion after an overnight fast.

Pericardial fat

All MR imaging and proton MR spectroscopy (^{1}H-MRS) studies were performed using a 1.5 Tesla whole body MR scanner (Gyroscan ACS-NT15; Philips Medical Systems, Best, The Netherlands) in supine position. All MR-measurements were made in the postprandial state (4 hours after the last meal). Pericardial fat was quantified using electrocardiographically gated breath-holds with a balanced turbo-field echo MR sequence (20). Imaging parameters included: echo time (TE) = 1.60 ms, repetition time (TR) = 3.2 ms, flip-angle = 50°, slice thickness = 10 mm. The whole four chamber view was analyzed. To quantify the pericardial fat volume, contours around the pericardial fat of the ventricles and atria were drawn manually at end systole and multiplied by the thickness of the slice to yield a volume. We used MASS analytical software (Medis, Leiden, the Netherlands) for post processing. Contours were drawn by two independent observers and we used the mean volume of the two observers. A representative image is given in Figure 1.

Figure 1. Pericardial fat (P) as visualized by a four chamber view of the heart on a 1.5 Tesla whole body MR scanner (Gyroscan ACS-NT15; Philips Medical Systems, Best, The Netherlands). To quantify the pericardial fat volume, contours around the pericardial fat of the ventricles and atria were drawn manually at end systole and multiplied by the thickness of the slice to yield a volume.



Visceral and subcutaneous fat

Abdominal fat was quantified with a turbo spin echo imaging (6). Imaging parameters were: TE = 11ms, TR =168ms, flip angle = 90°, slice thickness = 10mm. Three transverse images were obtained during one breath hold, at the level of the 5th lumbar vertebrae. With post-processing software (MASS analytical software, Medis, Leiden, The Netherlands), the volumes of the visceral and subcutaneous fat depots were quantified. The number of pixels were converted to square centimeters and multiplied by the slice thickness. The total fat volume was calculated by summing the fat volumes of the three individual slices.

Hepatic magnetic resonance spectroscopy

Hepatic ^{1}H-MRS was performed as previously described (21). The bodycoil was used for radio-frequency transmission and a 17 cm diameter circular coil for signal receiving. An 8 ml voxel was placed in the liver, carefully avoiding large vascular structures. To ascertain the same position of the voxel at all study days, we used the twelfth thoracic vertebrae as marker. A TR of 3000 ms and TE of 26 ms were used. A spectrum with water suppression to detect small TG peaks and a spectrum without water suppression as internal standard were obtained. Sixty-four averages were collected without water suppression. The spectra were fitted using Java-base MR user interfase software (jMRUI version 2.2), as previously described (21). The percentage of hepatic TG signals was calculated as: (signal amplitude of hepatic triglycerides)/ (signal amplitude of water) x 100.

Assays

Serum insulin and C-peptide were measured with an automated immunoluminometric assay (ILMA) on an Immulite 2500 analyzer of Siemens Diagnostics (Breda, The Netherlands, intra-assay coefficient of variant (CV) 6-7.5%, inter-assay CV 7-8.2% respectively). Serum glucose, cholesterol and triglycerides were measured using a Modular P800 chemistry analyzer of Roche Diagnostics (Mannheim, Germany, total CV for glucose, cholesterol and triglyceride <2%). Plasma FFA concentrations were measured by a commercial kit (FFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis

Data are expressed as mean values ± standard error of the mean (SEM) or as median (interquartile range) if not normally distributed. Differences between the three measurements were analyzed with a general linear model for repeated measures, with time as within-subject factor. LSD post-hoc tests were used in case of a significant F-ratio. The plots were created with GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, USA). Statistical analyses were performed using SPSS 17.0 (SPSS Inc.Chicago, Illinois, USA). A P-value of 0.05 or less was considered statistically significant.

RESULTS

Caloric intake and weight gain

The VLCD reduced caloric intake from 2471±115 kcal/day at baseline to 450 kcal/day, which resulted in a 22% weight reduction compared to baseline (P<0.05, Table 1). After discontinuation of the VLCD, after an additional 14 months of follow-up, the estimated caloric intake was 1889±62 kcal/day, P<0.05 vs. baseline). During follow-up patients regained weight to 90% of baseline (P<0.05 vs. baseline, Table 1).

Antidiabetic medication, glycemic control and lipids

At baseline, before start of the VLCD, all patients used insulin, 8 patients used metformin and 3 patients a sulfonylureum (SU)-derivate. During the 16 week-VLCD all patients had stopped insulin therapy and oral glucose-lowering medication. Directly after the intervention metformin was prescribed to all patients and 3 patients were prescribed an additional SU-derivate. After an additional 14 months of follow-up (patients were treated by their specialists according to the standard guidelines), four patients had restarted insulin therapy, 12 patients used metformin and 4 patients used a SU-derivate.

The VLCD significantly improved glycemic control, reflected in decreased fasting plasma glucose and HbA1c levels, even though all patients had stopped the use of insulin and oral blood glucose-lowering medication during the whole VLCD period (Table 1). At 18 months

Table 1. Metabolic changes and changes in fat distribution at baseline, after a 16-week VCLD and after an additional 14 months of follow-up on a regular diet.

	baseline	16 week VLCD		18 months		
Weight (kg)	107 ± 4	83 ± 4	*	96 ± 4	*	!
Body Mass Index (kg/m^2)	35.3 ± 1.1	27.5 ± 1.1	*	31.7 ± 1.1	*	!
Fasting plasma concentrations						
HbA1c (%)	8.4 ± 0.3	7.0 ± 0.4	*	7.7 ± 0.5		
Fasting glucose (mmol/L)	11.9 ± 0.6	7.8 ± 0.8	*	10.4 ± 1.0		!
Fasting insulin (mmol/L)	23.5(14.3-29.3)	10.0(6.8-12.0)	*	6.5(5.0-14.3)		
Fasting C-peptide (mmol/L)	2.8 ± 0.3	2.1 ± 0.3		1.0 ± 0.1	*	!
Total cholesterol (mmol/L)	6.0 ± 0.4	5.3 ± 0.3		5.2 ± 0.4		
Triglycerides (mmol/L)	2.3 ± 0.2	1.2 ± 0.1	*	2.8 ± 0.6		!
HDL (mmol/L)	1.13 ± 0.05	1.25 ± 0.09	*	1.29 ± 0.07	*	
LDL (mmol/L)	4.4 ± 0.4	3.7 ± 0.3	*	3.2 ± 0.2	*	
Fat distribution						
Hepatic TG content (%)	22.8 ± 3.9	3.6 ± 1.0	*	13.4 ± 2.2	*	!
Subcutaneous abdominal fat (ml)	1194 ± 105	701 ± 108	*	978 ± 89	*	!
Visceral abdominal fat (ml)	553 ± 37	228 ± 46	*	456 ± 51	*	!
Visceral/subcutaneous fat	0.52 ± 0.07	0.33 ± 0.04	*	0.53 ± 0.09		!
Pericardial fat (ml)	39 ± 4	31 ± 2	*	32 ± 2	*	

Data are mean ± SEM or median (interquartile range). * $p<0.05$ vs. baseline, ! $p<0.05$ 18 months vs. 16 weeks.
HbA1c: glycated hemoglobin; HDL: high density lipoprotein; LDL: low density lipoprotein, TG: triglyceride.

however, HbA1c and fasting plasma glucose returned to baseline values, albeit with less intensive anti-diabetic medication.

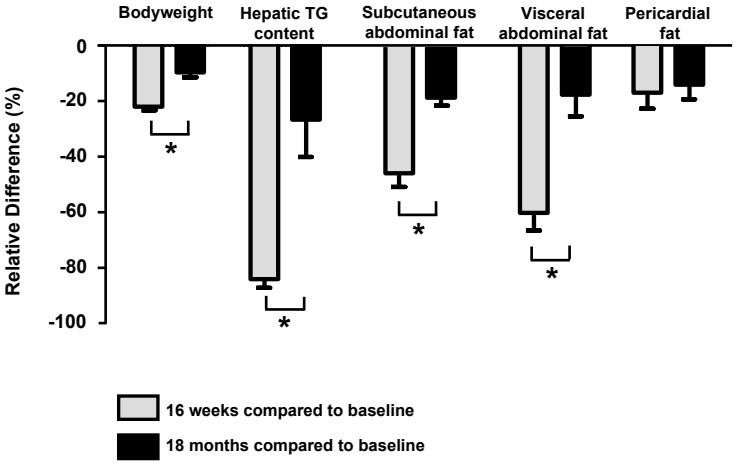
Total plasma cholesterol levels did not change after the VLCD. HDL-cholesterol levels were higher and LDL cholesterol levels lower after the VLCD which was sustained after 18 months of follow-up, despite the above described weight regain (Table 1).

Pericardial fat and other fat compartments

After the 16-week VLCD, pericardial fat significantly decreased by 17% (from 39±4 ml at baseline to 31±2 ml after 16 weeks). Fourteen months after discontinuation of the VLCD, pericardial fat volume remained stable, despite the considerable weight regain and deterioration of glycemic control.

A preferential loss of visceral fat compared to subcutaneous fat was observed after the 16-week VLCD (40% and 55% resp. of baseline values (both $P< 0.05$, Figure 2)). After an additional 14 months of follow-up, both visceral and subcutaneous fat volumes increased to 83% and 82% resp. of baseline values (both $P<0.05$ compared to baseline, Figure 2). Accordingly, the ratio between visceral and subcutaneous fat decreased significantly from 0.52 to 0.33 after 16

Figure 2. Relative changes in bodyweight, hepatic triglyceride (TG) content, subcutaneous and visceral abdominal fat and pericardial fat after 16 weeks of a very low calorie diet (grey box) and after 14 months of follow-up on a regular diet (black box) compared to baseline.



* p<0.05 for decrease in weight/fat between respectively 16 weeks and 18 months compared to baseline.

weeks, but returned to a ratio of 0.53 after an additional follow-up of 14 months. Therefore, the preferential loss of visceral fat had disappeared.

Due to technical problems abdominal fat and hepatic TG content could not be assessed in 1 patient at 18 months. The VLCD decreased hepatic TG content to 16% of baseline levels. However, 14 months after discontinuation, hepatic TG content increased to 73% of baseline values (P<0.05 vs. baseline, Figure 2).

DISCUSSION

This study is the first to assess the long-term effects of caloric restriction on pericardial fat and other fat compartments in patients with T2DM using state of the art MR imaging and spectroscopy. Our data reveal differential short- and long-term effects of a 16-week VLCD and 14 months follow-up on weight, pericardial fat and other fat compartments. In general, we found a substantial regain in (non)adipose TG compartments with as clear exception pericardial fat.

We found that the beneficial effects of a 16-week VLCD on weight and metabolic parameters partially deteriorated 14 months after reinstating a regular diet, which is in line with previous studies (22-25). However, in those studies, effects on fat compartments were not studied. Interestingly, despite the fact that the relative reduction in pericardial fat volume after the VLCD was the smallest of all fat compartments, this was the only fat compartment which did not expand during the additional follow-up of 14 months. Previous studies on caloric restriction in non-diabetic subjects, also reported a larger decrease in visceral fat volume

as compared to pericardial fat volume (19). Likewise, in a short-term caloric restriction study in overweight women, cross-sectional, but no longitudinal associations between pericardial fat volume and other fat compartments and metabolic parameters were found (26). However, in these studies, no long-term effects after reinstating a regular diet were assessed. We previously found that treatment with pioglitazone in patients with T2DM, increased pericardial fat, but did not change visceral fat volumes (20). The results of the previous and present studie(s) clearly suggest that pericardial fat and visceral fat volumes are differentially influenced by diet and drugs (like thiazolidinediones), and cannot be considered as metabolically identical compartments. As pericardial fat volume has been associated with cardiovascular risk in cross-sectional studies (11,13,27), it can be hypothesized that our finding is compatible with a sustained beneficial effect of a VLCD on cardiovascular risk.

We observed a preferential loss of visceral fat compared to subcutaneous fat volume immediately after the 16-week VLCD. After 14 months of follow-up this preferential loss of visceral fat was no longer present. This short-term effect can be explained by a higher susceptibility of visceral fat to lipolysis than subcutaneous fat via a diminished response to insulin (28) and a greater sensitivity to norepinephrine (29,30).

The weight reduction of 22% immediately after the VLCD observed in our study was associated with a major reduction of 84% in hepatic TG content. Reductions in hepatic TG content are strongly associated with improved hepatic insulin sensitivity and lipoprotein metabolism through different mechanisms, including effects of fatty acids metabolites and inflammatory intermediates on insulin receptor signaling and VLDL synthesis (8). Therefore, the regain in hepatic TG content after 14 months on a regular diet is likely related to the deterioration in fasting plasma glucose and TG levels, although remarkably, beneficial effects of the VLCD on HDL- and LDL cholesterol were preserved after long-term follow-up.

In conclusion, diet-induced weight loss and subsequent regain of weight during regular diet induces tissue-specific variations in (non)adipose TG stores in T2DM patients. Whereas visceral fat volume and hepatic TG content largely parallel changes in weight and metabolic parameters, sustained effects on pericardial fat are observed. These observations are most likely the consequence of differences in sensitivity to regulatory mechanisms, controlling TG accumulation in different (non)adipose tissue compartments.

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CHAPTER 9

Summary and conclusions

INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is rising steadily. The World Health Organization estimates that by the year 2030 more than 5% of the adult population worldwide is suffering from T2DM (1). Prevention is of utmost importance, as for the time being all interventions are insufficient. It is thus important for practical, economical as well as medical reasons to optimize treatment for T2DM patients. Eighty to ninety percent of the T2DM patients suffer from overweight or obesity. In obese T2DM patients insulin resistance plays a pivotal role in the pathophysiology of the disease and therapeutical interventions should aim at least to improve insulin resistance. Cornerstones in the treatment of T2DM are lifestyle interventions, oral glucose-lowering medication and insulin (2). Nowadays, it seems that pharmaceutical solutions are more important than lifestyle interventions while these have at least equal therapeutic strength.

Lifestyle interventions traditionally include diet-induced weight reduction. It has been shown that diet-induced weight loss can improve insulin resistance and its associated metabolic abnormalities (3-6). Exercise is still underutilized in the treatment of obese T2DM patients since the effects on both weight reduction and insulin resistance are not as large (7-9).

In this thesis we evaluated both short- and long-term effects of a 16-week very low calorie diet (VLCD)-only and in combination with an exercise program in obese insulin-dependent T2DM patients.

FIRST AIM: SYSTEMATIC REVIEW OF THE LITERATURE REGARDING THE EFFECT OF DIET-INDUCED WEIGHT REDUCTION AND EXERCISE ON ECTOPIC FAT DEPOSITIONS

In Chapter 2, we describe in a systematic review the literature regarding the effect of diet-induced weight loss and exercise on ectopic fat depositions. Ectopic fat deposition is defined as triglyceride (TG) droplets stored in tissue other than adipose tissue, such as the liver, skeletal muscle and the heart.

In skeletal muscle, ectopic fat (intramyocellular lipid (IMCL) accumulation) is associated with insulin resistance and T2DM (10-12). Moderate diet-induced weight loss (less than 10% bodyweight reduction) does not affect IMCL accumulation or peripheral insulin sensitivity (13-15). In contrast, substantial weight reduction does lead to a significant decrease in IMCLs and improvement in peripheral insulin sensitivity (6,16). The effect of exercise is less clear, exercise has been shown to be accompanied either by increased (17), unchanged (7,18), or decreased (9,19) IMCL accumulation in skeletal muscle.

In the liver, ectopic fat deposition (non alcoholic fatty liver disease (NAFLD)) is associated with insulin resistance and dyslipidemia (20). Even a relatively modest diet-induced weight

reduction (5-10 %) is associated with a substantial drop in hepatic TG content (15,21). The reduction of hepatic TG content is associated with an amelioration of hepatic insulin resistance (15,21). The effect of exercise on hepatic TG content is less clear since data are scarce and most studies combine exercise with a diet. In these studies diet already leads to weight reduction. Because a relatively modest decline in body weight already affects hepatic TG content the unique effect of exercise is difficult to determine.

Pericardial fat (the fat deposition around the heart) is positively associated with coronary heart disease and whole body insulin resistance (22). The exact function of the pericardial fat remains unknown. There is only limited data available, however, these data show that weight loss and exercise induce a significant decrease in pericardial fat. In one study this was accompanied by an improvement in diastolic cardiac function (23-25).

Cross-sectional data show that myocardial TG accumulation is associated with impaired diastolic function (26). Only one study showed that diet induced weight reduction is associated with a non-significant decrease in myocardial TG content (27).

In conclusion, ectopic fat depositions are associated with disruption of normal metabolic processes in the cell. Whether the ectopic fat depositions are a cause or consequence of insulin resistance is still under investigation. Lifestyle interventions affect ectopic fat depositions and ameliorate organ function (hepatic/peripheral insulin resistance or cardiac function).

In this thesis we describe the results of a 16-week randomized intervention study combining exercise and a VLCD to further investigate the effects on insulin sensitivity and ectopic fat depositions.

FIRST PART OF THE THESIS

In the first part of this thesis we evaluate and compare the effects of a 16-week VLCD and a 16-week VLCD combined with an exercise program. The study population, consisting of 27 sedentary obese (body mass index (BMI) 37.2 ± 0.9 kg/m^2) insulin-dependent T2DM patients (14 men, 13 women) with residual beta-cell capacity, all followed a 16-week VLCD. Three weeks before the intervention all oral glucose-lowering medication was discontinued, one day before the intervention insulin therapy was omitted, during the whole intervention period no blood glucose-lowering medication (including insulin) were prescribed. The diet contained a total of ~450 kcal/day divided over 3 sachets of liquid food providing all necessary vitamins and micronutrients (Modifast®). Thirteen subjects participated simultaneously in an exercise program which consisted of one hour in-hospital training weekly, primarily aerobic exercise, under supervision of a physiotherapist. And 4 training sessions at home on a cyclo-ergometer for 30 minutes at 70% of maximum aerobic capacity.

Before the start of the intervention, directly after, 6 months and 18 months after the start of the intervention several measurements were performed, such as height, weight, waist

circumference and blood pressure. At each time point fasting blood samples were drawn to determine among others fasting plasma glucose, insulin, leptin, cholesterol, cytokines and highly sensitive C reactive protein (hsCRP) levels. Also at the above ascribed time points quality of life (QoL) questionnaires were filled out by the participants. Only at baseline and directly after the intervention a hyperinsulinaemic euglycaemic clamp was performed with stable isotopes ([$^2H_5$]-glycerol en [6,6-$^2H_2$]-glucose) to determine peripheral and hepatic insulin sensitivity. During the clamp both under basal and hyperinsulinaemic conditions indirect calorimetry (to measure lipid and glucose oxidation rates) and muscle biopsies (to measure the insulin signaling pathway, muscle morphology and mitochondrial DNA content) were performed.

SECOND AIM: THE ADDITIONAL EFFECTS OF EXERCISE ON WEIGHT LOSS, INSULIN SENSITIVITY, INSULIN SIGNALING, MUSCLE MORPHOLOGY AND MITOCHONDRIAL DNA CONTENT

Baseline characteristics were identical in both groups. Substantial weight reduction occurred in both groups (-23.7 ± 1.7 kg VLCD-only vs. -27.2 ± 1.9 kg VLCD with exercise, p=NS). The VLCD with exercise group lost significantly more fat mass and waist circumference. Our a priori hypothesis was that the addition of exercise would further improve and might even normalize insulin sensitivity. However, glycemic control, hepatic and peripheral insulin sensitivity improved similarly in both intervention groups. Peripheral insulin sensitivity improved considerably in both intervention groups by approximately 150% (glucose disposal rate: baseline vs. after 16 weeks 15.5 ± 1.2 vs. 38.6 ± 4.6 µmol/kg_{LBM}/min (VLCD-only); 16.6 ±1.2 vs. 41.8 ± 3.66 µmol/kg_{LBM}/min (VLCD with exercise)).

The similar improvement in insulin sensitivity in both groups is in accordance with the observed identical amelioration in insulin signaling pathway as reflected by increased proline-rich AKT substrate (PRAS40) phosphorylation and increased levels of insulin receptor expression. AKT substrate 160 (AS160) showed the same trend however did not reach significance. The insulin receptor expression increased even further with the addition of exercise; however this was not accompanied by further improvement of glucoregulation.

As ascribed above, IMCL accumulation in the skeletal muscle is associated with insulin resistance and T2DM. We observed a significant decrease in IMCL accumulation in the skeletal muscle in both the VLCD-only and VLCD with exercise group after the 16-week intervention. The improvements in insulin signaling found in our study might be related to the observed decrement in IMCL accumulation and its metabolic products.

Only in the VLCD with exercise group, maximum aerobic capacity, mitochondrial copy number and basal lipid oxidation rate improved whereas in the VLCD-only group no change was found. However, this did not result in a further decrease in IMCL accumulation.

A low capacity to oxidize fat due to a low percentage of type 1 (oxidative) muscle fibers might lead to obesity and T2DM, although a causal relation has not been established. To the best of our knowledge we were the first to observe a significant identical increase in both intervention groups in type 1 (oxidative) muscle fibers after weight reduction.

Our a priori hypothesis that exercise would further improve insulin sensitivity is thus proven wrong. A possible explanation could be that the 16-week VLCD ameliorates insulin resistance to a maximal extent in these patients and that the additional effect of exercise is too small to detect in this relatively small patient population.

THIRD AIM: THE LONG-TERM EFFECTS OF A VLCD-ONLY AND COMBINED WITH AN EXERCISE PROGRAM ON GLYCEMIC CONTROL AND ANTHROPOMETRIC MEASUREMENTS

After the 16-week VLCD with or without exercise patients were given advice on a healthy diet, and regular exercise. After a weight-stabilizing period, in which normal meals were reintroduced, patients were referred back to their own health care provider at 6 months after the start of the intervention. During the time thereafter we were not able to influence treatment strategies, especially not with respect to bodyweight or glycemic control.

Eighteen months after the start of the intervention both groups had regained some weight and waist circumference. However, both weight and waist circumference were still significantly diminished compared to baseline values. Eighteen months after the start of the intervention there was still a decline in bodyweight of more than 10% compared to baseline in both intervention groups. We did not observe any difference in the amount of weight loss 18 months after the start of the intervention between the VLCD-only group and the VLCD with exercise group.

As stated above we could not adjust treatment strategies with regard to metabolic regulation. This may be one of the reasons that glycemic control was not as tightly regulated as guidelines require. Another reason is that patients probably feel reluctant to resume insulin therapy. Regrettably, 18 months after the start of the intervention both fasting plasma glucose and HbA1c levels had returned to baseline values. However, homeostatic model assessment of insulin resistance (HOMA-IR) (a measure for insulin resistance) and fasting insulin levels showed a sustained improvement in the VLCD with exercise group. Also, in none of the patients in the VLCD with exercise group insulin therapy was reinstituted, whereas in the VLCD-only group 6 patients received insulin therapy again. Altogether these results suggest less insulin resistance in the VLCD with exercise group 18 months after the start of the intervention. Eighteen months after the start of the intervention patients in the VLCD with exercise group were still exercising for more than 3 hours a week. This might partially explain the sustained improvements in insulin sensitivity in this group.

FOURTH AIM: SHORT- AND LONG-TERM EFFECTS ON QOL AFTER A VLCD-ONLY AND COMBINED WITH AN EXERCISE PROGRAM

QoL is reduced in patients with chronic diseases such as T2DM (28,29). It is very important to improve QoL in patients, since this can lead to better social functioning, compliance to therapy and adherence in self-management (29). In our study QoL was measured with four different questionnaires (short form-36 (SF-36), hospital anxiety and depression scale (HADS), multidimensional fatigue index-20 (MFI-20) and the Nottingham health profile (NHP)). All patients completed the questionnaires three times, at baseline, directly after the 16-week intervention and 18 months after the start of the intervention. Patients were compared to two (lean and obese) healthy well-matched (age, gender and race) control populations.

At baseline QoL was significantly impaired at scores for physical and social functioning, as well as fatigue and pain in the two patients groups, compared to both healthy lean and obese control subjects. Immediately after the 16-week intervention, we observed a significant and similar improvement in all QoL scores, especially those concerning physical functioning, fatigue, and self confidence in both intervention groups. Directly after the intervention, most of the QoL scores of the T2DM patients improved even to the level of that of the lean and obese control subjects. Only in the VLCD with exercise group improvements in QoL sustained up to 18 months after the start of the intervention, whereas in the VLCD-only group QoL returned to baseline values, despite persistent weight loss and reduced drug use compared to baseline.

Several factors might have contributed to the improvements in QoL of our patients such as the loss of a considerable amount of weight, discontinuation of insulin therapy, improvement of glycemic control and better physical condition. The last reason could explain the lasting amelioration in QoL in the VLCD with exercise group since these patients were still exercising for more than 3 hours a week 18 months after start of the intervention.

FIFTH AIM: SHORT- AND LONG-TERM EFFECTS ON LOW-GRADE INFLAMMATION AND RISK FACTORS FOR CARDIOVASCULAR DISEASE (CVD) AFTER A VLCD-ONLY AND COMBINED WITH AN EXERCISE PROGRAM

Visceral adipose tissue is characterized by large adipocytes, active adipocyte lipolysis, abundant adipose tissue macrophages, which secrete pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin (IL)6, IL8 and IL1, which subsequently promote hsCRP production by the liver (30). This chronic low-grade inflammatory state can interfere with the insulin signaling pathway in other tissues but can also be an additional risk factor for the development of premature atherosclerosis in T2DM patients (31-33).

Directly after the 16-week intervention classical risk factors for the development of CVD such as hypertension and dyslipidemia improved dramatically and similarly in both groups. However, this was not a lasting effect, since blood pressure returned to baseline values 18 months after the start of the intervention. The beneficial effect on cholesterol/HDL ratio and LDL-cholesterol were maintained in the long run in both intervention groups and only in the VLCD with exercise group, respectively.

The effects of the 16-week VLCD with or without exercise on low-grade inflammation became only apparent after patients were on a eucaloric diet after the weight stabilizing period, thus 6 months after the start of the intervention. Directly after the intervention no change was observed in low-grade inflammation, whereas 6 months after the start of the intervention the low-grade inflammatory state was completely abolished and values of hsCRP and pro-inflammatory cytokines (such as TNFα, IL6 and IL1) were decreased to the level of normal healthy lean control subjects. This effect persisted in the long run up to 18 months after the start of the intervention. The addition of an exercise program had a beneficial effect on hsCRP only, no other additional effects were observed.

The mechanism by which a VLCD can ameliorate the low-grade inflammatory status may be the decrease in visceral fat mass (and thereby the number of adipose tissue macrophages). Indeed in our study we observed a significant decrease in waist circumference (an indirect measure of visceral fat storage). During weight loss the number of adipocytes is the same, while the size decreases, therefore basal lipolysis remains high. This attracts macrophages, which phagocytose the excess lipids, leading to an increase concentration of macrophages in the adipose tissue (34,35). The lack of an immediate effect of the VLCD on low-grade inflammation might be explained by the increase in macrophages in the adipose tissue during the VLCD and the gradual decline in the weight stabilizing period thereafter.

SECOND PART OF THE THESIS

In the second part of this thesis we focused on ectopic fat depositions and the amount of visceral and subcutaneous fat mass. Fourteen patients (8 men and 6 women), a subpopulation of the above described study population of obese (BMI 35 ± 1 kg/m^2) insulin-dependent T2DM, who fitted in the magnetic resonance imaging (MRI) scanner, were studied at baseline, directly after and 18 months after the 16-week VLCD with or without the exercise program. In the liver and myocardium ectopic fat depositions were measured *in vivo* by proton magnetic resonance spectroscopy (^{1}H-MRS). Measuring myocardial ectopic fat deposition by ^{1}H-MRS is a new technique developed by our group (36). Pericardial, visceral, subcutaneous fat mass and cardiac function was measured using 1.5 Tesla MRI. IMCL accumulation in the skeletal muscle has been described previously and is not measured with the ^{1}H-MRS but in the muscle biopsies.

SIXTH AIM: SHORT- AND LONG-TERM EFFECTS OF A 16-WEEK VLCD WITH OR WITHOUT EXERCISE ON QUANTITY AND FUNCTIONAL EFFECTS OF ECTOPIC FAT DEPOSITIONS IN THE HEART

Even in well-controlled T2DM patients diastolic function is impaired compared to a healthy control population. In T2DM patients diastolic dysfunction is associated with increased risk of development of heart failure and mortality independently of coronary artery disease (37). In cross-sectional studies increased depositions of TG in the myocardium of T2DM patients were observed, which were negatively correlated with diastolic cardiac function (26).

The estimate of myocardial oxygen consumption (rate pressure product) (38,39) decreased significantly directly after the 16-week VLCD and the effect sustained up to 18 months after the start of the intervention, which is compatible with an improved cardiac efficiency.

T2DM patients have a decreased left ventricular (LV) end diastolic volume index compared to healthy controls, associated with diminished compliance (40). In this thesis we show sustained improvements in diastolic function and diastolic left ventricular distensibility up to 18 months after the start of the intervention, as reflected by an increase in LV end diastolic volume index at comparable estimated LV filling pressure and sustained improvement in the early (E) and atrial (A) diastolic filling phase ratio (E/A ratio). Our study is the first to show that weight reduction leads to sustained improvement in diastolic function and an increase in compliance of the left ventricle in T2DM patients.

Myocardial TG content decreased directly after the 16-week VLCD. However, 18 months after the start of the intervention myocardial TG content returned to baseline values. The study design does not allow us to assess the direct relation between myocardial TG stores and myocardial function. Apparently the regain in myocardial TG content does not immediately correspond with diastolic function. It could be hypothesized that the positive effect of the VLCD on glucotoxicity or oxidative stress may contribute more to diastolic function than the regain in myocardial TG content.

Left ventricular mass serves as an independent predictor of mortality. In this thesis a significant decrease in left ventricular mass was observed. Insulin is considered to be a growth factor and may therefore induce cardiac hypertrophy. The persistent decreased plasma insulin level, together with the significant amount of weight loss may be part of the explanation for the sustained decrease in left ventricular mass 18 months after the start of the intervention.

SEVENTH AIM: SHORT- AND LONG-TERM EFFECTS OF A 16-WEEK VLCD WITH OR WITHOUT EXERCISE ON QUANTITY OF VISCERAL AND SUBCUTANEOUS FAT MASS AND ECTOPIC FAT DEPOSITIONS IN THE LIVER AND PERICARDIUM

A 16-week VLCD significantly reduced hepatic TG content, visceral and subcutaneous abdominal fat mass and pericardial fat volume to respectively 16, 40, 53 and 83% of baseline values. Directly after the intervention we observed a preferential loss of visceral fat compared to subcutaneous fat volume. Eighteen months after the start of the intervention both visceral and subcutaneous fat mass had increased but there was still a significant reduction in fat mass in both compartments compared to baseline values. The preferential loss of visceral fat, however, did not sustain. Also, hepatic TG content tended to return to baseline values 18 months after the start of the intervention compared to values directly after the 16-week VLCD, although there was still a significant reduction compared to baseline.

Interestingly, pericardial fat was significantly reduced by the VLCD with or without an exercise program and it did not increase during the follow-up period. Therefore, pericardial fat is apparently less amendable to dietary interventions compared to other (non-)adipose tissue stores. As pericardial fat volume has been associated with increased cardiovascular risk (41) it can be hypothesized that our finding could point to a sustained beneficial effect of VLCD with or without exercise on cardiovascular risk.

OVERALL CONCLUSION

In T2DM patients, a VLCD with the discontinuation of all blood glucose-lowering agents can be safely used without the risk of severe hyperglycemia, provided that patients still have remaining endogenous insulin secretion (42). Using a VLCD, patients can lose substantial amounts of body weight. The weight loss consists mainly of the loss of fat mass (both visceral and subcutaneous (abdominal) fat) together with the loss of ectopic fat depositions in the heart, liver and skeletal muscle. The loss of ectopic fat depositions in the liver and skeletal muscle were accompanied by a significant improvement in both hepatic and peripheral insulin sensitivity, which is in accordance with an amelioration of the insulin signaling pathway in skeletal muscle biopsies. The loss of ectopic fat depositions in the heart was associated with a sustained improvement in diastolic function and LV distensibility, even up to 18 months after the start of the intervention.

The addition of an exercise program to a 16-week VLCD did not lead to a further improvement in insulin sensitivity, probably because the effect of the VLCD was so substantial, that the relatively modest effects of exercise in our patient population could not be detected. However we observed a beneficial effect on mitochondrial copy number, lipid oxidation and aerobic fitness in the exercise group. We also observed that it is difficult to maintain weight

reduction after cessation of the VLCD. It seemed that the addition of exercise to the VLCD had long-term beneficial effects on glycemic control and QoL but not on weight loss.

Our observations stress the importance of weight-reducing therapies in T2DM patients. Besides a VLCD, bariatric surgery can be used for this purpose. Bariatric surgery has the largest effect on weight, insulin sensitivity and QoL. However, surgery is expensive, invasive, has substantial morbidity and logistically it is impossible to operate all obese T2DM patients. Therefore, diet and lifestyle interventions remain the mainstay of treatment for most obese T2DM patients.

FURTHER RESEARCH

In this thesis we evaluated the effect of diet-induced weight loss with or without the addition of exercise. Because of the substantial beneficial effects of a 16-week VLCD, it is difficult to show additional effects of exercise. To really differentiate between the effects of exercise and diet a different study must be performed. We propose a study with 2 intervention groups of obese insulin-dependent T2DM patients. Both groups first follow a 10-week VLCD and thereafter a 10-week weight maintenance diet. During the weight maintenance diet, only one group simultaneously participates in a 10-week exercise-program, which consists of a one hour in hospital training and at least 4 trainings at home on a cycloergometer at 70% of maximum aerobic capacity. This study design would allow evaluation of the impact of exercise on insulin action in slimmed patients. We also examined the effect of diet-induced weight loss with or without exercise on the amount of mitochondria in skeletal muscle (mtDNA content), but we did not investigate mitochondrial function. Impaired fat oxidation and low metabolic rate are risk factors for body weight gain and insulin resistance. It would thus be interesting to not only measure the number but also the function of mitochondria in the new study.

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CHAPTER 10

Samenvatting

INTRODUCTIE

De wereldwijde prevalentie van type 2 Diabetes Mellitus (T2DM) stijgt nog altijd sterk. De Wereldgezondheidsorganisatie schat dat in 2030 meer dan 5% van de volwassen wereldpopulatie lijdt aan T2DM. Op de lange termijn is preventie van T2DM natuurlijk zeer belangrijk. Maar gezien het feit dat er weinig goede preventieprogramma's bestaan, worden alle interventies om de behandeling van T2DM te verbeteren toegejuicht. Het is van essentieel belang, voor zowel praktische, economische en medische redenen, dat de behandeling van T2DM verder verbeterd wordt.

Van de patiënten met T2DM hebben 80-90% overgewicht of obesitas. Insulineresistentie speelt een uitermate belangrijke pathofysiologische rol in obese patiënten met T2DM. Therapeutische interventies moeten vooral gericht zijn op het verbeteren van insulineresistentie in deze patiëntenpopulatie. Hoekstenen in de behandeling van T2DM zijn; leefstijlinterventies, orale glucoseverlagende middelen en insulinetherapie. Tegenwoordig ligt de nadruk echter vooral op medicamenteuze therapie, terwijl van leefstijlinterventies op zijn minst vergelijkbare therapeutische effecten te verwachten zijn.

Leefstijlinterventies bestaan uit dieet-geïnduceerd gewichtsverlies, inspanningsprogramma's en psychologische programma's gericht op gedragsveranderingen. Uit eerdere studies blijkt dat dieet-geïnduceerd gewichtsverlies insulineresistentie en de daarmee geassocieerde metabole veranderingen, kan verbeteren. Inspanningsprogramma's zijn nog steeds ondergewaardeerd in de behandeling van obese patiënten met T2DM. Dit omdat de effecten op zowel gewichtsreductie als behandeling van insulineresistentie iets minder uitgesproken zijn.

In dit proefschrift bestuderen we zowel de korte als de lange termijn effecten van een zestien weken durend zeer laagcalorisch dieet (very low calorie diet, VLCD), al dan niet in combinatie met een inspanningsprogramma in obese insuline-afhankelijke T2DM patiënten.

EERSTE DOELSTELLING: SYSTEMATISCHE SAMENVATTING VAN DE BESCHIKBARE LITERATUUR OVER HET EFFECT VAN DIEET-GEÏNDUCEERD GEWICHTSVERLIES EN INSPANNINGSPROGRAMMA'S OP ECTOPISCHE VETDEPOSITIES.

In hoofdstuk 2 wordt er een systematische samenvatting van de beschikbare literatuur gegeven over het effect van dieet-geïnduceerd gewichtsverlies en inspanningprogramma's op ectopische vetdeposities. Ectopische vetdeposities zijn gedefinieerd als triglyceriden stapeling (in vetdruppeltjes) in organen anders dan vetweefsel, zoals de lever, de skeletspier of het hart.

In cross-sectionele studies zijn ectopische vetdeposities in de skeletspier (intramyocellulaire lipiden (IMCL) accumulatie) geassocieerd met insulineresistentie en T2DM. Een relatief kleine hoeveelheid lichaamsgewichtreductie (<10%) geïnduceerd door dieet heeft geen effect op IMCL accumulatie en insulinegevoeligheid in de skeletspier. Een meer uitgesproken gewichtreductie leidt echter tot een significante daling in IMCL en een verbetering in insulinegevoeligheid in de skeletspier. Het effect van inspanningsprogramma's op de IMCL accumulatie is minder duidelijk. Inspanningsprogramma's gaan gepaard met òf een stijging òf een daling òf geen verandering in IMCL accumulatie in de skeletspier.

In cross-sectionele studies zijn ectopische vetdeposities in de lever geassocieerd met insulineresistentie en dyslipidemie. Zelfs een zeer kleine (<5%) door dieet-geïnduceerde afname in lichaamsgewicht gaat gepaard met een aanzienlijke vermindering van triglyceriden stapeling in de lever. Deze afname in triglyceriden stapeling in de lever is geassocieerd met een verbetering in insulinegevoeligheid in de lever. De effecten van inspanningsprogramma's op triglyceriden stapeling in de lever zijn minder duidelijk omdat er nauwelijks onderzoek naar is gedaan. In de beschikbare onderzoeken wordt een inspanningsprogramma gecombineerd met een dieet. In deze studies leidt alleen het dieet al tot een afname in lichaamsgewicht en derhalve tot een aanzienlijke daling in triglyceriden stapeling in de lever. Hierdoor is het moeilijk aan te tonen wat het unieke effect van een inspanningsprogramma is op de triglyceriden stapeling in de lever.

In cross-sectionele studies is pericard vet (dit zijn vetdeposities rond het hart) positief gecorreleerd met een toename van hart- en vaatziekte en insulineresistentie. De exacte functie van pericard vet is nog niet bekend. Dieet-geïnduceerd gewichtsverlies en inspanningsprogramma's leiden tot een significante afname in de hoeveelheid pericard vet. In één onderzoek ging dit gepaard met een verbetering van de diastolische functie van het hart.

Cross-sectionele data laten zien dat triglyceriden stapeling in het myocard geassocieerd is met een afname van de diastolische functie van het hart. Eén onderzoek laat zien dat dieet-geïnduceerd gewichtsverlies geassocieerd is met een niet-significante afname van triglyceriden stapeling in het myocard.

Concluderend zijn ectopische vetdeposities geassocieerd met negatieve veranderingen in de metabole processen in de desbetreffende organen. Of ectopische vetdeposities de oorzaak of het gevolg zijn van insulineresistentie is nog steeds onderwerp van studie. Het is in ieder geval duidelijk dat leefstijlinterventies zowel leiden tot een vermindering van ectopische vetdeposities als een verbetering van de orgaanfunctie (insulineresistentie in de lever en skeletspier en met name de diastolische functie van het hart).

In dit proefschrift worden de resultaten van een zestien weken durende gerandomiseerde interventie studie beschreven waarin een VLCD gecombineerd wordt met een inspanningsprogramma en gekeken wordt naar de effecten op insulinegevoeligheid en ectopische vetdeposities.

EERSTE GEDEELTE VAN HET PROEFSCHRIFT

In het eerste gedeelte van het proefschrift vergelijken we de effecten van een zestien weken durend VLCD en een zestien weken durend VLCD gecombineerd met een inspanningsprogramma. De studiepopulatie bestond uit zevenentwintig obese (body mass index (BMI) 37.2 ± 0.9 kg/m^2), niet sportende, insuline-afhankelijke T2DM patiënten (veertien mannen en dertien vrouwen). Allen hadden nog endogene insuline secretie uit de β-cel in de pancreas. Drie weken voor de start van de interventie werden alle orale glucoseverlagende middelen gestopt. Eén dag voor de interventie werd ook de insulinetherapie gestopt. Gedurende de gehele interventieperiode werd er geen glucoseverlagende medicatie (inclusief insuline) gebruikt.

Het VLCD bestond uit ~450 kcal/dag, verdeeld over drie vloeibare maaltijden en bevat alle essentiële vitamines en mineralen (Modifast®). Dertien patiënten namen naast het VLCD tegelijkertijd deel aan een inspanningsprogramma. Het inspanningsprogramma bestond wekelijks uit een één uur durende training in het ziekenhuis onder leiding van een fysiotherapeut (met name aerobics), met daarnaast minimaal vier trainingen van dertig minuten thuis op een fietsergometer, op 70% van maximale inspanningscapaciteit.

Voor, direct na, zes maanden na de start en achttien maanden na de start van de interventie werden er verschillende metingen verricht. Ten eerste werd er telkens lengte, gewicht, buikomvang en bloeddruk gemeten. Tevens werd op elk tijdspunt nuchter bloed afgenomen voor bepaling van glucose, insuline, leptine, cholesterolspectrum, cytokines en hoog sensitief C reactive protein (hsCRP). Daarnaast werd er door de patiënten op elk tijdspunt een kwaliteit van leven (KvL) vragenlijst ingevuld. Alleen voor de start van de interventie en direct erna werd een hyperinsulinemische euglycemische clamp uitgevoerd met stabiele isotopen ([$^2H_5$]-glycerol en [6,6-$^2H_2$]-glucose) om insulineresistentie in de lever en skeletspier te bepalen. Gedurende de clamp werd zowel onder basale als onder hyperinsulinemische condities een indirecte caloriemetrie (om vet- en glucoseoxidatie snelheid te bepalen) en spierbiopten (om de insuline signaaltransductie cascade, spiermorfologie en de hoeveelheid mitochondriaal DNA te bepalen) uitgevoerd.

TWEEDE DOELSTELLING: VASTSTELLEN VAN DE ADDITIONELE EFFECTEN VAN EEN INSPANNINGSPROGRAMMA OP GEWICHTSVERLIES, INSULINEGEVOELIGHEID, INSULINE SIGNAALTRANSDUCTIE CASCADE EN DE HOEVEELHEID MITOCHONDRIAAL DNA

Beide groepen waren voor start van de interventie gelijk in antropometrische waarden, insulineresistentie, duur van de T2DM en medicatiegebruik. Er trad in beide groepen een aanzienlijke maar gelijke gewichtsreductie op (-23.7 ± 1.7 kg VLCD alleen vs. -27.2 ± 1.9 kg VLCD

met inspanningsprogramma, p = niet significant). In de groep waar een VLCD gecombineerd werd met een inspanningsprogramma trad er een grotere vermindering op in vetmassa en buikomvang. De *a priori* hypothese luidde dat toevoegen van het inspanningsprogramma aan een VLCD de insulinegevoeligheid verder zou verbeteren en deze wellicht volledig zou normaliseren. Echter, in beide interventiegroepen traden er zowel in glucoregulatie als insulinegevoeligheid van de lever en de skeletspier dezelfde grote verbeteringen op. Insulinegevoeligheid van de skeletspier verbeterde het meeste (meer dan 150%) in beide groepen (de insuline gestimuleerde glucose opname in de skeletspier op baseline vs. na de zestien weken durende interventieperiode: 15.5 ± 1.2 vs. 38.6 ± 4.6 μmol/kg_{VVM}/min (VLCD alleen); 16.6 ± 1.2 vs. 41.8 ± 3.66 μmol/kg_{VVM}/min (VLCD gecombineerd met het inspanningsprogramma); (VVM: vetvrije massa)).

De vergelijkbare verbetering in insulinegevoeligheid in beide interventiegroepen, direct na de zestien weken durende interventie, ging logischerwijs ook gepaard met gelijke verbetering in de insuline signaaltransductie cascade in de cel. Er traden significante verbeteringen op in de fosforylatie van proline-rich AKT substraat (PRAS40) en de expressie van de insuline receptor. Ook de fosforylatie van AKT substraat 160 (AS160) verbeterde met dezelfde trend, echter dit bereikte net geen significantie. De expressie van de insuline receptor op het celmembraan nam nog verder toe met de toevoeging van het inspanningsprogramma aan het zestien weken durende VLCD. Dit laatste ging echter niet gepaard met een verdere verbetering in glucoregulatie en glucose opname.

Zoals reeds hierboven beschreven is IMCL accumulatie in de skeletspier geassocieerd met een toename van insulineresistentie en T2DM. In ons onderzoek trad er, zowel in de groep die alleen het VLCD volgde als in de groep die naast het VLCD ook het inspanningsprogramma volgde, een significante daling op in IMCL accumulatie in de skeletspier na de zestien weken durende interventie. De verbeteringen die gevonden waren in insulinegevoeligheid en in de insuline signaaltransductie cascade zouden gerelateerd kunnen zijn aan de daling van IMCL accumulatie in de skeletspier.

Alleen in de groep patiënten die naast het VLCD ook aan het inspanningsprogramma deelnam, trad een verbetering op in maximale aërobe capaciteit, de hoeveelheid mitochondrieën en de basale vetoxidatie op. De verbeteringen gingen echter niet gepaard met een verdere daling van IMCL accumulatie in de skeletspier. In de groep die alleen het VLCD volgde, werden er geen veranderingen gezien in deze parameters.

Theoretisch gezien zou een lagere vetoxidatie capaciteit, door een lager percentage aan type 1 (oxidatieve) spiervezels, kunnen leiden tot overgewicht en T2DM. Echter, er zijn nog geen onderzoeken die deze causale relatie bewijzen. In dit onderzoek laten we voor het eerst zien dat door gewichtsreductie een significante stijging in type 1 (oxidatieve) spiervezels op kan treden.

Onze *a priopi* hypothese dat het toevoegen van een inspanningsprogramma aan een VLCD leidt tot een verdere verbetering in insulinegevoeligheid bleek niet correct. Een mogelijke

verklaring kan zijn dat de verbeteringen in insulinegevoeligheid, ontstaan door een zestien weken durend VLCD, zo groot zijn dat de additionele effecten van inspanning te klein zijn om in deze relatief kleine patiëntenpopulatie waar te nemen.

DERDE DOELSTELLING: DE LANGE TERMIJN EFFECTEN VAN EEN ZESTIEN WEKEN DUREND VLCD ALLEEN EN GECOMBINEERD MET EEN INSPANNINGSPROGRAMMA OP GLUCOREGULATIE EN ANTROPOMETRISCHE WAARDEN.

Na de zestien weekse interventie (VLCD met of zonder het inspanningsprogramma) hebben patiënten bewegingsadviezen en adviezen voor gezonde voeding gekregen. In deze periode was het doel het gewicht te stabiliseren en langzaam normale maaltijden te herintroduceren. Zes maanden na start van de interventie zijn de patiënten terugverwezen naar de eigen behandelaar (dit was de huisarts of internist). Gedurende de daaropvolgende tijd hadden de onderzoekers geen invloed op behandelstrategieën met betrekking tot gewicht- en glucoregulatie.

Achttien maanden na de start van de interventie zijn beide groepen opnieuw onderzocht. Er was sprake van een lichte toename in gewicht en buikomvang. Echter, er bestond een blijvende gewichtsvermindering van meer dan tien procent ten opzicht van de uitgangswaarden in beide interventiegroepen. Wederom was er tussen de beide interventiegroepen geen verschil in gewichtsreductie ten opzichte van de uitgangswaarde.

Zoals hierboven beschreven, konden de onderzoekers geen invloed uitoefenen op behandelstrategieën met betrekking tot glucoregulatie. Dit was één van de redenen dat de glucoregulatie niet zo strikt gereguleerd was als in de richtlijnen is voorgeschreven. Een andere reden was dat de patiënten een aversie hadden tegen het herstarten van insulinetherapie. Achttien maanden na de start van de interventie waren de nuchtere glucose en HbA1c waarden weer op het niveau terug van de uitgangswaarden. Echter, in de groep die naast het VLCD ook deelnam aan het inspanningsprogramma was de nuchtere insulinewaarde en de HOMA-IR (een parameter voor insulineresistentie) blijvend verbeterd. Daarnaast was er in diezelfde groep niemand bij wie insulinetherapie hervat moest worden, terwijl er in de groep die alleen het VLCD gevolgd hadden zes patiënten waren bij wie de insulinetherapie hervat moest worden. Al deze resultaten samen suggereren dat achttien maanden na de start van de interventie, in de groep die naast het VLCD een inspanningsprogramma volgde, de insulineresistentie minder was. Eén van de verklaringen hiervoor kan zijn dat in deze groep de patiënten, achttien maanden na start van de interventie, nog steeds meer dan drie uur per week sportten.

DOELSTELLING VIER: KORTE EN LANGE TERMIJN EFFECTEN VAN EEN VLCD ALLEEN OF GECOMBINEERD MET EEN INSPANNINGSPROGRAMMA OP DE KWALITEIT VAN LEVEN.

De KvL is afgenomen bij patiënten met chronische ziekten zoals T2DM. Het is zeer belangrijk de KvL te verbeteren bij patiënten, omdat dit kan leiden tot een beter sociaal functioneren, betere compliantie van ingestelde behandeling en betere zelfcontrole. In ons onderzoek werd KvL gemeten met vier verschillende vragenlijsten. Alle patiënten vulden de KvL vragenlijsten drie keer in; voor, direct na en achttien maanden na de start van de interventie. Patiënten werden vergeleken met twee gezonde controlepopulaties van vergelijkbare leeftijd, geslacht en ras (één met een normaal gewicht en één met vergelijkbaar overgewicht als de patiënten maar zonder T2DM).

Voor de start van de interventie hadden patiënten een significant slechtere KvL in vergelijking met beide gezonde controlepopulaties. Met name op scores die betrekking hadden tot fysiek en sociaal functioneren, vermoeidheid en pijn. Direct na de interventie was er sprake van een significante maar gelijke verbetering in alle KvL-scores in beide interventiegroepen. Met name de scores voor fysiek functioneren, vermoeidheid en zelfvertrouwen waren direct na de interventie sterk verbeterd. De verbeteringen direct na de interventie waren zo uitgesproken dat de meeste van de KvL-scores gelijk waren aan die van zowel een normaal gewichtige als een obese controlepopulaties. Alleen de groep die naast het VLCD deelnam aan het inspanningsprogramma liet een blijvende verbetering zien in KvL, achttien maanden na start van de interventie. In de groep die alleen het VLCD volgde, verslechterden de KvL scores achttien maanden na start van de interventie naar de uitgangswaarden.

Verschillende factoren spelen een rol bij de verbeteringen van KvL van de patiënten. Zowel het verlies van een aanzienlijke hoeveelheid gewicht, het stoppen van insulinetherapie, verbetering in glucoregulatie en een betere fysieke conditie kunnen (mede) de oorzaak zijn van de verbeteringen in KvL. De laatste reden kan tegelijkertijd de verklaring zijn waarom er een blijvende verbetering optrad in de groep waarin het VLCD gecombineerd werd met een inspanningsprogramma. In deze groep sportten de patiënten nog steeds meer dan drie uur per week achttien maanden na start van de interventie.

DOELSTELLING VIJF: KORTE EN LANGE TERMIJN EFFECTEN VAN EEN VLCD ALLEEN OF GECOMBINEERD MET EEN INSPANNINGSPROGRAMMA OP LAAGGRADIGE INFLAMMATIE EN RISICOFACTOREN VOOR HART- EN VAATZIEKTEN.

Viscerale vetmassa wordt gekenmerkt door grote adipocyten, verhoogde lipolyse in de adipocyt en een groot aantal macrofagen tussen de adipocyten, die pro-inflammatoire cyto-

kines uitscheiden, zoals tumor necrosis factor alfa (TNFα), interleucine (IL) 6, IL8 en IL1. Deze pro-inflammatoire cytokines leiden tot een verhoogde productie van hsCRP in de lever en interveniëren in de insuline signaaltransductie cascade. Deze chronische laaggradige inflammatoire status kan, naast de traditionele risicofactoren, een additionele risicofactor zijn voor de ontwikkeling van hart- en vaatziekte in T2DM patiënten.

Direct na de zestien weken durende interventie waren klassieke risicofactoren voor de ontwikkeling van hart- en vaatziekten zoals hypertensie en dyslipidemie duidelijk verbeterd in beide groepen. Dit was echter geen blijvend effect. De bloeddruk verslechterde naar de uitgangswaarde, achttien maanden na start van de interventie. De gunstige effecten op de cholesterol/HDL ratio en LDL-cholesterol bleven echter wel bestaan op de lange termijn, respectievelijk in beide groepen en in de groep waarin het VLCD gecombineerd werd met een inspanningsprogramma.

De effecten van het zestien weken durende VLCD al dan niet gecombineerd met een inspanningsprogramma op laaggradige inflammatie werden pas duidelijk na de gewichtstabiliserende periode (zes maanden na start van de interventie). Direct na de interventie waren er geen veranderingen in laaggradige inflammatie. Daarentegen was zes maanden na de start van de interventie de laaggradige inflammatoire status volledig verdwenen en waren de waarden van hsCRP en de pro-inflammatoire cytokines (TNF-α, IL-6 en IL-1) vergelijkbaar met de waarden van beide gezonde controle populaties. Dit was een blijvend effect in ieder geval tot achttien maanden na start van de interventie. De toevoeging van het inspanningsprogramma had alleen toegevoegde waarde voor de daling in hsCRP, geen andere additionele effecten werden waargenomen.

Het onderliggende mechanisme, waardoor er een verbetering in laaggradige inflammatie kan ontstaan door een VLCD, is een afname in viscerale vetmassa (en daarmee de hoeveelheid macrofagen in het vetweefsel). In onze studie hebben we inderdaad een daling in buikomvang (een indirecte maat voor de hoeveelheid visceraal vet) gevonden. Gedurende de periode van gewichtsverlies blijft het aantal adipocyten gelijk, terwijl de grootte afneemt, hierdoor neemt de basale lipolyse snelheid toe. Dit trekt een grotere hoeveelheid macrofagen aan in het vetweefsel die de overmaat aan vetten fagocyteren. Het ontbreken van een direct effect van het VLCD op laaggradige inflammatie kan derhalve verklaard worden door een grotere concentratie macrofagen in het vetweefsel gedurende het VLCD. Deze macrofagen verdwijnen langzaam in de daaropvolgende gewichtstabiliserende periode.

TWEEDE GEDEELTE VAN HET PROEFSCHRIFT

In het tweede gedeelte van het proefschrift ligt de focus op ectopische vetdeposities en de hoeveelheid viscerale en subcutane vetmassa. De studiepopulatie bestond uit veertien patiënten (acht mannen en zes vrouwen). Dit is een subpopulatie van de eerder beschreven

studiepopulatie van obese (BMI 35 ± 1 kg/m^2) insuline-afhankelijke T2DM patiënten die in de magnetic resonance imaging (MRI) scanner pasten. Er werden drie keer metingen gedaan, namelijk voor, direct na en achttien maanden na de start van het zestien weken durende VLCD, al dan niet gecombineerd met het inspanningsprogramma. In de lever en het myocard werden ectopische vetdeposities gemeten *in vivo* met proton magnetic resonance spectroscopie (^{1}H-MRS). Het meten van ectopische vetdeposities in het myocard met ^{1}H-MRS is een nieuwe techniek, ontwikkeld door onze groep. Pericardiale, viscerale, subcutane vetmassa's en hartfunctie werd gemeten met een 1.5 Tesla MRI. IMCL accumulatie in de skeletspier is eerder beschreven en is niet gemeten met ^{1}H-MRS maar in spierbiopten.

DOELSTELLING ZES: KORTE EN LANGE TERMIJN EFFECTEN VAN EEN ZESTIEN WEKEN DUREND VLCD AL DAN NIET GECOMBINEERD MET EEN INSPANNINGSPROGRAMMA OP DE ECTOPISCHE VETDEPOSITIES IN HET HART EN DE HARTFUNCTIE.

In vergelijking met een gezonde controlepopulatie is zelfs bij goed ingestelde T2DM patiënten de diastolische hartfunctie verminderd. Diastolische dysfunctie van het hart is geassocieerd met een verhoogd risico op het ontwikkelen van hartfalen en mortaliteit, onafhankelijk van het al dan niet aanwezig zijn van hart- en vaatziekte. Verder wordt in cross-sectionele onderzoeken gezien dat er in T2DM patiënten sprake is van verhoogde triglyceriden stapeling in het myocard. Dit is geassocieerd met diastolische dysfunctie van het hart.

Door een zestien weken durend VLCD was de geschatte myocardiale zuurstofconsumptie (rate pressure product) significant verlaagd. Dit was een blijvend effect, in ieder geval tot achttien maanden na de start van de interventie. Dit duidt derhalve op een verbetering in efficiëntie van het hart.

Uit eerdere onderzoeken blijkt dat T2DM patiënten, in vergelijking met gezonde controles, een verminderde linker ventrikel eind diastolische volume index hebben. Dit is geassocieerd met een verminderde compliantie van het hart. In dit proefschrift beschrijven we een blijvende verbetering, in ieder geval tot achttien maanden na de start van het zestien weken durende VLCD, in diastolische functie en de distensibiliteit van de linker ventrikel gedurende de diastole. Ons onderzoek is het eerste dat laat zien dat gewichtsreductie leidt tot een blijvende verbetering in diastolische functie en een verbetering van de compliantie van de linker ventrikel in T2DM patiënten.

De stapeling van triglyceriden in het myocard nam af direct na de interventie. Achttien maanden na de start van de interventie was de triglyceriden stapeling echter weer gelijk aan de uitgangswaarde. In dit onderzoek is het niet mogelijk een directe relatie tussen triglyceriden stapeling in het myocard en myocardfunctie te leggen. Wat wel uit dit onderzoek blijkt, is dat een toename in triglyceriden stapeling in het hart niet gelijk leidt tot een verslechtering

in diastolische functie. Wellicht zijn de positieve effecten van een VLCD op glucoregulatie en/of oxidatieve stress belangrijker voor de verbetering in diastolische functie dan de toename in triglyceriden stapeling in het myocard.

Linker ventrikel massa is een onafhankelijke voorspeller van mortaliteit. In dit proefschrift werd een significante en blijvende daling in linker ventrikel massa gezien na de zestien weekse interventie in de T2DM patiënten. Insuline is een groeifactor en zou daardoor cardiale hypertrofie kunnen veroorzaken. De blijvende daling in de insulinewaarden, samen met de significante daling in gewicht, zouden een goede verklaring kunnen zijn voor de blijvende afname in linker ventrikel massa, achttien maanden na de start van de interventie.

DOELSTELLING ZEVEN: KORTE EN LANGE TERMIJN EFFECTEN VAN EEN ZESTIEN WEKEN DUREND VLCD AL DAN NIET GECOMBINEERD MET EEN INSPANNINGSPROGRAMMA OP DE HOEVEELHEID VISCERALE EN SUBCUTANE VETMASSA EN ECTOPISCHE VETDEPOSITIES IN HET LEVER EN PERICARD.

Een zestien weken durend VLCD leidt tot een significante vermindering in triglyceriden stapeling in de lever, viscerale en subcutane vetmassa en de hoeveelheid pericard vet. Ze namen respectievelijk af met 16, 40, 53 en 83% ten opzichte van de uitgangswaarden. Achttien maanden na de start van de interventie was de hoeveelheid viscerale en subcutane vetmassa lichtelijk toegenomen, maar was er een blijvende verbetering ten opzichte van de uitgangswaarden. Direct na de interventie is er een sterkere daling in viscerale vetmassa ten opzichte van de subcutane vetmassa. Achttien maanden na de start van de interventie was dit effect echter verdwenen. Ook de triglyceriden stapeling in de lever nam iets toe, achttien maanden na start van de interventie. Er was echter ook hier een blijvende verbetering in vergelijking met de uitgangswaarde.

De pericardiale vetmassa daalde significant direct na het zestien weken durend VLCD en nam daarna ook niet meer toe in de follow-up periode. Hieruit blijkt dat pericard vet blijkbaar minder fluctueert gedurende dieetinterventies in vergelijking met andere ectopische vetdeposities. Omdat een grotere hoeveelheid pericard vet geassocieerd is met een verhoogd risico op hart- en vaatziekte, kan men hypothetiseren dat onze bevinding, een daling in pericardvet, zou kunnen leiden tot een verminderd risico op hart- en vaatziekten, in onze patiënten.

CONCLUSIE

In T2DM patiënten is het gebruik van een VLCD, zonder het gebruik van orale glucoseverlagende middelen en/of insulinetherapie, veilig. Het risico op ernstige hyperglycemie is zeer klein, mits patiënten nog een endogene insulinesecretie hebben. Met het gebruik van

een VLCD kunnen patiënten een grote hoeveelheid gewicht verliezen. Het gewichtsverlies bestaat voornamelijk uit het verlies van vetmassa (zowel viscerale als subcutane vetmassa) en de vermindering van ectopische vet deposities in bijvoorbeeld het hart, de lever of de skeletspier. Het verlies van ectopische vetdeposities in de lever en in de skeletspier was geassocieerd met een significante verbetering in insulinegevoeligheid en in de skeletspier biopten met een verbetering van de insuline signaaltransductie cascade. Het verlies van ectopische vetdeposities in het hart was geassocieerd met een blijvende verbetering (tot achttien maanden na start van de interventie) in diastolische functie en distensibiliteit van de linker ventrikel gedurende de diastole.

Toevoeging van een inspanningsprogramma aan een zestien weken durend VLCD verbeterde de insulinegevoeligheid niet verder. Waarschijnlijk omdat de effecten van het VLCD zo groot waren dat de relatief kleine effecten van inspanning niet detecteerbaar waren in onze patiëntenpopulatie. Er werden echter wel verbeteringen gezien in lipiden oxidatie snelheid, de hoeveelheid mitochondrieën in de skeletspier en maximale aërobe capaciteit. Het is moeilijk op de lange termijn de hoeveelheid gewichtsreductie vast te houden. Toevoegen van het inspanningsprogramma aan het zestien weken durend VLCD had voor de lange termijn (achttien maanden na de start van de interventie) geen toegevoegde waarde voor de hoeveelheid gewichtsverlies, echter wel voor de verbeteringen in KvL en mogelijk insulineresistentie.

Onze observaties laten nogmaals zien hoe belangrijk gewichtreducerende therapieën zijn in patiënten met T2DM. Naast dieet kan voor dit doel natuurlijk ook bariatrische chirurgie gebruikt worden. Bariatrische chirurgie heeft de grootste effecten op gewicht, insulineresistentie en KvL. Maar bariatische chirurgie is duur, invasief en heeft een aanzienlijke mortaliteit en morbiditeit. Bovendien is het onmogelijk alle obese patiënten met T2DM te opereren. Daarom blijven dieet, inspanning en andere leefstijlinterventies een belangrijk onderdeel in de behandeling van de meeste obese patiënten met T2DM.

TOEKOMSTIG ONDERZOEK

In dit proefschrift kijken we naar de effecten van dieet-geïnduceerd gewichtsverlies met of zonder de toevoeging van een inspanningsprogramma. Door de zeer grote effecten van het zestien weken durend VLCD, is het moeilijk additionele effecten van inspanning aan te tonen. Om daar goed tussen te kunnen differentiëren, is een andere studie noodzakelijk. Wij stellen voor een onderzoek waarin twee interventie groepen van obese insuline-afhankelijke T2DM patiënten met elkaar vergeleken worden. Beide groepen volgen eerst een tien weken durend VLCD met daarna een tien weken durende gewichtstabiliserende periode. Gedurende de tien weken durende gewichtstabiliserende periode, neemt één groep gelijktijdig deel aan een inspanningsprogramma. Dit bestaat wekelijks uit een één uur durende training in het

ziekenhuis onder leiding van een fysiotherapeut (met name aerobics) en een minimum van 4 trainingen van 30 minuten thuis op een fietsergometer, op 70% van de maximale inspanningscapaciteit. In dit studieontwerp kan er beter gekeken worden naar het effect van een inspanningsprogramma op insulineresistentie in patiënten die al gewicht verloren hebben.

Daarnaast hebben we in dit proefschrift gekeken naar de effecten van dieet-geïnduceerd gewichtsverlies al dan niet in combinatie met een inspanningsprogramma op de hoeveelheid mitochondrieën in de skeletspier. We hebben niet gekeken naar de mitochondriële functie. Een verminderde vetoxidatie en een laag metabolisme zijn risicofactoren voor toename in gewicht en insulineresistentie. Het zou derhalve zeer interessant zijn om in de nieuwe studie niet alleen naar de hoeveelheid mitochondrieën te kijken maar ook naar de functie van de mitochondrieën.

CURRICULUM VITAE

Marieke Snel werd geboren op 20 juni 1979 te Delft. Na het behalen van haar VWO diploma aan 't Assink college te Haaksbergen, begon zij in 1997 aan de studie Biomedische Wetenschappen aan de Universiteit van Leiden. In 1999 begon zij vervolgens aan de studie Geneeskunde aan dezelfde universiteit. Het doctoraal examen van Biomedische wetenschappen en Geneeskunde werd respectievelijk behaald in 2003 en 2002. Haar artsexamen behaalde zij in 2005 cum laude. Hierna ging zij een jaar als arts-assistent niet in opleiding (ANIOS) werken op de afdeling algemene interne geneeskunde in het Medisch centrum Haaglanden (locatie Westeinde ziekenhuis) te Den Haag. In 2006 startte zij vervolgens met het promotieonderzoek in het Leids Universitair Medisch Centrum, onder begeleiding van Prof. dr. A.E. Meinders, Prof. dr. H. Pijl en dr. I.M. Jazet, waarvan de resultaten in dit proefschrift staan beschreven. In september 2009 startte zij met de opleiding interne geneeskunde in het Bronovo ziekenhuis te Den Haag (opleiders dr. J.W. van 't Wout, Prof. dr. J.W.A. Smit).

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Full Papers

1. Snel M, Hammer S, Lamb HJ, Jazet IM, van der Meer RW, Pijl H, Meinders AE, Romijn JA, de Roos A, Smit JWA. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J Am Coll Cardiol* 52: 1006-1012, 2008.

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1. Snel M, Jonker JT, Schoones J, Pijl H, Smit JWA, Meinders AE, Jazet, IM. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions

2. Snel M, Gastaldelli A, Ouwens DM, Hesselink MKC, Schaart G, Buzzigoli, Frolich M, Romijn JA, Pijl H, Meinders AE, Jazet IM. Adding exercise to a 16-week very low calorie diet in

obese, insulin-dependent type 2 diabetes mellitus patients improves metabolic flexibility, VO_{2max} and mitochondrial copy number in skeletal muscle

3. Snel M, van Diepen J, Pijl H, Romijn JA, Meinders AE, Voshol P, Jazet IM. Immediate and long-term effects of addition of exercise to a 16-week very low calorie diet on low-grade inflammation in obese, insulin-dependent type 2 diabetes mellitus patients

4. Snel M, Jonker JT, Hammer S, Jazet IM, van der Meer RW, Pijl H, Meinders AE, de Roos A, Smit JWA, Romijn JA, Lamb HJ. Sustained cardiac remodeling after a 16-week very low calorie diet in type 2 diabetes mellitus patients

5. Snel M, Jonker JT, Hammer S, Kerpershoek G, Lamb HJ, Meinders AE, Pijl H, de Roos A, Romijn JA, Smit JWA, Jazet IM. Long-term beneficial effect of a 16-week very low calorie diet on pericardial fat in obese type 2 diabetes mellitus patients

6. Snel M, Wijngaarden MA, Bizino MB, van der Grond J, Teeuwisse WM, van Buchem, Jazet IM, Pijl H. Visual and odorous food cues do not modulate the hypothalamic response to fasting in healthy humans

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