

Sebastiaan Hammer
Myocardial Triglycerides

Sebastiaan
Hammer
**Myocardial
Triglycerides**

Magnetic Resonance Spectroscopy in Health and Diabetes

Myocardial Triglycerides

***Magnetic Resonance Spectroscopy
in Health and Diabetes***

Cover Design: Geert Gratama.

Printed by: Optima Grafische Communicatie, Rotterdam.

ISBN: 978-90-8559-409-3.

© 2008, Sebastiaan Hammer, Leiden, The Netherlands. All rights reserved. No part of this thesis may be reproduced or transmitted in any form, by any means, without prior written permission of the author.

Myocardial Triglycerides

Magnetic Resonance Spectroscopy in Health and Diabetes

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 20 november 2008

klokke 15.00 uur

door

Sebastiaan Hammer

geboren te Utrecht

in 1981

PROMOTIECOMMISSIE

Promotores

Prof. Dr. J.W.A. Smit

Prof. Dr. J.A. Romijn

Prof. Dr. A. de Roos

Co-promotor

Dr. H.J Lamb

Referent

Prof. C.B. Higgins, M.D., Radiology, University of California, San Francisco, USA

Overige commissieleden

Prof. Dr. H. Pijl

Prof. Dr. J.H. Bolk

Prof. Dr. S.E. Papapoulos

Prof. Dr. Ir. L.M. Havekes

Financial support by the Netherlands Heart Foundation and the Dutch Diabetes Foundation for the publication of this thesis is gratefully acknowledged.

Additional financial support is provided by The J.E. Jurriaanse Foundation, Foundation Imago (Oegstgeest), Foundation of Image Processing, Philips BV, Astra Zeneca BV, GlaxoSmithKline BV, Medis medical imaging systems BV, Bristol-Myers-Squibb BV, Merck Sharp & Dohme BV, Solvay Pharmaceuticals BV, Guerbet Nederland BV, Sanofi-Aventis BV and Novo Nordisk BV.

Voor mijn ouders

Contents

1. General introduction 9

PART I: HEALTHY VOLUNTEERS

2. Metabolic imaging of myocardial triglyceride content: reproducibility of ¹H magnetic resonance spectroscopy with respiratory navigator gating in volunteers 27
Radiology 2007; 245(1):251-257
3. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects 41
Diabetes 2007; 56(12):2849-2853
4. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men 55
Journal of Clinical Endocrinology and Metabolism 2008; 93(2):497-503
5. Effects of short-term high-fat, high-energy diet on hepatic and myocardial triglyceride content in healthy men 69
Journal of Clinical Endocrinology and Metabolism 2008; 93(7):2702-2708

PART II: DIABETES MELLITUS

6. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus 87
American Journal of Physiology - Endocrinology and Metabolism 2008; 295(3):E714-E718
7. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function 101
Journal of the American College of Cardiology 2008; 52(12):1006-1012
8. Short-term hyperglycemic dysregulation in patients with type 1 diabetes mellitus does not change myocardial triglyceride content or myocardial function 115
Adapted from Diabetes Care 2008; 31(8):1613-1614

9.	General discussion	127
10.	Summary / Samenvatting	141
	List of publications	151
	Nawoord	155
	Curriculum Vitae	157

Chapter 1

General Introduction



Myocardial triglyceride (TG) content refers to the intracellular TG pool in cardiomyocytes. Myocardial TG stores *per se* are probably inert, but reflect non-oxidative energy pathways which may negatively influence myocardial function. Myocardial TG stores are tightly regulated by dietary TG intake, plasma TG levels and non-esterified fatty acids (NEFAs), and myocardial fatty acid uptake and oxidation. However, the physiological and pathophysiological relevance of myocardial TGs for cardiac function in humans, especially in metabolic disease, is largely unknown. In this introduction the cardiovascular risk in metabolic disease in general, and the specific potential role for myocardial TGs is discussed, in relation to TG metabolism.

CARDIOVASCULAR RISK IN METABOLIC DISEASE

Excessive caloric intake in combination with decreased physical exercise has led to an increase in the prevalence of obesity and type 2 diabetes mellitus (DM2) in the developed world (1). Obesity and DM2 are major risk factors for cardiovascular disease (2;3). In addition to the effects of insulin resistance and dyslipidemia on cardiovascular disease, a growing amount of evidence suggests a pathophysiological role of increased circulating levels of adipokines released by the adipose tissue (4) and activation of inflammatory pathways (5). Additional to atherosclerosis, obesity and DM2 also induce metabolic changes in the heart (6). The mechanisms by which these metabolic myocardial alterations in obesity and DM2 influence myocardial systolic and diastolic function are not fully elucidated. These metabolic alterations may be reflected in excessive TG accumulation in cardiomyocytes (7;8).

As early as in 1933, it was suggested in autopsy studies that fatty degeneration of the heart is a common finding in obesity, possibly associated with the development of dilated cardiomyopathy (9). However, only in the past decade a syndrome of cardiomyopathy induced by fat accumulation was described in rodents (7).

In human models, the current literature on this issue is limited, mainly due to the challenges faced by the measurement of myocardial lipid accumulation *in vivo*. There are, however, indications that alterations in metabolic pathways in the heart in obesity and DM2 are also present in humans and may affect myocardial function (10;11). Therefore, the studies presented in this thesis aim to clarify the pathophysiological relevance of myocardial TG accumulation on myocardial function in healthy subjects and in subjects with type 1 diabetes mellitus (DM1) and DM2.

TRIGLYCERIDE AND FATTY ACID METABOLISM

TGs in the circulation are derived from the diet after absorption in the intestines (packed into chylomicrons), and produced by the liver (packed in very low-density lipoproteins, VLDL-TGs).

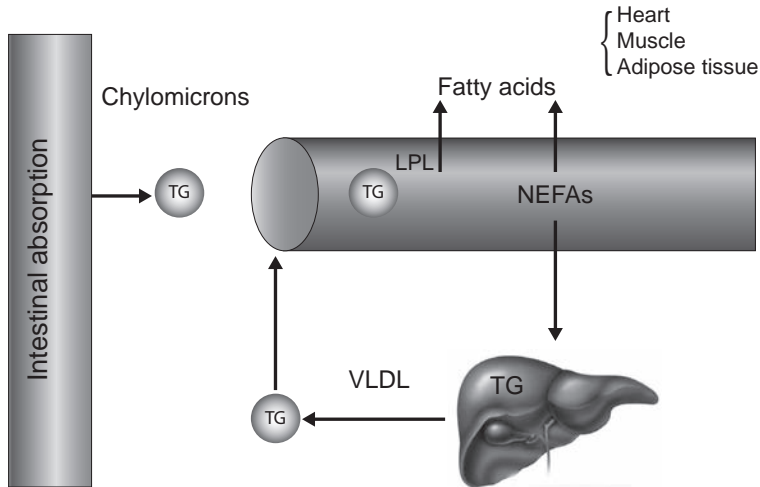


Figure 1.1. Representation of triglyceride metabolism.

Plasma triglyceride (TG) is contained within two forms of lipid particles. The liver produces very low-density lipoprotein (VLDL) TGs, whereas the intestines produce chylomicrons upon dietary intake of fat. These TG particles are lipolyzed by lipoprotein lipase (LPL) in the capillary wall to generate fatty acids, which are subsequently taken up by the cells. In addition, in the circulation there are fatty acids bound to plasma albumin (non-esterified fatty acids = NEFAs) derived from lipolysis of TGs stored in adipose tissue. These NEFAs and the TG derived-fatty acids are the sources for fatty acids for the cells to be used for energy requirements, or alternatively to be stored as intracellular TGs.

These particles are hydrolyzed by tissue-specific expression of endothelium-bound lipoprotein lipase (LPL) (12), resulting in TG derived fatty acids. These fatty acids enter the cells of the respective tissues, like myocardium, skeletal muscle and adipose tissue, where they are used for energy requirements, or in case of excessive uptake, the fatty acids are re-esterified and stored as TGs. Fatty acids are not only derived from plasma TGs, because fatty acids also circulate bound to albumin, the so called NEFAs. The balance between the utilization of different fatty acid sources is mainly substrate driven. In the fed state, fatty acids are derived from a mixture of chylomicrons and VLDL-TGs, whereas during fasting fatty acids are mainly derived from VLDL-TGs and from lipolysis of TGs stored within adipose tissue (13). A schematic overview of wholebody TG and fatty acid metabolism is provided in Figure 1.1. The heart is especially effective in hydrolyzing VLDL-TGs and chylomicron derived TGs by LPL (14-16).

In healthy conditions, almost all TGs present within the body are stored in adipose tissue, with only a small amount present in non-adipose tissues as the heart (17), the liver (18) and skeletal muscle (19). The amount of TGs stored in these non-adipose tissues is tightly regulated, but when this regulation is only slightly disrupted, TGs can accumulate in these non-adipose tissues. This accumulation is reflected in hepatic steatosis and accumulation of TGs in the pancreas (20), associate with beta cell failure in obesity and DM2 (21).

ENERGY SUBSTRATE METABOLISM IN THE HEART

The heart has a constant need for energy. The healthy heart is mostly dependent of mitochondrial oxidation of plasma fatty acids compared to glucose for energy requirements and adenosine-triphosphate (ATP) synthesis. These fatty acids account for >70% of ATP demand (22). Fatty acids enter the myocardium by passive diffusion or by protein-mediated transport, involving fatty acid transporters (mainly CD36) or fatty acid binding protein, FABP (23). Within the cardiomyocyte, the fatty acids are mainly bound to FABP and are then activated by esterification to fatty acyl-coenzyme A. These long-chain fatty acids can be redirected to TGs in the cardiomyocyte, or can be used for beta oxidation, predominantly in the mitochondria and to a lesser extent in the peroxisomes (24). The end product of beta oxidation (acetyl-coenzyme A) fuels the Krebs cycle, which ultimately generates ATP (22).

Glucose from the plasma is transported through the myocardial cellular membrane. This is regulated both by the gradient of glucose and the availability of glucose transporters (GLUT), mainly GLUT-4 (25). Acetyl-coenzyme A is formed from decarboxylation of pyruvate, which is derived from glycolysis and lactate oxidation (26). This acetyl-coenzyme A, together with acetyl-coenzyme A derived from beta oxidation of fatty acids, enters the Krebs cycle to generate ATP.

Differences in substrate delivery to the heart shift the balance between glucose and fatty acid utilization (26;27). In accordance with this concept, the rate of fatty acid uptake by the heart is primarily determined by the concentration of NEFAs in the blood (28), in addition to glucose concentrations, plasma insulin levels and factors including insulin resistance. Increased myocardial reliability on fatty acids is a hallmark of both DM1 and DM2 (29-31). A mismatch between excessive fatty acid uptake in relation to fatty acid utilization results in re-esterification of fatty acids into TGs. However, in obesity fatty acid oxidation is also increased (32-34). This increased oxidation is paralleled by a decrease in glucose utilization. Accordingly, in obesity and insulin resistance impaired fatty acid oxidation *per se* is not likely to contribute to the observed ectopic lipid accumulation (35). Excessive fatty acid supply to the myocardium increases fatty acid uptake, and this feature may result in TG accumulation.

Furthermore, the heart uses a small amount of ketone bodies for its energy requirements. Extraction of these ketones by the heart is increased, when the delivery of ketone bodies is increased (36;37), *i.e.* in poorly regulated diabetes and starvation, when insulin levels are relatively low and plasma NEFAs are increased (38-41). Oxidation of ketone bodies inhibits fatty acid oxidation (36) and can, consequently, contribute to myocardial TG accumulation. A simplified overview of myocardial substrate metabolism is provided in Figure 1.2.

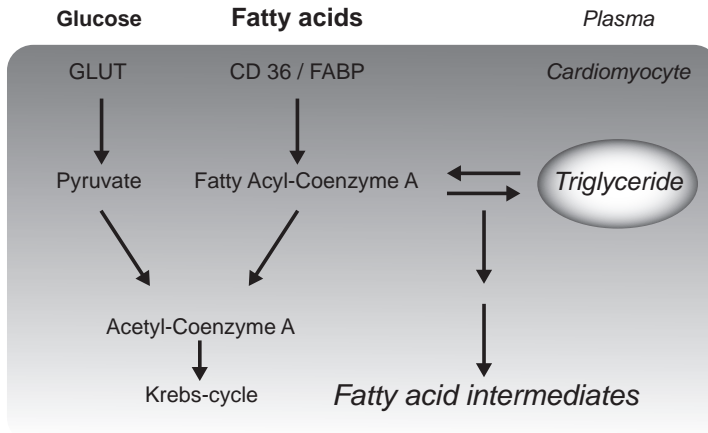


Figure 1.2. Simplified schematic overview of myocardial substrate metabolism.

A mismatch between excessive fatty acid uptake in relation to fatty acid oxidation results in excessive fatty acid re-esterification and accumulation of triglycerides (TGs). The TGs in the cytosol are a reflection of increased availability of fatty acid intermediates (see also Figure 1.3). These myocardial TGs can be quantified using ^1H magnetic resonance spectroscopy.

MECHANISMS OF MYOCARDIAL LIPOTOXICITY

An overload of cellular fatty acid uptake in relation to oxidative requirements may result in a process called lipotoxicity. The postulated pathways by which lipotoxicity induces alterations in myocardial function are diverse and are discussed in this paragraph.

The balance between cell division and cell death influences the cellular population of organs and, thereby, the functional capacity of these organs (42). A mismatch between the rate of cell death and the replacement of cells creates a functional deficit. The loss of beta cells in the pancreas in DM2 is an example of this concept, which ultimately results in insulin deficiency and hyperglycemia in DM2. This cell loss is induced by a process called programmed cell death or apoptosis. In addition to apoptotic stimuli like thermal- and chemical stress factors (43), metabolic alterations can contribute to apoptosis as well. In animal studies metabolic factors were involved in this so-called lipoapoptosis, associated with the development of pancreatic beta cell dysfunction and cardiomyopathy (7;21;44-47). Accordingly, obesity-related deposition of TGs in non-adipose tissues is associated with insulin resistance and the development of DM2 (48-53).

When fatty acid overload in cells exceeds the oxidative capacity, surplus fatty acids enter non-oxidative pathways. As mentioned above, this overload will lead to re-esterification of fatty acid derivatives into TGs within the cells, although TGs *per se* are probably not harmful. However, these TGs are the reflection of increased availability of fatty acid derivatives like diacylglycerol and fatty acyl-coenzyme A. Therefore, intracellular TG might be considered as an inert reflection of the potentially damaging pathways.

Different pathways may lead to cellular dysfunction upon increased availability of fatty acid derivatives. In addition to lipid peroxidation (47) and diacylglycerol, the ceramide pathway seems to be important (7;42). The increase in fatty acyl-coenzyme A levels, resulting from chronic lipid overload, induces *de novo* synthesis of tumor necrosis factor alpha and ceramide (46), which upregulates the expression of inducible nitric oxid synthase (21). Furthermore, fatty acyl-coenzyme A decreases Akt kinase activity (53), which ultimately decreases the translocation of the GLUT resulting in decreased glucose availability. Moreover, fatty acyl-coenzyme A activates the apoptotic process and serves as a ligand for transcription factors like peroxisomal proliferator-activated receptor alpha, which ultimately alters the structure and function of the heart.

Based on rodent studies, excessive myocardial fatty acid uptake and resulting TG accumulation may be causally involved in the development of disturbed myocardial function in diabetes mellitus (7;10;47;54). The amount of TGs is associated with alterations in myocardial function (7;47;55). Moreover, it may reflect long-chain fatty acid induced activation of calcium channels (56) which may alter cardiac function (57). The mechanisms of lipotoxicity are complex

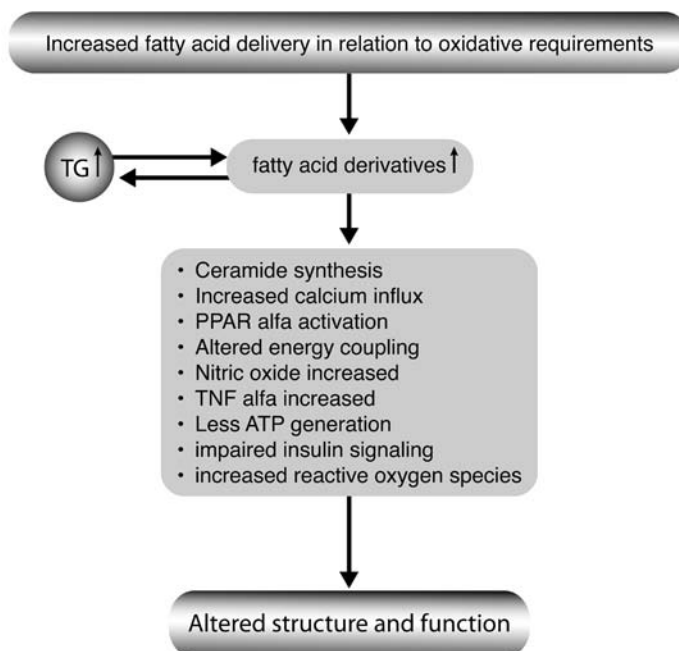


Figure 1.3. Proposed mechanism of lipotoxicity.

Increased fatty acid delivery in relation to oxidative requirements results in increased intracellular pools of fatty acid derivatives, providing substrates for non-oxidative pathways. This has different effects, which ultimately alter myocardial metabolism and myocardial function.

TG = triglyceride, PPAR = peroxisome proliferator-activated receptor, TNF = tumor necrosis factor, ATP = adenosine-triphosphate.

as glucose and fatty acid metabolism also interact with each other. For example, fatty acids inhibit Akt 1, resulting in altered insulin signaling and decreased glucose uptake. The involved pathways in lipotoxicity are summarized in Figure 1.3. Taken together, quantification of intracellular TGs may be a representation of these toxic, non-oxidative pathways. Accordingly, when obese rats are treated with troglitazone, myocardial TG accumulation is decreased, associated with a decrease in intracellular content of ceramides, DNA laddering and an improvement in myocardial contractility (7). Furthermore, hyperleptinemia in obese mice prevents the development of lipotoxic cardiomyopathy (54).

In humans, plasma TG levels are an independent predictor of left ventricular relaxation (58). Alterations in left ventricular function (59;60) are associated with altered myocardial (high-energy phosphate) metabolism in patients with DM2 (61) and in patients with hypertension (62). Furthermore, in obesity, plasma levels of NEFAs are associated with myocardial TG content (63) and with left ventricular diastolic function (63;64). These circumstantial lines of evidence indicate that the observations on the effects of fatty derivatives documented in rodent studies may also be applicable in human pathophysiology.

MYOCARDIAL TRIGLYCERIDES IN HUMANS

Although experimental studies in rodents suggest a causal relation between myocardial TG content and myocardial function, translational studies on this subject in humans are scarce. One important reason for this lack of human studies is that non-invasive measurement of myocardial TG content is challenging, mainly due to the confounding effects of cardiac and respiratory motion. However, recently, hydrogen 1 magnetic resonance spectroscopy (¹HMRS) became available to assess TG content of the myocardium in humans *in vivo* (17;63;65-67). An example of a ¹HMR spectrum of the myocardium is shown in Figure 1.4. This technique has been validated against histological samples for measurement of hepatic TG content (68;69) and skeletal muscle TG content (70).

The ¹HMRS measurement of myocardial TG content is technically challenging, and, therefore, not widely available. To obtain accurate measurements *in vivo* it is essential to minimize artifacts induced by cardiac and respiratory motion (66;67;71;72). Recent studies have shown that myocardial TG content correlates with histological verified TG content (17). Therefore, this technique also allows to measure myocardial TG content in the human heart *in vivo*.

MAGNETIC RESONANCE IMAGING OF THE HEART

Cardiovascular magnetic resonance (CMR) is perfectly suitable to assess myocardial systolic and diastolic function (73-75). CMR combined with metabolic imaging of TG content by ¹HMRS and

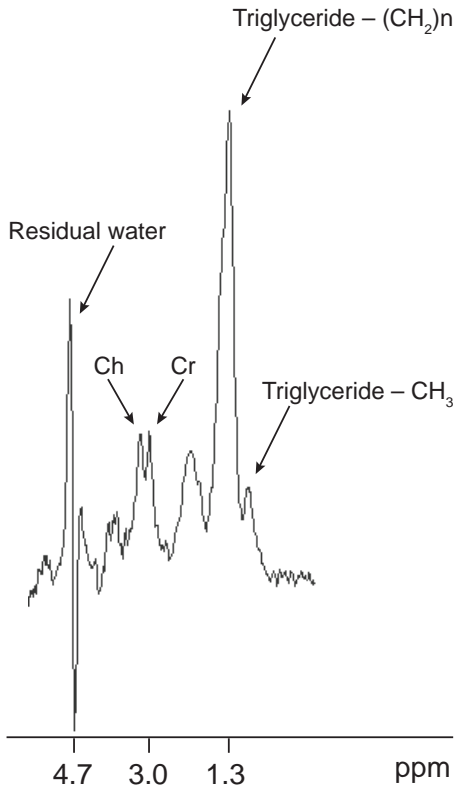


Figure 1.4. Myocardial ¹H magnetic resonance spectrum.

Typical example of a myocardial ¹H spectrum obtained in an 8-ml voxel, placed in the interventricular spectrum showing resonances of the myocardial lipids, creatine (Cr) and choline (Ch). ppm = parts per million.

phosphorus 31 (³¹P) spectroscopy provides a potential useful tool to study myocardial substrate metabolism in relation to myocardial function *in vivo*. The first studies on this subject indicate that myocardial TG content is indeed associated with plasma fatty acid levels and myocardial function (63;76). Moreover, it seems that fatty infiltration in the myocardium precedes the onset of systolic dysfunction and is, therefore, a potential parameter for the evaluation of treatment in the insulin resistant state, even before diabetic cardiomyopathy is present (76). However, the pathophysiological associations between myocardial TG accumulation and myocardial function in humans remain largely uncharacterized.

Therefore, the aim of this thesis is to study myocardial TG content in relation to cardiac function in a variety of metabolic interventions, in healthy subjects and in patients with DM1 and DM2.

OUTLINE OF THE THESIS

The studies in this thesis evaluate the relation between myocardial TG content and myocardial function under physiological and pathophysiological circumstances in humans *in vivo*.

Moreover, we evaluate tissue-specific effects by including the quantification of hepatic TG content, being an extra-cardiac location of ectopic deposition of TGs.

The first part of this thesis documents studies performed in healthy subjects. Chapter 2 evaluates the effects of compensation for the movement artifacts induced by cardiac and respiratory motion on ^1H MRS measurements. Chapter 3 describes a study on the effects of short-term caloric restriction on myocardial TG content and myocardial function. Furthermore, we evaluate whether extending the model of partial caloric restriction to complete starvation results in more pronounced alterations in Chapter 4. Chapter 5 describes a study on the effects of a hypercaloric, high-fat diet on plasma metabolic parameters, and myocardial TG content in relation to cardiac function.

Part two of this thesis evaluates the flexibility of TG content of the diabetic myocardium in relation to myocardial function in humans *in vivo*. Therefore, we evaluate the effects of partial caloric restriction on myocardial TG content and myocardial function in patients with DM2 in chapter 6. Moreover, we study the same subjects under the condition of partial caloric restriction during inhibition of adipose tissue lipolysis by administration of acipimox, to specifically assess the contribution of plasma NEFA levels to myocardial TG content and myocardial function. Chapter 7 describes the effects of prolonged partial caloric restriction in severely obese patients with DM2. According to our previous work, we hypothesize that this may result in decreased myocardial TG stores, associated with improved myocardial function as weight loss increases insulin sensitivity (77-79). In patients with DM1 glucoregulation is imperfect and frequent episodes of hyperglycemia and high plasma NEFA levels are frequently present. Therefore, these metabolic alterations may adversely affect myocardial metabolism, with accumulation of myocardial and hepatic TGs as well. Chapter 8 evaluates the effects of controlled partial insulin deprivation for 24 hours with resulting hyperglycemia and increased plasma NEFA levels in otherwise well-controlled patients with DM1 on myocardial TG content and myocardial function.

REFERENCES

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; 414(6865):782-787.
2. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006; 113(6):898-918.
3. Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Arch Intern Med* 2002; 162(16):1867-1872.
4. Rahmouni K, Correia ML, Haynes WG, Mark AL. Obesity-associated hypertension: new insights into mechanisms. *Hypertension* 2005; 45(1):9-14.
5. Mehta S, Farmer JA. Obesity and inflammation: a new look at an old problem. *Curr Atheroscler Rep* 2007; 9(2):134-138.
6. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *JAMA* 1979; 241(19):2035-2038.
7. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000; 97(4):1784-1789.
8. McGavock JM, Victor RG, Unger RH, Szczepaniak LS. Adiposity of the heart, revisited. *Ann Intern Med* 2006; 144(7):517-524.
9. Smith HL, Willius FA. Adiposity of the Heart. *Arch Intern Med* 1933; 52:811-931.
10. Sharma S, Adroque JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeyer H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* 2004; 18(14):1692-1700.
11. Witteles RM, Fowler MB. Insulin-resistant cardiomyopathy clinical evidence, mechanisms, and treatment options. *J Am Coll Cardiol* 2008; 51(2):93-102.
12. Havel RJ, Fielding CJ, Olivecrona T, Shore VG, Fielding PE, Egelrud T. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoproteins lipase from different sources. *Biochemistry* 1973; 12(9):1828-1833.
13. Barrows BR, Parks EJ. Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed states. *J Clin Endocrinol Metab* 2006; 91(4):1446-1452.
14. Goudriaan JR, Tacke PJ, Dahlmans VE, Gijbels MJ, van Dijk KW, Havekes LM, Jong MC. Protection from obesity in mice lacking the VLDL receptor. *Arterioscler Thromb Vasc Biol* 2001; 21(9):1488-1493.
15. Sakai J, Hoshino A, Takahashi S, Miura Y, Ishii H, Suzuki H, Kawarabayasi Y, Yamamoto T. Structure, chromosome location, and expression of the human very low density lipoprotein receptor gene. *J Biol Chem* 1994; 269(3):2173-2182.
16. Augustus AS, Kako Y, Yagy H, Goldberg IJ. Routes of FA delivery to cardiac muscle: modulation of lipoprotein lipolysis alters uptake of TG-derived FA. *Am J Physiol Endocrinol Metab* 2003; 284(2):E331-E339.

17. Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. *Am J Physiol* 1999; 276(5 Pt 1):E977-E989.
18. Ishii M, Yoshioka Y, Ishida W, Kaneko Y, Fujiwara F, Taneichi H, Miura M, Toshihiro M, Takebe N, Iwai M, Suzuki K, Satoh J. Liver fat content measured by magnetic resonance spectroscopy at 3.0 tesla independently correlates with plasminogen activator inhibitor-1 and body mass index in type 2 diabetic subjects. *Tohoku J Exp Med* 2005; 206(1):23-30.
19. Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Ma YZ, Savoye M, Rothman DL, Shulman GI, Caprio S. Assessment of skeletal muscle triglyceride content by (1)H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 2002; 51(4):1022-1027.
20. Tushuizen ME, Bunck MC, Pouwels PJ, Bontemps S, van Waesberghe JH, Schindhelm RK, Mari A, Heine RJ, Diamant M. Pancreatic fat content and beta-cell function in men with and without type 2 diabetes. *Diabetes Care* 2007; 30(11):2916-2921.
21. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A* 1998; 95(5):2498-2502.
22. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 2005; 85(3):1093-1129.
23. van der Vusse GJ, van Bilsen M, Glatz JF. Cardiac fatty acid uptake and transport in health and disease. *Cardiovasc Res* 2000; 45(2):279-293.
24. Kunau WH, Dommes V, Schulz H. beta-oxidation of fatty acids in mitochondria, peroxisomes, and bacteria: a century of continued progress. *Prog Lipid Res* 1995; 34(4):267-342.
25. Young LH, Coven DL, Russell III RR. Cellular and molecular regulation of cardiac glucose transport. *Journal of Nuclear Cardiology* 2000; 7(3):267-276.
26. Gertz EW, Wisneski JA, Stanley WC, Neese RA. Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. *J Clin Invest* 1988; 82(6):2017-2025.
27. Wisneski JA, Stanley WC, Neese RA, Gertz EW. Effects of acute hyperglycemia on myocardial glycolytic activity in humans. *J Clin Invest* 1990; 85(5):1648-1656.
28. Wisneski JA, Gertz EW, Neese RA, Mayr M. Myocardial metabolism of free fatty acids. Studies with 14C-labeled substrates in humans. *J Clin Invest* 1987; 79(2):359-366.
29. Perseghin G, Lattuada G, Danna M, Sereni LP, Maffi P, De CF, Battezzati A, Secchi A, Del MA, Luzi L. Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *Am J Physiol Endocrinol Metab* 2003; 285(6):E1174-E1181.
30. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. *Cardiovasc Res* 1997; 34(1):25-33.
31. Peterson LR, Herrero P, McGill J, Schechtman KB, Kisrieva-Ware Z, Lesniak D, Gropler RJ. Fatty acids and insulin modulate myocardial substrate metabolism in humans with type 1 diabetes. *Diabetes* 2008; 57(1):32-40.
32. Peterson LR, Herrero P, Schechtman KB, Racette SB, Waggoner AD, Kisrieva-Ware Z, Dence C, Klein S, Marsala J, Meyer T, Gropler RJ. Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. *Circulation* 2004; 109(18):2191-2196.

33. Buchanan J, Mazumder PK, Hu P, Chakrabarti G, Roberts MW, Yun UJ, Cooksey RC, Litwin SE, Abel ED. Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity. *Endocrinology* 2005; 146(12):5341-5349.
34. Mazumder PK, O'Neill BT, Roberts MW, Buchanan J, Yun UJ, Cooksey RC, Boudina S, Abel ED. Impaired Cardiac Efficiency and Increased Fatty Acid Oxidation in Insulin-Resistant ob/ob Mouse Hearts. *Diabetes* 2004; 53(9):2366-2374.
35. Lopaschuk GD, Folmes CD, Stanley WC. Cardiac energy metabolism in obesity. *Circ Res* 2007; 101(4): 335-347.
36. Chen V, Wagner G, Spitzer JJ. Regulation of substrate oxidation in isolated myocardial cells by beta-hydroxybutyrate. *Horm Metab Res* 1984; 16(5):243-247.
37. Forsey RG, Reid K, Brosnan JT. Competition between fatty acids and carbohydrate or ketone bodies as metabolic fuels for the isolated perfused heart. *Can J Physiol Pharmacol* 1987; 65(3):401-406.
38. Jensen MD, Ekberg K, Landau BR. Lipid metabolism during fasting. *Am J Physiol Endocrinol Metab* 2001; 281(4):E789-E793.
39. Klein S, Sakurai Y, Romijn JA, Carroll RM. Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. *Am J Physiol* 1993; 265(5 Pt 1):E801-E806.
40. Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. *Diabetes* 1988; 37(8):1020-1024.
41. Savendahl L, Underwood LE. Fasting increases serum total cholesterol, LDL cholesterol and apolipoprotein B in healthy, nonobese humans. *J Nutr* 1999; 129(11):2005-2008.
42. Unger RH, Orci L. Lipoapoptosis: its mechanism and its diseases. *Biochim Biophys Acta* 2002; 1585(2-3):202-212.
43. Feuerstein GZ, Young PR. Apoptosis in cardiac diseases: stress- and mitogen-activated signaling pathways. *Cardiovasc Res* 2000; 45(3):560-569.
44. Chiu HC, Kovacs A, Ford DA, Hsu FF, Garcia R, Herrero P, Saffitz JE, Schaffer JE. A novel mouse model of lipotoxic cardiomyopathy. *J Clin Invest* 2001; 107(7):813-822.
45. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 1994; 91(23):10878-10882.
46. Shimabukuro M, Higa M, Zhou YT, Wang MY, Newgard CB, Unger RH. Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. *J Biol Chem* 1998; 273(49):32487-32490.
47. Vincent HK, Powers SK, Dirks AJ, Scarpace PJ. Mechanism for obesity-induced increase in myocardial lipid peroxidation. *Int J Obes Relat Metab Disord* 2001; 25(3):378-388.
48. Krssak M, Falk PK, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ¹H NMR spectroscopy study. *Diabetologia* 1999; 42(1):113-116.
49. Machann J, Haring H, Schick F, Stumvoll M. Intramyocellular lipids and insulin resistance. *Diabetes Obes Metab* 2004; 6(4):239-248.
50. Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. *Physiology (Bethesda)* 2004; 19:183-190.

51. Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, Buzzigoli E, Sironi AM, Cersosimo E, Ferrannini E, DeFronzo RA. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 2007; 133(2):496-506.
52. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, Bain J, Stevens R, Dyck JR, Newgard CB, Lopaschuk GD, Muoio DM. Mitochondrial overload and incomplete Fatty Acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab* 2008; 7(1):45-56.
53. Zhou H, Summers SA, Birnbaum MJ, Pittman RN. Inhibition of Akt kinase by cell-permeable ceramide and its implications for ceramide-induced apoptosis. *J Biol Chem* 1998; 273(26):16568-16575.
54. Lee Y, Naseem RH, Duplomb L, Park BH, Garry DJ, Richardson JA, Schaffer JE, Unger RH. Hyperleptinemia prevents lipotoxic cardiomyopathy in acyl CoA synthase transgenic mice. *Proc Natl Acad Sci U S A* 2004; 101(37):13624-13629.
55. Christoffersen C, Bollano E, Lindgaard ML, Bartels ED, Goetze JP, Andersen CB, Nielsen LB. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003; 144(8):3483-3490.
56. Huang JM, Xian H, Bacaner M. Long-chain fatty acids activate calcium channels in ventricular myocytes. *Proc Natl Acad Sci U S A* 1992; 89(14):6452-6456.
57. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. *Circulation* 2002; 105(12):1503-1508.
58. de Las Fuentes L, Waggoner AD, Brown AL, Davila-Roman VG. Plasma Triglyceride Level is an Independent Predictor of Altered Left Ventricular Relaxation. *J Am Soc Echocardiogr* 2005; 18(12):1285-1291.
59. Ahmed SS, Jaferi GA, Narang RM, Regan TJ. Preclinical abnormality of left ventricular function in diabetes mellitus. *Am Heart J* 1975; 89(2):153-158.
60. Liu JE, Palmieri V, Roman MJ, Bella JN, Fabsitz R, Howard BV, Welty TK, Lee ET, Devereux RB. The impact of diabetes on left ventricular filling pattern in normotensive and hypertensive adults: the Strong Heart Study. *J Am Coll Cardiol* 2001; 37(7):1943-1949.
61. Diamant M, Lamb HJ, Groeneveld Y, Ender EL, Smit JW, Bax JJ, Romijn JA, de Roos A, Radder JK. Diastolic dysfunction is associated with altered myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2 diabetes mellitus. *J Am Coll Cardiol* 2003; 42(2):328-335.
62. Lamb HJ, Beyerbach HP, van der Laarse A, Stoel BC, Doornbos J, van der Wall EE, de Roos A. Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. *Circulation* 1999; 99(17):2261-2267.
63. Kankaanpaa M, Lehto HR, Parkka JP, Komu M, Viljanen A, Ferrannini E, Knuuti J, Nuutila P, Parkkola R, Iozzo P. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006; 91(11):4689-4695.
64. Leichman JG, Aguilar D, King TM, Vlada A, Reyes M, Taegtmeier H. Association of plasma free fatty acids and left ventricular diastolic function in patients with clinically severe obesity. *Am J Clin Nutr* 2006; 84(2):336-341.
65. den Hollander JA, Evanochko WT, Pohost GM. Observation of cardiac lipids in humans by localized 1H magnetic resonance spectroscopic imaging. *Magn Reson Med* 1994; 32(2):175-180.
66. Felblinger J, Jung B, Slotboom J, Boesch C, Kreis R. Methods and reproducibility of cardiac/respiratory double-triggered (1)H-MR spectroscopy of the human heart. *Magn Reson Med* 1999; 42(5):903-910.

67. Schar M, Kozerke S, Boesiger P. Navigator gating and volume tracking for double-triggered cardiac proton spectroscopy at 3 Tesla. *Magn Reson Med* 2004; 51(6):1091-1095.
68. Longo R, Pollesello P, Ricci C, Masutti F, Kvam BJ, Bercich L, Croce LS, Grigolato P, Paoletti S, de BB. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. *J Magn Reson Imaging* 1995; 5(3):281-285.
69. Thomsen C, Becker U, Winkler K, Christoffersen P, Jensen M, Henriksen O. Quantification of liver fat using magnetic resonance spectroscopy. *Magn Reson Imaging* 1994; 12(3):487-495.
70. Howald H, Boesch C, Kreis R, Matter S, Billeter R, Essen-Gustavsson B, Hoppeler H. Content of intramyocellular lipids derived by electron microscopy, biochemical assays, and (1)H-MR spectroscopy. *J Appl Physiol* 2002; 92(6):2264-2272.
71. Kozerke S, Schar M, Lamb HJ, Boesiger P. Volume tracking cardiac 31P spectroscopy. *Magn Reson Med* 2002; 48(2):380-384.
72. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.
73. Hartiala JJ, Mostbeck GH, Foster E, Fujita N, Dulce MC, Chazouilleres AF, Higgins CB. Velocity-encoded cine MRI in the evaluation of left ventricular diastolic function: measurement of mitral valve and pulmonary vein flow velocities and flow volume across the mitral valve. *Am Heart J* 1993; 125(4):1054-1066.
74. Paelinck BP, de Roos A, Bax JJ, Bosmans JM, van der Geest RJ, Dhondt D, Parizel PM, Vrints CJ, Lamb HJ. Feasibility of tissue magnetic resonance imaging: a pilot study in comparison with tissue Doppler imaging and invasive measurement. *J Am Coll Cardiol* 2005; 45(7):1109-1116.
75. Pattynama PM, Lamb HJ, van der Velde EA, van der Wall EE, de Roos A. Left ventricular measurements with cine and spin-echo MR imaging: a study of reproducibility with variance component analysis. *Radiology* 1993; 187(1):261-268.
76. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007; 116(10):1170-1175.
77. Jazet IM, Schaart G, Gastaldelli A, Ferrannini E, Hesselink MK, Schrauwen P, Romijn JA, Maassen JA, Pijl H, Ouwens DM, Meinders AE. Loss of 50% of excess weight using a very low energy diet improves insulin-stimulated glucose disposal and skeletal muscle insulin signalling in obese insulin-treated type 2 diabetic patients. *Diabetologia* 2008; 51(2):309-319.
78. Wing RR. Use of very-low-calorie diets in the treatment of obese persons with non-insulin-dependent diabetes mellitus. *J Am Diet Assoc* 1995; 95(5):569-572.
79. Wing RR, Blair EH, Bononi P, Marcus MD, Watanabe R, Bergman RN. Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. *Diabetes Care* 1994; 17(1):30-36.

Part I

Healthy Volunteers



Chapter 2

Metabolic Imaging of Myocardial Triglyceride Content: Reproducibility of ^1H MR Spectroscopy with Respiratory Navigator Gating in Volunteers

Radiology 2007; 245(1):251-257

R.W. van der Meer

J. Doornbos

S. Kozerke

M. Schär

J.J. Bax

S. Hammer

J.W.A. Smit

M. Diamant

L.J. Rijzewijk

A. de Roos

H.J. Lamb



SUMMARY

Objectives: The purpose of the study was to prospectively compare spectral resolution and reproducibility of ^1H magnetic resonance spectroscopy ($^1\text{HMRS}$), with and without respiratory motion compensation based on navigator echoes, in the assessment of myocardial triglyceride (TG) content in the human heart.

Materials and methods: In 20 volunteers (14 men, 6 women; mean age \pm standard error, 31 ± 2.8 years [range, 19-60 years]; body mass index, 19-30 kg/m 2) without history of cardiovascular disease, $^1\text{HMRS}$ of the myocardium was performed at rest, with and without respiratory motion compensation.

Results: Unsuppressed water signal linewidth changed from 11.9 Hz to 10.7 Hz ($P < 0.001$) with the use of the navigator, which indicated better spectral resolution. The navigator improved the intraclass correlation coefficient for the assessment of myocardial TG content from 0.32 to 0.81.

Conclusions: It is concluded that respiratory motion correction is essential for reproducible assessment of myocardial TGs.

INTRODUCTION

Hydrogen 1 magnetic resonance (MR) spectroscopy (¹HMRS) is a promising tool for metabolic imaging to assess triglyceride (TG) content of myocardial tissue in humans (1-3). Findings from rat studies have shown that there is a negative correlation between myocardial TG content and heart function, while treatment with insulin-sensitizing drugs reduced myocardial TG deposition and reversed contractile dysfunction in lipotoxic heart disease in obese Zucker rats (4-6). These findings suggest that intramyocardial TG accumulation is deleterious to the heart (7). Furthermore, myocardial TG content may be a marker of myocardial viability after coronary occlusion due to enhanced esterification and/or reduced oxidation of fatty acids in ischemically insulted but viable myocardium (8).

Motion artifacts from cardiac and respiratory motion have a negative effect on the reliability of myocardial ¹HMRS. Motion of the heart relative to the volume of interest may lead to reduced spectral resolution and contamination of the ¹HMR spectrum by, for example, epicardial fat. In addition, respiratory motion may negatively influence ¹HMR spectral resolution by preventing optimal shimming and water suppression. Several methods for respiratory gating have been proposed to improve repeatability and spectral resolution at ¹HMRS (2;3;9). Recently, respiratory navigator gating and volume tracking for double-triggered cardiac ¹HMRS became available (10). However, the influence of respiratory navigator gating on spectral resolution and on the reproducibility of myocardial TG measurements is unknown. Therefore, the purpose of our study was to prospectively compare spectral resolution and reproducibility of ¹HMRS, with and without respiratory motion compensation based on navigator echoes, to assess myocardial TGs in the human heart.

MATERIALS AND METHODS

One of the authors (M.S.) is an employee of Philips Medical Systems (Cleveland, Ohio). This author provided technical and intellectual input to the study. The authors who were not employed by Philips Medical Systems had full control of the inclusion of the data and information that might have presented a conflict of interest for this author. In 20 volunteers (14 men, 6 women; mean age \pm standard error, 31 ± 2.8 years [range, 19-60 years]; body mass index, 19-30 kg/m²) without a history of cardiovascular disease, ¹HMRS of the myocardium was performed at rest. Furthermore, one healthy male subject (age, 22 years; body mass index, 23 kg/m²) underwent ¹HMRS before and after a very low-calorie diet. This healthy subject had no history or clinical evidence of cardiovascular disease, diabetes, or any other chronic disease (screening visit consisted of a medical history, physical examination, electrocardiography [ECG], and screening laboratory tests such as fasting plasma glucose and lipid levels and an oral glucose tolerance test). The

medical ethical committee of our institution (Leiden University Medical Center, Leiden, The Netherlands) approved our study protocol, and all participants gave informed consent.

Study design

ECG-triggered ^1H MRS was performed twice during one session with the same parameters, without changing the position of the voxel, both with and without the use of respiratory navigator gating and volume tracking. Thereafter, the volunteer was removed from the imager. After 5 minutes, the volunteer was repositioned in the magnet bore, and ECG-triggered ^1H MRS was repeated with and without respiratory navigator gating and volume tracking after completing all preparation phases. No marking of coil position on the chest wall of subjects or other efforts to minimize variability were performed. To test the ability of respiratory navigator-gated ^1H MRS to demonstrate changes in myocardial TG content after metabolic interventions, ^1H MRS was performed in one volunteer before and after a 3-day very low-calorie diet. The low-calorie diet consisted of 471 kilocalories, 50.2 g of carbohydrates, and 6.9 g of fat (0.94 g saturated fat, Modifast Intensive; Nutrition & Santé Benelux, Breda, The Netherlands) per day. The volunteer was instructed to remain fasted for 4 hours prior to both ^1H MRS examinations.

Magnetic resonance technique

Cardiac MR examinations were performed at 1.5-Tesla (Gyrosan ACS/NT15; Philips, Best, The Netherlands). A 17-cm diameter circular surface coil, with a vitamin-A capsule in the center for visualization of the coil center on survey images, was positioned on the chest wall. Gradient-echo survey images were acquired to verify location of the ^1H MRS surface coil. When necessary, the coil was repositioned to place the coil center just below the mitral valve level of the heart (Figure 2.1). Once the coil was at the correct position, ECG-triggered MR imaging was performed to acquire multiphase gradient-echo images (repetition time ms/ echo time ms, 3.5/1.75; 35-40 heart phases) in the four-chamber and short-axis views to image the interventricular septum and to determine the time point of end-systole (Figure 2.1).

^1H magnetic resonance spectroscopy technique

ECG-triggered cardiac ^1H MRS spectra were obtained from the interventricular septum with subjects in the supine position. The body coil was used for radiofrequency transmission, and the 17-cm diameter circular surface coil was used for signal reception. An 8-ml voxel ($4 \times 2 \times 1$ cm) was positioned in the interventricular septum on the four-chamber and short-axis images at end-systole, thereby avoiding contamination from epicardial fat (Figure 2.1). A section-selective 90° pulse, followed by two section-selective refocusing pulses (a pointresolved spectroscopy sequence) was used to acquire single-voxel MR spectroscopic data (1). Spectra were acquired at end-systole, with an echo time of 26 ms and a repetition time of at least 3000 ms; 1024 data points were collected by using 1000-Hz spectral width and 128 signals acquired. The repetition time of 3000 ms was chosen to approach complete relaxation of the TG signals. A pencil

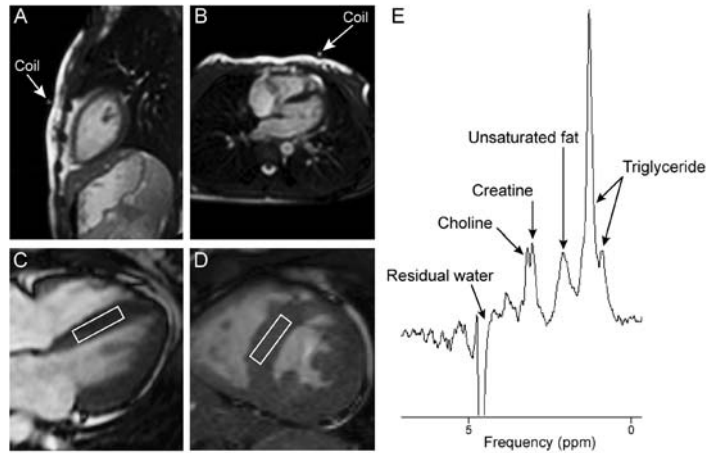


Figure 2.1.

Images show coil position and spectroscopic volume. The surface coil was positioned just below the mitral valve level of the heart on, A, sagittal and, B, transverse balanced steady-state free precession magnetic resonance (MR) images. Spectroscopic volume localization in the interventricular septum on, C, four-chamber and, D, short-axis views (ECG-triggered balanced steady-state free precession MR is demonstrated). Care was taken to avoid contamination from epicardial fat. E, Typical water-suppressed ^1H spectrum of myocardial tissue located in the interventricular septum (128 signals acquired; voxel size 8 ml). Peak heights are in arbitrary units, ppm = parts per million.

beam navigator was positioned on the lung-liver interface of the right hemidiaphragm (Figure 2.2) for respiratory motion gating and tracking (10-12) by one of the authors (R.W.v.d.M.). A two-dimensional spatially-selective radiofrequency pulse for pencil beam-shaped excitation was used. A pencil beam with a diameter of 25 mm and a length of 80 mm was selected. Respiratory navigator-gated spectroscopic data were accepted during data acquisition when the diaphragm-lung interface was within a predefined acceptance window of 5 mm around end expiration.

Motion tracking was used to compensate for any residual translational shifts of the diaphragm-lung interface within the predefined acceptance window. The assumed scale factor between diaphragmatic motion and cardiac motion in the feet-to-head direction was 0.6 (13). Automatic center frequency determination, gradient shimming, transmit power, receiver gain optimization, and water suppression were performed by using respiratory navigator gating and tracking. Without changing any parameter, a spectrum without water suppression was obtained, with a repetition time of 10000 ms (to approach complete relaxation of the water signal) and four signals acquired, to be used as an internal reference (see next section). Total acquisition time for both a watersuppressed and a water-unsuppressed spectrum, including (re-)positioning of the patient, shimming, and parameter adjustment for water suppression, was on average 25 minutes. Assessment of a single, water-suppressed spectrum with 128 signals acquired took approximately 10 minutes depending on the respiratory cycle of the volunteer and on the acceptance rate of the respiratory navigator.

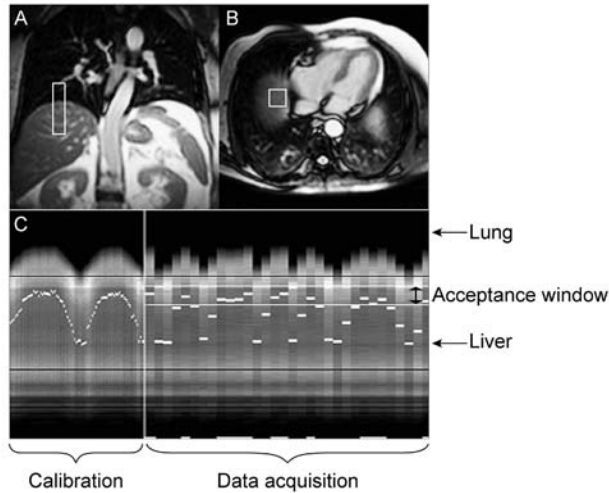


Figure 2.2.

Images show position of pencil beam on right hemidiaphragm. A, Coronal and B, transverse balanced steady-state free precession magnetic resonance images show positioning of pencil beam on right hemidiaphragm. C, White dots (left) represent the automatically traced position of diaphragm (pencil beam excitation pulse is applied in foot-head direction). Two respiratory cycles are used for calibrations (smooth white line); thereafter, during data acquisition, navigator samples are taken with lower temporal resolution (white points, right). White horizontal lines indicate acceptance window (end-expiration, 5 mm); whenever the detected motion state of the diaphragm is within the window, the spectroscopic measurement is accepted.

Spectral quantification

All ^1H MRS data were fitted in the time domain, directly on free induction decays by using Java-based MR user-interface software (jMRUI version 2.2; A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium) (14) in consensus by two authors (R.W.v.d.M. and H.J.L., with 2 and 15 years of experience in myocardial MR imaging, respectively). The Hankel-Lanczos filter (single-variable decomposition method) was used to remove residual water signal from spectra acquired with water suppression. Myocardial TG signal amplitudes were analyzed automatically by using the Advanced Magnetic Resonance, or AMARES, fitting algorithm within the jMRUI software (15). The AMARES fitting algorithm within jMRUI also provides the standard deviation of the amplitude (one time the Cramer-Rao standard deviation [CRSD]), which can be used as a measure of the accuracy of the fitted signal amplitude, reflecting the signal-to-noise ratio (16). The CRSD of the lipid signal was divided by the lipid signal amplitude, yielding a relative CRSD, which is inversely related to the signal-to-noise ratio. Resonance frequency estimates for intramyocardial lipids were described with the assumption of Gaussian line shapes at 0.9, 1.3, and 2.1 parts per million (ppm). (In keeping with the approach of Torriani *et al.* (17), we summed the amplitudes of lipid resonances at 0.9 and 1.3 ppm for TG quantification for statistical analysis). Prior knowledge was incorporated into the fitting algorithm by using previously published criteria (18-20). Fixed frequencies for TG peaks were used, and linewidths and amplitudes were

unconstrained. The zero-order phase correction was estimated by using the AMARES algorithm, and the first-order phase correction was fixed to 0.13 ms. The water signal from spectra without water suppression obtained from the same voxel was used as internal reference for relative quantification of lipid resonances. The water signal peak at 4.7 ppm was quantified and the linewidth (full width at half maximum) was calculated by using a Lorentzian line shape in the AMARES algorithm. The percentage of myocardial TG content relative to water was calculated as the signal amplitude of TGs divided by the signal amplitude of water, and multiplied by 100.

Statistical analysis

To compare reproducibility of percentage of myocardial TG content with and without respiratory navigator gating and volume tracking, the intraclass correlation coefficients were calculated by using a mixed-effects analysis of variance (with patients as random factor) for both conditions separately. Furthermore, the coefficients of variance were calculated for both conditions separately. Moreover, Bland-Altman plots were constructed. Statistical significance of differences was assessed by using two-tailed paired t-tests, and $P < 0.05$ was considered to indicate a significant difference. Mean values \pm standard errors are given. Statistical analyses were performed by using statistical software (SPSS, version 12.01; SPSS, Chicago, Ill).

RESULTS

The full width at half maximum value of the unsuppressed water signal changed from $11.9 \text{ Hz} \pm 0.4$ to $10.7 \text{ Hz} \pm 0.44$ (all data pooled, $P < 0.001$), without and with respiratory navigator gating, respectively. A decrease was observed in the calculated mean myocardial TG percentage with use of respiratory navigator gating compared with myocardial TG percentage assessed without use of the navigator (Table 2.1).

In all acquisition conditions, the CRSD was less than 1% of the lipids signal amplitude.

Bland-Altman plots of the observed percentage of myocardial TGs without and with respiratory navigator showed smaller limits of agreement (mean \pm 2 standard deviations) when respiratory navigator is used, indicating improved reproducibility (Figure 2.3). Without use of

Table 2.1. Reproducibility of human myocardial triglyceride content.

Data	Without navigator		With navigator	
	%TG*	Relative CRSD (%) [†]	%TG*	Relative CRSD (%) [†]
All data (n=40)	0.46 ± 0.02	0.80 ± 0.07	$0.38 \pm 0.02^{\ddagger}$	0.92 ± 0.09
Acquisition 1 (n=20)	0.43 ± 0.03	0.86 ± 0.12	$0.37 \pm 0.03^{\ddagger}$	0.95 ± 0.12
Acquisition 2 (n=20)	0.48 ± 0.03	0.74 ± 0.09	$0.40 \pm 0.03^{\ddagger}$	0.89 ± 0.12

Data are mean \pm standard error. * Relative to the water signal.

[†] CRSD (cramer-rao standard deviation) relative to the triglyceride (TG) signal amplitude. Relative CRSD was used as a representative for the signal-to-noise ratio. [‡] Percentage of TGs without navigator is significantly different from that with navigator (two-tailed paired t-tests, $P < 0.05$).

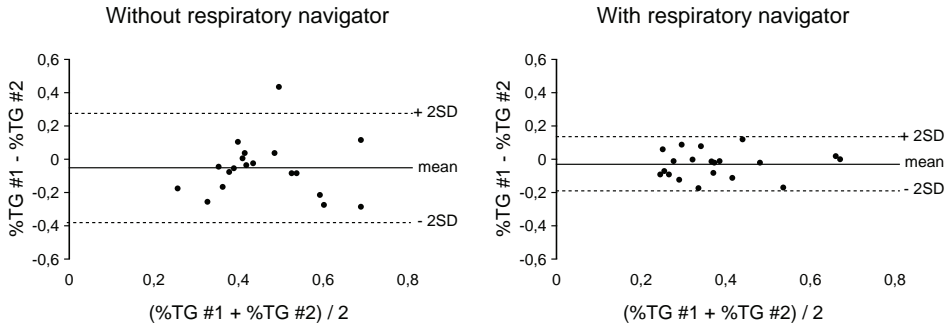


Figure 2.3

Bland-Altman plot of metabolic imaging of myocardial triglyceride (TG) content without (left) and with (right) respiratory navigator gating and volume tracking. Without respiratory navigator gating, the mean interacquisition difference (\pm standard error) of the percentage of TGs is $-0.05\% \pm 0.04$ ($P = 0.20$); with respiratory navigator gating, the difference of the two acquisitions in the same subjects is $-0.03\% \pm 0.02$ ($P = 0.11$). No trends are observed. Limits of agreement (mean \pm 2 standard deviations [2SD]) are smaller when respiratory navigator is used, indicating improved reproducibility.

%TG = myocardial TG percentage relative to unsuppressed water, #1 and #2 = acquisitions 1 and 2.

the respiratory navigator, the intraclass correlation coefficient was 0.32 (95% confidence interval: $-0.14, 0.66$; $P = 0.08$), and this coefficient improved to 0.81 (95% confidence interval: $0.58, 0.92$; $P < 0.001$) with use of navigator gating and volume tracking. Furthermore, the coefficient of variation of the assessment of myocardial TG percentage without use of the navigator was 14.5% higher than with the use of the navigator (32.4% vs 17.9%). The very low-calorie diet induced an 83% increase in myocardial TG content compared with baseline percentage of TGs (1.1% and 0.6 %TG content, respectively) (Figure 2.4) in one volunteer.

DISCUSSION

In our study, reproducibility of metabolic imaging findings by using ECG-triggered ^1H MRS to assess myocardial TG accumulation was assessed with and without the use of respiratory navigator echo-based motion compensation. Spectral resolution (defined by means of the linewidth of the unsuppressed water signal), which is a measure of spectroscopic quality, increased significantly ($P < 0.001$) with use of respiratory motion compensation. Furthermore, reproducibility of the assessment of myocardial TG content was improved when respiratory navigator gating was applied.

Respiratory motion causes a relative displacement of the acquisition volume in relation to the position of the human heart. Thereby, respiratory motion may hamper shimming and water suppression. In our study, the full width at half maximum values of the unsuppressed water signal decreased significantly with use of the respiratory navigator compared with acquisitions without respiratory navigator gating and tracking. The observed values in our study of full

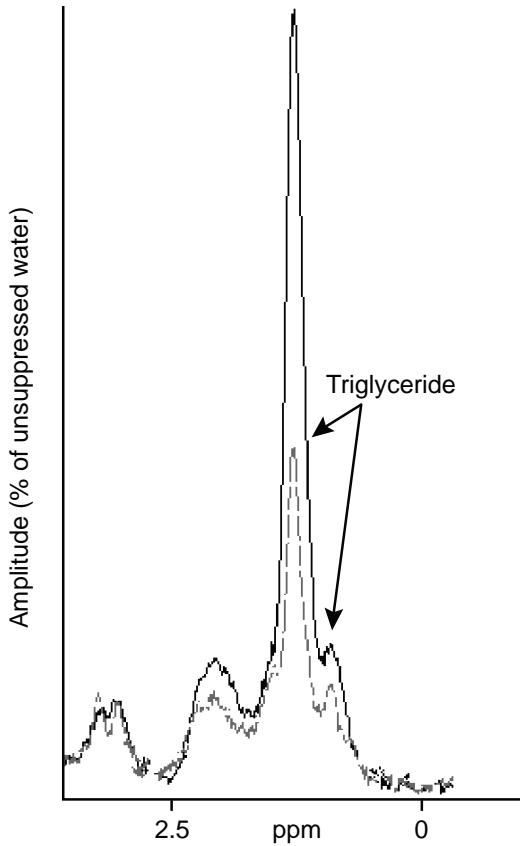


Figure 2.4

Water-suppressed magnetic resonance spectra from metabolic imaging show effect of a very low-calorie diet on triglyceride content in a healthy volunteer. Peak height is relative to the water signal in a reference spectrum without water suppression. Myocardial triglyceride peak height increased nearly twofold after a 3-day very low-calorie diet. Dashed line = baseline, solid line = very low-calorie diet, ppm = parts per million.

width at half maximum with use of the respiratory navigator technique correspond to values reported for the tibialis anterior muscle (17) and are lower than previously published values for myocardial ¹H MRS (1). Therefore, application of respiratory navigator gating and tracking improves spectral resolution for metabolic imaging of myocardial TGs of the human heart.

The mean percentages of myocardial TGs, assessed with and without respiratory motion compensation, were in accordance with previously published data from other studies (9), but with respiratory navigator, the percentage myocardial TG content was lower than the acquired values in our study without use of respiratory motion compensation.

The observed percentages of TGs are scattered over a relatively large range for all acquisition conditions. In all acquisition conditions, the CRSDs were less than 1% of the signal amplitude, and thus spectral noise was considered to have a negligible contribution to the uncertainty of our measurements. Therefore we assume that the observed range in myocardial TG percentages reflects differences in measurement conditions (i.e., presence or absence of navigator gating). The observed higher percentage of myocardial TGs without application of respiratory motion compensation is probably caused by contamination of epicardial fat. The contamination is most likely caused by the relative displacement of the acquisition volume in myocardial tissue,

due to respiratory motion causing contamination from outside the selected voxel and thereby to an increase in the apparent percentage of myocardial TGs.

Bland-Altman analysis showed improved agreement in myocardial TG assessment with use of respiratory navigator gating and tracking. No comparable data could be found in previous reports. In addition, with use of the respiratory navigator, reproducibility of myocardial TG assessment expressed as the intraclass correlation coefficient and the coefficient of variation improved significantly. In our study, use of respiratory navigator gating and tracking improved the intraclass correlation coefficient from 0.32 to 0.81 and decreased the coefficient of variation from 32.4% to 17.9% for assessment of myocardial TGs. A coefficient of variation of 17.9% for the assessment of myocardial TGs with use of respiratory motion compensation is in concordance with results of previous studies in which various other methods were used for cardiac and respiratory motion correction to increase spectroscopic quality (2;3). Szczepaniak *et al.* (3) showed a coefficient of variation for MR spectroscopic determination of myocardial TGs of 17%, with use of a pressure belt for respiratory gating, while others reported a coefficient of variation of 13% for TG determination by using double triggering based on the ECG signal (2).

In our study, an increase in myocardial TG content was found after a short-term very low-calorie diet in a healthy subject. Although this test was performed in only one volunteer, and thus is not representative of a proved finding, the result corresponds to the findings of Reingold *et al.* (9). The clinical interpretation of the above-mentioned finding needs to be established in a larger cohort study. This clinical example suggests that metabolic imaging of myocardial TG content may be a useful new tool for monitoring effects of dietary and/or medical interventions in metabolic and cardiac disorders, such as metabolic syndrome, diabetes, and myocardial lipotoxicity. Furthermore, metabolic imaging of myocardial TG content may provide new (patho-) physiologic insights of myocardial TG handling, also in relation to global and regional cardiac function.

Our study has some limitations. First, ^1HMR S was performed in healthy volunteers only. A patient who is experiencing any sort of stress due to a medical condition is possibly less cooperative with a longer acquisition time caused by the respiratory motion compensation. We think, however, that a clinical cardiac MR imaging examination time of approximately 25 minutes to acquire a cardiac spectrum is not any different from other clinical cardiac MR imaging applications. The more reliable results of respiratory motion-compensated spectroscopy compared with non-respiratory motion-compensated spectroscopy warrants the extra time investment. Second, the use of cardiac ^1HMR S currently has only limited clinical relevance. However, it is potentially a very useful tool in cardiac metabolic studies; for example, in the evaluation of diet and therapy effects. Third, ^1HMR spectra were obtained in the myocardial septum only. The use of ^1HMR S in other regions of the heart was not demonstrated. We think that the myocardial septum is the most favorable region for acquiring cardiac spectra in generalized disorders that affect the myocardium, such as diabetes mellitus. Motion in the myocardial septum is limited, and the myocardial septum is far from the free walls and from the pericardial fat, which could

contaminate the spectra. However, more work needs to be done to develop a reliable $^1\text{HMRS}$ technique for the lateral walls of the heart, which could be of interest in assessing myocardial lipid accumulation in localized disorders such as myocardial infarction.

CONCLUSIONS

Respiratory navigator-gated and ECG-triggered $^1\text{HMRS}$ of the human heart to assess myocardial TG content showed substantially better spectral resolution and reproducibility than ECG-triggered $^1\text{HMRS}$ without respiratory motion correction. Therefore, we believe that respiratory motion correction is essential for reproducible metabolic imaging of myocardial TG content of the human heart.

ACKNOWLEDGMENTS

We thank Jan van Ooijen (Philips Medical Systems) for his technical support.

REFERENCES

1. den Hollander JA, Evanochko WT, Pohost GM. Observation of cardiac lipids in humans by localized 1H magnetic resonance spectroscopic imaging. *Magn Reson Med* 1994; 32(2):175-180.
2. Felblinger J, Jung B, Slotboom J, Boesch C, Kreis R. Methods and reproducibility of cardiac/respiratory double-triggered (1)H-MR spectroscopy of the human heart. *Magn Reson Med* 1999; 42(5):903-910.
3. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbiqve D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.
4. Young ME, Guthrie PH, Razeghi P, Leighton B, Abbasi S, Patil S, Youker KA, Taegtmeyer H. Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese Zucker rat heart. *Diabetes* 2002; 51(8):2587-2595.
5. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000; 97(4):1784-1789.
6. Sharma S, Adroque JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeyer H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* 2004; 18(14):1692-1700.
7. Finck BN, Han X, Courtois M, Amond F, Nerbonne JM, Kovacs A, Gross RW, Kelly DP. A critical role for PPARalpha-mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. *Proc Natl Acad Sci U S A* 2003; 100(3):1226-1231.
8. Straeter-Knowlen IM, Evanochko WT, den Hollander JA, Wolkowicz PE, Balschi JA, Caulfield JB, Ku DD, Pohost GM. 1H NMR spectroscopic imaging of myocardial triglycerides in excised dog hearts subjected to 24 hours of coronary occlusion. *Circulation* 1996; 93(7):1464-1470.
9. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *Am J Physiol Endocrinol Metab* 2005; 89(5):E935-E939.
10. Schar M, Kozerke S, Boesiger P. Navigator gating and volume tracking for double-triggered cardiac proton spectroscopy at 3 Tesla. *Magn Reson Med* 2004; 51(6):1091-1095.
11. Kozerke S, Schar M, Lamb HJ, Boesiger P. Volume tracking cardiac 31P spectroscopy. *Magn Reson Med* 2002; 48(2):380-384.
12. Wang Y, Rossman PJ, Grimm RC, Riederer SJ, Ehman RL. Navigator-echo-based real-time respiratory gating and triggering for reduction of respiration effects in three-dimensional coronary MR angiography. *Radiology* 1996; 198(1):55-60.
13. Wang Y, Riederer SJ, Ehman RL. Respiratory motion of the heart: kinematics and the implications for the spatial resolution in coronary imaging. *Magn Reson Med* 1995; 33(5):713-719.
14. Naressi A, Couturier C, Devos JM, Janssen M, Mangeat C, de BR, Graveron-Demilly D. Java-based graphical user interface for the MRUI quantitation package. *MAGMA* 2001; 12(2-3):141-152.
15. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129(1):35-43.
16. Lamb HJ, Doornbos J, den Hollander JA, Luyten PR, Beyerbacht HP, van der Wall EE, de Roos A. Reproducibility of human cardiac 31P-NMR spectroscopy. *NMR Biomed* 1996; 9(5):217-227.

17. Torriani M. Measuring muscle lipids with ¹H-MR spectroscopy. *Skeletal Radiol* 2007; 36(7):607-608.
18. Boesch C, Slotboom J, Hoppeler H, Kreis R. In vivo determination of intra-myocellular lipids in human muscle by means of localized ¹H-MR-spectroscopy. *Magn Reson Med* 1997; 37(4):484-493.
19. Rico-Sanz J, Hajnal JV, Thomas EL, Mierisova S, la-Korpela M, Bell JD. Intracellular and extracellular skeletal muscle triglyceride metabolism during alternating intensity exercise in humans. *J Physiol* 1998; 510 (Pt 2):615-622.
20. Schick F, Eismann B, Jung WI, Bongers H, Bunse M, Lutz O. Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Magn Reson Med* 1993; 29(2):158-167.

Chapter 3

Short-term Caloric Restriction Induces Accumulation of Myocardial Triglycerides and Decreases Left Ventricular Diastolic Function in Healthy Subjects

Diabetes 2007; 56(12):2849-2853

R.W. van der Meer
S. Hammer
J.W.A. Smit
M. Fröhlich
J.J. Bax
M. Diamant
L.J. Rijzewijk
A. de Roos
J.A. Romijn
H.J. Lamb

SUMMARY

Objectives: Diabetes and obesity are associated with increased plasma non-esterified fatty acid (NEFA) levels, myocardial triglyceride (TG) accumulation, and myocardial dysfunction. Because a very low-calorie diet (VLCD) also increases plasma NEFA levels, we studied the effect of a VLCD on myocardial TG content and cardiac function in healthy subjects.

Materials and methods: Fourteen healthy nonobese men underwent ^1H magnetic resonance spectroscopy ($^1\text{HMRS}$) to determine myocardial and hepatic TG content, ^{31}P magnetic resonance spectroscopy ($^{31}\text{PMRS}$) to assess myocardial high-energy phosphate (HEP) metabolism (phosphocreatine/ATP), and magnetic resonance imaging of myocardial function at baseline and after a 3-day VLCD.

Results: After the dietary intervention, plasma NEFA levels increased compared with those at baseline (from mean \pm standard error 0.5 ± 0.1 to 1.1 ± 0.1 mmol/l, $P < 0.05$). Concomitantly, myocardial TG content increased by $\sim 55\%$ compared with that at baseline (from 0.38 ± 0.05 to $0.59 \pm 0.06\%$, $P < 0.05$), whereas liver TG content decreased by $\sim 32\%$ (from 2.2 ± 0.5 to $1.5 \pm 0.4\%$, $P < 0.05$). The VLCD did not change myocardial phosphocreatine-to-ATP ratio (2.33 ± 0.15 vs 2.33 ± 0.08 , $P < 0.05$) or systolic function. Interestingly, deceleration of the early diastolic flow across the mitral valve decreased after the VLCD (from 3.37 ± 0.20 to 2.91 ± 0.16 ml/s $^2 \times 10^{-3}$, $P < 0.05$). This decrease in diastolic function was significantly correlated with the increase in myocardial TG content.

Conclusions: Short-term VLCD induces accumulation of myocardial TGs. In addition, VLCD decreases left ventricular diastolic function, without alterations in myocardial HEP metabolism. This study documents diet-dependent physiological variations in myocardial TG content and diastolic function in healthy subjects.

INTRODUCTION

In diabetes and obesity, plasma non-esterified fatty acid (NEFA) levels are elevated because of excessive lipolysis in adipose tissue (1). In animal models of type 2 diabetes mellitus (DM2) and obesity, excessive plasma NEFA levels result in accumulation of myocardial triglycerides (TGs) (2;3). In these models, TG accumulation in cardiomyocytes is directly related to cardiac dysfunction (4-6) and an increased susceptibility for cardiac ischemia (7). This so-called “myocardial lipotoxicity” is due to complex mechanisms, most likely involving intermediates of NEFA metabolism and oxidative stress (2;6;8). Interestingly, in animal models, therapeutic interventions aimed at reducing myocardial TG accumulation reversed myocardial dysfunction (6). In addition to contributing to myocardial lipotoxicity, increased plasma NEFA levels may affect myocardial high-energy phosphate (HEP) metabolism (9).

Myocardial TG accumulation has been demonstrated *ex vivo* in myocardial tissue of patients with DM2 (10) and patients with heart failure (11). Recently, myocardial hydrogen 1 magnetic resonance spectroscopy (¹HMRS) has been developed and validated to measure myocardial TG content in humans *in vivo* (12;13). Using this technique, a relation between body mass index (BMI) and myocardial TG content was suggested (12;14). However, dynamic changes in myocardial TG content and myocardial function have not been documented within subjects. Because short-term exposure to a very low-calorie diet (VLCD) increases plasma NEFA levels (15), we hypothesized that this dietary intervention might be a model to study the flexibility of myocardial TG content and myocardial function in healthy subjects. Therefore, the purpose of the present study was to determine the effect of a VLCD on myocardial TG content and cardiac function in healthy subjects, using ¹HMRS, phosphorus 31 magnetic resonance spectroscopy (³¹PMRS), and cardiac magnetic resonance (MR) imaging. Each subject was studied twice, before and after 3 days of VLCD. ³¹PMRS was used to assess myocardial HEP metabolism. Cardiac MR imaging was used to assess myocardial function in detail. Furthermore, hepatic TG content was assessed concomitantly using ¹HMRS to study the tissuespecific effects of a VLCD.

MATERIALS AND METHODS

Fourteen healthy men participated in this study, which was approved by the local ethics committee. All volunteers provided written informed consent. Subjects were included if they were aged >18 years and had no known acute or chronic disease based on history, physical examination, standard laboratory tests (blood counts, fasting blood glucose, lipids, serum creatinine and electrocardiogram [ECG]). Exclusion criteria included drug treatment, smoking, substance abuse, hypertension, or impaired glucose tolerance (as confirmed by a 75-g oral glucose tolerance test (16)). Subjects underwent MR scanning in the afternoon on two different occasions. Before both visits, they were instructed to follow one of two different dietary regimes for 3 days

before the measurements. In the first regime, each subject used his normal diet, and this dietary condition was used for the collection of baseline data. During the second regime, subjects consumed a VLCD consisting of 471 kcal, 50.2 g carbohydrates, and 6.9 g fat (0.94 g saturated fat; Modifast Intensive, Nutrition & Santé Benelux, Breda, Netherlands) per day. The low-fat content was used to induce a physiological elevation of plasma NEFA levels.

Subjects were instructed to maintain a sufficient fluid intake (>1.5 l daily). Use of alcohol was not allowed during the 3-day diets. The last meal of each diet was consumed 4 h before venous blood sampling and subsequent cardiac MR imaging and spectroscopy measurements. Furthermore, ^1H MRS of the liver was performed to study the extracardiac effects of the VLCD. The effect of the VLCD on the study parameters was compared with the data obtained after the reference diet.

^1H magnetic resonance spectroscopy

All MR imaging and spectroscopy studies were performed with the use of a 1.5-Tesla whole-body MR scanner (Gyrosan ACS/NT15; Philips, Best, Netherlands) with subjects in supine position at rest. Myocardial ^1H MRS spectra were obtained from the interventricular septum. The body coil was used for radio-frequency transmission, and a 17-cm diameter circular surface coil was used for signal reception.

An 8-ml voxel was positioned in the interventricular septum on four-chamber and short-axis images in end-systole, carefully avoiding contamination from epicardial fat (Figure 3.1). A point-resolved, spatially localized spectroscopic pulse sequence was used to acquire single-voxel MR spectroscopic data (17). Spectroscopic data acquisition was double-triggered using ECG triggering and respiratory navigator echoes to minimize breathing influences (13;18). Spectra were acquired at end-systole, with an echo time (TE) of 26 ms and a repetition time (TR) of at least 3000 ms. A total of 1024 data points were collected using a 1000-Hz spectral width and averaged over 128 acquisitions. Without changing any parameter, spectra without water suppression with a TR of 10000 ms and four averages were obtained to be used as an internal standard.

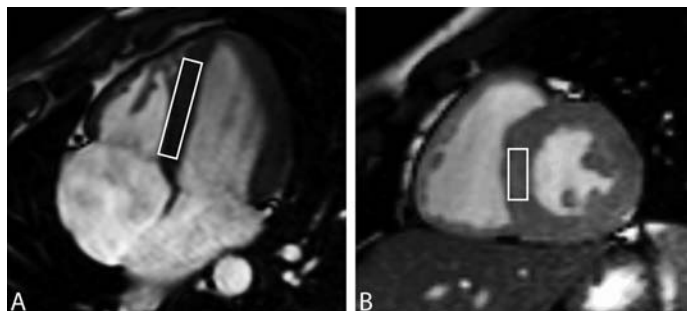


Figure 3.1. Myocardial voxel localization for ^1H magnetic resonance spectroscopy. Voxel position in four-chamber (A) and short-axis (B) views.

¹HMRS of the liver was performed with an 8-ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. The 12th thoracic vertebra was used as a landmark to ensure the same position of the voxel during both visits. Spectra were obtained using the same parameters as described above. Sixty-four averages were collected with water suppression. All ¹HMR spectroscopic data were fitted using Java-based MR user interface (jMRUI) software (version 2.2 [developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium]) (19).

Spectra were analyzed in the time domain directly on free-induction decays. For spectra acquired with water suppression, the Hankel-Lanczos filter was used to remove residual water signal, using the single-variable decomposition method. Myocardial TG signals were analyzed using the Advanced Magnetic RESonance (AMARES) fitting algorithm within jMRUI (20).

Resonance frequency estimates for intramyocardial lipids were described with the assumption of Gaussian line shapes at 0.9, 1.3, and 2.1 parts per million (ppm, only data from the peaks at 0.9 and 1.3 ppm were summated and used on statistical analysis (21)). Prior knowledge was incorporated into the fitting algorithm by using previously published criteria (22-24). The zero-order phase correction was estimated by using the AMARES algorithm, and the first-order phase correction was fixed to 0.13 ms. The water signal from spectra without water suppression obtained from the same voxel was used as an internal reference for relative quantification of lipid resonances. The water signal peak at 4.7 ppm was quantified using a Lorentzian line shape and analyzed using the AMARES algorithm. Myocardial and hepatic TG content was calculated as a percentage relative to water: TGs/water × 100.

Furthermore, peak estimates of the creatine signals of the heart spectrum at 3.0 ppm were derived from the water-suppressed spectrum using jMRUI, and the TG-to-creatine ratio and the percentage of creatine (creatine/ water × 100) were calculated.

³¹P magnetic resonance spectroscopy

A 10-cm diameter surface coil was used to acquire ECG-triggered ³¹PMR spectra of the left ventricular (LV) anterior wall with subjects in the supine position. Volumes of interest were selected by image-guided spectroscopy with three-dimensional image selected *in vivo* spectroscopy. Shimming was performed automatically and tuning and matching of the ³¹P surface coil was performed manually. Technical details of data acquisition and spectral quantification were similar as previously described (25). Shortly, spectroscopic volume size was typically 7 × 7 × 7 cm. Acquisitions were based on 192 averaged free-induction decays, and total acquisition time was 10 min. ³¹PMR spectra were quantified automatically in the time domain using prior spectroscopic knowledge and were corrected for partial saturation effects and for the adenosine-triphosphate (ATP) contribution from blood in the cardiac chambers. The phosphocreatine-to-ATP ratios of the spectra were calculated and used as a parameter representing myocardial HEP metabolism (26).

Left ventricular function

All images were analyzed quantitatively using dedicated software (FLOW[®] or MASS[®]; Medis, Leiden, Netherlands). The entire heart was imaged in short-axis orientation using an ECG-triggered, sensitivity-encoding balanced steady-state free precession sequence with breath holds. Imaging parameters included the following: TE = 1.7 ms, TR = 3.4 ms, flip angle = 35°, slice thickness = 10 mm with a gap of 0 mm, field of view = 400 mm², and reconstructed matrix size = 256 × 256. LV ejection fraction was assessed for the determination of LV systolic function. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding (Venc) was performed to measure blood flow across the mitral valve for the determination of LV diastolic function. Imaging parameters included the following: TE = 4.8 ms, TR = 14 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350 mm², matrix size = 256 × 256, Venc = 100 cm/s, and scan percentage = 80%. Early diastolic filling, mean deceleration of the early diastolic flow across the mitral valve, and an estimation of LV filling pressures (27) were used as parameters of LV diastolic function. During MR imaging, blood pressure and heart rate were measured.

Assays

Plasma glucose and TGs were measured on a fully automated P800 analyzer (Roche, Almere, Netherlands) and insulin on an Immulite 2500 random-access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA). Coefficients of variation were < 2% for glucose and TGs and < 5% for insulin. Leptin and adiponectin were measured with radioimmunoassays from Linco Research (St. Charles, MO). For leptin, the coefficient of variation varied from 3.0 to 5.1% and the sensitivity was 0.5 µg/l; for adiponectin, these data were 6.3 to 8.1%, with a sensitivity of 1 µg/l. Plasma NEFAs were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis

Statistical analysis was performed using SPSS for windows (version 12.0; SPSS, Chicago, Ill). Data are expressed as means ± standard error. Between-group differences were calculated using two-tailed dependent sample t-tests. Pearson-*r* values were used for correlations. Significance was assumed at *P* < 0.05 (two-tailed).

RESULTS

¹HMRs and myocardial function were successfully assessed in all fourteen subjects. In nine subjects, ³¹PMRS was successfully completed at both occasions. In the other five subjects, ³¹PMRS data at baseline or after the VLCD could not be assessed because of time constraints or technical problems. Mean age of the studied subjects was 25 ± 2 years. Characteristics at baseline and after the VLCD are shown in Table 3.1. All subjects performed exercise (walking,

Table 3.1. Characteristics of the study group at baseline and after the very low-calorie diet.

	Baseline	VLCD
Body mass index (kg/m ²)	23.6 ± 0.7	23.2 ± 0.7*
Systolic blood pressure (mmHg)	123 ± 4	118 ± 3
Diastolic blood pressure (mmHg)	66 ± 2	62 ± 2*
Heart rate (bpm)	60 ± 2	61 ± 3
Plasma glucose (mmol/l)	4.90 ± 0.09	4.26 ± 0.10*
Plasma insulin (mU/l)	9.14 ± 1.27	7.9 ± 1.16
Plasma triglycerides (mmol/l)	1.29 ± 0.09	0.82 ± 0.07*
Plasma non-esterified fatty acids (mmol/l)	0.5 ± 0.1	1.1 ± 0.1*
Plasma leptin (µg/l)	2.99 ± 0.49	1.41 ± 0.20*
Plasma adiponectin (mg/l)	7.81 ± 0.84	6.79 ± 0.61

* $P < 0.05$ compared with baseline (paired t-tests). Values are mean ± standard error.

VLCD = very low-calorie diet.

running, and/or biking) regularly (ranges 3-5 hours weekly), but none of the subjects engaged in high-performance sports.

Myocardial and hepatic spectroscopy

Typical myocardial ¹HMR and ³¹PMR spectra at baseline and after the VLCD of the same subject are shown in Figure 3.2. After the VLCD, myocardial TG content as well as the myocardial

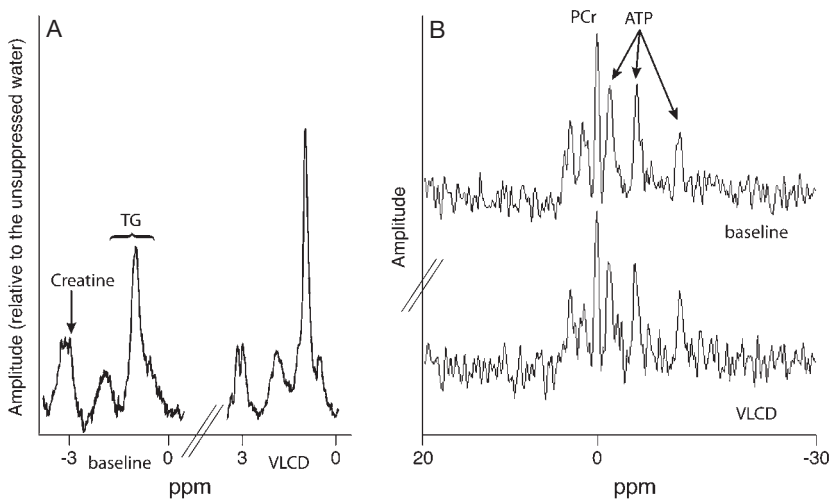


Figure 3.2. Typical ¹H magnetic resonance and ³¹P magnetic resonance spectra at baseline and after dietary intervention.

A: ¹H magnetic resonance (MR) spectra at baseline and after a very low-calorie diet (VLCD) diet are displayed. Note the increase of the triglyceride signal amplitude at 0.9 and 1.3 parts per million (ppm) after the VLCD without a change in the creatine signal amplitude at 3.0 ppm. B: ³¹PMR spectra of one volunteer at baseline and after a VLCD are displayed. Note an unchanged phosphocreatine (PCr) and adenosine-triphosphate (ATP) signal.

TG = triglyceride.

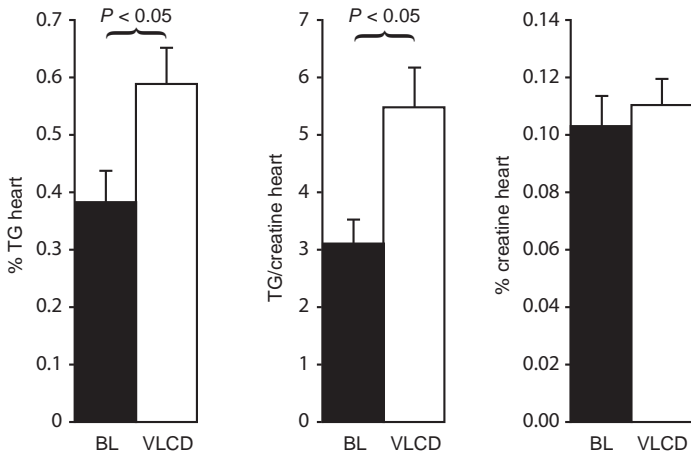


Figure 3.3. Influence of a short-term very low-calorie diet on myocardial triglyceride and creatine content.

A very low-calorie diet (VLCD) increased the percentage of myocardial triglycerides and the triglyceride-to-creatinine ratio without changing the creatine-to-water ratio. Therefore, the increase in myocardial percentage of triglycerides assessed by magnetic resonance spectroscopy is the effect of an increase of myocardial triglycerides rather than of decreased myocardial water content.

BL = baseline; TG = triglyceride.

TG-to-creatinine ratio were increased compared with those at baseline (from 0.38 ± 0.05 to $0.59 \pm 0.06\%$ and from 3.11 ± 0.39 to 5.42 ± 0.71 , respectively, $P < 0.05$), whereas the myocardial percentage of creatine did not change (Figure 3.3). The VLCD did not change the myocardial phosphocreatine-to-ATP ratio compared with baseline values (2.33 ± 0.15 vs 2.33 ± 0.08 , $P >$

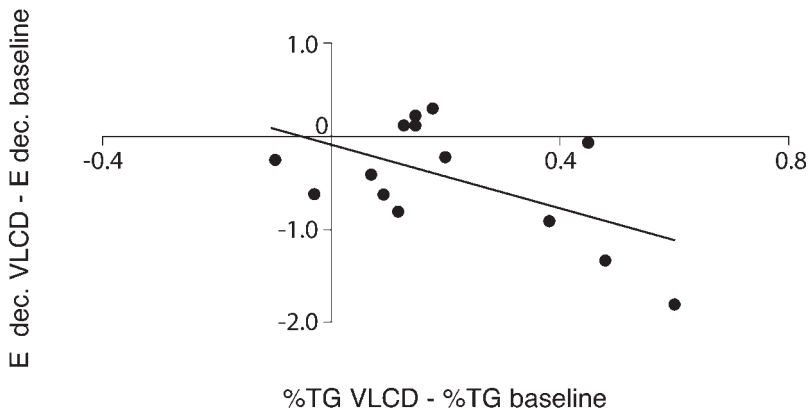


Figure 3.4. Correlation between diastolic function and myocardial triglycerides.

The decrease in left ventricular diastolic function after the very low-calorie diet (VLCD) was significantly correlated with the increase in myocardial triglyceride content after the VLCD ($r = -0.55$, $P < 0.05$).

%TG = myocardial percentage of triglycerides, E dec = deceleration of early diastolic flow across the mitral valve.

0.05). Hepatic TG content decreased during the VLCD compared with that at baseline (from 2.2 ± 0.5 to $1.5 \pm 0.4\%$, $P < 0.05$).

Myocardial function

LV systolic function, represented by the ejection fraction, did not change (60 ± 1 vs $60 \pm 1\%$, $P > 0.05$) after the VLCD. In contrast, the mean deceleration of the early diastolic flow across the mitral valve decreased after the VLCD compared with baseline values (from 3.37 ± 0.20 to $2.91 \pm 0.16 \text{ ml/s}^2 \times 10^{-3}$, $P < 0.05$). This decrease in mean deceleration of the early diastolic flow across the mitral valve after the VLCD was significantly correlated with the increase in myocardial TG content after the VLCD (Figure 3.4). Furthermore, there was no statistically significant change in LV filling pressures between the VLCD and baseline (estimation of LV filling pressures = 10.0 ± 1.3 vs 9.3 ± 0.7 , $P > 0.05$).

DISCUSSION

This study shows that in healthy subjects, a short-term consumption of a VLCD increases myocardial TG content and concomitantly decreases LV diastolic function without changing myocardial HEP metabolism. Moreover, this study shows that short-term caloric restriction exerts differential tissue-specific effects on TG content in liver and myocardium. These observations stress the physiological flexibility of ectopic TG pools.

Under normal conditions, myocardial energy is mainly derived from NEFAs (1). However, the rates of uptake and oxidation of NEFAs in cardiomyocytes are not tightly coupled. When NEFAs are taken up in excess of fatty acid oxidation, myocardial TG content increases. Apparently, myocardial fatty acid uptake is increased in relation to myocardial fatty acid oxidation during a VLCD.

Several animal models of DM2 and obesity demonstrated that excessive plasma NEFA levels result in accumulation of myocardial TGs (2;3). However, these diabetic models lead to very different metabolic changes compared with the physiologically increased NEFA concentrations seen in healthy subjects as a result of caloric restriction. Since very little is known about the flexibility of myocardial TG content and myocardial function in healthy subjects in reaction to increased NEFA levels, in our study a VLCD was used as a model for a short-term physiological increase of plasma NEFA levels. The present findings of an increase in myocardial TG content after a VLCD are in concordance with the findings of Reingold *et al.* (28), who showed increased myocardial TG content after 48 hours of fasting. Both conditions are associated with increased plasma NEFA levels. Since the myocardial TG content measured by MRS is expressed relative to water, the VLCD-induced increase in myocardial TG content may also be explained by a decrease of myocardial water content. Therefore, the myocardial TG-to-creatinine and creatinine-to-water ratios were assessed additionally. The increased myocardial TG-to-creatinine ratio in the presence

of the unchanged creatine-to-water ratio in our study supports our conclusion that the diet-related increase in myocardial TG content assessed by ¹HMRs is due to increased myocardial TG accumulation rather than decreased myocardial water content.

The increase in myocardial TG content can be derived from plasma NEFAs and/or plasma TGs. The heart is especially effective in removal of circulating TGs (29;30). Moreover, heart lipoprotein lipase activity increases during fasting (31). In contrast, however, VLCD decreased plasma TG levels, whereas plasma NEFA levels increased in our study. Therefore, it remains unclear to what extent the VLCD has altered the relative contribution of plasma NEFAs vs plasma TGs to myocardial TG stores.

In addition to increasing myocardial TG content, the short-term VLCD intervention was associated with altered myocardial function. Although myocardial systolic function and heart rate were not changed after a VLCD in our study, a significant impact on diastolic function was observed. The deceleration of the early filling phase of the left ventricle decreased significantly after the VLCD. Transmitral filling patterns can be influenced by LV filling pressure and myocardial relaxation capacity. Although we observed a change in diastolic blood pressures, tissue MR (27) showed no diet-induced changes in estimated LV filling pressures. We therefore hypothesize that changed relaxation of the left ventricle accounts for the observed change in the transmitral filling pattern. The mechanism(s) responsible for the change in diastolic function during a VLCD can not be derived from the present data. Short-term caloric restriction in mice causes remodeling of myocardial membranes through the activation of phospholipases (32). Altered membrane structure in a fatty acid-based metabolic system may lead to changes in calcium homeostasis (33;34) and thereby to altered LV diastolic function (35). Therefore, altered calcium uptake might be involved in the mechanisms causing the decreased diastolic function observed during VLCD. Another explanation for decreased myocardial diastolic function after the VLCD might be the lower plasma glucose levels and the higher plasma NEFA levels. As a consequence, the heart becomes relatively more reliable on NEFAs than on plasma glucose for its fuel supply. Carbohydrate oxidation, however, has potential salutary effects on myocardial function and efficiency (36;37). Based on the present data, we can not implicate myocardial TG accumulation as the mediator of decreased myocardial diastolic function. During a VLCD, many hormonal, metabolic, and biophysical changes occur within the myocardium that will impact myocardial function. Nonetheless, the increase in myocardial TG content is a reflection of these changes within the myocardium during a VLCD, which significantly correlated with the decrease in deceleration of the early diastolic flow across the mitral valve.

In the present study, the short-term VLCD did not affect myocardial HEP metabolism. This confirms findings in previous animal studies, in which an increase in myocardial TG content did not cause a significant decrease in HEP status (38). A possible explanation for the preserved myocardial HEP metabolism is that, in these healthy young men, myocardial ATP demand remains unchanged after a VLCD. A disturbance in the HEP metabolism might only be present when the heart is additionally stressed, e.g., by adenosine/exercise testing or ischemia.

In parallel to the increase in myocardial TG content, hepatic TG content decreased after the short-term VLCD. Westerbacka *et al.* (39) previously reported similar findings on the effects of dietary interventions on hepatic TG content. In their study, decreased dietary fat content in obese women reduced hepatic fat content within 2 weeks without changing plasma NEFA levels. This could be an indication that dietary fat is an important direct source of fatty acids for the liver separate from NEFAs. The decrease in hepatic fat after the VLCD in lean healthy subjects in our study, where plasma NEFA levels were increased after the VLCD, points in the same direction. However, obese subjects have a different metabolic profile than lean subjects and might therefore have shown other reactions to the VLCD. We think that further studies need to be conducted to evaluate the influence of different metabolic profiles on reactions to dietary fat content.

The opposite changes in myocardial and hepatic TG content indicate differential, organ-specific mechanisms underlying tissue-specific partitioning of plasma TGs and/or fatty acids among non-adipose organs, at least with respect to the liver and the heart. Unfortunately, the underlying mechanisms can not be derived from the methods used in our study.

CONCLUSIONS

In conclusion, short-term VLCD induces accumulation of myocardial TGs. In addition, VLCD decreases LV diastolic function without alterations in myocardial HEP metabolism. This study documents diet-dependent, physiological variations in myocardial TG content and diastolic function in healthy subjects.

ACKNOWLEDGMENTS

We would like to thank Michael Schär (Philips Medical Systems, Cleveland, OH and Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD) for his technical support.

REFERENCES

1. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 2005; 85(3):1093-1129.
2. Schaffer JE. Lipotoxicity: when tissues overeat. *Curr Opin Lipidol* 2003; 14(3):281-287.
3. Unger RH. Lipotoxic diseases. *Annu Rev Med* 2002; 53:319-336.
4. Christoffersen C, Bollano E, Lindegaard ML, Bartels ED, Goetze JP, Andersen CB, Nielsen LB. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003; 144(8): 3483-3490.
5. Ouwens DM, Boer C, Fodor M, de Galan P, Heine RJ, Maassen JA, Diamant M. Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. *Diabetologia* 2005; 48(6):1229-1237.
6. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000; 97(4):1784-1789.
7. Balschi JA, Hai JO, Wolkowicz PE, Straeter-Knowlen I, Evanochko WT, Caulfield JB, Bradley E, Ku DD, Pohost GM. ¹H NMR measurement of triacylglycerol accumulation in the post-ischemic canine heart after transient increase of plasma lipids. *J Mol Cell Cardiol* 1997; 29(2):471-480.
8. Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J* 2001; 15(2):312-321.
9. Scheuermann-Freestone M, Madsen PL, Manners D, Blamire AM, Buckingham RE, Styles P, Radda GK, Neubauer S, Clarke K. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. *Circulation* 2003; 107(24):3040-3046.
10. Regan TJ, Lyons MM, Ahmed SS, Levinson GE, Oldewurtel HA, Ahmad MR, Haider B. Evidence for cardiomyopathy in familial diabetes mellitus. *J Clin Invest* 1977; 60(4):884-899.
11. Sharma S, Adroque JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeier H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* 2004; 18(14):1692-1700.
12. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.
13. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of myocardial triglyceride content: reproducibility of ¹H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007; 245(1): 251-257.
14. Kankaanpaa M, Lehto HR, Parkka JP, Komu M, Viljanen A, Ferrannini E, Knuuti J, Nuutila P, Parkkola R, Izzo P. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006; 91(11):4689-4695.
15. Hirsch J, Goldrick RB. Serial studies on the metabolism of human adipose tissue. I. lipogenesis and free fatty acid uptake and release in small aspirated samples of subcutaneous fat. *J Clin Invest* 1964; 43:1776-1792.

16. Anon. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003; 26 Suppl 1:5S-20.
17. den Hollander JA, Evanochko WT, Pohost GM. Observation of cardiac lipids in humans by localized 1H magnetic resonance spectroscopic imaging. *Magn Reson Med* 1994; 32(2):175-180.
18. Schar M, Kozerke S, Boesiger P. Navigator gating and volume tracking for double-triggered cardiac proton spectroscopy at 3 Tesla. *Magn Reson Med* 2004; 51(6):1091-1095.
19. Naressi A, Couturier C, Devos JM, Janssen M, Mangeat C, de BR, Graveron-Demilly D. Java-based graphical user interface for the MRUI quantitation package. *MAGMA* 2001; 12(2-3):141-152.
20. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129(1):35-43.
21. Torriani M. Measuring muscle lipids with 1H-MR spectroscopy. *Skeletal Radiol* 2007; 36(7):607-608.
22. Boesch C, Slotboom J, Hoppeler H, Kreis R. In vivo determination of intra-myocellular lipids in human muscle by means of localized 1H-MR-spectroscopy. *Magn Reson Med* 1997; 37(4):484-493.
23. Rico-Sanz J, Hajnal JV, Thomas EL, Mierisova S, la-Korpela M, Bell JD. Intracellular and extracellular skeletal muscle triglyceride metabolism during alternating intensity exercise in humans. *J Physiol* 1998; 510 (Pt 2):615-622.
24. Schick F, Eismann B, Jung WJ, Bongers H, Bunse M, Lutz O. Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Magn Reson Med* 1993; 29(2):158-167.
25. Lamb HJ, Doornbos J, den Hollander JA, Luyten PR, Beyerbach HP, van der Wall EE, de Roos A. Reproducibility of human cardiac 31P-NMR spectroscopy. *NMR Biomed* 1996; 9(5):217-227.
26. Bottomley PA. MR spectroscopy of the human heart: the status and the challenges. *Radiology* 1994; 191(3):593-612.
27. Paelinck BP, de Roos A, Bax JJ, Bosmans JM, van der Geest RJ, Dhondt D, Parizel PM, Vrints CJ, Lamb HJ. Feasibility of tissue magnetic resonance imaging: a pilot study in comparison with tissue Doppler imaging and invasive measurement. *J Am Coll Cardiol* 2005; 45(7):1109-1116.
28. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *Am J Physiol Endocrinol Metab* 2005; 289(5):E935-E939.
29. Augustus AS, Kako Y, Yagyu H, Goldberg IJ. Routes of FA delivery to cardiac muscle: modulation of lipoprotein lipolysis alters uptake of TG-derived FA. *Am J Physiol Endocrinol Metab* 2003; 284(2):E331-E339.
30. Nelson RH, Prasad A, Lerman A, Miles JM. Myocardial uptake of circulating triglycerides in nondiabetic patients with heart disease. *Diabetes* 2007; 56(2):527-530.
31. Doolittle MH, Ben-Zeev O, Elovson J, Martin D, Kirchgessner TG. The response of lipoprotein lipase to feeding and fasting. Evidence for posttranslational regulation. *J Biol Chem* 1990; 265(8):4570-4577.
32. Han X, Cheng H, Mancuso DJ, Gross RW. Caloric restriction results in phospholipid depletion, membrane remodeling, and triacylglycerol accumulation in murine myocardium. *Biochemistry* 2004; 43(49):15584-15594.
33. Philipson KD, Ward R. Effects of fatty acids on Na⁺-Ca²⁺ exchange and Ca²⁺ permeability of cardiac sarcolemmal vesicles. *J Biol Chem* 1985; 260(17):9666-9671.
34. Huang JM, Xian H, Bacaner M. Long-chain fatty acids activate calcium channels in ventricular myocytes. *Proc Natl Acad Sci U S A* 1992; 89(14):6452-6456.

35. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. *Circulation* 2002; 105(12):1503-1508.
36. Ferrannini E, Santoro D, Bonadonna R, Natali A, Parodi O, Camici PG. Metabolic and hemodynamic effects of insulin on human hearts. *Am J Physiol* 1993; 264(2 Pt 1):E308-E315.
37. Korvald C, Elvenes OP, Myrnes T. Myocardial substrate metabolism influences left ventricular energetics in vivo. *Am J Physiol Heart Circ Physiol* 2000; 278(4):H1345-H1351.
38. Stewart LC, Kramer JK, Sauer FD, Clarke K, Wolynetz MS. Lipid accumulation in isolated perfused rat hearts has no apparent effect on mechanical function or energy metabolism as measured by ³¹P NMR. *J Lipid Res* 1993; 34(9):1573-1581.
39. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, Yki-Jarvinen H. Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 2005; 90(5): 2804-2809.

Chapter 4

Progressive Caloric Restriction Induces Dose-dependent Changes in Myocardial Triglyceride Content and Diastolic Function in Healthy Men

Journal of Clinical Endocrinology and Metabolism 2008; 93(2):497-503

S. Hammer
R.W. van der Meer
H.J. Lamb
M. Schär
A. de Roos
J.W.A. Smit
J.A. Romijn



SUMMARY

Objectives: In animal experiments, high plasma concentrations of non-esterified fatty acids (NEFAs) are associated with increased triglyceride (TG) stores in liver and heart, and impaired cardiac function. In humans caloric restriction increases plasma NEFA levels. Our objective was to assess the effects of progressive caloric restriction on myocardial and hepatic TG content and myocardial function.

Materials and methods: This study included 10 lean healthy men. Three-day partial (471 kcal/d) and complete starvation was performed. Plasma levels of NEFAs, myocardial and hepatic TG content (^1H magnetic resonance (MR) spectroscopy, and myocardial function (MR imaging) were calculated.

Results: Plasma NEFAs increased from mean \pm standard deviation 0.6 ± 0.4 mmol/l to 1.2 ± 0.4 and to 1.9 ± 0.7 mmol/l, after partial and complete starvation, respectively ($P < 0.001$). Myocardial TG content increased from $0.35 \pm 0.14\%$ to $0.59 \pm 0.27\%$, and $1.26 \pm 0.49\%$, respectively ($P < 0.01$). The ratio between the early diastole and atrial contraction decreased from 2.2 ± 0.4 to 2.1 ± 0.4 ($P = 0.7$) and 1.8 ± 0.4 , respectively ($P < 0.01$), and diastolic early deceleration from $3.4 \pm 0.7 \text{ ml/s}^2 \times 10^{-3}$ to 2.9 ± 0.5 and $2.8 \pm 0.9 \text{ ml/s}^2 \times 10^{-3}$, respectively ($P < 0.05$). Hepatic TG content decreased after partial starvation (from $2.23 \pm 2.24\%$ to $1.43 \pm 1.33\%$; $P < 0.05$) but did not change upon complete starvation.

Conclusions: Progressive caloric restriction induces a dose-dependent increase in myocardial TG content and a dose-dependent decrease in diastolic function in lean healthy men. Hepatic TG content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous TG stores is tissue-specific.

INTRODUCTION

Almost all endogenous triglycerides (TGs) are stored in adipose tissue to accommodate discrepancies between whole body fat uptake and fat oxidation. However, a very small proportion is stored in non-adipose tissues like the heart (1), the liver (2), and skeletal muscle (3), especially in obesity and type 2 diabetes mellitus. There are indications that this storage of TG in non-adipose tissues is not merely an inert phenomenon but is associated with more or less subtle physiological changes in organ-specific functioning (4-8). In animal models there is an inverse relation between myocardial TG content and myocardial function. For example, myocardial lipid accumulation is associated with a decrease in left ventricular systolic function in obese Zucker rats and treatment with thiazolidinediones reduces myocardial TG content and improves left ventricular function (7). The underlying mechanisms of the decrease in left ventricular function are complex, and are related to effects of fatty acid (FA)-derivatives, like fatty acyl-coenzyme A, ceramides and diacylglycerol (4;6;8).

High plasma concentrations of non-esterified fatty acids (NEFAs) may result in excessive FA uptake in non-adipose tissues, such as the liver and the heart, which may affect normal organ function (6;7). However, in humans the relation between myocardial TG accumulation and myocardial function was difficult to study by non-invasive methods, as measurement of myocardial TG content is challenging due to artifacts induced by cardiac and respiratory motion. Recently, hydrogen 1 magnetic resonance spectroscopy (¹HMRS) of the heart was developed which enables to measure myocardial TG content in humans *in vivo* (1;9-12). Using this method, Reingold *et al.* documented that fasting for 48 hours increases plasma NEFA levels and myocardial TG content in healthy subjects, whereas myocardial TG content did not change after a single high-fat meal (13). In another, cross-sectional study, Kankaanpää *et al.* showed that increased levels of plasma NEFAs in obese subjects correlate positively with myocardial TG content and inversely with cardiac function (11). However, both studies did not address the relation between myocardial function in relation to myocardial TG content within the same subjects. In a recent study we documented that the use of a very low-calorie diet increases plasma NEFAs and myocardial TG content, associated with a decrease in myocardial diastolic function (14). Therefore, it appears that myocardial TG content is not fixed, but varies within the same subject according to physiological conditions. It is yet unknown, whether our recent findings of myocardial flexibility can be extrapolated when caloric restriction is progressively increased. Therefore, the aim of the present study was to extend the conditions of partial caloric restriction to complete caloric restriction, i.e. complete starvation. For this purpose we compared baseline observations, with those obtained after 3 days of a partial starvation (471 kcal/day) and after 3 days of complete starvation with respect to plasma levels of NEFAs, myocardial TG content, myocardial function and hepatic TG content.

MATERIALS AND METHODS

Subjects

There were ten non-smoking, healthy men included in this study (age; mean \pm standard deviation: 23.7 ± 4.7 years, range 20.8-36.0 years, body mass index (BMI): 23.6 ± 0.9 kg/m²). Women were excluded, as the hormonal status or contraceptive use may affect lipid metabolism (15). The study population was partly based on a previous cohort (14). In each subject, medical history was obtained and physical examination was performed. An electrocardiogram (ECG) was made during the first visit. Subjects with any aberrations on the ECG were excluded. In addition, a two-hour 75 g oral glucose tolerance test was performed in the fasted state, to exclude subjects suffering from diabetes mellitus (16). Other exclusion criteria were: obesity (BMI > 30 kg/m²), liver disease (increased plasma levels of alanine aminotransferase, aspartate aminotransferase and/or gamma-glutamyl transferase > 2 standard deviations above the reference value of our institution), renal disease (defined by plasma creatinine levels > 2 standard deviations above the reference value of our institution), use of any medication, and a history of (congenital) heart disease. Specifically, subjects with prior or present coronary artery disease (based on medical history) or hypertension (defined as sitting systolic blood pressure > 130 mmHg and / or diastolic blood pressure > 85 mmHg) were excluded. From all participants written informed consent was obtained prior to the study. The local ethics committee approved the study.

Study design

The study consisted of 3 conditions. Baseline measurements were made, while subjects followed a normal diet, but abstained from alcohol for 3 days (mean intake 2065 kcal/day). Subjects were admitted 4 hours after the last meal for measurement of plasma concentrations of glucose, insulin, and lipids and for evaluation by magnetic resonance (MR) imaging and ¹HMRS. The second measurement was performed after a 3-day period of partial caloric restriction (471 kcal/day, Modifast Intensive, Nutrition & Santé Benelux, Breda, The Netherlands). The third measurement was performed after a 3-day period of complete starvation (0 kcal/day, only water was allowed), after which subjects were again admitted for blood sampling and MR imaging and ¹HMRS evaluation. Plasma concentrations of NEFAs and insulin were used to assess study compliance (17). Between all study occasions a washout period with a minimum of 14 days was acquired (18), and the sequence of the second and third occasions was determined by balanced assignment.

¹H magnetic resonance spectroscopy of the liver and the heart

All MR imaging and ¹HMRS measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, The Netherlands) in the supine position. Localized single-voxel ($2 \times 2 \times 2$ cm for the liver and $2 \times 4 \times 1$ cm for the heart) spectra were recorded using a body coil for radiofrequency transmission and a surface coil (\varnothing 17 cm) for

signal receiving. For the heart, the spectral volume was placed in the interventricular septum on four-chamber and short-axis images at end-systole, avoiding contamination with epicardial fat (Figure 4.1). Data collection was double-triggered by using ECG triggering and navigator echoes for compensation of respiratory motion as described earlier (12). For the liver, voxel sites were matched at both study occasions, carefully avoiding blood vessels and bile ducts. To detect weak lipid signals, water-suppressed spectra with 128 averages for the heart, and 64 for the liver were collected. Spectral parameters were: a repetition time (TR) of 3000 ms, echo time (TE) of 26 ms and 1024 data points over 1000-Hz spectral width. In the same voxel, using the same parameters except for a repetition time of 10000 ms, unsuppressed spectra with 4 averages were collected. Spectra were analyzed in the time domain, using Java-based MR user interface software and prior knowledge files (jMRUI version 2.2 (19)), as described earlier (12). Peak estimates of lipid resonances of myocardial TGs at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (TG content, TGs/water $\times 100$).

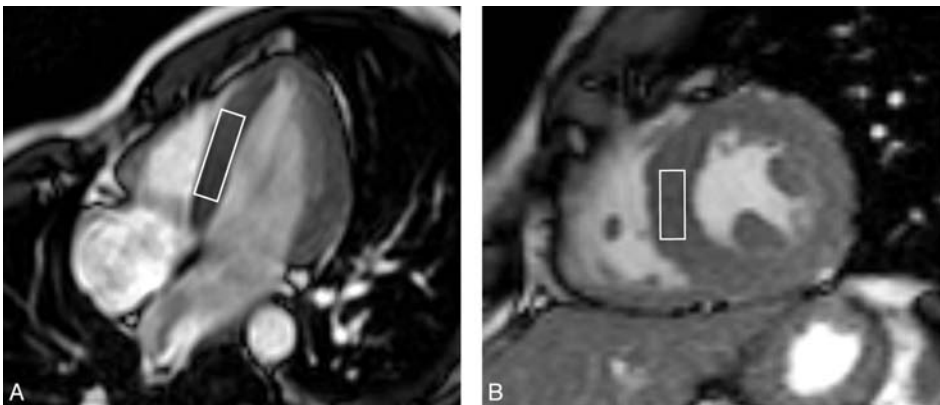


Figure 4.1. Myocardial spectroscopic volume.

Localization of the myocardial voxel in the four-chamber (A) and short-axis (B) views.

Magnetic resonance imaging of the heart

Imaging of the heart was performed using a body coil for radiofrequency transmission and a 5 elements synergy coil for signal receiving. In order to assess systolic function, the heart was imaged from apex to base with 12 to 14 imaging levels (dependent on the heart size) in short-axis view using an ECG-triggered, sensitivity-encoding balanced steady-state free precession sequence. Imaging parameters were a field of view of 400×320 mm, a matrix size of 256×256 , a slice thickness of 10 mm, a slice gap of 0 mm, a flip angle of 35° , a TE of 1.7 ms and a TR of 3.4 ms. Temporal resolution was 25 to 39 ms. End-diastolic and end-systolic images were identified on all slices and endocardial contours were drawn using MASS[®] post processing software (Medis, Leiden, The Netherlands) as described previously (20). Left ventricular ejection fraction

(LVEF) was calculated for assessment of systolic function. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for the determination of left ventricular diastolic function (21;22). Imaging parameters included the following: a TE of 4.8 ms, a TR of 14 ms, a flip angle of 20°, a slice thickness of 8 mm, a field of view of 350 mm², a matrix size of 256 × 256, a velocity encoding of 100 cm/s and a scan percentage of 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 (Medis, Leiden, The Netherlands) by defining a region of interest on the modulus images in all cardiac phases. Furthermore, the mean deceleration of the E wave and an estimation of left ventricular filling pressures (E/Ea) (23) were measured. All spectroscopic and functional analyses were performed by an experienced observer, blinded to the interventions. During MR imaging, blood pressure and heart rate were measured twice with an automatic device (Dinamap DPC100X, Freiburg, Germany) and averaged for analysis.

Assays

Glucose, total cholesterol (TC) and TGs were measured on a fully automated P800 analyzer (Roche, Almere, The Netherlands) and insulin on a Immulite 2500 random access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA, USA). Coefficients of variation were < 2% for glucose, TC and TG, and < 5% for insulin. Plasma NEFAs were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis

All statistical analyses were performed using SPSS, version 12.01 (SPSS Inc., Chicago, Ill, USA). Statistical comparisons between the three physiological conditions were made by repeated measures ANOVA. Pearson-*r* values were used for correlation analysis. Data are shown as mean ± standard deviation. *P* < 0.05 (two-tailed) was considered significant. Based on previous report we expected a decrease in diastolic early deceleration. Therefore *P* < 0.05 (one-tailed) was considered to be significant for this parameter (14).

RESULTS

Metabolic effects of progressive caloric restriction

Subject characteristics at baseline, after partial starvation, and after complete starvation are shown in Table 4.1. Postabsorptive plasma glucose levels decreased from 5.0 ± 0.3 mmol/l at baseline to 4.3 ± 0.4 mmol/l after partial (*P* = 0.001) and to 3.9 ± 0.5 mmol/l after complete starvation (*P* < 0.001). This was associated with a dose-dependent decrease in plasma insulin levels. Simultaneously, plasma concentrations of NEFAs increased dose-dependently from 0.6 ± 0.4 mmol/l to 1.2 ± 0.4 mmol/l after partial (*P* < 0.001) and to 1.9 ± 0.7 mmol/l after complete

Table 4.1. Metabolic response to progressive caloric restriction.

Plasma concentrations	Baseline	Partial starvation	Complete starvation
Glucose (mmol/l)	5.0 ± 0.3	4.3 ± 0.4*	3.9 ± 0.5*
Insulin (mU/l)	10.1 ± 5.3	8.0 ± 3.7	3.0 ± 1.8*
Non-esterified fatty acids (mmol/l)	0.6 ± 0.4	1.2 ± 0.4**	1.9 ± 0.7**
Triglycerides (mmol/l)	1.3 ± 0.4	0.9 ± 0.3*	1.3 ± 0.6
Total cholesterol (mmol/l)	5.0 ± 1.3	5.1 ± 1.4	5.9 ± 1.8*

* $P < 0.01$ and ** $P < 0.001$ vs baseline. Data are mean ± standard deviation.

Blood samples were collected 4 hours after the last meal.

starvation ($P < 0.001$). Plasma TG levels decreased after partial starvation (from 1.3 ± 0.4 mmol/l to 0.9 ± 0.3 mmol/l ($P = 0.009$), but did not change upon complete starvation ($P = 0.677$). TC increased from 5.0 ± 1.3 mmol/l at baseline to 5.1 ± 1.4 mmol/l after partial ($P = 0.810$) and to 5.9 ± 1.8 mmol/l after complete starvation ($P = 0.005$).

Effects of progressive caloric restriction on myocardial and hepatic triglyceride content

Myocardial TG content increased dose-dependently from $0.35 \pm 0.14\%$ at baseline to $0.59 \pm 0.27\%$ after partial ($P = 0.006$) and to $1.26 \pm 0.49\%$ after complete starvation ($P < 0.001$, Figure 4.2). Hepatic TG content correlated with BMI at baseline ($r = 0.67$, $P = 0.033$). Hepatic TG content significantly decreased after partial starvation (from $2.24 \pm 2.24\%$ to $1.43 \pm 1.33\%$, $P = 0.031$), whereas it did not change after complete starvation ($2.54 \pm 2.53\%$, $P = 0.378$, Figure 4.3).

Effects of progressive caloric restriction on myocardial function

Systolic and diastolic blood pressure, heart rate and myocardial LVEF did not change significantly during/after partial and complete starvation, compared to baseline (Table 4.2). Furthermore, estimated left ventricular filling pressures were unchanged after partial (8.8 ± 3.8 $P = 0.742$) and complete starvation (8.2 ± 2.5 , $P = 0.299$) compared to baseline (9.3 ± 2.6). Diastolic E/A ratio decreased dose-dependently from 2.2 ± 0.4 at baseline to 2.1 ± 0.4 after partial starvation ($P = 0.687$) and to 1.8 ± 0.4 after complete starvation ($P = 0.005$). E deceleration decreased dose-dependently from 3.4 ± 0.7 ml/s² × 10⁻³ at baseline to $2.9 \pm 0.5 \times 10^{-3}$ ml/s² after partial ($P = 0.036$) and to 2.8 ± 0.9 after complete starvation ($P = 0.032$).

Table 4.2. Effects of progressive caloric restriction on myocardial function.

	Baseline	Partial starvation	Complete starvation
Systolic blood pressure (mmHg)	120 ± 10	118 ± 9	122 ± 12
Diastolic blood pressure (mmHg)	64 ± 7	62 ± 7	61 ± 5
Heart rate (bpm)	62 ± 13	59 ± 10	65 ± 10
LVEF (%)	60 ± 4	59 ± 4	60 ± 6
E/A ratio	2.2 ± 0.4	2.1 ± 0.4	1.8 ± 0.4*
E deceleration (ml/s ² × 10 ⁻³)	3.4 ± 0.7	2.9 ± 0.5†	2.8 ± 0.9†

* $P < 0.01$, † $P < 0.05$ vs baseline. Data are mean ± standard deviation.

LVEF = left ventricular ejection fraction, E = early diastolic wave, A = atrial diastolic wave.

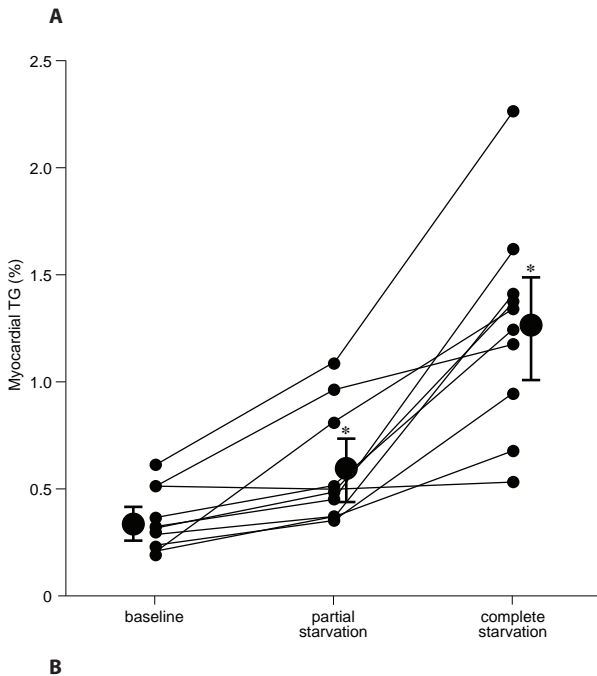
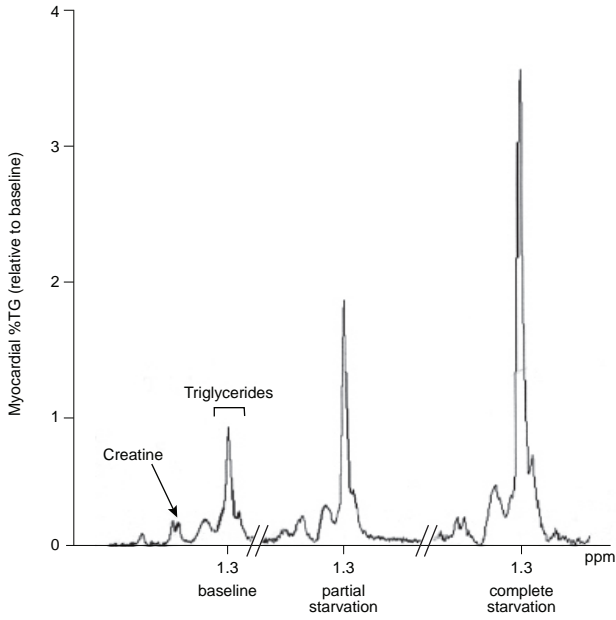


Figure 4.2. Myocardial triglyceride content at baseline and after partial and complete starvation.

Typical ^1H spectra of myocardial triglyceride (TG) content of one subject at baseline and after partial and complete starvation scaled relative to baseline (A) and individual changes in myocardial TG content upon complete starvation ($n = 10$) (B).

Vertical lines represent mean \pm standard deviation, * $P < 0.01$ vs baseline. ppm = parts per million.

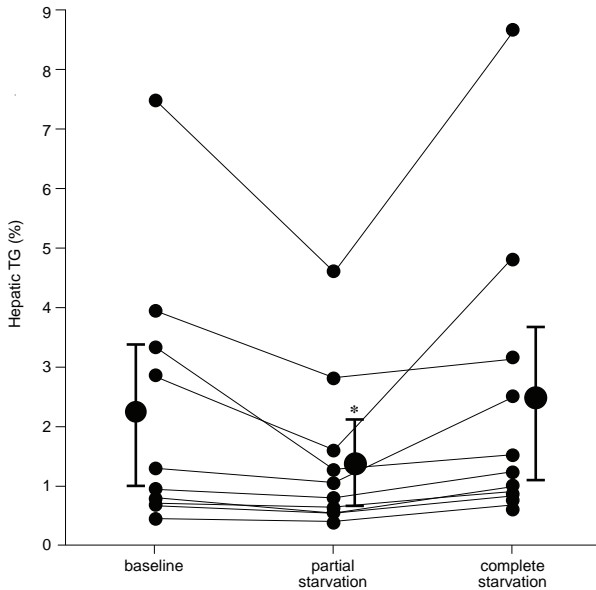


Figure 4.3. Hepatic triglyceride content at baseline and after partial and complete starvation.

Individual changes in hepatic triglyceride content upon starvation ($n = 10$).

Vertical lines represent mean \pm standard deviation, * $P < 0.05$ vs baseline.

ppm = parts per million, TG = triglyceride.

DISCUSSION

This study demonstrates that progressive caloric restriction increases myocardial TG content in lean healthy men. This increase is paralleled by decreased diastolic myocardial function. In addition, the results document a dose-dependent effect between the degree of caloric restriction and the myocardial effects.

These observations point to physiological variations in myocardial TG content and diastolic function. The effect of caloric restriction on redistribution of endogenous TG stores is tissue-specific, since we demonstrated differential effects of partial and complete starvation on liver TG content. Different degrees of starvation were associated with a considerable increase in plasma NEFA levels, in accordance with previous observations (24;25). These increased NEFA levels reflect increased lipolysis of TG content in adipose tissue. Apparently, during starvation myocardial FA uptake exceeds the requirements of myocardial FA oxidation, resulting in increased TG stores. Moreover, progressive caloric restriction has dose-dependent effects on myocardial TG accumulation and myocardial function. However, a causal relationship between myocardial TG content and myocardial function can not be derived from the present data.

Our data are supported by animal experiments. In those studies excessive exposure of the myocardium to plasma FA is accompanied by increased storage of myocardial TGs, resulting

in the production of FA intermediates, and ultimately deteriorations in myocardial function (7;26;27). Accordingly, it has been suggested that in obese subjects, subclinical diastolic dysfunction is due to changes in myocardial metabolism (28-31). Kankaanpää *et al.* reported that alterations in left ventricular function in moderate obese subjects are associated with increased myocardial TG content, compared to lean subjects (11). Moreover, Szczepaniak *et al.* showed increased myocardial TG content in overweight and obese subjects, which was accompanied by increased left ventricular mass (1). In accordance with our study, Reingold *et al.* documented that short-term fasting leads to myocardial TG accumulation, although they did not document effects on myocardial function (13). The current results, documenting dose-dependent effects of caloric restriction on levels of plasma NEFAs, myocardial TG content and diastolic function, extend these findings and support the general concept that increased myocardial TG content is associated with decreased myocardial function (32). Alternatively, starvation profoundly alters endogenous metabolic regulation and other, yet undefined, metabolic effects than merely increased levels of plasma NEFAs and myocardial TG content, which may be involved to explain the reduction in myocardial diastolic function. For example, caloric restriction might change calcium homeostasis in the myocardium (33), which affects myocardial diastolic function (34).

Transmitral flow velocities are load dependent and can be affected by changes in intravascular volume. However, estimated left ventricular filling pressures were unchanged upon progressive caloric restriction. Therefore, we believe the observed change in transmitral flow patterns results from a change in the relaxation of the left ventricle. Caloric restriction enhances adipose tissue lipolysis, reflected in increased levels of plasma NEFAs, due to reduced insulin levels. Similar to our results in the heart, others found corresponding results of increased TG content of skeletal muscle after fasting (18;24;25). Starvation affects more parameters of lipid metabolism, because plasma NEFAs stimulate the hepatic production of very low-density lipoprotein (VLDL), which is an important supplier for TG to the heart (35;36). Plasma NEFA levels also increase during starvation and most likely will contribute to increased myocardial TG levels. However, the relative contribution of albumin-bound fatty acids vs fatty acids derived from VLDL-TGs to myocardial TG stores during caloric restriction can not be derived from the present data.

We found a correlation between hepatic fat content and BMI, in accordance with previous observations (2;37). However, despite the increase in the flux of plasma NEFAs to the liver, considering the increased plasma NEFA levels, hepatic TG content was decreased after partial starvation but was unchanged after complete starvation. In line with our results, Westerbacka *et al.* previously documented that a low-fat diet in moderately obese women decreases hepatic TG content (38). Because hepatic TG content is tightly regulated by the balance of hepatic FA uptake, hepatic FA oxidation and output of VLDL-TG particles, it is possible that this hepatic balance between FA uptake and TG output is differentially affected by partial and complete starvation. Nonetheless, our data indicate that progressive caloric restriction differentially affects tissue-specific stores of TGs in heart and liver, and prove that myocardial TG content

and myocardial function vary depending on nutritional conditions, at least with respect to progressive degrees of starvation. Additional studies are required to elucidate to which extent these results can be extrapolated to clinically relevant conditions like type 2 diabetes mellitus and obesity.

CONCLUSIONS

In conclusion, progressive caloric restriction induces a dose-dependent increase in myocardial TG content and a dose-dependent decrease in diastolic function in lean healthy men. Hepatic TG content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous TG stores is tissue-specific, at least in lean healthy men.

REFERENCES

1. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.
2. Ishii M, Yoshioka Y, Ishida W, Kaneko Y, Fujiwara F, Taneichi H, Miura M, Toshihiro M, Takebe N, Iwai M, Suzuki K, Satoh J. Liver fat content measured by magnetic resonance spectroscopy at 3.0 tesla independently correlates with plasminogen activator inhibitor-1 and body mass index in type 2 diabetic subjects. *Tohoku J Exp Med* 2005; 206(1):23-30.
3. Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Ma YZ, Savoye M, Rothman DL, Shulman GI, Caprio S. Assessment of skeletal muscle triglyceride content by (1)H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 2002; 51(4):1022-1027.
4. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 1994; 91(23):10878-10882.
5. Shimabukuro M, Higa M, Zhou YT, Wang MY, Newgard CB, Unger RH. Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. *J Biol Chem* 1998; 273(49):32487-32490.
6. Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J* 2001; 15(2):312-321.
7. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000; 97(4):1784-1789.
8. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A* 1998; 95(5):2498-2502.
9. den Hollander JA, Evanochko WT, Pohost GM. Observation of cardiac lipids in humans by localized 1H magnetic resonance spectroscopic imaging. *Magn Reson Med* 1994; 32(2):175-180.
10. Felblinger J, Jung B, Slotboom J, Boesch C, Kreis R. Methods and reproducibility of cardiac/respiratory double-triggered (1)H-MR spectroscopy of the human heart. *Magn Reson Med* 1999; 42(5):903-910.
11. Kankaanpaa M, Lehto HR, Parkka JP, Komu M, Viljanen A, Ferrannini E, Knuuti J, Nuutila P, Parkkola R, Iozzo P. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006; 91(11):4689-4695.
12. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of myocardial triglyceride content: reproducibility of 1H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007; 245(1): 251-257.
13. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *Am J Physiol Endocrinol Metab* 2005; 289(5):E935-E939.

14. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007; 56(12):2849-2853.
15. Anon. Hormones and cardiovascular health in women. *Hum Reprod Update* 2006; 12(5):483-497.
16. Anon. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003; 26 Suppl 1:S5-20.
17. Klein S, Sakurai Y, Romijn JA, Carroll RM. Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. *Am J Physiol* 1993; 265(5 Pt 1):E801-E806.
18. Johnson NA, Stannard SR, Rowlands DS, Chapman PG, Thompson CH, O'Connor H, Sachinwalla T, Thompson MW. Effect of short-term starvation versus high-fat diet on intramyocellular triglyceride accumulation and insulin resistance in physically fit men. *Exp Physiol* 2006; 91(4):693-703.
19. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129(1):35-43.
20. Pattynama PM, Lamb HJ, van der Velde EA, van der Wall EE, de Roos A. Left ventricular measurements with cine and spin-echo MR imaging: a study of reproducibility with variance component analysis. *Radiology* 1993; 187(1):261-268.
21. Hartiala JJ, Mostbeck GH, Foster E, Fujita N, Dulce MC, Chazouilleres AF, Higgins CB. Velocity-encoded cine MRI in the evaluation of left ventricular diastolic function: measurement of mitral valve and pulmonary vein flow velocities and flow volume across the mitral valve. *Am Heart J* 1993; 125(4):1054-1066.
22. Lamb HJ, Beyerbacht HP, van der Laarse A, Stoel BC, Doornbos J, van der Wall EE, de Roos A. Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. *Circulation* 1999; 99(17):2261-2267.
23. Paelinck BP, de Roos A, Bax JJ, Bosmans JM, van der Geest RJ, Dhondt D, Parizel PM, Vrints CJ, Lamb HJ. Feasibility of tissue magnetic resonance imaging: a pilot study in comparison with tissue Doppler imaging and invasive measurement. *J Am Coll Cardiol* 2005; 45(7):1109-1116.
24. Stannard SR, Thompson MW, Fairbairn K, Huard B, Sachinwalla T, Thompson CH. Fasting for 72 h increases intramyocellular lipid content in nondiabetic, physically fit men. *Am J Physiol Endocrinol Metab* 2002; 283(6):E1185-E1191.
25. Wietek BM, Machann J, Mader I, Thamer C, Haring HU, Claussen CD, Stumvoll M, Schick F. Muscle type dependent increase in intramyocellular lipids during prolonged fasting of human subjects: a proton MRS study. *Horm Metab Res* 2004; 36(9):639-644.
26. Christoffersen C, Bollano E, Lindegaard ML, Bartels ED, Goetze JP, Andersen CB, Nielsen LB. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003; 144(8):3483-3490.
27. Ouwens DM, Boer C, Fodor M, de Galan P, Heine RJ, Maassen JA, Diamant M. Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. *Diabetologia* 2005; 48(6):1229-1237.
28. Sharma S, Adroque JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeyer H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* 2004; 18(14):1692-1700.
29. de Las Fuentes L, Waggoner AD, Brown AL, Davila-Roman VG. Plasma Triglyceride Level is an Independent Predictor of Altered Left Ventricular Relaxation. *J Am Soc Echocardiogr* 2005; 18(12):1285-1291.

30. Diamant M, Lamb HJ, Groeneveld Y, Endert EL, Smit JW, Bax JJ, Romijn JA, de Roos A, Radder JK. Diastolic dysfunction is associated with altered myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2 diabetes mellitus. *J Am Coll Cardiol* 2003; 42(2):328-335.
31. Peterson LR, Herrero P, Schechtman KB, Racette SB, Waggoner AD, Kisrieva-Ware Z, Dence C, Klein S, Marsala J, Meyer T, Gropler RJ. Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. *Circulation* 2004; 109(18):2191-2196.
32. McGavock JM, Victor RG, Unger RH, Szczepaniak LS. Adiposity of the heart, revisited. *Ann Intern Med* 2006; 144(7):517-524.
33. Han X, Cheng H, Mancuso DJ, Gross RW. Caloric restriction results in phospholipid depletion, membrane remodeling, and triacylglycerol accumulation in murine myocardium. *Biochemistry* 2004; 43(49):15584-15594.
34. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. *Circulation* 2002; 105(12):1503-1508.
35. Goudriaan JR, Tacke PJ, Dahlmans VE, Gijbels MJ, van Dijk KW, Havekes LM, Jong MC. Protection from obesity in mice lacking the VLDL receptor. *Arterioscler Thromb Vasc Biol* 2001; 21(9):1488-1493.
36. Sakai J, Hoshino A, Takahashi S, Miura Y, Ishii H, Suzuki H, Kawarabayasi Y, Yamamoto T. Structure, chromosome location, and expression of the human very low density lipoprotein receptor gene. *J Biol Chem* 1994; 269(3):2173-2182.
37. Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Hakkinen AM, Fredriksson J, Yki-Jarvinen H. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia* 2004; 47(8):1360-1369.
38. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, Yki-Jarvinen H. Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 2005; 90(5): 2804-2809.

Chapter 5

Effects of Short-term High-fat, High-energy Diet on Hepatic and Myocardial Triglyceride Content in Healthy Men

Journal of Clinical Endocrinology and Metabolism 2008; 93(7):2702-2708

S. Hammer
R.W. van der Meer
H.J. Lamb
M. Fröhlich
M. Diamant
L.J. Rijzewijk
A. de Roos
J.A. Romijn
J.W.A. Smit



SUMMARY

Objectives: An association has been suggested between elevated plasma non-esterified fatty acid (NEFA) levels, myocardial triglyceride (TG) accumulation and myocardial function. The objective of the present study was to investigate the effects of an elevation of plasma NEFAs by a high-fat, high-energy (HFHE) diet on hepatic and myocardial TG accumulation, and on myocardial function.

Materials and methods: Fifteen healthy males (mean \pm standard deviation age: 25.0 ± 6.6 years) were subjected to a 3-day HFHE diet consisting of their regular diet, supplemented with 800 ml of cream (280g fat) every day. ^1H magnetic resonance (MR) spectroscopy was performed for assessing hepatic and myocardial TGs. Furthermore, left ventricular function was assessed using MR imaging.

Results: The HFHE diet increased hepatic TGs compared to baseline (from 2.01 ± 1.79 to $4.26 \pm 2.78\%$, $P = 0.001$), in parallel to plasma TGs and NEFAs. Myocardial TGs did not change (0.38 ± 0.18 vs $0.40 \pm 0.12\%$, $P = 0.7$).

The HFHE diet did not change myocardial systolic function. Diastolic function, assessed by dividing the maximum flow across the mitral valve of the early diastolic filling phase, by the maximum flow of the atrial contraction (E/A ratio), decreased compared to baseline (from 2.11 ± 0.39 to 1.89 ± 0.33 , $P = 0.031$). This difference was no longer significant after adjustment for heart rate ($P = 0.12$).

Conclusions: Short-term HFHE diet in healthy males results in major increases in plasma TGs and NEFAs and hepatic TGs, whereas it does not influence myocardial TGs or myocardial function. These observations indicate differential, tissue-specific partitioning of TGs and/or fatty acids among non-adipose organs during HFHE diet.

INTRODUCTION

Dietary triglycerides (TGs) are absorbed for > 95% by the gut. After absorption, these TGs can either be oxidized or stored in adipose tissue. A minimal part of these dietary TGs may be stored in non-adipose tissue, such as the pancreas, liver, and myocardium. Storage of TGs in non-adipose tissues is very tightly regulated and disruption of this regulation is associated with functional and structural changes. In humans, high-fat (HF) diets rapidly raise plasma TG and non-esterified fatty acid (NEFA) levels, increase hepatic TG content and cause insulin resistance (1). Short-term HF diets also increase intramyocellular TG content in skeletal muscle accompanied by molecular adaptations that favor fat storage in muscle rather than oxidation (2).

In some conditions, the myocardium can also accumulate TGs. This increase in myocardial TG content may be of pathophysiological relevance. Patients suffering from type 2 diabetes mellitus (DM2) show increased myocardial TG content (3) and healthy volunteers, who were fed a very low-calorie diet for three days, showed increased plasma NEFA levels and accumulated myocardial TGs. The increase in myocardial TGs was associated with alterations in myocardial function (4). In addition, in an animal model, a HF diet for 7 weeks causes cardiac steatosis and myocardial dysfunction (5).

Increased plasma NEFA levels are also associated with abnormal myocardial energy metabolism. In patients with DM2, myocardial high-energy phosphate (HEP) metabolism was significantly impaired (6). Furthermore, obese men with preserved systolic and diastolic function showed abnormal myocardial HEP metabolism, which was associated with insulin resistance (7).

Hydrogen 1 magnetic resonance spectroscopy (¹H MRS), phosphorus (³¹P) MRS, and magnetic resonance (MR) imaging are imaging tools, perfectly capable of assessing hepatic and myocardial TG content, myocardial HEP metabolism, and myocardial function non-invasively (8-11).

In humans, a single high-fat containing meal had no influence on myocardial TG content and on hepatic TG content (12;13). This one-meal intervention might have been too subtle to initiate myocardial and hepatic TG accumulation. The effect of a prolonged disturbance of plasma lipids on myocardial TG accumulation remains to be investigated.

Therefore, the goal of the present study was to investigate the effect of a 3-day HF, high-energy (HE) diet on hepatic and myocardial TG accumulation, myocardial HEP metabolism, and on myocardial function in healthy subjects using MR spectroscopy and MR imaging.

MATERIALS AND METHODS

Subjects

There were fifteen healthy men who volunteered to participate in this study that was approved by the local ethics committee. Only males were included, because the hormonal status or use of contraceptives may affect lipid metabolism in women. Given the well documented effects of estrogens on lipid metabolism (including plasma lipid levels, adipose tissue) and the gender differences in expression of certain cell surface receptors/transporters of fatty acids, (14;15) we decided to exclude women at this stage to avoid possible confounding influences of potential fluctuation in lipid metabolism in women on hepatic and myocardial TG accumulation. All volunteers signed written informed consent. Subjects were included if they met the following criteria: 1) age older than 18 years; and 2) no known acute or chronic disease based on history, physical examination, and standard laboratory tests (blood counts, serum creatine, alanine aminotransferase, aspartate aminotransferase, and electrocardiogram [ECG]). Exclusion criteria included treatment with drugs, smoking, substance abuse, hypertension or impaired glucose tolerance (experienced with a two-hour oral glucose tolerance test) (16). All subjects performed exercise (walking, running, biking) regularly (ranges 3-5 hours weekly), but none of the subjects engaged in high-performance sports.

Study design

Subjects underwent MR scanning in the afternoon at two different occasions. Before both visits, they were instructed to follow different dietary regimes for 3 days prior to the measurements. Use of alcohol was not allowed during the 3-day diets. In the first regime each subject used his normal diet. Mean intake was approximately 2100 kcal/day. The calories were approximately divided as follows: carbohydrates 40%, fat 35%, protein 25%. This reference diet was used for the collection of baseline data. The last meal was consumed 4 hours before venous blood sampling and data collection. During the second regime, the subjects were placed on a 3-day hypercaloric diet characterized by HFHE content. The HFHE diet consisted of the same intake as the reference diet, complemented with 800 ml of cream every day. The cream added 2632 kcal/day (carbohydrates 3.5%, fat 94%, protein 2.5%). Therefore, during the HFHE diet, total energy intake was approximately 4732 kcal/day with the calories divided as: carbohydrates 20%, fat 69%, protein 11%. The last 200 ml of cream were taken 4 hours before data collection. The HFHE content was used to induce an elevation of plasma NEFA and TG levels. At each visit, after venous blood collection, MR imaging and MR spectroscopy of the heart and liver were performed.

¹H magnetic resonance spectroscopy

All MR imaging and MR spectroscopy studies were performed using a 1.5-Tesla whole-body MR scanner (Gyrosan ACS/NT15; Philips, Best, The Netherlands) with subjects in the supine position at rest.

Cardiac ¹HMR spectra were obtained from the interventricular septum as described before (9). The body coil was used for radiofrequency transmission and a 17-cm diameter circular surface coil was used for signal reception.

A point resolved spectroscopy sequence was used to acquire single-voxel MR spectroscopic data from an 8-ml voxel, located in the interventricular septum (Figure 5.1). Spectra were acquired at end-systole, with an echo time (TE) of 26 ms and a repetition time (TR) of at least 3000 ms. 1024 data points were collected using a 1000-Hz spectral width and averaged over 128 acquisitions. The spectroscopic data acquisition was ECG-triggered and respiratory gating based on navigator echoes was applied to minimize breathing influences (9). Without changing any parameter, spectra without water suppression with a TR of 10000 ms and 4 averages were obtained, to be used as an internal standard.

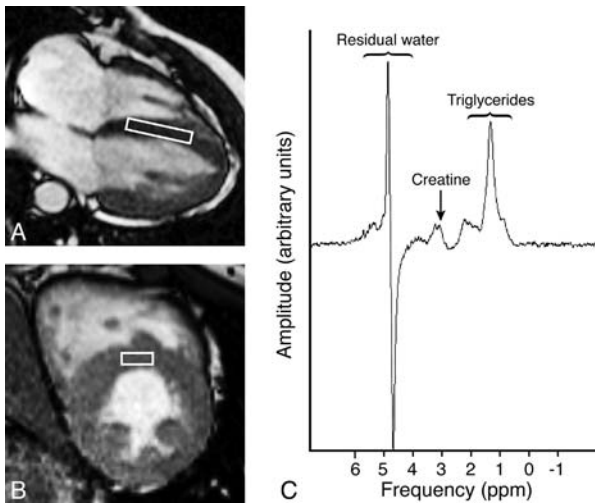


Figure 5.1. Myocardial voxel localization for ¹H magnetic resonance spectroscopy.

Voxel position in four-chamber (A) and short-axis (B) views. An 8-ml voxel was positioned in the interventricular septum in end-systole. Panel C shows a typical water-suppressed spectrum. ppm = parts per million.

¹HMRS of the liver was performed with an 8-ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. The twelfth thoracic vertebra was used as a landmark to ensure the same position of the voxel during both visits. Spectra were obtained without respiratory motion compensation using the same parameters as described above. Only 64 averages were collected with water suppression.

All ^1H MRS data were fitted using Java-based MR user interface software (jMRUI version 2.2; developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium) (17) as described before (9). Resonance frequency estimates for intramyocardial lipids were described with the assumption of Gaussian line shapes at 0.9, 1.3, and 2.1 parts per million (ppm, only data from the peaks at 0.9 and 1.3 ppm were summated and used on statistical analysis (18)). Prior knowledge was incorporated into the fitting algorithm by using previously published criteria. (19-21) The water signal from spectra without water suppression obtained from the same voxel was used as internal reference for relative quantification of lipid resonances. The water signal peak at 4.7 ppm was quantified by using a Lorentzian line shape and analyzed by using the AMARES algorithm. The percentage of myocardial and hepatic TG signal relative to the water signal was calculated as $(\text{signal amplitude of TGs})/(\text{signal amplitude of water}) \times 100$ (%TGs uncorrected for T2 decay times of the studied metabolites).

^{31}P magnetic resonance spectroscopy

A 10-cm diameter surface coil was used to acquire ECG-triggered ^{31}P MR spectra of the left ventricular (LV) anterior wall with subjects in the supine position. Volumes of interest were selected by image-guided spectroscopy with three-dimensional image selected *in vivo* spectroscopy. Shimming was performed automatically and tuning and matching of the ^{31}P surface coil was performed manually. Technical details of data acquisition and spectral quantification were similar as described before (11). Shortly, spectroscopic volume size was typically $7 \times 7 \times 7$ cm. Acquisitions were based on 192 averaged free induction decays, and total acquisition time was 10 min. ^{31}P MR spectra were quantified automatically in the time domain using prior spectroscopic knowledge and were corrected for partial saturation effects and for the adenosine-triphosphate (ATP) contribution from blood in the cardiac chambers. The phosphocreatine (PCr)/ATP ratios of the spectra were calculated and used as a parameter representing myocardial HEP metabolism (22).

Magnetic resonance imaging

The entire heart was imaged in short-axis orientation using ECG-gated breath-holds with a sensitivity-encoding balanced turbo-field echo sequence. Imaging parameters included the following: TE = 1.7 ms, TR = 3.4 ms, flip angle = 35 degrees, slice thickness = 10 mm with a gap of 0 mm, field of view = 400 mm^2 , reconstructed matrix size = 256×256 . The temporal resolution was 25-39 ms. All images were analyzed quantitatively using dedicated software (MASS[®], Medis, Leiden, The Netherlands). LV ejection fraction (EF) was assessed as measure of LV systolic function. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding was performed to measure bloodflow across the mitral valve for the determination of LV diastolic function. Imaging parameters included the following: TE = 4.8 ms, TR = 14 ms, flip angle = 20 degrees, slice thickness = 8 mm, field of view = 350 mm^2 , matrix size = 256×256 , velocity encoding = 100 cm/s, scan percentage = 80%. Analysis was performed by using dedicated

software (FLOW®, Medis, Leiden, The Netherlands). The early filling phase (E) and the atrial contraction (A) were analyzed and the ratio of the maximal flow rate of E and the maximal flow rate of A (E/A) was calculated. In addition, the peak deceleration gradient of E was assessed. Furthermore left ventricular filling pressures (E/Ea) were estimated (23). During MR imaging, blood pressure and heart rate were measured.

Assays

Plasma glucose and TGs were measured by a fully automated P800 analyzer (Roche, Almere, The Netherlands) and insulin using a Immulite 2500 random access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA, USA). Coefficients of variation were below 2% for glucose and TGs and below 5% for insulin. HOMA index was calculated as (glucose × insulin)/22.5. Plasma NEFAs were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany). C-reactive protein (CRP) was determined with a us-CRP Elisa (DSL, Webster, Texas, USA). The sensitivity was 1.6 µg/l and the interassay coefficients of variations range from 3 to 5%.

Statistical analysis

Statistical analysis was performed with SPSS for windows version 12.0 (SPSS Inc., Chicago, Ill, USA). Data are expressed as mean ± standard deviation. The two study conditions were compared by two-tailed paired t-tests. Linear mixed model was used for correcting within subjects differences when necessary. Significance was assumed when $P < 0.05$.

Table 5.1. Clinical and biochemical characteristics.

Variable	Baseline	High-fat, high-energy diet	P-value
Body mass index (kg/m ²)	23.4 ± 2.5	23.6 ± 2.5	0.098
Systolic blood pressure (mmHg)	123 ± 13	125 ± 13	0.673
Diastolic blood pressure (mmHg)	67 ± 8	64 ± 8	0.179
Heart rate (bpm)	60 ± 9	69 ± 11	0.008
Plasma glucose (mmol/l)	4.9 ± 0.3	5.0 ± 0.4	0.356
Plasma insulin (mU/l)	9.1 ± 4.6	21.4 ± 8.8	< 0.001
HOMA index	2.0 ± 1.2	4.9 ± 2.3	0.001
Plasma triglycerides (mmol/l)	1.3 ± 0.4	2.9 ± 1.1	< 0.001
Plasma non-esterified fatty acids (mmol/l)	0.54 ± 0.29	0.92 ± 0.33	0.002
Plasma alanine aminotransferase (mmol/l)	25 ± 16	28 ± 13	0.769
Plasma aspartate aminotransferase (mmol/l)	33 ± 10	33 ± 7	0.250
Gamma-glutamyl transferase (mmol/l)	20 ± 8	20 ± 6	0.849
Ultra-sensitive C-reactive protein (mg/l)	3.6 ± 4.8	1.4 ± 1.1	0.074

P-values were calculated using two-tailed paired t-tests. Values are mean ± standard deviation.

HOMA = homeostatic model assessment.

RESULTS

Clinical and biochemical characteristics

All participants completed the protocol uneventfully. The mean age of the studied subjects was 25.0 ± 6.6 years. Characteristics of the studied subjects at baseline and after the HFHE diet are shown in Table 5.1.

After the HFHE diet, postprandial plasma insulin levels increased significantly (from 9.1 ± 4.6 to 21.4 ± 8.8 mU/l, $P = 0.001$) as did plasma TGs (from 1.3 ± 0.4 to 2.9 ± 1.1 mmol/l, $P < 0.001$) and plasma NEFAs (from 0.54 ± 0.29 to 0.92 ± 0.33 mmol/l, $P = 0.002$) levels (Figure 5.2). Plasma glucose levels remained unchanged (4.9 ± 0.3 vs 5.0 ± 0.4 mmol/l).

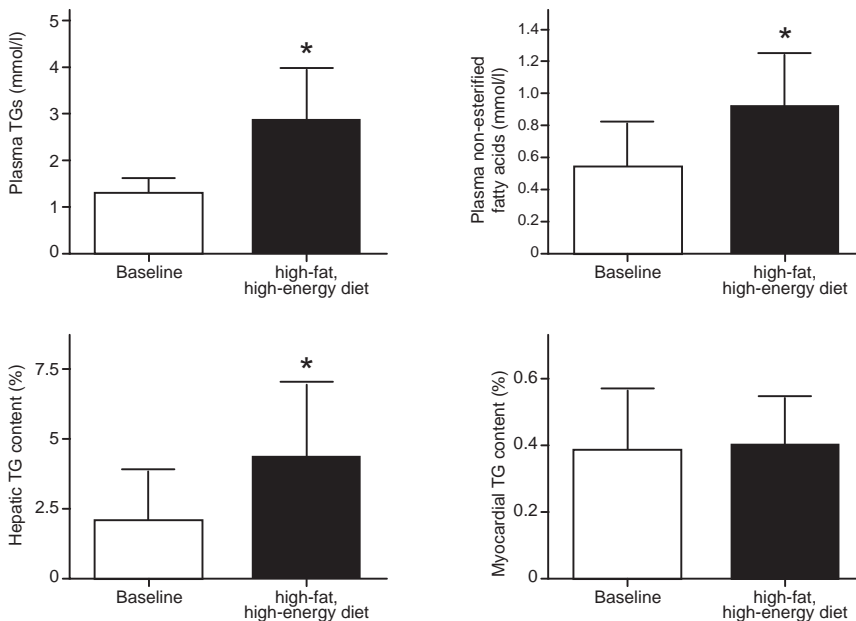


Figure 5.2. Lipid response to high-fat, high-energy diet.

Hepatic and myocardial triglyceride content are relative to the myocardial and hepatic water signals. Bars represent mean + standard deviation, * $P < 0.05$. TG = triglyceride.

Magnetic resonance spectroscopy

After the HFHE diet, ^1HMR revealed a significant increase in hepatic TG content compared to baseline ($4.26 \pm 2.78\%$ vs $2.01 \pm 1.79\%$, $P = 0.001$, Figure 5.2). Typical hepatic ^1HMR spectra of one volunteer before and after the HFHE diet are shown in Figure 5.3. No significant difference in myocardial TG content was detected after the HFHE diet compared to baseline ($0.40 \pm 0.12\%$ vs $0.38 \pm 0.18\%$, $P = 0.7$, Figure 5.2).

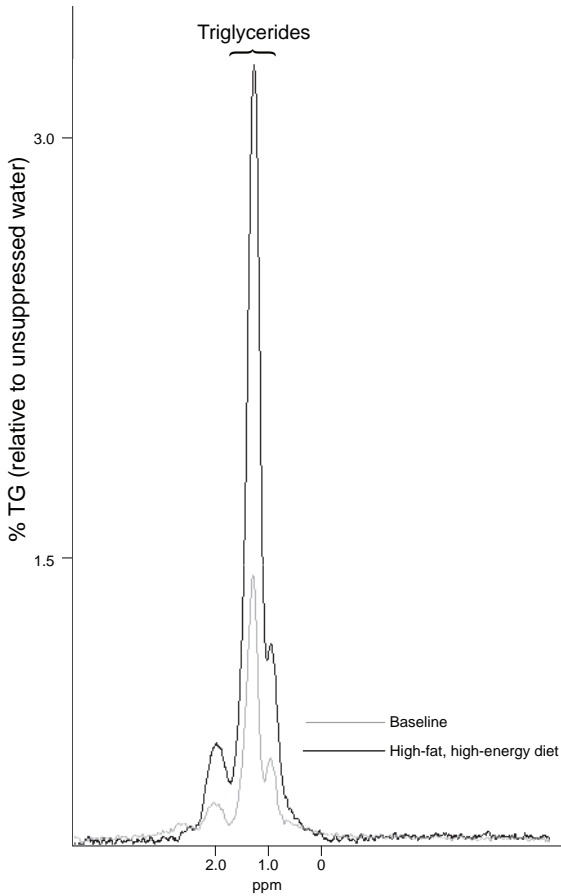


Figure 5.3. Typical ^1H magnetic resonance spectra of one subject before and after the high-fat, high-energy diet.

Only triglyceride region is displayed. Note the marked increase in hepatic triglycerides after the high-fat, high-energy diet. %TG is the amount of triglycerides relative to water $\times 100$.

In 8 subjects, ^{31}P MRS was successfully completed on both study occasions. In the other 7 subjects, ^{31}P MRS data at baseline or after the HFHE diet could not successfully be completed due to technical problems or insufficient spectral quality (relative Cramer-Rao standard deviation $>20\%$ (11)). After the HFHE diet, myocardial PCr/ATP ratio remained unchanged (2.37 ± 0.51 vs 2.35 ± 0.46 , $P = 0.95$).

Myocardial function by magnetic resonance imaging

The parameters of myocardial function are shown in Table 5.2. HFHE diet did not affect left ventricular systolic function. Myocardial work load, represented by the rate pressure product (RPP = heart rate \times systolic bloodpressure) was significantly increased after the HFHE diet compared to baseline (from 7312 ± 1354 to 8563 ± 1867 mmHg \times beats per minute (bpm), $P = 0.02$).

Table 5.2. The effects of high-fat, high-energy diet on metabolic and left ventricular functional parameters.

	Baseline	High-fat, high-energy diet	P-value
Triglyceride content liver (%)	2.01 ± 1.79	4.26 ± 2.78	0.001
Triglyceride content heart (%)	0.38 ± 0.18	0.40 ± 0.12	0.696
PCr/ATP	2.37 ± 0.51	2.35 ± 0.46	0.945
Ejection fraction (%)	60 ± 4	62 ± 5	0.100
Rate pressure product (mmHg × bpm)	7312 ± 1354	8563 ± 1867	0.023
E peak deceleration (ml/s ² × 10 ⁻³)	5.0 ± 1.0	5.1 ± 1.2	0.668
E/Ea	8.8 ± 2.0	9.1 ± 4.0	0.659
E (ml/s)	614 ± 89	630 ± 125	0.529
A (ml/s)	299 ± 64	340 ± 75	0.024
E/A	2.11 ± 0.39	1.89 ± 0.33*	0.031*

P-values were calculated using two-tailed paired t-tests. Values are mean ± standard deviation.

* Adjusted for heart rate, there was no significant difference in E/A ratio between the two diets.

PCr = phosphocreatine, ATP = adenosine-triphosphate, E = early filling phase, A = atrial filling phase, E/Ea = estimated left ventricular filling pressure.

The HFHE diet decreased E/A ratio, a measure of diastolic function, significantly compared to baseline (from 2.11 ± 0.39 to 1.89 ± 0.33, $P = 0.031$) and increased heart rate significantly from 60 ± 9 bpm to 69 ± 11 bpm ($P = 0.008$). After adjustment for heart rate, there were no significant differences in E/A ratios between the two diets ($P = 0.12$).

DISCUSSION

This study shows that in males, a short-term intervention with a hypercaloric, HF diet increases postprandial plasma NEFAs and TGs considerably, which is associated with a more than twofold increase in hepatic TG content. In contrast, this HFHE diet has no acute effects on myocardial TG content, myocardial HEP metabolism, or myocardial function, despite the increased supply of NEFAs and TGs to the heart. These observations stress the short-term physiological and tissue-specific flexibility of ectopic TG pools.

Increased plasma NEFA and TG levels after the 3-day hypercaloric HF diet, indicating good dietary compliance of the volunteers, were associated with a more than twofold increase in hepatic TG content. Westerbacka *et al.* previously reported similar findings on the effects of dietary interventions on hepatic TG content in women (24). The liver acts as a buffer for excessive postprandial flux of NEFAs and TGs (25). The current observation indicates that these hepatic TG stores already expand during very short-term HFHE diets. Based on the unchanged plasma levels of liver enzymes and CRP, short-term hepatic TG accumulation did not give rise to overt indications for hepatic cellular damage or steatohepatitis. Previously published data showed that hepatic liver steatosis is associated with insulin resistance (26). Our study supports these findings. However, as our study only involves a short-term exposure to an unphysiologically

high-fat, high-energy diet, we can not simply extrapolate the findings of our study to the longer term.

The HFHE diet was also associated with increased plasma insulin levels. Insulin promotes the synthesis and storage of TGs in the liver and inhibits the release of very low-density lipoprotein-TG into the circulation (27). In addition, insulin increases the expression or activity of enzymes that catalyze lipid synthesis, whereas insulin inhibits the activity or expression of those that catalyze degradation. Many of these processes require an insulin-induced increase of the transcription factor sterol-regulatory-element-binding protein 1c (28) which, in the liver, is increased by a HF diet (29).

In contrast to the accumulation of hepatic TGs, myocardial TG content remained unchanged after the HFHE diet. Apparently, increased plasma NEFA and TG levels after short-term consumption of a HFHE diet do not change the interrelationship between myocardial NEFA/TG uptake and oxidation in the healthy human heart. This absence of effects of a HFHE diet on myocardial TG content is also in contrast to the response of skeletal muscles which accumulated TGs under HF feeding conditions (1;2). We expected a similar response of the myocardium to a HFHE diet based on these reports. Apparently, increased plasma NEFA and TG levels are not a determinant of excessive myocardial fatty acid uptake, in excess of fatty acid oxidation during short-term HFHE diets in healthy male volunteers. This might be explained by the increased rate pressure product in our study after the HFHE diet. Since plasma glucose concentrations were constant and plasma NEFA and TG levels increased, the increased cardiac workload probably led to an increase in cardiac lipid oxidation rates (30;31) which compensated for the increased lipid availability resulting in no net change in myocardial TG content. In the conditions of our study, carbohydrate intake was not changed. It has been suggested (32) that in healthy, non-diabetic human subjects, dietary-induced intramyocellular TG accumulation and NEFA oxidation may be influenced by dietary carbohydrate intake, plasma glucose availability, (33) and muscular glycogen stores (34). We can not exclude the possibility that HF diets with decreased carbohydrate content may have resulted in changed myocardial TG content.

Chronically elevated plasma NEFA levels in patients with DM2 are associated with altered myocardial HEP metabolism (6). Increased fatty acid availability in these patients results in increased NEFA uptake in the mitochondria which decreases the amount of ATP produced per molecule of oxygen consumed in the mitochondrial electron transport chain (35). In the present study, the short-term HFHE diet and the associated increase in plasma NEFA levels did not affect myocardial HEP metabolism. In our opinion, these findings are in line with the unchanged myocardial TG content which may indicate no dysregulation of mitochondrial substrate handling.

We used MR imaging to study the impact of a HFHE diet induced increase of plasma NEFA levels on left ventricular function. Chronically elevated levels of plasma NEFAs have been demonstrated to be associated with decreased diastolic function in obesity (36). In the present study, short-term elevated plasma NEFA levels as a consequence of a HFHE diet did not affect myocardial systolic and diastolic function. Although there was a slight decrease in diastolic

E/A ratio after the HFHE diet, mainly caused by an increase in A peak flow rate, this change in E/A ratio was accompanied by an increased heart rate, which is a well known postprandial alteration, especially during HF feeding (37-39). An elevated heart rate accounts for an increased preload of the left ventricle which influences LV filling velocities. Adjusted for heart rate, the E/A ratio before and after the HFHE diet were not significantly different, indicating no change in LV diastolic function.

Caloric restriction also increases plasma NEFA levels which is accompanied by decreased plasma glucose and unchanged plasma insulin levels. This leads to myocardial TG accumulation, associated with decreased myocardial function (4). Increased plasma NEFA levels after the HFHE diet were accompanied by unchanged plasma glucose and increased plasma insulin levels. After a HFHE diet there are no changes in myocardial TG content or myocardial function. Apparently, increased plasma NEFA levels after caloric restriction or HFHE diets are associated with different metabolic states and therefore influence myocardial TG content and function differently. These findings are in line with the hypothesis that there might be an association between myocardial TG accumulation and diastolic function. Most likely, myocardial TG stores itself are inert, but rather are a reflection of increased intracellular concentrations of fatty acid intermediates that alter myocellular structure and function by complex molecular mechanisms (40). Further studies need to be conducted to unravel this hypothesis.

Some potential limitations of this study should be addressed. First, excluding women from the study and the narrow age range used in this study limit the generalizability of the present study. Further studies need to be initiated to extend the present finding to subjects from both genders and different ages.

Second, data on myocardial lipid uptake and oxidation rates would extend our findings. However, to approximate myocardial lipid uptake and oxidation rates, positron emission tomography using palmitate tracers should be performed, which is a complicated technique. Finally, only half of the volunteers completed ³¹PMRS measurements and therefore, sample size for this parameter is limited and should be interpreted with caution.

CONCLUSIONS

Short-term HFHE diet in healthy males results in major increases in plasma TGs and NEFAs and hepatic TG content, whereas it does not influence myocardial TG content or myocardial function. These observations indicate differential, tissue-specific partitioning of TGs and/or fatty acids among non-adipose organs during a HFHE.

REFERENCES

1. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, Loviscach M, Stumvoll M, Claussen CD, Schick F, Haring HU, Jacob S. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 2001; 50(11):2579-2584.
2. Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie DG, Saris WH, Nicolay K, Schrauwen P. Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet. *Obes Res* 2005; 13(12):2088-2094.
3. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007; 116(10):1170-1175.
4. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007; 56(12):2849-2853.
5. Ouwens DM, Boer C, Fodor M, de Galan P, Heine RJ, Maassen JA, Diamant M. Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. *Diabetologia* 2005; 48(6):1229-1237.
6. Scheuermann-Freestone M, Madsen PL, Manners D, Blamire AM, Buckingham RE, Styles P, Radda GK, Neubauer S, Clarke K. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. *Circulation* 2003; 107(24):3040-3046.
7. Perseghin G, Ntali G, De Cobelli F, Lattuada G, Esposito A, Belloni E, Canu T, Costantino F, Ragogna F, Scifo P, Del Maschio A, Luzi L. Abnormal left ventricular energy metabolism in obese men with preserved systolic and diastolic functions is associated with insulin resistance. *Diabetes Care* 2007; 30(6):1520-1526.
8. Lamb HJ, Beyerbach HP, van der Laarse A, Stoel BC, Doornbos J, van der Wall EE, de Roos A. Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. *Circulation* 1999; 99(17):2261-2267.
9. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of myocardial triglyceride content: reproducibility of 1H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007; 245(1): 251-257.
10. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.
11. Lamb HJ, Doornbos J, den Hollander JA, Luyten PR, Beyerbach HP, van der Wall EE, de Roos A. Reproducibility of human cardiac 31P-NMR spectroscopy. *NMR Biomed* 1996; 9(5):217-227.
12. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005; 288(2):E462-E468.
13. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *Am J Physiol Endocrinol Metab* 2005; 289(5):E935-E939.

14. D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS. Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem* 2005; 280(43):35983-35991.
15. Stahlberg N, Rico-Bautista E, Fisher RM, Wu X, Cheung L, Flores-Morales A, Tybring G, Norstedt G, Tollet-Egnell P. Female-predominant expression of fatty acid translocase/CD36 in rat and human liver. *Endocrinology* 2004; 145(4):1972-1979.
16. Anon. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003; 26 Suppl 1:S5-20.
17. Naressi A, Couturier C, Devos JM, Janssen M, Mangeat C, de Beer R, Graveron-Demilly D. Java-based graphical user interface for the MRUI quantitation package. *MAGMA* 2001; 12(2-3):141-152.
18. Torriani M, Thomas BJ, Halpern EF, Jensen ME, Rosenthal DI, Palmer WE. Intramyocellular lipid quantification: repeatability with 1H MR spectroscopy. *Radiology* 2005; 236(2):609-614.
19. Boesch C, Slotboom J, Hoppeler H, Kreis R. In vivo determination of intra-myocellular lipids in human muscle by means of localized 1H-MR-spectroscopy. *Magn Reson Med* 1997; 37(4):484-493.
20. Rico-Sanz J, Hajnal JV, Thomas EL, Mierisova S, Ala-Korpela M, Bell JD. Intracellular and extracellular skeletal muscle triglyceride metabolism during alternating intensity exercise in humans. *J Physiol* 1998; 510 (Pt 2):615-622.
21. Schick F, Eismann B, Jung WI, Bongers H, Bunse M, Lutz O. Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Magn Reson Med* 1993; 29(2):158-167.
22. Bottomley PA. MR spectroscopy of the human heart: the status and the challenges. *Radiology* 1994; 191(3):593-612.
23. Paelinck BP, de Roos A, Bax JJ, Bosmans JM, van der Geest RJ, Dhondt D, Parizel PM, Vrints CJ, Lamb HJ. Feasibility of tissue magnetic resonance imaging: a pilot study in comparison with tissue Doppler imaging and invasive measurement. *J Am Coll Cardiol* 2005; 45(7):1109-1116.
24. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, Yki-Jarvinen H. Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 2005; 90(5):2804-2809.
25. Frayn KN. Adipose tissue as a buffer for daily lipid flux. *Diabetologia* 2002; 45(9):1201-1210.
26. Hwang JH, Stein DT, Barzilai N, Cui MH, Tonelli J, Kishore P, Hawkins M. Increased intrahepatic triglyceride is associated with peripheral insulin resistance: in vivo MR imaging and spectroscopy studies. *Am J Physiol Endocrinol Metab* 2007; 293(6):E1663-E1669.
27. Sparks JD, Sparks CE. Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes. *J Biol Chem* 1990; 265(15):8854-8862.
28. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc Natl Acad Sci U S A* 1999; 96(24):13656-13661.
29. Biddinger SB, Almind K, Miyazaki M, Kokkotou E, Ntambi JM, Kahn CR. Effects of diet and genetic background on sterol regulatory element-binding protein-1c, stearyl-CoA desaturase 1, and the development of the metabolic syndrome. *Diabetes* 2005; 54(5):1314-1323.
30. Soto PF, Herrero P, Kates AM, Dence CS, Ehsani AA, Davila-Roman V, Schechtman KB, Gropler RJ. Impact of aging on myocardial metabolic response to dobutamine. *Am J Physiol Heart Circ Physiol* 2003; 285(5):H2158-H2164.

31. Zhou L, Huang H, Yuan CL, Keung W, Lopaschuk GD, Stanley WC. Metabolic Response to an Acute Jump in Cardiac Workload: Effects on Malonyl-CoA, Mechanical Efficiency, and Fatty Acid Oxidation an Acute Jump in Cardiac Workload. *Am J Physiol Heart Circ Physiol* 2007.
32. Johnson NA, Stannard SR, Rowlands DS, Chapman PG, Thompson CH, O'Connor H, Sachinwalla T, Thompson MW. Effect of short-term starvation versus high-fat diet on intramyocellular triglyceride accumulation and insulin resistance in physically fit men. *Exp Physiol* 2006; 91(4):693-703.
33. Sidossis LS, Wolfe RR. Glucose and insulin-induced inhibition of fatty acid oxidation: the glucose-fatty acid cycle reversed. *Am J Physiol* 1996; 270(4 Pt 1):E733-E738.
34. Schrauwen P, Marken Lichtenbelt WD, Saris WH, Westerterp KR. Role of glycogen-lowering exercise in the change of fat oxidation in response to a high-fat diet. *Am J Physiol* 1997; 273(3 Pt 1):E623-E629.
35. Taegtmeyer H, McNulty P, Young ME. Adaptation and maladaptation of the heart in diabetes: Part I: general concepts. *Circulation* 2002; 105(14):1727-1733.
36. Leichman JG, Aguilar D, King TM, Vlada A, Reyes M, Taegtmeyer H. Association of plasma free fatty acids and left ventricular diastolic function in patients with clinically severe obesity. *Am J Clin Nutr* 2006; 84(2):336-341.
37. Fagan TC, Sawyer PR, Gourley LA, Lee JT, Gaffney TE. Postprandial alterations in hemodynamics and blood pressure in normal subjects. *Am J Cardiol* 1986; 58(7):636-641.
38. Kelbaek H. Acute effects of alcohol and food intake on cardiac performance. *Prog Cardiovasc Dis* 1990; 32(5):347-364.
39. Vatner SF, Patrick TA, Higgins CB, Franklin D. Regional circulatory adjustments to eating and digestion in conscious unrestrained primates. *J Appl Physiol* 1974; 36(5):524-529.
40. Schaffer JE. Lipotoxicity: when tissues overeat. *Curr Opin Lipidol* 2003; 14(3):281-287.

Part II

Diabetes Mellitus



Chapter 6

Short-term Flexibility of Myocardial Triglycerides and Diastolic Function in Patients with Type 2 Diabetes Mellitus

American Journal of Physiology - Endocrinology and Metabolism 2008; 295(3):E714-E718

S. Hammer
R.W. van der Meer
H.J. Lamb
H.H. de Boer
J.J. Bax
A. de Roos
J.A. Romijn
J.W.A. Smit



SUMMARY

Objectives: Short-term caloric restriction increases plasma levels of non-esterified fatty acids (NEFAs) and is associated with increased myocardial triglyceride (TG) content and decreased myocardial function in healthy subjects. The objective of this study was to evaluate whether this flexibility of myocardial TG stores and myocardial function is also present in patients with type 2 diabetes mellitus (DM2).

Materials and methods: Myocardial TG content and left ventricular (LV) ratio between the early (E) and atrial (A) diastolic filling phase (E/A) were determined using ^1H magnetic resonance (MR) spectroscopy and MR imaging respectively, before and after a 3-day very low-calorie diet (VLCD) in 11 patients with DM2. In addition, we studied patients after a 3-day VLCD combined with the anti-lipolytic drug acipimox.

Results: The VLCD induced myocardial TG accumulation (from mean \pm standard error $0.66 \pm 0.09\%$ [baseline] to $0.98 \pm 0.16\%$, $P = 0.028$), and a decrease in E/A ratio (from 1.00 ± 0.05 [baseline] to 0.90 ± 0.06 , $P = 0.002$). This was associated with increased plasma NEFA levels (from 0.57 ± 0.08 mmol/l [baseline] to 0.92 ± 0.12 , $P = 0.019$). After the VLCD with acipimox, myocardial TG content, diastolic function and plasma NEFA levels were similar to baseline values.

Conclusions: In patients with DM2 a VLCD increases myocardial TG content and is associated with a decrease in LV diastolic function. These effects were not observed when a VLCD was combined with acipimox, illustrating the physiologic flexibility of myocardial TG stores and myocardial function in patients with DM2.

INTRODUCTION

Type 2 diabetes mellitus (DM2) and obesity are associated with elevated plasma levels of non-esterified fatty acids (NEFAs) (1-3) and ectopic accumulation of triglycerides (TGs), reflected in hepatic (4;5) and cardiac steatosis (6;7). This accumulation of TGs in the heart appears not to be an epiphenomenon, as it is associated with altered structure and function of the heart. For instance, increased plasma levels of NEFAs are associated with increased myocardial TG content and left ventricular (LV) mass (8). In rodents, cardiac TG accumulation induces lipoapoptosis and is associated with cardiac dysfunction (9;10). In humans parameters of myocardial fatty acid metabolism are predictors of LV mass in hypertension and diastolic dysfunction (11), and increased myocardial TG content may precede the onset of profound systolic dysfunction in patients with obesity and/ or DM2 (6).

Myocardial TG content is not fixed as it is modulated by dietary interventions, at least in healthy subjects. We and others have previously shown that short-term caloric restriction is associated with myocardial TG accumulation (12-14) and a decrease in LV diastolic function in healthy volunteers (13;14). As patients with uncomplicated DM2 show alterations in myocardial high-energy phosphate metabolism, illustrating the changes in normal myocardial substrate handling (15), we hypothesize this flexibility is diminished in patients with respect to myocardial TG content and LV diastolic function. As short-term caloric restriction increases adipose tissue lipolysis, it is a suitable research tool to stress myocardial substrate selection, and study the effects on myocardial TG stores in relation to myocardial function.

The objective of the present study was therefore to assess the effects of short-term caloric restriction (3 days of a very low-calorie diet, VLCD) on myocardial TG content and function in patients with DM2 compared with control observations with no dietary restriction. Furthermore we assessed whether the effects of a VLCD could be prevented by co-administration of the anti-lipolytic drug acipimox (16;17). Acipimox has been extensively used to decrease plasma fatty acid levels, and it therefore serves as a model to study the effects of fatty acids during the interventions. To study the effects on tissue-specific distribution of ectopic TG pools in patients with DM2, hepatic TG content was also measured in the three conditions.

MATERIALS AND METHODS

Patients

We included 11 well-controlled male patients with DM2 (mean age \pm standard deviation 57.6 \pm 4.7 years) in this prospective, cross-over intervention study. The sample size was based on our previous experiments in healthy subjects, in which we observed a statistical power of 0.89 for detecting a mean increase in myocardial TG content of 0.23% in 10 subjects (13). Patient characteristics are shown in Table 6.1. All patients used stable doses of metformin and glimepiride for

at least 3 months. The use of other antidiabetic drugs was prohibited. In each patient a medical history was obtained and a physical examination was performed. Furthermore, an electrocardiogram (ECG) was made and dobutamine-stress echocardiography was performed. Exclusion criteria were: a history of/ or present cardiac disease (any abnormality on the electrocardiogram and/ or wall motion abnormalities at rest or during dobutamine-stress echocardiography to exclude ischemic heart disease), and any endocrine, hepatic or renal disease (standard laboratory and urinary tests). All patients signed informed consent prior to participation. The local ethics committee approved the study.

Study design

The study consisted of 3 conditions. To obtain baseline measurements subjects followed their normal diet, only alcohol was restricted for a 3-day period. Four days prior to baseline measurements glimepiride was discontinued to avoid episodes of hypoglycemia during the second and third intervention period.

On the second occasion the subjects were studied either after a 3-day VLCD alone (471 kcal/day, 50.2 g carbohydrates, 6.9 g fat of which 0.94 was saturated, Modifast Intensive, Nutrition & Santé Benelux, Breda, The Netherlands) or after a VLCD for 3 days plus acipimox (VLCD+acipimox). Acipimox (Nedios, ALTANA Pharma BV, Hoofddorp, The Netherlands) 250 mg was administered p.o. at 6-hour intervals during the last 24 hours of the 3-day period of VLCD (i.e. 4, 10, 16 and 24 hours prior to blood sampling). The sequence of the interventions was randomly assigned to minimize influences caused by the sequence of the interventions. Both VLCD studies were separated by a wash-out phase of at least 14 days. For all study occasions patients used their last meal or last sachet of Modifast 4 hours prior to blood sampling. Blood samples were taken just before MR evaluation. The duration of the VLCD diet was chosen based on our previous experiments in healthy subjects (14).

Determination of myocardial and hepatic triglyceride content

All magnetic resonance (MR) imaging and hydrogen 1 MR spectroscopy (¹H MRS) measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, The Netherlands) in the supine position in the afternoon. Single-voxel (8-ml) spectra were obtained using a body coil for radiofrequency transmission and a circular surface coil (Ø 17 cm) for signal receiving. The myocardial voxel was placed in the interventricular septum on four-chamber and short-axis images at end-systole, carefully avoiding contamination from epicardial fat. Data collection was double-triggered by using ECG triggering and navigator echoes for compensation of respiratory motion (18). In short, an echo time (TE) of 26 ms and a repetition time (TR) of 3000 ms were used. 1024 Data points were collected using a spectral width of 1000 Hz, averaged over 128 acquisitions. To detect the resonances of the lipids, the water signal was suppressed. Furthermore, in the same voxel, the water signal (with an echo time of 10000 ms) was measured to be used as an internal standard. For the liver we used the

same parameters, except for 64 averages for the suppressed spectrum. Spectra were analyzed in the time domain on the free-induction decays with Java-based MR user interface software and incorporated prior knowledge files (jMRUI version 2.2 (19)), as described earlier (18). Peak estimates of lipid resonances of myocardial and hepatic TGs at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (TGs/water $\times 100$).

Evaluation of myocardial systolic and diastolic function

During MR imaging, systolic and diastolic blood pressure and heart rate were measured at rest with an automatic device (Dinamap DPC100X, Freiburg, Germany). To assess systolic function, the heart was imaged from apex to base with 12 to 14 imaging levels in short-axis view using an ECG-triggered sensitivity-encoding balanced steady-state free precession sequence with breath-holds (1 for each slice). Imaging parameters included: field of view (FOV) = 400×320 mm, matrix size = 256×256 , slice thickness = 10 mm, slice gap = 0 mm, flip angle = 35° , TE = 1.7 ms and TR = 3.4 ms. The temporal resolution was 25 to 39 ms depending on the heart rate. Left ventricular (LV) end-diastolic and end-systolic contours were drawn using dedicated software (MASS[®] post processing software, Medis, Leiden, The Netherlands) as described earlier (20). LV ejection fraction (LVEF) and cardiac index (defined as cardiac output divided by body surface area) were calculated for assessment of systolic function. MR imaging is accurate to assess diastolic function as compared to Doppler-derived results (21). Therefore, we measured blood flow across the mitral valve with an ECG-gated gradient-echo sequence with velocity encoding (21;22). Imaging parameters were: TE = 4.8 ms, TR = 14 ms, flip angle = 20° , slice thickness = 8 mm, FOV = 350 mm^2 , matrix size = 256×256 pixels and the velocity encoding = 100 cm/s. Flow velocities in early diastole (E) and during the atrial contraction (A) were measured. Analyses were performed using dedicated analysis software (FLOW[®] analytical software package, Medis, Leiden, The Netherlands). The peak slope of the deceleration of the E (E deceleration) and the ratio between the peak filling rate of the E (E-PFR) and A (A-PFR) were calculated (E/A ratio) as measures for diastolic function. The E/A ratio is load dependent and therefore the load independent E_a was measured and an estimation of LV filling pressure was calculated (E/E_a , (23)).

Visceral fat quantification

Abdominal visceral fat depots were quantified by a turbo spin echo imaging protocol. Imaging parameters were: TE = 11 ms, TR = 168 ms, flip angle = 90° , slice thickness = 10 mm. At the level of the fifth lumbar vertebrae, three transverse images were acquired during a breath hold. In post processing visceral fat depots of the slices were calculated by converting the number of pixels to square centimeters multiplied by the thickness of the slices (using MASS[®] analytical software, Medis, Leiden, The Netherlands). The volume of the fat was calculated by summing the volumes of the individual slices.

Assays

Plasma concentrations of glucose, total cholesterol (TC) and TGs were measured on a fully automated P800 analyzer (Roche, Almere, The Netherlands). Insulin concentrations were measured on a Immulite 2500 random access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA, USA). Coefficients of variation for glucose, TC and TGs were < 2%, and were < 5% for insulin. Plasma NEFA concentrations were measured by a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis

Statistical analyses were performed using SPSS, version 14.0.2 (SPSS Inc., Chicago, Ill, USA). Statistical comparisons between the conditions were made by paired t-tests. *P*-values reflect data compared to baseline unless indicated otherwise. Data are shown as mean ± standard error. *P* < 0.05 (two-tailed) was considered significant.

RESULTS

Metabolic changes

Metabolic changes are listed in Table 6.1. Plasma NEFA levels increased after the VLCD compared to baseline (from 0.57 ± 0.08 mmol/l to 0.92 ± 0.12 , *P* = 0.019). In contrast, plasma NEFA levels after the VLCD+acipimox were unchanged compared to baseline (*P* = 0.142), but decreased significantly compared to VLCD alone (0.35 ± 0.12 mmol/l, *P* = 0.006).

Table 6.1. Metabolic parameters at baseline, after the diet and after the diet+acipimox.

Variable	Baseline	VLCD	VLCD+ acipimox
HbA1c (%)	6.0 ± 0.2		
Body mass index (kg/m ²)	26.6 ± 0.9	25.8 ± 0.8*	25.9 ± 0.9*
Glucose (mmol/l)	6.0 ± 0.4	5.2 ± 0.3‡	4.9 ± 0.2‡
Insulin (mU/l)	6.6 ± 1.3	3.3 ± 0.6‡	2.3 ± 0.2‡
Triglycerides (mmol/l)	2.2 ± 0.4	1.3 ± 0.2‡	1.0 ± 0.1‡
Non-esterified fatty acids (mmol/l)	0.57 ± 0.08	0.92 ± 0.12‡	0.35 ± 0.12
Total cholesterol (mmol/l)	4.5 ± 0.4	4.7 ± 0.2	4.5 ± 0.3
Visceral adipose tissue (ml)	375 ± 55	295 ± 35‡	303 ± 39
Hepatic triglyceride content (%)	16.4 ± 1.4	14.2 ± 1.0	14.2 ± 1.2

* *P* < 0.001, † *P* < 0.01, ‡ *P* < 0.05 vs baseline. Data are mean ± standard error.

VLCD = very low-calorie diet, HbA1c = glycated hemoglobin.

Myocardial and hepatic triglyceride content

Myocardial TG content at baseline was $0.66 \pm 0.09\%$. After the VLCD myocardial TG content increased to $0.98 \pm 0.16\%$ (*P* = 0.028), whereas it returned to baseline values after the VLCD+acipimox (to $0.73 \pm 0.15\%$, *P* = 0.485 compared to baseline (Figures 6.1 and 6.2A).

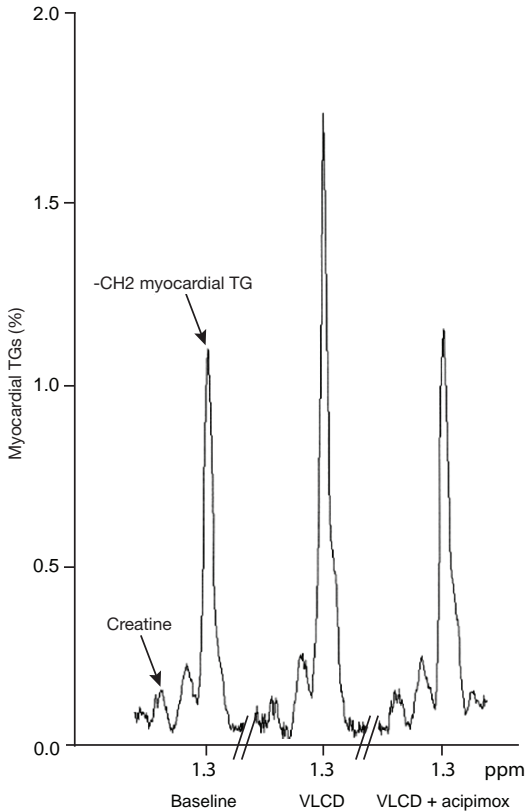


Figure 6.1. Myocardial ^1H magnetic resonance spectra.

^1H magnetic resonance spectra of one patient at baseline, after the VLCD and after the VLCD with acipimox (relative to the unsuppressed water).

VLCD = very low-calorie diet, TG = triglyceride, CH_2 = methyl groups of myocardial lipid content

Moreover, myocardial TG content was decreased after the VLCD+acipimox compared to the VLCD alone ($P = 0.044$). Myocardial ^1HMR spectra could not be obtained in 1 patient due to technical problems. Hepatic TG content did not change significantly upon both interventions (Table 6.1).

Myocardial function

Systolic function was unaffected by the dietary interventions (Table 6.2). Diastolic blood pressure was equally decreased after the VLCD and after the VLCD+acipimox. E deceleration decreased significantly from $3.6 \pm 0.2 \text{ ml/s}^2 \times 10^{-3}$ to $2.9 \pm 0.2 \text{ ml/s}^2 \times 10^{-3}$ after the VLCD compared to baseline ($P = 0.004$, Figure 6.2B). E/A peak ratio decreased from 1.00 ± 0.05 to 0.90 ± 0.06 after the VLCD compared to baseline ($P = 0.002$, Figure 6.2C). In contrast, after the VLCD+acipimox the E deceleration ($3.3 \pm 0.2 \text{ ml/s}^2 \times 10^{-3}$) and the E/A peak ratio (0.98 ± 0.06) were unchanged compared to baseline ($P = 0.270$ and $P = 0.590$ respectively, Figures 6.2B and 6.2C).

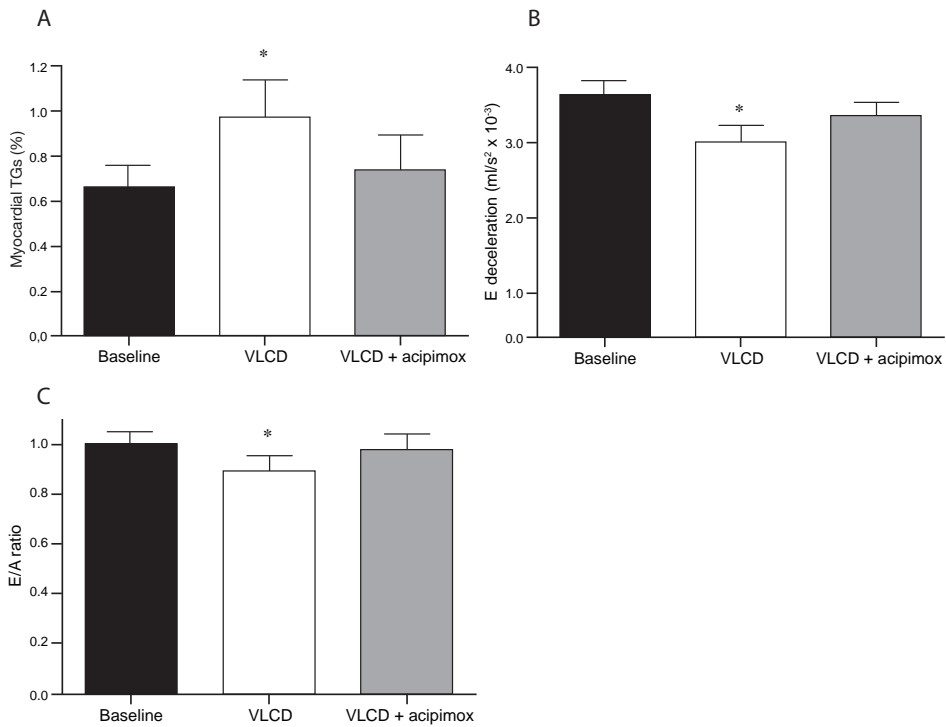


Figure 6.2.

Myocardial triglyceride (TG) content is significantly increased after the very low-calorie diet (VLCD) and unchanged after administration of acipimox during the VLCD (A) associated with changes in diastolic E deceleration (B) and E/A ratio (C).

E = early diastolic wave, A = atrial diastolic wave.

Bars represent mean + standard error, * $P < 0.05$.

DISCUSSION

This study shows that in well-controlled patients with DM2 short-term caloric restriction increases myocardial TG content by ~48%. This increase in myocardial TG content is accompanied by a decrease in myocardial diastolic function. A VLCD combined with acipimox has no effects on myocardial TG content and myocardial function. These data demonstrate the flexibility of the diabetic myocardium during short-term caloric restriction.

In the present study we show that a physiological increase in circulating NEFA levels is accompanied by increased myocardial uptake and re-esterification of fatty acids in patients with DM2. As patients with DM2 have altered myocardial metabolism (15), the short-term flexibility of myocardial TG stores is remarkable during caloric restriction. The patients with DM2 in our cohort were under good glycemic control and only moderately obese. Therefore, in more

Table 6.2. Parameters of myocardial function at baseline, after the diet and after the diet+acipimox.

Variable	Baseline	VLCD	VLCD+ acipimox
Systolic blood pressure (mmHg)	115 ± 5	114 ± 6	110 ± 5
Diastolic blood pressure (mmHg)	73 ± 2	69 ± 3‡	68 ± 2†
Heart rate (bpm)	64 ± 3	63 ± 2	64 ± 3
LVEF (%)	55 ± 1	58 ± 2	55 ± 2
Cardiac index (l/min/m ²)	2.8 ± 0.1	2.7 ± 0.1	2.8 ± 0.2
E peak filling rate (ml/s)	415 ± 27	342 ± 30‡	380 ± 19
A peak filling rate (ml/s)	415 ± 16	394 ± 33	395 ± 17
E/Ea	8.5 ± 0.8	9.1 ± 1.0	9.9 ± 0.9

† $P < 0.01$, ‡ $P < 0.05$ vs baseline. Data are mean ± standard error.

VLCD = very low-calorie diet, E = early diastolic wave, A = atrial diastolic wave, LVEF = left ventricular ejection fraction, E/Ea = estimated left ventricular filling pressure.

severe obesity and / or poor glycemic control the effects of a short term VLCD may have different effects. Moreover, future studies should address the differences in the response to a VLCD between patients with DM2 and healthy subjects matched for body mass index an age, as they influence myocardial TG content and diastolic function (24).

During caloric restriction, elevated plasma levels of NEFAs increase hepatic very low-density lipoprotein TG production (25), which is an important supplier of fatty acids to the myocardium (26;27). During the VLCD with acipimox no changes were observed in myocardial TG content. This supports the notion that there is a relationship between increased fatty acid fluxes from the adipose tissue and myocardial TG stores, although we can not exclude the possibility of a direct effect of acipimox. This appears however unlikely, as the anti-lipolytic effects would lead to an increase, rather than a decrease in myocardial TG content. Furthermore, as acipimox was added in a hypocaloric situation, its effects underline the potential of the heart to switch substrate metabolism, even in a situation of increased fatty acid dependence.

We hypothesize that the decrease in visceral adipose tissue contributes to the increased levels of circulating fatty acids and possibly to the myocardial TG accumulation after the VLCD.

Although our results can not be extrapolated to the long-term implications of chronic (hyper- or eucaloric) exposure to elevated NEFA levels in obesity and DM2, the data suggest that in general, interventions aiming to decrease plasma lipids or pathological elevated myocardial TG content seem promising. Accordingly, it was recently shown in insulin treated DM2 patients that adding pioglitazone to insulin therapy decreased myocardial TG stores (28).

We used MR velocity mapping to assess blood flow across the mitral valve. E-PFR, A-PFR and their ratio (E/A) obtained with MR velocity mapping are measures which are highly correlated to the same parameters when obtained with echocardiography (8). Early deceleration is an MR reflection of the early deceleration time which is used in echocardiography. Therefore, the observed changes in parameters of diastolic function as observed in the present study would be observed likewise when the study was performed with ultrasound. The flow measurements

can be affected by changes in preload. Furthermore, systemic effects of acipimox include vasodilatation (29). However, MR estimated LV filling pressures were unaffected after the interventions and therefore, the preload was unchanged. Accordingly, the observed changes in diastolic function are likely to be caused by changes in elastic recoil of the LV. This extends the previously documented relation between plasma NEFA levels and diastolic function in obesity (30). Furthermore, the results are in accordance with results obtained in animal models of obesity, documenting the association between myocardial TG accumulation and myocardial function (9;10;31). Alternatively, caloric restriction and increased plasma NEFA levels may change myocardial calcium handling and thereby influence diastolic function (32-34). A causal relationship between myocardial TG stores and diastolic function can therefore not be derived from the present data.

Although acipimox is not suitable for long-term administration regarding the rebound effects on plasma levels of NEFAs (35), the present data, however, warrant future studies in a clinical setting to study the effects of therapeutic interventions on myocardial TG content and myocardial function. We believe that the differences observed in diastolic function are too small to reflect clinically relevant diastolic dysfunction but merely reflect the interaction between short-term metabolic fluctuations and diastolic function. These mechanisms may be relevant for the pathogenesis of cardiac dysfunction in patients with DM2 (6), although this can not be concluded from the present data.

We also studied the effects of a VLCD on hepatic TG content in the patients with DM2. Hepatic TG content was 6-7 fold increased at baseline compared to our previous observations in healthy subjects (13;14). In contrast to myocardial TG content, hepatic TG content was not significantly affected by the short-term VLCD, either with or without acipimox. We postulate the duration of the VLCD is too short to induce reductions in hepatic TG content in subjects with DM2 with hepatic steatosis, since a prolonged VLCD in obese subjects with DM2 induces major reductions in hepatic TG content (36). Nonetheless, the present study documents that the heart and the liver have a differential response to short-term caloric restriction in patients with DM2.

Our study has some limitations. Although the study was powered to detect relevant differences in the patient and patients are their own controls, the number of patients in the study is still limited. Second, we evaluated the effects of a VLCD only with MR imaging and ¹H MRS. It would however be interesting to combine data on myocardial TG content with data obtained using positron emission tomography (PET) on fatty acid and glucose uptake because the balance between the use of glucose and plasma fatty acids determines myocardial energy supply and the cardiac function. Unfortunately, these data could not be obtained in the present study, since a PET scanner is unavailable at our institution.

CONCLUSIONS

In conclusion, in patients with well-controlled DM2 a VLCD increases myocardial TG content and is associated with a decrease in LV diastolic function. These effects were not observed when a VLCD was combined with acipimox. These data illustrate physiologic flexibility of myocardial TG stores and myocardial function in patients with DM2.

REFERENCES

1. Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. *Diabetes* 1988; 37(8):1020-1024.
2. Lopaschuk GD, Folmes CD, Stanley WC. Cardiac energy metabolism in obesity. *Circ Res* 2007; 101(4): 335-347.
3. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. *Cardiovasc Res* 1997; 34(1):25-33.
4. Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, Buzzigoli E, Sironi AM, Cersosimo E, Ferrannini E, DeFronzo RA. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 2007; 133(2):496-506.
5. Thomas EL, Hamilton G, Patel N, O'Dwyer R, Dore CJ, Goldin RD, Bell JD, Taylor-Robinson SD. Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut* 2005; 54(1):122-127.
6. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007; 116(10):1170-1175.
7. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbiqve D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.
8. Kankaanpaa M, Lehto HR, Parkka JP, Komu M, Viljanen A, Ferrannini E, Knuuti J, Nuutila P, Parkkola R, Iozzo P. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006; 91(11):4689-4695.
9. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000; 97(4):1784-1789.
10. Christoffersen C, Bollano E, Lindegaard ML, Bartels ED, Goetze JP, Andersen CB, Nielsen LB. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003; 144(8): 3483-3490.
11. de Las Fuentes L, Herrero P, Peterson LR, Kelly DP, Gropler RJ, Davila-Roman VG. Myocardial fatty acid metabolism: independent predictor of left ventricular mass in hypertensive heart disease. *Hypertension* 2003; 41(1):83-87.
12. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *Am J Physiol Endocrinol Metab* 2005; 289(5):E935-E939.
13. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW, Romijn JA. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008; 93(2):497-503.
14. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007; 56(12):2849-2853.

15. Diamant M, Lamb HJ, Groeneveld Y, Endert EL, Smit JW, Bax JJ, Romijn JA, de Roos A, Radder JK. Diastolic dysfunction is associated with altered myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2 diabetes mellitus. *J Am Coll Cardiol* 2003; 42(2):328-335.
16. Santomauro AT, Boden G, Silva ME, Rocha DM, Santos RF, Ursich MJ, Strassmann PG, Wajchenberg BL. Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. *Diabetes* 1999; 48(9):1836-1841.
17. Worm D, Vinten J, Vaag A, Henriksen JE, Beck-Nielsen H. The nicotinic acid analogue acipimox increases plasma leptin and decreases free fatty acids in type 2 diabetic patients. *Eur J Endocrinol* 2000; 143(3): 389-395.
18. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of myocardial triglyceride content: reproducibility of 1H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007; 245(1): 251-257.
19. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129(1):35-43.
20. Pattynama PM, Lamb HJ, van der Velde EA, van der Wall EE, de Roos A. Left ventricular measurements with cine and spin-echo MR imaging: a study of reproducibility with variance component analysis. *Radiology* 1993; 187(1):261-268.
21. Hartiala JJ, Mostbeck GH, Foster E, Fujita N, Dulce MC, Chazouilleres AF, Higgins CB. Velocity-encoded cine MRI in the evaluation of left ventricular diastolic function: measurement of mitral valve and pulmonary vein flow velocities and flow volume across the mitral valve. *Am Heart J* 1993; 125(4): 1054-1066.
22. Lamb HJ, Beyerbach HP, van der Laarse A, Stoel BC, Doornbos J, van der Wall EE, de Roos A. Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. *Circulation* 1999; 99(17):2261-2267.
23. Paelinck BP, de Roos A, Bax JJ, Bosmans JM, van der Geest RJ, Dhondt D, Parizel PM, Vrints CJ, Lamb HJ. Feasibility of tissue magnetic resonance imaging: a pilot study in comparison with tissue Doppler imaging and invasive measurement. *J Am Coll Cardiol* 2005; 45(7):1109-1116.
24. van der Meer RW, Rijzewijk LJ, Diamant M, Hammer S, Schar M, Bax JJ, Smit JWA, Romijn JA, de Roos A, Lamb HJ. The ageing male heart: myocardial triglyceride content as independent predictor of diastolic function. *Eur Heart J* 2008; 29(12):1516-22.
25. Lewis GF, Uffelman KD, Szeto LW, Weller B, Steiner G. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J Clin Invest* 1995; 95(1): 158-166.
26. Goudriaan JR, Tacke PJ, Dahlmans VE, Gijbels MJ, van Dijk KW, Havekes LM, Jong MC. Protection from obesity in mice lacking the VLDL receptor. *Arterioscler Thromb Vasc Biol* 2001; 21(9):1488-1493.
27. Sakai J, Hoshino A, Takahashi S, Miura Y, Ishii H, Suzuki H, Kawarabayasi Y, Yamamoto T. Structure, chromosome location, and expression of the human very low density lipoprotein receptor gene. *J Biol Chem* 1994; 269(3):2173-2182.
28. Zib I, Jacob AN, Lingvay I, Salinas K, McGavock JM, Raskin P, Szczepaniak LS. Effect of pioglitazone therapy on myocardial and hepatic steatosis in insulin-treated patients with type 2 diabetes. *J Investig Med* 2007; 55(5):230-236.

29. Pontiroli AE, Fattor B, Pozza G, Pianezzola E, Strolin BM, Musatti L. Acipimox-induced facial skin flush: frequency, thermographic evaluation and relationship to plasma acipimox level. *Eur J Clin Pharmacol* 1992; 43(2):145-148.
30. Leichman JG, Aguilar D, King TM, Vlada A, Reyes M, Taegtmeier H. Association of plasma free fatty acids and left ventricular diastolic function in patients with clinically severe obesity. *Am J Clin Nutr* 2006; 84(2):336-341.
31. Sharma S, Adroge JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeier H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* 2004; 18(14):1692-1700.
32. Han X, Cheng H, Mancuso DJ, Gross RW. Caloric restriction results in phospholipid depletion, membrane remodeling, and triacylglycerol accumulation in murine myocardium. *Biochemistry* 2004; 43(49):15584-15594.
33. Huang JM, Xian H, Bacaner M. Long-chain fatty acids activate calcium channels in ventricular myocytes. *Proc Natl Acad Sci U S A* 1992; 89(14):6452-6456.
34. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. *Circulation* 2002; 105(12):1503-1508.
35. Vaag AA, Beck-Nielsen H. Effects of prolonged Acipimox treatment on glucose and lipid metabolism and on in vivo insulin sensitivity in patients with non-insulin dependent diabetes mellitus. *Acta Endocrinol (Copenh)* 1992; 127(4):344-350.
36. Lewis MC, Phillips ML, Slavotinek JP, Kow L, Thompson CH, Toouli J. Change in liver size and fat content after treatment with Optifast very low calorie diet. *Obes Surg* 2006; 16(6):697-701.

Chapter 7

Prolonged Caloric Restriction in Obese Patients with Type 2 Diabetes Mellitus Decreases Myocardial Triglyceride Content and Improves Myocardial Function

Journal of the American College of Cardiology 2008; 52(12):1006-1012

S. Hammer
M. Snel
H.J. Lamb
I.M. Jazet
R.W. van der Meer
H. Pijl
A.E. Meinders
J.A. Romijn
A. de Roos
J.W.A. Smit

SUMMARY

Objectives: Myocardial triglyceride (TG) content is increased in patients with type 2 diabetes mellitus (DM2) and may reflect altered myocardial function. The purpose of this study was to assess the effects of prolonged caloric restriction in obese patients with DM2 on myocardial TG content and myocardial function.

Materials and methods: Myocardial TG content (^1H magnetic resonance (MR) spectroscopy), left ventricular myocardial function (MR imaging), plasma glycated hemoglobin (HbA1c) and body mass index (BMI), were measured in twelve obese, insulin-treated DM2 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: BMI decreased from mean \pm standard error 35.6 ± 1.2 (baseline) to 27.5 ± 1.3 kg/m² (after the VLCD, $P < 0.001$), associated with an improvement in HbA1c from 7.9 ± 0.4 (baseline) to $6.3 \pm 0.3\%$ (after the VLCD, $P = 0.006$). Myocardial TG content decreased from 0.88 ± 0.12 to $0.64 \pm 0.14\%$, respectively ($P = 0.019$), 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 patients with DM2 decreases BMI and improves glucoregulation associated with decreased myocardial TG content and improved diastolic function. Therefore, myocardial TG stores in obese patients with DM2 are flexible and amendable to therapeutic intervention by caloric restriction.

INTRODUCTION

Obesity and type 2 diabetes mellitus (DM2) are associated with increased deposition of triglycerides (TGs) in non-adipose tissue, like 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 fatty acid derivatives, such as fatty acyl-coenzyme A and diacylglycerol (7-9). In humans myocardial TG content can be measured non-invasively *in vivo* by hydrogen 1 magnetic resonance spectroscopy (¹HMRS) (10-14). These 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, whereas a single high-fat meal does not affect myocardial TG stores (12;15). 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 DM2. However, the effects of caloric restriction on myocardial TG content have not been studied in these patients.

Therefore, the primary aim of the present study was to evaluate the effects of prolonged caloric restriction by using a very low-calorie diet (VLCD) in obese patients with DM2 on myocardial TG content and left ventricular myocardial function in relation to metabolic regulation. In addition, DM2 is associated with ectopic deposition of TGs in the liver (17;18). To assess the tissue-specific effects of caloric restriction we also assessed liver TG content in these obese patients with DM2.

MATERIALS AND METHODS

Patients

We studied 12 obese (mean \pm standard error: body mass index (BMI) 35.6 ± 1.2 kg/m²) patients with DM2 (7 men, 5 women). The mean duration of DM2 was 9.6 ± 1.4 years. The age was 48.3 ± 2.8 years. Patients were recruited from the outpatient clinic. All subjects used insulin treatment (mean dosage 93 ± 21 units/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, 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 < twofold increase after glucagon stimulation (1.0 mg iv.). This criterion was included since we documented in a previous study that preservation of the capacity of beta cells to secrete insulin predicts a favourable metabolic response to a VLCD in obese patients with DM2 (19;20). Body weight was stable for at least three 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 prior to 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 three sachets Modifast per day (450 kcal/day, Nutrition & Santé, Antwerpen, Belgium), providing about 50 g protein, 50-60 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 hours 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 hours after the last meal) were repeated. Blood samples were taken after an overnight fast.

¹H magnetic resonance spectroscopy of the heart and the liver

All measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, The Netherlands) in the supine position. For ¹H 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 four-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 artefacts (14). For the liver, voxel sites were matched at the study occasions (by using the twelfth 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 a repetition time (TR) of at least 3000 ms and an echo time (TE) of 26 ms. 1024 Data points were collected over a 1000-Hz spectral width. Furthermore, unsuppressed spectra with 4 averages were acquired in the same voxel, using the same parameters except for a repetition time of 10000 ms. Spectra were analyzed in the time domain, using the advanced magnetic resonance algorithm in the Java-based MR

user interface software (jMRUI version 2.2 (21)), as described earlier (14). Peak estimates of lipid resonances of myocardial and hepatic TGs at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (%TGs, TGs/water \times 100) and used in further analysis.

Left ventricular function

Imaging was performed in a single session together with ^1H MRS 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 precession 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° , TE = 1.7 ms and TR = 3.4 ms. Temporal resolution was 25 to 39 ms (depending on the heart rate). End-diastolic and end-systolic images were identified on all slices and dedicated post processing software (MASS[®], Medis, Leiden, The Netherlands) was used to quantify LV ejection fraction, LV mass, cardiac output (CO) and stroke 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 assess 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: TE = 4.8 ms, TR = 14 ms, flip angle = 20° , slice thickness = 8 mm, field of view = 350 mm^2 , 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 (Medis, Leiden, The Netherlands). Furthermore, the down slope of the E (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 on an Immulite 2500 random access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA, USA). Coefficients of variation were $< 2\%$ for glucose and $< 5\%$ for insulin. Plasma levels of non-esterified fatty acids (NEFAs) were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany). Glycated hemoglobin (HbA1c) levels were measured with an HPLC system (Variant, Biomed, Hercules, CA, USA). Leptin and adiponectin were measured with a radioimmunoassay from Linco Research (St. Charles, MO, USA), with coefficients of variation ranging from 3.0 to 5.1% for leptin and 7 to 9% for adiponectin, and a sensitivity of $0.5 \mu\text{g/l}$. The high-sensitive C-reactive protein ELISA came from DSL, Webster, Texas, USA. The sensitivity was 0.03 mg/l and the coefficient of variation was between 3 and 6%.

Statistical analysis

All statistical analyses were performed with SPSS, version 14.0 (SPSS Inc., Chicago, Ill, USA). Statistical comparisons between baseline measurements and measurements after prolonged caloric restriction were made by paired t-tests. Data are shown as mean \pm standard error. $P < 0.05$ was considered to reflect significant differences.

RESULTS

Metabolic parameters

Caloric restriction reduced BMI from 35.6 ± 1.2 at baseline to 27.5 ± 1.3 kg/m² after the intervention period ($P < 0.001$, Figure 7.1). Metabolic parameters before and after prolonged caloric restriction are shown in Table 7.1 and Figure 7.2. After 16 weeks of caloric restriction, glycaemic control was significantly improved, as 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 $6.3 \pm 0.3\%$ at baseline and after prolonged caloric restriction respectively, $P = 0.006$.

Plasma NEFA 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 7.2A). Furthermore, liver enzymes, plasma total cholesterol and plasma TG levels were significantly decreased after the VLCD compared to baseline (Table 7.1, Figure 7.2).

Myocardial and hepatic triglyceride content

Typical myocardial ¹HMR spectra of a patient at baseline and after caloric restriction are shown in Figure 7.3. Myocardial TG content decreased from 0.88 ± 0.12 (baseline) to $0.64 \pm 0.14\%$ (after the VLCD, $P = 0.019$, Figure 7.2C, based on $n = 11$ successful myocardial spectral measurements).

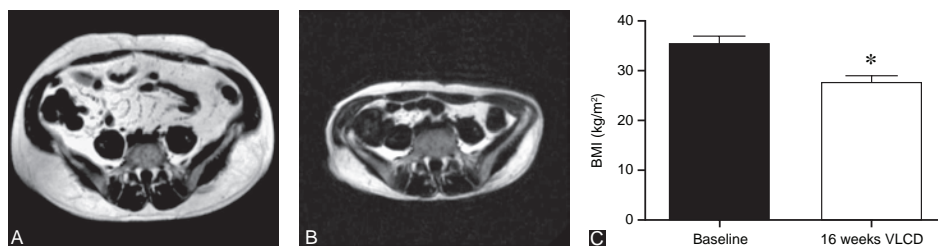


Figure 7.1. Fat stores and body mass index.

Example of a transversal slice at the level of the fifth lumbar vertebrae showing visceral and subcutaneous fat depots, illustrating the effects of 16 weeks of caloric restriction in the same patient (A and B). Body mass index (BMI) is decreased after prolonged caloric restriction (C).

Bars represent mean + standard error, * $P < 0.001$.

Table 7.1. Metabolic response to 16 weeks of caloric restriction in obese patients with type 2 diabetes mellitus.

Fasting plasma concentrations	Baseline	After 16 weeks of caloric restriction
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 ^a	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‡
Non-esterified 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 (mg/l)	5.2 ± 0.7	7.8 ± 1.1†
hs-CRP (mg/l)	18.5 ± 4.2	7.5 ± 2.0†

* $P < 0.001$, † $P < 0.01$ and ‡ $P < 0.05$ vs baseline. Data are mean ± standard error.

HbA1c = glycated hemoglobin, AST = aspartate aminotransferase, ALT = alanine aminotransferase, γGT = gamma-glutamyl transferase, TG = triglyceride, hs-CRP = high-sensitive C-reactive protein, ^a insulin (short acting) was stopped >12 hours.

Concomitantly, hepatic TG content decreased from 21.2 ± 4.2 to 3.0 ± 0.9%, respectively ($P < 0.001$, Figure 7.2D).

Myocardial systolic and diastolic function

Systolic blood pressure decreased from 144 ± 8 to 118 ± 6 mmHg at baseline and after substantial weight loss respectively ($P < 0.001$). Diastolic blood pressure decreased from 81 ± 2 at baseline to 71 ± 2 mmHg after weight loss ($P < 0.001$). Heart rate was significantly decreased after substantial weight loss (Table 7.2).

During caloric restriction myocardial function improved. Cardiac output decreased significantly from 7971 ± 601 ml/min at baseline to 6508 ± 401 ml/min after prolonged caloric restriction ($P = 0.001$). Furthermore, LV mass was significantly decreased as well (from 118 ± 7 to 99 ± 6 g respectively, $P < 0.001$, Figure 7.4A). 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 7.4B).

DISCUSSION

This study demonstrates 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 DM2. The data prove that myocardial TG stores in obese patients with DM2 are flexible and amendable to therapeutic intervention by caloric restriction.

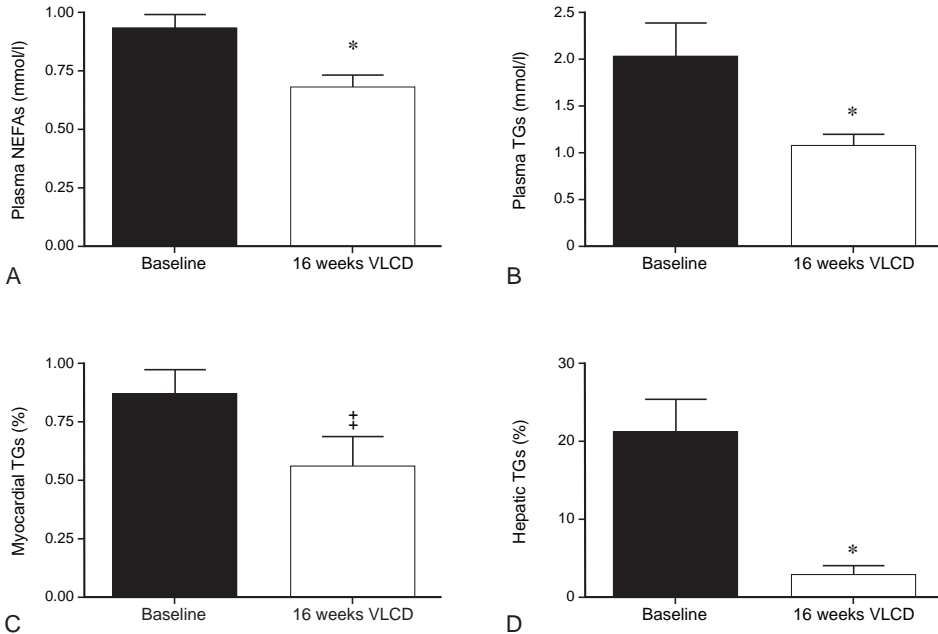


Figure 7.2. Metabolic changes in at baseline and after 16 weeks caloric restriction.

Changes in plasma NEFAs (A), plasma TGs (B), and myocardial (C) and hepatic (D) TGs upon prolonged caloric restriction.

VLCD = very low-calorie diet, TGs = triglycerides, NEFAs = non-esterified fatty acids.

Bars represent mean + standard error, * $P < 0.001$, ‡ $P < 0.05$.

Myocardial TG accumulation is the net result of excessive fatty acid uptake in relation to oxidative fatty acid 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, which was higher in subjects suffering from obesity and DM2 (25). Recently, McGavock *et al.* documented that in patients with DM2 myocardial TG content is increased, and suggested that myocardial TG accumulation precedes overt changes in systolic function (2). Therefore, myocardial TG content may be an interesting marker for the risk of non-ischemic heart disease, and a potential surrogate marker to assess 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 upon caloric restriction in the present study can not merely be ascribed to the decreased myocardial TG stores, because there were also major alterations in other factors that affect cardiac mass and function like BMI, and blood pressure.

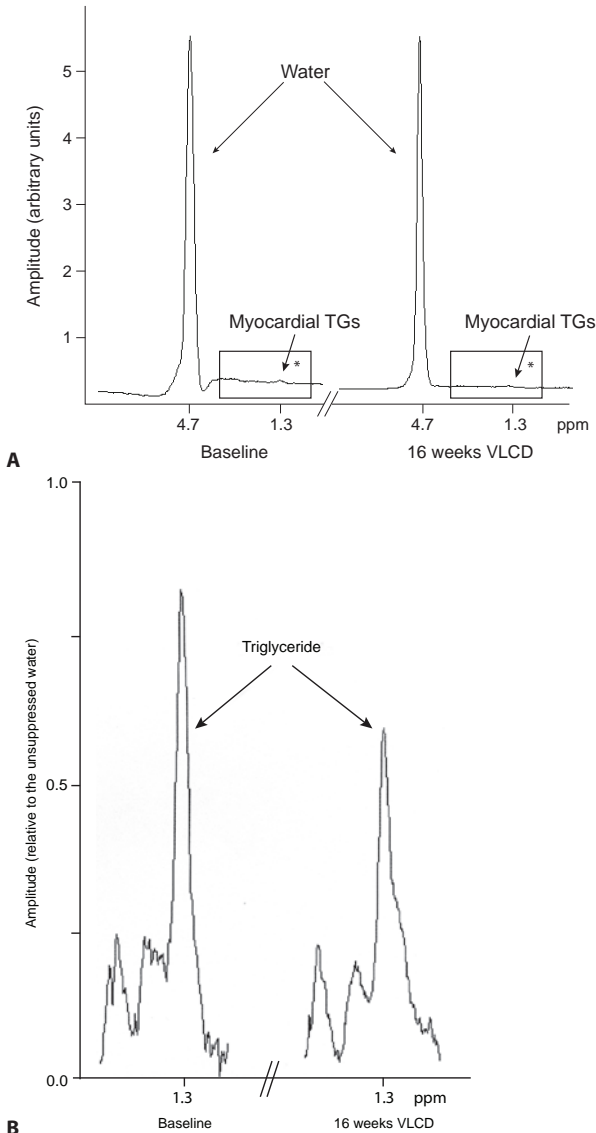


Figure 7.3. Myocardial ^1H magnetic resonance spectra.

Typical unsuppressed ^1H magnetic resonance spectra of the same patient at baseline and after 16 weeks of caloric restriction (A). The starred boxes indicate the part of the spectrum where the myocardial triglycerides (TGs) resonate, of which the suppressed spectra are shown in B.

VLCD = very low-calorie diet, ppm = parts per million.

Others reported beneficial effects of weight loss on cardiac function after bariatric surgery (27) or a VLCD (28). Moreover, we found a decline in heart rate, which is beneficial as 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

Table 7.2. Intra-individual effects of 16 weeks of caloric restriction on systolic and diastolic function in obese patients with type 2 diabetes mellitus.

	Baseline	After 16 weeks of caloric restriction
Systolic blood pressure (mmHg)	144 ± 8	118 ± 6*
Diastolic blood pressure (mmHg)	81 ± 2	71 ± 2*
Heart rate (bpm)	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

* $P < 0.001$, † $P < 0.01$ and ‡ $P < 0.05$ vs baseline. Data are mean ± standard error.

LVEF = left ventricular ejection fraction, LV = left ventricular, ED = end-diastolic, ES = end-systolic, E = early filling phase, A = atrial filling phase, E/Ea = estimated LV filling pressure.

data (30). 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). 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). Due to the dramatic changes in body size, some of the indexed values for LV dimensions were changed after the intervention period. LV mass index decreased, whereas end-diastolic index was increased.

The decrease in LV mass can directly influence left ventricular filling pressures, and, consequently, parameters of left ventricular 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 E/A ratio may be improved elastic properties of the LV, in line with results from animal models, documenting the relation between myocardial TG accumulation and myocardial function (5;6). One of the alternative mechanisms may be that changes in plasma fatty acids 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).

Our study has some limitations. First, the study is descriptive and does not establish a causal relation 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 control and the magnitude of the metabolic and functional changes is illustrative as it indicates dynamic features of myocardial TG and diastolic function.

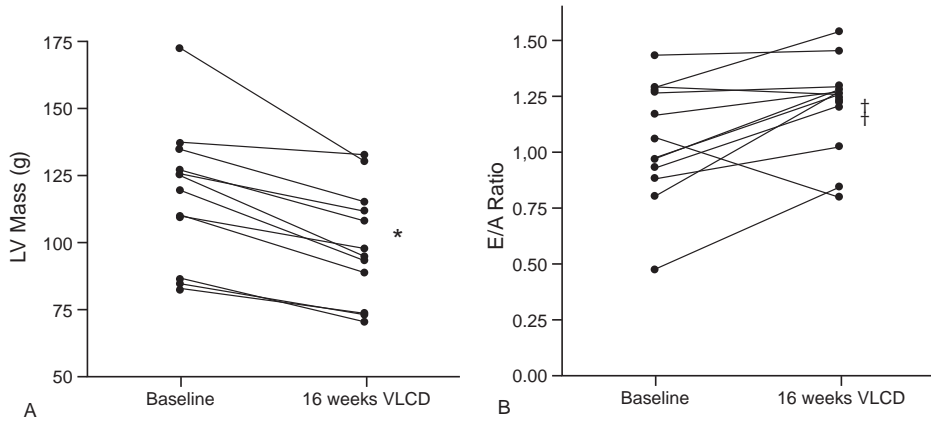


Figure 7.4. Changes in myocardial function.

Intra-individual changes in left ventricular (LV) mass (A) and the ratio between the early filling phase and the atrial filling phase (E/A ratio) upon progressive caloric restriction (B), * $P < 0.001$, † $P < 0.05$.

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 non-adipose tissues, including liver and the heart.

CONCLUSIONS

In conclusion, prolonged caloric restriction in obese patients with DM2 decreases BMI and improves glucoregulation associated with decreased myocardial TG content and improved LV diastolic function. Therefore, myocardial TG stores in obese patients with DM2 are flexible and amendable to therapeutic intervention by caloric restriction.

REFERENCES

1. Machann J, Haring H, Schick F, Stumvoll M. Intramyocellular lipids and insulin resistance. *Diabetes Obes Metab* 2004; 6(4):239-248.
2. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007; 116(10):1170-1175.
3. Tushuizen ME, Bunck MC, Pouwels PJ, Bontemps S, van Waesberghe JH, Schindhelm RK, Mari A, Heine RJ, Diamant M. Pancreatic fat content and beta-cell function in men with and without type 2 diabetes. *Diabetes Care* 2007; 30(11):2916-2921.
4. Thomas EL, Hamilton G, Patel N, O'Dwyer R, Dore CJ, Goldin RD, Bell JD, Taylor-Robinson SD. Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut* 2005; 54(1):122-127.
5. Christoffersen C, Bollano E, Lindegaard ML, Bartels ED, Goetze JP, Andersen CB, Nielsen LB. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003; 144(8): 3483-3490.
6. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000; 97(4):1784-1789.
7. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 1994; 91(23):10878-10882.
8. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A* 1998; 95(5):2498-2502.
9. Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J* 2001; 15(2):312-321.
10. den Hollander JA, Evanochko WT, Pohost GM. Observation of cardiac lipids in humans by localized 1H magnetic resonance spectroscopic imaging. *Magn Reson Med* 1994; 32(2):175-180.
11. Felblinger J, Jung B, Slotboom J, Boesch C, Kreis R. Methods and reproducibility of cardiac/respiratory double-triggered (1)H-MR spectroscopy of the human heart. *Magn Reson Med* 1999; 42(5):903-910.
12. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *Am J Physiol Endocrinol Metab* 2005; 289(5):E935-E939.
13. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbiqve D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.
14. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of myocardial triglyceride content: reproducibility of 1H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007; 245(1): 251-257.

15. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW, Romijn JA. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008; 93(2):497-503.
16. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007; 56(12):2849-2853.
17. Ishii M, Yoshioka Y, Ishida W, Kaneko Y, Fujiwara F, Taneichi H, Miura M, Toshihiro M, Takebe N, Iwai M, Suzuki K, Satoh J. Liver fat content measured by magnetic resonance spectroscopy at 3.0 tesla independently correlates with plasminogen activator inhibitor-1 and body mass index in type 2 diabetic subjects. *Tohoku J Exp Med* 2005; 206(1):23-30.
18. Teranishi T, Ohara T, Maeda K, Zenibayashi M, Kouyama K, Hirota Y, Kawamitsu H, Fujii M, Sugimura K, Kasuga M. Effects of pioglitazone and metformin on intracellular lipid content in liver and skeletal muscle of individuals with type 2 diabetes mellitus. *Metabolism* 2007; 56(10):1418-1424.
19. Jazet IM, Pijl H, Frolich M, Schoemaker RC, Meinders AE. Factors predicting the blood glucose lowering effect of a 30-day very low calorie diet in obese Type 2 diabetic patients. *Diabet Med* 2005; 22(1): 52-55.
20. Jazet IM, Schaart G, Gastaldelli A, Ferrannini E, Hesselink MK, Schrauwen P, Romijn JA, Maassen JA, Pijl H, Ouwens DM, Meinders AE. Loss of 50% of excess weight using a very low energy diet improves insulin-stimulated glucose disposal and skeletal muscle insulin signalling in obese insulin-treated type 2 diabetic patients. *Diabetologia* 2008; 51(2):309-319.
21. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129(1):35-43.
22. Pattynama PM, Lamb HJ, van der Velde EA, van der Wall EE, de Roos A. Left ventricular measurements with cine and spin-echo MR imaging: a study of reproducibility with variance component analysis. *Radiology* 1993; 187(1):261-268.
23. Hartiala JJ, Mostbeck GH, Foster E, Fujita N, Dulce MC, Chazouilleres AF, Higgins CB. Velocity-encoded cine MRI in the evaluation of left ventricular diastolic function: measurement of mitral valve and pulmonary vein flow velocities and flow volume across the mitral valve. *Am Heart J* 1993; 125(4): 1054-1066.
24. Paelinck BP, de Roos A, Bax JJ, Bosmans JM, van der Geest RJ, Dhondt D, Parizel PM, Vrints CJ, Lamb HJ. Feasibility of tissue magnetic resonance imaging: a pilot study in comparison with tissue Doppler imaging and invasive measurement. *J Am Coll Cardiol* 2005; 45(7):1109-1116.
25. Sharma S, Adroque JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeier H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* 2004; 18(14):1692-1700.
26. Lee Y, Naseem RH, Duplomb L, Park BH, Garry DJ, Richardson JA, Schaffer JE, Unger RH. Hyperleptinemia prevents lipotoxic cardiomyopathy in acyl CoA synthase transgenic mice. *Proc Natl Acad Sci U S A* 2004; 101(37):13624-13629.
27. Ikonomidis I, Mazarakis A, Papadopoulos C, Patsouras N, Kalfarentzos F, Lekakis J, Kremastinos DT, Alexopoulos D. Weight loss after bariatric surgery improves aortic elastic properties and left ventricular function in individuals with morbid obesity: a 3-year follow-up study. *J Hypertens* 2007; 25(2): 439-447.

28. Dhindsa P, Scott AR, Donnelly R. Metabolic and cardiovascular effects of very-low-calorie diet therapy in obese patients with Type 2 diabetes in secondary failure: outcomes after 1 year. *Diabet Med* 2003; 20(4):319-324.
29. Kannel WB, Kannel C, Paffenbarger RS, Jr., Cupples LA. Heart rate and cardiovascular mortality: the Framingham Study. *Am Heart J* 1987; 113(6):1489-1494.
30. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006; 113(6):898-918.
31. Leichman JG, Aguilar D, King TM, Mehta S, Majka C, Scarborough T, Wilson EB, Taegtmeier H. Improvements in systemic metabolism, anthropometrics, and left ventricular geometry 3 months after bariatric surgery. *Surg Obes Relat Dis* 2006; 2(6):592-599.
32. Benjamin EJ, Levy D. Why is left ventricular hypertrophy so predictive of morbidity and mortality? *Am J Med Sci* 1999; 317(3):168-175.
33. Himeno E, Nishino K, Nakashima Y, Kuroiwa A, Ikeda M. Weight reduction regresses left ventricular mass regardless of blood pressure level in obese subjects. *Am Heart J* 1996; 131(2):313-319.
34. Sasson Z, Rasooly Y, Bhesania T, Rasooly I. Insulin resistance is an important determinant of left ventricular mass in the obese. *Circulation* 1993; 88(4 Pt 1):1431-1436.
35. Alpert MA, Lambert CR, Terry BE, Cohen MV, Mukerji V, Massey CV, Hashimi MW, Panayiotou H. Influence of left ventricular mass on left ventricular diastolic filling in normotensive morbid obesity. *Am Heart J* 1995; 130(5):1068-1073.
36. Huang JM, Xian H, Bacaner M. Long-chain fatty acids activate calcium channels in ventricular myocytes. *Proc Natl Acad Sci U S A* 1992; 89(14):6452-6456.
37. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. *Circulation* 2002; 105(12):1503-1508.
38. Ridker PM. High-sensitivity C-reactive protein, inflammation, and cardiovascular risk: from concept to clinical practice to clinical benefit. *Am Heart J* 2004; 148(1 Suppl):S19-S26.
39. Wing RR, Blair EH, Bononi P, Marcus MD, Watanabe R, Bergman RN. Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. *Diabetes Care* 1994; 17(1):30-36.
40. Wing RR. Use of very-low-calorie diets in the treatment of obese persons with non-insulin-dependent diabetes mellitus. *J Am Diet Assoc* 1995; 95(5):569-572.

Chapter 8

Short-term Hyperglycemic Dysregulation in Patients with Type 1 Diabetes Mellitus Does Not Change Myocardial Triglyceride Content or Myocardial Function

Adapted from Diabetes Care 2008; 31(8):1613-1614

S. Hammer
J.T. Jonker
H.J. Lamb
R.W. van der Meer
W. Zondag
J.M. Sepers
A. de Roos
J.W.A. Smit
J.A. Romijn

SUMMARY

Objectives: Patients with type 1 diabetes mellitus (DM1) suffer from frequent episodes of hyperglycemia and high plasma levels of non-esterified fatty acids (NEFAs) despite insulin treatment. The aim of this study was to evaluate the effects of partial insulin deprivation with resulting hyperglycemia on myocardial triglyceride (TG) content and myocardial function in patients with DM1.

Materials and methods: Myocardial TG content and left ventricular (LV) systolic and diastolic function were measured in 10 patients with DM1 (mean \pm standard error glycated hemoglobin: $7.4 \pm 0.2\%$) using ^1H magnetic resonance (MR) spectroscopy and MR imaging respectively during optimal glucose regulation and after 24 hours of partial insulin deprivation to induce plasma glucose levels between 15 and 20 mmol/l.

Results: Mean insulin infusion rate was 45 ± 5 units per 24 hours at baseline, whereas it was 27 ± 5 units per 24 hours during hyperglycemic conditions ($P < 0.001$). During partial insulin deprivation plasma 24-hour glucose levels increased from 8.4 ± 0.6 to 15.9 ± 0.8 mmol/l ($P < 0.001$), and plasma NEFA levels from 0.31 ± 0.05 to 0.46 ± 0.07 mmol/l ($P = 0.015$). This hyperglycemic dysregulation had no effects on myocardial TG content (0.31 ± 0.04 and $0.34 \pm 0.06\%$, respectively, $P = 0.587$) and LV systolic or diastolic function.

Conclusions: Short-term hyperglycemic dysregulation, frequently observed in patients with DM1, does not modulate myocardial TG content or myocardial function, despite considerable metabolic adaptations. Apparently, the heart is protected from short-term metabolic effects of hyperglycemic dysregulation in patients with DM1 with respect to myocardial TG content and myocardial function.

INTRODUCTION

Intensive insulin treatment is the cornerstone of therapy of patients with type 1 diabetes mellitus (DM1). Nonetheless, this treatment is not fully able to restore glucoregulation to normal, and patients with DM1 suffer from frequent episodes of less optimal metabolic regulation, reflected in short- or longer lasting episodes of hyperglycemia. These problems in optimization of glucoregulation may also result in altered lipid metabolism. For instance, an increased amount of intramyocellular lipid content was observed in the soleus and tibialis anterior muscles in patients with DM1 compared to controls (1). In these patients with DM1 there was an association between intramyocellular lipid content and the degree of glucoregulation reflected by glycated hemoglobin (HbA1c) values (1). These findings suggest a major role of metabolic dysregulation in the induction of abnormal intramyocellular lipid accumulation in DM1.

The metabolic effects of DM1 also extend to the heart. Studies with positron emission tomography have documented that patients with DM1 exhibit increased myocardial fatty acid utilization and oxidation, whereas myocardial glucose utilization is reduced (2;3). Myocardial substrate metabolism in these patients is influenced by plasma insulin and non-esterified fatty acid (NEFA) levels (4). The authors studied patients with DM1 during euglycemia, hyperlipidemia, and a hyperinsulinemic-euglycemic clamp and concluded that insulin and plasma NEFA levels can regulate the intramyocardial fate of fatty acids in humans with DM1. Therefore, it is likely that cardiac metabolism is also affected by episodes of metabolic dysregulation in patients with DM1.

In several conditions there is a discrepancy between fatty acid uptake and fatty acid utilization in the heart, reflected in alterations in myocardial triglyceride (TG) content. For instance, we documented that caloric restriction induces a dose-dependent increase in plasma NEFA levels and myocardial TG content in healthy subjects (5). Therefore, myocardial TG content is not fixed, but can be modulated depending on metabolic conditions. Moreover, this increase in myocardial TG content during caloric restriction was associated with impaired diastolic function (5;6). In accordance, in animal experiments myocardial TG accumulation is associated with impaired myocardial function (7;8), via routes involving fatty acid derivatives (9-11). Therefore, we hypothesized that episodes of metabolic dysregulation in patients with DM1 due to insufficient insulin provision may also modulate myocardial TG content and possibly myocardial function.

The primary aim of the present study was to evaluate the effects of short-term metabolic dysregulation, caused by insufficient insulin provision, on myocardial TG content and myocardial function in patients with DM1, otherwise well-controlled by continuous insulin pumps. For this purpose, the subjects were studied twice, during intensive insulin treatment and, on a separate occasion, after 24 hours of a ~50% reduction in baseline insulin infusions, resulting in hyperglycemia. Myocardial TG content and myocardial function were measured using hydrogen 1 magnetic resonance spectroscopy (¹HMRS) and magnetic resonance (MR) imaging,

respectively. To assess the tissue-specificity of the potential changes in myocardial TG content, we also measured hepatic TG content on the two occasions with ¹HMRS.

MATERIALS AND METHODS

Patients

We studied 10 (mean age \pm standard error: 41 ± 11 years), C-peptide negative patients with DM1 (5 men, HbA1c $7.4 \pm 0.2\%$). The sample size was based on our previous experiments in healthy subjects, in which we observed a statistical power of 0.89 for detecting a mean increase in myocardial TG content of 0.23% in 10 subjects (5). The mean duration of DM1 was 21.7 ± 2.3 years. All subjects used insulin treatment by insulin pump therapy (continuous subcutaneous insulin infusion) and used frequent self monitoring of blood glucose levels. A screening visit was performed with a medical history, physical examination and routine laboratory tests. Exclusion criteria were: smoking, an abnormal electrocardiogram (ECG), hypertension, active retinopathy, the use of other medication known to influence lipolysis and/or glucose metabolism (especially thiazolidinediones) or renal (microalbuminuria <30 mg/24h, normal creatinin levels), hepatic or other endocrine disease. Furthermore, obese subjects (body mass index > 30 kg/m²) were excluded.

The experimental protocol was approved by the institutional ethical committee and all subjects signed informed consent prior to participation.

Study design

The study consisted of 2 study occasions separated by a washout period of at least 2 weeks. The baseline study was done after a period, in which subjects aimed at optimal blood glucose levels by intensive insulin treatment by insulin pump therapy, combined with frequent assessments of blood glucose levels. The subjects documented their (normal) caloric intake and were restricted from alcohol. Patients were also instructed to document their basal and bolus insulin infusions. The second occasion was performed after $\sim 50\%$ reduction in both basal and bolus insulin infusions during 24 hours, compared with the first study, in order to maintain hyperglycemia between 15 and 20 mmol/l. The 3 days prior to evaluation, patients were again instructed to maintain the same caloric intake as for baseline measurements.

Blood glucose levels were monitored from 3 days prior to baseline and hyperglycemic measurements with a continuous glucose monitoring system (Metronic MiniMed Inc., Northridge, CA, USA). Furthermore, patients self-measured and documented their plasma glucose levels and ensured blood glucose levels remained < 20 mmol/l during targeted partial insulin deprivation. When blood glucose levels raised above 20 mmol/l patients were instructed to infuse insulin to maintain levels between 15 mmol/l and 20 mmol/l. At each study occasion,

post absorptive blood samples were obtained and we performed ^1H MRS and MR imaging. The sequence between the 2 occasions was assigned by balanced assignment.

^1H magnetic resonance spectroscopy of the heart and the liver

All MR measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, The Netherlands) with patients in the supine position at rest. MR data was obtained in the afternoon. For ^1H MRS measurements, a body coil for radiofrequency transmission and a surface coil for signal receiving were used. A point resolved spatially localized spectroscopic pulse sequence was used to acquire single-voxel (8-ml) spectra. The myocardial voxel was placed in the myocardial septum on standard four-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 (12). For the liver, voxel sites were matched at the study occasions (by using the twelfth thoracic vertebra as an anatomical landmark), avoiding vascular structures and bile ducts. Water-suppressed spectra with 128 averages were collected to detect weak lipid signals from the heart, and suppressed spectra with 64 averages were acquired from the liver. Spectral parameters included: repetition time (TR) of at least 3000 ms, and an echo time (TE) of 26 ms. 1024 Data points were collected using a 1000-Hz spectral width. Unsuppressed spectra with 4 averages were acquired in the same voxel, using the same parameters except for a TR of 10000 ms to be used as an internal standard. Spectra were analyzed using the advanced magnetic resonance algorithm in the Java-based MR user interface software (jMRUI version 2.2 (13)), as described earlier (12). Peak estimates of lipid resonances of myocardial and hepatic TGs at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (TGs/water \times 100).

Left ventricular function

Imaging was performed using a body coil for radiofrequency transmission and a 5-element synergy coil for signal receiving. To assess systolic function, the heart was imaged in the short-axis orientation using an ECG-triggered, sensitivity-encoding balanced steady-state free precession sequence with breath-holds. Imaging parameters were: field of view = 400 \times 320 mm, reconstructed matrix size = 256 \times 256, slice thickness = 10 mm, slice gap = 0 mm, flip angle = 35°, TE = 1.7 ms, TR = 3.4 ms and 12 to 14 slices (dependent on the heart size). Temporal resolution was 25 to 39 ms, depending on the heart rate. Dedicated post processing software (MASS®, Medis, Leiden, The Netherlands) was used to assess left ventricular (LV) ejection fraction (EF) as described previously (14). To assess LV diastolic function, an ECG-gated, free-breathing gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve (15;16). Imaging parameters were: TE = 4.8 ms, TR = 14 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350 mm², matrix size = 256 \times 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 FLOW® (Medis, Leiden, The Netherlands). Furthermore, we calculated the deceleration of the early filling phase (E deceleration). During MR imaging, blood pressure and heart rate were measured with an automatic device (Dinamap DPC100X, Freiburg, Germany).

Assays

Plasma glucose concentrations were measured by a continuous glucose monitoring system (Metronic MiniMed Inc., Northridge, CA, USA) and/ or (when not applicable) by the patients own device (at least each 2 hours during daytime and at 4-hour intervals during night time).

Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), γ -glutamyl transferase (γ GT), total cholesterol (TC) and TG concentrations were measured on a fully automated P800 analyzer (Roche, Almere, The Netherlands). Plasma NEFA levels were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany). HbA1c levels were measured with an HPLC system (Variant, Biomed, Hercules, CA, USA).

Statistical analysis

Statistical comparisons were performed with SPSS, version 14.0 (SPSS Inc., Chicago, Ill, USA). Baseline measurements and measurements during partial insulin deprivation were compared by paired t-tests. Data are shown as mean \pm standard error. $P < 0.05$ was considered to reflect significant differences.

RESULTS

Metabolic effects

Patient characteristics at baseline and during hyperglycemia are shown in Table 8.1. Mean insulin infusion rate was 45 ± 5 units per 24 hours during the control study, whereas it was only 27 ± 5 units per 24 hours during partial insulin deprivation ($P < 0.001$). During partial insulin deprivation, hyperglycemic dysregulation was present in all patients. Mean plasma 24-hour glucose was 8.4 ± 0.6 mmol/l during the control study which increased to 15.9 ± 0.8 mmol/l during partial insulin deprivation ($P < 0.001$). Concomitantly, plasma NEFA levels increased from 0.31 ± 0.05 to 0.46 ± 0.07 mmol/l ($P = 0.015$). Furthermore, plasma AP concentrations increased from 79 ± 6 to 88 ± 4 U/l ($P = 0.008$) and plasma AST concentrations decreased from 37 ± 4 to 28 ± 3 U/l ($P = 0.004$).

Myocardial and hepatic triglyceride content

Myocardial TG content was $0.31 \pm 0.04\%$ at baseline and did not change during hyperglycemic dysregulation ($0.34 \pm 0.06\%$, $P = 0.587$). In addition, hepatic TG content did not change during

Table 8.1 Metabolic parameters at baseline and after insulin reduction.

Variable	Baseline	Hyperglycemia
Age (yrs)	41 ± 3	
HbA1c (%)	7.4 ± 0.2	
Body mass index (kg/m ²)	23.5 ± 0.6	23.0 ± 0.8
Plasma mean 24 hour glucose (mmol/l)	8.4 ± 0.6	15.9 ± 0.8*
Plasma mean 24 hour insulin (U/l)	45 ± 5	27 ± 5*
Plasma AP (mmol/l)	79 ± 6	88 ± 4†
Plasma AST (mmol/l)	37 ± 4	28 ± 3†
Plasma ALT (mmol/l)	24 ± 3	21 ± 3
Plasma γGT (mmol/l)	21 ± 2	22 ± 3
Plasma cholesterol (mmol/l)	4.3 ± 0.2	4.4 ± 0.2
Plasma non-esterified fatty acids (mmol/l)	0.31 ± 0.05	0.46 ± 0.07‡
Plasma TGs (mmol/l)	1.03 ± 0.24	0.85 ± 0.49
Liver TG content (%)	0.77 ± 0.09	0.84 ± 0.11
Myocardial TG content (%)	0.31 ± 0.04	0.34 ± 0.06

$P < 0.001$, † $P < 0.05$ vs baseline. Data are mean ± standard error.

HbA1c = glycated hemoglobin, AP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, γGT = gamma-glutanyl transferase, TG = triglyceride.

hyperglycemic dysregulation ($0.77 \pm 0.09\%$ at baseline vs $0.84 \pm 0.11\%$ under hyperglycemic conditions, $P = 0.400$).

Left ventricular function

Systolic and diastolic blood pressures and LVEF were unchanged during hyperglycemic dysregulation (Table 8.2). Furthermore, E deceleration did not change ($4.4 \text{ ml/s}^2 \times 10^{-3}$ at baseline vs $4.5 \text{ ml/s}^2 \times 10^{-3}$ during hyperglycemic conditions $P = 0.777$). E/A ratio was also unaffected (1.9 ± 0.2 at baseline vs 1.9 ± 0.3 during hyperglycemic dysregulation, $P = 0.854$).

Table 8.2. Parameters of myocardial function at baseline and during hyperglycemia.

Variable	Baseline	Hyperglycemia
Systolic blood pressure (mmHg)	114 ± 4	118 ± 5
Diastolic blood pressure (mmHg)	68 ± 3	73 ± 3
Heart rate (bpm)	63 ± 1	60 ± 3
LV Ejection fraction (%)	58 ± 1	59 ± 2
E peak filling rate (ml/s)	475 ± 21	467 ± 20
E deceleration ($\text{ml/s}^2 \times 10^{-3}$)	4.4 ± 0.4	4.5 ± 0.3
A peak filling rate (ml/s)	267 ± 21	254 ± 38
E/A peak ratio	1.9 ± 0.2	1.9 ± 0.3

Data are mean ± standard error.

LV = left ventricular, E = early filling phase, A = atrial filling phase.

DISCUSSION

The present study was designed to study clinically relevant episodes of hyperglycemic dysregulation, frequently observed in patients with DM1. The study shows that hyperglycemic dysregulation for 24 hours does not influence myocardial TG content or myocardial function, despite considerable metabolic alterations. Moreover, hepatic TG content was not affected by short-term partial insulin deprivation in patients with DM1.

Hyperglycemic dysregulation did not alter LV (diastolic) heart function in the present study. Others could not document conclusive effects of hyperglycemia on myocardial blood flow (17) and vascular function (18). To our knowledge, this is the first study to document the effects of short-term hyperglycemic dysregulation on LV myocardial function in humans *in vivo*. Although our results suggest that short-term hyperglycemia does not alter myocardial function or myocardial TG content, we can not exclude the possibility that prolongation of the duration of partial insulin deprivation beyond 24 hours might have resulted in changes in cardiac function and myocardial TG accumulation. Nonetheless, the present study reflects a realistic clinical situation, as short-term hyperglycemic dysregulation frequently occurs in patients with DM1.

The present study was initiated, since we anticipated that partial insulin deprivation would also result in changes in myocardial TG content, possibly associated with changes in myocardial function. Targeted hypoinsulinemia in patients with DM1 indeed increased plasma levels of NEFAs by increasing adipose tissue lipolysis, resembling the effects of caloric restriction on plasma NEFA levels in healthy subjects (5;6). During caloric restriction, the resulting increased availability of plasma NEFAs considerably exceeds the oxidative requirements of fatty acids (19). In accordance with those observations, caloric restriction induces myocardial TG accumulation in healthy subjects (5;6;20). Moreover, in obesity and type 2 diabetes mellitus chronically elevated plasma NEFA levels are associated with increased myocardial TG content (21). These increased TG stores are associated with impaired myocardial function (22;23). In the present study in patients with DM1, insulin deficiency was introduced by targeted reduction of intensive insulin therapy. This resulted in considerable hyperglycemia and increased NEFA levels, but this excess of plasma energy substrates apparently did not result in myocardial TG accumulation.

Patients with DM1 have considerably altered myocardial glucose and fatty acid metabolism. Myocardial fatty acid utilization is increased in patients with DM1 compared to healthy subjects (2). Moreover, a larger proportion of myocardial fatty acid utilization is oxidized in patients with DM1, whereas myocardial glucose uptake is considerably lower in patients compared to controls (2;4). These changes protect the heart to substrate overflow of the myocardium. Accordingly, in the present study, myocardial TG content was not different in patients with DM1 from the values we observed in previous studies in healthy subjects (5;6;12). However, in healthy subjects myocardial function and TG content rapidly adapt to changes in nutritional intake, associated with considerable changes in plasma levels of NEFAs.

Hepatic TG content was measured to study the potential organ differential distribution of fatty acids between the heart and the liver as has been observed before (5;6). Baseline values of hepatic TG content were in the normal range in this particular cohort of patients, probably due to the intensive insulin treatment in these subjects, also reflected in relatively low HbA1c levels. Nonetheless, partial insulin deficiency resulted in changes in hepatic functions although dissociated from the unchanged hepatic TG content. We observed an increase in plasma AP levels and a decrease in AST levels during hyperglycemic dysregulation. Apparently, in this study, hepatic TG content is not a good parameter reflecting changes in hepatic metabolism, other than reflecting the net balance between fatty acid uptake, de novo lipogenesis, fatty acid oxidation and very low-density lipoprotein TG secretion.

CONCLUSIONS

In conclusion, the present study shows that short-term hyperglycemic dysregulation, which is frequently observed in patients with DM1, does not alter myocardial TG content or LV function, despite considerable metabolic adaptations. The study for the first time documents the myocardial effects of hyperglycemic dysregulation in patients with DM1. Apparently, the heart is protected from short-term metabolic effects of hyperglycemic dysregulation in patients with DM1 with respect to myocardial TG content and myocardial function.

ACKNOWLEDGMENTS

We gratefully thank Marja Dijk-Schaap and Nathalie Masurel for their assistance with the study.

REFERENCES

1. Perseghin G, Lattuada G, Danna M, Sereni LP, Maffi P, De CF, Battezzati A, Secchi A, Del MA, Luzi L. Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *Am J Physiol Endocrinol Metab* 2003; 285(6):E1174-E1181.
2. Herrero P, Peterson LR, McGill JB, Matthew S, Lesniak D, Dence C, Gropler RJ. Increased myocardial fatty acid metabolism in patients with type 1 diabetes mellitus. *J Am Coll Cardiol* 2006; 47(3):598-604.
3. Monti LD, Lucignani G, Landoni C, Moresco RM, Piatti P, Stefani I, Pozza G, Fazio F. Myocardial glucose uptake evaluated by positron emission tomography and fluorodeoxyglucose during hyperglycemic clamp in IDDM patients. Role of free fatty acid and insulin levels. *Diabetes* 1995; 44(5):537-542.
4. Peterson LR, Herrero P, McGill J, Schechtman KB, Kisrieva-Ware Z, Lesniak D, Gropler RJ. Fatty acids and insulin modulate myocardial substrate metabolism in humans with type 1 diabetes. *Diabetes* 2008; 57(1):32-40.
5. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW, Romijn JA. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008; 93(2):497-503.
6. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007; 56(12):2849-2853.
7. Christoffersen C, Bollano E, Lindgaard ML, Bartels ED, Goetze JP, Andersen CB, Nielsen LB. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003; 144(8):3483-3490.
8. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000; 97(4):1784-1789.
9. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 1994; 91(23):10878-10882.
10. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A* 1998; 95(5):2498-2502.
11. Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J* 2001; 15(2):312-321.
12. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of myocardial triglyceride content: reproducibility of 1H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007; 245(1):251-257.
13. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129(1):35-43.
14. Pattynama PM, Lamb HJ, van der Velde EA, van der Wall EE, de Roos A. Left ventricular measurements with cine and spin-echo MR imaging: a study of reproducibility with variance component analysis. *Radiology* 1993; 187(1):261-268.

15. Hartiala JJ, Mostbeck GH, Foster E, Fujita N, Dulce MC, Chazouilleres AF, Higgins CB. Velocity-encoded cine MRI in the evaluation of left ventricular diastolic function: measurement of mitral valve and pulmonary vein flow velocities and flow volume across the mitral valve. *Am Heart J* 1993; 125(4): 1054-1066.
16. Lamb HJ, Beyerbach HP, van der Laarse A, Stoel BC, Doornbos J, van der Wall EE, de Roos A. Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. *Circulation* 1999; 99(17):2261-2267.
17. Sundell J, Laine H, Nuutila P, Ronnema T, Luotolahti M, Raitakari O, Knuuti J. The effects of insulin and short-term hyperglycaemia on myocardial blood flow in young men with uncomplicated Type I diabetes. *Diabetologia* 2002; 45(6):775-782.
18. Gordin D, Ronnback M, Forsblom C, Heikkila O, Saraheimo M, Groop PH. Acute hyperglycaemia rapidly increases arterial stiffness in young patients with type 1 diabetes. *Diabetologia* 2007; 50(9): 1808-1814.
19. Elia M, Zed C, Neale G, Livesey G. The energy cost of triglyceride-fatty acid recycling in nonobese subjects after an overnight fast and four days of starvation. *Metabolism* 1987; 36(3):251-255.
20. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *Am J Physiol Endocrinol Metab* 2005; 289(5):E935-E939.
21. Kankaanpaa M, Lehto HR, Parkka JP, Komu M, Viljanen A, Ferrannini E, Knuuti J, Nuutila P, Parkkola R, Iozzo P. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006; 91(11):4689-4695.
22. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007; 116(10):1170-1175.
23. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.

Chapter 9

General Discussion



In this thesis we evaluated the relation between myocardial triglyceride (TG) content and myocardial function in healthy subjects and in patients with type 1 diabetes mellitus (DM1) and patients with type 2 diabetes mellitus (DM2). We performed interventional studies to test the flexibility of myocardial TG stores in relation to myocardial function, using innovative magnetic resonance (MR) techniques. In this chapter, the following issues are addressed:

- Relevance and measurement of myocardial triglycerides
- Flexibility of ectopic triglyceride stores
- Relevance of myocardial triglycerides for myocardial function
- Myocardial triglycerides in clinical interventions

RELEVANCE AND MEASUREMENT OF MYOCARDIAL TRIGLYCERIDES

In a variety of animal models, myocardial TG accumulation is related to obesity and diabetes. Moreover, myocardial TG accumulation is associated with impaired myocardial function (1-6). Although increased TG stores *per se* are most likely inert, they are associated with increased levels of fatty acid derivatives. The exact mechanisms of the detrimental effects of accumulation of fatty acid derivatives on myocardial function are not fully elucidated, but include interactions with biochemical and molecular pathways and lipoapoptosis (3;4;7;8). Furthermore, cardiac function improves in rats upon treatment with drugs that decrease TG stores in the heart (4).

In humans, myocardial TG content is increased in obesity and DM2 (9-11), indicating that it may be an interesting marker for metabolic disease. However, the number of publications on myocardial TG stores in humans is limited, due to the challenging techniques needed to quantify myocardial TGs (12). Nonetheless, the evaluation of myocardial TG stores is of interest, as TGs provide a direct substrate for myocardial metabolism (13;14). Furthermore, metabolic alterations such as seen in DM1 and DM2 and obesity influence myocardial functional parameters by altering myocardial substrate utilization (10;15-19).

The first aim of this thesis was to optimize the technique of hydrogen 1 magnetic resonance spectroscopy (¹HMR) to make it suitable for the assessment of myocardial TG stores in humans *in vivo*. In **chapter 2** we describe the need for respiratory motion compensation using navigator echoes and volume tracking (20;21) for adequate spectral resolution (optimized shimming) and reproducible quantification of myocardial proton spectra in a study on reproducibility in healthy subjects. ¹HMR spectra are obtained from the interventricular spectrum to avoid spectral contamination with epicardial (extracellular) fat. Data selection is triggered on end-systole by using electrocardiogram (ECG) signals. Quantification is performed with dedicated software (22), with incorporated prior knowledge (23), and myocardial TG content can be quantified as a percentage of the completely relaxed (unsuppressed) water signal. The data provided in

chapter 2 indicate that this dual compensation for cardiac and respiratory motion considerably decreased the variability of the measurements of myocardial TG content.

Implications and perspective

Respiratory navigator-gated and ECG-triggered ¹HMRs of the human heart provides a tool to accurately assess myocardial TG stores and thereby allows using it in metabolic studies and relate it to parameters of myocardial function.

We developed ¹HMRs at 1.5-Tesla. It would, however, be interesting to optimize the method also for 3 Tesla, as this increased magnetic field strength will theoretically improve signal-to-noise ratio and allows further discrimination between different metabolites.

FLEXIBILITY OF ECTOPIC TRIGLYCERIDE STORES

Healthy subjects

In general, virtually all TGs are stored in adipose tissue. However, a very small fraction is stored in non-adipose tissues like the heart (10), liver (24) and skeletal muscle (25). In this thesis we have focused on myocardial TG stores, but in the interventional studies we also assessed hepatic TG content, being extra cardiac location of TG accumulation in non-adipose tissue. The tissue-specific distribution of TGs in non-adipose tissues is influenced by dietary factors like caloric restriction (26-28) and high-fat diet (29;30). Therefore, we aimed to study the metabolic flexibility of myocardial (and hepatic) TG stores during different dietary regimes in healthy subjects. We studied the effects of short-term partial (**chapter 3**) and complete (**chapter 4**) starvation and the effects of a short-term high-fat diet (**chapter 5**) on myocardial and hepatic TG stores. Changes in dietary composition influence myocardial substrate selection and may therefore influence myocardial TG stores. For the heart, we documented a dose-dependent increase in myocardial TG content upon progressive caloric restriction associated with a dose-dependent increase in plasma non-esterified fatty acids (NEFAs). Although the mechanisms by which caloric restriction induces myocardial TG accumulation can not be derived from the study design, it is likely that the heart has a need for a slightly physiologically increased TG pool, to accommodate sufficient adenosine-triphosphate (ATP) production when blood glucose levels are low. This is in line with results by Reingold *et al.* and Johnson *et al.*, who documented an increase in intracellular TG stores in the heart and in skeletal muscle after short-term fasting in healthy subjects (30;31). Increased ectopic TG stores in obesity and diabetes mellitus are associated with decreased insulin sensitivity and organ dysfunction (9;10;19;25;32), whereas in healthy subjects during caloric restriction it reflects a new equilibrium between the uptake and utilization of glucose and fatty acids. It is therefore of utmost importance to dissociate these physiological processes from the pathologically elevated plasma lipids and its consequences in metabolic disease. Interestingly, we also found tissue-specific effects of caloric restriction.

As hepatic TG content decreased after partial starvation, but was unchanged after complete starvation, we have shown that redistribution of endogenous TG stores is tissue-specific. Our results in the liver are in line with results of Westerbacka *et al.* who showed that a low-fat diet decreases hepatic TG stores (33).

Implications and perspective

Myocardial TG stores are not fixed, but flexible and amendable to caloric restriction in healthy subjects. Myocardial TG stores are physiologically and dose-dependently increased upon progressive caloric restriction. Redistribution of endogenous TG stores is tissue-specific, since the liver TG stores respond differentially compared to the myocardial TG stores. The data document physiological variations of TG stores in non-adipose tissues.

Future studies could address the myocardial and hepatic effects of diets of different (eu-caloric) nutritional composition. Furthermore, it would be interesting to study the reversibility of the effects of progressive caloric restriction in healthy subjects, which is likely to be present.

A high-fat diet is another model to increase plasma levels of TGs and NEFAs. A high-fat diet increases skeletal muscle TG stores (30) and hepatic TG content (33), associated with insulin resistance (34;35). In accordance, we documented an increase in plasma TG and NEFA concentrations and an increase in hepatic TG content after a 3-day hypercaloric, high-fat diet (**chapter 5**). In contrast, however, this high-fat diet did not alter myocardial TG content. Our results after 3 days of high-fat feeding are in concordance with the results obtained by Reingold *et al.*, who showed that a single high-fat meal did not alter myocardial TG content (31). Apparently, caloric restriction and high-fat feeding differentially affect myocardial TG content, even though these two conditions similarly increased plasma fatty acid levels. However, these two conditions were discrepant in dietary caloric content and macronutrient composition, which may underlie the discrepant effect on myocardial TG accumulation (36). A long term, hypercaloric, high-fat dietary intake induces obesity and, thereby, influences hepatic and myocardial TG stores (37;38). We can not exclude the possibility that during short-term high-fat feeding fatty acid oxidation in the heart increases, together with increased fatty acid uptake, with the net result of unchanged myocardial TG stores. In the condition of caloric restriction, the increased myocardial TG stores indicate that fatty acid uptake exceeds myocardial fatty acid oxidation rates. It is presently unclear what the factors are underlying these discrepancies between fatty acid uptake and oxidation during high-fat feeding and (partial) starvation.

Implications and perspective

A high-fat diet does not increase myocardial TG content in the short-term, whereas hepatic TG stores rapidly increase. Therefore, a high-fat diet induces differential, tissue-specific responses of TG and/or fatty acid partitioning among non-adipose organs.

In future studies it would be interesting to perform positron emission tomography studies with palmitate tracers during a high-fat diet, to obtain data on fatty acid uptake and oxidation in the myocardium. This would allow discriminating between the contribution of altered uptake and oxidation of fatty acids to myocardial TG stores.

Patients with type 2 diabetes mellitus

Myocardial metabolism is altered in patients with DM2 (15;18). Specifically, the heart of patients with DM2 relies more on fatty acids (6), primarily due to increased fatty acid levels (16;39), associated with increased lipolysis of TGs contained in adipose tissue. DM2 is associated with increased myocardial TG levels (9-11). In **chapter 6**, we evaluated the myocardial flexibility of these increased myocardial TG stores in patients with DM2. For this study we recruited patients with DM2 who were well-controlled and without comorbidities. This allowed us to study *in vivo* the effects of physiological dietary interventions in a clinically relevant group of patients. We applied short-term partial caloric restriction by a very low-calorie diet (VLCD) in these patients and found an increase in myocardial TG stores. Furthermore, in patients with DM2, the VLCD did not alter hepatic TG stores, indicating tissue-specific effects of a VLCD in patients with DM2. In the same group of patients we also evaluated the combination of VLCD with the anti-lipolytic drug acipimox. In patients with DM2 acipimox decreases plasma fatty acid levels (40-42) and skeletal muscle TG content (43;44) and may improve insulin sensitivity (40;41;43;45). However, acipimox is not suitable as therapy as there is a rebound effect on plasma fatty acid levels during long term administration (46). We used acipimox to decrease fatty acid levels during short-term caloric restriction by a VLCD to assess the contribution of increased plasma fatty acid levels to increased myocardial TG stores. We found that acipimox prevented myocardial TG accumulation during caloric restriction associated with the targeted decrease in plasma fatty acid levels. This observation indicates that the effect of caloric restriction on myocardial TG stores is mediated, at least in part, by the increase in plasma fatty acid levels induced by caloric restriction.

Implications and perspective

Upon short-term partial caloric restriction, myocardial TG stores increase in patients with DM2. These effects are at least in part mediated by the increase in plasma fatty acid levels induced by caloric restriction. These data illustrate that myocardial TG stores in patients with DM2 are not fixed, but flexible upon physiological, nutritional interventions.

Future studies should address the effects of lipid lowering therapy in patients with DM2 as our studies suggest a possible role for lipid lowering therapy in patients with elevated plasma fatty acid levels.

RELEVANCE OF MYOCARDIAL TRIGLYCERIDES FOR MYOCARDIAL FUNCTION

It has been suggested that increased myocardial TG accumulation in patients with impaired glucose tolerance and DM2 precedes the onset of profound systolic dysfunction (10;47). Furthermore, in animal studies myocardial TG content has been linked to myocardial function in various models, including hyperleptinemia (1), obesity (4) and heart failure (2). In this thesis we have shown that changes in myocardial TG content are associated with changes in diastolic left ventricular function. During progressive caloric restriction in healthy subjects, MR parameters of diastolic function decrease dose-dependently, associated with progressive myocardial TG accumulation, providing circumstantial evidence in humans for the observations in animal models of myocardial lipotoxicity. For example, myocardial TG content may affect myocardial function via changes in calcium homeostasis and lipoapoptosis induced by accumulation of damaging ceramides (7). Moreover, myocardial function can be affected also directly by caloric restriction as it may induce membrane remodeling (48) which affects myocardial diastolic function (49). However, during partial starvation the decrease in diastolic function was correlated with the increase in myocardial TG content. In line with this, others reported myocardial TG accumulation in obesity, associated with changes in left ventricular mass (10;11). The main question in relation to our observations is to which extent increased availability of fatty acid derivatives, reflected in increased myocardial TG stores, contribute to the observed alterations in myocardial function.

In patients with DM2 we found that administration of acipimox during a VLCD prevents the myocardial metabolic and functional alterations induced by caloric restriction, suggesting a role for lipid lowering therapy in patients with elevated levels of plasma lipids. This might lead to a decrease myocardial TG stores and possibly improve myocardial function. In skeletal muscle, for example, acipimox decreases intracellular TG content associated with improvements in insulin sensitivity (44). Furthermore, in selected patient groups with DM2, treatment with pioglitazone has salutary effects on hepatic and myocardial TG stores (50).

Implications and perspective

Changes in myocardial TG content are associated with changes in myocardial function in healthy subjects and in patients with DM2. These data indicate that myocardial TG content is a relevant metabolic marker for myocardial function. As anti-lipolytic therapy with acipimox during caloric restriction prevents the negative effects of partial caloric restriction on diastolic myocardial function, there may be a role for anti-lipolytic therapy in myocardial dysfunction in patients with DM2.

Future studies should be performed to assess the effects of anti-lipolytic therapy on the relation between myocardial TG stores and myocardial function. Measurement of myocardial TGs may provide an interesting marker for risk assessment of myocardial function in different patient groups.

MYOCARDIAL TRIGLYCERIDES IN CLINICAL INTERVENTIONS

Effects of prolonged caloric restriction in patients with type 2 diabetes mellitus

The last part of this thesis describes the effects of two clinical interventions on myocardial TGs and myocardial function. In obese patients with DM2 myocardial TG stores are increased (10) and obesity negatively influences myocardial diastolic function (51). In these patients, therapy should be aimed at decreasing body weight. In accordance, improvements in myocardial geometrics have been documented after bariatric surgery (52;53). Another possibility to achieve substantial weight loss is by using a VLCD (54;55). In **chapter 7** we describe the effects of prolonged caloric restriction using a VLCD in obese patients with DM2. This treatment induces substantial weight loss in these patients, associated with considerable metabolic improvements and improved myocardial diastolic function. This dietary intervention resulted in a decrease in ectopic TG stores, including the liver and the heart. The study design does not allow to assess the direct relation between myocardial TG stores and myocardial function. However, as TG stores are also flexible in severely obese, dysregulated patients with DM2, quantification of myocardial TG content may be an interesting new marker to assess the effects of metabolic interventions on the heart. Furthermore, the data suggest that even in obese, hyperglycemic patients myocardial TG stores are amendable to caloric restriction.

Implications and perspective

Myocardial TG content decreases upon prolonged caloric restriction in obese patients with DM2, associated with functional improvements.

As myocardial TG content is amendable to therapeutic interventions, measurement of myocardial TGs may be used in future studies to assess the metabolic effects of different interventions on the heart.

Hyperglycemic dysregulation in patients with type 1 diabetes mellitus

Patients with DM1 suffer from frequent episodes of hyperglycemia. The metabolic consequences also involve changes in lipid metabolism. Specifically, hyperglycemic dysregulation in DM1 is the result of relative hypoinsulinemia. During hyperglycemia, plasma levels of NEFAs also increase as adipose tissue lipolysis increases during insulin deficiency. These metabolic alterations influence myocardial substrate selection (56). However, the functional consequences of these metabolic alterations are not fully elucidated. Some studies reported alterations in vascular function during hyperglycemia (57) whereas others could not document changes in myocardial blood flow (58). Nonetheless, in our study described in **chapter 8** we aimed to mimic the clinically relevant condition of short-term hyperglycemia in patients with DM1 by decreasing the infused exogenous insulin for one day. Despite considerable increases in plasma glucose levels and plasma levels of NEFAs this hyperglycemic dysregulation had no effects on myocardial TG content and myocardial left ventricular function. We can not exclude

the possibility that the duration of hyperglycemic dysregulation for one day is not long enough to induce detectable changes in myocardial TG content and myocardial function. Nonetheless, the results are clinically relevant, since this duration mimics short-term hyperglycemia such as frequently observed in patients with DM1. Apparently, the heart is protected from deleterious effects of short-term hyperglycemic dysregulation in these patients with DM1, at least with respect to myocardial TG content and left ventricular function. This is also supported by the fact that myocardial TG content was not different in the patients with DM1 in **chapter 8** compared to the healthy subjects in **chapter 3, 4** and **5**. Apparently, the imperfections of glucoregulation present in patients with DM1, reflected in higher levels of glycated hemoglobin than those present in healthy subjects, are not associated with increased hepatic and myocardial TG stores.

Implications and perspective

The heart of patients with DM1 is protected from the short-term effects of hyperglycemic dysregulation, at least with respect to myocardial TG content and myocardial function.

Our results can not be extrapolated to hyperglycemic dysregulation that exists for a longer period. Therefore, it would be interesting to assess the effects of hyperglycemia that exists for more than one day.

GENERAL CONCLUSIONS

The studies described in this thesis aimed to clarify the relation between myocardial TG content and left ventricular function in different metabolic conditions, in healthy subjects and in patients with DM1 and patients with DM2.

We have shown that:

1. Myocardial TG content can accurately and reproducibly be quantified with ¹HMRS.
2. Myocardial TG content is not fixed, as we have shown flexibility upon different dietary interventions, in healthy subjects and in patients with DM2.
3. Caloric restriction and a high-fat diet induce tissue-specific effects on TG redistribution in the heart and the liver, indicating organ specific adaptations.
4. Changes in myocardial TG content are associated with changes in left ventricular function.
5. The increases in myocardial TG stores induced by partial caloric deprivation are at least in part caused by increased plasma NEFA levels, since acipimox prevents myocardial TG accumulation and decreased diastolic function during short-term caloric restriction in patients with DM2.
6. Prolonged caloric restriction in obese, hyperglycemic patients with DM2 decreases myocardial TG content and improves myocardial function.

7. Short-term hyperglycemic dysregulation, which is frequently present in patients with DM1, does not change myocardial TG content or myocardial function, suggesting that the heart is protected from these short-term metabolic alterations.

Therefore, increased myocardial TG content is associated with altered myocardial function. It reflects a discrepancy between fatty acid uptake and fatty acid oxidation and most likely reflects increased intracellular availability of fatty acid derivatives, which alter structure and function of the myocardium. The observations described in this thesis indicate that these studies on the relation between myocardial TG content and myocardial function should be extended to patients with myocardial dysfunction in order to establish to which extent metabolic interventions may improve myocardial function in these patients.

REFERENCES

1. Lee Y, Naseem RH, Duplomb L, Park BH, Garry DJ, Richardson JA, Schaffer JE, Unger RH. Hyperleptinemia prevents lipotoxic cardiomyopathy in acyl CoA synthase transgenic mice. *Proc Natl Acad Sci U S A* 2004; 101(37):13624-13629.
2. Sharma S, Adrogue JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeier H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* 2004; 18(14):1692-1700.
3. Vincent HK, Powers SK, Dirks AJ, Scarpace PJ. Mechanism for obesity-induced increase in myocardial lipid peroxidation. *Int J Obes Relat Metab Disord* 2001; 25(3):378-388.
4. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000; 97(4):1784-1789.
5. Unger RH. Lipotoxic diseases. *Annu Rev Med* 2002; 53:319-336.
6. Christoffersen C, Bollano E, Lindegaard ML, Bartels ED, Goetze JP, Andersen CB, Nielsen LB. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003; 144(8):3483-3490.
7. Unger RH, Orci L. Lipoapoptosis: its mechanism and its diseases. *Biochim Biophys Acta* 2002; 1585(2-3):202-212.
8. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A* 1998; 95(5):2498-2502.
9. Kankaanpaa M, Lehto HR, Parkka JP, Komu M, Viljanen A, Ferrannini E, Knuuti J, Nuutila P, Parkkola R, Iozzo P. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006; 91(11):4689-4695.
10. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007; 116(10):1170-1175.
11. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003; 49(3):417-423.
12. Bottomley PA. MR spectroscopy of the human heart: the status and the challenges. *Radiology* 1994; 191(3):593-612.
13. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 2005; 85(3):1093-1129.
14. Korvald C, Elvenes OP, Myrmel T. Myocardial substrate metabolism influences left ventricular energetics in vivo. *Am J Physiol Heart Circ Physiol* 2000; 278(4):H1345-H1351.
15. Lopaschuk GD, Folmes CD, Stanley WC. Cardiac energy metabolism in obesity. *Circ Res* 2007; 101(4):335-347.
16. Peterson LR, Herrero P, Schechtman KB, Racette SB, Waggoner AD, Kisrieva-Ware Z, Dence C, Klein S, Marsala J, Meyer T, Gropler RJ. Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. *Circulation* 2004; 109(18):2191-2196.

17. Poirier P, Eckel RH. Obesity and cardiovascular disease. *Curr Atheroscler Rep* 2002; 4(6):448-453.
18. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. *Cardiovasc Res* 1997; 34(1):25-33.
19. McGavock JM, Victor RG, Unger RH, Szczepaniak LS. Adiposity of the heart, revisited. *Ann Intern Med* 2006; 144(7):517-524.
20. Kozerke S, Schar M, Lamb HJ, Boesiger P. Volume tracking cardiac 31P spectroscopy. *Magn Reson Med* 2002; 48(2):380-384.
21. Schar M, Kozerke S, Boesiger P. Navigator gating and volume tracking for double-triggered cardiac proton spectroscopy at 3 Tesla. *Magn Reson Med* 2004; 51(6):1091-1095.
22. Naressi A, Couturier C, Devos JM, Janssen M, Mangeat C, de BR, Graveron-Demilly D. Java-based graphical user interface for the MRUI quantitation package. *MAGMA* 2001; 12(2-3):141-152.
23. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129(1):35-43.
24. Ishii M, Yoshioka Y, Ishida W, Kaneko Y, Fujiwara F, Taneichi H, Miura M, Toshihiro M, Takebe N, Iwai M, Suzuki K, Satoh J. Liver fat content measured by magnetic resonance spectroscopy at 3.0 tesla independently correlates with plasminogen activator inhibitor-1 and body mass index in type 2 diabetic subjects. *Tohoku J Exp Med* 2005; 206(1):23-30.
25. Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Ma YZ, Savoye M, Rothman DL, Shulman GI, Caprio S. Assessment of skeletal muscle triglyceride content by (1)H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 2002; 51(4):1022-1027.
26. Jensen MD, Ekberg K, Landau BR. Lipid metabolism during fasting. *Am J Physiol Endocrinol Metab* 2001; 281(4):E789-E793.
27. Klein S, Sakurai Y, Romijn JA, Carroll RM. Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. *Am J Physiol* 1993; 265(5 Pt 1):E801-E806.
28. Savendahl L, Underwood LE. Fasting increases serum total cholesterol, LDL cholesterol and apolipoprotein B in healthy, nonobese humans. *J Nutr* 1999; 129(11):2005-2008.
29. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, Yki-Jarvinen H. Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 2005; 90(5):2804-2809.
30. Johnson NA, Stannard SR, Rowlands DS, Chapman PG, Thompson CH, O'Connor H, Sachinwalla T, Thompson MW. Effect of short-term starvation versus high-fat diet on intramyocellular triglyceride accumulation and insulin resistance in physically fit men. *Exp Physiol* 2006; 91(4):693-703.
31. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *Am J Physiol Endocrinol Metab* 2005; 289(5):E935-E939.
32. Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. *Physiology (Bethesda)* 2004; 19:183-190.
33. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, Yki-Jarvinen H. Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 2005; 90(5):2804-2809.
34. Schrauwen-Hinderling VB, Hesselink MK, Schrauwen P, Kooi ME. Intramyocellular lipid content in human skeletal muscle. *Obesity (Silver Spring)* 2006; 14(3):357-367.

35. Yki-Jarvinen H. Fat in the liver and insulin resistance. *Ann Med* 2005; 37(5):347-356.
36. Vatner SF, Patrick TA, Higgins CB, Franklin D. Regional circulatory adjustments to eating and digestion in conscious unrestrained primates. *J Appl Physiol* 1974; 36(5):524-529.
37. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005; 288(2):E462-E468.
38. Thomas EL, Hamilton G, Patel N, O'Dwyer R, Dore CJ, Goldin RD, Bell JD, Taylor-Robinson SD. Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut* 2005; 54(1):122-127.
39. Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. *Diabetes* 1988; 37(8):1020-1024.
40. Qvigstad E, Mostad IL, Bjerve KS, Grill VE. Acute lowering of circulating fatty acids improves insulin secretion in a subset of type 2 diabetes subjects. *Am J Physiol Endocrinol Metab* 2003; 284(1):E129-E137.
41. Santomauro AT, Boden G, Silva ME, Rocha DM, Santos RF, Ursich MJ, Strassmann PG, Wajchenberg BL. Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. *Diabetes* 1999; 48(9):1836-1841.
42. Worm D, Vinten J, Vaag A, Henriksen JE, Beck-Nielsen H. The nicotinic acid analogue acipimox increases plasma leptin and decreases free fatty acids in type 2 diabetic patients. *Eur J Endocrinol* 2000; 143(3): 389-395.
43. Vaag A, Skott P, Damsbo P, Gall MA, Richter EA, Beck-Nielsen H. Effect of the antilipolytic nicotinic acid analogue acipimox on whole-body and skeletal muscle glucose metabolism in patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 1991; 88(4):1282-1290.
44. Bajaj M, Suraamornkul S, Romanelli A, Cline GW, Mandarino LJ, Shulman GI, DeFronzo RA. Effect of a sustained reduction in plasma free fatty acid concentration on intramuscular long-chain fatty Acyl-CoAs and insulin action in type 2 diabetic patients. *Diabetes* 2005; 54(11):3148-3153.
45. Worm D, Henriksen JE, Vaag A, Thye-Ronn P, Melander A, Beck-Nielsen H. Pronounced blood glucose-lowering effect of the antilipolytic drug acipimox in noninsulin-dependent diabetes mellitus patients during a 3-day intensified treatment period. *J Clin Endocrinol Metab* 1994; 78(3):717-721.
46. Vaag AA, Beck-Nielsen H. Effects of prolonged Acipimox treatment on glucose and lipid metabolism and on in vivo insulin sensitivity in patients with non-insulin dependent diabetes mellitus. *Acta Endocrinol (Copenh)* 1992; 127(4):344-350.
47. Szczepaniak LS, Victor RG, Orzi L, Unger RH. Forgotten but not gone: the rediscovery of fatty heart, the most common unrecognized disease in America. *Circ Res* 2007; 101(8):759-767.
48. Han X, Cheng H, Mancuso DJ, Gross RW. Caloric restriction results in phospholipid depletion, membrane remodeling, and triacylglycerol accumulation in murine myocardium. *Biochemistry* 2004; 43(49):15584-15594.
49. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. *Circulation* 2002; 105(12):1503-1508.
50. Zib I, Jacob AN, Lingvay I, Salinas K, McGavock JM, Raskin P, Szczepaniak LS. Effect of pioglitazone therapy on myocardial and hepatic steatosis in insulin-treated patients with type 2 diabetes. *J Investig Med* 2007; 55(5):230-236.

51. Alpert MA, Lambert CR, Terry BE, Cohen MV, Mukerji V, Massey CV, Hashimi MW, Panayiotou H. Influence of left ventricular mass on left ventricular diastolic filling in normotensive morbid obesity. *Am Heart J* 1995; 130(5):1068-1073.
52. Leichman JG, Aguilar D, King TM, Mehta S, Majka C, Scarborough T, Wilson EB, Taegtmeier H. Improvements in systemic metabolism, anthropometrics, and left ventricular geometry 3 months after bariatric surgery. *Surg Obes Relat Dis* 2006; 2(6):592-599.
53. Ikonomidis I, Mazarakis A, Papadopoulos C, Patsouras N, Kalfarentzos F, Lekakis J, Kremastinos DT, Alexopoulos D. Weight loss after bariatric surgery improves aortic elastic properties and left ventricular function in individuals with morbid obesity: a 3-year follow-up study. *J Hypertens* 2007; 25(2): 439-447.
54. Jazet IM, Schaart G, Gastaldelli A, Ferrannini E, Hesselink MK, Schrauwen P, Romijn JA, Maassen JA, Pijl H, Ouwens DM, Meinders AE. Loss of 50% of excess weight using a very low energy diet improves insulin-stimulated glucose disposal and skeletal muscle insulin signalling in obese insulin-treated type 2 diabetic patients. *Diabetologia* 2008; 51(2):309-319.
55. Wing RR. Use of very-low-calorie diets in the treatment of obese persons with non-insulin-dependent diabetes mellitus. *J Am Diet Assoc* 1995; 95(5):569-572.
56. Peterson LR, Herrero P, McGill J, Schechtman KB, Kisrieva-Ware Z, Lesniak D, Gropler RJ. Fatty acids and insulin modulate myocardial substrate metabolism in humans with type 1 diabetes. *Diabetes* 2008; 57(1):32-40.
57. Gordin D, Ronnback M, Forsblom C, Heikkila O, Saraheimo M, Groop PH. Acute hyperglycaemia rapidly increases arterial stiffness in young patients with type 1 diabetes. *Diabetologia* 2007; 50(9): 1808-1814.
58. Sundell J, Laine H, Nuutila P, Ronnema T, Luotolahti M, Raitakari O, Knuuti J. The effects of insulin and short-term hyperglycaemia on myocardial blood flow in young men with uncomplicated Type I diabetes. *Diabetologia* 2002; 45(6):775-782.

Chapter 10

Summary

Samenvatting



Summary

In this thesis we focused on the functional and metabolic consequences of myocardial triglyceride (TG) accumulation in healthy subjects and in patients with diabetes mellitus. Ectopic accumulation of TGs is associated with organ dysfunction in metabolic disease in experimental animal studies. These organs include the heart, the liver and skeletal muscle. For the heart, translational studies in humans are scarce, mainly due to the difficulty of the assessment of myocardial TG content in humans, *in vivo*.

Therefore, it remains unclear to what extent the observations in animal experiments can be extended to humans. Furthermore, the physiological and pathophysiological relevance of myocardial TG accumulation for myocardial function is unknown.

In Chapter 2 we describe a non-invasive method, using hydrogen 1 magnetic resonance spectroscopy (¹HMRS), to accurately and reproducibly measure myocardial TG content in humans, *in vivo*. We observed improved spectral resolution and an improved intraclass correlation coefficient for the assessment of myocardial TG content when spectroscopic measurements were performed with respiratory motion correction compared to spectra obtained without respiratory motion compensation.

Diabetes mellitus and obesity are associated with increased plasma non-esterified fatty acid (NEFA) levels, myocardial TG accumulation, and myocardial dysfunction. Because a very low-calorie diet (VLCD) also increases plasma NEFA levels, we studied the effect of a short-term VLCD on myocardial TG content and cardiac function in healthy subjects in Chapter 3. We found increased myocardial TG content and a decrease in left ventricular diastolic function. Moreover, hepatic TG content decreased, indicating organ-specific effects of a VLCD.

In animal studies high plasma levels of NEFAs are associated with increased myocardial TG stores and impaired myocardial function. Caloric restriction increases the delivery of fatty acids to the myocardium. We have therefore evaluated the effects of progressive caloric restriction in healthy subjects in Chapter 4. Upon progressive caloric restriction we documented a dose-dependent increase in plasma levels of NEFAs and myocardial TG content, and a dose-dependent decrease in left ventricular diastolic function.

Short-term high-fat diets increase TG content in skeletal muscle. Moreover, a high-fat diet induces myocardial TG accumulation and myocardial dysfunction in animal models. We studied the effects of a short-term high-fat diet in healthy individuals in Chapter 5. We found no changes in myocardial TG content and no effects on left ventricular function. However, hepatic TG content increased. The data document physiological and organ-specific adaptation of TG content during a high-fat diet.

Myocardial metabolism in patients with type 2 diabetes mellitus (DM2) is heavily dependent on fatty acids. Furthermore, in animals and in humans this increased fatty acid reliability has been associated with structural changes in the diabetic myocardium and with myocardial dysfunction. Therefore we have evaluated the effects of a short-term VLCD in patients with DM2 in Chapter 6, to test the myocardial flexibility in these patients. We have shown that myocardial TGs increase after a VLCD, associated with a decrease in left ventricular diastolic function. Furthermore, anti-lipolytic therapy with acipimox during the VLCD prevented these changes in myocardial TG stores and myocardial function. Hepatic TG content was unchanged after both the interventions. The study illustrates the flexibility of myocardial TG stores and myocardial function in patients with DM2. Moreover, the data implicate the relevance of plasma NEFAs as mediators of the cardiac effects of a VLCD in patients with DM2.

In Chapter 7 we evaluated the effects of therapeutic weight loss in obese, insulin-treated patients with DM2. Obesity and DM2 are major risk factors for cardiovascular disease, and prolonged caloric restriction has shown to induce weight loss and improve glycemic control. In this study we evaluated the effects of prolonged caloric restriction on myocardial and hepatic TG content and on myocardial function. Upon substantial weight loss there were considerable metabolic improvements in glucose and fat metabolism, associated with decreased myocardial TG content and a decrease in hepatic TG stores. Furthermore, myocardial diastolic function improved. The data show that in these obese patients with DM2, myocardial TG stores are flexible and amendable to therapeutic intervention by caloric restriction.

Patients with type 1 diabetes mellitus (DM1) suffer from frequent episodes of hyperglycemic dysregulation, due to imperfections in exogenous insulin treatment, which mimics endogenous insulin secretion. These episodes of hyperglycemia are accompanied by perturbations in lipid metabolism as well. We have therefore evaluated the effects of controlled, short-term hyperglycemia in patients with DM1 in Chapter 8. Despite hyperglycemic dysregulation by partial insulin deprivation and the increase in plasma NEFA levels, myocardial TG content and myocardial function did not change. Apparently, the heart is protected from short-term metabolic effects of partial insulin deprivation in patients with DM1.

In conclusion, myocardial TGs can be accurately measured in humans with ^1H MRS. Myocardial TG stores are flexible in healthy subjects and in patients with DM2 upon differences in dietary nutritional intake. Changes in myocardial TG content are associated with changes in left ventricular function. Myocardial TGs reflect the discrepancy between fatty acid uptake and fatty acid oxidation and most likely reflect increased intracellular availability of fatty acid derivatives, which alter structure and function of the myocardium. Redistribution of TGs is tissue-specific, since TGs in the heart and the liver do not always show the same responses to physiological interventions. In patients with DM1, the heart is protected from short-term metabolic effects

of hyperglycemic dysregulation, with respect to myocardial TG accumulation and alterations in myocardial function.

Samenvatting

Dit proefschrift beschrijft de functionele en metabole gevolgen van triglyceriden (TG) stapeling in het hart, in gezonde proefpersonen en in patiënten met diabetes mellitus. Ectopische stapeling van TG is geassocieerd met orgaandysfunctie bij metabole ziekten in experimentele dierstudies, onder andere in het hart, de lever en skeletspieren. Weinig is echter bekend over de gevolgen van myocardiale TG stapeling bij mensen, met name door de moeilijkheid van de bepaling van TG in het hart *in vivo*.

Het is om deze reden onduidelijk, in hoeverre de bevindingen gedaan in dierstudies kunnen worden doorgetrokken naar mensen. Daarnaast is het fysiologische en pathofysiologische belang van TG stapeling in het hart in relatie tot de hartspierfunctie onbekend.

Hoofdstuk 2 beschrijft een niet-invasieve methode, met ^1H magnetic resonance spectroscopy ($^1\text{HMRS}$), waarmee in mensen, *in vivo*, betrouwbaar en reproduceerbaar de hoeveelheid myocardiale TG kan worden bepaald. De spectrale resolutie en de intraklasse correlatie coëfficiënt waren beter als de spectra werden gemeten met ademhalingscorrectie, vergeleken met spectra gemeten zonder ademhalingscorrectie.

Diabetes mellitus en overgewicht zijn geassocieerd met verhoogde vrije vetzuren (VZ) in het plasma, stapeling van TG in het hart en cardiale dysfunctie. Omdat een zeer laag calorisch dieet (ZLCD) de VZ in het plasma ook verhoogt, is in Hoofdstuk 3 het effect van een kortdurend ZLCD op de hoeveelheid TG in het hart en de linker ventrikel functie in gezonde vrijwilligers bestudeerd. De hoeveelheid myocardiale TG nam toe, samen met een afname in de diastolische functie. Daarnaast nam de hoeveelheid hepatische TG af, wijzend op orgaan-specifieke effecten van een ZLCD.

In diermodellen zijn hoge spiegels van VZ in het plasma geassocieerd met een toegenomen hoeveelheid myocardiale TG en een afgenomen diastolische functie. Calorier restrictie verhoogt het aanbod van vetzuren aan het hart. Daarom is in Hoofdstuk 4 gekeken naar de effecten van progressieve calorier restrictie in gezonde vrijwilligers. Tijdens deze progressieve calorier restrictie namen de VZ in het plasma en de TG in het hart dosis-afhankelijk toe. Tegelijkertijd nam de diastolische functie dosis-afhankelijk af.

Kortdurende diëten met veel vet verhogen de hoeveelheid TG in skeletspieren. Daarnaast verhoogt een dieet met veel vet de hoeveelheid TG in het hart en induceert het myocardiale dysfunctie in diermodellen. De effecten van een kortdurende hoge vetbelasting in gezonde vrijwilligers zijn bestudeerd in Hoofdstuk 5. Het kortdurende dieet had geen effect op de hoeveelheid TG in het hart en de linker ventrikel functie. De hepatische TG namen wel toe. De data

beschrijven fysiologische en orgaan-specifieke aanpassingen van de hoeveelheid TG tijdens een vetbelasting.

Het cardiale metabolisme van patiënten met type 2 diabetes mellitus (DM2) is vooral afhankelijk van vetzuren. In dieren en mensen is deze toegenomen afhankelijkheid van vetzuren geassocieerd met structurele veranderingen in het diabetische hart en met cardiale dysfunctie. Om de flexibiliteit van TG in het hart te bestuderen is in Hoofdstuk 6 gekeken naar de effecten van een kortdurend ZLCD in patiënten met DM2. De hoeveelheid myocardiale TG nam toe, terwijl de diastolische functie van de linker ventrikel afnam. Daarnaast bleek dat anti-lipolytische therapie met acipimox tijdens het ZLCD deze effecten op de hoeveelheid TG en de hartspierfunctie kon voorkomen. De hoeveelheid TG in de lever was onveranderd na beide interventies. De studie illustreert de flexibiliteit van myocardiale TG en hartspierfunctie in patiënten met DM2. Daarnaast benadrukken de data de relevantie van de effecten van vetzuren in het bloed op het hart tijdens een ZLCD bij patiënten met DM2.

In Hoofdstuk 7 worden de effecten van therapeutisch gewichtsverlies in insuline-behandelde patiënten met DM2 en overgewicht beschreven. Overgewicht en DM2 zijn belangrijke risicofactoren voor cardiovasculaire ziekten, en langdurige calorierestrictie induceert gewichtsverlies en verbetert de glycemische conditie. In deze studie zijn de effecten van langdurige calorierestrictie op de hoeveelheid myocardiale TG, de hoeveelheid hepatische TG en op de hartspierfunctie geëvalueerd. Tijdens substantieel gewichtsverlies traden er duidelijke metabole verbeteringen op, samen met een afname van de hoeveelheid TG in het hart en een afname van de hoeveelheid hepatische TG. Daarnaast verbeterde de diastolische functie van de linker ventrikel. De data laten zien dat de myocardiale TG en de hartspierfunctie in obese patiënten met DM2 flexibel zijn, en zich aanpassen tijdens een therapeutische interventie bestaande uit calorierestrictie.

Patiënten met type 1 diabetes mellitus (DM1) ondervinden regelmatig episodes van hyperglycemische dysregulatie, door de suboptimale behandeling met exogeen insuline, wat de endogene insulinesecretie nabootst. De hyperglycemische episodes gaan samen met veranderingen in het vetmetabolisme. Om deze reden is in Hoofdstuk 8 gekeken naar de effecten van gecontroleerde, kortdurende hyperglycemie in patiënten met DM1. Ondanks de hyperglycemische dysregulatie, geïnduceerd door partiële insuline deprivatie, en de toename van VVZ in het plasma, waren de hoeveelheid myocardiale TG en de hartspierfunctie onveranderd. Blijkbaar is het hart van patiënten met DM1 beschermd tegen de kortdurende effecten van partiële insuline deprivatie.

Concluderend kunnen myocardiale TG betrouwbaar gemeten worden met ¹HMRS. Myocardiale TG zijn flexibel in gezonde vrijwilligers en in patiënten met DM2 tijdens veranderingen in de

dietaire inname. Veranderingen in myocardiale TG zijn geassocieerd met veranderingen in de functie van de linker ventrikel. Myocardiale TG zijn een reflectie van de discrepantie tussen de opname en oxidatie van vetzuren in het hart, en waarschijnlijk van de intracellulaire beschikbaarheid van vetzuur intermediären, welke de structuur en functie van het hart kunnen aantasten. Redistributie van TG is orgaan-specifiek, omdat TG in het hart en in de lever onafhankelijk een respons laten zien tijdens fysiologische interventies. Het hart van patiënten met DM1 is beschermd tegen de metabole effecten van kortdurende hyperglycemische dysregulatie, met betrekking tot de hoeveelheid myocardiale TG en de linker ventrikel functie.

List of publications

FULL PAPERS

1. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A, Romijn JA, Smit JWA. Short-term Flexibility of Myocardial Triglycerides and Diastolic Function in Patients with Type 2 Diabetes Mellitus. *Am J Physiol Endocrinol Metab* 2008; 295(3):E714-E718.
2. Hammer S, Snel M, 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* 2008; 52(12):1006-1012.
3. Hammer S, Jonker JT, Lamb HJ, van der Meer RW, Zondag W, Sepers JM, de Roos A, Smit JWA, Romijn JA. Short-term Hyperglycemic Dysregulation in Patients with Type 1 Diabetes Mellitus does not Change Myocardial Triglyceride Content or Myocardial Function. *Diabetes Care* 2008; 31(8):1613-1614.
4. Hammer S, van der Meer RW, Lamb HJ, Fröhlich M, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Smit JWA. Effects of Short-term High-fat, High-energy Diet on Hepatic and Myocardial Triglyceride Content in Healthy Men. *J Clin Endocrinol Metab* 2008; 93(7):2702-2708.
5. Hammer S, van der Meer RW, Lamb HJ, Schär M, Smit JWA, de Roos A, Romijn JA. Progressive Caloric Restriction Induces Dose-dependent Changes in Myocardial Triglyceride Content and Diastolic Function in Healthy Men. *J Clin Endocrinol Metab* 2008; 93(2):497-503.
6. van der Meer RW, Hammer S, Smit JWA, Frölich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term Caloric Restriction Induces Accumulation of Myocardial Triglycerides and Decreases Left Ventricular Diastolic Function in Healthy Subjects. *Diabetes* 2007; 56(12):2849-2853.
7. van der Meer RW, Doornbos J, Kozerke S, Schär M, Bax JJ, Hammer S, Smit JWA, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic Imaging of Myocardial Triglyceride: Reproducibility of ¹HMR Spectroscopy Using Respiratory Navigator Gating. *Radiology* 2007; 245(1):251-257.
8. Rijzewijk LJ, van der Meer RW, Smit JWA, Diamant M, Bax JJ, Hammer S, Romijn JA, de Roos A, Lamb HJ. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J Am Coll Cardiol*, in press.

9. van der Meer RW, Rijzewijk LJ, Diamant M, Hammer S, Schär M, Bax JJ, Smit JWA, Romijn JA, de Roos A, Lamb HJ. The Aging Male Heart: Myocardial Triglyceride Content as Independent Predictor of Diastolic Function. *Eur Heart J* 2008; 29(12):1516-1522.
10. Lamb HJ, Smit JWA, van der Meer RW, Hammer S, Doornbos J, de Roos A, Romijn JA. Metabolic MR Imaging of Myocardial and Hepatic Triglyceride Content. *Curr Opin Clin Nutr MetabCare*, 2008; 11(5):573-579.
11. van der Schee MPC, Hammer S, Schot R, Dragonieri S, Mertens BJA, Romijn JA, Pijl H, Sterk PJ. Assessment of Metabolic State in Type 1 Diabetes Mellitus by Electronic Nose. *Submitted*.
12. Dekkers OM, Hammer S, de Keizer RJW, Roelfsema F, Schutte PJ, Smit JWA, Romijn JA, Pereira AM. The Natural Course of Non-functioning Pituitary Macroadenomas. *Eur J Endocrinol* 2007; 156(2):219-226.

ABSTRACTS (first author)

1. Hammer S, Snel M, Lamb HJ, Jazet IM, van der Meer RW, 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. 68th Scientific Sessions, American Diabetes Association, San Francisco, USA, 2008.
2. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, de Roos A, Romijn JA, Smit JWA. Acipimox Reverses Myocardial Triglyceride Accumulation and Decreased Diastolic Function in Patients with Type 2 Diabetes Mellitus after a Very low-calorie Diet. 68th Scientific Sessions, American Diabetes Association, San Francisco, USA, 2008.
3. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, de Roos A, Romijn JA, Smit JWA. Acipimox Reverses Myocardial Triglyceride Accumulation and Decreased Diastolic Function in Patients with Type 2 Diabetes Mellitus after a Very low-calorie Diet. *European Congress of Endocrinology, Berlin, Germany, 2008*.
4. Hammer S, Snel M, Lamb HJ, Jazet IM, van der Meer RW, Meinders AE, Romijn JA, de Roos A, Smit JWA. Weight Loss in Type 2 Diabetes Mellitus is Associated with Decreased Myocardial Triglyceride Content and Improved Left Ventricular Function. *NVDO jonge onderzoekers bijeenkomst, Amersfoort, 2008*.
5. Hammer S, Snel M, Lamb HJ, Jazet IM, van der Meer RW, Meinders AE, Romijn JA, de Roos A, Smit JWA. Weight Loss in Type 2 Diabetes Mellitus is Associated with Decreased Myocardial Triglyceride Content and Improved Left Ventricular Function. *Society for Cardiovascular Magnetic Resonance, 11th Annual meeting, Los Angeles, USA, 2008*.

6. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, de Roos A, Romijn JA, Smit JWA. Metabolic Imaging in Diabetes: Modulations in Non-esterified fatty Acids are Associated with Myocardial Triglyceride Content and Diastolic Function. *Society for Cardiovascular Magnetic Resonance, 11th Annual meeting, Los Angeles, USA, 2008.*
7. Hammer S, van der Meer RW, Lamb HJ, Smit JWA, de Roos A, Romijn JA. Metabolic Imaging in Diabetes: Modulations in Non-esterified fatty Acids are Associated with Myocardial Triglyceride Content and Diastolic Function. *LVM Wetenschapsdag, LUMC, 2007.*
8. Hammer S, van der Meer RW, Lamb HJ, Schär M, Smit JWA, de Roos A, Romijn JA. Lipotoxicity in Humans: Effects of a Very low-calorie Diet and Starvation on Myocardial Triglyceride Content and Cardiac Function. *67th Scientific Sessions, American Diabetes Association, Chicago, USA, 2007.*
9. Hammer S, van der Meer RW, Lamb HJ, Schär M, Smit JWA, de Roos A, Romijn JA. Lipotoxicity in Humans: Effects of a Very low-calorie Diet and Starvation on Myocardial Triglyceride Content and Cardiac Function. *25th Annual Meeting of the Anglo-Danish-Dutch Diabetes Group, Copenhagen, Denmark, 2007.*
10. Hammer S, van der Meer RW, Lamb HJ, Schär M, Smit JWA, de Roos A, Romijn JA. Dose-dependent Effects of Caloric Restriction on Myocardial TG Accumulation, Assessed with ¹HMRS. *Society for Cardiovascular Magnetic Resonance, 10th Annual meeting, Rome, Italy, 2007.*
11. Hammer S, van der Meer RW, Lamb HJ, Schär M, Smit JWA, de Roos A, Romijn JA. Lipotoxicity in Humans: Effects of Caloric Restriction on Myocardial Lipid Accumulation Assessed with ¹HMRS". *NVDO jonge onderzoekers bijeenkomst, Amersfoort, 2007.*
12. Hammer S, van der Meer RW, Lamb HJ, Schär M, Smit JWA, de Roos A, Romijn JA. Hepatic and Myocardial TG Content, Assessed by ¹HMRS, have a Short-term Flexibility, but show Opposite Changes during Short-term Low Calorie Diet in Healthy Subjects. *LVM-ICar-VU wetenschapsdag, Vumc, 2006.*

Nawoord

In de eerste plaats gaat mijn dank uit naar alle patiënten en gezonde vrijwilligers die hebben deelgenomen aan de studies. Daarnaast de vrijwilligers (het waren er te veel om bij te houden) die hielpen de scantechnieken te optimaliseren.

Alle collega's (met name de kamergenoten van K5) en het ondersteunend personeel van de endocrinologie, radiologie en algemene interne geneeskunde bedank ik voor de samenwerking en de gezelligheid op borrels en congressen. In het bijzonder het PIRAMID team; het was een ervaring op zich een schakel in de keten te zijn.

Rutger, de eerste decepties van de mislukte spectro staan in mijn geheugen gegrift. Ik lag er zo nu en dan wakker van. Dat duurde gelukkig niet al te lang, ik heb veel van je geleerd. Naast alle praktische samenwerking heb ik het relativeren van je afgekeken. Het fietsen heeft daarbij geholpen en alle hobbels die volgden tijdens mijn promotie kon ik met meer gemak nemen. Ik ben er trots op tegelijk met jou te mogen promoveren.

Marieke, onze onderzoekslijnen hebben tot een mooie samenwerking geleid, ondanks dat de studie MRI-technisch verre van gladjes is verlopen. Het was een feest met je samen te werken.

Voor de studie met patiënten met type 1 diabetes heb ik veel steun gehad aan Wendy, die tijdens haar wetenschapsstage veel werk uit handen heeft genomen, en aan diabetesverpleegkundige Marja Dijk-Schaap. Jouw betrokkenheid bij de patiënten heeft er in belangrijke mate voor gezorgd dat zij met vertrouwen hun insulines halveerden.

Jacqueline, het is een veelbelovend onderzoeksveld en de voortzetting zie ik graag tegemoet!

De hechte vriendschappen en de echte betrokkenheid maakten het mij onmogelijk om niet met enige regelmaat een biertje te drinken, met CN'88 of Kartel. Zoals het hoort, komen de echte discussies na een paar glazen bier. We zetten het voort. Hans-Henk, je hebt mij ontzettend geholpen, ik vertrouw er op dat we binnen enkele jaren jouw promotiefeest vieren.

Vincent en Thijs, jullie zijn de personificatie van twee prachtige periodes. Ik ben er trots op dat jullie mijn paranimfen willen zijn.

Pap en mam. Jullie hebben mij alle ruimte geboden deze weg te kiezen. Dit proefschrift is het resultaat en ik draag het met trots aan jullie op. Femme en Sander, Ben en Francisca, ieder van ons volgt zijn eigen weg. Ik vind het prachtig dat we samen inmiddels zo'n breed maatschappelijk veld weten te dekken.

Maartje, jouw luisterend oor heeft het zo nu en dan zwaar te verduren gehad. Je bent van onschatbare waarde voor mij en ik ben blij dat zwart op wit nog eens te kunnen benadrukken.

Sebastiaan Hammer
Leiden, november 2008

Curriculum Vitae

Sebastiaan Hammer werd geboren op 31 december 1981 te Utrecht. Na het Atheneum (Adriaen Pauw College te Heemstede en Kaj Munk College te Hoofddorp) startte hij in 2000 met de opleiding Geneeskunde aan de Universiteit Leiden.

In 2002-2003 nam hij zitting in het bestuur van de Medische Faculteit der Leidse Studenten. In 2004 werd de Honours Class van de Universiteit Leiden "Stress, from biology to public issue" gevolgd. Daarnaast volgde hij de "Join the Board" stage bij de Raad van Bestuur van het Leids Universitair Medisch Centrum. Onder begeleiding van Dr. A.M. Pereira verrichtte hij in 2004 als student onderzoek op de afdeling Endocrinologie van het Leids Universitair Medisch Centrum naar het natuurlijk beloop van niet-functionerende hypofyse macroadenomen.

Zijn afstudeeronderzoek vond plaats op de afdelingen Endocrinologie en Radiologie en beschreef de effecten van calorierestrictie op stapeling van triglyceriden in het hart. In 2005 behaalde hij het doctoraalexamen. In januari 2006 startte hij vervolgens met het promotieonderzoek, onder begeleiding van Prof. Dr. J.W.A. Smit, Prof. Dr. J.A. Romijn, Prof. Dr. A. de Roos en Dr. H.J. Lamb, waarvan de resultaten in dit proefschrift staan beschreven. In maart 2008 startte hij met co-schappen.

