Christophe Noll,[1] Margaret Kunach,[1] Frédérique Frisch,[1] Lucie Bouffard,[1] Stéphanie Dubreuil,[1] Farrah Jean-Denis,[1] Serge Phoenix,[2] Stephen C. Cunnane,[3] Brigitte Guérin,[2] Eric E. Turcotte,[2] and André C. Carpentier[1,2]

Seven-Day Caloric and Saturated Fat Restriction Increases Myocardial Dietary Fatty Acid Partitioning in Impaired Glucose-Tolerant Subjects

Diabetes 2015;64:3690–3699 | DOI: 10.2337/db15-0337

METABOLISM

Subjects with impaired glucose tolerance (IGT) have increased myocardial partitioning of dietary fatty acids (DFAs) with left ventricular dysfunction, both of which are improved by modest weight loss over 1 year induced by lifestyle changes. Here, we determined the effects of a 7-day hypocaloric diet (−500 kcal/day) low in saturated fat (<7% of energy) (LOWCAL study) versus isocaloric with the usual amount saturated fat (~10% of energy) diet (ISOCAL) on DFA metabolism in subjects with IGT. Organ-specific DFA partitioning and cardiac and hepatic DFA fractional uptake rates were measured in 15 IGT subjects (7 males/8 females) using the oral 14(*R*,*S*)-[^{18}F]-fluoro-6-thia-heptadecanoic acid positron emission tomography method after 7 days of an ISOCAL diet versus a LOWCAL diet using a randomized crossover design. The LOWCAL diet led to reductions in weight and postprandial insulin area under the curve. Myocardial DFA partitioning over 6 h was increased after the LOWCAL diet (2.3 ± 0.1 vs. 1.9 ± 0.2 mean standard uptake value, *P* < 0.04). However, the early (90–120 min) myocardial DFA fractional uptake was unchanged after the LOWCAL diet (0.055 ± 0.025 vs. 0.046 ± 0.009 min^{-1}, *P* = 0.7). Liver DFA partitioning was unchanged, but liver fractional uptake of DFA tended to be increased. Very short-term caloric and saturated fat dietary restrictions do not lead to the same changes in organ-specific DFA metabolism as those associated with weight loss in subjects with IGT.

In industrialized and developing countries, reductions in dietary quality and physical activity strongly contribute to the development of chronic diseases such as obesity, type 2 diabetes, and cardiovascular disorders (1,2). While physical activity has decreased in the past few years, the energy intake, principally from fat and refined carbohydrate, has markedly increased. Abnormal fatty acid metabolism plays a major role in the pathophysiology of type 2 diabetes by altering insulin sensibility and glucose homeostasis (3,4). Adipose tissue samples from prediabetic and diabetic subjects show inefficient storage of dietary fatty acids (DFAs) (4,5), increasing the "spillover" of triglycerides (TGs) and nonesterified fatty acid (NEFA) in circulation during the postprandial state. This fatty acid spillover appears to be directly related to abdominal obesity in subjects with type 2 diabetes (6), resulting in an increased exposition of some, but not all, lean tissues to DFA (7).

There is strong experimental support for a deleterious effect of fatty acids on insulin sensitivity and β-cell function in humans (3). Short-term dietary trials in humans (8–10) have shown that saturated fatty acid (SFA) substitution for monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs) may lead to insulin resistance and more β-cell dysfunction. A change in myocardial fatty acid metabolism is also associated with diabetic cardiomyopathy (11). In animal and human dietary trials, the replacement of TG-raising carbohydrates with monounsaturated or n3 polyunsaturated fats (12) improved cardiac function. Recently, using the noninvasive method of positron emission tomography (PET) imaging with the oral administration of 14(*R*,*S*)-[^{18}F]-fluoro-6-thia-heptadecanoic acid ([^{18}F]-FTHA), a long-chain fatty

[1]Division of Endocrinology, Department of Medicine, Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke, Université de Sherbrooke, Sherbrooke, Quebec, Canada
[2]Department of Nuclear Medicine and Radiobiology, Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke, Université de Sherbrooke, Quebec, Canada
[3]Research Center on Aging, Université de Sherbrooke, Sherbrooke, Quebec, Canada

Corresponding author: André C. Carpentier, andre.carpentier@usherbrooke.ca.

Received 10 March 2015 and accepted 17 July 2015.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0337/-/DC1.

© 2015 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

acid analog (13), we have shown that subjects with impaired glucose tolerance (IGT) display an increase in myocardial DFA uptake and partitioning (defined as the relative biodistribution of DFA among all organs) that is associated with reduced left ventricular function (14). These metabolic and functional cardiac abnormalities were corrected after a 1-year lifestyle intervention with modest weight loss (15). As this lifestyle intervention included an individually tailored change in diet and physical activity, it was not possible to determine whether weight loss, change in insulin sensitivity, change in diet, or change in physical activity was responsible for correction of the cardiac DFA metabolic abnormalities.

The aim of the current study was to determine the effect of a 7-day caloric restriction with reduction of SFA intake, an intervention known to significantly improve insulin sensitivity (9), on cardiac and whole-body DFA metabolism. The hypothesis was that a short-term, low-calorie, low-SFA diet, with improvement in insulin sensitivity, when compared with an isocaloric (ISOCAL) diet, would be associated with a reduction in cardiac DFA uptake and partitioning and improvement in white adipose tissue DFA partitioning in individuals with IGT.

RESEARCH DESIGN AND METHODS

Study Participants

Fifteen Caucasian subjects (seven men and eight women between 44 and 65 years of age) with glucose intolerance, which was defined as having results from a 2-h post–75-g oral glucose tolerance test, undertaken after a 12-h overnight fast, of between 7.8 and 11.1 mmol/L on two occasions, underwent two postprandial metabolic studies using a randomized crossover design. Subjects with a history or clinical evidence of any cardiac disorder; any evidence of kidney, liver, or thyroid dysfunction; or any uncontrolled medical or surgical condition were excluded. Subjects who had received any antidiabetic medication (except metformin), β-blockers, or fibrates; had a history of any dietary or severe past allergic reaction; or had participated in any research trial involving radiation exposure within the past 12 months were also excluded. One male patient was removed from postprandial analyses because of technical problems with postprandial data acquisition during one of the two protocols. Those subjects who had dyslipidemia and were receiving treatment with a statin (three participants) or had hypertension and were treated with antihypertensive agents (four participants) had to stop taking these medications 3 weeks and 7 days, respectively, prior to the metabolic assessments. No women involved in this study were receiving hormonal contraceptive agents or menopausal hormone therapy. All metabolic studies performed in premenopausal women were performed during the follicular phase of the menstrual cycle.

Experimental Protocols

The subjects followed two different diets: an ISOCAL diet (0% alcohol, 17% proteins, 50% carbohydrates, and 33% fats [34% saturated fat, 66% monounsaturated fat and polyunsaturated fat]) and a hypocaloric diet low in saturated fat (LOWCAL diet) (<7% of total energy compared with ~10% for the ISOCAL diet; 500 kcal/day less than the ISOCAL diet; 0% alcohol, 17% proteins, 50% carbohydrates, and 33% fats [20% saturated fat, 80% monounsaturated fat and polyunsaturated fat]) for 7 days before undergoing each experimental protocol. Diets were administered in random order, and a washout period of 3–4 weeks was observed between the two diets. An experienced research nutritionist prescribed and supervised dietary intake every other day during the ISOCAL and LOWCAL interventions. The participants were asked to report food intake using a food diary with a scale to weigh the food ingested. Nutritional characteristics of the ISOCAL and LOWCAL diets were determined using Nutrific software (https://nutrific.fsaa.ulaval.ca). The subjects were instructed to maintain their regular physical activities and avoid strenuous exercise during the dietary intervention. During the 7 days of the ISOCAL and LOWCAL diets, each participant wore an accelerometer (GT3X+; ActiGraph, Pensacola, FL) to control for any variation in their physical activity.

On arrival at the test center on the morning of both meal tests, after a 12-h overnight fast, body weight, height, and waist circumference were measured, and lean body mass was determined with a Body Composition Analyzer (TBF 300A; Tanita Corporation of America, Arlington Heights, IL). An intravenous catheter was placed in one forearm for infusions, and another was placed in a distal vein in the contralateral arm and was maintained in a heating pad (55°C) for blood sampling.

Each participant underwent a 6-h postprandial experimental protocol after both the 7-day LOWCAL and ISOCAL diets, in which a standard liquid meal prepared as described (6) was consumed over 20 min (400 mL for a total of 906 kcal, 33 g or 33% as fat, 34 g or 17% as proteins, and 101 g or 50% as carbohydrates). Blood samples were collected in tubes containing Na_2 EDTA and orlistat (30 mg/mL; Roche, Mississauga, Ontario, Canada) to prevent in vitro TG lipolysis.

From time −60 min in the fasting state of the protocol to time 360 min after meal intake, a constant infusion of [7,7,8,8-D_4]-potassium palmitate (0.01 $\mu mol \cdot L^{-1} \cdot kg^{-1} \cdot min^{-1}$ in 100 mL 25% human serum albumin; Cambridge Isotopes Laboratories Inc.) and [1,1,2,3,3-^2H]-glycerol (0.05 $\mu mol \cdot L^{-1} \cdot kg^{-1} \cdot min^{-1}$ in saline; Cambridge Isotopes Laboratories Inc.) were administered, preceded by an intravenous bolus of [1,1,2,3,3-^2H]-glycerol (1.6 $\mu mol \cdot L^{-1} \cdot kg^{-1}$) and $NaHCO_3$ (1.2 $\mu mol \cdot L^{-1} \cdot kg^{-1}$; Cambridge Isotopes Laboratories Inc.) to prime the bicarbonate pool. In the liquid meal, [U-^{13}C]-palmitate (10.8 $\mu mol \cdot L^{-1} \cdot kg^{-1}$; Cambridge Isotopes Laboratories Inc.) was added.

Each subject was placed in the supine position and scanned using a 16-slice PET/computed tomography (CT) scanner (Gemini TF; Philips, Eindhoven, the Netherlands) (13). At time −60 min before meal intake, [^{11}C]-acetate (~185 MBq) was administered intravenously,

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

and electrocardiogram gating was performed, allowing the application of PET ventriculography to determine cardiac function, as previously published (14,15).

We used our novel oral [^{18}F]-FTHA method (13) to determine whole-body DFA partitioning. [^{18}F]-FTHA given orally is absorbed through chylomicron-TG, delivered into the circulation from the thoracic duct, and redistributes normally into other circulating fatty acid pools (13). This method assumes [^{18}F]-FTHA-containing chylomicron-TG hydrolysis and uptake into local tissue microcirculation, which are similar to those of chylomicron-TGs not containing [^{18}F]-FTHA. At time 0 min, ~70 MBq [^{18}F]-FTHA, produced as previously described (16), was mixed into Intralipid 20% (Baxter, Mississauga, Ontario, Canada), incorporated into gel capsules (T U B Enterprises), and given orally with the liquid meal. A dynamic list-mode PET scan centered on the thoracoabdominal segment was performed between time 90 and 120 min, and a whole-body PET/CT acquisition at time 360 min was performed as previously described (13). The maximal gastrointestinal tract radioactivity exposure for oral [^{18}F]-FTHA administration was estimated at 2.35 mSv in the stomach. The total radioactivity exposure to the participants was 19.54 mSv, including the two protocols. All tracers were tested for sterility and pyrogenicity.

PET/CT Image Analyses

For dynamic PET acquisitions, myocardial and hepatic fractional DFA uptakes (K_i) were determined using Patlak linearization (17) as previously described (14,15). For whole-body scans, the mean value of pixels (mean standard uptake value [SUV]) for all tissues of interest was recorded (14,15). Liver volume was extrapolated by drawing the liver surface on each 5 mm slice of the whole-body CT scan at time 360 min using OsiriX 5.8.2 software (Pixmeo SARL, Bernex, Switzerland).

The cardiac blood flow index (K_1 in min^{-1} of [^{11}C]-acetate, with correction for first-pass tissue extraction [18]) and oxidative metabolism index (K_2 in min^{-1} of [^{11}C]-acetate) were estimated from [^{11}C]-acetate using a three-compartment model (19), as previously published (7). For analysis of ventricular function, PET data from [^{11}C]-acetate images were analyzed as previously described (14,15).

Laboratory Assays and Assessment of Insulin Resistance

Glucose, insulin, C-peptide, total NEFA, and TG levels were measured as previously described (6). Chylomicrons and plasma lipids were separated by ultracentrifugation and assayed for [^{18}F] activity and TG concentration (13). The Matsuda index was determined as previously described (20).

Calculations of Plasma Palmitate, NEFA, Glycerol Appearance Rates, and Lipid Oxidative Rates

The plasma palmitate appearance rate ($Ra_{palmitate}$), plasma NEFA appearance rate (Ra_{NEFA}), plasma glycerol appearance rate ($Ra_{glycerol}$), fractional palmitate oxidation ($FOx_{palmitate}$), plasma palmitate oxidation ($Ox_{palmitate}$), and net total body carbohydrate oxidation (CHOox) and fatty acid oxidation (FA_{ox}) were calculated as previously described (21). Total energy expenditure was estimated by indirect calorimetry during the postprandial state.

Statistical Analyses

Data are expressed as the mean ± SEM. A Wilcoxon test, Mann-Whitney *U* test, and two-way ANOVA for repeated measures were used when appropriate. Spearman correlations were performed to examine the association between variables. A two-tailed *P* value <0.05 was considered significant. All analyses were performed with Prism version 6.0 for Macintosh (GraphPad, San Diego, CA).

Study Approval

Informed written consent was obtained, in accordance with the Declaration of Helsinki, and the protocol received approval from the Human Ethics Committee of the Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke.

RESULTS

Dietary Intake During the ISOCAL and LOWCAL Diets

The aim of this study was to induce a decrease in energy intake of ~500 kcal/day during 1 week with a saturated fat intake of <7% of energy (Table 1). The participants reduced their energy intake by 546.8 ± 20.8 kcal/day ($P < 0.0001$) and their total postprandial energy expenditure by 34.9 ± 14.8 kcal · 370 min^{-1} ($P < 0.02$) in the LOWCAL diet versus the ISOCAL diet. Saturated fat intake was 6.6 ± 0.1% during the 7-day LOWCAL diet (−4.5 ± 0.2% vs. ISOCAL diet, $P < 0.0001$). Although we attempted to keep the percentage of energy from proteins, carbohydrates, and total fats the same between the LOWCAL and ISOCAL diets, there was a marginal but statistically significant increase in the percentage of energy from carbohydrates during the LOWCAL diet (0.6 ± 0.2%, $P < 0.004$). The decrease in the percentage of energy from saturated fats was compensated by an increase in the percentage of energy from MUFAs and PUFAs (MUFA+PUFA; 4.2 ± 0.3%, $P < 0.0001$) during the LOWCAL diet. There was also a significant decrease in fiber content during the LOWCAL diet (−3.1 ± 0.6 g/day, $P < 0.0009$).

Effect of Caloric and Saturated Fat Restriction on Anthropometric Parameters, Postprandial Plasma Metabolites, and Insulin Levels

After the LOWCAL diet, the participants had lost a significant amount of weight (−0.9 ± 0.3 kg, $P < 0.002$) and BMI (−0.3 ± 0.1 kg · m^{-2}, $P < 0.002$), but had no significant variation in their waist circumference (Table 2). The participants lost almost as much lean mass (−0.4 ± 0.2 kg, $P < 0.08$) as nonlean mass (−0.5 ± 0.2 kg, $P = 0.14$). Furthermore, IGT subjects had a significant reduction in their liver volume after the LOWCAL diet (−124.5 ± 49.3 cm^3, $P < 0.04$). We found no variation in fasting plasma glucose, NEFA, and adiponectin levels. However, we found a significant decrease in fasting levels of insulin (−8.1 ± 4.1 pmol · L^{-1},

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

Table 1—Dietary intake

	ISOCAL diet	LOWCAL diet	P*
Energy intake, kcal · day^{-1}	2,348 ± 95	1,801 ± 96	<0.0001
AUC$_{0–370}$ TEE, kcal · min^{-1} · 370 min^{-1}	439.0 ± 21.2	404.1 ± 20.2	<0.02
Carbohydrates, g · day^{-1}	293.2 ± 11.8	227.6 ± 12.4	<0.0001
Carbohydrates, % of energy	49.8 ± 0.1	50.4 ± 0.1	<0.004
Proteins, g · day^{-1}	101.7 ± 4.0	77.1 ± 4.1	<0.0001
Proteins, % of energy	17.3 ± 0.1	17.1 ± 0.1	0.19
Fats, g · day^{-1}	86.2 ± 3.5	65.4 ± 3.4	<0.0001
Fats, % of energy	33.0 ± 0.1	32.7 ± 0.1	<0.09
SFA, g · day^{-1}	29.2 ± 1.1	13.5 ± 0.9	<0.0001
SFA, % of energy	11.2 ± 0.1	6.6 ± 0.1	<0.0001
SFA, % of total fat	33.9 ± 0.4	20.4 ± 0.5	<0.0001
MUFA+PUFA, g · day^{-1}	57.0 ± 2.5	51.9 ± 2.5	0.0001
MUFA+PUFA, % of energy	21.8 ± 0.2	26.0 ± 0.2	<0.0001
MUFA+PUFA, % of total fat	66.0 ± 0.4	79.6 ± 0.5	<0.0001
Cholesterols, mg	246.6 ± 19.3	150.4 ± 17.5	0.0001
Fibers, g · day^{-1}	25.3 ± 1.7	22.1 ± 1.7	0.0009

Values are mean ± SEM. TEE, total energy expenditure. *P values are derived from a Wilcoxon test.

$P < 0.05$), TG (-0.3 ± 0.1 mmol · L^{-1}, $P < 0.05$), and leptin (-2.8 ± 0.8 ng · mL^{-1}, $P < 0.002$). Postprandial excursion levels of plasma glucose, insulin, C-peptide, NEFA, TG, and chylomicron-TG are shown in Fig. 1. During the postprandial state, we found a significant decrease in postprandial area under the curve for insulin at 60–360 minutes (AUC$_{60–360}$ insulin) ($-22,144 \pm 7,458$ pmol · L^{-1} · 300 min^{-1}, $P < 0.02$) and a trend toward reduction in AUC$_{60–360}$ C-peptide (-85.2 ± 87.8 ng · mL^{-1} · 300 min^{-1}, $P < 0.06$). We also found a significant decrease in AUC$_{60–360}$ TG (-77.3 ± 24.3 mmol · L^{-1} · 300 min^{-1}, $P < 0.02$) and a trend toward a reduction in AUC$_{120–360}$ chylomicron-TG (-15.8 ± 7.8 mmol · L^{-1} · 240 min^{-1}, $P < 0.07$). After the LOWCAL diet, no variation was found for the total postprandial plasma [^{18}F] activity, [^{18}F]-NEFA activity, total [^{18}F]-TG activity, and [^{18}F]-chylomicron activity (Fig. 2*A–D*). Finally, the participants did not change their physical activity (-22.8 ± 35.8 kcal · day^{-1}, P = 0.78).

Organ-Specific Partitioning of DFAs and Myocardial and Hepatic DFA Uptake

After the LOWCAL diet, we found a significant 16.4% increase in myocardial partitioning (0.32 ± 0.14 SUV, $P < 0.04$) (Fig. 3*C*) and a significant 28.6% increase in skeletal muscle (quadriceps femoris) partitioning (0.11 ± 0.05 SUV, $P < 0.05$) (Fig. 3*E*). The increase in myocardial DFA partitioning with LOWCAL tended to be higher in women (ΔSUV 0.037 ± 0.272 vs. 0.537 ± 0.099 in men, P = 0.13) (Supplementary Fig. 2A). No variations in DFA partitioning were found in the liver (Fig. 3*D*) and in subcutaneous (Fig. 3*F* and *G*) and perirenal (Fig. 3*H*) adipose tissue depots. No variation in myocardial DFA fractional uptake was found between diets (Fig. 4*A*), but hepatic DFA fractional uptake tended to be higher (0.017 ± 0.009 min^{-1}, $P < 0.09$) (Fig. 4*B*) after the LOWCAL diet. No gender difference was observed in the response of myocardial and hepatic DFA fractional uptake between the two diets (Supplementary Fig. 3*A* and *B*).

Palmitate, NEFA, and Glycerol Appearance Rates; Palmitate Concentration, and Net Substrate Oxidation Rates

After the LOWCAL diet, we found a significant decrease in AUC$_{0–360}$ Ra$_{palmitate}$ ($-10,290 \pm 6,577$ μmol · min^{-1} · 360 min^{-1}, $P < 0.04$) (Supplementary Fig. 1A). No variation was found for Ra$_{NEFA}$, Ra$_{glycerol}$, palmitate concentration, Fox$_{palmitate}$, Ox$_{palmitate}$, CHO$_{ox}$, and FA$_{ox}$ with two-way ANOVA or Mann-Whitney *U* test for AUC (Supplementary Fig. 1*B–H*).

Left Ventricular Function, Blood Flow, and Oxidative Metabolism

Left ventricular ejection fraction (LVEF) was significantly decreased after the LOWCAL diet when compared with the ISOCAL diet (-2.9 ± 0.5%, $P < 0.008$) (Table 3). We also found a trend toward an increase in end-systolic volume after the LOWCAL diet (2.4 ± 1.1 mL, $P < 0.07$) and a trend toward decrease in cardiac output (-384 ± 156 mL · min^{-1}, $P < 0.06$), cardiac index (-0.19 ± 0.08 mL · min^{-1} · m^{-2}, $P < 0.06$), and diastolic blood pressure (-2.8 ± 1.3 mmHg, $P < 0.06$). No variations were found in blood flow index and oxidative metabolism.

Correlation

We found a strong negative correlation between the change in MUFA+PUFA, the change in dietary fiber intake or leptin level, and the change in myocardial DFA level partitioning (Table 4). We also found that the reduction

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

Table 2—Characteristics of the participants and effect of the LOWCAL diet

	ISOCAL diet	LOWCAL diet	P*
Age, years	57.6 ± 1.7		
Sex, *n*			
Male	7		
Female	8		
Weight, kg	84.2 ± 3.6	83.3 ± 3.6	<0.002
BMI, kg · m^{-2}	30.0 ± 1.0	29.7 ± 1.0	<0.002
Waist circumference, cm	97.7 ± 3.0	97.3 ± 2.9	0.2
Lean mass, kg	53.2 ± 2.6	52.8 ± 2.6	<0.08
Nonlean mass, kg	30.9 ± 2.2	30.5 ± 2.1	0.14
Matsuda index	9.5 ± 1.2	14.2 ± 3.9	<0.06
Energy expenditure from activities, kcal · day^{-1}	513.0 ± 61.8	490.1 ± 60.4	0.78
Fasting glucose, mmol · L^{-1}	5.1 ± 0.2	5.1 ± 0.2	0.89
Fasting insulin, pmol · L^{-1}	103.5 ± 12.8	95.4 ± 13.5	<0.05
Fasting NEFA, μmol · L^{-1}	573 ± 46	589 ± 58	0.99
Fasting TG, mmol · L^{-1}	1.6 ± 0.2	1.3 ± 0.2	<0.03
Fasting leptin, ng · mL^{-1}	13.3 ± 3.0	10.5 ± 2.3	<0.002
Fasting adiponectin, ng · mL^{-1}	5,902 ± 832	5,013 ± 682	0.13
$AUC_{60–360}$ glucose, mmol · L^{-1} · 300 min^{-1}	2,050 ± 76	2,064 ± 74	0.89
$AUC_{60–360}$ insulin, pmol · L^{-1} · 300 min^{-1}	115,953 ± 12,393	93,585 ± 11,778	<0.02
$AUC_{60–360}$ C-peptide, ng · mL^{-1} · 300 min^{-1}	2,152 ± 184	2,067 ± 199	<0.06
$AUC_{60–360}$ NEFA, μmol · L^{-1} · 300 min^{-1}	87.7 ± 8.9	99.4 ± 11.3	0.19
$AUC_{60–360}$ TG, mmol · L^{-1} · 300 min^{-1}	690.5 ± 59.8	613.2 ± 58.9	<0.02
$AUC_{120–360}$ chylomicron-TG, mmol · L^{-1} · 240 min^{-1}	87.4 ± 8.1	71.6 ± 10.8	<0.07
Liver radiodensity, Hounsfield units	46.1 ± 2.5	45.8 ± 2.4	0.79
Liver volume, cm^3	1,567 ± 73	1,442 ± 57	<0.04

Values are reported as the mean ± SEM. **P* values are derived from a Wilcoxon test.

in liver radio-density correlated with the increase in myocardial DFA partitioning of DFA.

DISCUSSION

We previously found that subjects with IGT display an increase in myocardial DFA uptake that is associated with decreased left ventricular function (14,22), an abnormality that was corrected by modest weight loss after a 1-year individually tailored lifestyle intervention (15). Here, we found that the improvement of insulin resistance with a 7-day hypocaloric, low-SFA diet did not change early 2-h postprandial myocardial DFA uptake in subjects with IGT. In contrast to our initial hypothesis, we found that a LOWCAL diet increased cardiac DFA partitioning over 6 h after meal intake despite significant improvement in insulin sensitivity, a small but significant degree of weight loss, and excellent compliance of the subjects with the dietary interventions. DFA partitioning in white adipose tissue also did not change significantly with the LOWCAL diet, which was reflected by the unchanged postprandial total plasma NEFA concentration and Ra_{NEFA}, and the unchanged oxidation rate, although the plasma $Ra_{palmitate}$ was slightly reduced.

The mechanism for the LOWCAL diet–induced increase in cardiac DFA partitioning is not clear, but we can rule out an increase in cardiac chylomicron-TG uptake and an increase in NEFA spillover as possible causes. Taken together, the observation of a higher early liver fractional DFA uptake without an increase in liver DFA partitioning at 6 h suggests an increase in hepatic turnover of DFA. As fasting plasma TG levels were significantly reduced, this may suggest an increase in VLDL-TG clearance that is associated with an increased secretion rate. This could in turn lead to increased transport of DFA to the heart via VLDL-TG uptake (23). As insulin is known to suppress VLDL-TG secretion (24), it is possible that lower postprandial insulin levels led to an increase in VLDL-TG secretion. However, we found no correlation between either the change in insulin level or the change in insulin sensibility (by Matsuda index) and the change in cardiac DFA partitioning. Unfortunately, the transport of [^{18}F]-FTHA into VLDL-TG was not measured in the current study. In contrast, the increase in cardiac DFA partitioning was significantly correlated with reduced leptin level. Leptin is known to increase cardiac fatty acid oxidation in animals (25). On the other hand, in *db/db* mice, a murine model of nonfunctional leptin receptor, TGs accumulate in myocardial cells (26) through an increase of peroxisome proliferator–activated receptor α target genes, including lipoprotein lipase (27). [^{18}F]-FTHA

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

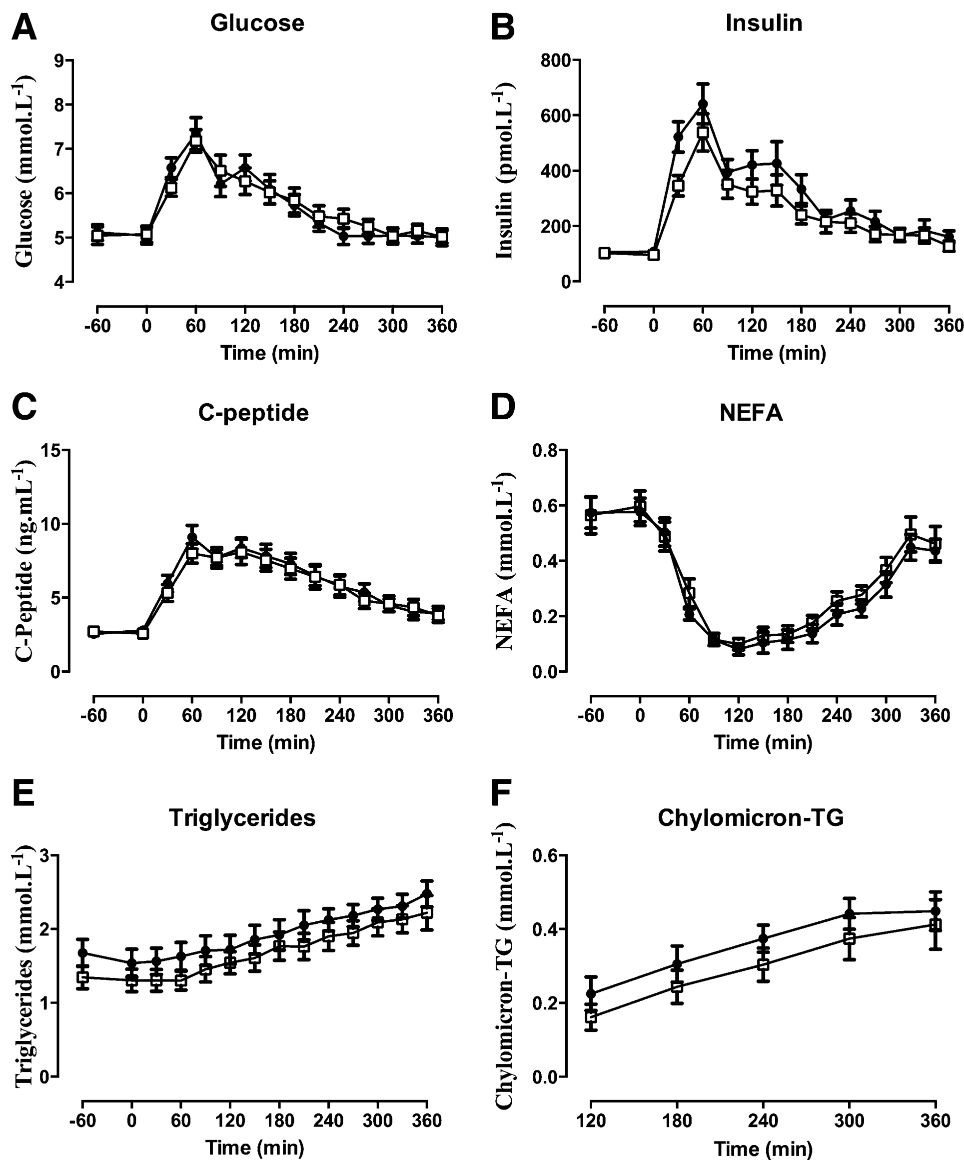

Figure 1—Postprandial plasma levels of glucose (*A*), insulin (*B*), C-peptide (*C*), NEFAs (*D*), TGs (*E*), and chylomicron-TG (*F*) after the ISOCAL diet (closed circles) and after the LOWCAL diet (open squares). Data are reported as the mean ± SEM.

accumulates in oxidative and nonoxidative metabolic pathways in the heart (16). Therefore, a shift toward nonoxidative fatty acid metabolism would not be detectable by our method.

Diabetes is an important risk factor for heart failure: each 1% elevation of hemoglobin A_{1c} is associated with a 15% increase in the risk of heart failure (28,29). At least part of this risk can be attributed to diabetic cardiomyopathy that is characterized by reduced myocardial energetic efficiency (17,30) (i.e., increased oxidative metabolism in the face of a similar or reduced mechanical work). Type 2 diabetes is associated with increased cardiac NEFA uptake and oxidation during the fasting state both in men (31) and in women (32). Prediabetic and diabetic subjects are also characterized by an increase in intramyocardial TG content (33–36). An increase in myocardial fatty acid use and oxidation and a decrease in myocardial energetic efficiency can predispose subjects to ischemic damage and cardiomyocyte apoptosis (30,35). Cardiac lipoprotein lipase–mediated lipolysis is also thought to play a role in diabetic and metabolic cardiomyopathies in animals (37–39). In the current study, the increase in cardiac DFA partitioning was associated with reduced LVEF and a trend toward reduction in cardiac output. This is consistent with our previous studies showing an association between increased cardiac DFA partitioning and reduced left ventricular function in humans (14,15) and rats (17). However, diastolic blood pressure was also reduced together with reduced postprandial energy expenditure, a likely explanation for the reduction in cardiac output. Furthermore, a reduction in plasma NEFA levels with acipimox treatment over 1 week was shown to reduce left ventricular function

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

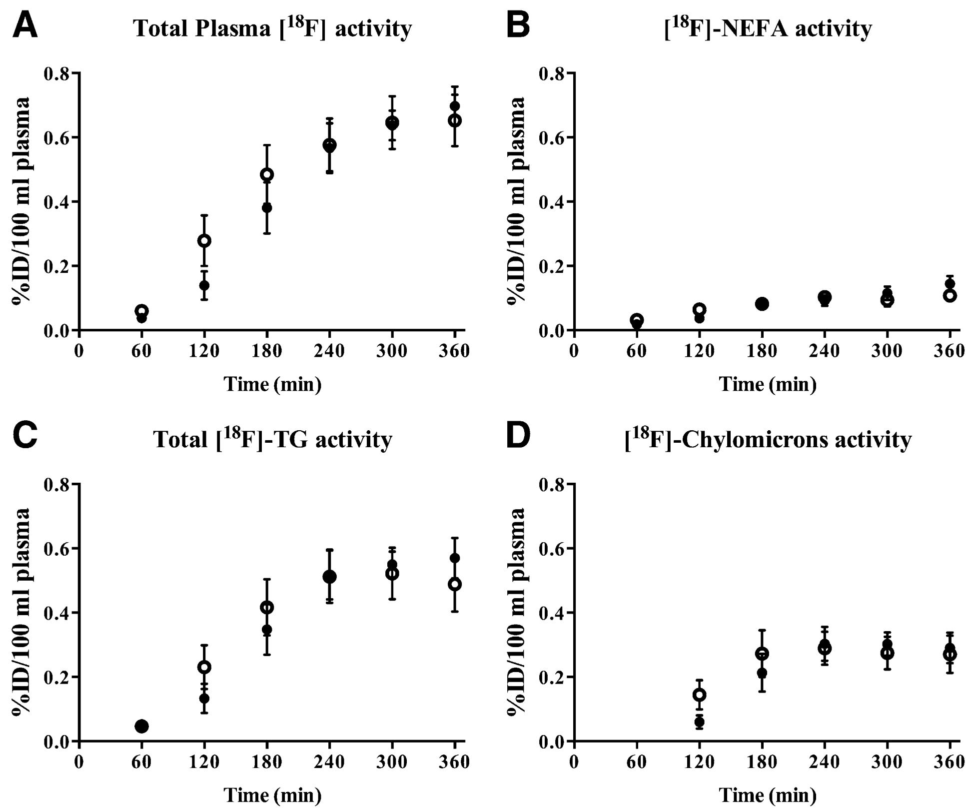

Figure 2—Postprandial levels of total plasma (*A*), NEFAs (*B*), total TGs (*C*), and chylomicron (*D*) [^{18}F] activity after the ISOCAL diet (closed circles) and after the LOWCAL diet (open squares). %ID, percentage ingested dose. Data are reported as the mean ± SEM.

(40). In subjects with type 2 diabetes, a 3-day very low-calorie diet (471 kcal/day, but with SFA >13%) induced an increase in myocardial TG levels and reduced diastolic function, but with no change in LVEF measured by MRI (41). Fatty acid uptake by the heart is essential for intramyocellular TG repletion and the maintenance of cardiac function (42,43). It is therefore premature to postulate that enhanced cardiac DFA partitioning can be mechanistically linked to a reduction in LVEF in the current study. The small 2–3% reduction in LVEF within the normal range that we observed in the current study is likely not clinically significant. Whether these changes may be clinically relevant in patients with cardiomyopathies remains to be determined.

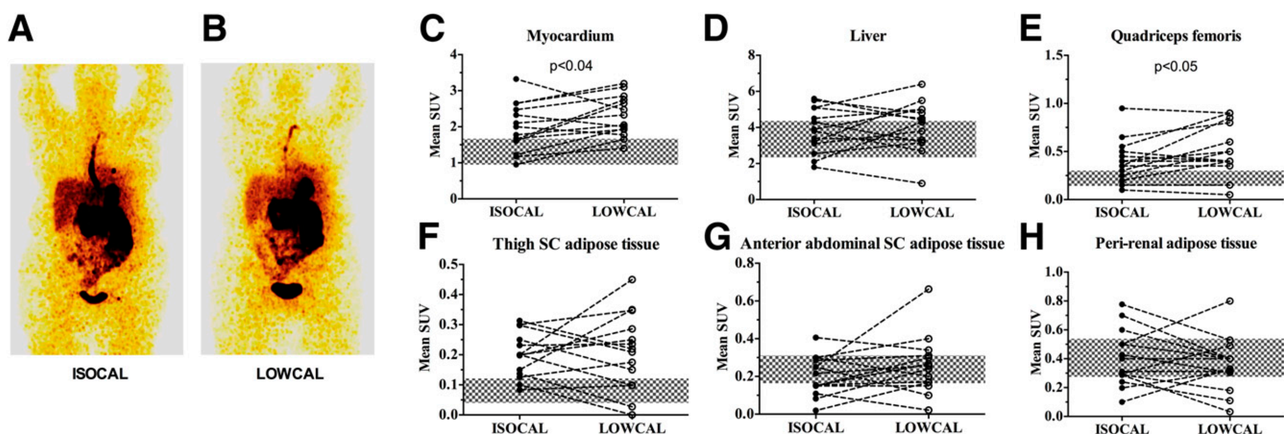

Figure 3—Organ-specific partitioning of DFAs. Anteroposterior coronal whole-body PET acquisition performed 6 h after oral ingestion of [^{18}F]-FTHA after the ISOCAL diet (*A*) and after the LOWCAL diet (*B*). SUVs from whole-body PET scans in the heart (*C*), liver (*D*), skeletal muscle (*E*), thigh subcutaneous (SC) adipose tissue (*F*), anterior SC abdominal adipose tissue (*G*), and perirenal adipose tissue (*H*). *P* values are derived from a Wilcoxon test.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

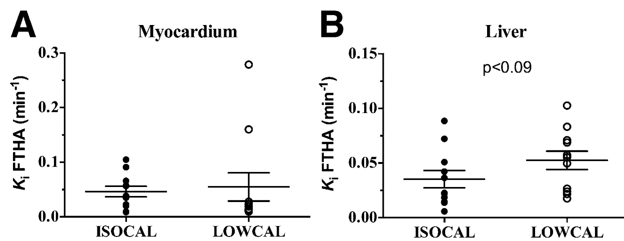

Figure 4—Fractional DFA uptake in the heart and the liver. Myocardial (*A*) and hepatic (*B*) fractional DFA uptake rate (K_i) after the ISOCAL diet (closed circles) and after the LOWCAL diet (open circles). *P* values are derived from a Wilcoxon test.

The dietary fiber content was not exactly the same between the LOWCAL and ISOCAL diets. Dietary fibers are plant constituents that are resistant to digestion and absorption by the human small intestine with complete or partial fermentation in the large intestine and are classified into the following two types: water soluble, which represents ~20% of total dietary fiber intake, and water insoluble, which represents the remaining 80% (44). In the current study, we found that a reduction in total dietary fibers intake was associated with increased cardiac DFA partitioning. Water-soluble fibers are associated with a decrease in cardiovascular events by lowering LDL cholesterol levels (44,45). Serum TG concentration is not modified by water-soluble fibers (45), but a study (46) in type 2 diabetes subjects has shown that high intake of water-soluble plus insoluble fibers could decrease serum TG concentration. However, the amount of total dietary fiber intake that was administered in the latter study was twice the amount that our subject consumed during the dietary interventions, and plasma TG levels fell concomitantly with the reduction in total fiber intake. Bacterial fermentation of dietary fibers in the colon induces biosynthesis of short-chain fatty acids such as acetate, propionate, and butyrate (47). Butyrate decreases the synthesis of adipose TG lipase (48), which contributes to myocardial uptake and the oxidation of fatty acids (49). An increase in acetate production by the intestinal microbiota could induce an increase in the production of acetyl-CoA in myocardial cells, which in turn could induce an increase in malonyl-CoA, an allosteric inhibitor of carnitine palmitoyltransferase 1, the rate-limiting enzyme of β-oxidation (49). We did not measure plasma or stool levels of butyrate and acetate in the current study. Future studies will be needed to address the effect of the change in dietary fiber intake, microbiota metabolism, and cardiac DFA metabolism.

An important limitation of our work is the small number of participants. This limitation is due to the complexity and costs of the metabolic protocols used. Despite the small number of participants, the a priori power of our study for most outcomes ranged between 70% and 95%. The small number of men and women limits our power to detect sex differences in the contribution of adipose tissues versus direct chylomicron-TG uptake to cardiac DFA partitioning (22). Another limitation of our study is that the PET venticulography method may not be as sensitive as cardiac MRI or echocardiography to detect changes in left ventricular diastolic function.

In conclusion, the current study demonstrates that a short-term, moderate caloric restriction with low SFA levels and improvement of insulin resistance induces an increase in cardiac DFA partitioning, which is associated with a decrease in LVEF in individuals with IGT. Neither the change in direct chylomicron-TG uptake nor the change in NEFA spillover from white adipose tissue can explain these findings. Therefore, short-term caloric and SFA dietary restrictions do not lead to the same changes in cardiac and adipose tissue DFA metabolism as those associated with weight loss induced by lifestyle changes in subjects with IGT. Lower total fiber intake was closely

Table 3—Left ventricular volumes, function, blood flow index, and oxidative metabolism index

	ISOCAL diet	LOWCAL diet	*P**
ESV, mL	40.1 ± 6.1	42.5 ± 6.0	<0.07
EDV, mL	102.9 ± 10.2	102.1 ± 10.2	0.83
Stroke volume, mL	62.8 ± 4.5	59.5 ± 4.5	0.13
LVEF, %	62.6 ± 2.4	59.8 ± 2.2	<0.008
Heart rate, bpm	73.2 ± 3.2	71.2 ± 2.8	0.29
Cardiac output, mL · min^{-1}	4,668 ± 512	4,284 ± 435	<0.06
Cardiac index, L · min^{-1} · m^{-2}	2.46 ± 0.20	2.26 ± 0.16	<0.06
Systolic blood pressure, mmHg	134 ± 3	133 ± 3	0.45
Diastolic blood pressure, mmHg	82 ± 3	79 ± 3	<0.06
RPP, mmHg · min^{-1}	8,871 ± 450	8,635 ± 476	0.18
Myocardial blood flow index, min^{-1}	0.780 ± 0.046	0.744 ± 0.044	0.49
Myocardial oxidative metabolism index (K_2), min^{-1}	0.072 ± 0.033	0.039 ± 0.010	0.42

Values are reported as the mean ± SEM. EDV, end-diastolic volume; ESV, end-systolic volume; RPP, rate pressure product. **P* values are derived from a Wilcoxon test.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

Table 4—Correlations

	Heart (ΔSUV)
Caloric intake (Δkcal · day^{-1})	ρ = 0.334
Fiber (Δg)	ρ = −0.713*
SFA (Δg)	ρ = 0.220
MUFA+PUFA (Δg)	ρ = −0.515*
Fasting insulin (Δpmol · L^{-1})	ρ = 0.000
Fasting leptin (Δng · mL^{-1})	ρ = −0.579*
$AUC_{60–360}$ insulin (Δpmol · L^{-1} · 300 min^{-1})	ρ = −0.039
Matsuda index	ρ = −0.044
Liver radiodensitometry (ΔHU)	ρ = −0.810*

HU, Hounsfield units. *$P < 0.05$, Spearman correlation test.

associated with increased cardiac DFA partitioning in the current study. Future studies will be needed to test the effect of dietary fiber intake on cardiac DFA metabolism.

Funding. This work was supported by a grant from the Canadian Institutes of Health Research (MOP 53094 to A.C.C.) and was performed at Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke, a research center funded by Fonds de Recherche du Québec–Santé. C.N. is the recipient of a Canadian Institutes of Health Research postdoctoral fellowship.

Duality of Interest. The Canadian Institutes of Health Research-GlaxoSmithKline Chair in Diabetes, which is held by A.C.C., was created in part through a donation of $1 million by GlaxoSmithKline to the Université de Sherbrooke. A.C.C. holds the Canadian Institutes of Health Research–GlaxoSmithKline Chair in Diabetes. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. C.N. was responsible for the collection, analysis, and interpretation of the data and drafted the article or revised it critically for important intellectual content. M.K., F.F., L.B., S.D., F.J.-D., S.P., S.C.C., B.G., and E.E.T. were responsible for the collection, analysis, and interpretation of the data. A.C.C. was responsible for the collection, analysis, and interpretation of the data; drafted the article or revised it critically for important intellectual content; and was responsible for the conception and design of the experiments. A.C.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. Lancet 2014;383:1999–2007
2. Mozaffarian D, Benjamin EJ, Go AS, et al.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2015 update: a report from the American Heart Association. Circulation 2015;131:e29–e322
3. Giacca A, Xiao C, Oprescu AI, Carpentier AC, Lewis GF. Lipid-induced pancreatic β-cell dysfunction: focus on in vivo studies. Am J Physiol Endocrinol Metab 2011;300:E255–E262
4. Carpentier AC, Labbe SM, Grenier-Larouche T, Noll C. Abnormal dietary fatty acid metabolic partitioning in insulin resistance and type 2 diabetes. Clin Lipidol 2011;6:703–716
5. Bickerton AST, Roberts R, Fielding BA, et al. Adipose tissue fatty acid metabolism in insulin-resistant men. Diabetologia 2008;51:1466–1474
6. Normand-Lauzière F, Frisch F, Labbé SM, et al. Increased postprandial nonesterified fatty acid appearance and oxidation in type 2 diabetes is not fully established in offspring of diabetic subjects. PLoS One 2010;5:e10956
7. Labbé SM, Croteau E, Grenier-Larouche T, et al. Normal postprandial nonesterified fatty acid uptake in muscles despite increased circulating fatty acids in type 2 diabetes. Diabetes 2011;60:408–415
8. Vessby B, Uusitupa M, Hermansen K, et al.; KANWU Study. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 2001;44:312–319
9. Bachmann OP, Dahl DB, Brechtel K, et al. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. Diabetes 2001;50:2579–2584
10. Xiao C, Giacca A, Carpentier A, Lewis GF. Differential effects of monounsaturated, polyunsaturated and saturated fat ingestion on glucose-stimulated insulin secretion, sensitivity and clearance in overweight and obese, non-diabetic humans. Diabetologia 2006;49:1371–1379
11. Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. Diabetologia 2014;57:660–671
12. Stanley WC, Dabkowski ER, Ribeiro RF Jr, O'Connell KA. Dietary fat and heart failure: moving from lipotoxicity to lipoprotection. Circ Res 2012;110:764–776
13. Labbé SM, Grenier-Larouche T, Croteau E, et al. Organ-specific dietary fatty acid uptake in humans using positron emission tomography coupled to computed tomography. Am J Physiol Endocrinol Metab 2011;300:E445–E453
14. Labbé SM, Grenier-Larouche T, Noll C, et al. Increased myocardial uptake of dietary fatty acids linked to cardiac dysfunction in glucose-intolerant humans. Diabetes 2012;61:2701–2710
15. Labbé SM, Noll C, Grenier-Larouche T, et al. Improved cardiac function and dietary fatty acid metabolism after modest weight loss in subjects with impaired glucose tolerance. Am J Physiol Endocrinol Metab 2014;306:E1388–E1396
16. Ci X, Frisch F, Lavoie F, et al. The effect of insulin on the intracellular distribution of 14(R,S)-[^{18}F]Fluoro-6-thia-heptadecanoic acid in rats. Mol Imaging Biol 2006;8:237–244
17. Ménard SL, Croteau E, Sarrhini O, et al. Abnormal in vivo myocardial energy substrate uptake in diet-induced type 2 diabetic cardiomyopathy in rats. Am J Physiol Endocrinol Metab 2010;298:E1049–E1057
18. van den Hoff J, Burchert W, Börner AR, et al. [1-(11)C]Acetate as a quantitative perfusion tracer in myocardial PET. J Nucl Med 2001;42:1174–1182
19. Klein LJ, Visser FC, Knaapen P, et al. Carbon-11 acetate as a tracer of myocardial oxygen consumption. Eur J Nucl Med 2001;28:651–668
20. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–1470
21. Brassard P, Frisch F, Lavoie F, et al. Impaired plasma nonesterified fatty acid tolerance is an early defect in the natural history of type 2 diabetes. J Clin Endocrinol Metab 2008;93:837–844
22. Kunach M, Noll C, Phoenix S, et al. Effect of sex and impaired glucose tolerance on organ-specific dietary fatty acid metabolism in humans. Diabetes 2015;64:2432–2441
23. Teusink B, Voshol PJ, Dahlmans VEH, et al. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. Diabetes 2003;52:614–620
24. 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;95:158–166
25. Lee Y, Naseem RH, Duplomb L, et al. Hyperleptinemia prevents lipotoxic cardiomyopathy in acyl CoA synthase transgenic mice. Proc Natl Acad Sci USA. 2004;101:13624–13629
26. Hall ME, Maready MW, Hall JE, Stec DE. Rescue of cardiac leptin receptors in db/db mice prevents myocardial triglyceride accumulation. Am J Physiol Endocrinol Metab 2014;307:E316–E325
27. Finck BN, Lehman JJ, Leone TC, et al. The cardiac phenotype induced by PPARalpha overexpression mimics that caused by diabetes mellitus. J Clin Invest 2002;109:121–130

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024

28. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405–412
29. Iribarren C, Karter AJ, Go AS, et al. Glycemic control and heart failure among adult patients with diabetes. Circulation 2001;103:2668–2673
30. van de Weijer T, Schrauwen-Hinderling VB, Schrauwen P. Lipotoxicity in type 2 diabetic cardiomyopathy. Cardiovasc Res 2011;92:10–18
31. Rijzewijk LJ, van der Meer RW, Lamb HJ, et al. Altered myocardial substrate metabolism and decreased diastolic function in nonischemic human diabetic cardiomyopathy: studies with cardiac positron emission tomography and magnetic resonance imaging. J Am Coll Cardiol 2009;54:1524–1532
32. McGill JB, Peterson LR, Herrero P, et al. Potentiation of abnormalities in myocardial metabolism with the development of diabetes in women with obesity and insulin resistance. J Nucl Cardiol 2011;18:421–429
33. Kankaanpää M, Lehto H-R, Pärkkä JP, et al. 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;91:4689–4695
34. McGavock JM, Lingvay I, Zib I, et al. Cardiac steatosis in diabetes mellitus: a ^{1}H-magnetic resonance spectroscopy study. Circulation 2007;116:1170–1175
35. Szczepaniak LS, Victor RG, Orci L, Unger RH. Forgotten but not gone: the rediscovery of fatty heart, the most common unrecognized disease in America. Circ Res 2007;101:759–767
36. Iozzo P. Viewpoints on the way to the consensus session: where does insulin resistance start? The adipose tissue. Diabetes Care 2009;32(Suppl. 2): S168–S173
37. Ghosh S, An D, Pulinilkunnil T, et al. Role of dietary fatty acids and acute hyperglycemia in modulating cardiac cell death. Nutrition 2004;20:916–923
38. Goldberg IJ, Trent CM, Schulze PC. Lipid metabolism and toxicity in the heart. Cell Metab 2012;15:805–812
39. Yagyu H, Chen G, Yokoyama M, et al. Lipoprotein lipase (LpL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. J Clin Invest 2003;111:419–426
40. Lehto H-R, Pärkkä J, Borra R, et al. Effects of acute and one-week fatty acid lowering on cardiac function and insulin sensitivity in relation with myocardial and muscle fat and adiponectin levels. J Clin Endocrinol Metab 2012;97:3277–3284
41. Hammer S, van der Meer RW, Lamb HJ, et al. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. Am J Physiol Endocrinol Metab 2008;295:E714–E718
42. Guzzardi MA, Hodson L, Guiducci L, et al. Independent effects of circulating glucose, insulin and NEFA on cardiac triacylglycerol accumulation and myocardial insulin resistance in a swine model. Diabetologia 2014;57:1937–1946
43. Lahey R, Wang X, Carley AN, Lewandowski ED. Dietary fat supply to failing hearts determines dynamic lipid signaling for nuclear receptor activation and oxidation of stored triglyceride. Circulation 2014;130:1790–1799
44. Theuwissen E, Mensink RP. Water-soluble dietary fibers and cardiovascular disease. Physiol Behav 2008;94:285–292
45. Erkkilä AT, Lichtenstein AH. Fiber and cardiovascular disease risk: how strong is the evidence? J Cardiovasc Nurs 2006;21:3–8
46. Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. N Engl J Med 2000;342:1392–1398
47. Weickert MO, Pfeiffer AFH. Metabolic effects of dietary fiber consumption and prevention of diabetes. J Nutr 2008;138:439–442
48. Ohira H, Fujioka Y, Katagiri C, et al. Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages. J Atheroscler Thromb 2013;20:425–442
49. Lopaschuk GD, Ussher JR, Folmes CDL, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. Physiol Rev 2010;90:207–258

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/64/11/3690/577341/db150337.pdf by guest on 09 September 2024