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

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OBJECTIVE—Diabetes and obesity are associated with increased plasma nonesterified fatty acid (NEFA) levels, myocardial triglyceride 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 triglyceride content and cardiac function in healthy subjects.

RESEARCH DESIGN AND METHODS—Fourteen healthy nonobese men underwent ^{1}H-magnetic resonance spectroscopy (MRS) to determine myocardial and hepatic triglyceride content, ^{31}P-MRS 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 0.5 ± 0.1 to 1.1 ± 0.1 mmol/l, $P < 0.05$). Concomitantly, myocardial triglyceride content increased by ~55% compared with that at baseline (from 0.38 ± 0.05 to 0.59 ± 0.06%, $P < 0.05$), whereas liver triglyceride content decreased by ~32% (from 2.2 ± 0.5 to 1.5 ± 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 × 10^{-3}, $P < 0.05$). This decrease in diastolic function was significantly correlated with the increase in myocardial triglyceride content.

CONCLUSIONS—Short-term VLCD induces accumulation of myocardial triglycerides. In addition, VLCD decreases left ventricular diastolic function, without alterations in myocardial HEP metabolism. This study documents diet-dependent physiological variations in myocardial triglyceride content and diastolic function in healthy subjects. ***Diabetes* 56:2849–2853, 2007**

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Received for publication 6 June 2007 and accepted in revised form 21 August 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 23 August 2007. DOI: 10.2337/db07-0768.

AMARES, Advanced Magnetic RESonance; ECG, electrocardiogram; HEP, high-energy phosphate; jMRUI, Java-based magnetic resonance user interface; LV, left ventricular; MR, magnetic resonance; MRI, MR imaging; MRS, MR spectroscopy; NEFA, nonesterified fatty acid; TR, repetition time; VLCD, very low–calorie diet.

In diabetes and obesity, plasma nonesterified fatty acid (NEFA) levels are elevated because of excessive lipolysis in adipose tissue (1). In animal models of type 2 diabetes and obesity, excessive plasma NEFA levels result in accumulation of myocardial triglycerides (2,3). In these models, triglyceride 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 triglyceride 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 triglyceride accumulation has been demonstrated ex vivo in myocardial tissue of type 2 diabetic patients (10) and patients with heart failure (11). Recently, myocardial ^{1}H-magnetic resonance spectroscopy (MRS) has been developed and validated to measure myocardial triglyceride content in humans in vivo (12,13). Using this technique, a relation between BMI and myocardial triglyceride content was suggested (12,14). However, dynamic changes in myocardial triglyceride 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 triglyceride content and myocardial function in healthy subjects. Therefore, the purpose of the present study was to determine the effect of a VLCD on myocardial triglyceride content and cardiac function in healthy subjects, using ^{1}H-MRS, ^{31}P-MRS, and cardiac magnetic resonance imaging (MRI). Each subject was studied twice, before and after 3 days of VLCD. ^{31}P-MRS was used to assess myocardial HEP metabolism. Cardiac MRI was used to assess myocardial function in detail. Furthermore, hepatic triglyceride content was assessed concomitantly using ^{1}H-MRS to study the tissue-specific effects of a VLCD.

RESEARCH DESIGN 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,

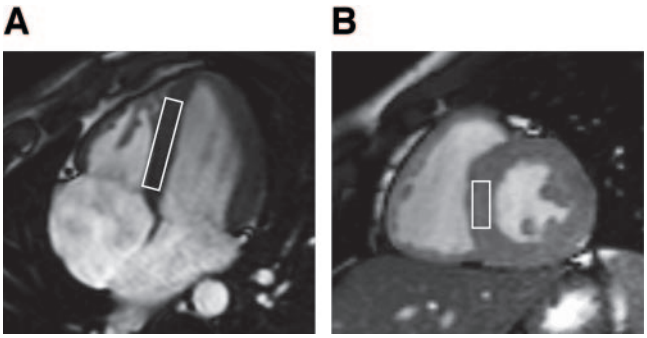

FIG. 1. Myocardial voxel localization for ^{1}H-MRS. Voxel position in four-chamber (*A*) and short-axis (*B*) views.

alanine aminotransferase, aspartate aminotransferase, 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 magnetic resonance (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 MRI and MRS measurements. Furthermore, 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.

Proton MRS. All MRI/MRS studies were performed with the use of a 1.5-T whole-body MR scanner (Gyroscan ACS/NT15; Philips, Best, Netherlands) with subjects in supine position at rest. Myocardial ^{1}H-MR 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 (Fig. 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 of 26 ms and a repetition time (TR) of at least 3,000 ms. A total of 1,024 data points were collected using a 1,000-Hz spectral width and averaged over 128 acquisitions. Without changing any parameter, spectra without water suppression with a TR of 10 s and four averages were obtained to be used as an internal standard.

^{1}H-MRS 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 ^{1}H-MR 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 triglyceride 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 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 triglyceride content was calculated as a percentage relative to water: triglyceride/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 triglyceride-to-creatine ratio and the percentage of creatine (creatine/water × 100) were calculated.

Phosphorus MRS. A 100 mm–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 3D-ISIS. 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 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. ^{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 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).

LV 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 ECG-gated breath holds, with a sensitivity-encoding balanced turbofield echo sequence. Imaging parameters included the following: echo time = 1.67 ms, TR = 3.3 ms, flip angle = 35°, slice thickness = 10 mm with a gap of 0 mm, field of view = 400 mm^2, 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: echo time = 5 ms, TR = 14 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350 mm, 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 MRI, blood pressure and heart rate were measured.

Assays. Plasma glucose and triglyceride 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 (CVs) were <2% for glucose and triglycerides and <5% for insulin. Leptin and adiponectin were measured with radioimmunoassays from Linco Research (St. Charles, MO). For leptin, the CV 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, IL). Data are expressed as means ± SE. Between-group differences were calculated using a two-tailed dependent sample *T* test. Pearson *r* values were used for correlations. Significance was assumed at $P < 0.05$ (two tailed).

RESULTS

^{1}H-MRS and myocardial function were successfully assessed in all 14 subjects. In nine subjects, ^{31}P-MRS was successfully completed at both occasions. In the other five subjects, ^{31}P-MRS 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 1. All subjects performed exercise (walking, running, and/or biking) regularly (ranges 3–5 h weekly), but none of the subjects engaged in high-performance sports.

Myocardial and hepatic spectroscopy. Typical myocardial ^{1}H- and ^{31}P-MR spectra at baseline and after the VLCD of the same subject are shown in Fig. 2. After the VLCD, myocardial triglyceride content as well as the myocardial triglyceride-to-creatine ratio were increased compared with those at baseline (from 0.38 ± 0.05 to 0.59 ± 0.06% and from 3.11 ± 0.39 to 5.42 ± 0.71, respectively; $P < 0.05$), whereas the myocardial percentage of creatine did

TABLE 1
Characteristics of the study group at baseline and after VLCD

	Baseline	VLCD
BMI (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 nonesterified 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

Data are means ± SE. *$P < 0.05$ compared with baseline (paired t test).

not change (Fig. 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, $P > 0.05$).

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

Myocardial function. LV systolic function, represented by the ejection fraction, did not change (60 ± 1 vs. 60 ± 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 ± 0.16 ml/s^2 × 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 triglyceride content after the VLCD (Fig. 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, $P > 0.05$).

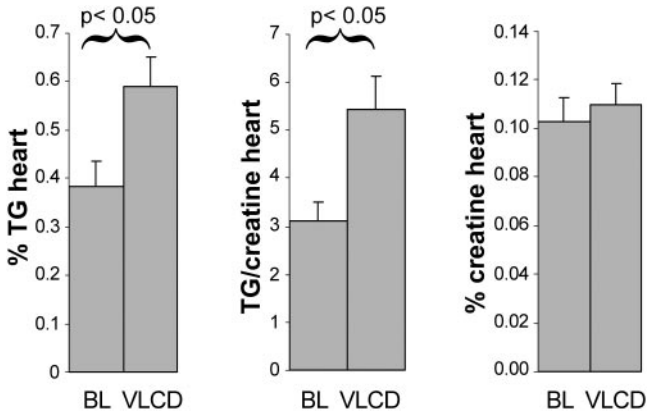

FIG. 3. Influence of a short-term VLCD on myocardial triglyceride and creatine content. A VLCD increased the percentage of myocardial triglycerides and the triglyceride-to-creatine ratio without changing the creatine-to-water ratio. Therefore, the increase in myocardial percentage of triglycerides assessed by MRS is the effect of an increase of myocardial triglycerides rather than of decreased myocardial water content. BL, baseline; TG, triglyceride.

DISCUSSION

This study shows that in healthy subjects, a short-term consumption of a VLCD increases myocardial triglyceride 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 triglyceride content in liver and myocardium. These observations stress the physiological flexibility of ectopic triglyceride 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 triglyceride content increases. Ap-

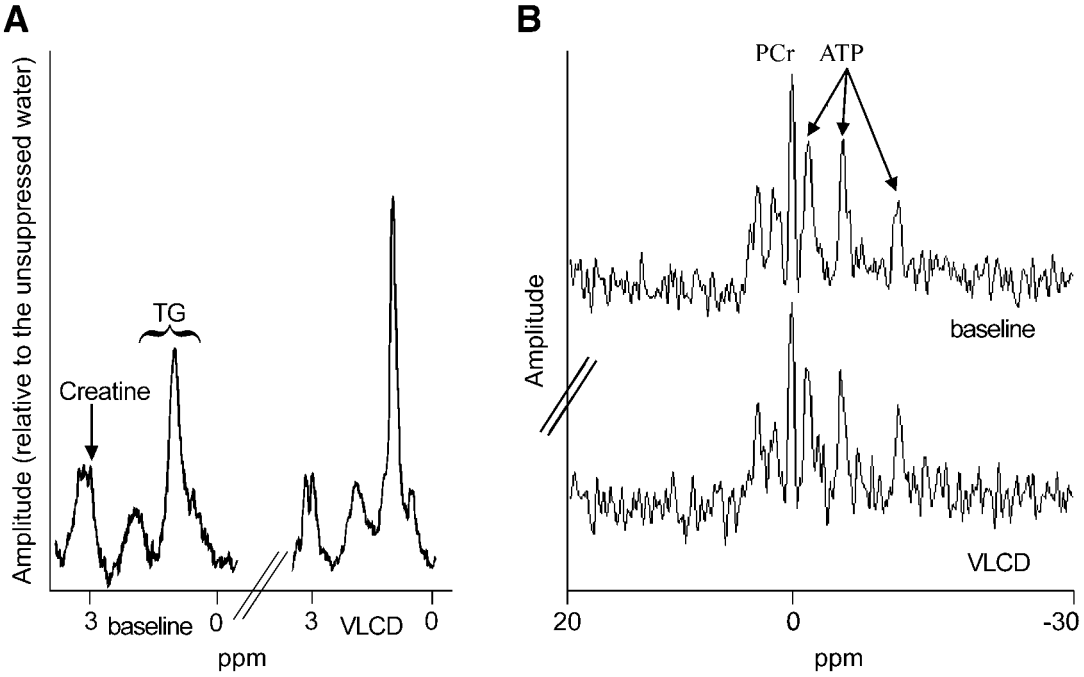

FIG. 2. Typical ^{1}H- and ^{31}P-MR spectra at baseline and after dietary intervention. *A*: ^{1}H-MR spectra at baseline and after a VLCD diet are displayed. Note the increase of the triglyceride signal amplitude at 0.9 and 1.3 ppm after the VLCD without a change in the creatine signal amplitude at 3.0 ppm. *B*: ^{31}P-MR spectra of one volunteer at baseline and after a VLCD are displayed. Note an unchanged phosphocreatine (PCr) and ATP signal. TG, triglyceride.

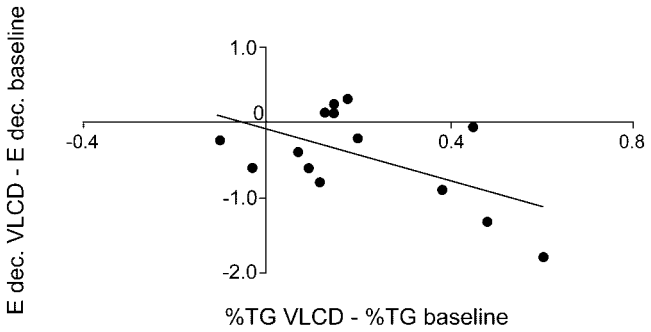

FIG. 4. Correlation between diastolic function and myocardial triglycerides. The decrease in LV diastolic function after the VLCD was significantly correlated with the increase in myocardial triglyceride content after the VLCD (Pearson $r = -0.55$, $P < 0.05$). %TG, myocardial percentage of triglycerides; E dec, deceleration of early diastolic flow across the mitral valve.

parently, myocardial fatty acid uptake is increased in relation to myocardial fatty acid oxidation during a VLCD.

Several animal models of type 2 diabetes and obesity demonstrated that excessive plasma NEFA levels result in accumulation of myocardial triglycerides (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 triglyceride 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 triglyceride content after a VLCD are in concordance with the findings of Reingold et al. (28), who showed increased myocardial triglyceride content after 48 h of fasting. Both conditions are associated with increased plasma NEFA levels. Since the myocardial triglyceride content measured by MRS is expressed relative to water, the VLCD-induced increase in myocardial triglyceride content may also be explained by a decrease of myocardial water content. Therefore, the myocardial triglyceride–to–creatine and creatine-to-water ratios were assessed additionally. The increased myocardial triglyceride-to-creatine ratio in the presence of the unchanged creatine-to-water ratio in our study supports our conclusion that the diet-related increase in myocardial triglyceride content assessed by MRS is due to increased myocardial triglyceride accumulation rather than decreased myocardial water content.

The increase in myocardial triglyceride content can be derived from plasma NEFA and/or plasma triglycerides. The heart is especially effective in removal of circulating triglycerides (29,30). Moreover, heart lipoprotein lipase activity increases during fasting (31). In contrast, however, VLCD decreased plasma triglyceride 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 NEFA versus plasma triglycerides to myocardial triglyceride stores.

In addition to increasing myocardial triglyceride 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 MRI (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 cannot 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 homeastosis (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 NEFA 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 cannot implicate myocardial triglyceride 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 triglyceride 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 triglyceride 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 triglyceride, hepatic triglyceride content decreased after the short-term VLCD. Westerbacka et al. (39) previously reported similar findings on the effects of dietary interventions on hepatic triglyceride 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 NEFA. 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 triglyceride content indicate differential, organ-specific mechanisms underlying tissue-specific partitioning of plasma triglycerides and/or fatty acids among nonadipose organs, at least with respect to the liver and the heart. Unfortu-

nately, the underlying mechanisms cannot be derived from the methods used in our study.

In conclusion, short-term VLCD induces accumulation of myocardial triglycerides. In addition, VLCD decreases LV diastolic function without alterations in myocardial HEP metabolism. This study documents diet-dependent, physiological variations in myocardial triglyceride 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.

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