

# Desaturation of Skeletal Muscle Structural and Depot Lipids in Obese Individuals during a Very-Low-Calorie Diet Intervention

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## Abstract

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**Objective:** This study investigated whether a very-low-calorie dietary intervention (VLCD) may influence composition of skeletal muscle cell membrane phospholipid and composition and concentration of intramyocellular triglyceride (IMTG) in obese subjects. The working hypothesis proposed that a VLCD would decrease saturated fatty acids (FAs) and increase long-chain polyunsaturated FAs (LCPUFAs) in muscular structural lipids, as such changes have been associated with improved insulin sensitivity.

**Research Methods and Procedures:** Skeletal muscle biopsies (vastus lateralis) were obtained from 13 obese subjects (nine women) before and after 8 weeks on VLCD (~600 to 800 kcal/d). FA composition in muscle cell membrane phospholipid and concentration and FA composition of IMTG were determined by gas-liquid chromatography.

**Results:** Baseline BMI was  $36.0 \pm 3.4$  kg/m<sup>2</sup>. Weight loss was  $9.3 \pm 1.1$  kg ( $8.8 \pm 1.1\%$ ;  $p < 0.0001$ ); loss of adipose tissue was  $5.9 \pm 0.9$  kg ( $p < 0.0001$ ). Insulin resistance (by homeostasis model assessment) decreased ( $-44 \pm 7\%$ ;  $p <$

$0.001$ ). Muscle cell membrane phospholipid saturated FAs decreased ( $-3.2 \pm 1.3\%$ ;  $p < 0.05$ ), whereas monounsaturated FAs ( $4.3 \pm 1.7\%$ ;  $p < 0.05$ ), LCPUFAs ( $11 \pm 6\%$ ;  $p < 0.05$ ), and the ratio of LCPUFAs to saturated FAs ( $12 \pm 5\%$ ;  $p < 0.05$ ) increased. IMTG decreased, but not significantly ( $-5\%$ ). IMTG-saturated FAs decreased ( $-3.3 \pm 1.5\%$ ;  $p < 0.05$ ), whereas LCPUFAn-3 ( $29 \pm 9\%$ ;  $p < 0.01$ ), LCPUFAn-6 ( $33 \pm 9\%$ ;  $p < 0.01$ ), and the ratio of LCPUFAs to saturated FAs ( $34 \pm 8\%$ ;  $p < 0.001$ ) increased. Plasma total cholesterol ( $-15 \pm 6\%$ ;  $p < 0.05$ ), low-density lipoprotein-cholesterol ( $-16 \pm 5\%$ ;  $p < 0.01$ ), high-density lipoprotein-cholesterol ( $-8 \pm 2\%$ ;  $p < 0.01$ ), and plasma triglyceride ( $-19 \pm 12\%$ ;  $p = 0.10$ ) all decreased during the VLCD.

**Discussion:** Desaturation of both muscle cell membrane phospholipid and IMTG was significant but modest during a VLCD in obese subjects. Further research must delineate whether such changes in skeletal muscle structural and depot lipid composition themselves are enough to promote the observed improvements in insulin action.

**Key words:** dietary intervention, membrane phospholipids, intramyocellular triglyceride, insulin sensitivity, weight loss

## Introduction

A very-low-calorie dietary intervention program (VLCD)<sup>1</sup> is used as an initial treatment modality to provide a significant and fast weight loss in obese and pre-diabetic individuals before more prolonged and traditional dietary intervention strategies are initiated (1–3). A VLCD, which normally is carried on for 4 to 8 weeks, improves insulin

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<sup>1</sup> Nonstandard abbreviations: VLCD, very-low-calorie dietary intervention; PUFA, polyunsaturated fatty acid; LCPUFA, long-chain PUFA; IMTG, intramyocellular triglyceride; <sup>1</sup>H-MRS, proton magnetic resonance spectroscopy; FA, fatty acid; HDL-C, high-density lipoprotein-cholesterol; HbA<sub>1c</sub>, glycosylated hemoglobin; LDL-C, low-density lipoprotein-cholesterol; HOMA<sub>IR</sub>, homeostasis model assessment of insulin resistance.

sensitivity (4). But the mechanisms by which VLCD improves insulin sensitivity are not yet fully clarified.

The composition of structural lipids of the skeletal muscle cell membrane, i.e., the phospholipids, may play a role for whole-body insulin action (5–9). In vivo data from the rodent model and in vitro data have suggested that changes in the phospholipid composition of cell membranes are associated with the number of insulin receptors (10), the affinity of insulin to the insulin receptor (11), membrane glucose transport (12), and the fact that a diet enriched with polyunsaturated fatty acids (PUFAs, i.e.,  $\geq 18$  carbon units) may increase long-chain PUFAs (LCPUFAs, i.e.,  $\geq 20$  carbon units) in muscle cell membrane and decrease fasting plasma insulin (13). We have recently shown that obese subjects during a 6-month hypocaloric dietary intervention program may considerably increase the content of LCPUFAs, especially those of n-3 origin, in skeletal muscle phospholipids and that such changes correlated strongly and positively with changes in insulin sensitivity (6).

Intramyocellular triglyceride (IMTG) in skeletal muscle has been implicated in insulin sensitivity (14–22). Indirect methods to measure IMTG by proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ) and by computed tomographic scanning have demonstrated that an excess of IMTG is associated with insulin resistance (14,16,17,20,22). In accordance, studies that used more direct methods to estimate IMTG, i.e., by analyzing skeletal muscle biopsies with oil red O staining or by combining thin-layer chromatography and gas-liquid chromatographic analyses, have concluded that increased IMTG is related to impaired insulin action (15,18,19,21). However, studies that have investigated the role of IMTG fatty acid (FA) composition in insulin sensitivity are scarce (23). Moreover, the importance, if any, of the IMTG composition for insulin action, remains unclear (7,24).

Rodents placed on a caloric restriction program have shown improved insulin sensitivity and altered composition of skeletal muscle phospholipids (25). It may be hypothesized that this is also the case in obese human subjects during a VLCD. As to whether a VLCD might alter the concentration and/or composition of skeletal muscle triglyceride of obese subjects remains, to the best of our knowledge, to be clarified. To address these issues, we measured the composition of structural and depot FAs, including the concentration of the latter in skeletal muscle biopsies harvested from obese subjects before and at the end of an 8-week VLCD.

## Research Methods and Procedures

### Study Subjects

Seventeen volunteers (12 women) were recruited from an ongoing dietary intervention study at our outpatient university-based diabetes and obesity clinic. Selection criteria were

abdominally obese patients [BMI between 30 and 40 kg/m<sup>2</sup> and waist circumference  $\geq 92$  cm (women) or  $\geq 102$  cm (men)], age  $\geq 18$  years and  $< 65$  years, and with at least one of the following risk factors: 1) early (i.e., only diet-treated) type 2 diabetes mellitus (fasting plasma glucose  $\geq 7$  mM) or impaired fasting plasma glucose (fasting plasma glucose between 6.1 and 7 mM); 2) dyslipidemia, with high-density lipoprotein-cholesterol (HDL-C)  $\leq 0.9$  mM (men) or  $\leq 1.1$  mM (women) or serum triglycerides  $\geq 2.3$  mM but  $< 10$  mM. Patients with a glycosylated hemoglobin (HbA1c) of  $> 10\%$  were excluded. Any ongoing medication for dyslipidemia and diabetes prohibited participation in the study, as did supplements of n-3 FAs (e.g., fish oil), except from cod oil in dietary doses. Informed written consent was obtained in accordance with Helsinki Declaration II. The local ethics committee approved the present substudy, and the study was approved by the ethical committee of the cities of Copenhagen and Frederiksberg, Denmark (trial no. 01-363-98).

### Dietary Intervention

The recommended energy level during the VLCD period was 600 to 800 kcal/d, which was provided by Nutrilett Intensive energy powder (Nycomed Pharma AS, Oslo, Norway) (3). The Nutrilett formula provides 318 kcal per 100 g of powder, i.e., proteins 36.8 g, carbohydrates 30.7 g, and FAs 4.9 g (1.3 g saturated FAs, 1.9 g linoleic [C18:2(n-6)], and 0.3 g linolenic [C18:3(n-3)] FAs). The patients consulted a registered dietitian 6 times during the 8-week VLCD (i.e., at Weeks 0, 1, 3, 5, 7, and 8) for the purpose of weight control and guidance.

### Anthropometric Measurements

Body weight and height were measured on a calibrated scale. Waist circumference was measured in the standing position between the top of the iliac crest and the lower rib margin on each side, while the patient exhaled, and with the tape parallel to the floor. Hip circumference was measured in the horizontal plane at the level of the maximal extension of the buttocks. Measurements of weight, height, waist, and hip were carried out in duplicate, and mean values were noted. BMI was calculated as weight/height<sup>2</sup> (kg/m<sup>2</sup>). Total body fat mass and lean body mass were estimated by DXA scanning (Model XR-36; Norland Medical Systems, Fort Atkinson, WI).

### Blood Sampling and Assays

Blood samples were collected after an overnight fast ( $\geq 8$  hours) and were handled and analyzed by commercially available kits in accordance with the standard procedures for the central laboratory, Medi-Laboratory (Copenhagen, Denmark). The following blood variables were analyzed: HbA1c, plasma glucose, plasma insulin, plasma

C-peptide, total cholesterol, HDL-C, low-density lipoprotein-cholesterol (LDL-C), and serum triglyceride.

### **Muscle Biopsy**

A percutaneous muscle biopsy was obtained under local anesthesia using a Bergström needle (Depuy, Tempe, AZ) from the vastus lateralis muscle before and after the 8 weeks of VLCD. The specimen was immediately and carefully dissected free of visible connective tissue, lipid, and blood, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until assayed.

### **Skeletal Muscle Phospholipids and Triglycerides**

Extraction of skeletal muscle phospholipids and triglycerides in general followed the principle described by Folch et al. (26). Internal standards of C15:0 phosphatidylcholine and C15:0 triglycerides were added to samples of skeletal muscle tissue, followed by extraction of the total lipid material with chloroform/methanol, 2/1, vol/vol, during homogenization with an Ultra-Turrax homogenizer (Ika Labortechnik, Staufen, Germany). The extracted lipids were separated into phospholipids and triglycerides by thin-layer chromatography using a pre-manufactured silica plate (E. Merck, Darmstadt, Germany). The kiesel gel bands containing the phospholipids and the triglycerides were scraped off the thin-layer chromatography plate and extracted from the kiesel gel. The FA profiles of phospholipids and triglycerides were determined by gas-liquid chromatography of the FA methyl esters using a Hewlett-Packard 6890 instrument (Hewlett-Packard, Böblingen, Germany) equipped with an SP2380 capillary column (internal diameter, 60 m  $\times$  0.25 mm; film thickness, 0.2  $\mu\text{m}$ ; Supelco, Bellefonte, PA) operated with temperature programming and using helium as carrier gas. Detection was by flame ionization. FA methylesters were identified by comparing their retention times with those of actual standards (Sigma, Inc., St. Louis, MO). The individual fatty esters were quantified and reported as their percentage of the total peak area. Only fatty esters constituting  $>0.1\%$  of total peak area are reported. Content of triglycerides in each sample was calculated from the internal standard.

### **Calculations**

The homeostasis model assessment of insulin resistance index ( $\text{HOMA}_{\text{IR}}$ ) derives an estimate of whole-body insulin sensitivity from fasting glucose and insulin concentrations (27). Changes, when given in percentages, were calculated as the value after the VLCD minus the value before the VLCD, divided by the latter value and multiplied by 100%.

### **Statistical Analysis**

All data are presented as means  $\pm$  standard error of the mean, if not otherwise indicated. The paired Student's *t* test was used to compare distribution of paired data sets. Sta-

tistical analyses were performed using SPSS software (version 12.0; SPSS, Inc., Chicago, IL). Statistical significance was accepted for  $p < 0.05$ .

## **Results**

Four patients dropped out because of unwillingness to have a second muscle biopsy performed after the 8-week VLCD period. Other than a trend for younger age of those who dropped out, none of the measured baseline variables, including skeletal muscle FA composition and content, differed significantly between those who dropped out and those who underwent both muscle biopsies (data not shown). Thus, only data from the 13 patients (nine women) who completed the study are given. Two of these patients had diabetes mellitus, and six patients displayed impaired fasting glucose at baseline. All participants were of white ethnicity.

### **Anthropometric and Metabolic Characteristics of Study Subjects**

Table 1 shows data on anthropometric variables, glucose metabolism, and lipidemia at baseline and after 8 weeks on VLCD from the 13 patients who completed the study. It seemed that the VLCD induced a highly significant weight loss ( $\sim 9\%$ ), most of which was attributable to loss of adipose tissue. Waist circumference was highly significantly reduced. Notably, both fasting plasma glucose and insulin decreased significantly, which improved insulin sensitivity almost 2-fold (i.e., a reduction in  $\text{HOMA}_{\text{IR}}$  of 44%). The two patients who displayed diabetes before intervention displayed normoglycemia after the VLCD. Although a steady-state of HbA1c may not have been reached after 8 weeks on VLCD, this important glycemic marker was significantly reduced during the study period. Plasma C-peptide, which is a surrogate marker for pre-hepatic insulin secretion, was reduced, but slightly less than plasma insulin; this may be due to an increase in hepatic insulin extraction, which has been shown to correlate positively with insulin sensitivity (28). During the VLCD, plasma total cholesterol, HDL-C, LDL-C, and triglyceride levels decreased.

### **Skeletal Muscle Phospholipids and Triglycerides**

Table 2 presents data on skeletal muscle phospholipid composition at baseline and after the VLCD. The sum of all individual identified FAs was 98.6% at baseline and 98.5% after the VLCD. Significant changes were observed in saturated FAs (reduction), monounsaturated FAs (increase), and LCPUFAs (increase). Among the LCPUFAn-6 class, most importantly, the fraction of arachidonic acid [C20:4(n-6)] increased considerably, and linoleic acid [C18:2(n-6)] was significantly reduced. On average, the LCPUFAn-3 did not change; however, docosapentaenoic acid C22:5(n-3) increased. The ratio of LCPUFAs to saturated FAs increased.

**Table 1.** Anthropometric and metabolic characteristics of study subjects before and after VLCD

Characteristic	Baseline		Intervention		% change	<i>p</i>
	Mean	SEM	Mean	SEM		
Age (years)	50.3	2.8				
Height (cm)	171.8	2.7				
BMI (kg/m <sup>2</sup> )	36.0	0.9	32.8	1.0	-8.8	<0.001
Waist (cm)	114.1	2.8	106.9	2.6	-6.3	<0.001
Hip (cm)	124.2	2.7	117.6	2.8	-5.3	<0.001
Fat mass (kg)	44.6	2.7	38.7	3.0	-13.3	<0.001
Lean mass (kg)	58.3	3.8	55.3	3.7	-5.1	<0.01
Weight (kg)	106.4	4.1	97.0	4.3	-8.8	<0.001
Fp-glucose (mM)	6.3	0.4	5.4	0.2	-14.8	<0.05
Fp-insulin (pM)	104	14	59	5	-43.5	<0.01
Fp-C-peptide (pM)	1075	97	793	69	-26.2	<0.001
HbA1c (%)	6.5	0.3	6.0	0.1	-7.9	<0.05
HOMA <sub>IR</sub>	2.0	0.2	1.1	0.1	-44.2	<0.01
Fp-cholesterol (mM)	6.0	0.3	5.1	0.3	-14.9	<0.01
Fp-LDL-C (mM)	3.9	0.2	3.3	0.3	-15.8	<0.05
Fp-HDL-C (mM)	1.3	0.1	1.2	0.1	-8.3	<0.01
Fs-triglyceride (mM)	1.8	0.2	1.4	0.1	-19.6	NS

Data are from 13 patients (9 women). SEM, standard error of the mean; % change, the change relative to baseline level after 8 weeks on VLCD; Fp, fasting plasma; Fs, fasting serum; NS, not significant.

Table 3 shows data from skeletal muscle triglyceride of both FA composition and content before and after the VLCD. All individual FAs of IMTG were identified. In parallel with the findings on membrane phospholipids, saturated FAs, on average, were reduced during the VLCD in the IMTG. Mono-unsaturated FAs as a class did not change significantly, whereas both LCPUFAs and the ratio of LCPUFAs to saturated FAs increased. The increase in LCPUFAs was found both in the LCPUFAn-6 class and in the LCPUFAn-3 class. The IMTG content showed an insignificant decrease of 5%.

No significant correlation was observed between changes in HOMA<sub>IR</sub> and changes in muscle structural and depot lipids, plasma lipids, and anthropometric measures (data not shown). Although average changes of LCPUFAs and saturated FAs in IMTG and muscle membrane phospholipids went in the same direction during the VLCD, these changes did not correlate (all  $p > 0.3$ ; data not shown). Female patients ( $n = 9$ ), as compared with male patients ( $n = 4$ ), displayed an increased percentage of body fat ( $48 \pm 2\%$  vs.  $34 \pm 2\%$ ;  $p < 0.01$ ). Moreover, a trend toward increased IMTG was observed in women. IMTG-saturated FAs were increased in men ( $35.2 \pm 1.0\%$  vs.  $30.9 \pm 0.6\%$ ;  $p < 0.05$ ), which was attributable to an increased concentration of C16:0. Therefore, the ratio of polyunsaturated to saturated

FAs in IMTG was lower in men ( $36 \pm 3\%$  vs.  $46 \pm 2\%$ ;  $p < 0.05$ ). No other sex differences were detected at baseline. The direction of the changes in muscle lipid composition was independent of sex.

## Discussion

Desaturation of structural and depot FAs in skeletal muscle was observed in obese insulin-resistant subjects who exhibited a negative energy balance for 8 weeks. The desaturation of these lipid entities appeared with an ambient significant increase in LCPUFAs. On the other hand, the magnitude of changes was small. Interestingly, the present data suggest that 8 weeks of a VLCD may not influence significantly the concentration of depot triglyceride in skeletal muscle. The VLCD induced significant improvements in insulin sensitivity, glycemia, insulin secretion, and anthropometric measures, including more modest improvements in lipidemia in obese subjects.

A negative energy balance for up to 29 months was shown to significantly increase docosahexaenoic acid [C22:6(n-3)] of skeletal muscle phospholipids in rodents; this increase was found to be associated with improvements in insulin sensitivity (25). Among the membrane LCPUFAn-3s, docosapentaenoic

**Table 2.** Skeletal muscle phospholipids

	Baseline		Intervention		% change	<i>p</i>
	Mean	SEM	Mean	SEM		
C14:0	0.45	0.02	0.41	0.02	-8.5	NS
C16:0	17.48	0.87	16.60	0.61	-5.0	NS
C17:0	0.26	0.02	0.22	0.01	-14.8	NS
C18:0	17.91	0.41	17.72	0.27	-1.0	NS
Saturated	36.12	0.66	34.98	0.51	-3.2	<0.05
C16:1(n-7)	0.79	0.07	0.80	0.05	2.2	NS
C18:1tr	0.55	0.09	0.55	0.07	0.0	NS
C18:1(n-9)	7.73	0.41	7.97	0.31	3.1	NS
C18:1(n-7)	2.10	0.08	2.28	0.10	8.6	<0.01
Monounsaturated	11.35	0.43	11.83	0.33	4.3	<0.05
C18:2(n-6)	31.52	0.72	30.45	0.66	-3.4	<0.05
C18:3(n-3)	0.40	0.05	0.31	0.04	-21.1	NS
C20:3(n-9)	0.27	0.03	0.31	0.02	13.6	NS
C20:3(n-6)	1.53	0.08	1.55	0.08	1.0	NS
C20:4(n-6)	12.54	0.57	14.07	0.49	12.2	<0.01
C20:5(n-3)	0.92	0.11	0.79	0.09	-14.2	<0.05
C22:4(n-6)	0.47	0.05	0.59	0.06	24.2	<0.01
C22:5(n-3)	1.08	0.07	1.24	0.07	15.2	<0.05
C22:6(n-3)	2.16	0.20	2.09	0.17	-3.3	NS
PUFA	50.77	1.00	51.24	0.63	0.9	NS
LCPUFA	19.26	0.79	20.94	0.63	8.7	<0.05
LCPUFAn-3	4.16	0.36	4.12	0.31	-0.9	NS
LCPUFAn-6	14.69	0.65	16.36	0.57	11.3	<0.01
PUFA/S ratio	142	5	147	4	3.9	NS
LCPUFA/S ratio	54	3	60	2	11.8	<0.05

Data are from 13 patients (9 women). Each FA is given as the percentage of the sum of all identified FAs (from skeletal muscle membrane phospholipids). SEM, standard error of the mean; % change, the change relative to baseline level after 8 weeks on VLCD; S, saturated FAs; NS, not significant.

acid [C22:5(n-3)] increased significantly at the expense of eicosapentaenoic acid [C20:5(n-3)] in the present study. Thus, LCPUFAn-3 remained unchanged, as C22:6(n-3) did not change significantly. C20:5(n-3) may be implicated positively in insulin action and glucose uptake *in vitro* (29), although C22:5(n-3) was the only LCPUFAn-3 structural FA that correlated with insulin sensitivity in patients with coronary artery disease (5). It cannot be ruled out, therefore, that increased C22:5(n-3) level may have a positive influence on insulin action in the present setting. Other studies have indicated that total LCPUFAn-3, and C22:6(n-3) in particular, are the structural FAs associated with insulin sensitivity and improved glycemia (6,13,30). However, changes in and absolute concentrations of both total LCPUFAs and saturated FAs in muscle membrane phospholipids correlated significantly with insulin

action in three human studies [two interventional studies (6,7) and a cross-sectional study (8)], such that increased LCPUFAs and reduced saturated FAs were associated with improved insulin action.

Whether a certain IMTG FA composition may have any physiological significance in terms of influence on insulin action remains unclear. Only a few studies have found a relatively higher unsaturation-to-saturation ratio of IMTG to be associated with improved insulin sensitivity (23,24), whereas this relationship failed in most such studies (7,9,19,31). An excess of IMTG has consistently been associated with insulin resistance and type 2 diabetes mellitus in humans, which has been documented by use of the <sup>1</sup>H-MRS technique (16,17,20,22), by computed tomographic scans (14), and, more directly, by biochemical

**Table 3.** Skeletal muscle triglycerides

	Baseline		Intervention		% change	<i>p</i>
	Mean	SEM	Mean	SEM		
C12:0	0.64	0.06	0.62	0.08	-2.7	NS
C14:0	2.80	0.08	2.67	0.10	-4.7	<0.05
C16:0	23.93	0.41	23.08	0.45	-3.6	<0.01
C17:0	0.22	0.02	0.22	0.01	3.3	NS
C18:0	4.62	0.36	4.59	0.39	-0.8	NS
Saturated	32.26	0.76	31.21	0.87	-3.3	<0.05
C14:1	0.35	0.04	0.38	0.03	7.8	NS
C16:1(n-9)	0.91	0.23	0.77	0.07	-15.9	NS
C16:1(n-7)	5.42	0.35	5.26	0.42	-2.9	NS
C17:1	0.23	0.04	0.34	0.05	48.6	<0.05
C18:1tr	1.14	0.12	1.25	0.08	10.0	NS
C18:1(n-9)	42.37	0.39	43.07	0.44	1.6	<0.05
C18:1(n-7)	2.84	0.23	2.63	0.09	-7.4	NS
C20:1	0.71	0.09	0.91	0.02	28.0	<0.05
Monounsaturated	53.98	0.64	54.61	0.76	1.2	NS
C18:2(n-6)	10.62	0.27	10.47	0.29	-1.4	NS
C18:3(n-3)	1.07	0.12	1.03	0.08	-3.8	NS
C20:2	0.17	0.02	0.20	0.01	22.6	<0.05
C20:3(n-6)	0.25	0.04	0.27	0.02	8.5	NS
C20:4(n-6)	0.40	0.04	0.50	0.04	23.9	<0.01
C22:3(n-3)	0.29	0.06	0.45	0.07	52.2	<0.05
C22:4(n-6)	0.13	0.03	0.27	0.03	105.2	<0.01
C22:5(n-3)	0.34	0.05	0.44	0.04	29.6	<0.05
C22:6(n-3)	0.45	0.09	0.52	0.09	14.4	NS
PUFA	13.59	0.54	13.98	0.42	2.8	NS
LCPUFA	2.07	0.24	2.68	0.20	29.6	<0.01
LCPUFAn-3	1.12	0.18	1.44	0.16	28.6	<0.01
LCPUFAn-6	0.78	0.09	1.04	0.07	32.5	<0.01
PUFA/S ratio	42.7	2.4	45.4	2.3	6.5	<0.05
LCPUFA/S ratio	6.5	0.8	8.7	0.8	33.5	<0.001
Total IMTG mg/g wet tissue	19.4	2.7	18.3	3.6	-5.3	NS

Data are from 13 patients (9 women). Each FA is given as the percentage of the sum of all identified FAs (from skeletal muscle triglycerides). SEM, standard error of the mean; % change, the change relative to baseline level after 8 weeks on VLCD; S, saturated FAs; NS, not significant.

analysis of muscle tissue (15,18,19,21,24). The mechanism accounting for increased IMTG being associated with poor insulin sensitivity is thought to be an increased level of substrate for mitochondrial  $\beta$ -oxidation, in turn, reducing the need for glucose uptake and storage (32). Isolated muscle cells, which were harvested from non-diabetic and type 2 diabetic obese subjects, showed increased transmembrane FA transport and re-esterification capacity, suggesting mechanisms for the increased IMTG observed in these states (33).

It remains to be explained why IMTG desaturated in the present study and why IMTG content was not reduced. We speculate that increased lipolysis of the subcutaneous and intra-abdominal fat depots during the negative energy balance induced by VLCD may reduce the turnover of the muscular depot fat, such that the ubiquitous FA enzymes, i.e., desaturases and elongases (34), may have desaturated and elongated the FAs of depot triglyceride in situ simply because of a low turnover of these depots' lipids. This

inference may also explain the lack of a significant reduction in intramyocellular fat content. Alternatively, more dynamic changes in IMTG content during the 8-week study may have taken place; i.e., during the first few days of catabolism, IMTG may have increased in parallel with a temporary impaired insulin sensitivity, as has recently been demonstrated during short-term (~3 days) starvation (35). A positive correlation between plasma concentration of free FAs, which increases during fasting, and IMTG has been described (21,35,36). Thus, after an initial period of increasing IMTG, the improved insulin sensitivity, which was facilitated during the VLCD, may have increased the flexibility of substrate metabolism in the study subjects (32). During this new metabolic “set point,” a net mobilization of muscle triglyceride may have occurred, neutralizing the net effect on IMTG content of the VLCD.

Several studies have indicated that dietary FA composition is likely to be reflected in skeletal muscle lipids (13, 37–39). Thus, the relatively high fraction of both linoleic [C18:2(n-6), ~38% of total fat] and linolenic [C18:3(n-3), ~6% of total fat] FAs in the present VLCD and the low content of saturated FAs (~26%) compared with an average Western diet may have contributed to the overall desaturation of skeletal muscle phospholipids and triglycerides. Although both C18:2(n-6) and C18:3(n-3) tended to decrease in phospholipids and triglycerides during the study, this may just signify that these essential dietary FAs were elongated and desaturated before or after incorporation into skeletal muscle phospholipids and triglycerides (40,41). Notably, total fat content was only 4.9% (weight percentage) in this VLCD. A reduced relative intake of dietary fat has also been suggested to be associated with an increased degree of desaturation and elongation of structural FAs in humans (6). The lack of effect seen in C22:6(n-3) of IMTG and membrane phospholipids in this 8-week study may be due to the longer retroconversion step of peroxisomal  $\beta$ -oxidation in docosahexaenoic acid formation (42) and rapid use/turnover of whatever docosahexaenoic acid is/was present.

Overall, the modification in FA levels in muscle lipids was modest; however, exact value(s) (e.g., LCPUFA-to-saturated FA ratio, saturated FAs, LCPUFAs) required for modification to have an effect on insulin action have not been determined to date. Therefore, the modest changes in lipid composition and concentration observed, themselves, may be enough to elicit the improved insulin action. Several other mechanisms may contribute to improve insulin sensitivity during a VLCD. However, recent data have indicated that adiponectin, which has been shown to correlate strongly with insulin action, may not change during a VLCD (43). Another important feature associated with insulin action is low-grade inflammation in obese subjects (44). Thus, changes in proinflammatory markers both in blood and in other relevant tissues (e.g., skeletal muscle, liver, pancreatic

$\beta$  cells, and adipose tissue) may potentially play a role in the changes in insulin sensitivity during a VLCD. Finally, the HOMA model that was used in the present study may more closely reflect insulin sensitivity at the hepatic level rather than at the muscular level (27). Recently, a weight loss program of 1200 kcal/d over ~8 weeks improved primarily hepatic insulin sensitivity, as compared with skeletal muscle insulin sensitivity, in type 2 diabetes patients (diet- and/or sulfonylurea-treated only) (45). Improved insulin sensitivity in that study was found to correlate with loss of hepatocellular triglyceride, measured by the  $^1\text{H}$ -MRS technique, whereas intramyocellular triglyceride was not affected (45). It may merit research, therefore, to address whether non-alcoholic steatohepatitis would ameliorate during a VLCD and, if so, whether this would correlate with improvement in insulin sensitivity.

Among the limitations of the present study is the lack of a healthy normal-weight control group. We may, therefore, not be able to delineate whether the concentration and composition of structural and depot FAs were normal or abnormal in our study group. Also, the small number of participants and the somewhat non-homogeneous fasting glycemia may limit the interpretation of data. However, of note, none of the study subjects was on antidiabetic medication at study entry, and the relative impact of weight loss on insulin sensitivity was quite similar in those with and those without diabetes mellitus and impaired fasting glucose, as defined by fasting plasma glucose at study entry (data not shown). The coefficient of variance for the estimation of IMTG in repeated biopsies may be as high as 24% (46), in particular when microdissection is not performed (47). Thus, inherent imprecision in IMTG measurement may have exceeded any effect size, causing no change in IMTG during the intervention. Finally, of course, the small number of participants may have hampered the likelihood of achieving significant correlations between changes in insulin sensitivity and changes in structural and depot lipids, including plasma lipids and anthropometric measures. Larger studies are needed to investigate such correlations.

In conclusion, the present findings indicate that an 8-week VLCD may increase LCPUFAs in the skeletal muscle membrane of obese subjects. Eight-week VLCD may not induce a major reduction in the skeletal muscle content of depot triglyceride, but a modest desaturation of this lipid entity should be expected, the importance of which remains to be elucidated. Taken together, the VLCD induced significant but modest improvements in structural lipids and depot fat of skeletal muscle. Further research is needed to delineate whether such changes in skeletal muscle structural and depot lipid composition themselves are enough to promote the observed improvements in insulin action.

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