The effect of dietary fat content on phospholipid fatty acid profile is muscle fiber type dependent

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Janovská A, Hatzinikolas G, Mano M, Wittert GA. The effect of dietary fat content on phospholipid fatty acid profile is muscle fiber type dependent. Am J Physiol Endocrinol Metab 298: E779–E786, 2010. First published January 19, 2010; doi:10.1152/ajpendo.00356.2009.—A high-saturated-fat diet (HFD) induces obesity and insulin resistance (IR). IR has been linked to alterations and increased saturation in the phospholipid composition of skeletal muscles. We aimed to determine whether HFD feeding affects fatty acid (FA) membrane profile in a muscle fiber type-specific manner. We measured phospholipid FAs and expression of FA synthesis genes in oxidative soleus (SOL) and glycolytic extensor digitorum longus (EDL) muscles from rats fed either standard chow (standard laboratory diet, SLD) or a HFD. The HFD increased fat mass, plasma insulin, and leptin levels. Compared with EDL, SOL muscles preferentially accumulated C18 over C16 FAs and n-6 over n-3 polyunsaturated FAs (PUFAs) on either diet. With the HFD, SOL muscles contained more n-9 monounsaturated FAs (MUFAs) and n-6 PUFAs and less n-7 MUFAs and n-3 PUFAs than EDL muscles and had lower unsaturation index, a pattern known to be associated with IR. Stearoyl-CoA desaturase-1 expression was ~13-fold greater in EDL than in SOL muscles but did not change with the HFD in either muscle. The expression of Elongase-5 was higher, and that of Elongase-6 (Elovl6) was lower in EDL compared with SOL muscles with both diets. In EDL muscles, the expression of Elovl6 was lower in the HFD than in the SLD. The pattern of FA uptake, expression, and diet-induced changes in FA desaturating and elongating enzymes maintained higher FA unsaturation in EDL muscles. Accordingly, the fiber type composition of skeletal muscles and their distribution may be important in the development and progression of obesity and IR.

skeletal muscle; stearoyl-coenzyme A desaturase; elongases; obesity

HIGH-FAT DIET (HFD) feeding leads to obesity in rodents as well as in humans and, in skeletal muscles, to an increase in the intracellular content of lipids and resistance to the actions of insulin and leptin.

Skeletal muscle represents 40–50% of the total body mass and is the major tissue responsible for whole body insulin-stimulated glucose disposal. Skeletal muscle fibers are divided into three major categories, types I, IIa, and IIb. Type I fibers are slow-twitch, oxidative (SO) fibers with a high mitochondrial content; type IIa fibers are fast-twitch, oxidative-glycolytic (FOG) fibers that are high in both mitochondrial and glycolenogenic enzymes; type IIb fibers are fast-twitch, glycolytic (FG) fibers that are high in the enzymes of glycogenolysis (22, 59). Although the oxidative muscle fibers are highly dependent on lipids as a substrate, the glycolytic or oxidative-glycolytic fibers utilize either predominantly glucose or a fuel mixture with an important contribution of glucose as a substrate under normal feeding conditions (46). Oxidative muscles are more insulin sensitive and have a greater insulin binding capacity (5), kinase activity of insulin receptor (25), content of glucose transporter (GLUT)4 (26), and insulin-stimulated glucose uptake (35) than glycolytic muscles. In obesity and IR, the distribution of fiber types is shifted from oxidative to glycolytic muscle fibers (21).

In skeletal muscle, saturated fatty acids (SFAs) are associated with IR and induce diacylglycerol (DAG) and ceramide synthesis (10), whereas polyunsaturated FAs (PUFAs, particularly n-3 PUFAs) improve insulin-stimulated glucose uptake, probably by channeling excess SFAs into an inert triglyceride (TG) lipid pool rather than into the synthesis of bioactive lipids (30, 32). Insulin action in skeletal muscle has been linked, not only to levels of intracellular lipids that can affect the insulin signaling pathway and thus glucose uptake (48), but also to alterations in the composition of structural lipids, i.e., phospholipids, leading to alterations in the function of membrane proteins, e.g., insulin receptors and glucose transporters (6, 52).

A HFD enriched in n-3 PUFAs maintained tyrosine phosphorylation of the insulin receptor and the insulin receptor substrate-1, phosphatidylinositol 3'-kinase (PI3K) activity, and the GLUT4 content in muscle, whereas, in a HFD enriched in n-6 PUFAs, the phosphorylations, PI3K activity, and GLUT-4 content were reduced (53).

The purpose of this study was to compare the profile of phospholipid FAs (PLs) and the expression of the genes involved in FA synthesis in two different muscle fiber types, oxidative soleus (SOL; 84% SO, 16% FOG, and 0% FG fibers) and glycolytic extensor digitorum longus muscles (EDL; 3% SO, 59% FOG, and 38% FG fibers) (2) under conditions of low and high dietary fat content.

MATERIALS AND METHODS

Animals. Male Wistar rats, at 6 wk of age and of an average weight of 180 g, were housed individually and maintained under a 12-h:12-h light/dark cycle at a constant room temperature of 24 ± 1°C. After 2 wk of acclimatization, the rats were divided into two groups and fed with either the standard laboratory diet (SLD; 18.4 kJ/g, 11.4% fat, 71.4% carbohydrate, 17.2% protein), or with a high fat palatable (cafeteria) diet composed of crushed chocolate, salted peanuts, powered meatmeal, condensed milk, and lard and enriched with vitamins, i.e., the high-fat group (HFD; 19.5 kJ/g, 59.8% fat, 21.8% carbohydrate, and 18.4% protein) ad libitum for 20 wk. The FA composition of the diets is presented in Table 1. Water was available ad libitum. Food intake was monitored daily and body weight twice per week. All experimental procedures in this study were approved by the Univer-
Table 1. **Fatty acid composition of the diets**

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>SLD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>—</td>
<td>1.3</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.7</td>
<td>1.1</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>17.8</td>
<td>19.7</td>
</tr>
<tr>
<td>C16:1 n-7</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>C18:0</td>
<td>7.1</td>
<td>14.1</td>
</tr>
<tr>
<td>C18:1 n-9</td>
<td>32.6</td>
<td>43.1</td>
</tr>
<tr>
<td>C18:2 n-6</td>
<td>32.2</td>
<td>16.2</td>
</tr>
<tr>
<td>C18:3 n-3</td>
<td>5.0</td>
<td>0.5</td>
</tr>
<tr>
<td>LC-PUFAs</td>
<td>3.1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

SLD, standard laboratory diet; HFD, high-fat diet; LC-PUFAs, long-chain polyunsaturated fatty acids.

**RESULTS**

**TG quantification.** Plasma TGs were measured by the use of the enzymatic-colorimetric method and the Cobas Bio analyzer (Roche Diagnostics, Castle Hill, NSW, Australia).

**Analysis of total plasma FAs and PLs.** Lipids were extracted from the plasma and muscle tissue according to the method of Folch (16), centrifuged (at 1,000 revolution/min for 2 min for PLs and 2,000 revolution/min for 10 min for plasma FAs), and the lower organic layer was dried under a stream of nitrogen (N2) at 40°C. For PLs, it was sequentially dissolved in chloroform-methanol (65:25, vol/vol) and separated by a thin-layer chromatography plate using acetone-petroleum spirit (1:3, vol/vol) with butylated hydroxytoluene as the developing solvent. PLs, scraped from the plate, and the lower organic layer with plasma FAs were methylated in 1% H2SO4 in dry methanol (Merck) for 1 h at 50°C. The FAMEs were extracted with petroleum spirit, dried under N2, dissolved in hexane, then purified by the use of Florisil in a Pasteur pipette plugged with glass wool, and eluted with hexane-diethylether (90:10, vol/vol) (11). The solvent was evaporated under N2 and the FAMES dissolved in iso-octane for analysis by gas chromatography using an Agilent 6890 gas chromatograph. A portion (0.2 µl) of the sample was injected (using a cool on-column injector) onto a BPX70 capillary column (70% cyanopropyl polysilphenylene-siloxane, 30 m × 0.53 mm × 0.5 µm film thickness; SGE Analytical Sciences, Ringwood, Victoria, Australia) using hydrogen as the carrier gas. The oven temperature program was 120°C to 180°C (6°C/ min) to 230°C (3°C/min) and the flame ionization detection set at 300°C. FAMES were identified by comparison of retention times with a standard mix (Supelco 37 Component FAME mix). 1,2-diheneicosanoyl-sn-glycero-3-phosphocholine (C21:0 PC) was added as the internal standard for the quantification of PLs, and a triheptadecanoin (C17:0) was added as the internal standard for the quantification of plasma FAs.

**RNA isolation and qRT-PCR.** The total RNA was extracted from 10–15 mg of muscle tissue with Trizol (Invitrogen, Mulgrave, Victoria, Australia). By the use of one 5-mm stainless steel bead and the TissueLyser for 1.5 min at 30 Hz (Qiagen), quantified using nanodrop spectrometry at 260 nm, and reverse-transcribed into cDNA with the Quantitect Reverse Transcription kit. Synthesized cDNA was mixed with 2× Quantitect SYBR Green PCR Master Mix and with various sets of 10× Quantitect primer assays and subjected in triplicates to real-time PCR quantification using the Rotor-Gene RG-3000 (Corbett Research, Sydney, Australia). All reactions were carried out according to the manufacturer’s protocols. By performing three to six different serial dilutions over the range of the amount of mRNA expected in the experimental samples, standard curves were generated to quantify relative concentrations of target and housekeeping genes. To compensate for RNA input variation and the efficiency of reverse transcription, results were normalized to the mRNA abundance of housekeeping genes. Ppib (cyclophilin B) and Polr2c were used as internal controls, as levels of their absolute expressions did not show any changes with the dietary intervention.

**Statistical analysis.** All data are reported as means ± SE. Differences among groups were compared using ANOVA or a nonparametric Kruskal-Wallis test if a normal distribution of the variable was not confirmed. The linear regression model was used to determine the relation between the concentration of plasma leptin, insulin, and the weight of adipose tissue and the concentration of individual PLs or the activities of desaturases. For evaluation of these linear correlations, the Pearson or Spearman’s correlation coefficient was used, respectively, if an assumption of normality was not confirmed. Differences among groups were considered as statistically significant if P ≤ 0.05. All statistical analyses were performed using a commercially available statistical package (SPSS 15.0 for Windows).

**RESULTS**

**Body weight, fat depots, leptin, and insulin concentrations.** The body weights and the weight of white adipose tissue of the
The effect of muscle fiber type on PLs with different diets. The difference in SFAs between SOL and EDL muscles depended on chain length. Irrespective of diet, C16:0 was lower in SOL than in EDL muscles. However, C18:0, C20:0, C22:0, and C24:0 were higher in SOL than in EDL muscles (Fig. 2). Likewise, the n-9 monounsaturated FA (MUFA), C18:1, was greater in SOL than in EDL muscles, irrespective of diet. Of the n-6 PUFAs, the main one was C18:2, and its levels were higher in SOL than in EDL muscles on each diet. However, levels of its metabolite, C20:4 n-6, were the same in both muscles, regardless of diet. The main n-3 PUFA was C22:6, and its levels were lower in SOL than in EDL muscles on each diet. Furthermore, in SOL when compared with EDL muscles, C22:5 n-3 was higher in the SLD group but lower in the HFD group (Fig. 2).

The HFD increased concentrations of SFAs in both muscles, with the exception of C16:0, which was reduced in SOL muscles. In response to the HFD, n-7 MUFAs and n-3 PUFAs were lower in both muscles, whereas n-9 MUFAs and n-6 PUFAs (C18:2 and C20:2) were higher only in SOL muscles (Fig. 2). Diet altered the differences between fiber types in certain FAs. In SOL when compared with EDL muscles, the levels of C18:1 n-7 and n-3 PUFAs were lower on the HFD compared with the SLD. This was reversed for C18:1 n-9 and C18:2 n-6 (Table 3).

The levels of C20-22 PUFAs and the ratio of n-3:n-6 PUFAs were lower in SOL muscles, irrespective of diet. In response to the HFD, the ratio of n-3:n-6 PUFAs was further reduced in both muscles, and the IU was lower in SOL muscles, thus showing a significantly reduced membrane unsaturation in SOL when compared with EDL muscles (Fig. 2).

Relationships between HFD-induced obesity and levels of PLs in EDL and SOL muscles. We performed linear regressions between muscle FAs and the levels of plasma insulin and leptin and the weight of adipose tissue in HFD-induced obesity. We found positive correlations between the levels of SFAs (C18:0, C20:2, and C24:0) and n-6 PUFAs (C20:2 and C20:4) and negative correlations between the levels of n-3 PUFAs (C18:3, C20:5, and C22:6) and n-7 MUFAs (C16:1 and C18:1), and plasma insulin and leptin levels as well as adipose tissue weight in both muscles. The levels of n-9 MUFAs (C18:1 and C20:1) and n-6 PUFAs (C18:2) exhibited positive

![Fig. 1. Concentration of individual fatty acids (FAs) in the plasma from rats fed with standard laboratory diet (SLD) and high-fat diet (HFD).](http://ajpendo.physiology.org/)

Table 2. Weight of body and adipose tissue and concentrations of plasma leptin, insulin, FAs, and TGs in rats fed with SLD and HFD for a period of 20 wk

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight, g</th>
<th>White adipose tissue, g</th>
<th>Leptin, ng/ml</th>
<th>Insulin, ng/ml</th>
<th>FA, μmol/ml</th>
<th>TGs, μmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLD</td>
<td>627.3 ± 9.6</td>
<td>66.8 ± 4.9</td>
<td>107.5 ± 16.6</td>
<td>1.70 ± 0.36</td>
<td>3.28 ± 0.18</td>
<td>2.04 ± 0.14</td>
</tr>
<tr>
<td>HFD</td>
<td>692.1 ± 26.7</td>
<td>99.3 ± 9.6</td>
<td>206.2 ± 15.5</td>
<td>4.99 ± 0.97</td>
<td>2.35 ± 0.09</td>
<td>0.92 ± 0.08</td>
</tr>
</tbody>
</table>

Data represent means ± SE; n = 10 per group for body weight, adipose tissue, n = 9–10 per group for leptin and insulin levels and n = 12 per group for FAs and triglycerides (TGs); *P ≤ 0.05; †P ≤ 0.01; ‡P ≤ 0.001.

The levels of total and individual FAs and TGs in the plasma. In obese animals fed ad libitum, the levels of total FAs and TGs were significantly higher in the HFD than in the SLD group (Table 2).

Leptin levels in skeletal muscles. The expression levels of leptin, the adipose tissue marker, were not higher in either muscle dissected from animals fed with the HFD. Although the levels were slightly higher in SOL than in EDL muscles, the difference was not significant (SLD vs. HFD, 0.04 vs. 0.043 for SOL muscles). It demonstrates that the adipose tissue surrounding the muscles together with their vessels and nerves was successfully removed and that the results obtained were not compromised because of contamination of muscle tissue by fat cells.

Levels of total and individual FAs and TGs in the plasma. In obese animals fed ad libitum, the levels of total FAs and TGs were significantly lower (P ≤ 0.001), the concentrations of C18:0, C20:0, C20:2 n-6 and C20:4 n-6 were higher (P ≤ 0.01), of C22:0 and C24:0 were unchanged, and of all other measured FA species (C16:0, C16:1 n-7, C18:1 n-7, C18:1 n-9, C18:2 n-6, C18:3 n-3, C20:1 n-9, C20:5 n-3, C22:5 n-3, and C22:6 n-3) were lower (P ≤ 0.01). The ratio of n-3:n-6 PUFAs and the index of unsaturation (IU) were lower (P ≤ 0.01 and P ≤ 0.05, respectively) in the blood of the animals fed with the HFD (Table 2, Fig. 1).
correlations and C22:5 n-3 negative correlations with insulin and leptin levels and adipose tissue weight only in SOL muscles. The correlation coefficients (r) and levels of significance are presented in Table 4.

Expression of genes involved in FA synthesis. On either diet, SCD1 mRNA expression was 11–14.7 times greater (P ≤ 0.001) in EDL than in SOL muscles. By contrast, SCD2 mRNA expression did not differ between muscles in either dietary group. There was no effect of diet on SCD1 or SCD2 expression in either muscle type (Fig. 3).

Elongase-5 (Elov5, rELO1) mRNA expression was higher in EDL than in SOL muscles in both dietary groups (P ≤ 0.05), with no effect of the HFD on either muscle. Elongase-6 (Elov6, rELO2) mRNA expression was lower in EDL than in SOL muscles in both dietary groups (P ≤ 0.05) and lower (P ≤ 0.05) in EDL muscles in response to the HFD (Fig. 3).

DISCUSSION
Consistent with previous studies (12, 31), we found a significant increase in body weight, adipose tissue mass, plasma leptin, and insulin levels in response to the HFD. An effect of insulin stimulation to reduce plasma FAs and TGs in obese animals occurred. The plasma FA profile reflected the FA composition of the diets, with the exception of C16:0 and C18:1 n-9. The lower levels of FAs and TGs, C16:0, and C18:1 n-9 FAs in the plasma reflected the consequence of fat deposition in an expanded adipose tissue mass.

In response to HFD-induced obesity, muscle phospholipid levels of long-chain SFAs and n-6 PUFAs increased, and those of n-7 MUFAs and n-3 PUFAs decreased with increasing levels of plasma leptin and insulin and weight of adipose tissue. There was a positive correlation of n-9 MUFAs with insulin, leptin, and adipose tissue in SOL muscles. There were distinct patterns of accumulation of certain FAs in each muscle type. Irrespective of diet, EDL muscles contained more phospholipid C22:6 n-3 and C16:0 in preference to C18:0 when compared with SOL muscles, whereas SOL muscles had a preference for C18 FAs, C18:0, C18:1 n-9, and C18:2 n-6. This is consistent with previous studies (18, 27). In addition, the content of n-3 and long-chain PUFAs was lower in SOL muscles. The HFD further amplified these differences in the FA pattern between EDL and SOL muscles, resulting in a lower membrane unsaturation in SOL muscles (despite the higher incorporation of C18:1 n-9 and C18:2 n-6) than in EDL muscles. Diets high in SFAs, n-9 MUFAs, and n-6 PUFAs, but not in n-3 PUFAs, and the increase in saturation of membrane
FA in extensor digitorum longus (EDL) muscles from that in soleus (SOL) muscles on each diet. a

Relationships between plasma insulin and leptin and the weight of adipose tissue and levels of individual
muscles can be explained by a muscle fiber type-dependent
their ratio to other FAs.

Difference in the effect of n-6 PUFAs may relate to method-
long-chain SFAs (7, 10, 51). In rats, diets rich in n-6 PUFAs
exposure to a HFD (9), and n-3 PUFAs have been known to
insulin; however, its levels in adipose tissue are reduced on
The differential susceptibility of muscle fibers to IR with HFD
fiber type specificity in their accumulation may further explain
FAs are known to be associated with IR (50, 51, 55). The
n-3 PUFAs
n-7 MUFAs
n-6 PUFAs
C20:2 n6
C20:4 n6
C20:5 n3
C22:5 n3
C22:6 n3
C24:0
C22:5 n6
C24:0
C22:6 n6
C24:2
C25:2
C25:4
n-3 PUFAs
C18:3 n3
C20:3 n9
C20:4 n9
C22:4 n9
C22:6 n9
C24:6 n9
C24:2
C25:2
C25:4

Differences in levels of phospholipid FAs between EDL and SOL muscles with different diets

Table 3. Differences in levels of phospholipid FAs between EDL and SOL muscles with different diets

<table>
<thead>
<tr>
<th></th>
<th>SLD EDL</th>
<th>SLD SOL</th>
<th>HFD EDL</th>
<th>HFD SOL</th>
<th>HFD SOL – EDL</th>
<th>SLD SOL – EDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-7 MUFAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1 n-7</td>
<td>0.26 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.02*</td>
</tr>
<tr>
<td>n-3 PUFAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:3 n-3</td>
<td>8.84 ± 0.93</td>
<td>11.1 ± 1.34</td>
<td>1.9 ± 0.47</td>
<td>1.79 ± 0.19</td>
<td>2.29 ± 0.9</td>
<td>0.11 ± 0.48*</td>
</tr>
<tr>
<td>C22:5 n-3</td>
<td>0.13 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.08 ± 0.00</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.01b</td>
</tr>
<tr>
<td>C22:6 n-3</td>
<td>1.14 ± 0.04</td>
<td>0.90 ± 0.04</td>
<td>0.80 ± 0.06</td>
<td>0.41 ± 0.02</td>
<td>0.24 ± 0.04</td>
<td>0.40 ± 0.06*</td>
</tr>
<tr>
<td>n-9 MUFAs and n-6 PUFAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1 n-9</td>
<td>0.37 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.45 ± 0.04</td>
<td>0.67 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>0.22 ± 0.06*</td>
</tr>
<tr>
<td>C18:2 n-6</td>
<td>1.39 ± 0.08</td>
<td>1.67 ± 0.08</td>
<td>1.38 ± 0.08</td>
<td>1.96 ± 0.08</td>
<td>0.28 ± 0.08</td>
<td>0.58 ± 0.12*</td>
</tr>
</tbody>
</table>

Values are in μmol/g wet weight. Data represent means ± SE; n = 11 per group. Values were calculated as a subtraction of the concentration of a particular FA in extensor digitorum longus (EDL) muscles from that in soleus (SOL) muscles on each diet. *P ≤ 0.05; †P ≤ 0.001; ‡P = 0.052; §P = 0.065; *values for C18:3, n-3 were adjusted (real value × 10³). MUFAs, monounsaturated FAs.

FA profiles and levels between EDL and SOL muscles can be explained by a muscle fiber type-dependent uptake of FAs from the plasma as well as dissimilar expression of desaturases and elongases. The muscle content of PLs broadly reflected the FA composition of the diets, as has been reported previously (1, 3). It has been shown that plasma-muscle concentration gradient depends not only on muscle type but also on the specific acid (18, 54). In oxidative compared with glycolytic muscles, there is a greater abundance of long-chain FA transporters (FA-binding protein, FA translocase/CD36, and FA transport protein) (4). Therefore, a greater free FA pool came from the plasma via the transporters in SOL than in EDL muscles. The abundance of long-chain FA transporters is further elevated after the HFD (8). With HFD feeding, higher FA levels in oxidative SOL compared with glycolytic EDL muscles have also been reported previously (14). In obesity, there is also an increase in FA uptake and esterification (19).

SCD1 but not SCD2 expression was higher in EDL when compared with SOL muscles in both dietary groups. SCD catalyses the synthesis of either n-7 or n-9 MUFAs via desatu-

Table 4. Relationships between plasma insulin and leptin and the weight of adipose tissue and levels of individual phospholipid FAs in EDL and SOL muscles

<table>
<thead>
<tr>
<th>SFAs</th>
<th>Insulin</th>
<th>Leptin</th>
<th>Fat</th>
<th>Insulin</th>
<th>Leptin</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>−0.476§</td>
<td>NS</td>
</tr>
<tr>
<td>C18:0</td>
<td>+0.505*</td>
<td>+0.620†</td>
<td>+0.445*</td>
<td>+0.647†</td>
<td>+0.673†</td>
<td>+0.530†</td>
</tr>
<tr>
<td>C20:0</td>
<td>NS</td>
<td>+0.501*</td>
<td>NS</td>
<td>+0.552*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C22:0</td>
<td>+0.504*</td>
<td>+0.574†</td>
<td>+0.492*</td>
<td>+0.473*</td>
<td>NS</td>
<td>+0.603†</td>
</tr>
<tr>
<td>C24:0</td>
<td>+0.528*</td>
<td>+0.595†</td>
<td>+0.475*</td>
<td>+0.631†</td>
<td>+0.648†</td>
<td>+0.476*</td>
</tr>
<tr>
<td>n-7 MUFAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1 n-7</td>
<td>−0.582†</td>
<td>−0.706†</td>
<td>−0.490*</td>
<td>−0.490*</td>
<td>−0.571†</td>
<td>−0.406*</td>
</tr>
<tr>
<td>C18:1 n-7</td>
<td>NS</td>
<td>−0.527*</td>
<td>NS</td>
<td>−0.566*</td>
<td>−0.778†</td>
<td>−0.438*</td>
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<tr>
<td>n-9 MUFAs</td>
<td></td>
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<td></td>
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<tr>
<td>C18:1 n-9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>+0.481*</td>
<td>+0.558†</td>
<td>+0.481*</td>
</tr>
<tr>
<td>C20:1 n-9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>+0.724†</td>
<td>+0.663†</td>
<td>+0.672†</td>
</tr>
<tr>
<td>n-6 PUFAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:2 n6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>+0.493*</td>
<td>NS</td>
<td>+0.405*</td>
</tr>
<tr>
<td>C20:2 n6</td>
<td>+0.423*</td>
<td>+0.594†</td>
<td>+0.576*</td>
<td>+0.621†</td>
<td>+0.434*</td>
<td></td>
</tr>
<tr>
<td>C20:4 n6</td>
<td>+0.634†</td>
<td>+0.705†</td>
<td>+0.714†</td>
<td>+0.763†</td>
<td>+0.608†</td>
<td></td>
</tr>
<tr>
<td>n-3 PUFAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:3 n3</td>
<td>−0.678†</td>
<td>−0.782†</td>
<td>−0.537*</td>
<td>−0.537*</td>
<td>−0.653†</td>
<td>−0.449*</td>
</tr>
<tr>
<td>C20:5 n3</td>
<td>−0.644†</td>
<td>−0.735†</td>
<td>−0.546*</td>
<td>−0.658†</td>
<td>−0.743†</td>
<td>−0.481*</td>
</tr>
<tr>
<td>C22:5 n3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>−0.671†</td>
<td>−0.671†</td>
<td>−0.573*</td>
</tr>
<tr>
<td>C22:6 n3</td>
<td>NS</td>
<td>−0.446*</td>
<td>−0.456*</td>
<td>−0.464*</td>
<td>−0.646†</td>
<td>−0.525†</td>
</tr>
</tbody>
</table>

Correlation coefficients (r); n = 22–24 per group. For evaluation of these linear correlations, the Pearson correlation coefficient was used. If an assumption of normality was not confirmed, Spearman’s correlation coefficient was used instead. *P ≤ 0.05; †P ≤ 0.01. NS, nonsignificant; SFAs, saturated FAs.
Fig. 3. Effect of muscle fiber types on expression levels of desaturases and elongases with different diets. Nomenclature: stearoyl-CoA desaturase 1 (SCD1) and SCD2 (Δ9 desaturases), Elovl5 (rELO1), Elovl6 (rELO2). Data represent means ± SE; n = 11–12 per group; *values differ significantly between muscle types (EDL vs. SOL) with the same diet (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001); †values differ significantly between diets (SLD vs. HFD) with the same muscle type (†P ≤ 0.05, ††P ≤ 0.01).

ration of C16:0-CoA and C18:0-CoA to C16:1 n-7 and C18:1 n-9, respectively (reviewed in Ref. 40). Despite the higher SCD1 expression in EDL muscles, these muscles did not contain more C16:1 n-7 or C18:1 n-9 in the phospholipid or intracellular lipid fraction when compared with SOL muscles in the SLD group (FA fraction, containing free FAs, monoglycerides, diglycerides, and TGs; 0.066 ± 0.014 and 0.448 ± 0.074 μmol/g wet weight vs. 0.203 ± 0.050 and 1.828 ± 0.347 μmol/g wet weight; P ≤ 0.01 for C16:1 n-7 and C18:1 n-9 FAs, respectively, in EDL vs. SOL muscles). With the HFD, in the FA fraction, levels of C16:1 n-7 were significantly lower in EDL muscles, and levels of C18:1 n-9 were significantly higher in both muscles when compared with lower C16:1 n-7 levels in both muscles and higher C18:1 n-9 levels only in SOL muscles in the PL fraction (0.024 ± 0.006 and 0.100 ± 0.022 μmol/g wet weight vs. 0.865 ± 0.138 and 4.591 ± 0.991 μmol/g wet weight, C16:1 n-7 and C18:1 n-9 FAs, respectively, in EDL vs. SOL muscles). To evaluate changes in FAs, which are involved in the desaturation reaction with the HFD, and to adjust them to muscle type-dependent lipid levels, we calculated increase in the ratios of C16:1 n-7/C16:0 and C18:1 n-9/C18:0 with the HFD (values in the HFD were divided by values in the SLD) in both lipid fractions. The only significant difference was found in the lower value of C18:1 n-9/C18:0 ratio between EDL and SOL muscles in the PL fraction; no other values were dissimilar (FA fraction, 0.38 ± 0.15 vs. 0.32 ± 0.04 for C16:1 n-7/C16:0 and 1.26 ± 0.36 vs. 1.13 ± 0.16 for C18:1 n-9/C18:0 in EDL vs. SOL muscles, respectively; PL fraction, 0.37 ± 0.04 vs. 0.40 ± 0.13 for C16:1 n-7/C16:0 and 0.89 ± 0.07 vs. 1.07 ± 0.04, P ≤ 0.05, for C18:1 n-9/C18:0 in EDL vs. SOL muscles). Higher levels of both MUFAs in SOL muscles are in agreement with the statement that oxidative SOL muscles contain, and with HFD feeding accumulate, more FAs (14) and take up more C16:1 n-7 and C18:1 n-9 from the plasma than, for example, white gastrocnemius muscles (18); therefore, higher levels of these FAs were not expected in EDL than in SOL muscles even with the high SCD1 expression. The comparison of levels of FAs and the ratios of desaturation product/substrate between muscles showed that, with the HFD, EDL muscles preferably channeled C18:1 n-9 into TG than into the PL fraction because the TG fraction represents the largest proportion of intracellular lipids (18). The reason for the low levels of C16:1 n-7 with the HFD despite the high SCD expression is not clear. It can be suggested that C16:1 n-7 in skeletal muscles was either predominantly of a dietary origin rather than a result of desaturation of C16:0 or that C16:1 n-7 was preferentially utilized by carnitine palmitoyltransferase 1, which has a higher affinity for this FA (44).

It has been suggested that the presence of several SCD isoforms in rodents is related to their regulation through tissue-specific expression (reviewed in Ref. 40). The expression of SCD1 but not SCD2 (36) is induced by a high-carbohydrate diet or glucose oversupply in skeletal muscle and in the heart (23, 29, 36). Glycolytic EDL muscles rely more on glucose supply for their main source of energy and have lower rates of FA oxidation and lower insulin sensitivity than SOL muscles (46, 49). In addition, mRNA expression of SCD1 was higher in SOL muscle from animal models in which the fiber types were shifted from high-oxidative toward low-oxidative fibers (38), and SCD activity of microsomes of fast-twitch muscle has been found to be lower than that of slow-twitch muscle (45). Therefore, it may be speculated that the high SCD1 expression in EDL muscles is related to the glucose metabolism and high palmitate levels (C16:0) in glycolytic muscles and to a compensation for the lower SCD activity of the sarcoplasmic reticulum membrane, thus allowing these muscles to deal with increased levels of SFAs.

In response to the HFD, there was no increase in the expression of either SCD isoform in both muscles. This is in contrast to studies that have reported either an increase (30, 43) or a decrease (28) in the expression of SCD1 but is in agreement with a study in which no alteration in SCD expression was found with HFD feeding (41). The explanation lies in the regulation of SCD1 gene expression by dietary manipulation. Although SCD1 expression is upregulated in response to carbohydrates (23, 29, 36), palmitate (42), and SFAs (30), it is not altered after diets high in MUFAs and C18:2 n-6 (41) and is suppressed by diets high in PUFAs (reviewed in Ref. 39). In our study, whereas the HFD was high in C18:1 n-9, the plasma concentration of C18:1 n-9 was lower in obese animals, indicating increased uptake of this MUFA. The high levels of C18:1 n-9 in the HFD group were probably of dietary origin as well as from desaturation of C18:0, as has been reported previously (58). The high availability of C18:1 n-9 from the HFD probably blunted the increase in the SCD expression in both muscles and, in SOL, when compared with EDL muscles, led to the higher C18:1 n-9 levels in accordance with a greater
abundance of long-chain FA transporters and lipids in SOL muscles (4, 14).

The high SCD1 concentration in EDL muscles may therefore play a role in protecting these muscles against “lipotoxicity”-induced impairment of insulin signaling and consequent IR via the channeling of SFAs away from the synthesis of bioactive lipids into TGs. In SCD1 knockout mice, levels of C16:0 and C18:0 are elevated, whereas levels of C16:1 and C18:1 are decreased (32), a situation that is disadvantageous to insulin sensitivity (7, 8) because accumulation of C16:0, followed by C18:0, induces ceramide and DAG synthesis (10). C18:0 is a poor substrate for TG synthesis, whereas C18:1 leads to TG accumulation (47) and does not induce lipotoxicity. In addition, C16:1 n-7 acts as an insulin-sensitizing hormone, improving glucose metabolism (9). Overexpression of SCD1 results in TG esterification but attenuation of ceramide synthesis, improving glucose metabolism (9). Overexpression of SCD1 and elongation of SFAs and MUFAs leads to TG accumulation (47) and does not induce lipotoxicity. In addition, C16:1 n-7 acts as an insulin-sensitizing hormone, improving glucose metabolism (9).

Overexpression of SCD1 in EDL muscles is concordant with lower levels of C16:0 and higher levels of C18:0 and consequently of C18:1 n-9 in SOL muscles from both diets because the overexpression of rat Elovl6 has been shown to increase C18:1 n-9 content (24). On the contrary, the lower expression of Elovl6 with the HFD in EDL muscles probably restricted the increase in the synthesis of n-9 MUFAs in these muscles because Elovl6 deficiency decreases C18:0 and C18:1 n-9 levels in murine liver (33, 34). The higher Elovl5 expression in EDL muscles corresponds to higher levels of n-3 PUFAs, PUFAs with a chain length of C20-22 carbons, greater FA unsaturation, as well as the higher C20:4/C18:2 n-6 ratio when compared with SOL muscles because Elovl5 plays a role in endogenous PUFA synthesis (24) and the decline in its expression was found to be consistent with a failure to convert C18:2 to C20:4 n-6 in the liver (56). It has been suggested that changes in Elovl5 activity regulate hepatic glucose storage and production (57). In EDL muscles, the higher Elovl5 expression seems to be related to the glycolytic muscle type; nevertheless, the role of Elovl5 in carbohydrate metabolism in skeletal muscle remains to be further investigated.

The expression profile of elongases also suggests that SOL muscles may be more prone to IR with HFD feeding than EDL muscles because Elovl6 plays a role in the development of obesity, hyperinsulinemia, and hyperleptinemia (34). In the C16:0 intake, accompanied by elevations in TG, and it has been suggested that the resulting high ratio of C16:1/C16:0 and the reduction in C18 FAs might have protected HFD-fed mice against hepatic lipotoxicity and IR (34).

In summary, the study shows a muscle fiber type-dependent response to HFD feeding, consistent with the known effects of dietary FA composition on insulin sensitivity. Our data emphasize the potential importance of the fiber type composition of skeletal muscles and of their distribution in the development and progression of obesity and subsequent IR.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

MUSCLE FA PROFILE IS FIBER TYPE DEPENDENT


