Effect of Training on Muscle Triacylglycerol and Structural Lipids
A Relation to Insulin Sensitivity?

Jørn W. Helge and Flemming Dela

We studied whether endurance training impacts insulin sensitivity by affecting the structural and storage lipids in humans. Eight male subjects participated (age 25 ± 1 years, height 178 ± 3 cm, weight 76 ± 4 kg [mean ± SE]). Single-leg training was performed for 30 min/day for 4 weeks at ~70% of single-leg maximal oxygen uptake. After 8, 14, and 30 days, a two-step hyperinsulinemic-euglycemic glucose clamp, combined with catheterization of an artery and both femoral veins, was performed. In addition, a muscle biopsy was obtained from vastus lateralis of both legs. Maximal oxygen uptake increased by 7% in the trained leg (T), and training workload increased (P < 0.05) from 79 ± 12 to 160 ± 15 W. At day 8, glucose uptake was higher (P < 0.01) in the trained (0.8 ± 0.2, 6.0 ± 0.8, 13.4 ± 1.2 mg · min⁻¹ · kg⁻¹ · leg wt) than the untrained leg (0.5 ± 0.2, 3.7 ± 0.6, 10.5 ± 1.5 mg · min⁻¹ · kg⁻¹ · leg wt) at basal and the two succeeding clamp steps, respectively. After day 8, training did not further increase leg glucose uptake. Individual muscle triacylglycerol fatty acid composition and total triacylglycerol content were not significantly affected by training and thus showed no relation to leg glucose uptake. Individual muscle phospholipid fatty acids were not affected by training, but the content of phospholipid polyunsaturated fatty acids was higher (P < 0.06) after 30 than 8 days in T. Furthermore, after 30 days of training, the sum of phospholipid long-chain polyunsaturates was correlated to leg glucose uptake (r = 0.574, P < 0.04). Endurance training did not influence muscle triacylglycerol content or total triacylglycerol fatty acid composition. In contrast, training induced a minor increase in the content of phospholipid fatty acid membrane polyunsaturates, which may indicate that membrane lipids may have a role in the training-induced increase in insulin sensitivity. Diabetes 52:1881–1887, 2003

The fatty acid composition of the muscle membrane is linked to two major lifestyle diseases, obesity and diabetes (1). A higher proportion of saturated fatty acids in the sarcolemma is linked to adverse outcomes, such as insulin resistance and excessive accumulation of body fat (1). The incorporation of fatty acids into the muscle membrane in humans is influenced by the dietary fatty acid profile and dietary fat content per se (1,2). However, the composition of the phospholipid fatty acids in muscle membranes can also be modulated by endurance training (3,4). In parallel, endurance training leads to an improved insulin sensitivity of the skeletal muscle (5–7). However, the degree to which the training-induced improvement in insulin sensitivity is related to and influenced by changes in membrane fatty acid composition is inadequately described.

In recent years, studies have reported an inverse relationship between insulin sensitivity and both total muscle triacylglycerol (8,9) and intramyocellular triacylglycerol storage (10,11); however, between extramyocellular triacylglycerol storage and insulin sensitivity, most studies do not find a relationship (12). There is evidence that endurance training maintains (13) or even increases muscle triacylglycerol storage (14–16) and that muscle triacylglycerol stores around mitochondria are increased (17,18). Because endurance training improves insulin sensitivity, these observed adaptations therefore seem to be in conflict (19).

We have now studied the effect of endurance training on insulin sensitivity in leg skeletal muscle and compared the data with the muscle structural and storage fatty acid composition and the lipid content. The design included the single-leg training model in which the other leg served as control, which excludes the influence of dietary fatty acid composition and diet fat content, and thus serves as a very specific instrument to study the selective effect of training. Furthermore, to elucidate a time-course effect, we carried out experiments after 8, 14, and 30 days of single-leg training.

RESEARCH DESIGN AND METHODS
Materials and methods. Eight young (25.5 ± 1.0 years), healthy men were studied. They were lean (weight 76.4 ± 4.0 kg; BMI 25.4 ± 1.0 kg/m²) and without any family history of diabetes. The study was approved by the Ethical Committee for Copenhagen and Frederiksborg (KF 01-091/99), and all subjects gave informed consent.

Before training began and on one of the last days of the training period,
maximal oxygen uptake was measured on an ergometer bicycle (Ergoline 900, Blitz, Germany) during a graded exercise test, according to the leveling-off principle. Oxygen consumption and carbon dioxide production were measured by use of an online system (Oxycon Champion, Erich Jaeger, Hoechberg, Germany). Measurements were performed with the subject using both legs for biking and with each leg alone. All subjects trained one leg only on an ergometer bicycle for 30 min/day for 4 weeks at a work rate corresponding to ~70% of the predetermined single-leg maximal oxygen uptake (Fig. 1). During all training sessions, heart rate was continuously monitored and the work rate was adjusted upward every 2–3 days as progress in fitness occurred (Fig. 1). After 8, 14, and 30 days of training, muscle biopsies were obtained and a two-step hyperinsulinemic glucose clamp was performed. The subjects were always studied postabsorptive (10 h), ~18 h after the last exercise session. In the 3 days preceding the clamps, a minimum of 250 g of carbohydrates was consumed each day. After arrival in the laboratory, subjects were weighed and their height was measured and they went to bed. Precordial electrodes monitored electrocardiogram and heart rate. Cannulas were inserted into a cubital vein and the brachial or radial artery for later infusion of insulin and glucose and for monitoring of blood pressure and blood sampling, respectively. After application of local anesthesia, Teflon catheters were inserted into both femoral veins (Seldinger technique) 2 cm distal to the inguinal ligament and advanced so that the tip of the catheter was located ~2 cm distal to the inguinal ligament. The catheters were conical, with the hole at the tip being just wide enough for a thermistor to pass through. Four small (0.3 mm in diameter) side holes were drilled 1.5 cm from the tip, allowing blood drawing and injection of cold saline. A thermistor (Edslab probe 94-030-2.5F, Baxter) was inserted into the catheter and advanced 6–8 cm beyond the catheter tip. All cannulas were kept patent with a slow drip of saline (artery) and Na/KCl (femoral veins). Muscle biopsies were then taken from the vastus lateralis from both the trained and the untrained leg. The muscle biopsies were quickly cleaned from visible blood and/or fat and thereafter (~10 s) frozen in liquid nitrogen. After a 45-min rest, basal blood samples were drawn simultaneously from the arterial and venous catheters. This was done twice with an interval of 10 min. Immediately after, blood flow was measured using the thermodilution technique as described in detail by Dela et al. (20). A two-step (designated 1, physiologic insulin level; and 2, pharmacologic insulin level) sequential hyperinsulinemic glucose clamp was then performed. For each subject, a 50-ml insulin infusion had been prepared for each clamp step from insulin (Actrapid, Novo-Nordisk; 100 IU/ml), saline, and ~2.5 ml of the subject’s own plasma. At the start of each clamp step, insulin was given as a 2-ml bolus followed by a constant infusion for 120 min, using a Braun precision pump at a rate of 258 μl/min. Insulin infusion rates were 28 and 480 ml·min⁻¹·m⁻². Arterial plasma glucose concentration was measured at each of 5 min, and glucose (20%) was given to maintain plasma glucose concentration fasting level throughout the clamp. For each subject, the glucose concentration was clamped at the concentration measured at basal on day 8. At $t = 85$, 100, and 115 min in each clamp step, blood samples and flow measurements were obtained.

**Analyses and calculations.** Leg volume was measured by water displacement and was calculated as total leg volume minus volume of the foot, and subsequently leg weight was calculated from leg volume by assuming a specific gravity of 1. Blood sampled for analysis of glucose was collected in heparinized tubes and immediately high-speed centrifuged, whereupon plasma glucose concentration was measured by an automated glucose analyzer (YSI 23 AM, Yellow Springs Instruments). Insulin was measured by ELISA (Dako, Ely, U.K.). Blood oxygen saturation, hemoglobin, and PO₂ were determined using conventional methods (ABL 625, Radiometer, Copenhagen, Denmark).

The biopsies were divided into two parts, and one part was freeze-dried and, under a microscope, dissected free of blood, fat, and connective tissue. A sample of the muscle powder was analyzed for glycogen content by the hexokinase method (21). The other part of the muscle biopsy was used for extraction, derivatization, and quantification of the fatty acid components of muscle phospholipids and triacylglycerol as described by Pan et al. (22), with some modifications. In brief, muscle tissue, ~50 mg wet weight, was homogenized in 2:1 (vol/vol) chloroform:methanol, and total lipid extracts were prepared according to Folch et al. (23). Lipids fractions were then separated by thin-layer chromatography as described by Sacchetti et al. (24). Fatty acids from the triacylglycerol fraction or the phospholipid fraction were then transmethylated, and the methyl fatty acids were separated, identified, and quantified by gas chromatography. An internal standard TG tritertadecanoin was used to quantify individual triacylglycerol fatty acid content and subsequent total triacylglycerol content (24).

The content of individual fatty acids in the phospholipids and triacylglycerol extracted from the muscle was expressed as a percentage of the total fatty acids that were identified. Individual fatty acids that made up <1% of the total in all groups are not shown in the tables. Several indexes—the sum of polyunsaturated fatty acids, the sum of monounsaturated fatty acids, the ratio between n-6 fatty acids and n-3 fatty acids (n-6/n-3), and the total percentage of long-chain polyunsaturated fatty acids (PUFAs) with ≥20 carbon units (2C20–22 PUFAs)—were derived. Uptake and release of glucose and O₂ were calculated as arteriovenous difference, and whole-blood concentration differences were multiplied by plasma or blood flow for the trained and untrained legs separately.

**Statistical analysis.** Differences as a result of endurance training and time were tested with a two-way repeated measurements variance analysis. Whenever ANOVA revealed significant effects, a Tukey’s post hoc test was used to discern differences between groups. Pearson correlation was performed to determine linear relationships between variables. Values are expressed as means ± SE. Although biopsies from both legs were always taken from all subjects, there were differences in the amount of muscle tissue obtained. Thus, the datasets do not always include all eight subjects. The actual number of samples is indicated in the legends to figures and tables.

**RESULTS**

**Training markers.** Body weight tended to decrease from day 8 (76.4 ± 4.0 kg) to day 14 (75.8 ± 3.8 kg; $P < 0.08$), but no change was seen at day 30 (76.6 ± 4.1 kg). Maximal oxygen uptake was increased when measurements were done with the trained leg alone, but no increase could be detected with two-legged exercise testing (Table 1). The absolute work load performed by the subjects increased ($P < 0.05$) with training (Fig. 1). At the start of training, it was 79 ± 12 W and in the end had increased ($P < 0.05$) to 160 ± 15 W, but the heart rate during each training session remained constant (Fig. 1). Fluctuations in heart rate and work load from day 15 and onward are because each subject was allowed to have 1 day off at his own choice (not during the last 3 days). Therefore, the number of subjects behind each data point in this part of the training program may vary. Glycogen content in the muscle did not differ between trained and untrained legs until on days 14 and 30 (Table 1). The difference between the two legs in glycogen content increased ($P < 0.05$) progressively during the training period (Table 1). In the untrained leg, first a decrease then an increase in glycogen content was seen on days 8–14 and days 14–30, respectively (both, $P <
and 10.8

le.

acids was higher (biopsy, the fraction of polyunsaturated phospholipid fatty

affected by training (Table 3). However, in the muscle

Whole-body glucose

concentrations did not change with training and were

Fasting plasma glucose and insulin

value was increased (from days 8, 14, and 30). However, at clamp step 2, the

Maximal oxygen uptake and muscle glycogen and muscle triacylglycerol after 8, 14, and 30 days of adaptation to single-leg

TABLE 1

Maximal oxygen uptake and muscle glycogen and muscle triacylglycerol after 8, 14, and 30 days of adaptation to single-leg training

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Trained</th>
<th>Both legs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal oxygen uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ml · min⁻¹ · kg⁻¹ body wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>37.9 ± 1.8</td>
<td>37.8 ± 1.8</td>
<td>46.5 ± 1.5</td>
</tr>
<tr>
<td>After</td>
<td>NA</td>
<td>40.3 ± 1.8*</td>
<td>46.5 ± 2.1</td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/kg dry wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 8</td>
<td>436 ± 25</td>
<td>482 ± 39</td>
<td>—</td>
</tr>
<tr>
<td>Day 14</td>
<td>341 ± 25*</td>
<td>438 ± 40†</td>
<td>—</td>
</tr>
<tr>
<td>Day 30</td>
<td>442 ± 34*</td>
<td>617 ± 65*†</td>
<td>—</td>
</tr>
<tr>
<td>Muscle triacylglycerol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/kg wet wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 8</td>
<td>8.5 ± 1.1</td>
<td>6.8 ± 1.2</td>
<td>—</td>
</tr>
<tr>
<td>Day 14</td>
<td>11.5 ± 2.6</td>
<td>11.5 ± 3.4</td>
<td>—</td>
</tr>
<tr>
<td>Day 30</td>
<td>13.9 ± 3.8</td>
<td>7.8 ± 1.6</td>
<td>—</td>
</tr>
</tbody>
</table>

Testing was performed with two legs and with each leg separately. Maximal oxygen uptake *after* the training was measured in both legs after 25 days and in the trained leg after 27 days. NA, not available. *P < 0.05 vs. previous value; †(P < 0.05) vs. untrained leg.

0.05). In the trained leg muscle, glycogen was markedly increased (P < 0.05) after 30 days (Table 1).

Glucose and insulin. Fasting plasma glucose and insulin concentrations did not change with training and were 5.4 ± 0.1 mmol/l and 42 ± 9 pmol/l, respectively (pooled data from days 8, 14, and 30). During insulin infusion, plasma insulin concentration averaged 296 ± 18 pmol/l and 10.8 ± 0.8 μmol/l in clamp steps 1 and 2, respectively (pooled data from days 8, 14, and 30). Whole-body glucose uptake (M value) at clamp step 1 was not different on the 3 days (6.3 ± 0.6 mg · min⁻¹ · kg⁻¹ body wt; pooled data from days 8, 14, and 30). However, at clamp step 2, the M value was increased (P < 0.05) at day 30 (16.0 ± 0.9 mg · min⁻¹ · kg⁻¹ body wt) vs. day 8 (15.0 ± 0.9 mg · min⁻¹ · kg⁻¹ body wt), whereas the M value at day 14 (15.8 ± 0.8 mg · min⁻¹ · kg⁻¹ body wt) was not significantly different from the other two days. Across the leg, basal and insulin-mediated glucose uptake rates increased (P < 0.05) with training already at day 8 and remained increased in trained versus untrained leg throughout the study period (Fig. 2). There was no further increase in glucose uptake rates in trained leg beyond day 8. Furthermore, in untrained legs no effect of time on glucose uptake rates could be detected. The training-induced increase in glucose uptake rates was attributable to increases in glucose delivery (i.e., blood flow). Thus, leg blood flow was generally higher (P < 0.05) in the trained than in the untrained leg at both basal (21.8 ± 2.5 vs. 18.0 ± 1.6 ml · min⁻¹ · kg⁻¹ body wt), step 1 (31.0 ± 2.9 vs. 24.9 ± 1.8 ml · min⁻¹ · kg⁻¹ body wt), and step 2 (47.0 ± 4.1 vs. 41.3 ± 4.2 ml · min⁻¹ · kg⁻¹ body wt). In contrast, glucose extraction was not significantly different between trained and untrained legs, although there was a tendency (P = 0.088, main effect) for trained > untrained (Table 2).

Individual muscle phospholipid fatty acids were not affected by training (Table 3). However, in the muscle biopsy, the fraction of polyunsaturated phospholipid fatty acids was higher (P < 0.06) at day 30 than at day 8 (Table 3). Furthermore, after 30 days, the sum of phospholipid long-chain polyunsaturates (ΣC20–22, PUFA) was directly correlated to the glucose uptake at step 1 (in which insulin was in the physiologic range, r = 0.574, P < 0.04; Fig. 3A), a correlation that was not present at day 8 or day 14. In line with this observation, significant inverse correlations were also observed at day 30 between the fraction of stearic...
Palmitic (16:0) 18.7
Stearic (18:0) 18.4
Linoleic (18:2 n-6) 34.3
Dihomo-20:4 n-6 1.6
Docosahexanoeic (22:6 n-3) 1.6
C20–22, PUFA 16.8
Docosapentaenoic (22:5 n-3) 0.3
Docosatrienoic (22:3 n-3) 0.2

Because of the limited number of subjects, there was no correlation between the total muscle triacylglycerol content (Table 1) and the total muscle triacylglycerol content (Table 1) were not affected by training and showed no relation to leg glucose uptake. Because of the limited number of subjects, we cannot exclude that the lack of an increase in total muscle triacylglycerol with training could be a type 2 error. Furthermore, the apparently random variation in triacylglycerol content over time in trained and untrained legs may be due to heterogeneity of triacylglycerol storage. There was no correlation between the total muscle triacylglycerol content and leg glucose uptake rates at basal, step 1, or step 2 (Fig. 4).

In skeletal muscle, a range of techniques has been applied to quantify muscle triacylglycerol storage, the latest addition being the nuclear magnetic resonance (NMR) technique (28,29). With the application of these techniques, there is evidence that triacylglycerol stores in skeletal muscle, both intramyocellular TG measured by NMR and histochemical Oil red O and total myocellular TG measured by the biopsy method, are inversely correlated to insulin sensitivity in obese subjects (8,11,30), in insulin-resistant subjects (12,31), and in studies in which both

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Day 8 Untrained</th>
<th>Day 8 Trained</th>
<th>Day 14 Untrained</th>
<th>Day 14 Trained</th>
<th>Day 30 Untrained</th>
<th>Day 30 Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic (16:0)</td>
<td>18.7 ± 0.5</td>
<td>19.3 ± 0.5</td>
<td>19.2 ± 0.5</td>
<td>19.4 ± 0.4</td>
<td>19.2 ± 0.4</td>
<td>19.1 ± 0.6</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>18.4 ± 0.5</td>
<td>18.0 ± 0.3</td>
<td>17.7 ± 0.4</td>
<td>18.0 ± 0.3</td>
<td>18.1 ± 0.3</td>
<td>18.0 ± 0.3</td>
</tr>
<tr>
<td>Oleic (18:1n-9)</td>
<td>6.3 ± 0.4</td>
<td>7.0 ± 0.2</td>
<td>6.1 ± 0.2</td>
<td>6.8 ± 0.4</td>
<td>6.3 ± 0.4</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>Vaccenic (18:1n-7)</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Linoleic (18:2 n-6)</td>
<td>34.3 ± 0.6</td>
<td>33.1 ± 0.7</td>
<td>33.7 ± 0.3</td>
<td>32.8 ± 0.5</td>
<td>33.8 ± 0.4</td>
<td>34.1 ± 0.7</td>
</tr>
<tr>
<td>Dihomo-γ-linoleic (20:3 n-6)</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Arachidonic (20:4 n-6)</td>
<td>11.8 ± 0.3</td>
<td>11.8 ± 0.3</td>
<td>12.1 ± 0.3</td>
<td>11.9 ± 0.3</td>
<td>11.9 ± 0.3</td>
<td>12.1 ± 0.4</td>
</tr>
<tr>
<td>Docosahexanoeic (22:6 n-3)</td>
<td>1.6 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>ΣPolyunsaturated FA</td>
<td>52.5 ± 0.5</td>
<td>51.3 ± 0.6</td>
<td>53.0 ± 0.3</td>
<td>51.7 ± 0.5</td>
<td>52.3 ± 0.4</td>
<td>52.9 ± 0.4†</td>
</tr>
<tr>
<td>ΣMonounsaturated FA</td>
<td>9.0 ± 0.4</td>
<td>9.7 ± 0.2</td>
<td>8.9 ± 0.3</td>
<td>9.6 ± 0.4</td>
<td>9.0 ± 0.4</td>
<td>9.0 ± 0.5</td>
</tr>
<tr>
<td>Σn-6/2n-3</td>
<td>12.8 ± 1.0</td>
<td>14.6 ± 2.0</td>
<td>11.3 ± 0.9</td>
<td>12.7 ± 1.5</td>
<td>13.3 ± 1.8</td>
<td>13.6 ± 2.0</td>
</tr>
<tr>
<td>ΣC20–22, PUFA</td>
<td>16.8 ± 0.6</td>
<td>17.1 ± 0.5</td>
<td>17.8 ± 0.5*</td>
<td>17.5 ± 0.5*</td>
<td>17.3 ± 0.5</td>
<td>17.6 ± 0.6</td>
</tr>
</tbody>
</table>

Values are % of fatty acids. Mean ± SE, n = 7. *P < 0.05 vs. day 8; †P < 0.06 vs. day 8 (within the trained leg).
lean and obese subjects were pooled (9,11,32). However, in single, homogeneous groups of subjects (9,33–35) in the present study and in normoglycemic obese subjects (31), the inverse relationship between muscle triacylglycerol stores (measured by muscle biopsy, NMR, and Oil red O) and insulin sensitivity is normally not present, although exceptions exist (10,36). As for the extramyocellular triacylglycerol stores (fat and adipose cells stored outside the myofibrils but within the muscle fascia), most but not all studies (9) find no relation to insulin sensitivity (11,12,37).

Endurance-trained subjects have a maintained (13) or even increased muscle triacylglycerol storage (14,16,38). At the same time, endurance training significantly improves insulin sensitivity, which is obviously in direct conflict with the above-mentioned presence of an inverse relationship between muscle triacylglycerol and insulin resistance (19). In the present study, we found an increased insulin sensitivity after training and a maintained, not increased, total muscle triacylglycerol concentration. The reason that we were unable to detect an increase in total muscle triacylglycerol content may be the relative small number of subjects, i.e., the risk of type 2 error. However, with endurance training, muscle triacylglycerol stores around mitochondria are enhanced (17,18), whereas in obese and diabetic subjects, muscle triacylglycerol stores may have a more scattered location. This is an explanation that intrinsically gains strength from the fact that obese and diabetic subjects overall have a relatively smaller mitochondrial capacity (39,40) and higher muscle triacylglycerol stores (31,40,41) and thus less storage capacity around the mitochondria. However, if the observed coupling between muscle triacylglycerol and insulin sensitivity is caused by triacylglycerol metabolites, such as long-chain FA-CoA or diacylglycerol rather than triacylglycerol per se (42), then it is possible that the higher fat oxidative capacity in endurance-trained subjects abrogates the apparent conflicting data. In mice that overexpress human muscle lipoprotein lipase, intramuscular triacylglycerol stores increase without a concomitant increase in insulin resistance (43), indicating that the relationship between muscle triacylglycerol and insulin resistance is not simple and causal. It is interesting that fatty acid–induced insulin resistance has been linked to a reduction in insulin receptor substrate–1–associated phosphatidylinositol 3-kinase activity and an increase in both diacylglycerol content and protein kinase C activity (44–46), all of which could well bear a mechanistic relation to the coupling between muscle triacylglycerol and insulin resistance through an increased intracellular long-chain FA-CoA concentration.

Apart from the content of triacylglycerol in the muscle, the composition of the triacylglycerol may also affect insulin action. In the present study, muscle triacylglycerol fatty acid composition was not influenced by regular exercise training, a finding that is in line with previous observations (3,4), but we did find an increase in insulin sensitivity in the muscle after training. This lack of correlation between muscle triacylglycerol fatty acid composition and insulin sensitivity has previously been reported in some (8,47,48) but not all studies (32). Thus, as insulin sensitivity is changed by training, muscle triacylglycerol

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**TABLE 4**

Individual triacylglycerol fatty acid composition in vastus lateralis muscle in a trained and an untrained leg after 8, 14, and 30 days of adaptation to single-leg training

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Day 8 Untrained</th>
<th>Day 8 Trained</th>
<th>Day 14 Untrained</th>
<th>Day 14 Trained</th>
<th>Day 30 Untrained</th>
<th>Day 30 Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (14:0)</td>
<td>3.3 ± 0.3</td>
<td>3.1 ± 0.2</td>
<td>3.3 ± 0.4</td>
<td>3.2 ± 0.2</td>
<td>3.6 ± 0.6</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>32.9 ± 2.2</td>
<td>29.8 ± 1.1</td>
<td>32.4 ± 1.8</td>
<td>28.9 ± 1.8</td>
<td>29.9 ± 1.3</td>
<td>28.1 ± 1.2</td>
</tr>
<tr>
<td>Palmitoleic (16:1 n-7)</td>
<td>3.3 ± 0.4</td>
<td>4.0 ± 0.7</td>
<td>4.0 ± 0.5</td>
<td>5.4 ± 0.7</td>
<td>4.2 ± 0.6</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>8.0 ± 1.0</td>
<td>7.2 ± 0.9</td>
<td>9.6 ± 3.0</td>
<td>6.1 ± 1.2</td>
<td>7.2 ± 0.9</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td>Oleic (18:1 n-9)</td>
<td>41.6 ± 2.9</td>
<td>46.7 ± 0.7</td>
<td>41.2 ± 3.2</td>
<td>46.0 ± 2.8</td>
<td>45.9 ± 2.4</td>
<td>47.7 ± 0.8</td>
</tr>
<tr>
<td>Linoleic (18:2 n-6)</td>
<td>8.4 ± 0.9</td>
<td>8.1 ± 1.3</td>
<td>8.0 ± 0.5</td>
<td>9.8 ± 0.4</td>
<td>8.8 ± 0.4</td>
<td>8.8 ± 1.4</td>
</tr>
<tr>
<td>∑Saturated FA</td>
<td>44.2 ± 2.9</td>
<td>40.0 ± 2.0</td>
<td>45.3 ± 3.5</td>
<td>38.3 ± 3.1</td>
<td>40.7 ± 2.3</td>
<td>38.7 ± 2.1</td>
</tr>
<tr>
<td>∑Monounsaturated FA</td>
<td>44.9 ± 3.2</td>
<td>50.7 ± 0.9</td>
<td>45.2 ± 3.6</td>
<td>51.3 ± 3.0</td>
<td>50.1 ± 0.3</td>
<td>51.7 ± 1.1</td>
</tr>
<tr>
<td>∑Polyunsaturated, PUFA</td>
<td>11.0 ± 0.5</td>
<td>9.2 ± 1.4</td>
<td>9.5 ± 0.4</td>
<td>10.4 ± 0.2</td>
<td>9.2 ± 0.5</td>
<td>9.6 ± 1.3</td>
</tr>
</tbody>
</table>

Values are % of total fatty acids. Mean ± SE, n = 8.
fatty acid composition (at least in that situation) is not causally related to insulin sensitivity.

In this study, 30 days of regular, single-leg exercise training induced a small change in the muscle membrane phospholipid fatty acid profile, which coincided with the appearance of a significant relationship between polyunsaturated long-chain membrane phospholipid fatty acids and insulin sensitivity (Fig. 3A). As a similar relationship was not found on days 8 and 14, it is evident that an increase in long-chain PUFAs in the muscle membrane cannot be a major determinant of leg glucose uptake but possibly a significant contributor. However, our finding of a significant relationship between content of long-chain PUFAs in the membrane and leg glucose uptake after 30 days of training is supported by data from two studies in which changes in membrane phospholipid fatty acid composition were present after 6 weeks of low-intensity exercise training (3) or 4 weeks of one-leg knee extensor training (4). In these studies, training induced an increase in the muscle membrane phospholipid fatty acid fraction of oleic acid (18:1n-9) and an increased fraction of PUFAs (3,4). In the present study, we also found an increased fraction of PUFAs after training, and on closer inspection, the oleic acid fraction shows a similar trend (P < 0.09).

Insulin sensitivity has been related to muscle triacylglycerol content or composition in several studies (1,47,49). The oleic acid fraction shows a similar trend (P = 0.09). That phospholipase activity–induced release of different diacylglycerol molecules could have modulatory effects on cellular signaling cascades, e.g., protein kinase C activity (1), and that membrane-related changes in energy expenditure may influence the triacylglycerol accumulation (50).

Training-induced changes in insulin sensitivity are not related to muscle triacylglycerol content or composition in an experimental model in which the influence of diet is excluded. This may suggest that muscle triacylglycerol content and composition are merely reflections of the metabolic events that lead to decreased insulin sensitivity rather than causal factors that exert a direct influence on insulin sensitivity. In contrast, the small changes in muscle membrane phospholipid fatty acid composition after training reflected a pattern that is compatible with an improvement of insulin sensitivity.

ACKNOWLEDGMENTS

This study was supported by the Danish National Research Foundation (grant no. 504-14), the Danish Diabetes Association, Team Denmark, the Novo Nordisk Foundation, the Foundation of 1870, and Jacob Madsens & Oiga Madsens Foundation. J.W.H. was supported by the Danish Heart Association, ref. 99-1-3-48-22930.

Regitze Kraunsoe and Jeppe Bach performed excellent technical assistance.

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