Fatty acid profile of skeletal muscle phospholipids in trained and untrained young men

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Andersson, Agneta, Anders Sjödin, Anu Hedman, Roger Olsson, and Bengt Vessby. Fatty acid profile of skeletal muscle phospholipids in trained and untrained young men. Am J Physiol Endocrinol Metab 279: E744–E751, 2000.—Endurance trained (n = 14) and untrained young men (n = 15) were compared regarding the fatty acid profile of the vastus lateralis muscle after 8 wk on diets with a similar fatty acid composition. The skeletal muscle phospholipids in the trained group contained lower proportions of palmitic acid (16:0) (−12.4%, P < 0.001) and di-homo-γ-linolenic acid [20:3(n-6)] (−15.3%, P = 0.018), a lower n-6-to-n-3 ratio (−42.0%, P = 0.015), higher proportions of stearic acid (18:0) (+9.8%, P = 0.004) and sum of n-3 polyunsaturated fatty acids (+33.8%, P = 0.009), and a higher ratio between 20:4(n-6) to 20:3(n-6) (+18.4%, P = 0.006) compared with those in the untrained group. The group differences in 16:0, 20:3(n-6), 18:0/16:0, and 20:4(n-6)/20:3(n-6) were independent of fiber-type distribution. The trained group also showed a lower proportion of 16:0 (−7.9%, P < 0.001) in skeletal muscle triglycerides irrespective of fiber type. In conclusion, the fatty acid profile of the skeletal muscle differed between trained and untrained individuals, although the dietary fatty acid composition was similar. This difference was not explained by different fiber-type distribution alone but appears to be a direct consequence of changes in fatty acid metabolism due to the higher level of physical activity.

higher proportions of saturated fatty acids, particularly palmitic acid (16:0), in skeletal muscle phospholipids are associated with insulin resistance. Insulin resistance is associated with lower proportions of oxidative slow-twitch type I fibers and higher proportions of glycolytic fast-twitch type IIb fibers (25). Moreover, a greater proportion of n-3 PUFA and a smaller proportion of palmitic acid (16:0) have been observed in membrane phospholipids of type I fibers compared with type IIb fibers (22). Genetic factors are probably of importance both for the fatty acid composition of skeletal muscle phospholipids (4) and for the fiber-type distribution (33). Skeletal muscle characteristics are also influenced by environmental factors such as diet and physical activity. Changes in the fatty acid composition of skeletal muscle phospholipids in response to changes in dietary fat quality have been demonstrated in rats (2, 34). Furthermore, recent data indicate that dietary fat has influence on the skeletal muscle fatty acid profile in humans as well (3).

Long-term endurance training has been shown to modify the muscle fiber distribution, with a shift away from the insulin-resistant type IIb fibers (30). The effect of training on the fatty acid composition of skeletal muscle membrane phospholipids is less understood. In a diet-controlled randomized intervention study in sedentary men (1), we have recently observed changes in the fatty acid composition of skeletal muscle phospholipids after 6 wk of regular low-intensity exercise. The principal aim of the present study was to compare the fatty acid composition of skeletal muscle phospholipids and triglycerides between endurance-trained young athletes and untrained young men receiving diets with an identical fatty acid profile. Another aim was to investigate whether the fatty acid pattern of the skeletal muscle in the two groups was related to variation in muscle fiber-type distribution. The fatty acid composition of serum, serum lipoprotein concentrations, and insulin sensitivity were also determined.

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SKELETAL MUSCLE plays an essential role in whole body energy metabolism and is the major tissue responsible for insulin-mediated glucose uptake (10). Insulin resistance is closely associated with the development of several diseases, including diabetes type 2, obesity, and atherosclerosis (9), but the underlying mechanisms are not completely understood. The fiber-type distribution, triglyceride storage, and the fatty acid composition of membrane phospholipids in skeletal muscle have been linked to insulin sensitivity (20). Studies both in animals (34) and in humans (6, 8, 28, 36) have shown that lower proportions of long-chain polyunsaturated fatty acids (PUFA) and physical activity; fiber type; triglycerides; dietary fat; insulin sensitivity

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MATERIALS AND METHODS

Subjects. Sixteen untrained young men (UNT group) with low habitual physical activity and 15 endurance-trained male athletes (TRA group) volunteered for the present study. Both groups consisted mainly of students from Uppsala University. Two subjects did not complete the study, one in the UNT group for personal reasons and one in the TRA group because of intercurrent illness. The UNT group had at the most been engaged in organized sport activity 1 day per week during the last year. The TRA group (cyclists, cross-country skiers, and orienteers) had trained systematically and had actively competed during the last 2 yr. All of them had regularly been engaged in sport activities for 6 yr or more. All participants were healthy and did not use any medications. The subjects were asked to refrain from all kinds of dietary supplementation (i.e., vitamins, minerals, and fatty acids) for at least 2 wk before the study. None of the subjects were tobacco users. They received free food-stuffs during the study but no monetary payment. All subjects gave their informed consent. The study was approved by the Ethical Committee of the Medical Faculty of Uppsala University.

Study design and diet. A partly controlled diet was given to all subjects for 8 wk (standardized period) to standardize the dietary fat quality before muscle biopsy was performed. All participants were given detailed dietary instructions (both written and oral) by the same dietitian. They were supplied with a margarine with a standardized fat composition once a week. The participants were instructed to use this margarine both as a spread on bread and for cooking their habitual diet. In addition, they received a portion of standardized lunch meal that was included daily in their diet. They were asked to reduce the intake of other food items with a high fat content. During the standardized period, the participants recorded their food intake twice as weighed food records (3 days each time: 2 weekdays and 1 day during the weekend). The body weight was checked regularly during the 8-wk standardized period to keep the subjects weight stable. Baseline data, collected 1 wk before the standardized period, included habitual dietary intake (3 days weighed food record), peak oxygen uptake (\( \dot{V}O_2 \) peak), insulin sensitivity, and body composition, and blood analyses. The blood analyses and measurements of insulin sensitivity and body composition were repeated at the end of the standardized period. During the standardized period, the members of the TRA group trained according to their usual training schedule and kept a training diary. The members of the UNT group continued their ordinary life with low physical activity and were allowed to exercise at most once a week. At the end of the standardized period, a muscle biopsy was performed.

Analyses of dietary intake. The average dietary intake at baseline and during the standardized period was calculated from weighed-food records using the database of the Swedish National Food Administration (PC-Kost 1996) and a computerized calculation program (Stor MAT’s, Rudans Låttdata, Västerås, Sweden). The margarine given to the participants during the standardized period and the fat used in preparing the lunch meals were analyzed for their fat content and fatty acid composition. The fatty acid compositions of the margarine and the cooking fat were then added to the database of the calculation program to obtain more exact information about the dietary fatty acid intake.

Aerobic capacity. \( \dot{V}O_2 \) peak was determined during an incremental exercise test on an electronic bicycle ergometer (Monark 829E, Varberg, Sweden), with simultaneous measurements of respiratory gas exchanges (SensorMedics 2900Z, Anaheim, CA).

Blood samples and biochemical analyses. Venous blood samples were drawn in the morning after an overnight fast. Very low density lipoproteins, low-density lipoproteins, and high-density lipoproteins (HDL) were isolated by a combination of preparative ultracentrifugation (17) and precipitation with a sodium phosphotungstate and magnesium chloride solution (31). Triglyceride and cholesterol concentrations were measured in serum and in the isolated lipoprotein fractions by enzymatic methods, with the IL Test Cholesterol Method 181618–10 and IL Test Triglyceride Enzymatic-Colorimetric Method 181610–60 for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, MA).

Fatty acid composition. The fatty acid compositions of serum phospholipids, cholesterol esters, skeletal muscle phospholipids, and triglycerides were determined by gas-liquid chromatography, as previously described (1), and were expressed as a percentage of the total fatty acids identified. The identity of 14 individual fatty acid peaks was ascertained by comparing each peak’s retention time relative to the retention times of fatty acids in synthetic standards of known fatty acid composition. The relative amount of each fatty acid (% of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids. The coefficient of variation for determination of the proportions of fatty acids in skeletal muscle phospholipids has also been reported previously (1). The activities of certain enzymes involved in fatty acid biosynthesis were estimated as the product-to-precursor ratios of the percent-ages of individual fatty acids. The estimated enzyme activities included those of elongase, calculated as the stearic acid (18:0)-to-palmitic acid (16:0) ratio; \( \Delta 5 \) desaturase, calculated as the arachidonic acid [20:4(n-6)]-to-di-homo-\( \gamma \)-linolenic acid [20:3(n-6)] ratio; \( \Delta 6 \) desaturase, calculated as the di-homo-\( \gamma \)-linolenic acid [20:3(n-6)]-to-linoleic acid [18:2(n-6)] ratio (assuming that \( \Delta 6 \) desaturase and not elongase is rate limiting); and \( \Delta 9 \) desaturase, calculated as the oleic acid [18:1(n-9)]-to-stearic acid (18:0) ratio. The total percentage of long-chain PUFA with \( \geq 20 \) carbon units (C20–22 PUFA); the sum of n-3 PUFA [18:3(n-3), 20:5(n-3), 22:5(n-3), and 22:6(n-3)]; and the sum of n-6 PUFA [18:2(n-6), 20:3(n-6), and 20:4(n-6)] were calculated from the primary data.

Muscle sample. Muscle biopsy samples were obtained from the vastus lateralis muscle, under local anesthesia, with a Bergstrom needle and were immediately frozen in liquid nitrogen and stored at \(-70^\circ C\). One part of the sample (15–30 mg) was homogenized, extracted overnight, and separated by thin-layer chromatography for analysis of fatty acid composition. The remaining part of the sample was examined to determine the muscle fiber distribution.

Fiber-type analyses. To determine the relative proportions of different fiber types, the standard histochemical myosin adenosine triphosphatase method was used (7). The sections were preincubated at \( pH \) 4.3, and fibers were classified in type I or II as described by Brooke and Kaiser (7). As large an area as possible was counted in each biopsy sample (206 \( \pm \) 71 fibers/sample). All morphological measurements were performed by the same person, who was blinded for the case status, with the use of a computerized image analysis system, designed for analysis of skeletal muscle morphology (Bio-Rad Scan Beam, Hadsund, Denmark), linked to an optical microscope (Leitz, Germany) by a videocamera (DAGE_MTI, CCD-72). The muscle fiber distribution was successfully evaluated in 8 subjects in the TRA group and 11 subjects in the UNT group. The reproducibility of muscle fiber distribution analyzed in our laboratory as duplicate biopsy samples from the same site of right vastus lateralis in
a group of 23 subjects corresponds to a coefficient of variation of ~20–30% (18).

Insulin sensitivity. Insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp technique (11). The insulin (Actrapid R Hum: Novo, Copenhagen, Denmark) infusion rate during the clamp study was 56 mU·m−2·min−1, resulting in a mean plasma insulin concentration of ~100 mU/L. The glucose uptake (M value, mg·kg body wt−1·min−1) was measured during the last 60 min of the clamp test. The insulin sensitivity index (M/I, mg·kg body wt−1·min−1 per mU/L × 100) was measured by dividing the mean glucose uptake by the mean insulin concentration during the last 60 min of the total 120 min of the clamp test. The measurements of insulin sensitivity were performed after an overnight fast in the “habitual state,” which meant that the members of the TRA group trained as usual according to their training program on the day before the clamp test and the UNT group remained sedentary. The serum insulin concentration was measured by an enzymatic immunological assay (Boehringer Mannheim, Germany) performed in an ES 300 automatic analyzer. Blood glucose concentrations were measured by the glucose oxidase assay.

Body composition. Body mass index (BMI) was calculated as body weight (kg) divided by squared height (m²). The body fat content was estimated by means of a three-compartment model (14) based on underwater weighing and bioelectrical impedance analysis.

Physical activity. Physical activity level (PAL) is defined as total energy turnover divided by basal metabolic rate (BMR) and reflects the habitual level of physical activity (32). As an approximate measure of the average level of physical activity in the present study, the estimated physical activity level (PALest) was obtained by dividing the energy intake (EI) by estimated basal metabolic rate (BMRest): PALest = EI / BMRest. EI was based on three 3-day weighed-food records. BMRest was calculated using equations from Westerterp et al. (37), taking both fat-free mass and fat mass into account.

Statistical analyses. The variables are presented as means ± SD. The data were analyzed statistically with use of SAS and JMP software (SAS Institute). Differences in group means were analyzed with Student’s unpaired t-test (in some cases after logarithmic transformation of data). Changes over time within groups were analyzed with Student’s paired t-test. Relationships between variables were analyzed by simple and partial correlations (Pearson’s correlation coefficients). When normality was not achieved by logarithmic transformation of data, nonparametric tests were used (Mann-Whitney’s U test, Wilcoxon matched paired signed rank test, or Spearman’s correlation). Correlations were calculated for all the subjects (TRA group and UNT group combined). Group differences in fatty acid composition of skeletal muscle phospholipids and triglycerides were adjusted for fiber-type distribution, body weight, and percentage of body fat, respectively, with an analysis of covariance model. All tests were two tailed, and the level of significance was 0.05. Statistical analyses where fiber-type distribution was included are presented only for the proportion of type I fibers, because the percentage of type I and type II fibers was strongly interrelated (r = −1.0).

RESULTS

Subject characteristics. Subject characteristics at baseline are shown in Table 1. The TRA group had higher values than the UNT group for VO2peak and insulin sensitivity and lower values for the percentage of body fat and serum triglycerides. Age, BMI, waist-to-hip-ratio, serum cholesterol, and HDL cholesterol were similar in the groups. The subject characteristics at the end of the standardized period were similar to those at baseline for both groups, except for HDL cholesterol, which was increased in the TRA group (probably as a result of a higher total fat intake during the standardized period), resulting in a group difference at the end of the standardized period (TRA group 1.30 ± 0.29 vs. UNT group 1.11 ± 0.21 mmol/l, P = 0.04).

Exercise. According to their training diaries, the members of the TRA group had a mean training time of 74 ± 24 min/day during the 8-wk standardized period. During this period (54 ± 4 days), they were actually training 37 ± 7 days and resting 17 ± 5 days, which resulted in a mean duration of exercise on the training days of 106 ± 30 min.

Dietary intake. The average dietary intake before (baseline) and during the standardized period, estimated from weighed food records, is presented in Table 2. The absolute intake of total energy was higher in the TRA group than in the UNT group, both at baseline and during the standardized period. The relative intake of macronutrients was similar in the groups both at baseline and during the standardized period, except for a slightly higher relative energy intake of carbohydrates in the TRA group compared with the UNT group during the standardized period. The energy percentage of fat was higher during the standardized period than at baseline in both groups. The UNT group reported a lower relative intake of saturated fatty acids and a higher relative intake of monounsaturated fatty acids and PUFA during the standardized period than at baseline. The TRA group reported a higher relative intake of monounsaturated fatty acids and PUFA but a similar intake of saturated fatty acids during the standardized period compared with baseline.

Fatty acid composition of skeletal muscle phospholipids. Table 3 summarizes the fatty acid profile of skeletal muscle phospholipids in the two groups at the end of the standardized period. The proportions of palmitic

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TRA Group</th>
<th>UNT Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>21 ± 4</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.9 ± 5.7</td>
<td>181.9 ± 7.6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.1 ± 6.8</td>
<td>76.4 ± 8.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.9 ± 1.5</td>
<td>23.1 ± 2.6</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.89 ± 0.03</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>13.3 ± 3.3</td>
<td>18.7 ± 7.2*</td>
</tr>
<tr>
<td>VO2peak, ml·min⁻¹·kg⁻¹</td>
<td>64.2 ± 4.6</td>
<td>49.4 ± 6.0‡</td>
</tr>
<tr>
<td>S-cholesterol, mmol/l</td>
<td>3.76 ± 0.57</td>
<td>4.08 ± 0.71</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/l</td>
<td>1.14 ± 0.17</td>
<td>1.05 ± 0.21</td>
</tr>
<tr>
<td>S-triglyceride, mmol/l</td>
<td>0.75 ± 0.39</td>
<td>1.35 ± 0.60‡</td>
</tr>
<tr>
<td>M/I</td>
<td>14.1 ± 4.1</td>
<td>9.8 ± 3.6†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 14 for trained (TRA) group and n = 15 for untrained (UNT) group. BMI, body mass index; VO2peak, peak oxygen uptake; M/I, insulin sensitivity index; HDL, high-density lipoprotein. *P < 0.05, ‡P < 0.01, †P < 0.001 for differences between groups.
Table 2. Calculated average dietary intake before (baseline) and during standardized period in TRA and UNT groups

<table>
<thead>
<tr>
<th></th>
<th>Baseline TRA group</th>
<th>Baseline UNT group</th>
<th>Standardized TRA group</th>
<th>Standardized UNT group</th>
<th>P value for diff. between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ</td>
<td>15.6 ± 2.2</td>
<td>10.6 ± 3.0</td>
<td>15.4 ± 2.3</td>
<td>9.6 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein, E%</td>
<td>14 ± 2</td>
<td>15 ± 3</td>
<td>14 ± 2</td>
<td>15 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrates, E%</td>
<td>60 ± 2</td>
<td>57 ± 4</td>
<td>58 ± 3</td>
<td>55 ± 4</td>
<td>0.042</td>
</tr>
<tr>
<td>Fat, E%</td>
<td>26 ± 2</td>
<td>28 ± 5</td>
<td>28 ± 2*</td>
<td>30 ± 4†</td>
<td>NS</td>
</tr>
<tr>
<td>SAFAs, E%</td>
<td>11 ± 1</td>
<td>11 ± 2</td>
<td>10 ± 1</td>
<td>9 ± 2†</td>
<td>NS</td>
</tr>
<tr>
<td>MUFAs, E%</td>
<td>9 ± 1</td>
<td>10 ± 2</td>
<td>11 ± 1†</td>
<td>12 ± 2‡</td>
<td>NS</td>
</tr>
<tr>
<td>PUFAs, E%</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 1†</td>
<td>6 ± 1†</td>
<td>NS</td>
</tr>
<tr>
<td>Dietary fiber, g</td>
<td>34 ± 9</td>
<td>25 ± 9</td>
<td>34 ± 7</td>
<td>20 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>423 ± 149</td>
<td>309 ± 172</td>
<td>358 ± 126</td>
<td>240 ± 136</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 14 for TRA group and n = 15 for UNT group. Average dietary intake at baseline is based on one 3-day weighed-food record. Dietary intake during the standardized period is based on two 3-day weighed food records. E%, percentage of total energy intake; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; NS, nonsignificant. *P ≤ 0.05, †P ≤ 0.01, ‡P ≤ 0.001 for differences between baseline and standardized period in the respective groups.

Fatty acid composition of skeletal muscle triglycerides. At the end of the standardized period, the proportions of palmitic acid (16:0) and palmitoleic acid (16:1(n-7)) were lower and those of heptadecanoic acid (17:0) and α-linolenic acid (18:3(n-3)) higher in the TRA group than in the UNT group (Table 4). These results were all independent of body weight, but only the difference in 16:0 remained significant after correction for differences in body fat proportion and fiber-type distribution.

Table 3. Fatty acid composition (%) of skeletal muscle phospholipids in TRA and UNT groups at end of standardized period

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>TRA Group</th>
<th>UNT Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.84 ± 0.11</td>
<td>0.86 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>15:0</td>
<td>0.21 ± 0.03</td>
<td>0.23 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>16:0</td>
<td>19.3 ± 1.1</td>
<td>22.1 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>17:0</td>
<td>0.29 ± 0.03</td>
<td>0.32 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td>18:0</td>
<td>14.7 ± 0.9</td>
<td>13.4 ± 1.3</td>
<td>0.004</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>0.64 ± 0.23</td>
<td>0.79 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>11.2 ± 1.5</td>
<td>10.5 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>31.1 ± 1.7</td>
<td>31.6 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.74 ± 0.14</td>
<td>0.68 ± 0.28</td>
<td>NS</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>1.10 ± 0.17</td>
<td>1.30 ± 0.22</td>
<td>0.018</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>13.1 ± 1.3</td>
<td>12.9 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>2.05 ± 0.45</td>
<td>1.84 ± 0.48</td>
<td>NS</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>1.80 ± 0.27</td>
<td>1.75 ± 0.26</td>
<td>NS</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>3.76 ± 0.51</td>
<td>2.86 ± 1.09</td>
<td>0.024</td>
</tr>
<tr>
<td>sum n-6</td>
<td>45.2 ± 1.7</td>
<td>45.8 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>sum n-3</td>
<td>8.2 ± 1.1</td>
<td>6.1 ± 2.3</td>
<td>0.009</td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>5.6 ± 1.0</td>
<td>9.7 ± 7.1</td>
<td>0.015</td>
</tr>
<tr>
<td>sum C20-22</td>
<td>21.0 ± 2.8</td>
<td>19.8 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>20:4(n-6)/20:3(n-6) (Δ5 activity)</td>
<td>12.1 ± 1.2</td>
<td>10.2 ± 1.9</td>
<td>0.006</td>
</tr>
<tr>
<td>20:3(n-6)/18:2(n-6) (Δ6 activity)</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>18:1/18:0 (Δ9 activity)</td>
<td>0.76 ± 0.10</td>
<td>0.79 ± 0.15</td>
<td>NS</td>
</tr>
<tr>
<td>18:0/16:0 (elongase activity)</td>
<td>0.76 ± 0.07</td>
<td>0.61 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 14 for TRA group and n = 15 for UNT group.

Table 4. Fatty acid composition (%) of skeletal muscle triglycerides in TRA and UNT groups at end of standardized period

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>TRA Group</th>
<th>UNT Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>3.10 ± 0.55</td>
<td>3.48 ± 0.43</td>
<td>NS</td>
</tr>
<tr>
<td>15:0</td>
<td>0.40 ± 0.09</td>
<td>0.41 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>16:0</td>
<td>21.9 ± 1.3</td>
<td>23.7 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>17:0</td>
<td>0.35 ± 0.11</td>
<td>0.26 ± 0.05</td>
<td>0.021</td>
</tr>
<tr>
<td>18:0</td>
<td>7.50 ± 2.31</td>
<td>5.86 ± 2.10</td>
<td>NS</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>4.80 ± 2.02</td>
<td>6.39 ± 1.99</td>
<td>0.044</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>48.4 ± 1.8</td>
<td>47.0 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>11.7 ± 0.56</td>
<td>11.1 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>1.70 ± 0.42</td>
<td>1.34 ± 0.31</td>
<td>0.012</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.57 ± 0.16</td>
<td>0.60 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 14 for TRA group and n = 15 for UNT group. ND, not detectable.
illustrated in Table 5. The fatty acids that we found in lower proportions [16:0 and 20:3(n-6)] in the TRA group than in the UNT group were in general inversely correlated with those variables, whereas the fatty acids and ratios of fatty acids that we found in higher proportions [18:0, 22:6(n-3), sum n-3, 20:4(n-6) to 20:3(n-6)] were positively correlated with those variables.

Fatty acid composition of serum phospholipids and cholesterol esters. There were no differences in fatty acid profiles of serum phospholipids and cholesterol esters between the study groups, either at baseline or at the end of the standardized period, except for the proportion of palmitic acid (16:0) in serum phospholipids, which was lower in the TRA group than in the UNT group at the end of the standardized period (29.6 ± 0.6 vs. 30.5 ± 1.1%, P = 0.016). The similarity of the changes in the fatty acid patterns in serum phospholipids and cholesterol esters in the study groups, which was observed during the standardized period, reflects the similarity of the dietary fat quality during the standardized period.

**DISCUSSION**

The main findings in the present study were the differences in the fatty acid profiles of skeletal muscle phospholipids and triglycerides between the TRA group and the UNT group despite their similar dietary fatty acid composition. The fatty acid pattern in muscle phospholipids is probably partly genetically determined (4) but has also been shown to be influenced by environmental factors such as diet (2, 3) and physical activity (1, 19, 35). In the present study, the fatty acid composition of skeletal muscle was determined after 8 wk of diet with a controlled fatty acid composition. According to the food records, the study groups had similar proportions of fatty acids in the diet both at baseline and during the standardized period. The compliance to the controlled diet was verified by the similarity between TRA and UNT groups regarding the fatty acid compositions of serum cholesterol esters and serum phospholipids. Thus the observed dissimilarity in the fatty acid profile of skeletal muscle was not influenced by dietary fat quality but did reflect the difference in fatty acid metabolism presumably due to the difference in the degree of physical activity.

A lower proportion of palmitic acid (16:0) in the skeletal muscle membrane in trained compared with sedentary men, as seen in the present study, was also observed in a study by Thomas and colleagues (35). In that study, however, the intake of dietary fat was not controlled. In a randomized intervention study on sedentary men with a fully controlled diet, we have recently observed a decrease in the proportion of palmitic acid (16:0) in the skeletal muscle phospholipids in the training group during a 6-wk period of regular low-intensity exercise (1). However, this change was not significantly different from that in the control group. Regular exercise has also been found to induce a reduction in the proportion of 16:0 in red quadriceps muscle in the rat (19). The proportion of palmitic acid (16:0) in skeletal muscle phospholipids has been found to be negatively related to insulin sensitivity (36). Concomitantly, with the lower percentage of palmitic acid (16:0) in the TRA group, we observed a higher proportion of stearic acid (18:0) than in the UNT group, resulting in a higher 18:0-to-16:0 ratio. In the aforementioned study by Thomas and colleagues, the proportion of stearic acid (18:0) in skeletal muscle membrane was found to be positively correlated with the amount of weekly mileage run in trained individuals. The 18:0-to-16:0 ratio in skeletal phospholipids, reflecting the activity of the enzyme elongase in the biosynthesis of fatty acids, has also been reported to be positively related to insulin sensitivity (8).

In our previous study in sedentary men (1), regular physical training seemed to affect the contents of n-6 PUFA and n-3 PUFA in different ways with a significant decrease in n-6 PUFA and a tendency toward a reduction of the n-6-to-n-3 PUFA ratio in the skeletal muscle phospholipids. This was even more obvious in the present study, where the n-6-to-n-3 PUFA ratio was significantly lower in the TRA group than in the group of UNT subjects. The lower n-6-to-n-3 PUFA ratio was mainly explained by a higher proportion of docosahexaenoic acid [22:6(n-3)] and a lower proportion of di-homo-γ-linolenic acid [20:3(n-6)]. A higher proportion of total n-3 PUFA, particularly docosahexaenoic acid [22:6(n-3)], in skeletal muscle phospholipids has previously been associated with a lower fasting plasma glucose level in young children (3) and higher insulin action in rats (34). In addition, in the present study, the ratio of 20:4(n-6) to 20:3(n-6) (reflecting the Δ5 desaturase activity), which has previously been shown to be positively related to insulin sensitivity in humans (6, 28), was higher in the TRA group than in the UNT group. In this study, we did not find any group differences in the proportion of arachidonic acid [20:4(n-6)] in skeletal muscle phospholipids. This is in contrast to the findings in our previous training study and in a rat study by Helge and colleagues (19), showing that regular exercise leads to a decreased proportion of 20:4(n-6) independent of diet.

A number of fatty acids as well as the ratios between 20:4(n-6) to 20:3(n-6) and 18:0 to 16:0 in skeletal mus-
cle phospholipids have been shown to be inversely related to the percentage of body fat (28). In the present study, the observed difference in fatty acid profile between the TRA group and UNT group was independent of body weight and body fat content.

The fatty acid pattern in skeletal muscle phospholipids seen in the TRA group corresponds to a fatty acid profile associated with a higher insulin sensitivity. This is in contrast to the findings by Helge and colleagues (19) of a lower proportion of 22:6(n-3) and of total C20–22 PUFA and lower Δ5-desaturase activity in trained rats compared with untrained, which is related to impaired insulin action. Whether the fatty acid composition of skeletal muscle phospholipids could explain the higher insulin sensitivity in the TRA group compared with the UNT group could not be determined in the present study, because the high insulin sensitivity in the TRA group could be attributable to both acute and long-term effects of training. Several underlying mechanisms such as increased blood flow, activation of the glucose transport system, increased muscle glycogen synthase activity, and increased capillary density are probably involved in the improved insulin action during exercise (13, 25). However, modifications in the membrane fatty acid composition of cultured cells are shown to accompany changes in membrane fluidity and an altered insulin-binding capacity (15).

In the present study, we also found that the fatty acid composition of skeletal muscle triglycerides differed between the TRA group and the UNT group. To our knowledge, this has not been reported previously. This is in contrast to our earlier observation in sedentary men who showed no changes in the fatty acid profile of muscle triglycerides during 6 wk of exercise (1). In that study, there was a much smaller difference in physical activity between the TRA group and UNT group, which may explain those different findings between our studies. The difference in fatty acid composition between skeletal muscle membrane phospholipids and intramuscular triglycerides might reflect different sources of fatty acids. The fatty acid pattern in muscle triglycerides is very similar to that of the adipose tissue triglycerides, whereas the fatty acid composition of muscle phospholipids is more similar to that of serum phospholipids. Thus the fatty acid composition of skeletal muscle phospholipids may be more directly influenced by the availability of dietary fat and by the interaction between fat oxidation and exercise.

The time scale of changes in skeletal muscle fatty acid profile in response to training and dietary fat is not accurately known. According to a dietary studies in rats, a turnover of muscle phospholipids occurs in less than 2 (2–4 wk (19). In humans, the dietary fatty acid profile is reflected in serum phospholipids after 2–3 wk (5), but the turnover time of skeletal muscle phospholipids is not known. We have estimated that a diet period of 8 wk probably would be sufficient to demonstrate an effect of change in dietary fat quality on skeletal muscle phospholipids.

In accordance with previous studies (30), we observed a higher proportion of type I fibers and a lower proportion of type II fibers in the endurance TRA group than in the UNT group. In the present investigation, we found the distribution of type I fibers to be negatively correlated with the percentage of palmitic acid (16:0) and positively correlated with the percentage of docosahexaenoic acid [22:6(n-3)]. These findings support earlier observations both in rats (22) and in humans (21). In isolated rat muscle, there has been observed a greater incorporation of palmitic acid in phospholipids in fast, glycolytic type II fibers than in slow, oxidative type I fibers (12). The fiber-type distribution is genetically determined (33) but is also influenced by training. A shift toward more type I and type IIa fibers away from type IIB has been documented as due to endurance long-term training (30). Physical inactivity can cause a decrease in the proportion of type I fibers and altered relative proportion of type IIa and IIB fibers (16). It is not clear whether these changes are accompanied by changes in the fatty acid composition of the skeletal muscle phospholipids. One recent experimental study (22) has shown that an alteration in fiber composition in exercising rats was not accompanied by changes in muscle membrane phospholipids. In the present study, the fiber-type distribution only partly explained the differences in fatty acid composition of skeletal muscle phospholipids between the TRA group and the UNT group. The differences in the proportions of palmitic acid (16:0) and di-homo-γ-linolenic acid [20:3(n-6)] and in the ratios of 20:4(n-6) to 20:3(n-6) and 18:0 to 16:0 were still significant after adjustment for fiber-type distribution.

The calculated correlations in present study, which are analyzed for all the subjects (TRA group and UNT group combined), clearly illustrate a similar fatty acid pattern for higher VO2peak, higher PALest, higher proportion of type I fibers, and improved insulin sensitivity, as shown in Table 5. All four variables are characteristics for the TRA group.

The observed difference in fatty acid composition of skeletal muscle phospholipids between the TRA and UNT subjects might be due either to a direct effect of the physical exercise or to a difference in fiber-type distribution or both. A training-induced effect on the fatty acid profile, independent of fiber-type changes, could probably be explained by several mechanisms. A higher total energy intake, and, hence, a higher intake of fat (55% in present study) and essential fatty acids due to a higher level of physical activity, will result in greater availability of essential fatty acids for incorporation into cell membranes. At the same time, the endurance training will increase the use of fat as an energy substrate. A higher “energy flux” including a more rapid turnover of endogenous and exogenous fatty acids in physically active people and a selective oxidation and incorporation of different fatty acids due to their chain length and desaturation (23, 24, 27, 29) may influence the fatty acid composition in skeletal muscle lipids. During exercise, an increased ratio of unsaturated to saturated fatty acids in plasma has
been reported (26) as being a result of a selective mobilization and/or a selective uptake of different fatty acids. Alterations in the activity of desaturase and elongase enzymes (estimated as product-to-precursor ratios of fatty acids in skeletal muscle phospholipids) could probably also influence fatty acid profile in skeletal muscle. The relative difference in fatty acid profile in TRA and UNT subjects may also reflect absolute changes of the amount of certain fatty acids due to training, and as we have no data on the total amount of the lipid pools within the skeletal muscle, this could not be determined in the present study.

In summary, the findings in the present study, in conjunction with our earlier results, suggest that regular aerobic physical activity influences the fatty acid composition of skeletal muscle phospholipids independently of the dietary fatty acid profile. In addition, we found that the fatty acid profile of skeletal muscle triglycerides differed between TRA and UNT individuals. These differences in the fatty acid patterns in the skeletal muscle are apparently independent of the difference in fiber-type distribution and might be due to changes in fatty acid metabolism in the TRA group resulting from the higher degree of physical activity than in the UNT group. Exactly how the changes in the fatty acid composition may interact with insulin sensitivity remains to be determined.

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