Lower activity of oxidative key enzymes and smaller fiber areas in skeletal muscle of postobese women

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Raben, Anne, Elsebeth Mygind, and Arne Astrup. Lower activity of oxidative key enzymes and smaller fiber areas in skeletal muscle of postobese women. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E487–E494, 1998.—Muscle fiber morphology and activities of four key enzymes, as well as energy metabolism, were determined in nine normal-weight postobese women and nine matched control subjects. No differences in fiber type composition, but a smaller mean fiber area and area of fiber types I and IIb, were found in postobese compared with control subjects (P < 0.05). The activities of \( \beta \)-hydroxyacyl-CoA dehydrogenase (HADH) and citrate synthase (CS) were 20% lower in postobese than in control subjects (P < 0.05). However, the activities of lactate dehydrogenase and lipoprotein lipase were not significantly different between postobese and control subjects. Basal metabolic rate and respiratory exchange ratio were also similar, but maximal oxygen uptake (\( V_{\text{O}2_{\text{max}}} \)) tended to be lower in postobese than in control subjects (P = 0.06). When adjustments were made for differences in \( V_{\text{O}2_{\text{max}}} \), HADH and CS were not different between postobese and control subjects. In conclusion, these data suggest that smaller fiber areas and lower enzyme activities, i.e., markers of aerobic capacity of skeletal muscle, but not fiber composition, may be factors predisposing to obesity.

The lower fat oxidation observed in postobese subjects may, however, also relate to a reduced capacity in skeletal muscle to take up and/or oxidize the circulating lipids. Several studies have been published on the relation between obesity and muscle fiber types and biochemical characteristics, although with conflicting results (19, 20, 22, 37, 40, 44, 47). Some studies have reported that obesity is related to a relative reduction in oxidative muscle fibers (type I) (20, 22, 44) and/or a relative increase in glycolytic muscle fibers (type IIb) (19, 20, 22). However, no such relations were found in another study (41). As for enzyme activities, the activity of citrate synthase, malate dehydrogenase, and oxoglutarate dehydrogenase, all oxidative key enzymes in the tricarboxylic acid cycle (i.e., markers of muscle aerobic-oxidative capacity), was found to be negatively related to body fat or weight gain (19, 37, 40). Also, a low activity of \( \beta \)-hydroxyacyl-CoA dehydrogenase (a key enzyme in fatty acid oxidation) was found to correlate with a high 24-h respiratory quotient (RQ) in Pima Indians and, thereby, to an increased risk of weight gain (47).

Together these studies indicate that muscle morphology and oxidative capacity are important in the etiology of obesity. To our knowledge, data on muscle morphology and enzyme activities in normal-weight subjects [body mass index (BMI) <25.0 kg/m\(^2\)] with the genetic predisposition to obesity have not yet been reported. The aim of the present study was therefore to measure muscle morphology and activities of selected key enzymes in normal-weight postobese subjects and compare these data with those of normal-weight, never-obese, matched control subjects.

METHODS

Subjects. Eighteen healthy, normal-weight women [9 postobese (PO) and 9 matched control (C) subjects] participated in the study (Table 1). The PO women had a family history of obesity (≥1 obese parent or sibling), had been >20% overweight [means ± SE = 47 ± 6.4% (29)], and had been weight stable (±3 kg) for ≥2 mo. All had dieted, and some also exercised, but none had undergone surgical operations to become of normal weight. The weight loss had taken place gradually. Two PO and two C subjects smoked regularly, and one C subject smoked only on social occasions.

The two groups were matched according to age, height, weight, BMI, body composition by bioimpedance [fat mass (FM\(_{\text{imp}}\)) and fat-free mass (FFM\(_{\text{imp}}\))], waist-to-hip ratio, sagittal height, and blood pressure (Table 1). These were initially measured on a random day when subjects were nonfasting, after voiding (for PO on days 1–14 and for C subjects on days 5–20 of the menstrual cycle). The measurements were performed again before assessment of the subjects' basal metabolic rate (BMR), which was done in the morning after 10 h of

A REDUCED CAPACITY FOR FAT OXIDATION, with a resulting positive fat balance, is an important etiological factor of obesity (4, 36). This, among other factors, is evidenced by studies in obesity-prone normal-weight subjects who have been found to have a lower lipid oxidation than never-obese subjects when subjected to a fat-rich diet (2, 32). The reasons for this lower oxidative capacity are, however, still unclear. They may relate to abnormalities in the adipose tissue (storage, release) and/or in skeletal muscle (uptake, oxidation). With regard to the adipose tissue, we found that fat was stored more efficiently and lipolysis was more suppressed after a fat-rich meal in postobese subjects (32). In contrast, the release of fat from adipose tissue for oxidation in skeletal muscle postabsorptively or during exercise may be intact in postobese subjects. Thus fat mobilization was found to be similar in postobese and matched control subjects during and after 1 h of exercise at 50% maximal oxygen uptake (\( V_{\text{O}2_{\text{max}}} \)) (35).

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Postobese</th>
<th>Control</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>40 ± 3</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Height, m</td>
<td>168.6 ± 2.0</td>
<td>166.7 ± 1.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64.9 ± 19</td>
<td>62.7 ± 12</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.9 ± 0.6</td>
<td>22.6 ± 0.5</td>
</tr>
<tr>
<td>Maximal weight, kg</td>
<td>92 ± 4</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>Fat massimp kg</td>
<td>18.0 ± 1.0</td>
<td>16.3 ± 0.8</td>
</tr>
<tr>
<td>%</td>
<td>27.5 ± 1.1</td>
<td>26.0 ± 0.8</td>
</tr>
<tr>
<td>Fat-free massimp kg</td>
<td>47.0 ± 1.2</td>
<td>46.3 ± 0.6</td>
</tr>
<tr>
<td>%</td>
<td>72.5 ± 1.1</td>
<td>74.0 ± 0.8</td>
</tr>
<tr>
<td>WH</td>
<td>0.76 ± 0.01</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>Sagittal height, cm</td>
<td>17.2 ± 0.4</td>
<td>17.3 ± 0.4</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>116 ± 6</td>
<td>111 ± 3</td>
</tr>
<tr>
<td>Systolic</td>
<td>74 ± 3</td>
<td>70 ± 3</td>
</tr>
<tr>
<td>Diastolic</td>
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</table>

Values are means ± SE of 9 female subjects in each group. Fat massimp and fat-free massimp were measured by bioimpedance. WH, waist-to-hip ratio. *P < 0.0001, postobese vs. control subjects by unpaired t-test.

The activity of four enzymes was determined. Three were key enzymes in the major energy-generating pathways: β-hydroxyacyl-CoA dehydrogenase (HADH), a key enzyme in the β-oxidation of free fatty acids; citrate synthase (CS), the first and rate-limiting enzyme in the tricarboxylic acid cycle, i.e., the energy-disposal for glucose; and lactate dehydrogenase (LDH), a marker enzyme for glycolysis under anaerobic conditions. Determination of HADH, CS, and LDH was based on the principle described by Lowry and Passonneau (26) with fluorometric determination of the NAD-NADP-coupled reaction as described by Essén et al. (10) and expressed per gram dry weight of the muscle tissue. The fourth enzyme was lipoprotein lipase (LPL), a major enzyme responsible for the hydrolysis of triglycerides to free fatty acids and monoaecylglycerol before uptake by the muscle (9). LPL was determined (per gram wet weight muscle) by a method described by Lithell and Boberg (24), modified by Lithell et al. (25). One milliunit of LPL signifies an activity equivalent to the release of 1 nmol fatty acid/min.

BMR, BMI and respiratory exchange ratio (RER) were measured by indirect calorimetry with an open-circuit, computerized ventilated hood system (12). The measurements were performed in the morning on days 7–10 of the menstrual cycle after >10 h of fasting. Subjects arrived at the department by car, train, or bus; they voided and rested for 30 min in the supine position. BMI and RER were measured during the subsequent 30 min. Carbon dioxide was measured with an infrared analyzer (Uras 10P, Hartmann & Braun, Frankfurt, Germany) and oxygen with a paramagnetic analyzer (model 1100A, Servomex, Sussex, UK). Ventilation through the hood was determined by a Hastings mass flowmeter (type HFM 201–100; Teledyne Hastings-Raydist, Hampton, VA). BMR was calculated by the Weir formula (45). RER was calculated as the ratio between CO₂ excretion and O₂ uptake. There was no correction for urinary nitrogen, but this would introduce an error of <2% (28).

Training status. The subjects’ Vo₂max was determined on an exercise bike. Subjects initially warmed up for 3 min at 70 rpm and 0.5 kg, corresponding to a workload of 35 W. The workload was increased by 0.5 kg (35 W) every 3 min until exhaustion, i.e., when the subjects could no longer continue exercising (<210 W). Gas exchange was continuously monitored by an on-line system (Medical Graphics). Heart rate was simultaneously monitored by a telemetric system (Polar Sport Tester). The highest oxygen uptake, measured during the test was considered to be the subject’s Vo₂max.

Subjects also completed a questionnaire on their weekly exercise habits. The questionnaire was developed at the Copenhagen Muscle Research Center, Rigshospitalet, Copenhagen, Denmark, in the morning after >10 h of fasting; they rested in the supine position. Twenty to fifty milligrams of muscle tissue were taken by needle biopsy technique (5) from the vastus lateralis, 12–16 cm above the knee. A bundle of muscle fibers was dissected out, mounted for histochemical studies in a plastic material (Tissue-Tec), and frozen in nitrogen-cooled isopentane (−130°C). The remaining portion of the muscle piece was carefully freed from visible fat, connective tissue, and blood and was frozen in liquid nitrogen. An additional piece of muscle was quickly washed in saline, dried on filter paper, and frozen in liquid nitrogen. This piece was used for determination of lipoprotein lipase activity. All samples were stored at −80°C until analyzed. Water content of the muscle samples was obtained by a weighing-drying procedure (26). Water content was in the normal range, i.e., 76.7 ± 0.4% (SE) for PO and 76.7 ± 1.0% for C subjects (P = 0.98).

For fiber type determination, the muscle sample was cut at −20°C. Serial sections were stained for capillaries with Schiff’s reagent (amylase-periodic acid Schiff stain method, Ref. 1) and for myofibrillar ATPase after preincubations at pH 10.3, 4.6, and 4.3 (7, 31). Hereafter, the relative number (percentage) and area of slow-twitch oxidative (type I), fast-twitch, glycolytic oxidative (type IIa), and fast-twitch glycolytic (type IIb) muscles were calculated. A small number of fibers could not easily be classified into any of the above fiber groups and were therefore termed intermediary fiber types. The number of capillaries around each fiber type and the total fiber area per capillary were calculated.

The measurement of oxygen uptake was considered to be the subject’s V̇O₂max. For the gas exchange test, subjects were monitored in random order. To determine the subjects’ normal activity, all subjects gave written consent after the experimental procedure had been explained to them. Matching measurements. Body weight was measured by a digital scale to the nearest 0.1 kg (Seca model 708, Seca Medizintechnik, Bogel & Halke, Hamburg, Germany) and oxygen with a paramagnetic analyzer (model 1100A, Hartmann & Braun, Frankfurt, Germany) and carbon dioxide was measured with an infrared analyzer (Uras 10P, Hartmann & Braun, Frankfurt, Germany) and oxygen with a paramagnetic analyzer (model 1100A, Servomex, Sussex, UK). Ventilation through the hood was determined by a Hastings mass flowmeter (type HFM 201–100; Teledyne Hastings-Raydist, Hampton, VA). BMR was calculated by the Weir formula (45). RER was calculated as the ratio between CO₂ excretion and O₂ uptake. There was no correction for urinary nitrogen, but this would introduce an error of <2% (28).
Habitual diet. The subjects recorded their habitual diet using a 7-day weighed-food record after having received careful oral and written instructions by a dietitian. The energy intake was compared with the subjects' calculated energy needs (see above), and the percentage of under- or overreporting was calculated. Energy and macronutrient intake was calculated by the computer database of foods Dankost 2.0 from the National Food Agency of Denmark (Søborg, Denmark) (30). Statistical analysis. Data are expressed as means ± SE. An unpaired t-test was used to test for differences between PO and C subjects. The significance level was set at P < 0.05. Simple correlation analyses were performed between the different measures. All significant correlations were plotted and, in case of extreme outliers (>95% confidence limits), reanalyzed and reported without this/these value(s). Forward stepwise selection analyses were performed for the four enzymes. The variables included were selected from the simple correlation analyses (P < 0.20), and an F value of 4.00 was the limit for a variable to enter the model. The adjusted R² values are given (the adjustment compensates for the expected chance prediction when the null hypothesis is true). Statgraphics Software version 4.2 (Graphic Software Systems, Rockville, MD) was used in the statistical calculations.

RESULTS

Muscle fibers and capillaries. The percentage of the different fiber types was similar in PO and C subjects (PO vs. C, type I: 53.4 ± 3.9% vs. 52.0 ± 4.7%; type IIa: 20.4 ± 1.6% vs. 19.7 ± 3.2%; type IIb: 9.3 ± 3.1% vs. 9.3 ± 2.7%; intermediary: 16.8 ± 2.0% vs. 19.1 ± 3.0%; P > 0.50) (Fig. 1). However, the area of fiber types I and IIb and mean fiber area were significantly smaller in PO than in C (PO vs. C, area I: 4,450 ± 200 vs. 5,429 ± 366 µm²; area IIb: 2,118 ± 467 vs. 4,121 ± 576 µm²; mean area: 3,910 ± 203 vs. 4,909 ± 372 µm²; P < 0.05) (Fig. 2). No significant differences were observed in the area of fiber type IIa (3,686 ± 246 vs. 4,297 ± 702 µm², P = 0.42) or intermediary fiber area (3,210 ± 349 vs. 4,246 ± 447 µm², P = 0.09). There were no differences in number of capillaries around each fiber type (Table 2), but fiber area per capillary was significantly smaller in PO than in C subjects (1,222 ± 64 vs. 1,555 ± 107 µm², P < 0.05) (Fig. 2).

Simple correlation analyses showed that kilometers of FFM_DXA correlated positively with fiber type I (r = 0.66, P < 0.01) and negatively with fiber type IIb (r = −0.53, P < 0.05). Percent FFM_DXA correlated negatively with area of intermediary fiber types (r = −0.46, P = 0.05; Fig. 3) and mean fiber area (r = −0.43, P = 0.07).

Muscle enzyme activities. The activities of HADH and CS were about 20% lower in PO than in C subjects (P < 0.05) (Fig. 4). These differences still remained after inclusion of smoking as a covariate in the analyses (HADH: P < 0.05, CS: P = 0.058). The HADH-to-CS ratio, a proposed expression for the ratio between fat oxidation and total oxidation (17), was not different between the groups (Table 2). The activity of LDH was 30% lower in PO, but the difference was not significant (P = 0.08). Without one extreme value in C (972 µmol·g⁻¹·min⁻¹), this difference diminished (344 in PO vs. 429 µmol·g⁻¹·min⁻¹ in C, P = 0.12). All correlations with LDH are reported without this outlier (n = 17).

Table 2. Number of capillaries per fiber type and adjusted enzyme activities

<table>
<thead>
<tr>
<th>No. capillaries/fiber type</th>
<th>Post-obese</th>
<th>Control</th>
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<tbody>
<tr>
<td>I</td>
<td>3.59 ± 0.14</td>
<td>3.56 ± 0.15</td>
</tr>
<tr>
<td>IIa</td>
<td>3.09 ± 0.19</td>
<td>2.90 ± 0.39</td>
</tr>
<tr>
<td>IIb</td>
<td>1.91 ± 0.39</td>
<td>2.44 ± 0.19</td>
</tr>
<tr>
<td>Intermediary</td>
<td>2.64 ± 0.14</td>
<td>2.65 ± 0.18</td>
</tr>
<tr>
<td>Mean no. capillaries</td>
<td>3.23 ± 0.15</td>
<td>3.28 ± 0.12</td>
</tr>
<tr>
<td>HADH/CS</td>
<td>1.21 ± 0.05</td>
<td>1.18 ± 0.05</td>
</tr>
<tr>
<td>HADH adj. V_{O2max}, µmol·g⁻¹·min⁻¹</td>
<td>31.1 ± 2.3</td>
<td>35.8 ± 2.1</td>
</tr>
<tr>
<td>HADH adj. V_{O2max} and age, µmol·g⁻¹·min⁻¹</td>
<td>32.2 ± 2.1</td>
<td>34.7 ± 2.1</td>
</tr>
<tr>
<td>CS adj. V_{O2max}, µmol·g⁻¹·min⁻¹</td>
<td>26.6 ± 2.2</td>
<td>30.1 ± 1.5</td>
</tr>
<tr>
<td>CS adj. V_{O2max} and age, µmol·g⁻¹·min⁻¹</td>
<td>27.7 ± 2.0</td>
<td>29.0 ± 1.3</td>
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</table>

Values are means ± SE of 9 female subjects in each group. HADH/CS, ratio of β-hydroxyacyl-CoA dehydrogenase to citrate synthase. V_{O2max}, maximal O₂ uptake. HADH adj. V_{O2max} and CS adj. V_{O2max}, HADH and CS adjusted for differences in V_{O2max}. There were no significant differences between postobese and control subjects.
LPL activity was similar in the two groups ($P = 0.85$) (Fig. 4).

Simple correlation analyses showed that age correlated negatively with HADH ($r = -0.64$, $P < 0.01$) and CS ($r = -0.73$, $P < 0.001$). $\dot{V}O_{2max}$ correlated positively with HADH ($r = 0.54$, $P < 0.05$), CS ($r = 0.61$, $P < 0.01$), and LPL ($r = 0.42$, $P = 0.08$) (Fig. 5). When adjustments were made for differences in $\dot{V}O_{2max}$, the differences in HADH and CS became nonsignificant ($P = 0.16$ and 0.20). The same was true when adjustments were made for both $\dot{V}O_{2max}$ and age ($P = 0.40$ and 0.59).

As for measures of body composition, HADH, CS, and LPL correlated negatively with %FFM$_{DXA}$ ($r = -0.44$, $P = 0.07$; $r = -0.53$, $P < 0.05$; $r = -0.42$, $P = 0.08$, respectively) (Fig. 4). Enzyme activities did not correlate with total body weight, maximal body weight, BMR, or RER.

Between enzymes and fiber types, HADH correlated positively with fiber type I ($r = 0.49$, $P < 0.05$), area of intermediary fiber types ($r = 0.75$, $P < 0.001$), and mean fiber area ($r = 0.61$, $P < 0.01$) (Fig. 6). CS and LPL also correlated positively with area of intermediary fiber types ($r = 0.64$, $P < 0.01$ and $r = 0.65$, $P < 0.01$) and mean fiber area ($r = 0.61$, $P < 0.01$ and $r = 0.48$, $P < 0.05$) (Fig. 6). When number of capillaries was examined, LPL correlated positively with number of capillaries around fiber type I ($P < 0.01$), intermediary fiber types ($P < 0.00001$), and mean number of capillaries ($P < 0.001$).

Forward stepwise selection analysis resulted in the following models [other models, but with lower $R^2$ values than those presented, were also found]. Age, %FFM$_{DXA}$, and area of intermediary fibers explained 75% of the variation in HADH (coefficients = $-0.32$, $1.26$, and 0.0026, respectively). Age and %FFM$_{DXA}$ explained 64% of the variation in CS (coefficients = $-0.49$ and $-0.77$, respectively). For LDH, BMI and area of fiber type I were included, explaining 41% of the variation in LDH (coefficients = $-38.1$ and 0.05). The number of capillaries around intermediary fiber types explained 69% of the variation in LPL (coefficient = 15.4).

BMR and training status. BMR, RER, and $\dot{V}O_{2max}$ were not different between the two groups (Table 3). However, $\dot{V}O_{2max}$ expressed per kilogram body weight tended to be lower in PO than in C subjects ($P = 0.06$; Table 3). $\dot{V}O_{2max}$ correlated negatively with age ($r = -0.59$, $P < 0.01$).

The duration and intensity of weekly exercise, as well as the estimated activity factor, were not significantly different between PO and C subjects (duration = 4.9 ± 1.9 and 7.1 ± 1.3 h; intensity = 1.6 ± 0.4 and 2.2 ± 0.1; and activity factor = 1.65 ± 0.03 and 1.70 ± 0.04). Positive correlations were found between the intensity of weekly exercise and $\dot{V}O_{2max}$ (total and per kg body wt, $P < 0.01$) and between the activity factor and $\dot{V}O_{2max}$ (per kg body wt, $P < 0.05$).

Calculated daily energy needs were not significantly different between the groups (Table 3).
There was a tendency for kilograms of FM_{DXA} and %FM_{DXA} to be greater in PO than in C subjects (P < 0.06; Table 3). Kilograms of FF_MDXA were, however, similar in the two groups, as was total bone mineral content (Table 3).

Habitual diet. Reported habitual energy intake was 22% lower in PO than in C subjects (P < 0.05) (Table 4). No significant differences in macronutrient intake were found between the groups. Compared with energy needs calculated from the BMR measurements, PO underreported by 6.6% and C subjects overreported by 17.6% (groups differ, P = 0.06).

Correlation analysis showed negative relations between BMI or %FM_{DXA} and intake of total energy, carbohydrate (g), and protein (g) (P < 0.05). Because this indicates underreporting, only relations with energy percent (E%) are relevant. Alcohol intake (in E%) correlated negatively with LPL (r = −0.48, P < 0.05).

DISCUSSION

The present study discloses several interesting and potentially important findings in muscle tissue of PO subjects. First, a 20% lower activity of both HADH and CS was observed in the PO compared with the C subjects. HADH is a key enzyme in β-oxidation of fatty acids in the muscle, and a reduced activity of this enzyme therefore points toward reduced lipid-oxidizing capacity in the PO subjects. This finding supports...
previous reports of reduced fat-oxidizing capacity of PO subjects when on a fat-rich diet (2, 32). In line with this are also the negative correlation between HADH and fat mass (%) found here and the previously reported negative correlation between 24-h RQ and HADH activity (low fat oxidation at a low HADH activity) (47). CS is the first enzyme in the tricarboxylic acid cycle and is rate limiting for the cycle. The reduced activity of CS found here is therefore a sign of reduced capacity for oxidative disposal of glucose in the PO subjects. In the present study we saw no significant differences in the activity of LDH between PO and C subjects. This indicates that nonoxidative disposal of glucose is not different in the two groups. Controversies exist, however, when the literature is examined. Thus a lower activity of LDH between PO and C subjects. This was surprising, because a low LPL activity has been suggested to be a risk factor for obesity. Accordingly, LPL was found to correlate inversely with %body fat, here and previously (46), and with a low ratio of fat to carbohydrate oxidation (high 24-h RQ) in Pima males (13). Our findings, however, indicate that skeletal muscle LPL activity may not be a rate-limiting step for muscle fat oxidation in this group of PO subjects. As pointed out by Ferraro et al. (13), the flux of fatty acids into skeletal muscle might instead depend on mitochondrial oxidative metabolism, which is the key determinant of the intracellular lipid pool turnover rate. This is also supported by recent findings of reduced cellular capacity for fat oxidation rather than reduced fatty acid uptake or intracellular fat availability in obese compared with lean subjects (18).

A third interesting finding was the identical fiber type composition in PO and C subjects. This was also not entirely expected from the significant positive relations between obesity and type I fibers and the negative relations between obesity and type IIb fibers previously reported (19, 20, 22, 44). However, the finding is in agreement with the aforementioned larger study of Simoéneau and Bouchard (37). They also agree with a recent study by Geerling et al. (14), which refutes the proposal by Wade et al. (44) that the proportion of oxidative type I muscle fibers relates to body fatness. If a certain fiber type composition is a predisposing, genetically determined risk factor for obesity, we should have observed differences in fiber types in our two study groups; for example, the PO group should have had relatively less of fiber type I and more of fiber type IIb. We also found no correlations between fiber type and fat mass, waist, or waist-to-hip ratio as found before (19, 20, 22, 44). The reason for this may be 1) that there is no important relationship or 2) the different methodology, i.e., a great variation in number and types of subjects (Caucasians or Pima Indians, lean or obese, predisposed to obesity or not, children or adults, young or old) used in the different studies.

A fourth finding of interest here was the smaller mean fiber area, area of muscle fibers I and IIb, and fiber area per capillary in PO compared with C subjects. Area of fiber type I and mean fiber area have been found to correlate positively with relative body weight and %body fat, i.e., the larger the body weight or fat mass, the greater the fiber area (22, 25, 44). In the present study we saw a negative relation between mean fiber area and %fat mass DXA. Thus the smaller the fiber area, the greater the fat mass. This would therefore indicate that a small fiber area predisposes to obesity and corresponds to our PO subjects being more predisposed to weight gain than our C subjects. The smaller fiber area and area per capillary also imply that the PO should have a better insulin action than C.
subjects (25). This was not measured in the present study. We have reported earlier that insulin sensitivity in PO subjects was similar to that in C subjects as measured by the euglycemic hyperinsulinemic glucose clamp technique (41). In another study, however, we observed lower postprandial glucose and insulin responses after 2 wk on three different ad libitum diets in PO subjects, indicating higher insulin sensitivity in this group (34). The latter would therefore be in line with the present findings. Furthermore, it would correspond to data from Pima Indians showing that increased insulin sensitivity was a risk factor for weight gain (36).

The reasons for the lower fiber area in PO subjects can be several. Thus the previous weight reduction (23), a lower $V_{O_2max}$ or different genes may be involved (15, 39). Training has been shown to influence oxidative enzyme activity, LPL activity, muscle fiber areas, and sometimes also fiber composition (15, 38, 42). We cannot know whether the PO subjects here were less physically fit (lower $V_{O_2max}$) because of different genes or different training levels, although the activity diaries indicated no differences in the latter. In twin studies, the response to both endurance training ($V_{O_2max}$ and endurance performance) and high-intensity intermittent training (several enzyme activities) was shown to be genotype dependent (15, 39). Furthermore, a recent study in obesity-prone, sedentary women suggested that skeletal muscle maximal oxidative capacity (measured by ATP production from oxidative phosphorylation) is an inherent characteristic of skeletal muscle within the individual (21). Genetic factors may also be involved in the variation of regulatory enzymes of the glycolytic and citric acid cycles, reaching 25–50% of the total phenotypic variation (after adjustment for age and sex differences) (6). Because of the cross-sectional design of the present study, however, we cannot conclude whether the differences observed here are causes (genetic make-up or low physical activity level) or consequences of obesity and the foregoing weight loss.

There were no differences in BMR (total or per kg FFM) or fasting RER between our groups of PO and C women. This corresponds to some but not all previous studies of PO and C subjects (8, 32). The lack of difference may, however, be due to the relatively small sample size used here and before and, therefore, to a lack of statistical power. Thus Astrup et al. (3) showed that, when pooling data from different studies ($n = 28$), BMR was in fact $8\%$ lower in PO than in C subjects ($P < 0.05$).

Dietary records showed that our PO subjects consumed $22\%$ less energy than C subjects. This corresponds to our previous study of dietary intake in PO women and C subjects (33). The lower intake is most likely not due to lower energy needs but rather to underreporting, because both BMR and calculated energy needs were similar in our two groups. This assumption is supported by the negative correlation between energy intake and BMI1 or FM found here. It is also supported by our previous study, in which measured 24-h energy expenditure was similar in PO and C subjects but PO subjects were still reported to consume $21\%$ less energy than C subjects (33).

Smoking may influence some of the measurements in the present study (e.g., enzyme activities). There were two regular smokers in each study group, but analyses of the data with smoking as a covariate did not alter our findings.

In conclusion, we found reduced activities of HADH and CS and smaller fiber areas in PO compared with C subjects. This suggests a reduced aerobic capacity of skeletal muscle in this group of subjects and implies that this may be an important factor predisposing to obesity. Conversely, the present data do not support the contention that muscle fiber composition is important for the development of obesity.

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