Exercise training decreases the concentration of malonyl-CoA and increases the expression and activity of malonyl-CoA decarboxylase in human muscle


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Kuhl, Jeanette E., Neil B. Ruderman, Nicolas Musi, Laurie J. Goodyear, Mary Elizabeth Patti, Sarah Crunkhorn, Deepthi Dronamraju, Anders Thorell, Jonas Nygren, Olle Ljungkvist, Marie Degerblad, Agnete Stahle, Torkel B. Brismar, Kirstine L. Anderssen, Asish K. Saha, Suad Efendic, and Peter N. Bavenholm. Exercise training decreases the concentration of malonyl-CoA and increases the expression and activity of malonyl-CoA decarboxylase in human muscle. Am J Physiol Endocrinol Metab 290: E1296–E1303, 2006. First published January 24, 2006; doi:10.1152/ajpendo.00341.2005.—The study was designed to evaluate whether changes in malonyl-CoA and the enzymes that govern its concentration occur in human muscle as a result of physical training. Healthy, middle-aged subjects were studied before and after a 12-wk training program that significantly increased VO2 max by 13% and decreased intra-abdominal fat by 17%. Significant decreases (25–30%) in the concentration of malonyl-CoA were observed after training, 24–36 h after the last bout of exercise. They were accompanied by increases in both the activity (88%) and mRNA (51%) of malonyl-CoA decarboxylase (MCD) in muscle but no changes in the phosphorylation of AMP kinase (AMPK, Thr172) or of acetyl-CoA carboxylase. The abundance of peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α), a regulator of transcription that has been linked to the mediation of MCD expression by PPARγ, was also increased (3-fold). In studies also conducted 24–36 h after the last bout of exercise, no evidence of increased whole body insulin sensitivity or fatty acid oxidation was observed during an euglycemic hyperinsulinemic clamp. In conclusion, the concentration of malonyl-CoA is diminished in muscle after physical training, most likely because of PGC-1α-mediated increases in MCD expression and activity. These changes persist after the increases in AMPK activity and whole body insulin sensitivity and fatty acid oxidation, typically caused by an acute bout of exercise in healthy individuals, have dissipated.

In muscle, malonyl-CoA allosterically inhibits carnitine palmitoyltransferase (CPT) I and thereby decreases the transfer of long-chain fatty acyl-CoA from the cytosol into mitochondria, where they are oxidized (22). A large body of evidence indicates that the concentration of malonyl-CoA in skeletal muscle responds to acute changes in cellular fuel availability and energy expenditure (EE) (35). Thus increases in malonyl-CoA have been described in muscle after provision of glucose, such as that which occurs during an euglycemic-hyperinsulinemic clamp (6) or refeeding after a fast (30), whereas decreases in the concentration of malonyl-CoA have been observed in muscle after exercise, which increases fat oxidation (7, 9, 15, 40). Exercise (muscle contraction) lowers the concentration of malonyl-CoA by activating AMP kinase (AMPK), which phosphorylates (Ser79) and inhibits acetyl-CoA carboxylase (ACC), the enzyme that catalyzes the formation of malonyl-CoA from cytosolic acetyl-CoA and CO2 (7, 12, 40, 49). Studies in animals and humans suggest that sustained elevations of the concentration of malonyl-CoA in skeletal muscle may play a role in the development of insulin resistance (3, 20, 36). Conversely, decreases in the concentration of malonyl-CoA, caused by an acute bout of exercise (9, 15) or the administration of a single dose of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (16), are accompanied by increases in insulin sensitivity (17, 33). If speculative, changes in malonyl-CoA have been attributed to alterations in the activity of ACC.

Recent data in rats (26, 38, 39) suggest that the concentration of malonyl-CoA in skeletal and cardiac muscle may also be regulated by AMPK-induced increases in the activity of malonyl-CoA decarboxylase (MCD), a key enzyme responsible for malonyl-CoA turnover in these tissues. AMPK might act to increase MCD activity chronically by increasing its abundance (39). In addition, it has been reported (26, 38) that AMPK can acutely phosphorylate and activate MCD in rodent tissues; however, this has not been a universal finding (14).

In the present study, we investigated the effects of physical training on malonyl-CoA levels in skeletal muscle of middle-aged human volunteers 24–36 h after the last bout of exercise. Specifically, we examined whether physical training concurrently alters malonyl-CoA and AMPK and the two enzymes that directly regulate malonyl-CoA turnover, ACC and MCD. In addition, we measured the expression of peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α), a molecule implicated in the regulation of mitochondrial biogenesis (24, 27) and activation of transcription of MCD through coactivation of PPARα (21, 29). All subjects were investigated and...
before and after a 12-wk program of combined aerobic and dynamic strength training, and the results were related to measures of insulin sensitivity.

MATERIALS AND METHODS

Participants. Eleven Swedish subjects (7 women and 4 men) were invited to participate in the study. They were recruited by advertising in a local morning newspaper. Individuals with body mass indexes (BMIs) between 22 and 32 kg/m² were considered eligible for the study. None of the subjects had previously participated in a regular exercise program with more than one training session per week, and they were free of chronic disease. Three subjects did not complete the study; one subject did not finish the study because an injury of the ankle joint prevented her from exercising, and two others dropped out because of poor compliance with the training program. The three dropouts were women. Overall, compliance to the training sessions was 86%. All participants received written and oral information regarding the nature and potential risks of the study and gave their informed consent. The experimental protocol was approved by the Ethics Committee at Karolinska Hospital and was carried out in accordance with the Declaration of Helsinki.

Study protocol. Metabolic investigations were performed at 8 AM after a 12-h overnight fast (baseline). After the training period, the insulin clamp studies and muscle biopsies were performed 24–36 h after the last bout of exercise. The subjects were instructed not to engage in any physical activity after the last bout of exercise, i.e., during the 24–36 h before the biopsy, and they were instructed to eat their usual balanced diet during this time. They were reminded of this individually as well as during three separate group meetings, one before entering the study, a second meeting during the course of study, and the third meeting before final examinations. Other clinical tests and metabolic investigations were generally performed within a week or on the same day as the clamp study. They were never performed on the first day after a weekend or holiday.

Hyperinsulinemic euglycemic clamp. At the beginning of the experiment the basilic vein in each arm was cannulated, one for blood sampling and the other for infusion. Insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was administered intravenously. During sampling and the other for infusion. Insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was administered intravenously. During the 24–36 h before the biopsy, and they were instructed to eat their usual balanced diet during this time. They were reminded of this individually as well as during three separate group meetings, one before entering the study, a second meeting during the course of study, and the third meeting before final examinations. Other clinical tests and metabolic investigations were generally performed within a week or on the same day as the clamp study. They were never performed on the first day after a weekend or holiday.

Hyperinsulinemic euglycemic clamp. At the beginning of the experiment the basilic vein in each arm was cannulated, one for blood sampling and the other for infusion. Insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was administered intravenously. During the first 10 min, insulin was infused at decreasing rates, and in a stepwise manner and adjusted for body weight, and then at 1.0 μU·kg⁻¹·min⁻¹ for 110 min. Glucose (20%) was simultaneously infused intravenously at a variable rate to maintain the blood glucose concentration at 4.8 mmol/l (10). Blood glucose was analyzed every 5 min.

Indirect calorimetry. The Deltatrac II Metabolic Monitor (Datex-Ohmeda, Helsinki, Finland) was used to measure oxygen consumption and carbon dioxide production, and from these data the respiratory quotient (RQ) and the rates of fatty acid and glucose oxidation were calculated (11). For this purpose, at 30 min before the clamp and at the end of the clamp, a transparent plastic hood was placed over the subject’s head for 30 min to determine O₂ consumption and CO₂ production. Timed samples of urine were collected for analysis of urinary urea excretion, and from this changes in urea pool size were calculated to correct for amino acid oxidation (47).

Body composition and physical fitness. Lean body mass (LBM), total body fat, and total truncal (abdominal and thoracic regions) fat mass were calculated using dual-energy X-ray absorptiometry (Lunar DPX-L X-ray bone densitometer, version 1.3Z; Lunar, Madison, WI). Computerized tomography (Siemens Somatom Plus, Iselin, NJ) was used to determine intra-abdominal fat mass. All subjects were examined in the morning before the insulin clamp. One 10-mm slice was exposed and examined at the level of the upper part of the iliac crest, which coincided with the disc between the fourth and fifth lumbar vertebrae and the umbilicus. A density range between ~150 and ~40 Hounsfield units was used to define fat mass. Total abdominal adipose tissue (subcutaneous plus intra-abdominal fat mass, measured in square centimeters) in this interval was calculated by the computer. The intra-abdominal cavity, including the retroperitoneal space, was outlined, and the area was estimated separately. Subcutaneous fat mass was calculated as the difference between total and intra-abdominal fat mass area.

Maximal oxygen consumption (V˙O₂ max) was determined during an exercise test performed on an electrically braked cycle ergometer. The initial resistance was set at 40/50 W (females/males) and was increased stepwise every minute by 10/20 W (2). Subjects reported their rate of perceived exertion (RPE) according to Borg’s RPE scale at the end of every second step increase (4). The test was terminated due to dyspnea or exhaustion after an RPE score >15 out of a maximum of 20 was attained. A 12-lead electrocardiogram was recorded during the test.Expired gases were measured for oxygen content. Calculations were performed by a Jaeger Oxycon computer software program (Jaeger, Hoechberg, Germany).

Exercise training. All subjects participated in a combined aerobic and dynamic strength training program three times per week over a period of 3 mo. As described previously (44), each training session lasted 50 min and included warm-up (6 min), jogging (4 min), flexibility exercises (stretching; 4 min), and strength training of the arms and back (4 min), abdomen (4 min), and large muscle groups (3 min). This was followed by jogging (3 min), flexibility exercises (3 min), strength exercises of large muscle groups (7 min), jogging (3 min), further strength exercises of large muscle groups (4 min), flexibility exercises, and stretching and cool down (5 min) (44). The lower extremities (including the vastus lateralis muscle) were active during at least two-thirds of the training session. A specialized physiotherapist supervised all of these activities. To increase adherence to the program, four training sessions at different times during the week were offered. Two specific exercise targets were used during the program: 1) an exercise intensity of >50% of the individuals’ maximal exercise capacity (on the basis of maximal heart rate reached during the baseline exercise test), maintained for at least 40 min; and 2) an exercise intensity >80% of the individuals’ maximal exercise capacity during three periods of 3–4 min each, engaging large muscle groups for training and adaptation of the central circulation (1a). The intensity of exercise during the training program was individually adjusted and was based on the performance at the baseline exercise test. The subjects were encouraged to reach an exertion of 13–15 of 20 rated on Borg’s RPE scale to obtain the exercise target of >80% of maximal working capacity during the more intense parts of the training program and 9–11 of 20 on the RPE scale (corresponding to >50% of maximal exercise capacity) during the remainder of the session.

The complete program was supported by music, which guided the intensity of the performance during the session. The training sessions were followed by 10 min of relaxation, also supported by music. To ascertain that the target exercise intensity was reached, heart rate was assessed at one of the three weekly training sessions during weeks 1 and 12 of the training period with a portable heart rate recorder (Sport Tester; Polar Electro Oy, Kempele, Finland). The subjects also rated their perceived exertion using the RPE scale during the more intense parts of the program.

Muscle biopsies. Both before and after training, the initial muscle biopsy was obtained immediately after the indirect calorimetric studies and before the insulin infusion. After instillation of 5–15 ml of prilocain (Citanest), 5 mg/ml for local anesthesia of the skin and subcutaneous tissue, an open biopsy of the vastus lateralis muscle (~1 g) was performed through a 4-cm incision ~12 cm above the knee joint, as described previously (13). The muscle was immediately frozen in liquid nitrogen and stored for further analysis. After closure of the fascia and skin, the insulin infusion was started. The second muscle biopsy was done through the same incision immediately after the insulin infusion was stopped. The tissue was taken from an area close to, but distinct from, the muscle biopsy taken during the first biopsy. All muscle samples were analyzed at the same time.
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EXERCISE INCREASES HUMAN MUSCLE MCD ACTIVITY

Table 1. Phenotype of subjects before and after exercise

<table>
<thead>
<tr>
<th></th>
<th>Before (n = 11)</th>
<th>After (n = 8)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>51.0 ± 2.2</td>
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<tr>
<td>Weight, kg</td>
<td>72.4 ± 3.7</td>
<td>73.8 ± 4.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.3 ± 0.7</td>
<td>25.4 ± 0.6</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.88 ± 0.02</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>M value, mg·kg⁻¹·min⁻¹</td>
<td>6.1 ± 0.5</td>
<td>6.4 ± 0.9</td>
</tr>
<tr>
<td>V̇O₂ max, ml·min⁻¹·LBM⁻¹</td>
<td>45.7 ± 1.2</td>
<td>51.3 ± 0.8</td>
</tr>
<tr>
<td>Intra-abdominal fat area, cm²</td>
<td>113 ± 11</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>DEXA Total fat, kg</td>
<td>23.1 ± 1.9</td>
<td>20.2 ± 1.3</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>48.2 ± 3.3</td>
<td>51.1 ± 4.4</td>
</tr>
<tr>
<td>Truncal fat, kg</td>
<td>10.8 ± 0.9</td>
<td>9.3 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; V̇O₂ max, maximal oxygen consumption; LBM, lean body mass; DEXA, dual-energy X-ray absorptiometry; NS, not significant. P, paired t-test was performed to evaluate significance of differences between subjects before and after exercise. Only subjects who had completed the study (n = 8) were used for this comparison.

Determination of malonyl-CoA and MCD. Measurement of malonyl-CoA muscle was homogenized and deproteinized with 10% perchloric acid, and the filtrate was neutralized as described previously (37). Malonyl-CoA was determined radioenzymatically in the neutralized filtrate by a modification of the methods of McGarry et al. (23) and Saha et al. (35). MCD was assayed spectrophotometrically, as described previously (38).

Determination of PGC-1α. Fifty micrograms of crude muscle homogenate were electrophoresed and transferred to polyvinylidene difluoride membrane (Bio-Rad Laboratories). After being transferred, the membranes were blocked with 5% BSA in Tris-buffered saline (TBS; 25 mM Tris, 135 mM NaCl, 2 mM KCl)-0.05% Tween 20 (TBST) for 1 h at room temperature. The membranes were incubated with PGC-1 antibody purchased from Calbiochem (San Diego, CA) and then with secondary antibodies conjugated to horseradish peroxidase from Amersham Pharmacia. Bands were visualized by enhanced chemiluminescence (ECL) and were quantitated by laser densitometry.

Determination of AMPK and ACC phosphorylation and protein expression. Freeze-clamped muscle samples were homogenized as previously described, and lysates were used for Western blotting (25). For Western blotting analysis, proteins (40 µg) from the lysates were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes. After the membranes were blocked with 2.5% BSA-TBST, they were probed overnight at 4°C with antibodies against phospho-

Thr172 AMPK (1:1,000; Cell Signaling, Beverly, MA), anti-phospho-ACC (recognizes both the Ser79 of ACC1 and Ser218 of ACC2) (1:1,000; Upstate, Waltham, MA), anti-AMPKα2 (raised against the sequence CMDDSAMHPGLPKPH-NH₂ of α2) (25), anti-AMPKα1/2 (raised against the sequence CAEKQKHDDGRVKIGHYNH₂ of both α1 and α2), or streptavidin for ACC detection (2 µg/ml; Roche, Indianapolis, IN). Bound antibodies were detected using anti-rabbit immunoglobulin-horseradish peroxidase-linked antibody and ECL reagents (NEN Life Science Products, Boston, MA). The bands were quantified using ImageQuant software (Molecular Dynamics).

Gene expression. Muscle biopsies were homogenized in TRIzol. Total RNA was purified with RNeasy (Qiagen). Expression of selected genes was determined using two-step real-time quantitative PCR (ABI Prism 7700; Applied Biosystems). Total RNA was treated with DNase I; cDNA was synthesized using random hexamer primers (Advantage; BD Biosciences). Primer and probe sequences were selected using Primer Express. Target gene and endogenous control amplicons were labeled with FAM and VIC, respectively. Expression was normalized to that of cyclophilin. Primer and probe sequences are available on request.

Plasma analyses. Plasma glucose was determined by the glucose oxidase method using a glucose analyzer (YSI, Yellow Springs, OH).

Table 2. Substrate utilization rates during clamps

<table>
<thead>
<tr>
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<th>Basal (n = 11)</th>
<th>Insulin (n = 11)</th>
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<tbody>
<tr>
<td>Fat ox</td>
<td>1.25 ± 0.12</td>
<td>0.80 ± 0.08</td>
</tr>
<tr>
<td>CHO ox</td>
<td>0.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>RQ</td>
<td>0.79 ± 0.01</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>EE</td>
<td>17.5 ± 0.7</td>
<td>17.7 ± 0.7</td>
</tr>
<tr>
<td>FFA</td>
<td>0.59 ± 0.091</td>
<td>0.017 ± 0.006</td>
</tr>
</tbody>
</table>

Values are means ± SE. CHO, carbohydrate; ox, oxidation; RQ, respiratory quotient; EE, energy expenditure; FFA, nonesterified (free) fatty acids. Differences before and after training were all not significant. P, paired t-test was performed to determine the significance of differences between parameters before and after the clamp. Eleven subjects were evaluated pretraining and 8 postraining. Only subjects that had completed the study (n = 8) were used for this comparison.

![Fig. 1: A] basal concentration of malonyl-CoA was significantly decreased after subjects were trained. Insulin infusion significantly increased malonyl-CoA both before and after subjects were trained. Results are means ± SE for 8 subjects. Statistical significance was assessed by a paired t-test. B: individual malonyl-CoA basal values before and after subjects were trained are presented.
Plasma nonesterified (free) fatty acids (FFA) were determined using a commercially available kit (Wako Pure Chemical Industries, Richmond, VA).

Statistical analyses. All values are presented as means ± SE. Logarithmic transformation was performed on all skewed variables to obtain a normal distribution before statistical computations and significance testing were undertaken. A paired t-test was used to compare 1) parameters measured during basal conditions before and after training, 2) parameters measured before and after insulin infusion during the clamps, and 3) insulin-stimulated samples before and after training. The nonparametric Mann-Whitney U-test was used for skewed variables that did not obtain a normal distribution after logarithmic transformation. Statistical significance was set at \( P < 0.05 \). Data processing was performed using the Statistica software package, version 6.1 (StatSoft).

RESULTS

Phenotype of the study population. The characteristics of the study subjects before and after they were trained are presented in Table 1. The subjects were middle aged (mean age 51) and slightly overweight (mean BMI 25.3) at the start of the study. Physical training significantly increased \( \dot{V}O_2 \text{max} \) (13%) and decreased intra-abdominal fat area (17%). No effect of training was seen on total body weight, BMI, waist-to-hip ratios, total or truncal fat mass, or LBM. The glucose infusion rates (\( M \) value) required to maintain euglycemia during the clamp, a measure of whole body insulin sensitivity, were similar before and after training [6.1 ± 0.5 to 6.4 ± 0.9 mg·kg\(^{-1}\)·min\(^{-1}\), \( P = \) not significant (NS)]. When men and women were analyzed separately, the decrease in intra-abdominal fat area was more pronounced in men (147 ± 25 to 111 ± 30 cm\(^2\), \( P = 0.02 \)) than in women (86 ± 17 to 76 ± 13 cm\(^2\), \( P = \) NS). Sex had no significant impact on any other parameter (data not shown), although a sex difference could have been missed because of the small number of men and women studied.

Substrate utilization, RQ, and plasma FFA levels. Training had no effect on postabsorptive plasma FFA levels, substrate utilization rates, or systemic EE (Table 2). Insulin infusion led to a 34–38% suppression of fat oxidation, and it caused a similar decrease in plasma FFA levels before and after training. Carbohydrate oxidation rates and RQs were increased during the clamps in all subjects; however, the difference was significant only in the pretrained (sedentary) state.

Malonyl-CoA, MCD, ACC, AMPK, and PGC-1\( \alpha \). In muscle biopsied before the clamp, training decreased the concentration of malonyl-CoA by 26% (\( P = 0.04 \); Fig. 1). This was associated with increases in MCD activity of 88% and MCD mRNA...
expression of 51% (Fig. 2). In contrast, no significant effect of training was observed on protein content or phosphorylation of ACC or the AMPKα subunit (Table 3). PGC-1 protein abundance was increased threefold after the subjects were trained ($P = 0.02$; Fig. 3), and PGC-1α mRNA expression was increased by 45% (NS; data not shown). Compared with basal values, malonyl-CoA content was increased after the euglycemic-hyperinsulinemic clamp pre- as well as postraining: pretraining 26%, $P = 0.03$; postraining 29%, $P = 0.01$ (Fig. 1). The phosphorylation of ACC was decreased by 28–48% after the clamps, suggesting an increase in its activity (Table 3), although only the change postraining was statistically significant. Correlations between these changes in ACC phosphorylation and the increases in malonyl-CoA concentration after the clamps (Fig. 1) were not significant (data not shown).

**DISCUSSION**

In the present study, we examined the effect of a long-term, combined aerobic and dynamic strength training program, without caloric restriction, on the regulation of malonyl-CoA in human skeletal muscle. The results indicate that 24–36 h after the last bout of exercise, the concentration of malonyl-CoA in muscle is significantly decreased and that this is associated with increased MCD expression and activity. These observations, plus the absence of changes in ACC that could have accounted for this, strongly suggest that MCD could be the principal regulator of malonyl-CoA in muscle at this time.

The mechanisms governing the regulation of malonyl-CoA in human muscle after long-term exercise training are unknown. To our knowledge, the present study is the first to report that MCD activity is increased by exercise training in human skeletal muscle. MCD phosphorylation was not assessed; however, the finding that MCD mRNA was increased suggests that the change in activity was due, at least in part, to an increase in enzyme abundance. The observation that PGC-1α abundance was also increased is in keeping with this conclusion because PGC-1α is a coactivator of PPARα, which activates the transcription of MCD in skeletal and cardiac muscle (46). An increase in MCD abundance has also been observed in cultured H9c2 cells overexpressing MCD mRNA when a constitutively active AMPK adenovirus is coexpressed (39). Whether acute increases in MCD due to phosphorylation, an effect observed by some investigators (26, 38) but not by others (14), contributed to the changes in MCD in the trained individuals was not studied.

In contrast to ACC, much of the activity of MCD activity in cells is located in mitochondria and peroxisomes, and except for liver (18) the percentage of enzyme in the cytosol is unclear (34, 50). Likewise, the precise roles of MCD in the three locations is unknown, as is the locus of the MCD activated chronically by changes in abundance. In liver, expression of MCD has been shown to lower the concentration of malonyl-CoA and diminish hepatic lipid accumulation and insulin resistance in fat-fed rats (1), and in cultured insulin-secreting cells it diminishes the concentration of malonyl-CoA and enhances the stimulation of insulin secretion by various secretagogues (31).

In the present study AMPK activity (phosphorylation of Thr172) was not increased 24–36 h after the last bout of exercise when the concentration of malonyl-CoA was diminished, and MCD and PGC-1α abundance were increased. The most likely explanation for these findings is that AMPK activation in muscle and some of its acute effects, such as ACC

![Fig. 3.](http://ajpendo.physiology.org/)

**Table 3. ACC and AMPKα subunit phosphorylation and protein content before and after training and during insulin stimulated conditions**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Before (n = 8)</th>
<th>After (n = 8)</th>
<th>P</th>
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<tbody>
<tr>
<td>AMPK protein α2</td>
<td>814 ± 53</td>
<td>716 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>AMPK protein α2</td>
<td>743 ± 55</td>
<td>690 ± 49</td>
<td>NS</td>
</tr>
<tr>
<td>AMPK protein α1 + α2</td>
<td>1,093 ± 178</td>
<td>1,133 ± 64</td>
<td>NS</td>
</tr>
<tr>
<td>AMPK protein α1 + α2</td>
<td>1,179 ± 102</td>
<td>1,132 ± 46</td>
<td>NS</td>
</tr>
<tr>
<td>p-AMPK basal</td>
<td>237 ± 38</td>
<td>211 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>p-AMPK insulin</td>
<td>222 ± 45</td>
<td>200 ± 22</td>
<td>NS</td>
</tr>
<tr>
<td>ACC protein basal</td>
<td>238 ± 32</td>
<td>187 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>ACC protein insulin</td>
<td>257 ± 36</td>
<td>191 ± 35</td>
<td>NS</td>
</tr>
<tr>
<td>p-ACC basal</td>
<td>563 ± 104</td>
<td>532 ± 82</td>
<td>NS</td>
</tr>
<tr>
<td>p-ACC insulin</td>
<td>435 ± 88</td>
<td>275 ± 61</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE and are in arbitrary units. AMPK, AMP kinase; p-AMPK, phosphorylated AMPK; ACC, acetyl-CoA carboxylase; p-ACC, phosphorylated ACC. Biopsies were analyzed only in subjects who completed the study. P, paired t-test was performed to determine significance of differences between parameters before and after exercise and before and after insulin infusion. *P < 0.05 vs. basal value.
phosphorylation, had dissipated during the 24–36 h since the subjects’ last bout of exercise, whereas some of the long-term effects of AMPK activation, such as increased PGC-1α and MCD expression, had persisted (48, 51). In a recent study of well-trained athletes (8), 3 wk of intensified exercise training did not alter basal AMPKα1 or -α2 activities in skeletal muscle 24 h after the last bout of exercise compared with pretraining values. However, in another study of healthy male volunteers (12), endurance training for 3 wk induced significant increases in AMPK activity in muscle 15 and 55 h after the last bout of exercise. The reason for the different effects of physical training on AMPK activity in these three studies is not clear but may, at least in part, be related to differences in study design and population studied.

Cross-sectional and longitudinal studies (5, 19) have typically shown that trained healthy subjects are more insulin sensitive and have a higher VO2 max than untrained subjects. The increase in VO2 max after training, observed in the present study, was similar to that reported by others (28, 32, 41–43). The increases in VO2 max in these other studies were associated with increased insulin sensitivity in some instances (28, 43) but not others (32, 41, 42). In general, the effect of training on insulin sensitivity has been less pronounced in middle-aged and elderly people (32, 42). In one study, Short et al. (42) demonstrated that 4 mo of exercise training induces significant increases in skeletal muscle mitochondrial biogenesis and glucose transporter protein expression and decreases abdominal fat mass in men and women aged 21–87 yr. Insulin sensitivity, however, was improved in the younger people but not in the middle-aged and the elderly groups. Thus insulin action in muscle may respond differently to exercise training depending on age. Similarly, in the present study, middle-aged subjects did not improve insulin sensitivity after long-term physical training despite significant changes in VO2 max, intra-abdominal fat, malonyl-CoA concentration, MCD activity, and PGC-1α abundance. In addition to the age of the subjects, the lack of change in whole body insulin sensitivity in the present study could be related to the design of the training program, which engaged leg muscles more than the rest of the body, or to the fact that we studied a cohort of mixed sex that, from the beginning, had normal insulin sensitivity. In favor of the latter possibility, we have found that the same training program markedly improved insulin sensitivity in a group of insulin-resistant patients with type 2 diabetes of similar age, weight, and sex (77% increase in M value), (J. Kuhl and P. Bavenholm, unpublished data).

We also found no changes in whole body EE or fat oxidation rates under postabsorptive conditions after the subjects were trained (respiratory exchange ratio, 0.89 vs. 0.87 pretraining) despite lower levels of malonyl-CoA in muscle. One possible explanation for this is that the subjects were studied 24–36 h after the last bout of exercise when the acute stimulatory effect of prior physical activity on fat oxidation in muscle had diminished. Another is that the whole body measurements of fat oxidation at this time did not reflect changes in muscle. Direct measurements of the RQ of muscle, its concentration of palmityl carnitine, or its oxidation of radioactive fatty acids could answer this question. In this context, Schrauwen et al. (40) have demonstrated that low-intensity endurance training tends to increase fat oxidation rates in resting muscle in healthy, nonobese volunteers when measured directly with the use of palmitate tracer, whereas whole body fat oxidation rates, measured by indirect calorimetry, were unchanged. Another explanation for the lack of increase in fat oxidation could be that the inhibitory effect of malonyl-CoA on CPT I is greater in muscle of trained than of untrained individuals (45).

In summary, healthy, middle-aged subjects participating in a 12-wk combined aerobic and dynamic strength training program without caloric restriction improved their physical fitness and reduced intra-abdominal fat mass without a concomitant increase in insulin sensitivity. An intriguing and novel finding was that in skeletal muscle, long-term training increased expression and protein content of PGC-1α and AMPKα1 subunit was unchanged. We suggest that these changes in PGC-1α and MCD are effects of prior activation of AMPK by exercise training that persist after other AMPK-mediated events such as ACC phosphorylation and inhibition have dissipated. To our knowledge, this is the first demonstration that MCD activity is regulated in human muscle by exercise. The results also reveal that MCD may play a key role in regulating the concentration of malonyl-CoA in this setting.

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