Lipid oxidation is reduced in obese human skeletal muscle

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Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol Endocrinol Metab 279: E1039–E1044, 2000.—The purpose of this study was to discern cellular mechanisms that contribute to the suppression of lipid oxidation in the skeletal muscle of obese individuals. Muscle was obtained from obese [body mass index (BMI), 38.3 ± 3.1 kg/m²] and lean (BMI, 23.8 ± 0.9 kg/m²) women, and fatty acid oxidation was studied by measuring 14CO₂ production from 14C-labeled fatty acids. Palmitate oxidation, which is at least partially dependent on carnitine palmitoyltransferase-1 (CPT-1) activity, was depressed (P < 0.05) by ~50% with obesity (6.8 ± 2.2 vs. 13.7 ± 1.4 nmole CO₂·g⁻¹·h⁻¹). The CPT-1-independent event of palmitoyl carnitine oxidation was also depressed (P < 0.01) by ~45%. There were significant negative relationships (P < 0.05) for adiposity with palmitate (r = −0.76) and palmitoyl carnitine (r = −0.82) oxidation. Muscle CPT-1 and citrate synthase activity, an index of mitochondrial content, were also significantly (P < 0.05) reduced (~35%) with obesity. CPT-1 (r = −0.48) and citrate synthase (r = −0.65) activities were significantly (P < 0.05) related to adiposity. These data suggest that lesions at CPT-1 and post-CPT-1 events, such as mitochondrial content, contribute to the reduced reliance on fat oxidation evident in human skeletal muscle with obesity.

SKELETAL MUSCLE PLAYS AN IMPORTANT role in whole body lipid oxidation. During the fasting (postabsorptive) condition, lipid oxidation is the predominant metabolic activity of resting skeletal muscle (7), with ~40% of the fatty acids supplied being cleared with one circuit through the skeletal muscle capillary system (30). At rest, the oxidation of lipid contributes significantly to overall energy needs: up to 90% of the energy requirements of resting muscle are obtained from fatty acid oxidation (5, 7). Skeletal muscle, by virtue of its mass, is therefore quantitatively a factor in terms of the fate of circulating lipid.

Several observations suggest that muscle lipid oxidation is reduced with obesity in the postabsorptive state. Intramuscular triglyceride content is elevated in obese individuals (12, 15, 20, 21, 28). It has been hypothesized that this increase is associated with a reduction in muscle lipid oxidation promoting lipid accretion (6, 14, 20, 24, 25, 29). Such a relationship would be fundamentally important, as an increase in intramuscular lipid content is linked with insulin resistance in obese individuals (12, 14, 20, 29) despite little to no differences in total muscle-associated (intramuscular) lipid concentration (9). Another possible consequence of a reduction in muscle lipid oxidation is the partitioning of ingested nutrients toward storage in adipocytes. A reduction in the rate of muscle lipid oxidation has therefore been postulated to promote positive fat balance and fat mass gain (2, 6, 19). In support of a persistent defect, a reduced ability to oxidize fatty acids has been reported in the skeletal muscle of obese individuals both before and after weight loss (14).

The cellular mechanism(s) responsible for this decrement in muscle lipid oxidation with obesity is, however, not evident. One candidate is a reduction in the activity of carnitine palmitoyltransferase (CPT-1), an enzyme that regulates the transport of long-chain fatty acids across mitochondrial membranes (6, 14, 16–18, 30). However, a decrement in the activities of enzymes indicative of muscle mitochondrial content has also been reported in obese human skeletal muscle (6, 14, 22, 28, 29, 33). The role, if any, of each of these alterations on muscle lipid oxidation has not been directly examined. The purpose of the current study was therefore to discern if defects at the CPT-1 and post-CPT-1 levels play a role in the reduction in lipid oxidation in obese human skeletal muscle. This was accomplished by examining the ability of obese human skeletal muscle homogenates to oxidize either long- (palmitate and palmitoyl carnitine) or medium-chain (octanoate) fatty acids. Our findings suggest that both CPT-1 and post-CPT-1 events are responsible for the decrement in muscle lipid oxidation with obesity.
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METHODOLOGY

**Experimental design and subjects.** Skeletal muscle from nine lean (body mass index (BMI) 28.0 k/m²) and nine obese (BMI >28.0 k/m²) women were compared. Women were screened for metabolic abnormalities or medications that could affect lipid oxidation with a health history questionnaire. No subjects were smokers. All women were sedentary and had not participated in a regular exercise program in the previous 12 mo. Subjects provided informed consent before participation. Lean and obese subjects were matched for age and race. Each group consisted of eight Caucasians and one African American. One woman in each group was postmenopausal and receiving estrogen treatment. One woman in each group was also consuming oral contraceptives. Muscle lipid metabolism did not differ in these subjects, and they were thus included in data analyses.

Skeletal muscle (50–100 mg) was obtained from the vastus lateralis of each subject with the percutaneous needle biopsy technique (10). Subjects were studied between 0700 and 0800 after a 12-h overnight fast (postabsorptive). Subjects were instructed to consume their usual diet and perform normal daily activities for 3 days before the biopsy. Body mass and height were obtained on the morning of the biopsy to the nearest 0.1 kg and 1.0 cm, respectively.

**Biochemical methods.** Fresh muscle was immediately placed into ice-cold medium containing 250 mM sucrose, 1 mM EDTA, 10 mM Tris-HCl, and 10 mM Tris-HCl (pH 7.4) (SETH). Muscle was then dried on blotting paper, weighed, and minced thoroughly with scissors in 2 ml of SETH medium per milligram of muscle. The mixture was homogenized in a glass homogenization tube with a motor-driven teflon pestle. This method has been found to provide intact mitochondria for metabolic studies (27). Protein content in the homogenates did not differ (P > 0.05) between the lean and obese groups (49.6 _±_ 3.4 vs. 46.1 _±_ 1.8 mg/g, respectively).

Muscle fatty acid oxidation rate was determined in the fresh muscle homogenate using a modification of the method of Dohm et al. (8). The oxidation rate of palmitate, octanoate, and palmitoyl carnitine was measured by collecting and counting the _14CO_2 produced during incubation. Forty microliters of a 20-fold diluted muscle homogenate were preincubated with a 95% _O_2-5% _CO_2 mixture at 30°C for 10 min. The incubation mixture (pH 7.4) contained 0.2 mM [1-14C]palmitate (0.5 μCi, 4.4 mM ATP, 4.4 mM MgCl₂, 16.7 mM KCl, 2.2 mM KCN, 40 mg/l rotenone, 0.1% BSA, and 50 μM palmitoyl-CoA. The substrate was 0.2 mM [3H]carnitine (0.5 μCi). The reaction was terminated with 60 μl of 1.2 mM ice-cold HCl. The formed [3H]palmitoyl carnitine was extracted with water-saturated butanol and counted by liquid scintillation. Muscle citrate synthase activity was determined spectrophotometrically from the homogenate using the method of Srere (26). There was insufficient muscle for some analyses; subject number for each analysis is presented in RESULTS.

We performed an additional experiment to confirm a reduction in muscle oxidative capacity with obesity in a non-ambulatory muscle group. Citrate synthase, β-hydroxyacyl-CoA dehydrogenase (β-HAD), and phosphofructokinase (PFK) activity were measured in rectus abdominis samples from lean (n = 25; BMI, 22.6 _±_ 0.6) and obese (n = 28; BMI, 41.3 _±_ 2.0) patients who had presented themselves for abdominal surgery (hysterectomies, gastric bypass). The methodology for obtaining a biopsy of the rectus abdominis muscle and characteristics of this general patient population have been described previously (9). Briefly, a muscle strip was obtained during surgery and transported to the laboratory in oxygenated buffer. A portion of this sample was freeze clamped and stored at –80°C for subsequent analyses of β-HAD and PFK activities (28, 29). These enzymes were studied as β-HAD activity influences the capacity to oxidize free fatty acids (11, 30), while PFK activity has been used as an index of glycolytic capacity (28, 29).

Statistics. Descriptive data, muscle lipid oxidation, and muscle enzymes in the lean and obese groups were compared with factorial analysis of variance. Data from the vastus lateralis muscle in the lean and obese groups were also combined and examined with Pearson product correlations. Statistical significance was denoted at the P < 0.05 level.

RESULTS

**Subjects.** In the groups where the vastus lateralis was studied, the obese subjects had a significantly (P < 0.001) higher mean BMI than the lean subjects (means ± SE, 38.3 _±_ 3.1 vs. 23.8 ± 0.9 kg/m², respectively). Body mass was also significantly (P < 0.01) greater in the obese subjects (102.2 _±_ 9.3 vs. 66.1 _±_ 2.7 kg). There were no significant differences between the obese and lean groups, respectively, in either age (36.7 _±_ 3.9 vs. 29.7 _±_ 4.7 yr) or stature (1.63 _±_ 0.03 vs. 1.67 ± 0.02 m).

**Muscle lipid oxidation.** Muscle lipid oxidation was reduced in the vastus lateralis muscle of the obese group. As presented in Fig. 1, palmitate oxidation was significantly (P < 0.05) lower by ~50% with obesity (6.8 ± 2.2 vs. 13.7 ± 1.4 nmol CO_2·g⁻¹·h⁻¹, 8 obese and 7 lean, respectively). Palmitoyl carnitine (7 obese and 4 lean) oxidation was depressed (P < 0.01) with obesity by ~45% (21.1 ± 3.3 vs. 38.5 ± 3.6 nmol CO_2·g⁻¹·h⁻¹), whereas octanoate oxidation (8 obese
and 7 lean) was reduced by \( \approx 60\% \) (4.4 \( \pm \) 1.3 vs. 11.7 \( \pm \) 1.0 nmol CO\(_2\)·g\(^{-1}\)·h\(^{-1}\)). As presented in Fig. 2, a reduction in muscle lipid oxidation with obesity was also evident in the correlational data. There were significant negative relationships (\( P < 0.05 \)) for adiposity (BMI) with palmitate (\( r = -0.76 \)), palmitoyl carnitine (\( r = -0.82 \)), and octanoate (\( r = -0.82 \)) oxidation. An interesting observation was that muscle lipid oxidation was relatively depressed once obesity was attained (BMI \( > 30 \)) but was not markedly influenced by the degree of obesity (Fig. 2).

**Muscle enzymes.** As presented in Fig. 3, CPT-1 activity in the vastus lateralis was reduced (\( P < 0.05 \)) by \( \approx 35\% \) with obesity (18.2 \( \pm \) 2.4 vs. 27.4 \( \pm \) 3.1 nmol·g\(^{-1}\)·h\(^{-1}\)). This was also evident by the negative correlation between muscle CPT-1 activity and BMI (\( r = -0.48 \); 8 obese and 9 lean subjects). As presented in Fig. 4, citrate synthase activity in the vastus lateralis was (35\%) reduced (\( P < 0.01 \)) with obesity (34.5 \( \pm \) 3.7 vs. 53.8 \( \pm \) 4.5 nmol·g\(^{-1}\)·h\(^{-1}\)). There was a significant negative relationship between muscle citrate syn-
that activity and obesity, as presented in Fig. 4 (8 obese and 9 lean subjects).

In an attempt to discern the relative contributions of the defects in CPT-1 and mitochondrial content (i.e., citrate synthase activity), we compared the subjects with complete oxidation and enzyme data (7 obese and 4 lean). Palmitate oxidation was decreased ($P < 0.01$) by 62% ($4.9 \pm 1.3$ vs. $12.9 \pm 1.9$ nmol CO$_2$/g·h$^{-1}$), palmitoyl carnitine ($P < 0.01$) by 45% ($21.1 \pm 3.3$ vs. $38.5 \pm 3.6$ nmol CO$_2$/g·h$^{-1}$), and octanoate ($P < 0.01$) by 69% ($3.8 \pm 1.4$ vs. $12.3 \pm 1.4$ nmol CO$_2$/g·h$^{-1}$). Palmitate oxidation, after correction for mitochondrial volume (palmitate oxidation/citrate synthase activity), tended ($P = 0.07$) to be reduced with obesity ($0.13 \pm 0.03$ vs. $0.25 \pm 0.05$ arbitrary units) by 48%.

As presented in Table 1, data from the rectus abdominis muscle also indicated a significant decrease in muscle oxidative capacity with obesity. Muscle citrate synthase activity was reduced ($P < 0.05$) by $\approx 15\%$ and β-HAD activity by $\approx 65\%$ with obesity. On the other hand, muscle PFK activity was elevated by $\approx 30\%$ with obesity. The PFK-to-citrate synthase ratio, an index of muscle glycolytic capacity (28), was significantly ($P < 0.01$) elevated in obese skeletal muscle.

**DISCUSSION**

An inability to effectively oxidize circulating lipids appears to play a role in the etiology of human obesity. An elevated resting respiratory quotient, indicative of increased reliance on carbohydrate oxidation, has been reported to predict subsequent weight gain in Pima Indians (32, 33). An increased respiratory quotient or reduced reliance on lipid oxidation has also been observed in obese compared with lean subjects (2, 6, 14).

**Table 1. Enzyme activities in rectus abdominis samples from lean and obese subjects**

<table>
<thead>
<tr>
<th>Enzyme, U/g wet wt</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase</td>
<td>$5.2 \pm 0.3$</td>
<td>$4.5 \pm 0.3^*$</td>
</tr>
<tr>
<td>β-HAD</td>
<td>$2.3 \pm 0.1$</td>
<td>$0.8 \pm 0.1^*$</td>
</tr>
<tr>
<td>PFK</td>
<td>$39.2 \pm 3.4$</td>
<td>$50.1 \pm 3.6^*$</td>
</tr>
<tr>
<td>PFK/citrate synthase</td>
<td>$7.9 \pm 0.8$</td>
<td>$11.2 \pm 0.7^*$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE. β-HAD, β-hydroxyacyl-coenzyme A dehydrogenase; PFK, phosphofructokinase. * $P < 0.05$; † $P < 0.01$ vs. lean.
With weight loss, this reliance on carbohydrate rather than fat oxidation does not dissipate, which may predispose previously obese individuals to weight recidivism via the partitioning of nutrients toward fat storage (3, 14). In support of a role of skeletal muscle in reducing total body fat oxidation with obesity, Kelley et al. (14) reported a low rate of fatty acid oxidation across the thigh in obese individuals both before and after weight loss. The cellular mechanism(s) responsible for this persistent defect in lipid metabolism in obese human skeletal muscle is not, however, evident.

The main finding of the present study was that both long- and medium-chain fatty acid oxidation was depressed at the level of the mitochondria in the skeletal muscle of obese individuals. The rate of long-chain (palmitate) fatty acid oxidation is at least partially dependent on transport across the mitochondrial membranes via CPT-1 (16–18, 24, 25, 30). The current data indicate that palmitate oxidation was markedly reduced in conjunction with a decrement in CPT-1 activity in the skeletal muscle of obese subjects (Figs. 1, 2). Although others have reported a decrement in CPT-1 activity in obese human skeletal muscle (6, 14), this reduction has not been directly linked with an alteration in mitochondrial long-chain fatty acid oxidation. The current data thus imply a role for CPT-1 in accounting for at least some of the decrement in long-chain fatty acid oxidation reported in human skeletal muscle in vivo with obesity (6, 14). This reduction in muscle CPT-1 activity is relevant, as it does not appear to be reversed with weight loss (14). Obese skeletal muscle thus appears to be prone to partitioning long-chain fatty acid toward storage within the muscle cell, at least in part, through a reduction in CPT-1 activity.

One hypothesis that may explain the reduction in CPT-1 activity with obesity involves malonyl-CoA. Malonyl-CoA directly inhibits CPT-1 activity, thereby reducing long-chain fatty acid oxidation (1, 16–18). An increase in malonyl-CoA has been reported in rodent models of obesity/insulin resistance in conjunction with a decrement in muscle lipid oxidation (24, 25). It is also conceivable that sensitivity to malonyl-CoA may differ between lean and obese individuals. We cannot, however, discern if the decrement in CPT-1 activity we observed was due to regulation by malonyl-CoA or some other mechanism (i.e., reduced expression) with human obesity.

Although a decrement in CPT-1 can contribute to a reduction in lipid oxidation, the current data suggest that other facets of muscle lipid metabolism are altered in the mitochondria of obese individuals. Palmitoyl carnitine is thought to be oxidized independently of CPT-1 (16–18, 24, 25, 30). WE utilized this characteristic to discern whether defects in lipid metabolism in obese skeletal muscle were specific to CPT-1 or subsequent steps. As indicated by our findings (Figs. 1 and 2), long-chain acylcarnitine oxidation was markedly reduced in obese human skeletal muscle. These data suggest that post-CPT-1 mechanisms are also responsible for the decrement in muscle lipid oxidation in obese subjects.

To provide a possible explanation, we measured muscle citrate synthase activity. Citrate synthase is involved in oxidative ATP production and is found in direct proportion to muscle mitochondrial content (11, 13). As presented in Fig. 4, muscle citrate synthase activity was significantly reduced in the vastus lateralis of obese subjects. This observation was validated in a nonlocomotive muscle, the rectus abdominus (Table 1). In addition, β-HAD, a key enzyme in β-oxidation, was also markedly reduced, while the PFK-to-citrate synthase ratio, a surrogate for muscle glycolytic capacity (28), increased significantly with obesity.

Together, these findings suggest that a decrement in muscle oxidative capacity, via a decrease in mitochondrial content, contributes to the inability to oxidize long-chain fatty acid in obese human skeletal muscle. Other investigators have also reported a decrement in the activities of enzymes involved with the aerobic catabolism of lipid (i.e., β-oxidation, tricarboxylic acid cycle) in human obesity (6, 22, 28, 29). The findings of the current study, however, provide new information, in that the reduction in oxidative capacity was specifically linked to a dampened ability to oxidize long-chain fatty acids (Figs. 1 and 2). Moreover, reductions in mitochondrial capacity were evident in two distinct muscle groups (Fig. 4; Table 1), indicating a consistent defect.

The interaction between CPT-1 and post-CPT-1 events in producing a cumulative decrement in lipid oxidation provides important new information about obese human skeletal muscle. The possible contribution of each of these steps was evident when comparing the relative decreases in muscle lipid oxidation with obesity. Palmitate oxidation, which requires CPT-1, was depressed with obesity by ≈62%. In contrast, muscle palmitoyl carnitine oxidation, which is CPT-1 independent, was depressed by ≈45% (see RESULTS). In addition, muscle palmitate oxidation tended (P = 0.07) to remain depressed by ≈48% after correction for mitochondrial content (citrate synthase activity). These findings, in conjunction with the reductions in enzymatic activities reported (Figs. 3 and 4; Table 1), suggest that lesions in both CPT-1 and mitochondrial content contribute at least partially to the decrement in muscle fatty acid oxidation in obese humans.

It is not evident why octanoate oxidation was depressed with obesity. In humans and under the current experimental conditions, medium-chain triglycerides may not be metabolized independently of carnitine (23). The decrease in CPT-1 activity with obesity may thus also have impeded octanoate oxidation (Figs. 1–3). The reduction in octanoate oxidation in the current study is also not in agreement with other findings. An in vivo study indicated that although long-chain oxidation was depressed, medium-chain triacylglycerol oxidation did not differ with obesity (4). The findings of Binnert et al. (4) are, however, difficult to compare with the current data, as ingested medium-chain fatty acids may be largely metabolized in the liver, whereas the current experiment examined intact skeletal muscle mitochondria. It is, however, possible that other yet undefined facets of octanoate oxidation in human skeletal muscle are limited with obesity.
In conclusion, the oxidation of both long- (palmitate and palmitoyl carnitine) and medium- (octanoate) chain fatty acids were reduced in skeletal muscle homogenates from obese subjects. Muscle CPT-1 activity was reduced with obesity, which may at least partially explain the decrement in palmitate oxidation. The decrease in palmitoyl carnitine, however, implies a post-CPT-1 defect such as a reduction in oxidative capacity via reduced mitochondrial content. In support of a reduction in mitochondrial content, muscle citrate synthase and β-HAD activities were lower, while PFK activity was elevated in the skeletal muscle of obese individuals. These data suggest that there are defects at several levels in the catabolic process for lipids in obese skeletal muscle mitochondria. These findings also provide a possible explanation for the reduced reliance on lipid oxidation seen in obese individuals.

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