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Lipid oxidation is reduced in obese human skeletal muscle

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Kim, Jong-Yeon, Robert C. Hickner, Ronald L. Cortright, G. Lynis Dohm, and Joseph A. Houmard. 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/m^2] and lean (BMI, 23.8 ± 0.9 kg/m^2) women, and fatty acid oxidation was studied by measuring $^{14}CO_2$ production from ^{14}C-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 $CO_2 \cdot g^{-1} \cdot h^{-1}$). 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.

carnitine palmitoyltransferase; fatty acids; malonyl-coenzyme A

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 (intra- plus intermuscular) 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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METHODS

Experimental design and subjects. Skeletal muscle from nine lean [body mass index (BMI) <28 k/m^2] and nine obese (BMI >28 k/m^2) 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 μl 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 $^{14}CO_2$ 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 15 min. A 160-μl reaction mixture (pH 7.4) was then added to the preincubated muscle homogenate. Final concentrations of the incubation mixture were in millimoles per liter: sucrose, 100; Tris·HCl, 10; potassium phosphate, 5; potassium chloride, 80; magnesium chloride, 1; L-carnitine, 2; malate, 0.1; ATP, 2; coenzyme A (CoA), 0.05; dithiothreitol, 1; EDTA, 0.2; and bovine serum albumin, 0.3%. The substrates used were 0.2 mM [1-^{14}C]palmitate (0.5 μCi), 0.2mM [1-^{14}C]octanoate (0.5 μCi), or 0.2 mM [1-^{14}C]palmitoyl carnitine (0.5 μCi) with 0.5% BSA. After 60 min of incubation at 30°C, 100 μl of 4 N sulfuric acid were injected to stop the reaction. CO_2 produced during the 60-min incubation was trapped with 200 μl of 2M sodium hydroxide. Briefly, the incubation wells and $^{14}CO_2$ trap consisted of a modified 48-well microtiter plate (Costar, Cambridge, MA). Each system consisted of two adjacent wells with a fabricated groove between each to allow the acid-driven $^{14}CO_2$ from the homogenate to be trapped by the sodium hydroxide. Adjoining well pairs were sealed from each other by a rubber gasket. One hundred fifty microliters of the sodium hydroxide trap were counted for evolved $^{14}CO_2$ by liquid scintillation and oxidation rate expressed as nanomoles of CO_2 per gram of wet weight per hour.

Muscle enzyme activities were determined from an aliquot of the muscle homogenate. Muscle CPT-1 was measured using modification of the methods of McGarry et al. (18) and Zierz and Engel (31). This method measures the rate of formation of palmitoyl carnitine from palmitoyl-CoA and carnitine. Ten microliters of a 20-fold diluted muscle homogenate were preincubated for 20 min at 30°C in a microcentrifuge tube. Reactions were initiated when 90 μl of reaction mixture were added to preincubated muscle homogenate at 30°C for 10 min. The incubation mixture (pH 7.4) contained 117 mM Tris·HCl, 0.28 mM reduced glutathione, 4.4 mM ATP, 4.4 mM $MgCl_2$, 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 [^{3}H]carnitine (0.5 μCi). The reaction was terminated with 60 μl of 1.2 mM ice-cold HCl. The formed [^{3}H]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 nonlocomotive muscle group. Citrate synthase, β-hydroxyacyl-CoA dehydrogenase (β-HAD), and phosphofructokinase (PFK) activity were measured in rectus abdominus 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 abdominus 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/m^2, 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 $CO_2 \cdot g^{-1} \cdot h^{-1}$, 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 $CO_2 \cdot g^{-1} \cdot h^{-1}$), whereas octanoate oxidation (8 obese

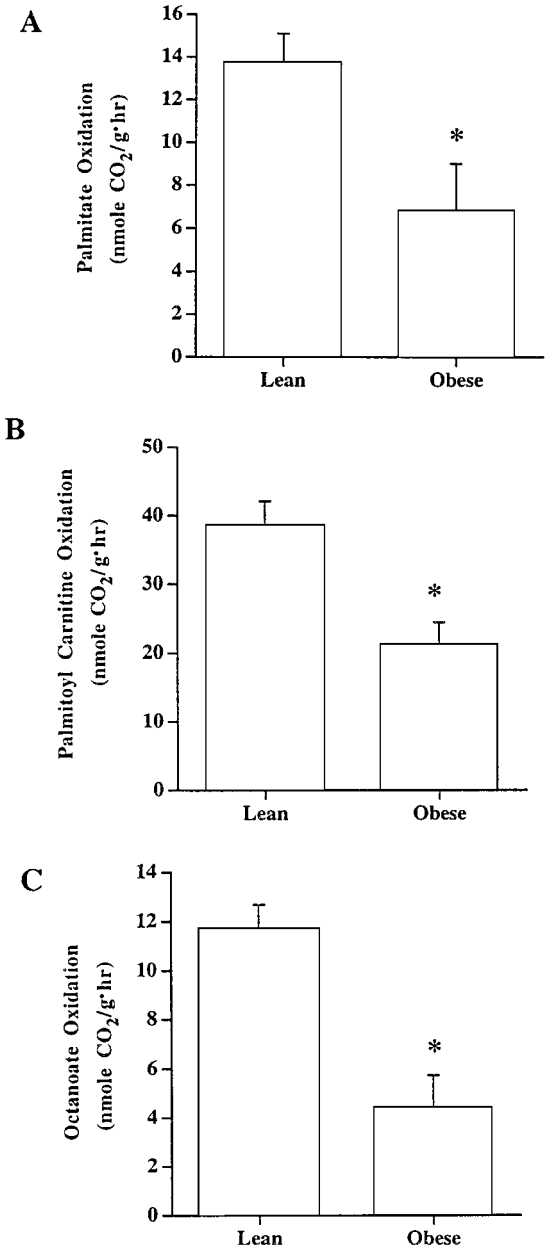

Fig. 1. Oxidation of ^{14}C-labeled palmitate (*A*), palmitoyl carnitine (*B*), and octanoate (*C*) in skeletal muscle homogenates from lean and obese subjects. *Significantly ($P < 0.05$) different between lean and obese subjects.

and 7 lean) was reduced by ≈60% (4.4 ± 1.3 vs. 11.7 ± 1.0 nmol $CO_2 \cdot g^{-1} \cdot h^{-1}$). 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 ≈35% with obesity (18.2 ± 2.4 vs. 27.4 ± 3.1 nmol $\cdot g^{-1} \cdot h^{-1}$). 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 ± 3.7 vs. 53.8 ± 4.5 nmol $\cdot g^{-1} \cdot h^{-1}$). There was a significant negative relationship between muscle citrate syn-

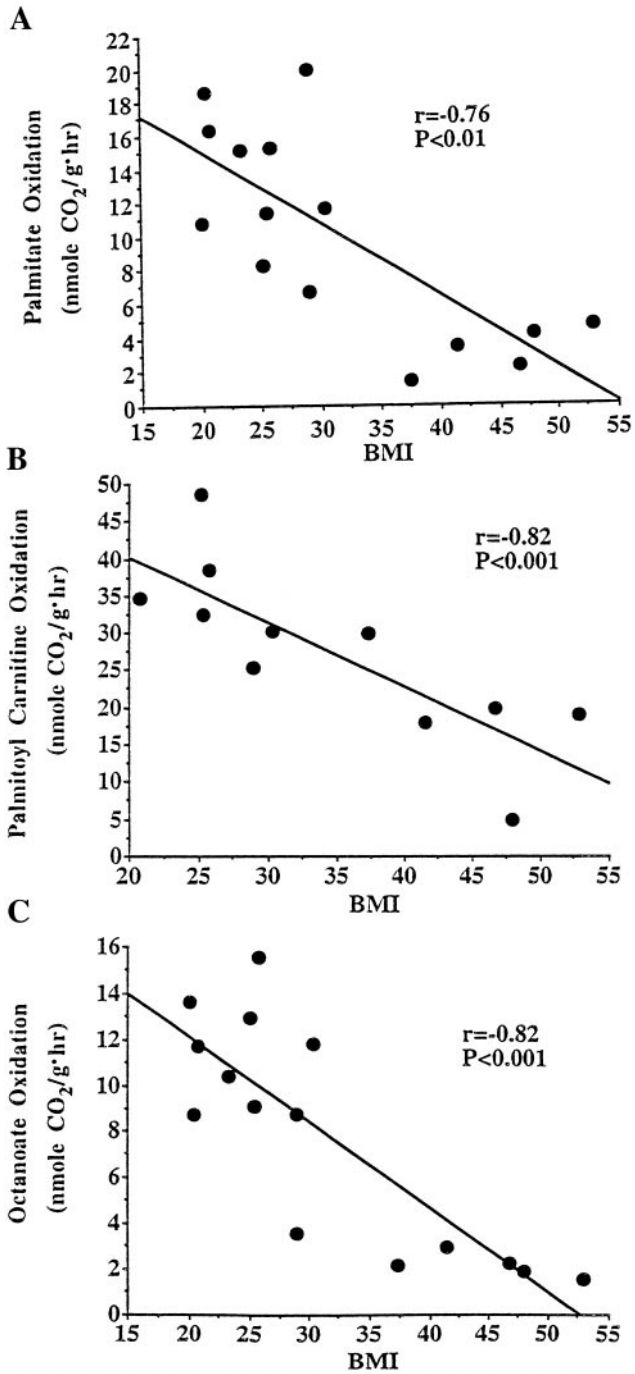

Fig. 2. Relationships between adiposity [body mass index (BMI)] and muscle palmitate (*A*), palmitoyl carnitine (*B*), and octanoate (*C*) oxidation in skeletal muscle homogenates from lean and obese subjects.

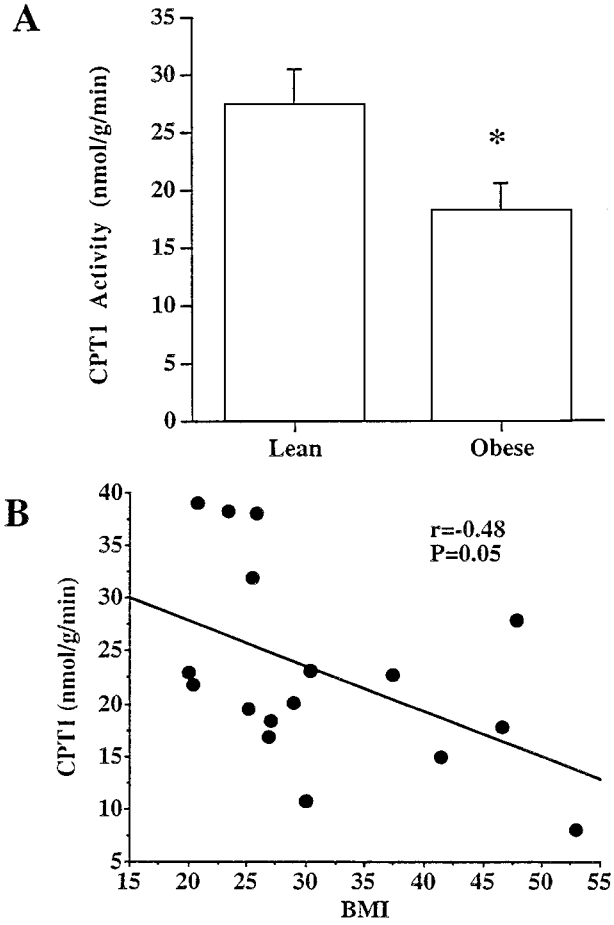

Fig. 3. Carnitine palmitoyltransferase-1 (CPT-1) activity in lean and obese human skeletal muscle (*A*) and relationship between adiposity (BMI) and CPT-1 activity in skeletal muscle from lean and obese subjects (*B*). *Significantly ($P < 0.05$) different between lean and obese subjects.

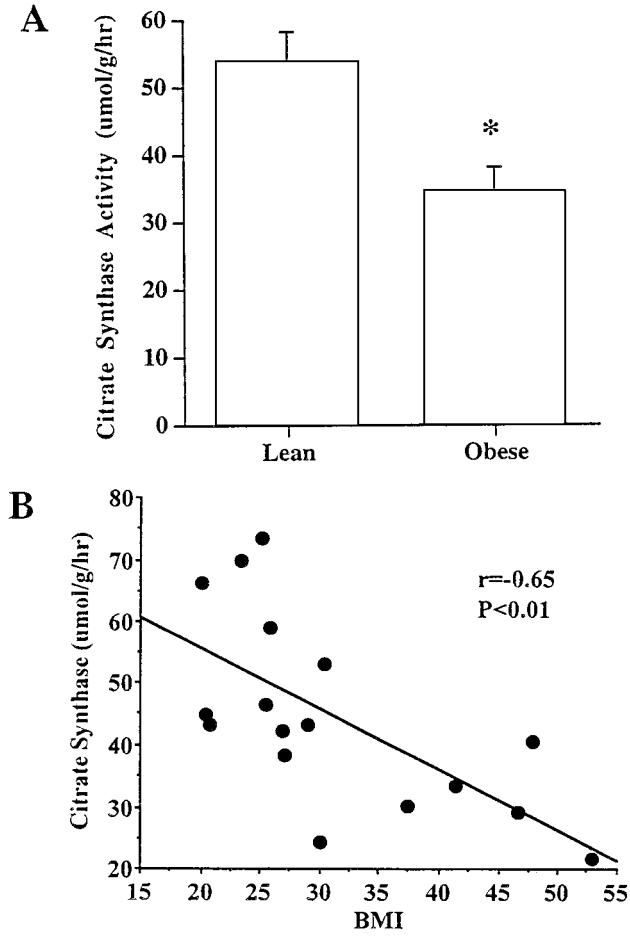

Fig. 4. Citrate synthase activity in lean and obese human skeletal muscle (*A*) and relationship between adiposity (BMI) and citrate synthase activity in skeletal muscle from lean and obese subjects (*B*). *Significantly ($P < 0.05$) different between lean and obese subjects.

thase 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 ± 1.3 vs. 12.9 ± 1.9 nmol $CO_2 \cdot g^{-1} \cdot h^{-1}$), palmitoyl carnitine ($P < 0.01$) by 45% (21.1 ± 3.3 vs. 38.5 ± 3.6 nmol $CO_2 \cdot g^{-1} \cdot h^{-1}$), and octanoate ($P < 0.01$) by 69% (3.8 ± 1.4 vs. 12.3 ± 1.4 nmol $CO_2 \cdot g^{-1} \cdot h^{-1}$). Palmitate oxidation, after correction for mitochondrial volume (palmitate oxidation/citrate synthase activity), tended ($P = 0.07$) to be reduced with obesity (0.13 ± 0.03 vs. 0.25 ± 0.05 arbitrary units) by 48%.

As presented in Table 1, data from the rectus abdominus muscle also indicated a significant decrease in muscle oxidative capacity with obesity. Muscle citrate synthase activity was reduced ($P < 0.05$) by ≈15% and β-HAD activity by ≈65% with obesity. On the other hand, muscle PFK activity was elevated by ≈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 abdominus samples from lean and obese subjects*

Enzyme, U/g wet wt	Lean	Obese
Citrate synthase	5.2 ± 0.3	4.5 ± 0.3*
β-HAD	2.3 ± 0.1	0.8 ± 0.1†
PFK	39.2 ± 3.4	50.1 ± 3.6*
PFK/citrate synthase	7.9 ± 0.8	11.2 ± 0.7†

Values are means ± SE. β-HAD, β-hydroxyacyl-coenzyme A dehydrogenase; PFK, phosphofructokinase. * $P < 0.05$; † $P \leq 0.01$ 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 thus 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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REFERENCES

1. **Alam N and Saggerson ED.** Malonyl-CoA and the regulation of fatty acid oxidation in soleus muscle. *Biochem J* 334: 233–241, 1998.
2. **Astrup A, Raben A, Buemann B, and Toubro S.** Fat metabolism in the predisposition to obesity. *Ann NY Acad Sci* 827: 417–430, 1997.
3. **Ballor DL, Harvey-Berino JR, Ades PA, Cryan J, and Calles-Escandon J.** Decrease in fat oxidation following a meal in weight-reduced individuals: a possible mechanism for weight recidivism. *Metabolism* 45: 174–178, 1996.
4. **Binnert C, Pachiaudi C, Beylot M, Hans D, Vandermander J, Chantre P, Riou J-P, and Laville M.** Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. *Am J Clin Nutr* 67: 595–601, 1998.
5. **Bulow J.** Lipid mobilization and utilization. *Principles of Exercise Biochemistry*. Basel: Karger, 1988, p. 140–163.
6. **Colberg SR, Simoneau JA, Theate FL, and Kelley DE.** Skeletal muscle utilization of FFA in women with visceral obesity. *J Clin Invest* 95: 1846–1853, 1995.
7. **Dagenais GR, Tancredi RG, and Zierlier KL.** Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in human forearm. *J Clin Invest* 58: 421–431, 1976.
8. **Dohm GL, Huston RL, Askew EW, and Weiser PC.** Effects of exercise on activity of heart and muscle mitochondria. *Am J Physiol* 223: 783–787, 1972.
9. **Dohm GL, Tapscott EB, Pories WJ, Dabbs DJ, Flinkinger EG, Meelheim D, Fushiki T, Atkinson SM, Elton CW, and Caro JF.** An in-vitro human muscle preparation suitable for metabolic studies. Decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. *J Clin Invest* 82: 486–494, 1988.
10. **Evans WJ, Phinney SD, and Young VR.** Suction applied to a muscle biopsy maximizes sample size. *Med Sci Sports Exerc* 14: 101–102, 1982.
11. **Gollnick PD and Saltin B.** Significance of skeletal muscle oxidative enzyme enhancement with endurance training: hypothesis. *Clin Physiol* 2: 1–12, 1982.
12. **Goodpaster BH, Kelley DE, Wing RR, Meier A, and Thaete FL.** Effects of weight loss on insulin sensitivity in obesity: influence of regional adiposity. *Diabetes* 48: 839–847, 1999.
13. **Howald H, Hoppeler H, Claasen H, Mathieu O, and Straub R.** Influence of endurance training on the ultrastructural composition of the different muscle fiber types in humans. *Pflügers Arch* 403: 369–376, 1985.
14. **Kelley DE, Goodpaster B, Wing RR, and Simoneau JA.** Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol Endocrinol Metab* 277: E1130–E1141, 1999.
15. **Landin K, Lindgarde F, Saltin B, and Wilhelmsen L.** Decreased muscle potassium in obesity. *Acta Med Scand* 223: 507–513, 1988.
16. **McGarry JD.** The mitochondrial carnitine palmitoyl transferase system: its broadening role in fuel homeostasis and new insights into its molecular features. *Biochem Soc Trans* 23: 321–324, 1995.
17. **McGarry JD and Brown NF.** The mitochondrial carnitine palmitoyltransferase system from concept to molecular analysis. *Eur J Biochem* 244: 1–14, 1997.
18. **McGarry JD, Mills SE, Long CS, and Foster DW.** Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. *Biochem J* 214: 21–28, 1983.
19. **Pagliassotti MJ, Gayles EC, and Hill JO.** Fat and energy balance. *Ann NY Acad Sci* 827: 431–448, 1997.
20. **Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, and Storlien LH.** Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46: 983–988, 1997.
21. **Phillips D, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, and Taylor R.** Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism* 45: 947–950, 1996.
22. **Raben A, Mygind E, and Astrup A.** Lower activity of oxidative key enzymes and smaller fiber areas in skeletal muscle of postobese women. *Am J Physiol Endocrinol Metab* 275: E487–E494, 1998.
23. **Rossle C, Carpentier YA, Richelle M, Dahlan W, D'Attellis NP, Furst P, and Elwyn DH.** Medium-chain triglycerides induce alterations in carnitine metabolism. *Am J Physiol Endocrinol Metab* 258: E944–E947, 1990.
24. **Ruderman NB, Saha AK, Vavvas D, Heydrick SJ, and Kurowksi TG.** Lipid abnormalities in muscle of insulin-resistant rodents. *Ann NY Acad Sci* 827: 221–230, 1997.
25. **Ruderman NB, Saha AK, Vavvas D, Kurowski T, Laybutt DR, Schmitz-Peiffer C, Biden T, and Kraegen EW.** Malonyl CoA as a metabolic switch and a regulator of insulin sensitivity. In: *Skeletal Muscle Metabolism in Exercise and Diabetes*, edited by Richter EA. New York: Plenum, 1998, p. 263–270.
26. **Srere PA.** Citrate synthase. *Methods Enzymol* 13: 3–26, 1969.
27. **Sholte HR, Yu Y, Ross JD, Oosterkamp II, Boonman AMC, and Busch HFM.** Rapid isolation of muscle and heart mitochondria, the lability of oxidative phosphorylation and attempts to stabilize the process in vitro by taurine, carnitine and other compounds. *Mol Cell Biochem* 174: 61–66, 1997.
28. **Simoneau JA, Colberg SR, Thaete FL, and Kelley DE.** Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *FASEB J* 9: 273–278, 1995.
29. **Simoneau JA and Kelley DE.** Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. *J Appl Physiol* 83: 166–171, 1997.
30. **Van der Vusse GJ and Reneman RS.** Lipid metabolism in muscle. *Handbook of Physiology: Regulation and Integration of Multiple Systems.* Bethesda, MD: Am. Physiol. Soc., 1996, sect. 12, chapt. 21, p. 952–994.
31. **Zierz S and Engel AG.** Different sites of inhibition of carnitine palmitoyletransferase by malonyl-CoA, and by acetyl-CoA and CoA, in human skeletal muscle. *Biochem J* 245: 205–209, 1987.
32. **Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF, Swinburn BA, Knowler WC, Bogardus C, and Ravussin E.** Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *Am J Physiol Endocrinol Metab* 259: E650–E657, 1990.
33. **Zurlo F, Nemeth PM, Choski RM, Sesodia S, and Ravussin E.** Whole body energy metabolism and skeletal muscle biochemical characteristics. *Metabolism* 43: 481–486, 1994.