

Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women

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Submitted 22 November 2006; accepted in final form 4 February 2007

Holloway GP, Thrush AB, Heigenhauser GJ, Tandon NN, Dyck DJ, Bonen A, Spriet LL. Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women. *Am J Physiol Endocrinol Metab* 292: E1782–E1789, 2007. First published February 20, 2007; doi:10.1152/ajpendo.00639.2006.— A reduction in fatty acid oxidation has been associated with lipid accumulation and insulin resistance in the skeletal muscle of obese individuals. We examined whether this decrease in fatty acid oxidation was attributable to a reduction in muscle mitochondrial content and/or a dysfunction in fatty acid oxidation within mitochondria obtained from skeletal muscle of age-matched, lean [body mass index (BMI) = 23.3 ± 0.7 kg/m²] and obese women (BMI = 37.6 ± 2.2 kg/m²). The mitochondrial marker enzymes citrate synthase (–34%), β-hydroxyacyl-CoA dehydrogenase (–17%), and cytochrome *c* oxidase (–32%) were reduced ($P < 0.05$) in obese participants, indicating that mitochondrial content was diminished. Obesity did not alter the ability of isolated mitochondria to oxidize palmitate; however, fatty acid oxidation was reduced at the whole muscle level by 28% ($P < 0.05$) in the obese. Mitochondrial fatty acid translocase (FAT/CD36) did not differ in lean and obese individuals, but mitochondrial FAT/CD36 was correlated with mitochondrial fatty acid oxidation ($r = 0.67$, $P < 0.05$). We conclude that the reduction in fatty acid oxidation in obese individuals is attributable to a decrease in mitochondrial content, not to an intrinsic defect in the mitochondria obtained from skeletal muscle of obese individuals. In addition, it appears that mitochondrial FAT/CD36 may be involved in regulating fatty acid oxidation in human skeletal muscle.

obesity; mitochondria; fatty acid translocase/CD36; transport proteins

IT HAS BEEN DEMONSTRATED that obese individuals have increases in intramuscular triacylglycerol (IMTG) storage, and this has been associated with insulin resistance (14–16, 23, 34). However, it appears unlikely that increases in IMTG levels represent a dysfunction in lipid metabolism (for review, see Ref. 39), and more reactive lipids, namely, diacylglycerol (DAG) and ceramides, may be important for the development of insulin resistance (18, 29, 34). It has been suggested that reductions in fatty acid oxidation in obese individuals contribute to the intramuscular lipid accumulation (20, 22, 24, 25, 31, 38). Based on reductions in carnitine palmitoyltransferase I (CPT I) activity in obese skeletal muscle, it has been proposed that fatty acid transport into the mitochondria is diminished. This may account for the observed reduction in fatty acid oxidation (22, 25), although other proteins may also be involved in this process.

The transport of fatty acids across the plasma membrane is generally believed to be largely mediated by fatty acid transporters, among which fatty acid translocase (FAT/CD36) appears to have a prominent role. This protein is located in several subcellular domains (5), and recently FAT/CD36 has also been found on rat and human muscle mitochondria (4, 10, 19). At this subcellular location, FAT/CD36, along with CPT I, contributes to regulation of mitochondrial fatty acid oxidation in skeletal muscle at rest, since blocking mitochondrial FAT/CD36 has been shown to almost completely inhibit fatty acid oxidation (4, 10, 19). During exercise, mitochondrial fatty acid oxidation is upregulated and is accompanied by an increase in mitochondrial FAT/CD36 content (10, 19). The exercise-induced increase in fatty acid oxidation is also completely inhibited when FAT/CD36 is pharmacologically blocked (10, 19). In other work, an increase in mitochondrial FAT/CD36 has been associated with exercise-induced weight loss and an improvement in whole body fatty acid oxidation (32). Thus it appears that changes in mitochondrial FAT/CD36 are associated with changes in mitochondrial fatty acid oxidation.

Fatty acid binding protein (FABPpm) is another well-recognized plasma membrane fatty acid transport protein that has also been found on mitochondria (3, 37). However, the role of FABPpm with respect to mitochondrial fatty acid oxidation remains unknown.

The net accumulation of lipids in obese skeletal muscle may result from a combination of increased whole muscle fatty acid uptake and/or decreased whole muscle oxidation. Although the concept of impaired fatty acid oxidation as a mechanism to increase intramuscular lipid species has gained attention in recent years, the exact mechanism remains unknown. However, two plausible explanations exist: either mitochondrial content is decreased, and/or there is a dysfunction in fatty acid oxidation within mitochondria. Work in skeletal muscle has indicated a reduction in mitochondrial content with obesity, along with a concomitant decrease in fatty acid oxidation (24). Although ratios of electron transport chain capacity to mitochondrial DNA and mitochondrial size have been used to infer dysfunction (24, 31), recently the ability of mitochondria to oxidize fatty acids was directly measured in skeletal muscle from type 2 diabetics and, controversially, was shown to be increased, although the underlying mechanism remains unknown (1).

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The focus of the present study was to examine whether the obesity-related decreases in skeletal muscle lipid oxidation are attributable to 1) a reduction in mitochondrial content and/or 2) an intrinsic defect in mitochondria, and also 3) whether there are reductions in the content of mitochondrial fatty acid transport proteins. We hypothesized that obesity would be associated with decreases in skeletal muscle mitochondrial content, as well as decreases in the ability of mitochondria to oxidize fatty acids. In addition, we speculated that impairments in mitochondrial oxidation would be associated with reductions in the content of mitochondrial FAT/CD36 and FABPpm.

METHODS

The participants were nine lean [body mass index (BMI) < 27 kg/m²; mean: 23.3 ± 0.7 kg/m²] and nine obese (BMI > 30 kg/m²; mean: 37.6 ± 2.2 kg/m²) nondiabetic women (Table 1). Subjects were admitted to McMaster Health Sciences Centre for abdominal surgery and gave informed written consent before participating in the study. The University of Guelph and McMaster University Ethics Committees approved the experimental procedures. Before participation, individuals were screened and excluded from the study if any known diseases or medications, as well as if weight fluctuations in the 6 mo preceding surgery, were reported, similar to procedures previously reported (7, 8).

Following an overnight fast (12–18 h), general anesthesia was induced with a short-acting barbiturate and maintained as required by a fentanyl and rocuronium volatile anesthetic mixture. A venous blood sample was sampled directly into a 5-ml heparinized tube, inverted, and placed on ice for future processing. A portion of the rectus abdominus muscle was sampled (~350 mg) and immediately placed in ice-cold oxygenated buffer (modified Krebs-Henseleit buffer containing 8 mM glucose) for transport to the laboratory.

Mitochondrial enzyme activities. A portion of the muscle (~10 mg) was immediately homogenized in 100 vol/wt of a 100 mM potassium phosphate buffer and used for the measurements of maximal β -hydroxyacyl-CoA dehydrogenase (β -HAD) and citrate synthase (CS) activities. Total muscle β -HAD activity was measured in Tris·HCl buffer (50 mM Tris·HCl, 2 mM EDTA, and 250 μ M NADH, pH 7.0) and 0.04% Triton-X. The reaction was started by addition of 100 μ M acetoacetyl-CoA, and absorbance was measured at 340 nm over a 2-min period (37°C) (2). The CS activity was assayed spectrophotometrically at 37°C by measuring the disappearance of NADH at 412 nm (2).

CS activity was also determined in the isolated mitochondrial preparations. Intact mitochondrial CS activity was determined by first assaying the extramitochondrial fraction in the suspension (1:20 dilution) and then assaying the total CS activity of the suspension (1:20 dilution) after lysing the mitochondria with 0.04% Triton X-100 and repeated freeze-thawing. The net difference provided a measure of the viability of the mitochondrial preparation, as well as a measure of the mitochondria recovered during the isolation procedure (com-

pared with the total muscle CS activity) (4). The mitochondrial recovery and viability were comparable to those reported previously by our group (4, 19).

Isolation of mitochondria from skeletal muscle. Differential centrifugation was used to obtain pure and intact mitochondria containing both intermyofibrillar (IMF) and subsarcolemmal (SS) fractions (10). All procedures were identical to those previously published by our group (4, 10, 19). Briefly, muscle (~350 mg) was homogenized with a tight-fitting Teflon pestle. The homogenate was centrifuged at 800 g for 10 min to separate the SS and IMF mitochondria. The IMF mitochondria were treated with a protease (0.025 ml/g; Sigma, St. Louis, MO) for exactly 5 min to digest the myofibrils. Further centrifugation was used to remove the myofibrils and recombine the IMF with the SS mitochondria. The combined samples were centrifuged twice at 10,000 g for 10 min. The pellet was resuspended in 1 μ l of buffer per milligram of tissue. Following the oxidation measurements, the remaining mitochondria were further purified using a Percoll gradient for Western blot analyses. Samples were centrifuged at 20,000 g for 1 h, and the mitochondrial layer was removed. The Percoll was removed from the sample by further centrifugation at 20,000 g for 5 h.

Mitochondrial palmitate oxidation. Labeled CO₂ production and acid-soluble trapped ¹⁴C from palmitate oxidation were measured following a 30-min incubation of viable mitochondria in a sealed system, as described previously (4, 10, 19). Briefly, viable mitochondria (100 μ l) were added to a system containing a pregassed modified Krebs-Ringer buffer supplemented with 5 mM ATP, 1 mM NAD⁺, 0.5 mM D,L-carnitine, 0.1 mM coenzyme A, 25 μ M cytochrome c, and 0.5 mM malate. A microcentrifuge tube, containing 500 μ l of 1 M benzethonium hydroxide inserted into a 1.5-ml centrifuge tube, was placed in the system to capture ¹⁴CO₂ produced during the oxidation reaction. The system was then sealed with a rubber cap and further sealed with parafilm. The reaction was initiated by the addition of a 6:1 palmitate-BSA complex (containing 10 μ Ci of [1-¹⁴C]palmitate, for a final palmitate concentration of 77 μ M) administered by syringe through the rubber cap. The reaction continued for 30 min at 37°C and was terminated with the addition of ice-cold 12 M perchloric acid (PCA) by syringe through the rubber cap.

A fraction of the reaction medium was removed through the cap and analyzed for isotopic fixation. Gaseous CO₂ produced from oxidation of [1-¹⁴C]palmitate was measured by acidifying the remaining reaction mixture. Liberated ¹⁴CO₂ was trapped by the benzethonium hydroxide over a 90-min incubation period at room temperature. The tube containing the ¹⁴CO₂ was put in a scintillation vial, and radioactivity was counted.

Whole muscle palmitate oxidation. Whole muscle palmitate oxidation rates, expressed per gram wet weight, were calculated from mitochondrial palmitate oxidation rates, expressed per milligram of mitochondrial protein. To accomplish this, the mitochondrial recovery was first applied to the mitochondrial oxidation value, and subsequently this value was divided by the starting wet weight value.

Determination of blood metabolites. Venous blood was sampled directly into vacutainers containing heparin and partitioned into two fractions. An aliquot of 200 μ l of whole blood was added to 1 ml of 0.6 M PCA and centrifuged. The deproteinized supernatant was stored at -80°C and later analyzed for glucose (2). A second aliquot of whole blood was immediately centrifuged, and the plasma was removed and stored at -80°C. The plasma was later analyzed for free fatty acids (Wako NEFA C test kit; Wako Chemicals, Richmond, VA) and insulin (RIA insulin kit; Cedarlane Laboratories, Hornby, ON, Canada).

Western blot analysis. Whole muscle crude membranes were generated as previously described (5, 6, 27) and analyzed for total protein (BCA protein assay), and 35 μ g of denatured protein were loaded for Western blotting. Purified isolated mitochondrial fractions were analyzed for total protein (BCA protein assay), and 25 μ g of denatured protein from each sample were loaded for Western blotting. All

Table 1. Subject characteristics

Characteristics	Lean	Obese
Age, yr	47 ± 3	45 ± 3
Body mass, kg	59.7 ± 2.0	99.2 ± 6.8*
BMI, kg/m ²	23.3 ± 0.7	37.6 ± 2.2*
Fasting blood glucose, mM	4.1 ± 0.1	4.5 ± 0.4
Fasting plasma insulin, pM	24.8 ± 1.9	35.9 ± 4.9*
Fasting free fatty acids, mM	0.87 ± 0.09	0.86 ± 0.06

Values are presented as means ± SE (*n* = 9), except for blood measurements (*n* = 6). BMI, body mass index. **P* < 0.05. Significantly different from lean.

samples were separated by electrophoresis on 8% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. The FABPpm polyclonal antibody used was produced in the laboratory of Dr. J. Calles-Escandon (11, 26). The MO-25 antibody used to detect FAT/CD36 was produced in the laboratory of Dr. N. N. Tandon (28). This antibody has also been used previously in our work (5, 10, 19). Commercially available antibodies were used to detect cytochrome *c* oxidase IV (Cox-IV; Invitrogen, Burlington, ON, Canada), GLUT4 (Chemicon International, Temecula, CA), caveolin-3 (Cav-3; BD Biosciences, Mississauga, ON, Canada), sarcoplasmic reticulum calcium-ATPase (SERCA; Affinity Bioreagents, Golden, CO), fatty acid transport protein 4 (FATP-4; Santa Cruz Biotechnology, Santa Cruz, CA), and hormone-sensitive lipase (HSL; ProSci, Poway, CA). An internal control of previously extracted human muscle crude membrane was used in each gel. Blots were quantified using chemiluminescence and the ChemiGenius 2 Bioimaging system (SynGene, Cambridge, UK).

Statistics. All data are presented as means \pm SE. Differences between lean and obese participants were analyzed with an unpaired, two-tailed *t*-test. Associations between variables were investigated using Pearson correlation analyses, as appropriate. Correlations were done by including both lean and obese individuals. Statistical significance was accepted at $P < 0.05$.

RESULTS

Subject characteristics. There were no differences between lean and obese participants in mean age, fasting blood glucose, or fasting free fatty acid concentrations (Table 1). In contrast, there was a significant ($P < 0.05$) increase in body mass, BMI, and fasting insulin concentrations in the obese participants (Table 1).

Effect of obesity on whole muscle fatty acid transport protein contents and fatty acid oxidation enzymes. Obesity did not alter the whole muscle protein expression of FAT/CD36, FABPpm, FATP-4 (Fig. 1, A–C, respectively), or HSL (Fig. 2A). In contrast, the whole muscle activity of β -HAD and CS, as well as Cox-IV protein content, were reduced ($P < 0.05$) by 17, 34, and 32% (Fig. 2, B–D, respectively). CS activity negatively correlated with BMI ($r = -0.53$; $P < 0.05$); however, β -HAD ($r = -0.31$), HSL ($r = 0.38$), and Cox-IV ($r = -0.26$) did not.

Effect of obesity on skeletal muscle mitochondrial proteins. The mitochondrial recovery was not different between lean and obese participants (21 ± 1 vs. $23 \pm 1\%$), nor was the viability of the mitochondrial preparation (92 ± 1 vs. $94 \pm 1\%$). The absence of SERCA (110 kDa), GLUT4 (45 kDa), and Cav-3

proteins (23 kDa) indicated that the isolation procedures successfully yielded highly purified mitochondria without contamination from other membrane or intracellular sources. In addition, the isolated mitochondria exhibited significant expression of FAT/CD36, FABPpm, and Cox-IV contents (Fig. 3, A–C, respectively) that did not differ with obesity.

Effect of obesity on whole muscle and mitochondrial fatty acid oxidation. Obesity did not alter the rate of palmitate oxidation in isolated mitochondria (Fig. 4A). In contrast, palmitate oxidation calculated in the whole muscle was reduced in obese individuals (Fig. 4B). The rate of palmitate oxidation in isolated mitochondria did not correlate with BMI ($r = 0.02$; Fig. 5A). However, independently of BMI, palmitate oxidation in isolated mitochondria significantly ($P < 0.05$) correlated with mitochondrial FAT/CD36 content ($r = 0.67$; Fig. 5B) but not with mitochondrial FABPpm content ($r = 0.42$). In addition, calculated whole muscle palmitate oxidation was significantly correlated ($P < 0.05$) with whole muscle CS activity ($r = 0.53$) and β -HAD activities ($r = 0.50$).

DISCUSSION

Mitochondrial dysfunction has been proposed to explain reductions in fatty acid oxidation in skeletal muscle of obese individuals, leading to elevations in fatty acid storage and ultimately insulin resistance (14–16, 23–25, 34). Although there is evidence in support of this hypothesis (22, 24, 25, 31, 35), these findings are not uniformly supported (7, 8, 30, 36, 40). The main novel findings of the present study were as follows: 1) mitochondrial fatty acid oxidation rates were not different in obese vs. lean individuals; 2) the mitochondrial contents of FAT/CD36 and FABPpm were similar in the two groups; 3) whole muscle fatty acid oxidation rates and mitochondrial content (whole muscle CS, β -HAD, and Cox-IV) were reduced in obese individuals; 4) total muscle expression of FAT/CD36, FABPpm, and FATP-4 were not altered with obesity; and 5) mitochondrial fatty acid oxidation rates were highly associated with the content of FAT/CD36 on mitochondrial membranes. Therefore, we suggest that obesity-related impairments in fatty acid oxidation are a result of reductions in mitochondrial content and not an inherent dysfunction of the mitochondria to oxidize fatty acids. In addition, at the level of the mitochondria, the capacity to oxidize fatty acid is related to the availability of FAT/CD36.

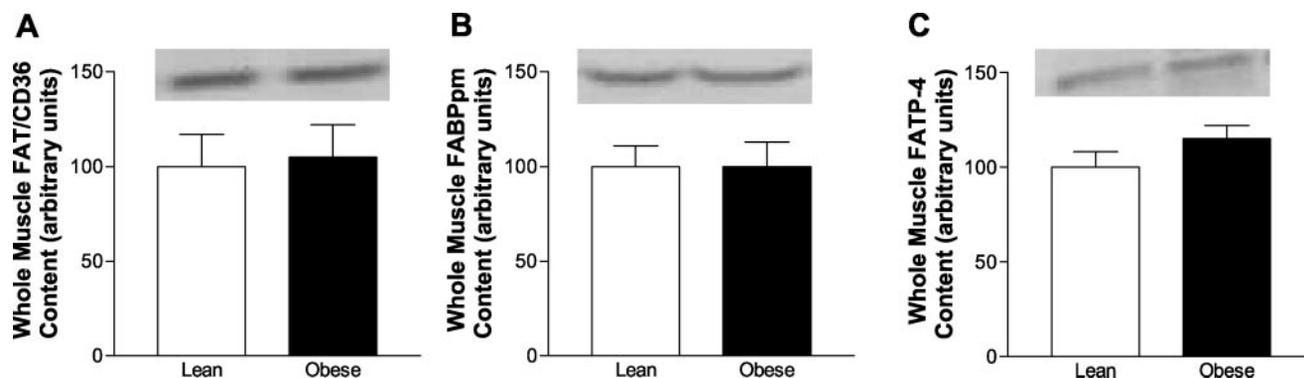


Fig. 1. Representative Western blots displaying the effects of obesity on the expression of fatty acid transporters. Values are means \pm SE ($n = 9$). A: whole muscle fatty acid translocase (FAT/CD36) content. B: whole muscle plasma membrane fatty acid binding protein (FABPpm) content. C: whole muscle fatty acid transport protein 4 (FATP-4) content.

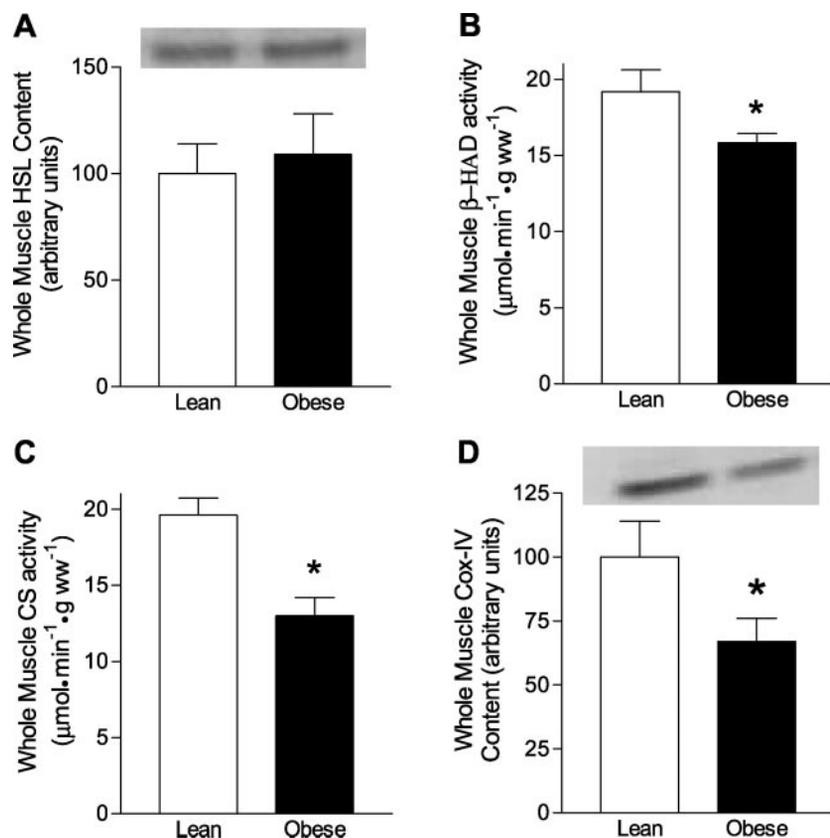


Fig. 2. Effects of obesity on the expression of enzymes involved in the lipolysis and oxidation of fatty acids. Values are means \pm SE ($n = 9$). A: representative Western blot of whole muscle hormone-sensitive lipase (HSL) content. B: homogenate β -hydroxyacyl-CoA dehydrogenase (β -HAD) activity. C: homogenate citrate synthase (CS) activity. D: representative Western blot of whole muscle cytochrome *c* oxidase complex IV (Cox-IV) content. * $P < 0.05$, significantly different from lean.

Whole muscle protein content. In the present study we found that whole muscle FAT/CD36 and FABPpm are unaltered with obesity. This corroborates our earlier finding that whole muscle FAT/CD36 is not decreased with obesity (7). However, our group previously reported that obesity resulted in a small (~25%) reduction in whole muscle FABPpm and increased with type 2 diabetes (7). This discrepancy might have arisen because of sample population variation as a result of small sample sizes, and the current data suggesting that obesity does not alter homogenate FABPpm fits with the literature pertaining to other fatty acid transporter proteins (1, 7). In addition, for the first time we report that whole muscle FATP-4 in lean and obese individuals does not differ, and this is in agreement with Bandyopadhyay et al. (1). Previously, we reported that obese individuals re-partition FAT/CD36 to the plasma membrane, thereby increasing fatty acid transport into the muscle (7). Although Bandyopadhyay et al. (1) also reported an increased amount of FAT/CD36 on plasma membranes in obese type 2 diabetics, their results are more difficult to interpret, because they also showed a proportional increase in FAT/CD36 in the intracellular compartment. This inconsistency might result from the different sexes studied or the skeletal muscle sampled. In the present study females were used exclusively and the rectus abdominus was sampled, whereas Bandyopadhyay et al. (1) used primarily male participants (20 male and 1 female) and sampled from an unspecified muscle biopsy (probably the vastus lateralis) (1). Regardless of these discrepancies, it is becoming increasingly clear that the transport of fatty acids into skeletal muscle is increased with obesity and type 2 diabetes. Interestingly, FATP-4 appears

to follow a similar trend, since type 2 diabetics also have increased levels of FATP-4 on the plasma membrane fraction (1).

Whole muscle fatty acid oxidation, enzymes, and mitochondrial volume. The current data suggest that obese individuals have an impairment at the whole muscle level to oxidize fatty acids, corroborating the findings of others (20, 25, 38). In addition, our whole muscle palmitate oxidation values correlated with whole muscle CS and β -HAD activities, further suggesting that mitochondrial content determines the rate of whole muscle fatty acid oxidation. For fatty acid oxidation to be impaired in obese individuals, either mitochondrial content must be substantially reduced and/or the ability of the mitochondria to oxidize fatty acids must be impaired. We corroborate the findings of others that obesity is related to reductions in CS and β -HAD activities, as well as a decrease in Cox-IV content (24, 25, 31, 35). Although various cofactors and covalent and allosteric regulators can influence the kinetics of enzymes, the maximal activity of an enzyme is indicative of the total amount of the protein. Therefore, reductions in CS and β -HAD maximal activities indicate a loss of the mitochondrial enzymes in skeletal muscle of obese individuals. Collectively, these data indicate that the mitochondrial content in skeletal muscle of obese individuals is reduced (21). Although a decrease in mitochondrial content can explain the observed reduction in whole muscle fatty acid oxidation, further measurements were required to determine whether this mechanism was solely responsible or whether mitochondrial dysfunction further exacerbated the impairment in skeletal muscle lipid oxidation in obese individuals.

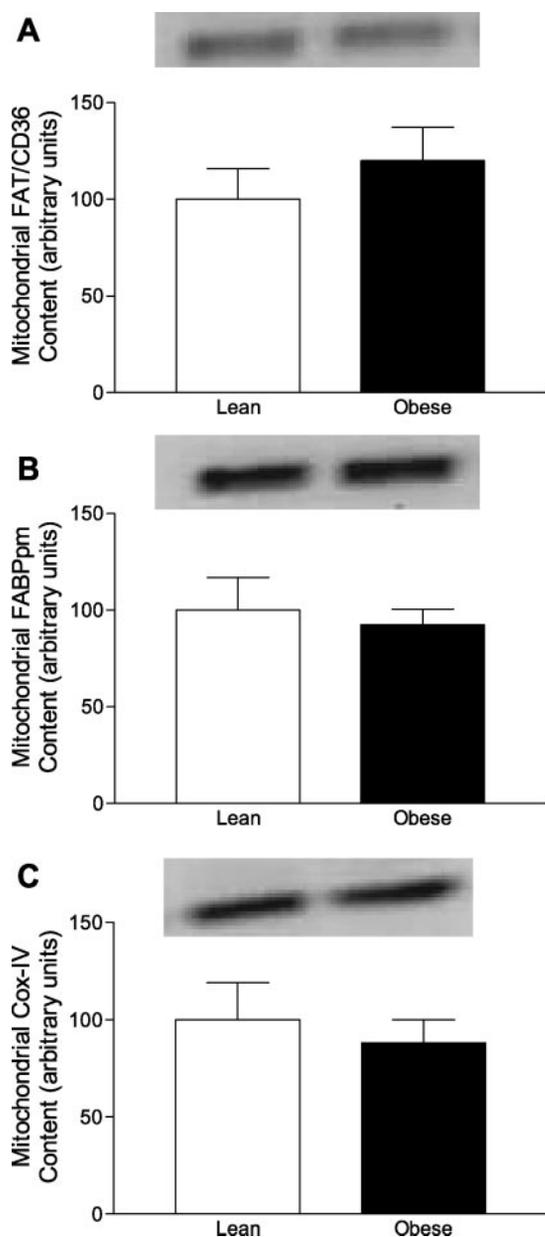


Fig. 3. Representative Western blots displaying the effects of obesity on the expression of mitochondrial proteins. Values are means \pm SE ($n = 9$). A: mitochondrial FAT/CD36 content. B: mitochondrial FABPpm content. C: mitochondrial Cox-IV content.

Mitochondrial fatty acid oxidation and transport proteins. Contrary to our hypothesis, there were no differences in the ability of mitochondria isolated from obese individuals to oxidize fatty acids. This suggests that mitochondrial dysfunction is not a mechanism for the observed reduction in whole muscle fatty acid oxidation and that a decrease in mitochondrial content is primarily responsible. We originally hypothesized that mitochondria isolated from obese participants would have a diminished ability to oxidize fatty acids, possibly as a result of a decrease in the content of FAT/CD36 (4, 10, 19) and perhaps FABPpm. Since mitochondria isolated from the obese did not differ in their ability to oxidize fatty acids, one would not expect the content of both transporters to be different. In agreement, the content of both mitochondrial FAT/CD36 and

FABPpm did not differ with obesity. In addition, although mitochondrial palmitate oxidation rates in lean and obese individuals did not correlate with BMI, the rates did correlate highly with FAT/CD36 mitochondrial content. Interestingly, FABPpm did not correlate with mitochondrial oxidation, suggesting that it is not rate limiting for the transport of long-chain fatty acid (LCFA) into mitochondria. Although this needs further investigation, the current belief is that the main function of FABPpm is to transport reducing equivalents into the mitochondria, since it is identical to mitochondrial aspartate amino transferase (3, 37).

Previous work from our group using a specific inhibitor of FAT/CD36 suggested that FAT/CD36 is necessary for fatty acid oxidation at the level of the mitochondria (4, 10, 19). In addition, we have previously shown that the content of FAT/CD36 on mitochondria can predict the ability of mitochondria to oxidize fatty acids during exercise (19). Others have also shown that mitochondrial FAT/CD36 can be altered with training, and the training-induced increases in mitochondrial FAT/CD36 correlated significantly with changes in resting fatty acid oxidation (32). The current work further strengthens the evidence that FAT/CD36 has a vital role in the transport of fatty acids into mitochondria. We recognize that CPT I also has an important role in this level of regulation (4, 19), and further research is required to determine exactly how CPT I and FAT/CD36 interact to facilitate LCFA transport.

Previous data suggest that there may be a threshold required for impairments in whole muscle fatty acid oxidation to be manifested, i.e., BMI between 40 and 50 (20, 38). In the

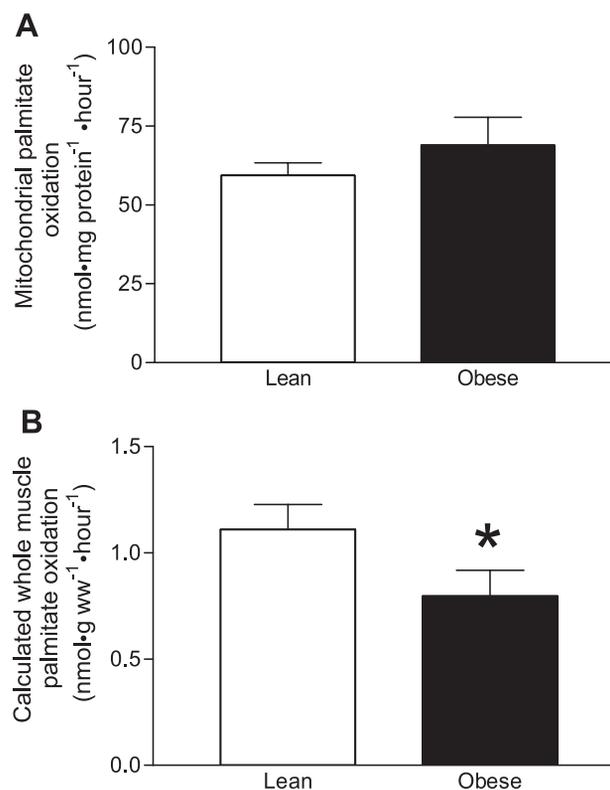


Fig. 4. Effects of obesity on the ability of skeletal muscle to oxidize palmitate. Values are means \pm SE ($n = 9$). A: isolated mitochondrial palmitate oxidation. B: calculated whole muscle palmitate oxidation. * $P < 0.05$, significantly different from lean.

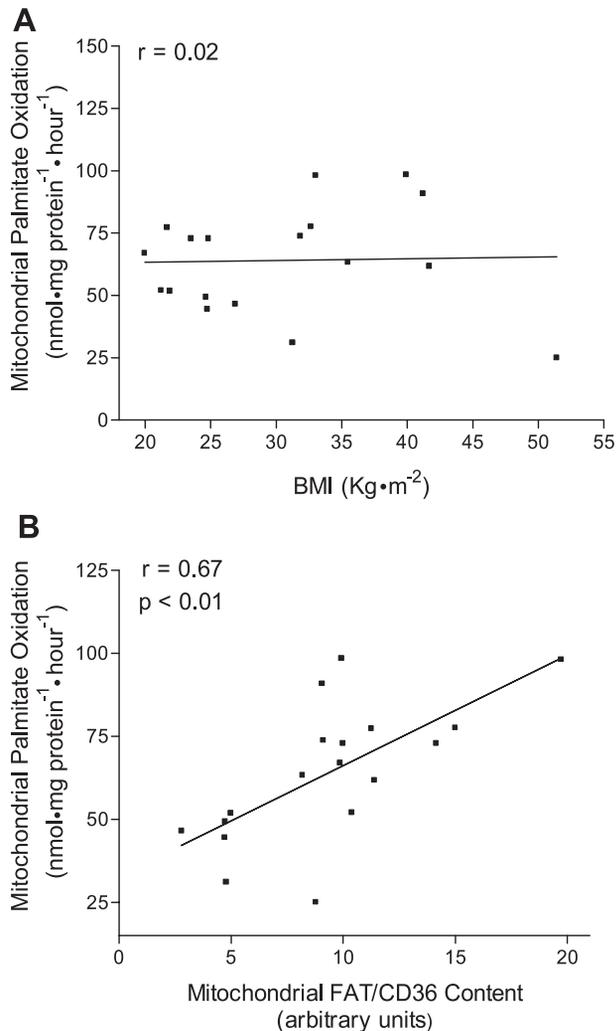


Fig. 5. Pearson correlations ($n = 18$; 9 lean and 9 obese individuals). A: correlation between mitochondrial palmitate oxidation and body mass index (BMI). B: correlation between mitochondrial palmitate oxidation and the mitochondrial FAT/CD36 content.

current study, our BMI value in the obese group was relatively high (mean: 37.6 kg/m²), but since they did not display mitochondrial dysfunction, it is possible that they were below this threshold. However, it was not directly determined in these previous studies whether mitochondrial function was impaired. In our study, the obese group displayed reductions in whole muscle CS and β -HAD activities, reduced Cox-IV content, and reduced whole muscle fatty acid oxidation rates, all of which indicate lipid oxidation was compromised. Thus the current data suggest that the reductions in fatty acid oxidation that have been associated with obesity result from reductions in mitochondrial content, and not from intrinsic mitochondrial alterations. This is an important distinction, because it suggests that interventions are required to increase mitochondrial biogenesis, but these do not have to “remodel” existing mitochondria. Indeed, previous work has shown that regular exercise can increase mitochondria volume in the obese population, as shown by increases in CS, β -HAD, and CPT I activity (9). In addition, associated with these changes in obese skeletal muscle are an increase in fatty acid oxidation and, subsequently,

reductions in total DAG and total ceramides contents, as well as improved insulin sensitivity (9).

In the current study, obese participants displayed a trend ($P = 0.12$) toward an increase in the capacity of mitochondria to oxidize fatty acids. Previously, Bandyopadhyay et al. (1) showed that the capacity of mitochondria to oxidize fatty acids in obese type 2 diabetics (BMI = 36.93 kg/m²) was actually increased. Together, these studies suggest that a “reverse continuum” may exist in mitochondrial oxidation with obesity; that is, although whole muscle fatty acid oxidation may decrease with increasing BMI as a result of reductions in mitochondrial content, the capacity of the remaining mitochondria to oxidize fatty acids might actually compensate by increasing oxidation in an undetermined way to counteract this effect. Interestingly, the BMI of our obese participants was almost identical to that of Bandyopadhyay et al. (1), suggesting that the continuum might be based on insulin sensitivity, and not BMI. Importantly, it remains to be determined whether the diabetic increase in mitochondrial capacity to oxidize fatty acids is a beneficial response or, in combination with a decrease in electron transport chain capacity, will lead to increases in lipid peroxide species and, subsequently, oxidative damage that could exacerbate the diabetic phenotype (13, 17, 33).

We originally hypothesized that mitochondria from obese individuals would display an impaired ability to oxidize fatty acids. It has been suggested that the SS mitochondria from obese individuals may display a disproportionate impairment in fatty acid oxidation, since a greater reduction in the ratio of electron transport chain activity to mitochondrial DNA was observed in this mitochondrial fraction (31). The SS fraction represents only ~25% of the total mitochondria in skeletal muscle (12). In the current study we pooled the SS mitochondria with the IMF mitochondria to ensure that adequate protein was recovered for our functional assays. Although this approach limited our ability to detect impairments in the SS mitochondria, in the previously mentioned study a significant reduction in the ratio of electron transport chain activity to mitochondrial DNA was also observed in IMF mitochondria and at the whole muscle level (31). Although this suggested dysfunction in all mitochondria, the present data suggest that mitochondrial dysfunction in fatty acid oxidation is not required for reductions in whole muscle fatty acid oxidation. It should be noted that only a small fraction of mitochondria were recovered during the isolation procedures (~20%), and therefore it is possible that a dysfunction is present in the mitochondria not recovered. Since the various subcellular mitochondria were pooled in the current study, future research should focus on examining the potential differences in the SS and IMF mitochondria of obese individuals.

In conclusion, we have shown that obesity does not alter the ability of skeletal muscle mitochondria to oxidize fatty acids. We observed that obesity-related reductions in skeletal muscle fatty acid oxidation are attributable to reductions in mitochondrial content and not to intrinsic alterations or dysfunction within the mitochondria. We further showed that the content of FAT/CD36 on mitochondria is not different in obese muscle. However, FAT/CD36 significantly predicts the ability of mitochondria to oxidize LCFA independently of BMI status.

ACKNOWLEDGMENTS

We acknowledge the contributions of the surgeons, Drs. Lightheart, Loopstra, and Sibley.

GRANTS

This research was funded by the National Sciences and Engineering Research Council (NSERC) of Canada (to A. Bonen, D. J. Dyck, and L. L. Spriet) and by the Canadian Institutes of Health Research (to A. Bonen, D. J. Dyck, G. J. F. Heigenhauser, and L. L. Spriet). A. Bonen is also a recipient of a Canada Research Chair in Metabolism and Health, and G. Holloway is a recipient of an NSERC Canadian Graduate Scholarship.

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