

Regulation of skeletal muscle mitochondrial fatty acid metabolism in lean and obese individuals^{1–4}

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ABSTRACT

A reduction in fatty acid (FA) oxidation has been associated with lipid accumulation and insulin resistance in skeletal muscle of obese individuals. Numerous reports suggest that the reduction in FA oxidation may result from intrinsic mitochondrial defects, although little direct evidence has been offered to support this conclusion. This brief review summarizes recent work from our laboratory that reexamined whether this decrease in skeletal muscle FA oxidation with obesity was attributable to a dysfunction in FA oxidation within mitochondria or simply to a reduction in muscle mitochondrial content. Whole-muscle mitochondrial content and FA oxidation was reduced in the obese, but there was no decrease in the ability of isolated mitochondria to oxidize FA. The mitochondrial content of the transport protein, FA translocase (FAT/CD36), did not differ between lean and obese women but was correlated with mitochondrial FA oxidation. It was concluded that the reduced FA oxidation in obesity is attributable to decreased muscle mitochondrial content and not intrinsic defects in mitochondrial FA oxidation, and that mitochondrial FAT/CD36 is involved in regulating FA oxidation in human skeletal muscle. The reduced skeletal muscle mitochondrial content with obesity may result from impaired mitochondrial biogenesis. However, this did not result from decreased protein contents of various transcription factors, because peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1 α), PGC1 β , peroxisome proliferator-activated receptor α (PPAR α), and mitochondrial transcription factor A (TFAM) were not reduced with obesity. In contrast, it appears that obesity is associated with altered regulation of cofactors (PGC1 α and PGC1 β) and their downstream transcription factors (PPAR α , PPAR δ/β , and TFAM), because relations among these variables were present in muscle from lean individuals but not from obese individuals. These findings imply that obese individuals would benefit from interventions that increase the skeletal muscle mitochondrial content and the potential for oxidizing FAs. *Am J Clin Nutr* 2009;89(suppl):455S–62S.

INTRODUCTION

Skeletal muscle metabolism at rest and during exercise represents a balance between carbohydrate and fatty acid (FA) oxidation. Detailed regulatory mechanisms determine the relative utilization of these 2 substrates. The regulatory sites that control carbohydrate metabolism have been identified and examined in skeletal muscle over the past several decades, but it was believed that fewer sites of regulation exist for FA metabolism. Recently, however, it has become apparent that there are also multiple regulatory sites controlling FA metabolism in skeletal muscle (1, 2). Specifically, skeletal muscle long-chain FA oxidation involves the following: 1) lipolysis

and long-chain FA release from the adipose tissue, 2) delivery of long-chain FA to the skeletal muscle and possibly dissociation from albumin, 3) transport across the plasma membrane, 4) lipolysis of intramuscular triacylglycerol (IMTG), 5) activation of long-chain FA with the addition of a coenzyme A thioester and transport through the cytosol to the mitochondria, and 6) transport across the mitochondrial membranes and ultimately oxidation.

This brief review focuses on recent literature from our laboratory that has expanded the understanding of the regulation of FA metabolism in skeletal muscle. Particular emphasis is placed on the transport of FA across the mitochondrial membranes for ultimate oxidation and the effects of obesity on mitochondrial function in human skeletal muscle.

FA TRANSPORT ACROSS THE MUSCLE AND MITOCHONDRIAL MEMBRANES

For many years, it was believed that the transport of FA across the plasma membrane in skeletal muscle occurred only via passive diffusion. However, in recent years, a substantial body of literature has emerged indicating that FA uptake into skeletal muscle cells also occurs via a protein-mediated process [for review see Bonen et al (3)]. A number of proteins have been shown to facilitate the uptake of FA into cells, including FA translocase, the homolog of human CD36 (FAT/CD36) (4); a family of FA transport proteins (FATP1-6) (5); and plasma membrane-associated FA binding protein (FABPpm) (6). Considerable evidence has accumulated to indicate that FAT/CD36 and FABPpm are present on the muscle membrane and are important in regulating the uptake of FA into skeletal muscle. Little is currently known about the importance of FATP1-6.

In skeletal muscle, the long-chain FA transport proteins are located in several subcellular domains (7), and recently FAT/

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CD36 was found on rat and human muscle mitochondria (8–10). At this location, FAT/CD36, along with carnitine palmitoyl-transferase I (CPTI), contributes to regulating mitochondrial FA transport and oxidation in skeletal muscle at rest, since blocking mitochondrial FAT/CD36 has been shown to almost completely inhibit FA oxidation (8–10). During exercise, mitochondrial FA oxidation is up-regulated and accompanied by an increase in mitochondrial FAT/CD36 content (8, 10). The exercise-induced increase in FA oxidation is also completely inhibited when FAT/CD36 is pharmacologically blocked (8, 10). In other work, an increase in mitochondrial FAT/CD36 has been associated with exercise-induced weight loss and an improvement in whole-body FA oxidation (11). Thus, it appears that changes in mitochondrial FAT/CD36 are associated with changes in mitochondrial FA oxidation. These studies do not negate the well-known role of CPTI activity in mitochondrial FA oxidation. However, it appears that FAT/CD36 works in conjunction with CPTI, because together these 2 proteins predict rates of mitochondrial FA oxidation (9), and FAT/CD36 and CPTI are colocalized in mitochondria (8, 11).

FABPpm is another well-recognized plasma membrane FA transport protein that has also been found on mitochondria (12, 13). However, the role of FABPpm with respect to mitochondrial FA oxidation remains unknown. The current thinking is that the main function of FABPpm is to transport reducing equivalents into the mitochondria, because the protein is identical to mitochondrial aspartate aminotransferase, a key enzyme in the malate-aspartate shuttle system (12, 13).

OBESITY AND MITOCHONDRIAL FUNCTION

Obese individuals have increases in IMTG storage, and this has been associated with insulin resistance (14–16). However, it appears likely that increases in IMTG levels simply represent a dysfunction in lipid metabolism (17) and that the more reactive lipid species, diacylglycerol (DAG), and ceramides may be important for the development of insulin resistance (18, 19). It has been shown in skeletal muscle from nondiabetic lean and obese women that the transport of FA into giant sarcolemmal vesicles was increased 4-fold with obesity and coincided with an increase in the plasma membrane content of FAT/CD36 (20). There were no increases in the total muscle expression of FAT/CD36 or FABPpm with obesity, suggesting that a permanent redistribution of FAT/CD36 to the plasma membrane occurred (20). This also provided evidence for a potential mechanism for elevating intramuscular lipid species and inducing insulin resistance.

It has also been suggested that reductions in FA oxidation in obese individuals contribute to IMTG accumulation (21–24). On the basis of reductions in whole-muscle CPTI activity in skeletal muscle from obese individuals, it has been proposed that FA transport into the mitochondria is diminished. This may account for the observed reduction in whole-muscle FA oxidation (22). It is possible that this reflects a decrease in mitochondrial content, although other proteins may also be involved.

Are mitochondria from skeletal muscle from obese individuals dysfunctional?

The net accumulation of lipids in obese skeletal muscle may result from a combination of increased whole-muscle FA uptake and/or decreased whole-muscle oxidation. Although the concept

of impaired FA oxidation as a mechanism to increase intramuscular lipid species has gained attention in recent years, the exact mechanisms remain unknown. However, 3 plausible explanations exist: 1) mitochondrial content is decreased, 2) there is a dysfunction in FA oxidation within mitochondria, or 3) both of these conditions occur. Previous work in skeletal muscle indicated a reduction in mitochondrial content with obesity along with a concomitant decrease in FA oxidation (21), suggesting that mitochondria from obese individuals contain an inherent dysfunction to oxidize FA. In some studies the ratios of electron transport chain capacity to mitochondrial DNA (mtDNA) and size have been used to infer mitochondrial dysfunction for FA oxidation (21, 23). It has also been suggested that the relative proportion of FAs that are completely oxidized is decreased with obesity (25). Because β oxidation is regulated by substrate and product concentrations, an imbalance among β oxidation, the tricarboxylic acid cycle, and electron transport chain activities would increase the amount of acyl-CoAs located within the cell. This may have deleterious effects and could induce oxidative damage in mitochondria and induce apoptosis, although this remains speculative. In addition, more FAT/CD36 is located on the plasma membrane without total expression being altered in the obese state. It is therefore possible that less would be available for mitochondria, potentially compromising oxidation and explaining the previously observed “mitochondrial dysfunction” in the obese state.

It should be mentioned that not all studies have reported decreased mitochondrial function with obesity. A recent study that measured the ability of mitochondria to oxidize FA directly in skeletal muscle from obese patients with type 2 diabetes reported the controversial finding of increased FA oxidation (26). However, this finding may not be directly applicable to the obese nondiabetic population and the underlying mechanism remains unknown.

Skeletal muscle mitochondria are not dysfunctional in obesity

Recent work in our laboratory focused on examination of whether obesity-related decreases in skeletal muscle lipid oxidation were attributable to a reduction in mitochondrial content or an intrinsic defect in mitochondria, and whether there were reductions in the content of mitochondrial FA transport proteins. We hypothesized that nondiabetic obesity would be associated with decreases in skeletal muscle mitochondrial content, as well as decreases in the ability of mitochondria to oxidize FA. In addition, we speculated that impairments in mitochondrial oxidation would be associated with reductions in the content of mitochondrial FAT/CD36 and FABPpm.

In age-matched lean [body mass index (BMI; in kg/m^2): 23.3 ± 0.7] and obese (BMI: 37.6 ± 2.2) individuals, citrate synthase (CS) and β -hydroxyacyl-CoA dehydrogenase (β -HAD) activities, common markers of total muscle mitochondrial volume and the capacity for FA oxidation, were decreased in muscle from obese individuals (27). This suggested that a decrease in mitochondrial volume was present and appeared to account for the observed reductions in FA oxidation, because isolated mitochondrial palmitate oxidation was not altered in muscle from obese subjects (Figure 1). In addition, mitochondrial FAT/CD36 and FABPpm content did not differ between



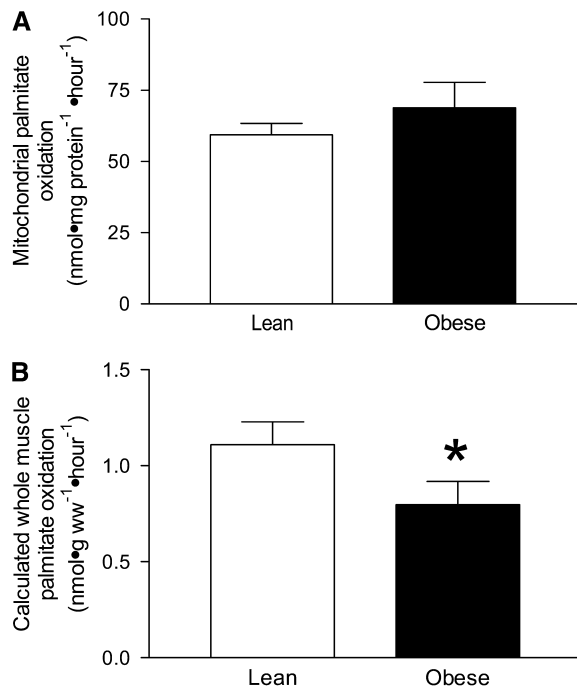


FIGURE 1. Effects of obesity on the ability of skeletal muscle to oxidize palmitate. Values are means \pm SEs ($n = 9$). A: Isolated mitochondrial palmitate oxidation (expressed in $\text{nmol} \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$). B: Calculated whole-muscle palmitate oxidation (expressed in $\text{nmol} \cdot \text{g wet weight (ww)}^{-1} \cdot \text{h}^{-1}$). *Significantly different from lean, $P < 0.05$.

lean and obese individuals when normalized to mitochondrial protein content (Figure 2). In summary, the mitochondria isolated from obese individuals were not dysfunctional. There simply was less mitochondrial volume and lower rates of whole-muscle FA oxidation.

An interesting finding was that, although mitochondrial palmitate oxidation rates in lean and obese individuals did not correlate with BMI, the rates strongly correlated with mitochondrial FAT/CD36 content (Figure 3). On the other hand, FABPpm did not correlate with mitochondrial oxidation, suggesting that it is not rate limiting for FA mitochondrial transport. Although this requires additional investigation, the main function of FABPpm appears to be related to transporting reducing equivalents into the mitochondria (12, 13).

Data from a previous study suggested that mitochondrial FA oxidation impairment may only be manifested above a certain threshold (ie, BMI of 40–50), although mitochondrial function was not directly measured in that study (24). In our study, the BMI in the obese group was relatively high (\bar{x} : 37.6) but there was no mitochondrial dysfunction, so it is possible that the subjects were below this threshold. The obese group displayed reductions in whole-muscle CS and β -HAD activities and reduced whole-muscle FA oxidation rates, indicating that lipid oxidation was compromised. Thus, the current data again suggest that the reductions in FA 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 overall mitochondrial biogenesis, without any “remodeling” of existing mitochondria. Indeed, previous work showed that regular exercise increased CS, β -HAD, and

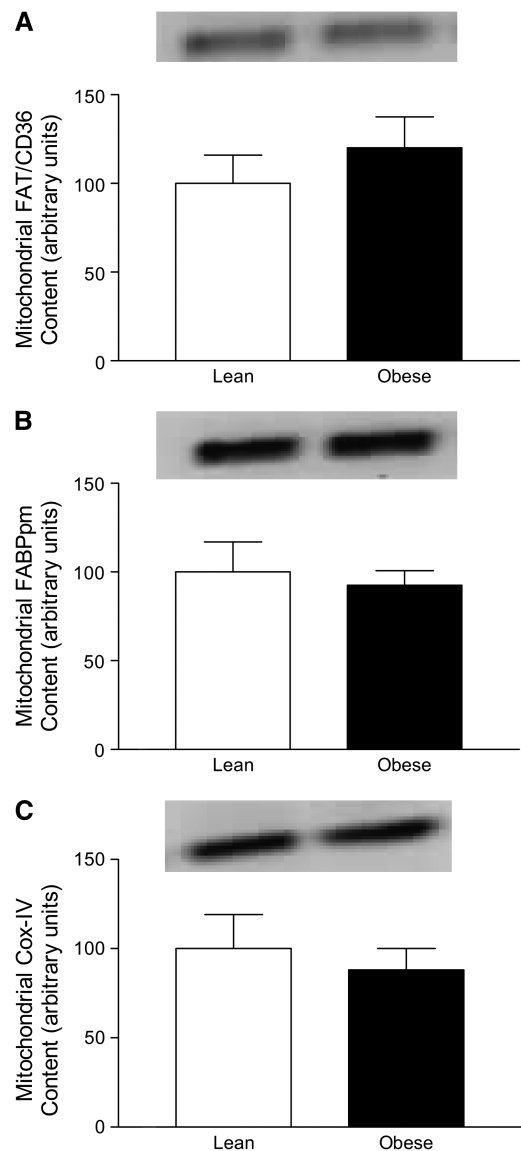


FIGURE 2. Representative Western blots displaying the effects of obesity on the expression of mitochondrial proteins. Values are means \pm SEs, expressed in arbitrary units ($n = 9$). Data are normalized to mitochondrial protein content. A: Mitochondrial FA translocase (FAT/CD36) content. B: Mitochondrial plasma membrane-associated FA binding protein (FABPpm) content. C: Mitochondrial Cox-IV content.

CPTI activities and therefore mitochondria volume in an obese population (28). Associated with these changes were increases in FA oxidation, reductions in total DAG and total ceramide contents, and improved insulin sensitivity (28).

We originally hypothesized that mitochondria from obese individuals would display an impaired ability to oxidize FA. It has been suggested that subsarcolemmal (SS) mitochondria from obese individuals may display a disproportionate impairment in FA oxidation, because a greater reduction in the ratio of electron transport chain activity to mtDNA was observed in this mitochondrial fraction (23). The SS fraction represents only $\approx 25\%$ of the total mitochondria in skeletal muscle (29). In our study we pooled the SS mitochondria with the intermyofibrillar (IMF) mitochondria to ensure that adequate protein was recovered for our functional assays (27). Although this approach limited our



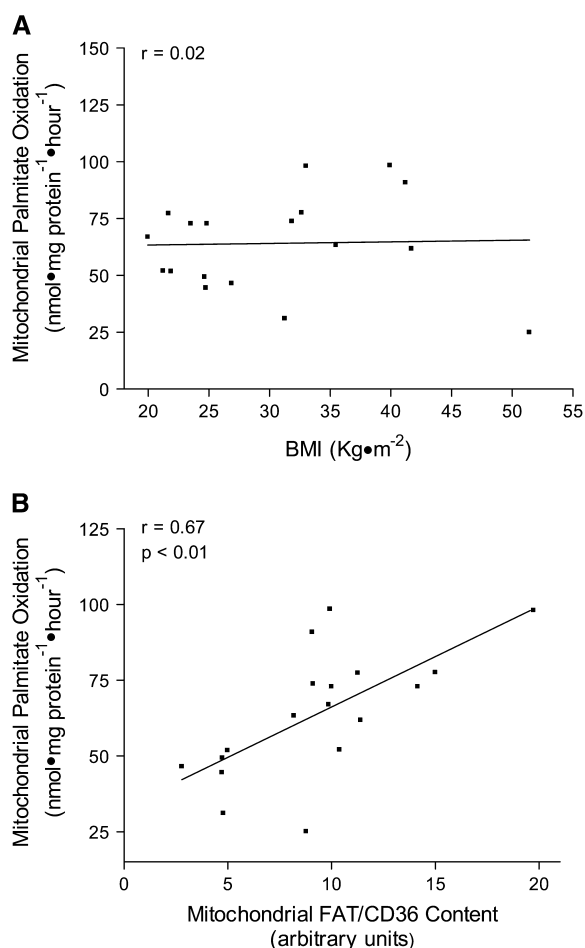


FIGURE 3. Pearson correlations ($n = 18$; 9 lean and 9 obese individuals). A: Correlation between mitochondrial palmitate oxidation and BMI. B: Correlation between mitochondrial palmitate oxidation and the mitochondrial content of FA translocase (FAT/CD36).

ability to detect impairments in the SS mitochondria, in the previously mentioned study (23) a significant reduction in the ratio of electron transport chain activity to mtDNA was also observed in IMF mitochondria and at the whole-muscle level. Although this suggested dysfunction in all mitochondria, the current data suggest that mitochondrial dysfunction in FA oxidation is not required for reductions in whole-muscle FA oxidation. It should be noted that only a small fraction of mitochondria were recovered during the isolation procedures ($\approx 20\%$), and therefore it is possible that a metabolic dysfunction was present in the mitochondria that were not recovered. Because the various subcellular mitochondria were pooled in the current study, future research will need to examine the potential differences in the SS and IMF mitochondria of obese individuals.

In the study by our laboratory, obese participants actually displayed a trend ($P = 0.12$) toward an increase in the capacity of mitochondria to oxidize FA (27). Previously, Bandyopadhyay et al (26) reported that the mitochondrial capacity to oxidize FA in obese patients with type 2 diabetes (BMI: 36.9) was actually increased. Together, these studies suggest that a “reverse continuum” may exist in mitochondrial oxidation with obesity. That is, whereas whole-muscle FA oxidation may decrease with increasing BMI as a result of reductions in mitochondrial content,

the capacity of the remaining mitochondria to oxidize FA may actually compensate by increasing oxidation in an undetermined manner to counteract this effect. Although a clear increase in mitochondrial FA oxidation in muscle from obese and diabetic populations has not been clearly established, there is no suggestion of a decrease.

Other evidence that mitochondrial function is normal in obesity

Two additional recent studies that directly measured the oxygen consumption of mitochondria isolated from lean and obese diabetic individuals concluded that there was no mitochondrial dysfunction in skeletal muscle from obese individuals. Boushel et al (30) demonstrated that whole-muscle FA oxidation was impaired with obesity. However, when the data were normalized to CS activity or mtDNA, this finding was lost, again indicating that obese diabetic individuals simply have less mitochondria in their skeletal muscle. Of importance, Boushel et al (30) measured oxygen consumption instead of radioactively labeled substrate consumption, which enables mitochondrial respiration to be measured in state 4 (quiescent) and state 3 (ADP-stimulated) conditions. This provided an indication of the metabolic responsiveness of the mitochondria. These measures provided additional evidence that mitochondria do not have an intrinsic dysfunction, because the respiratory control ratio (RCR, state 3/state 4) was similar between patients with diabetes and lean controls (30). Of interest, in another laboratory, Mogensen et al (31) reported a reduction in oxygen consumption in mitochondria isolated from individuals with type 2 diabetes compared with obese individuals, and associated with this was a reduction in the RCR value. However, these results were only observed when pyruvate and malate were used as substrates. When palmitoylcarnitine was used (the product of CPTI, thereby removing any differences in this enzyme from compromising the interpretation), no dysfunction was observed in oxygen consumption or in the RCR values, again implying normal functioning mitochondria (31).

It appears that at least 4 publications have now demonstrated that mitochondria isolated from obese (nondiabetic and diabetic) individuals do not contain an inherent dysfunction (26, 27, 30, 31). However, we must remember that the mitochondrial isolation techniques used in 3 of these studies may alter the morphology of mitochondria and that only $\approx 20\%$ of the total mitochondria are recovered (26, 27, 31). Therefore, it is possible that a mitochondrial dysfunction affecting FA oxidation may be present in vivo, and newer methodologies will be needed to fully confirm the data supporting the findings that mitochondrial function in the skeletal muscle of obese individuals is normal. However, Boushel et al (30) used a permeabilized-fiber technique that avoids some of these problems and reported similar findings.

WHY IS MITOCHONDRIAL CONTENT REDUCED IN OBESITY?

The reduced ability of skeletal muscle to oxidize FA with obesity appears to be a function of reduced mitochondrial content. This may result from lower physical activity levels in obese individuals and ultimately from reduced or impaired mitochondrial biogenesis. It is possible that decreases in the general transcription factors that regulate mitochondrial biogenesis are

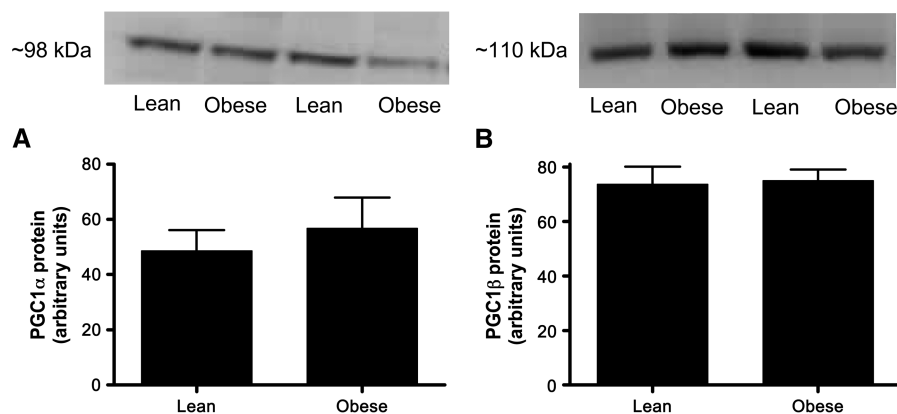


FIGURE 4. The impact of obesity on the protein content of (A) peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1 α) and (B) PGC1 β . Values are expressed in arbitrary units, normalized to Ponceau staining ($n = 9$ for lean and obese).

responsible for the obesity phenotype as a result of a genetic predisposition and/or a sedentary lifestyle.

The coordination between mtDNA and nuclear DNA transcription is a key component of mitochondrial biogenesis. Peroxisome proliferator activated receptor γ coactivator 1 α (PGC1 α) has been considered the “master regulator” coordinating these events (32),

because overexpression of PGC1 α results in increases in both nuclear and mitochondrial proteins (33). It has also become apparent that PGC1 α coordinates these events by activating downstream transcription factors, including nuclear respiratory factor 1 (NRF-1) (34) and NRF-2 (35). Peroxisome proliferator activated receptor γ coactivator 1 β (PGC1 β) has not been studied to the

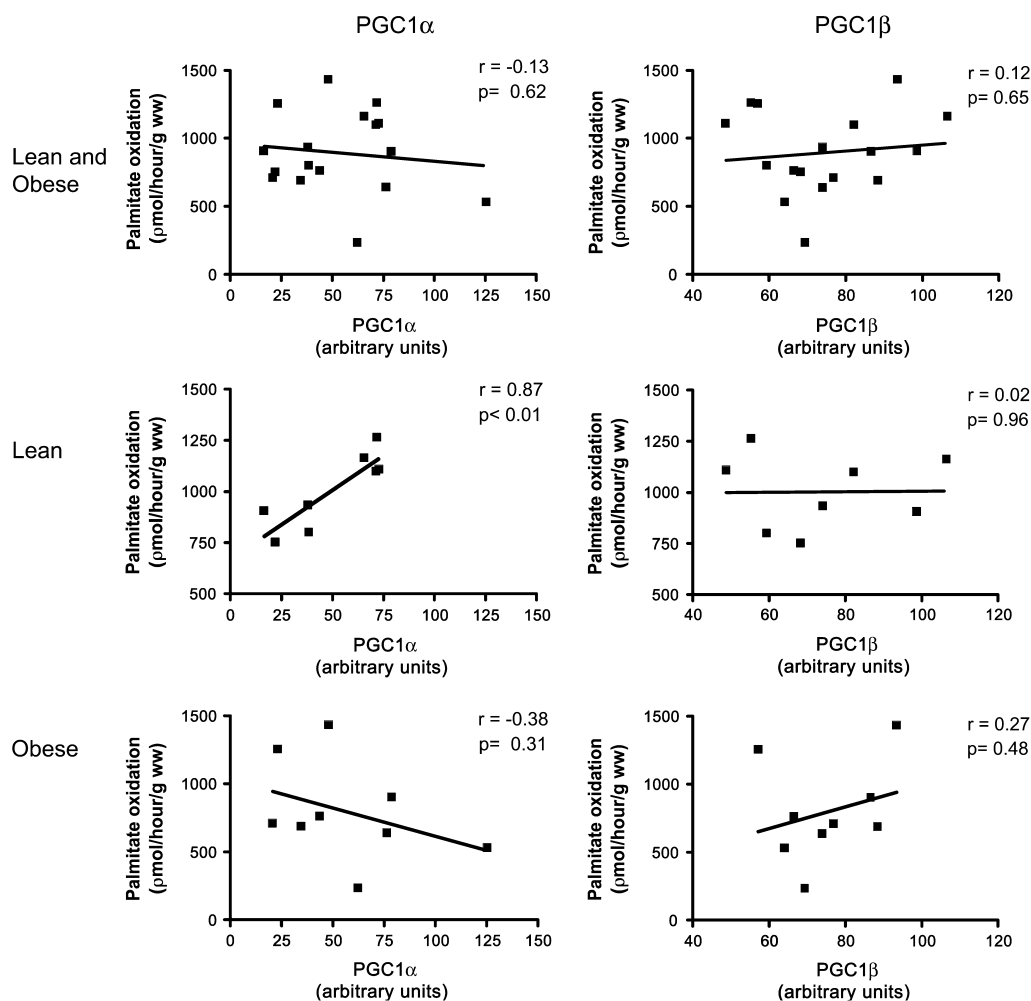


FIGURE 5. Relations among peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1 α), PGC1 β , and palmitate oxidation in lean and obese individuals. Values for palmitate oxidation are $\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ wet weight (ww), and Western blots are expressed in arbitrary units, normalized to Ponceau staining ($n = 8$ for lean, $n = 9$ for obese).

same extent as PGC1 α . However, it has also been implicated in the proliferation of mitochondria in skeletal muscle (36) and its overexpression prevented diet-induced insulin resistance (37).

Reductions in PGC1 α and PGC1 β have been linked to the presence of skeletal muscle insulin resistance because the mRNAs of these proteins were decreased with diabetes (38, 39), and single nucleotide polymorphisms in PGC1 α and PGC1 β (40, 41) have been reported in obesity and diabetes. However, the picture is not totally clear because reductions in PGC1 α and PGC1 β expression are not always present with insulin resistance (42), and research in humans has been limited mainly to mRNA data and not protein measurements. The role of PGC1 α protein in obesity-related insulin resistance in rodent muscle is also unclear, because ablating PGC1 α unexpectedly improved glucose tolerance and insulin sensitivity in mice consuming a high-fat diet (43), and overexpressing PGC1 α in mice unexpectedly induced insulin resistance (44). This may

be attributable to a massive PGC1 α up-regulation that increases the accumulation of intramuscular lipids to a greater extent than the increased capacity for their oxidation (45).

Given the prominent roles of PGC1 α and PGC1 β and their downstream targets [eg, peroxisome proliferator-activated receptors (PPARs) and mitochondrial transcription factor A (TFAM)] in regulating mitochondrial biogenesis and the capacity to oxidize FA in muscle, we sought to determine 1) whether PGC1 α and PGC1 β protein contents are reduced in the skeletal muscle of obese individuals, and 2) whether the protein content of other transcription factors located downstream of PGC1 α and β (PPAR γ , PPAR δ/β , PPAR α , and TFAM) are also reduced in the skeletal muscle of obese individuals.

Of interest, obesity did not alter the skeletal muscle protein content of PGC1 α and PGC1 β (Figure 4), PPAR α , or TFAM (46). In contrast, there was a significant 22% increase in PPAR γ and a nonsignificant 31% increase ($P = 0.13$) in PPAR δ/β .

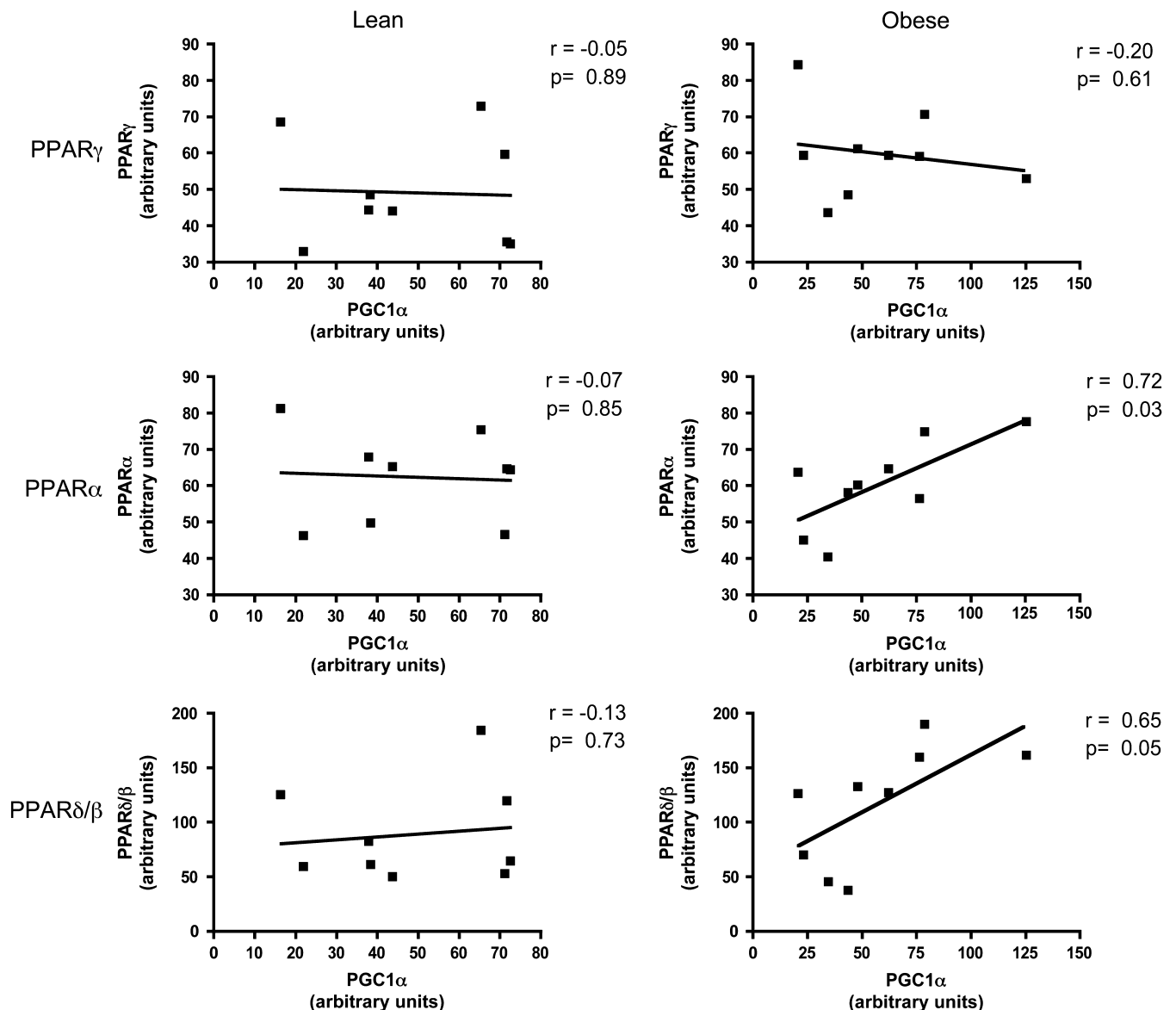


FIGURE 6. Relations between peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1 α) and peroxisome proliferator activated receptors (PPARs) in lean and obese individuals. Values are expressed in arbitrary units, normalized to Ponceau staining ($n = 9$ for lean and obese).



PGC1 α was significantly correlated with palmitate oxidation in lean participants, but this relation was lost in obese individuals (Figure 5). In contrast, PGC1 β protein content did not correlate with palmitate oxidation in lean and obese individuals.

PGC1 α protein content did not correlate with PGC1 β in lean and obese individuals. PGC1 α protein expression did not correlate with PPAR γ , PPAR α , or PPAR δ/β in lean participants, but did significantly correlate with both PPAR α and PPAR δ/β in obese individuals (Figure 6).

The novel findings of this study were that obesity did not decrease the protein content of PGC1 α , PGC1 β , PPAR α , PPAR δ/β , or TFAM, and that compensatory increases in PPAR γ occurred with obesity. In addition, PGC1 α correlated with palmitate oxidation in the lean individuals only, and PGC1 β correlated with PPAR γ and PPAR δ/β in lean individuals only. The latter 2 findings suggest that the coordinated regulation of selected transcription factors involved in mitochondrial biogenesis was lost with obesity. The concept that reduced PGC1 α expression is the explanation for the observed reduction in FA oxidation with insulin resistance associated with obesity is not supported by these recent data. The PGC1 α protein was not reduced in the skeletal muscle of obese individuals, whereas the muscle mitochondrial content was markedly reduced (27). Although these findings contradict much of the literature regarding PGC1 α mRNA data, mRNA changes are only weakly associated with changes in PGC1 α protein (47) and there is only a modest relation between skeletal muscle insulin sensitivity and PGC1 α mRNA (48). In support of our findings, a recent publication reported no change in either PGC1 α mRNA or total protein with obesity (49). These observations underscore the complexity of mechanisms that regulate protein expression, including the influence of phosphorylation on increasing PGC1 α mRNA stability (50) or activation (51), the ability of other proteins to suppress PGC1 α activity (52), and the importance of nuclear import (53). Puigserver and colleagues (54) have proposed a 3-step model of activation that includes PGC1 α interacting and docking with transcription factors, undergoing a conformational change that enables binding of additional cofactors, and subsequent induction of transcription. Clearly this is a complex process, and one should use caution when interpreting data that provide insight into only one of these 3 steps. However, the current data suggest that the reduction in mitochondrial content associated with obesity does not involve diminished PGC1 α protein, and may be located downstream of PGC1 α and involve activation or repression.

CONCLUSIONS

In conclusion, recent literature demonstrates that obesity does not alter the ability of skeletal muscle mitochondria to oxidize FA. In our laboratory, we observed that obesity-related reductions in skeletal muscle FA oxidation are attributable to reductions in mitochondrial content and not to intrinsic alterations, or dysfunction, within the mitochondria. We further demonstrated that the content of FAT/CD36 on mitochondria is not different in obese muscle. However, FAT/CD36 significantly predicted the ability of mitochondria to oxidize FA, independently of BMI status. We also revealed that obesity is not associated with reductions in the protein contents of PGC1 α or PGC1 β in skeletal muscle, and that the PPARs (γ and potentially δ/β) may be up-

regulated in response to the decreases in lipid oxidation associated with obesity. The relation between PGC1 α and palmitate oxidation was also lost with obesity, as were the relations among PGC1 β , PPAR γ , and PPAR δ/β . These data suggest that the regulation of PGC1 α and PGC1 β , and not total protein, is altered with obesity. These findings imply that obese individuals would benefit from interventions that increase the skeletal muscle mitochondrial content and the potential for oxidizing FAs. (Other articles in this supplement to the Journal include references 55 and 56.)

All authors contributed equally to this work and the writing of the paper. The authors stated that they have no conflicts of interest with respect to the published work contained in this paper.

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