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Metabolic challenges reveal impaired fatty acid metabolism and translocation of FAT/CD36 but not FABPpm in obese Zucker rat muscle

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Han X-X, Chabowski A, Tandon NN, Calles-Escandon J, Glatz JF, Luiken JJ, Bonen A. Metabolic challenges reveal impaired fatty acid metabolism and translocation of FAT/CD36 but not FABPpm in obese Zucker rat muscle. *Am J Physiol Endocrinol Metab* 293: E566–E575, 2007. First published May 22, 2007; doi:10.1152/ajpendo.00106.2007.—We examined, in muscle of lean and obese Zucker rats, basal, insulin-induced, and contraction-induced fatty acid transporter translocation and fatty acid uptake, esterification, and oxidation. In lean rats, insulin and contraction induced the translocation of the fatty acid transporter FAT/CD36 (43 and 41%, respectively) and plasma membrane-associated fatty acid binding protein (FABPpm; 19 and 60%) and increased fatty acid uptake (63 and 40%, respectively). Insulin and contraction increased lean muscle palmitate esterification and oxidation 72 and 61%, respectively. In obese rat muscle, basal levels of sarcolemmal FAT/CD36 (+33%) and FABPpm (+14%) and fatty acid uptake (+30%) and esterification (+32%) were increased, whereas fatty acid oxidation was reduced (−28%). Insulin stimulation of obese rat muscle increased plasmalemmal FABPpm (+15%) but not plasmalemmal FAT/CD36, blunted fatty acid uptake and esterification, and failed to reduce fatty acid oxidation. In contracting obese rat muscle, the increases in fatty acid uptake and esterification and FABPpm translocation were normal, but FAT/CD36 translocation was impaired and fatty acid oxidation was blunted. There was no relationship between plasmalemmal fatty acid transporters and palmitate partitioning. In conclusion, fatty acid metabolism is impaired at several levels in muscles of obese Zucker rats; specifically, they are *1*) insulin resistant with respect to FAT/CD36 translocation and fatty acid uptake, esterification, and oxidation and *2*) contraction resistant with respect to fatty acid oxidation and FAT/CD36 translocation, but, conversely, *3*) obese muscles are neither insulin nor contraction resistant at the level of FABPpm. Finally, *4*) there is no evidence that plasmalemmal fatty acid transporters contribute to the channeling of fatty acids to specific metabolic destinations within the muscle.

insulin; contraction; hindlimb perfusion; palmitate; oxidation; esterification

THERE IS A STRONG ASSOCIATION between obesity and the development of insulin resistance. Based on studies in skeletal muscle of obese animals and humans (27–30, 32), it appears that a possible underlying mechanism may be a reduction in fatty acid oxidation (27–30, 32), which may lead to an accumulation of lipid products that interfere with insulin signaling (cf. Ref. 47). Accumulation of intramuscular lipids can also occur when rates of fatty acid transport into muscle exceed the capacity for their oxidation (9, 37).

Fatty acid transport into skeletal muscle occurs primarily via a protein-mediated mechanism, involving the fatty acid transporters fatty acid translocase (FAT/CD36), plasma membrane-associated fatty acid binding protein (FABPpm), and a family of fatty acid transporters, FATP1–6 (for review, see Ref. 5). Insulin-induces the translocation of FAT/CD36, whereas muscle contraction and contraction-mimetic stimuli induce the translocation of both FAT/CD36 and FABPpm (cf. Ref. 5). Recently, our group (7) showed that the ablation of skeletal muscle FAT/CD36 almost fully blunts *1*) insulin-stimulated palmitate uptake and esterification and *2*) 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR)-stimulated palmitate uptake and oxidation, despite some compensatory upregulation of FATP1 and FATP4. In addition, it appears that FABPpm and FAT/CD36 interact with each other (39, 41). However, it seems unlikely that these fatty acid transporters channel fatty acids to particular metabolic fates in muscle, as has been proposed on the basis of studies in cell lines (2, 24, 45, 59), since these preparations lack normal physiological regulation. Moreover, in mammalian muscle, it is presumably the intracellular insulin- and contraction-mediated signals, downstream of the plasmalemmal fatty acid transporters, that regulate whether fatty acids are primarily esterified or oxidized. Altogether, FAT/CD36 and FABPpm would seem to be the key components contributing to the regulating of fatty acid transport into skeletal muscle, whereas beyond these transporters, intracellular signals direct the metabolic fate of fatty acids.

The obese Zucker rat is a well-established animal model of human skeletal muscle insulin resistance. In skeletal muscle of these obese animals and obese humans, the insulin-induced GLUT4 translocation is impaired, whereas contraction-stimulated GLUT4 translocation is normal (10–12, 21, 31, 33, 46). There are also parallel disturbances in skeletal muscle fatty acid transport and metabolism in obese Zucker rats and in obese humans, including an increased plasmalemmal FAT/CD36 content (9, 37), an increased rate of fatty acid transport (9, 37), and an accumulation of intramuscular triacylglycerols (9, 52), although there is disagreement whether fatty acid oxidation rates are altered (17, 27, 42, 52). In contrast to a large

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body of work examining insulin- and contraction-stimulated glucose transport and GLUT4 translocation in obese Zucker rats, disturbances in the subcellular localization of fatty acid transporters and in fatty acid uptake and metabolism in this animal model of insulin resistance have only been examined under basal conditions. It is not known whether in obese Zucker rat muscle *1*) insulin and/or muscle contraction induce a further increase in the plasmalemmal content of FAT/CD36, beyond its preexisting increased basal plasmalemmal content, and *2*) whether insulin and/or muscle contraction induce the translocation of FABPpm. In addition, it is also unknown *3*) to what extent fatty acid esterification and oxidation are altered by insulin and/or muscle contraction in obese Zucker rat muscle. Examination of all these parameters during metabolic challenges may reveal *4*) whether plasmalemmal FAT/CD36 and FABPpm are associated with channeling fatty acids to esterification or oxidation.

We hypothesized that insulin-induced fatty acid transporter translocation is impaired in insulin-resistant muscles of obese Zucker rats, whereas the contraction-induced translocation of fatty acid transporters is not impaired in these muscles. These different responses to insulin and contraction may also contribute to altered rates of fatty acid uptake and metabolism (esterification and/or oxidation) in muscles of obese Zucker rats. Therefore, we examined whether fatty acid uptake, esterification, and oxidation and fatty acid transporter translocation differ in skeletal muscles of lean and obese Zucker rats *1*) under basal conditions, *2*) during insulin stimulation, and *3*) during muscle contraction. Because of the differential responses observed, we were also able to examine *4*) whether fatty acid transporters appear to channel fatty acids to different metabolic fates within muscle.

METHODS

Materials and Supplies

[1-^{14}C]palmitate was obtained from GE Healthcare (Baie D'urfe, QC, Canada). Silica gel plates (250 mm) were obtained from Mandel Scientific (Guelph, ON, Canada). All other chemicals and reagents were obtained from Sigma-Aldrich (Oakville, ON, Canada). Glucose (Sigma-Aldrich, St. Louis, MO) and insulin (Linco, St. Charles, MO) were determined using commercially available procedures. Female lean (180 g) and obese (300 g) Zucker rats were purchased from Charles River (Charles River, Wilmington, MA) and housed in a room with 12:12-h light-dark reversed light cycles. Animals had free access to rat chow and water ad libitum. Ethical approval for this work was obtained from the committee on animal care at the University of Guelph.

Hindlimb Perfusion Experiments

Palmitate metabolism (esterification and oxidation) and the subcellular distribution of fatty acid transporters were examined in perfused hindlimb muscles during *1*) basal conditions, *2*) insulin stimulation, and *3*) muscle contraction in three groups of lean and obese Zucker rats. For these purposes, 15 lean rats and 15 obese Zucker rats were used for the metabolism studies ($n = 5$ lean and obese animals in each of the 3 treatments: basal, insulin, and contraction experiments). To determine the subcellular distribution of fatty acid transporters, we used an additional 15 lean rats and 15 obese Zucker rats ($n = 5$ lean and obese animals in each of the 3 treatments: basal, insulin, and contraction experiments). One week after arrival in the animal care facility, fed rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt) and surgically prepared for perfusion as we have done previously (23, 36, 38). During surgery, blood samples were obtained from the inferior vena cava and analyzed for glucose and insulin, as we have previously reported (37).

Hindlimb perfusion, under basal and insulin-stimulated conditions, as well as during muscle contraction, were performed as we have previously reported (23, 36, 38). Briefly, in all experiments, the hindquarter of rats were preperfused (gassed continuously with 95% O_2 and 5% of CO_2 at 37°C) with 0.1% BSA in Krebs-Henseleit buffer (pH 7.4) for 10 min, after which the outflow was discarded. Before the insulin experiments, insulin (20 mU/ml) was included during the 10-min preperfusion period. During the experimental period, muscles from the hindlimbs were perfused (recirculating mode) for 60 min at a flow rate of 18 ml/min, pH 7.4, with Krebs-Henseleit buffer containing 6 mM glucose, [1-^{14}C]palmitate (0.1 μCi/ml; 1 mM), and 4% BSA. Perfusion with insulin was conducted using a concentration of 20 mU/ml. For the muscle contraction studies, the sciatic nerve from the leg was exposed just before the 10-min preperfusion period. A few minutes before electrical stimulation was introduced, the knee joint was fixed with a steel pin beneath the tibiopatellar ligament and the Achilles tendon of the limb was connected to a calibrated force transducer. Muscle was adjusted to resting length immediately before and during the initial contractions to optimize production of muscle tension during muscle stimulation. Hindlimb muscles contracted isometrically throughout the perfusion period via electrical stimulation of the sciatic nerve (train delivery, 100 Hz/3 s at 10–20 V; train duration, 100 ms; pulse duration, 10 ms). Force production in lean and obese rats was recorded throughout using an eight-channel chart recorder. Muscle force production during the contraction experiments did not differ in lean and obese animals ($P > 0.05$, data not shown). In all experiments (basal, insulin, contraction) arterial and venous perfusate samples were collected every 5 min during the last 30 min of the 60-min perfusion period and kept in tightly sealed vials for later analysis of $^{14}CO_2$. At the point that the experiments were stopped (60 min), red gastrocnemius muscle was quickly harvested, rapidly frozen in liquid nitrogen, and stored at −80°C until analyzed for palmitate accumulation in the intramuscular triacylglycerol depot and for determining the subcellular location of FAT/CD36 and FABPpm.

Fatty Acid Metabolism

Palmitate incorporation into intramuscular triacylglycerol. Palmitate esterification was determined with thin-layer chromatography, as we have reported previously (7, 19, 38). Briefly, muscle (50 mg) was homogenized (Polytron; Kinematica, Brinkmann, Littau-Lucerne, Switzerland) in 2 ml of 1:1 chloroform-methanol on ice for 2 × 15 s at speed setting of 8, separated by a 15-s interval. Solvent solution was recovered by centrifugation at 6,000 *g* for 10 min at 4°C and transferred to a new tube. This solution was washed with 2 ml of H_2O, and the lipid-containing chloroform phase was separated from the aqueous phase with another centrifugation step. Thereafter, 500 μl of the chloroform phase were dried under nitrogen, and samples were reconstituted with 100 μl of 2:1 chloroform-methanol (vol/vol). Samples (50 μl) were then spotted onto 250-mm silica gel plates and resolved (60:40:3 heptane-isopropylether-acetic acid) for 50 min. Afterwards, the plate was air-dried and sprayed with chlorofluorescein dye (0.02% wt/vol in ethanol). The triacylglycerol lipid band was visualized under long-wave UV light against standards that were included with each separation. To determine the rate of palmitate incorporation into triacylglycerol depots, the silica gel powder of the individual band was carefully scraped off the plate and transferred into scintillation vials for determining the radioactive counts.

We also determined the intramuscular triacylglycerol concentration in resting muscle. For these purposes, muscle lipids were again separated with thin-layer chromatography. The resulting triacylglycerol bands were quantified against known standards that were included with the samples.

CO_2 production. In hindlimb perfusion studies, it is not possible to determine fatty acid oxidation rates in individual hindlimb muscles. Therefore, it was necessary to determine the rates of palmitate oxidation across the perfused rat hindquarter. Measurement of palmitate oxidation was performed using procedures that we have previously reported (38). Briefly, 0.5 ml of both arterial and venous perfusate samples acidified with 1 M H_2SO_4 (1 ml) in a tightly capped, sealed vial. Liberated $^{14}CO_2$ was captured in benzethonium hydroxide (0.5 ml) kept in a small Eppendorf tube, which was securely centered in the sealed vial. After 60 min, the tube containing benzethonium hydroxide with the released $^{14}CO_2$ was quickly transferred to a scintillation vials and counted for radioactivity. Palmitate oxidation was adjusted for radioactive label fixation (19, 54). For these purposes acid soluble ^{14}C products in hindlimb muscles were obtained during the extraction of the lipids from the hindlimb muscles. This method has been widely used in isolated muscles (4, 6, 9, 19, 20, 43, 48), in hindlimb perfused muscles (7, 38), and in studies of fatty acid oxidation by isolated mitochondria (13, 35). Standard calculations were used to determine CO_2 production across the perfused hindquarter (38).

Fatty acid uptake by skeletal muscle. It was not possible to also determine rates of fatty acid transport into giant vesicles prepared from individual red gastrocnemius muscles, since the red gastrocnemius muscle was used for subcellular fractionation. Therefore, we estimated the rates of fatty acid uptake during the hindlimb perfusion. For these purposes, we calculated the sum of palmitate esterfication (i.e., palmitate incorporation into phospholipids + diacylglycerol + triacylglycerol) in each muscle. From this sum, the total palmitate esterification by the rat hindquarter was calculated, taking into account the muscle mass and the fiber composition of hindlimb muscles (Bonen A, unpublished data). This hindquarter esterification rate was then summed with the palmitate oxidized by the hindquarter to obtain an estimate of the fatty acids taken up into the muscle. We (36, 38) have previously used a similar approach to estimate glucose transport (36) as well as fatty acid uptake by perfused rat (38) and mouse hindquarters (7).

Subcellular Fractionation of Muscle

We used the highly oxidative muscle red gastrocnemius muscle to examine the translocation of fatty acid transporters, FAT/CD36, and FABPpm. This type of highly oxidative muscle, unlike highly glycolytic muscles, metabolizes fatty acid at a high rate (7, 19, 38). Skeletal muscle plasma membrane and the low-density microsomal isolation procedure was based on the subcellular fractionation procedures described elsewhere (22, 34). Briefly, frozen sections of red gastrocnemius muscle (600 mg) were placed in 6 ml of cold (4°C) buffer (pH 7.4) containing 10 mM $NaHCO_3$, 0.25 M sucrose, 5 mM sodium azide, 0.1 mM phenylmethylsulfonyl fluoride, and protease inhibitors, including 5 mg/ml leupeptin, 5 mg/ml aprotinin, and 1 mg/ml pepstatin. After thawing, the tissue was homogenized using a Polytron (Kinematica). The resulting muscle homogenate was subsequently centrifuged at 1,000 *g* for 10 min; the supernatant fraction was saved, and the pellet was resuspended in the cold buffer, rehomogenized in a glass homogenizer (10 strokes), and combined with the first supernatant fraction. This was then centrifuged at 9,000 *g* for 10 min. The resulting supernatant was then centrifuged at 190, 000 *g* for 60 min. The pellet from this centrifugation (crude membranes) was resuspended in the cold buffer and applied to a discontinuous sucrose density gradient containing 25, 30, and 35% sucrose (wt/vol) solutions, respectively. The gradient was centrifuged in a swinging-bucket rotor (SW-41; Beckman, Mississauga, ON, Canada) in an ultracentrifuge (LE-80K, Beckman, Mississauga, ON, Canada) at 190,000 *g* for 16 h. Afterwards, the membranes were collected from the top of the 25% (plasma membrane, PM) and 35% gradients (low-density membranes, LDM). These were resuspended and diluted in the old buffer, pelleted by centrifugation at 190,000 *g* for 60 min, resuspended at the appropriate concentration in buffer, and frozen for later analyses of proteins by Western blotting as we have previously reported (9, 37). Quantification of blots was performed with Gene Tools (SynGene, PerkinElmer, Woodbridge, ON, Canada).

Statistics

Data were analyzed with analyses of variance [treatments (basal, insulin, contraction) vs. groups (lean vs. obese)]. A Fisher's least significant difference post hoc test was used to compare differences between conditions and groups when appropriate. All data are means ± SE.

RESULTS

As expected, body weights (180 ± 4.6 vs. 310 ± 6.8 g) and circulating insulin concentrations (0.33 ± 0.2 vs. 2.1 ± 0.5 nM) were each lower in lean than in obese rats ($P < 0.05$). Circulating glucose concentrations did not differ in lean (10.5 ± 0.9 mM) and obese rats (11.8 ± 1.5 mM). These results correspond to our previous observations in these animals (37). The intramuscular triacylglycerol concentration in red gastrocnemius muscle was greater in obese (3.25 ± 0.20 mg/g) than in lean animals (1.72 ± 0.10 mg/g) ($P < 0.05$).

Fatty Acid Uptake and Fatty Acid Transporters

Fatty acid uptake. Under basal conditions fatty acid uptake was greater in obese (+30%) than in lean muscle (Fig. 1). This is consistent with previous studies in obese Zucker rats under basal conditions (37, 52). In lean muscle, fatty acid uptake was increased when perfused with insulin (+63%, $P <$

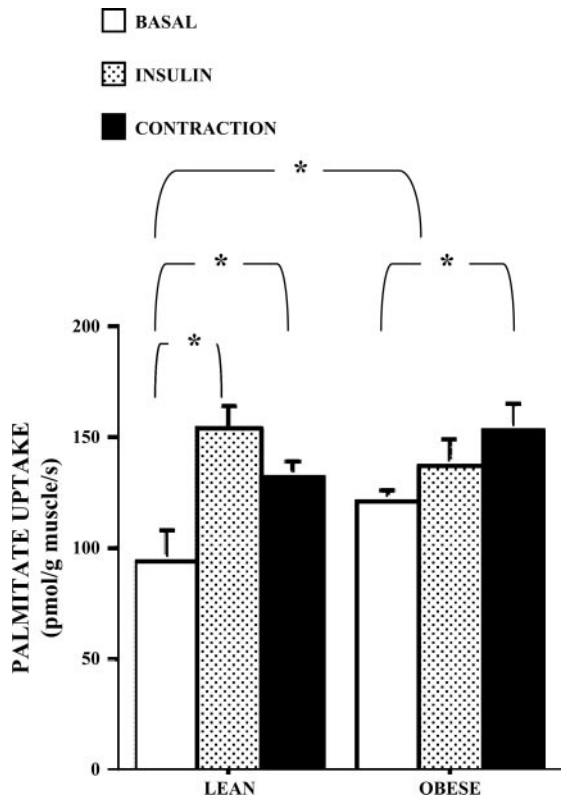

Fig. 1. Rates of fatty acid uptake by perfused red gastrocnemius muscle. Values are means ± SE; $n = 4$–5 independent experiments for each treatment. *$P < 0.05$ between comparisons indicated.

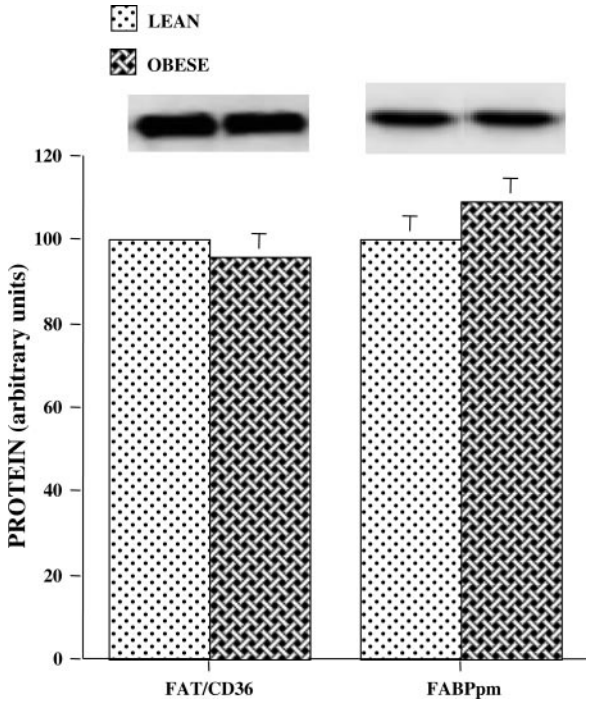

Fig. 2. Comparison of fatty acid transporter FAT/CD36 and plasma membrane-associated fatty acid binding protein (FABPpm) protein expression in lean and obese Zucker rat muscle. Values are means ± SE; $n = 4$ independent determinations in each group.

0.05) and during contraction (+40%, $P < 0.05$). In contrast, in obese muscle, insulin failed to stimulate fatty acid uptake (Fig. 1, $P > 0.05$). However, muscle contraction did increase fatty acid uptake over the rates observed under basal conditions in both lean and obese muscles ($P < 0.05$). The absolute contraction-induced increase (Δ) in fatty acid uptake was similar in lean ($\Delta = 38$ pmol·g^{-1}·s^{-1}) and obese muscle ($\Delta = 32$ pmol·g^{-1}·s^{-1}).

Fatty acid transporters FAT/CD36 and FABPpm. The protein expression of FAT/CD36 and FABPpm did not differ in lean and obese red gastrocnemius muscle (Fig. 2). This confirms our previous work in skeletal muscle of lean and obese Zucker rats (37).

Subcellular Distribution of FAT/CD36

Basal. The plasma membrane FAT/CD36 was 33% greater in obese than in lean muscle ($P < 0.05$, Fig. 3*A*). At the same time, the intracellular depot of FAT/CD36 was consistently lower (−14%) in obese muscle compared with lean muscle ($P < 0.05$, Fig. 3*B*).

Insulin. The lean and obese muscles responded very differently to insulin. In lean muscle, insulin induced the translocation of FAT/CD36 to the plasma membrane (+43%, $P < 0.05$, Fig. 3*A*) from its intracellular depot (−28%, $P < 0.05$, Fig. 3*B*). In marked contrast, in obese muscle, in which plasmalemmal FAT/CD36 was already 33% greater under basal conditions, insulin failed to induce a further increase in plasmalemmal FAT/CD36 ($P > 0.05$, Fig. 3*A*) or to further reduce the intracellular FAT/CD36 depot ($P > 0.05$, Fig. 3*B*). Thus, during insulin stimulation, the lean muscle plasmalemmal FAT/CD36 was increased to the levels observed during the basal and insulin-stimulating conditions in the obese muscle (Fig. 3*A*).

Contraction. The responses to muscle contraction also differed in lean and obese muscle. In lean muscle, contraction increased the plasma membrane FAT/CD36 (+41%, $P < 0.05$, Fig. 3*A*) and reduced the intracellular FAT/CD36 (−18%, $P < 0.05$, Fig. 3*B*). In the obese muscle, in which plasmalemmal FAT/CD36 was already upregulated under basal condition, contraction did not induce any further changes in plasmalemmal FAT/CD36 ($P > 0.05$, Fig. 3*A*) or in intracellular

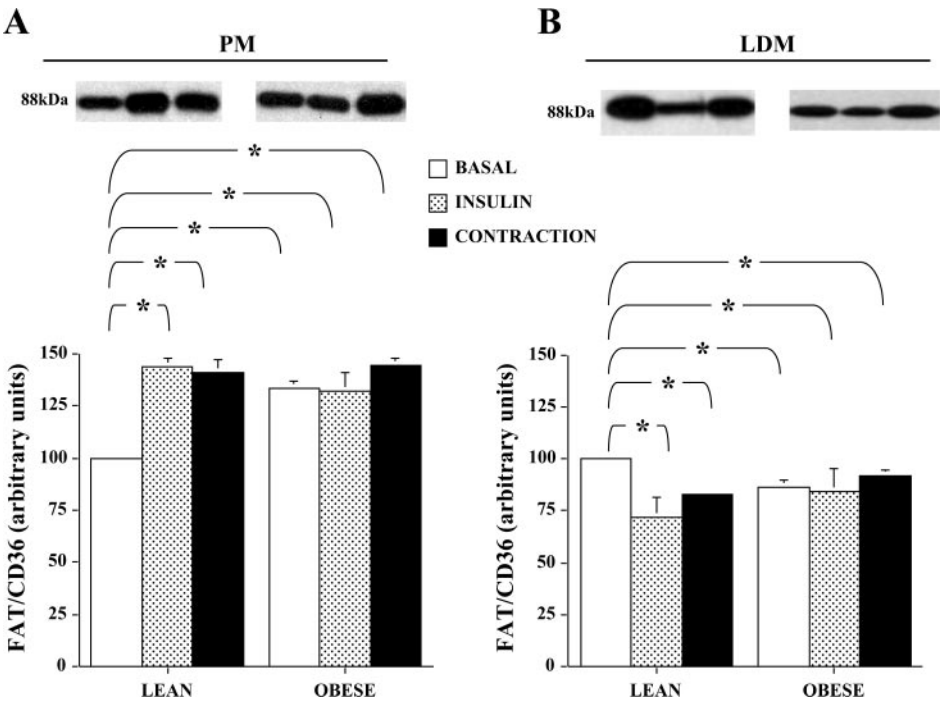

Fig. 3. Subcellular distribution of FAT/CD36 in lean and obese Zucker rat muscle under basal conditions and during insulin stimulation and muscle contraction. Values are means ± SE; $n = 4$ independent experiments for each treatment. LDM, low-density microsomal depot; PM, plasma membrane. *$P < 0.05$ between comparisons indicated.

FAT/CD36 ($P > 0.05$, Fig. 3*B*). Thus, during muscle contraction, the lean muscle plasmalemmal FAT/CD36 was increased to the levels observed during the basal and contraction-stimulating conditions in the obese muscle (Fig. 3*A*).

Subcellular Distribution of FABPpm

Basal. Under basal conditions, plasmalemmal FABPpm was slightly greater in obese muscle (+14%) compared with lean muscle ($P < 0.05$, Fig. 4*A*). Concomitantly, there was a reduction (−30%) in the intracellular FABPpm in obese muscle ($P < 0.05$, Fig. 4*B*).

Insulin. Stimulation with insulin increased plasmalemmal FABPpm in lean (+19%) and obese muscles (+15%) ($P < 0.05$, Fig. 4*A*). The intracellular FABPpm in lean muscle was reduced during insulin treatment (−44%, $P < 0.05$, Fig. 4*B*), but no further reduction occurred in intracellular FABPpm in obese muscle ($P > 0.05$, Fig. 4*B*), in which intracellular FABPpm was already reduced in the basal state.

Contraction. With muscle contraction, plasmalemmal FABPpm was increased in both lean (+67%) and obese muscle (+60% over obese basal, $P < 0.05$, Fig. 4*A*). In the lean muscle, there was a concomitant reduction in intracellular FABPpm (−24%, $P < 0.05$, Fig. 4*B*). However, no further reduction occurred in obese muscle intracellular FABPpm ($P > 0.05$, Fig. 4*B*), which was already reduced in the basal state.

Palmitate Incorporation Into Triacylglycerols

Basal. The rate of palmitate incorporation into intramuscular triacylglycerol depots was 32% greater in obese muscle compared with lean muscle ($P < 0.05$, Fig. 5).

Insulin. In lean muscle, insulin stimulated the rates of triacylglycerol formation (+72%, $P < 0.05$, Fig. 5). In contrast, in the obese muscles, insulin failed to further stimulate the rate of triacylglycerol formation ($P > 0.05$, Fig. 5), which was already upregulated in the basal state. Therefore, in the presence of insulin, the absolute rates of triacylglycerol formation did not differ between lean and obese muscles ($P > 0.05$, Fig. 5).

Contraction. The rate of palmitate incorporation into triacylglycerol depots was increased during contraction in lean muscle (+38%, $P < 0.05$) and obese muscle (+22%) ($P < 0.05$, Fig. 5). These relative (%) differences reflect the already greater rate of esterification in lean muscle under basal conditions, since the absolute increase in esterification over basal was similar in lean (+120 nmol·g^{-1}·60 min^{-1}) and obese muscles (+112 nmol·g^{-1}·60 min^{-1}). Therefore, during muscle contraction, the rate of palmitate esterfication was greater in obese muscle than in lean muscles ($P < 0.05$, Fig. 5).

Palmitate Oxidation

Basal. Under basal conditions the rate of palmitate oxidation was 28% lower in obese muscle than in lean muscle ($P < 0.05$, Fig. 6).

Insulin. Perfusion with insulin reduced the rate of palmitate oxidation in lean muscle (−52%, $P < 0.05$, Fig. 6). In contrast, insulin failed to reduce the rate of palmitate oxidation in obese muscle ($P > 0.05$, Fig. 6). Thus, during insulin stimulation, the rate of palmitate oxidation was greater (+43%) in obese muscles than in lean muscles (Fig. 6, $P < 0.05$).

Contraction. In contracting muscles, the rate of palmitate oxidation was increased in lean (+61%, $P < 0.05$, Fig. 6) and obese muscles (+37%, $P < 0.05$, Fig. 6). However, the absolute increase over basal rates of fatty acid oxidation was 2.2-fold greater in lean (+6.4 nmol·hindquarter^{-1}·min^{-1}) than obese muscle (+2.9 nmol·hindquarter^{-1}·min^{-1}). Thus, during muscle contraction, the rate of palmitate oxidation was lower (−40%) in obese muscle ($P < 0.05$, Fig. 6).

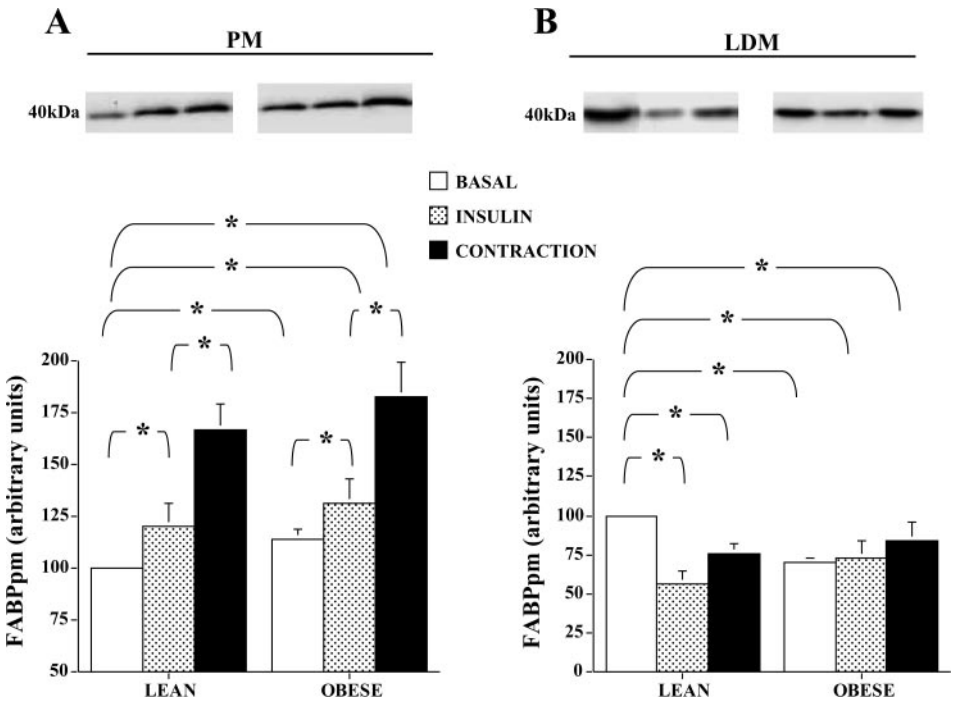

Fig. 4. Subcellular distribution of FABPpm in lean and obese Zucker rat muscle under basal conditions and during insulin stimulation and muscle contraction. Values are means ± SE; $n = 4$ independent experiments for each treatment. *$P < 0.05$ between comparisons indicated.

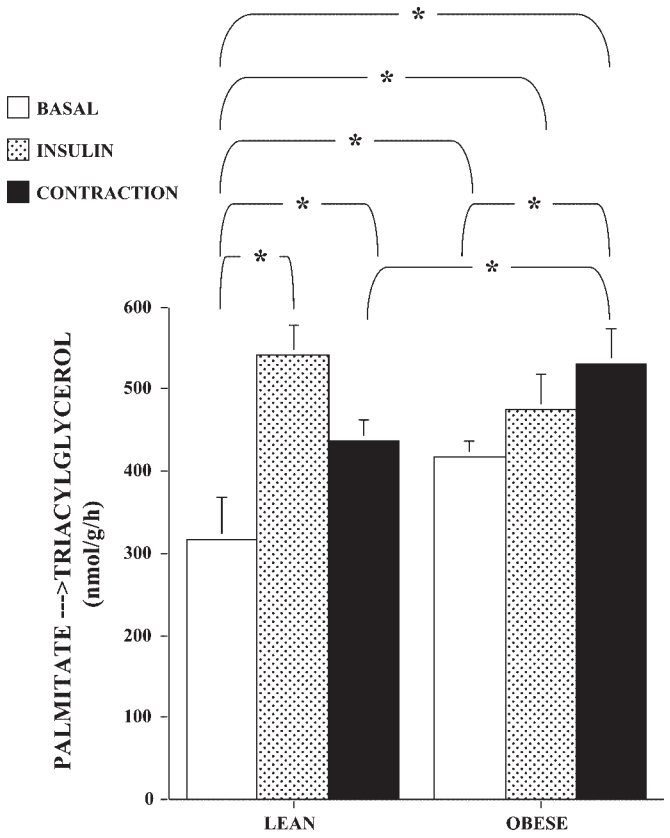

Fig. 5. Rates of palmitate esterification in perfused hindlimb muscle of lean and obese Zucker rats under basal conditions and during insulin stimulation and muscle contraction. Values are means ± SE; $n = 4–5$ independent experiments for each treatment. *$P < 0.05$ between comparisons indicated.

Relationship Between Plasmalemmal Fatty Acid Transporters and Fatty Acid Metabolism

A number of investigators have proposed, on the basis of experiments in cell lines, that fatty acid transporters may channel long-chain fatty acids to a particular metabolic fate (2, 24, 45, 59). To examine this possibility in mammalian skeletal muscle, we compared the changes in plasmalemmal fatty acid transporters with changes in fatty acid esterification and changes in fatty acid oxidation induced by insulin and muscle contraction. It is evident that there was no relationship between the changes in plasmalemmal fatty acid transporters and the changes in palmitate esterification (Fig. 7). Similarly, no relationship was observed between the changes in plasmalemmal fatty acid transporters and the changes in palmitate oxidation (data not shown).

DISCUSSION

We have examined, in perfused skeletal muscle of lean and obese Zucker rats, the effects of metabolic stimuli (insulin and muscle contraction) on *1*) the subcellular distribution of fatty acid transporters (FAT/CD36 and FABPpm) and *2*) fatty acid uptake and metabolism (esterification and oxidation). The novel findings include the following: skeletal muscles from obese Zucker rats are *1*) insulin resistant with respect to FAT/CD36 translocation and fatty acid uptake, esterfication, and oxidation, and *2*) they are contraction resistant with respect to FAT/CD36 translocation and fatty acid oxidation. In contrast, obese muscles are *3*) neither insulin nor contraction resistant at the level of FABPpm. Finally, *4*) there is no evidence that plasmalemmal fatty acid transporters contribute to channeling fatty acids to specific metabolic destinations within lean or obese skeletal muscle.

Fatty Acid Transporters and Fatty Acid Uptake in Lean and Obese Muscles

We confirmed previous observations (37, 52) that under basal conditions, FAT/CD36 and FABPpm are permanently relocated to the plasma membrane in skeletal muscle of obese Zucker rats. This would seem to account for the greater basal rates of fatty acid uptake in obese muscle, whether in rodents (present study and Ref. 37) or in humans (9).

Insulin and muscle contraction. In lean rats, the insulin- and muscle contraction-induced translocation of FAT/CD36 from an intracellular depot to the sarcolemma has been observed previously (5, 8, 38, 51). We observed, for the first time, that muscles from obese animals are insulin resistant and contraction resistant with respect to FAT/CD36 translocation. Such resistances in obese muscle were not evident for FABPpm, although we also report for the first time that insulin and muscle contraction induce the translocation of FABPpm in skeletal muscles, albeit with a considerable difference in magnitude (contraction >> insulin). Although the complete signaling pathways for the insulin- and contraction-induced translocation of FAT/CD36 and FABPpm remain to be identified, it is known that insulin-stimulated activation of phosphatidylinositol 3-kinase is required to induce the translocation of FAT/CD36 and that the translocation of FAT/CD36 and FABPpm can be induced by the activation of AMP-activated protein kinase (AMPK) and ERK (for review, see Ref. 5), proteins that

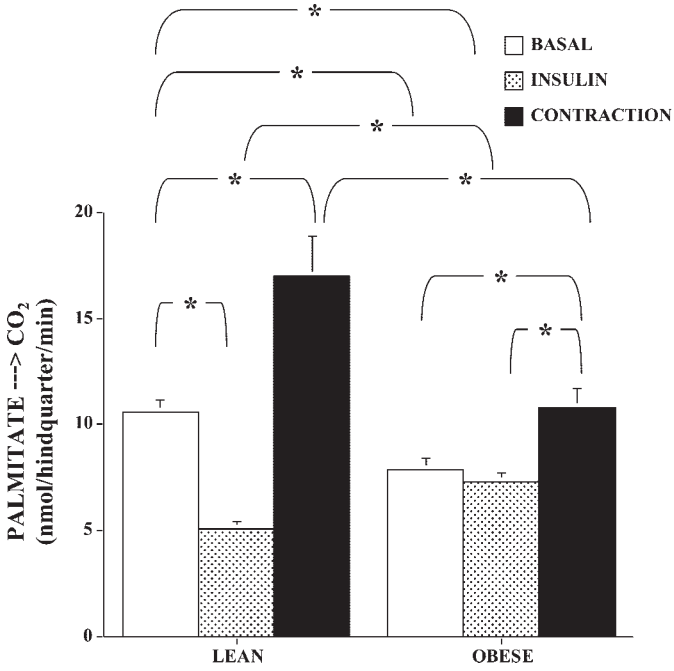

Fig. 6. Rates of palmitate oxidation in perfused hindlimb muscles of lean and obese Zucker rats under basal conditions and during insulin stimulation and muscle contraction. Values are means ± SE; $n = 4–5$ independent experiments for each treatment. *$P < 0.05$ between comparisons indicated.

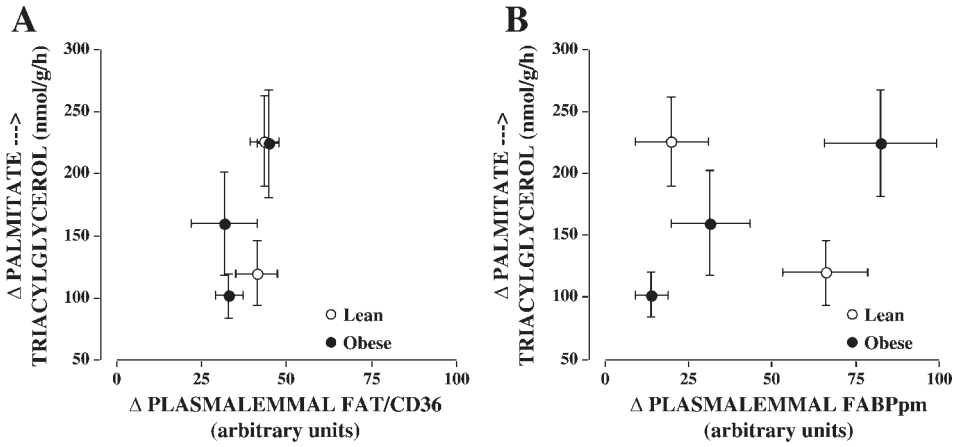

Fig. 7. Relationship between changes (Δ) in plasmalemmal FAT/CD36 (*A*) and plasmalemmalFABPpm (*B*) and changes in palmitate esterification. Values are means ± SE. Differences were derived by subtracting the basal levels of the fatty acid transporters and rates of esterification in the lean animals from the respective data in all other remaining conditions (i.e., insulin and contraction stimulation in lean muscle, and basal and insulin and contraction stimulation in obese muscle). Data are derived from data in Figs. 3, 4, and 5.

are also activated during muscle contraction. Our studies suggest strongly that components of both the insulin- and contraction-signaling pathways for FAT/CD36 are impaired in muscles from obese animals, whereas in these same muscles, components of the insulin- and contraction-signaling pathways for FABPpm are not disturbed.

We speculate that the increased circulating insulin levels in obese Zucker rats induced the permanent relocation of FAT/CD36 to the plasma membrane that has been observed under basal conditions (present study and Refs. 16, 37), and therefore, no more of the intracellular FAT/CD36 can be induced to translocate, either with insulin stimulation or with muscle contraction. Our data appear to support this suggestion, because in lean muscle, insulin and muscle contraction brought the plasmalemmal FAT/CD36 to the same level as was observed in the hyperinsulinemic obese animals in the basal state.

A perplexing observation in obese muscle was the absence of any FABPpm reduction in the LDM depot when insulin or contraction increased the FABPpm at the plasma membrane. FABPpm does not appear to traffic from the mitochondria to the sarcolemma (Bonen A, unpublished observations), as had been suggested elsewhere (14). Whether cytosolic FABPpm (44, 55, 57) can traffic to the plasma membrane is unknown.

FATTY ACID UPTAKE. During perfusion with insulin, the rates of fatty acid uptake did not differ between lean and obese animals. The reason seems to be that during these experimental conditions, the plasmalemmal content of FAT/CD36 and FABPpm were brought to similar levels in lean and obese animals, despite the differing insulin-induced effects on FAT/CD36 translocation in obese and lean muscle.

In lean and obese animals, the contraction-stimulated fatty acid uptake appears to be dependent on both FAT/CD36 and FABPpm, not just FAT/CD36. For example, the increased rates of fatty acid uptake in both lean and obese muscles were associated with similar levels of plasmalemmal FAT/CD36 and FABPpm. However, in lean muscle, this was associated with contraction-induced translocation of both FAT/CD36 and FABPpm, whereas in obese muscle, in which plasmalemmal FAT/CD36 was already upregulated, there was a substantial contraction-induced increase in plasmalemmal FABPpm only. A far more modest insulin-stimulated increase in FABPpm, however, had no impact on increasing fatty acid uptake in obese muscle. Together, these observations begin to suggest that the increase in plasmalemmal FABPpm, along with FAT/CD36, is important in promoting fatty acid uptake into contracting muscles. Presumably, FABPpm acts in conjunction with FAT/CD36 at the plasma membrane, because there is evidence that these two proteins appear to interact to facilitate the rate of fatty acid transport (39, 41) and because they coimmunoprecipitate (Chabowski A and Bonen A, unpublished data).

Palmitate Esterification and Oxidation

Under basal conditions, we confirmed other reports showing an increased rate of palmitate incorporation into triacylglycerols in skeletal muscle of obese Zucker rats (52) and obese humans (9, 49). There is, however, some disagreement as to whether basal rates of fatty acid oxidation in muscles of obese Zucker rats are altered (present study and Refs. 17, 42, 52). Nevertheless, the reduced basal rate of fatty acid oxidation in muscle of Zucker obese rats (present study) concurs with observations in skeletal muscle of severely obese individuals (body mass index = 54 kg/m^2) (27, 32). The greater rate of basal palmitate esterification is attributable, in part, to the increased levels of plasmalemmal FAT/CD36 and FABPpm, which facilitate a greater rate of uptake of palmitate (cf. Ref. 5).

Insulin and muscle contraction. Insulin stimulation in lean muscle induced a temporary, obesity-like effect, namely, an increase in palmitate esterification and a reduction in palmitate oxidation, as has been observed previously (20, 40, 53, 58). The insulin-stimulated rate of palmitate esterification in lean muscle was achieved, in part, by *1*) an increased rate of fatty acid uptake facilitated by the insulin-induced translocation of fatty acid transporters to the plasma membrane and *2*) by a concomitant reduction in fatty acid oxidation, likely as a result of the increased insulin-stimulated glucose uptake, which can inhibit palmitate oxidation (58). Together, these insulin-mediated effects likely channeled more fatty acids into the triacylglycerol depots in lean muscle. In contrast, in the insulin-perfused obese muscles, the rates of palmitate esterification and oxidation, which were already up- and downregulated, respectively, under basal conditions, were not altered. This likely occurred because insulin failed to further increase plasmalemmal FAT/CD36 in obese muscle, which then prevented a further increase in palmitate uptake. This is the first report to show that muscles from obese Zucker rats are insulin resistant with respect to palmitate oxidation and esterification, likely as

a result of the concomitant insulin resistance in FAT/CD36 translocation and fatty acid uptake in the obese muscles.

Contraction of lean and obese muscles stimulated palmitate oxidation and esterification, as has been observed previously in healthy animals (18, 50). Presumably, these effects were attributable, in part, to the increased rates of contraction-stimulated fatty acid uptake. In obese muscle, the contraction-stimulated increase in fatty acid uptake along with the impaired fatty acid oxidation combined to channel more of the fatty acid into the triacylglycerol depots. The impaired contraction-stimulated palmitate oxidation in obese muscle cannot be attributed to differences in altered mitochondrial density, reduced AMPKα2 activation, reduced muscle mass, or contraction-stimulated rates of glucose uptake, because these are all similar in muscles of lean and obese Zucker rats (Refs. 1, 11, 15, 56 and unpublished data). This suggests that there are alterations in the mitochondrial machinery regulating fatty acid oxidation in obese muscle, possibly involving FAT/CD36. Recently, FAT/CD36 was also implicated in regulating mitochondrial fatty acid oxidation in skeletal muscle in conjunction with carnitine palmitoyltransferase-1 (CPT1) (3, 13, 25, 26). It remains to be determined whether in contracting obese muscle there is also a failure to translocate FAT/CD36 to the mitochondria, which might then limit fatty acid oxidation.

Relating Fatty Acid Transporters to the Metabolic Fate of Fatty Acids

From studies in cell lines, it has been suggested that selected fatty acid transporters link with intracellular proteins to channel fatty acids to esterification or to oxidation (2, 24, 45, 59). Whether this is physiologically relevant is questionable. In these in vitro models, normal physiological regulation is absent, the cells have extremely low rates of fatty acid metabolism, and the regulation of fatty acid uptake and metabolism may not parallel that of metabolically important mammalian tissues. Moreover, we found no supporting evidence for intracellular fatty acid channeling by fatty acid transporters in skeletal muscle (see Fig. 7). This is not surprising. Although insulin and muscle contraction will each induce an increase in plasmalemmal fatty acid transporters, the additional fatty acids taken up are then channeled to esterification and oxidation, processes that are presumably regulated by intracellular insulin- and contraction-mediated metabolic signals. This is clearly illustrated in the present studies, in which insulin and contraction induced almost similar increases in plasmalemmal fatty acid transporters and fatty acid uptake into muscle, but the partitioning of the intracellular fatty acids to triacylglycerol or oxidation differed markedly depending on whether insulin or muscle contraction was used.

Summary

It has long been known that skeletal muscles of obese Zucker rats are insulin resistant with respect to glucose transporter (GLUT4) translocation and glucose transport, whereas these same muscles are not contraction resistant with respect to glucose transporter (GLUT4) translocation and glucose transport. Our studies have shown that when presented with metabolic challenges, lean muscles are insulin and contraction responsive, at the level of inducing fatty acid transporter translocation and stimulating fatty acid uptake and metabolism (esterification and/or oxidation). However, in skeletal muscle of obese Zucker rats, some of these processes are upregulated under basal conditions (i.e., plasmalemmal FAT/CD36 and fatty acid uptake and esterification). In addition, these obese muscles are also *1*) contraction resistant with respect to FAT/CD36 translocation and fatty acid oxidation and *2*) insulin resistant at the level of FAT/CD36 translocation and fatty acid uptake, esterification, and oxidation. Conversely, *3*) obese muscles are neither insulin nor contraction resistant at the level of FABPpm. Finally, *4*) we can find no evidence that, in vivo, fatty acid transporters at the plasma membrane contribute to the channeling of fatty acids to specific metabolic destinations within lean or obese skeletal muscle.

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