

## Chronic Leptin Administration Decreases Fatty Acid Uptake and Fatty Acid Transporters in Rat Skeletal Muscle\*

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Chronic leptin administration reduces triacylglycerol content in skeletal muscle. We hypothesized that chronic leptin treatment, within physiologic limits, would reduce the fatty acid uptake capacity of red and white skeletal muscle due to a reduction in transport protein expression (fatty acid translocase (FAT/CD36) and plasma membrane-associated fatty acid-binding protein (FABPpm)) at the plasma membrane. Female Sprague-Dawley rats were infused for 2 weeks with leptin (0.5 mg/kg/day) using subcutaneously implanted miniosmotic pumps. Control and pair-fed animals received saline-filled implants. Leptin levels were significantly elevated (~4-fold;  $p < 0.001$ ) in treated animals, whereas pair-fed treated animals had reduced serum leptin levels (approximately -2-fold;  $p < 0.01$ ) relative to controls. Palmitate transport rates into giant sarcolemmal vesicles were reduced following leptin treatment in both red (-45%) and white (-84%) skeletal muscle compared with control and pair-fed animals ( $p < 0.05$ ). Leptin treatment reduced FAT mRNA (red, -70%,  $p < 0.001$ ; white, -48%,  $p < 0.01$ ) and FAT/CD36 protein expression (red, -32%;  $p < 0.05$ ) in whole muscle homogenates, whereas FABPpm mRNA and protein expression were unaltered. However, in leptin-treated animals plasma membrane fractions of both FAT/CD36 and FABPpm protein expression were significantly reduced in red (-28 and -34%, respectively) and white (-44 and -56%, respectively) muscles ( $p < 0.05$ ). Across all experimental treatments and muscles, palmitate uptake by giant sarcolemmal vesicles was highly correlated with the plasma membrane FAT/CD36 protein ( $r = 0.88$ ,  $p < 0.01$ ) and plasma membrane FABPpm protein ( $r = 0.94$ ,  $p < 0.01$ ). These studies provide the first evidence that protein-mediated long chain fatty acid transport is subject to long term regulation by leptin.

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The development of obesity and insulin resistance in both humans and rodents is associated with abnormalities in lipid metabolism, involving impaired fatty acid oxidation and increased storage as intramuscular triacylglycerol (1, 2). Whereas the association of insulin resistance with increased concentrations of intramuscular triacylglycerol is well recognized (1–3), the underlying mechanisms are unknown. In *ob/ob* mice, the absence of leptin results in a phenotype characterized by obesity and insulin resistance, and treatment with recombinant leptin results in a rapid reduction in body adiposity and the restoration of insulin sensitivity (4). In skeletal muscle, an essential tissue responsible for regulating whole body lipid and glucose metabolism, leptin has been shown to increase fatty acid oxidation and intramuscular triacylglycerol hydrolysis acutely (<1 h) (5–7) while decreasing fatty acid esterification (5).

The effects of chronic (>7 days) leptin treatment on skeletal muscle fatty acid uptake have not been examined. Several studies have demonstrated that chronic leptin treatment in lean and diabetic rats as well as *ob/ob* mice leads to reduced body mass and results in significant reductions in circulating insulin, independent of reduced food intake (8–10), suggesting an improved insulin sensitivity. Chronic hyperleptinemia has also been shown to increase glucose uptake in skeletal muscle (3, 8). However, this is not due to increased GLUT-4 expression, suggesting that leptin may be altering the transporter's intrinsic activity and/or translocation to the sarcolemma (9). Another possible mechanism for improved insulin sensitivity may be the decrease of intramuscular triacylglycerols observed in red skeletal muscle following leptin treatment (3), since intramuscular triacylglycerol accumulation is correlated with insulin resistance (1–3, 11). Thus, it is important to understand the mechanisms by which chronic leptin treatment alters intramuscular triacylglycerol concentrations. A possible mechanism contributing to the insulin-induced reductions in intramuscular triacylglycerols may occur at the level of fatty acid transport into the muscle cell.

Fatty acids traverse the plasma membrane via passive diffusion and a protein-mediated mechanism (12). Several proteins have been shown to facilitate fatty acid transport, including the fatty acid translocase (FAT/CD36)<sup>1</sup> and plasma membrane-associated fatty acid-binding protein (FABPpm) (12). While fatty acid transport protein 1 (FATP1) was initially thought to be a fatty acid transporter (13), recent studies have

<sup>1</sup> The abbreviations used are: FAT, fatty acid translocase; FABPpm, plasma membrane-associated fatty acid-binding protein; FATP1, fatty acid transport protein 1; FABPc, cytosolic, 15-kDa fatty acid-binding protein; FA, fatty acid.

shown that FATP1 is a very long chain fatty acyl-CoA synthetase (14). The cytosolic, 15-kDa fatty acid-binding protein (FABPc) is also an important feature of the fatty acid transport system, since it acts as a fatty acid sink once fatty acids have crossed the plasma membrane (12).

In muscle, FABPc is present in great excess and therefore does not limit fatty acid uptake (12). However, increasing the expression of FAT/CD36 in skeletal muscle increases fatty acid transport and oxidation (15), whereas in FAT/CD36 null mice the uptake of fatty acids is reduced (16). Other mechanisms besides altered FAT/CD36 expression can also regulate fatty acid transport. In contracting muscles, FAT/CD36 is translocated, within minutes, from an intracellular pool to the plasma membrane, resulting in an increased rate of fatty acid transport (17). Thus, skeletal muscle fatty acid transport can be affected in a number of ways, by altering the expression of FAT/CD36 and/or relocating this protein to the plasma membrane.

A link between leptin-induced reductions in intramuscular triacylglycerol depots and improved insulin sensitivity in skeletal muscle may be the reduced uptake of fatty acids into the muscle cell. Fatty acids are known to induce insulin resistance in muscle (18), and limiting their entry into the muscle cell may be expected to reduce fatty acids available for esterification, thereby improving insulin sensitivity. We therefore hypothesized that chronic leptin treatment can lead to a reduced rate of fatty acid transport into muscle due to reductions in skeletal muscle fatty acid transporters, FAT/CD36 and FABPpm. We have examined the effects of chronic hyperleptinemia (2 weeks) on fatty acid transport, the expression of fatty acid transporters (FAT/CD36 and FABPpm), and their localization in the plasma membrane. The present studies have shown that leptin treatment (2 weeks) repressed FAT/CD36 expression in muscle and reduced plasma membrane FAT/CD36 and FABPpm, which resulted in a reduced fatty acid transport across the sarcolemmal membrane.

#### EXPERIMENTAL PROCEDURES

**Animals**—Female Sprague-Dawley rats ( $247.6 \pm 2.6$  g) were randomly assigned to one of three groups (*ad libitum* fed saline-treated (control), pair-fed saline-treated (pair-fed), or leptin-treated ( $n = 8$  per group)). In anesthetized (halothane) animals, miniosmotic pumps (2ML2; Durect Corp., Cupertino, CA) were implanted subcutaneously, slightly posterior to the scapulae. Pumps were filled with either sterile, phosphate-buffered saline (control, pair-fed) or leptin (Amgen, Thousand Oaks, CA). A leptin dosage of 0.5 mg/kg/day was used, since this had been previously demonstrated to induce moderate hyperleptinemia (10, 19). Animals, assigned to individual cages, were kept on a reverse 12 h/12 h light/dark cycle. Water was freely accessible for all groups. Food intake was *ad libitum* for both the control and leptin-treated animals, whereas pair-fed treated animals were fed the same amount of chow as the leptin-treated animals consumed. Body mass was monitored weekly over the 2-week treatment period. The committees on animal care at the Universities of Waterloo and Guelph approved all procedures.

**Blood and Tissue Sampling**—Blood samples were collected at the completion of treatment (2 weeks) via cardiac puncture after excision of red and white skeletal muscle. Samples were taken in the fed state between 0900 and 1100 to eliminate diurnal variability. Serum leptin and insulin concentrations were assayed in duplicate using radioimmuno assay kits (Linco, St. Charles, MO) specific for rat leptin and insulin. Fatty acids were assayed using a Wako NEFA kit (Wako Chemical, Richmond, VA). Serum glucose levels were determined fluorometrically (20). Soleus muscle intramuscular triacylglycerol content was determined on freeze-dried samples, which were dissected free of all visible connective tissue and blood, as previously outlined (21).

**Giant Sarcolemmal Vesicles**—Vesicles from red (vastus intermedius, red vastus lateralis, red gastrocnemius, red tibialis anterior) and white muscles (plantaris, white vastus lateralis, white gastrocnemius, white tibialis anterior) were prepared as we have described in detail previously (12, 15, 17, 22, 23). Vesicles were immediately used for transport experiments. In addition, some of the vesicles were placed in a blood cell

counting chamber and were photographed under a phase-contrast microscope to determine vesicle size and density. Remaining vesicles were stored at  $-80^{\circ}\text{C}$  for determination of plasma membrane FAT/CD36 and FABPpm.

**Fatty Acid Transport**—Palmitate uptake has been shown to be linear for up to 25 s in vesicles from red and white muscles, due in part to the large intravesicular sink of FABPc, which is present in great excess in giant vesicles derived from red and white muscles (12). The content of FABPc in muscles was determined by sandwich-type enzyme-linked immunosorbent assay as previously described (12). In the present experiments, palmitate ( $15\ \mu\text{M}$ ) uptake by giant sarcolemmal vesicles ( $80\ \mu\text{g}$  of protein) was determined over a 15-s period, as we have previously described in detail (12).

**Western and Northern Blotting**—The putative fatty acid transporters FAT/CD36 and FABPpm were measured in muscle homogenates as well as in plasma membranes of giant sarcolemmal vesicles. To detect FAT/CD36 and FABPpm, we used antibodies and procedures that have been described previously (12, 15, 17, 23). Messenger RNA for FAT and FABPpm were measured in red and white vastus muscle using procedures previously described (24).

#### RESULTS

**Body Composition and Food Intake**—Food intakes were significantly reduced in leptin-treated animals ( $-33\%$ ;  $p < 0.01$ ) compared with *ad libitum* fed controls (Fig. 1A). In pair-fed treated animals, food intake was matched with leptin-treated animals. Over the 2-week treatment period, food intake was constant in all groups. Body mass was reduced in both leptin and pair-fed treated animals ( $-12.5\%$ ,  $p < 0.05$ ) compared with controls following 2 weeks of treatment (Fig. 1B).

**Circulating Concentrations of Leptin, Insulin, Glucose, and Fatty Acids**—Chronic leptin treatment increased circulating leptin ( $8.75 \pm 0.75$  ng/ml) compared with control ( $1.72 \pm 0.30$  ng/ml) and pair-fed animals ( $0.5 \pm 0.10$  ng/ml) ( $p < 0.05$ ). In contrast, leptin treatment reduced circulating insulin ( $0.20 \pm 0.05$  ng/ml) and fatty acids ( $0.18 \pm 0.4$  mM) compared with control (insulin,  $1.30 \pm 0.04$  ng/ml; FA,  $0.35 \pm 0.05$  mM) and pair-fed animals (insulin,  $1.17 \pm 0.30$  ng/ml; fatty acids,  $0.18 \pm 0.10$  mM) ( $p < 0.05$ ). Glucose concentration did not differ among the three groups of animals ( $5.05$ – $5.20$  mM) ( $p > 0.05$ ).

**Intramuscular Triacylglycerols**—Intramuscular triacylglycerols (Fig. 2) in soleus muscle was significantly reduced in leptin-treated animals, relative to control ( $-41\%$ ,  $p = 0.03$ ) and pair-fed animals ( $-33\%$ ,  $p = 0.05$ ). Intramuscular triacylglycerols of pair-fed animals was not significantly different from controls.

**FAT/CD36 and FABPpm mRNA and Protein Expression**—With leptin treatment, FAT mRNA abundance was significantly reduced in both red ( $-70\%$ ;  $p < 0.001$ ) and white ( $-48\%$ ;  $p < 0.01$ ) muscles (Fig. 3A), while FABPpm mRNA abundance was unchanged (Fig. 3B). We measured the protein expression of FAT/CD36 and FABPpm in both red and white muscle homogenates (intracellular plus plasma membrane pools) as well as in plasma membrane only fractions derived from giant sarcolemmal vesicles. Chronic leptin treatment reduced FAT/CD36 protein in red ( $-32\%$ ;  $p < 0.01$ ) and white muscle homogenates ( $-15\%$ ;  $p > 0.05$ ) (Fig. 4A). FABPpm protein in both red and white muscle homogenates was unchanged with leptin treatment (Fig. 4B). Plasma membrane FAT/CD36 (Fig. 5A) and FABPpm (Fig. 5B) were significantly reduced following leptin treatment in both red and white muscles (FAT/CD36: red  $-49\%$ , white  $-57\%$ ; FABPpm: red  $-26\%$ , white  $-43\%$ ;  $p < 0.05$ ).

**Palmitate Transport in Giant Sarcolemmal Vesicles**—To determine whether leptin affected fatty acid transport in muscle, giant sarcolemmal vesicles obtained from red and white skeletal muscle were used. We have previously characterized fatty acid transport in red and white muscle (12). The giant vesicles from both red and white muscle were spherical in appearance and averaged  $13.8 \pm 0.05\ \mu\text{m}$  ( $n = 120$ ) in diameter, and vesicle size was similar in all groups ( $p > 0.05$ ). As previously demonstrated (12), red muscle contained a greater sink for incorpo-

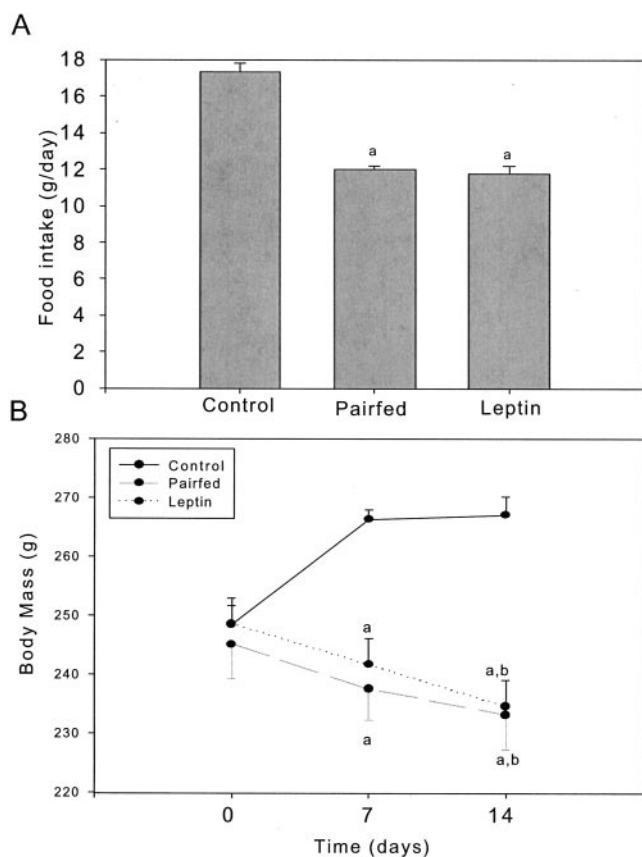


FIG. 1. Daily food consumption of animals during a 2-week period (A) and change in body mass of animals during a 2-week period (B). Control, *ad libitum* fed, sedentary animals; Pairfed, pair-fed sedentary animals (pair feeding occurred with leptin-treated animals); Leptin, continuous leptin infusion for 2 weeks. a, significantly different from control; b, significantly different from time 0.

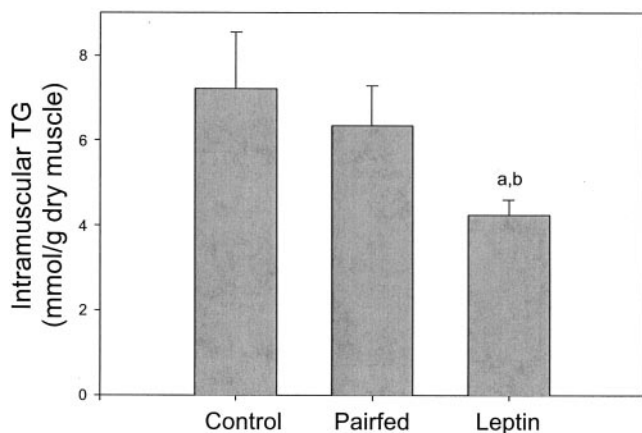


FIG. 2. Intramuscular triacylglycerol concentrations after a 2-week period. Control, *ad libitum* fed, sedentary animals; Pairfed, pair-fed sedentary animals (pair feeding occurred with leptin-treated animals); Leptin, continuous leptin infusion for 2 weeks. a, significantly different from control; b, significantly different from pair-fed.

rated palmitate due to an elevated FABPc content (red,  $1.53 \pm 0.25$  mg/g, wet weight; white,  $0.23 \pm 0.05$  mg/g, wet weight;  $p < 0.001$ ). There was no difference in FABPc content among treatments. As we have demonstrated previously (12), palmitate uptake was greater in red *versus* white skeletal muscle (+58%,  $p < 0.001$ ; Fig. 6). Palmitate uptake was significantly reduced in leptin-treated *versus* control animals in both red and white skeletal muscle (-33 and -46%, respectively;  $p < 0.05$ ; Fig. 6) but was not different between pair-fed and control animals.

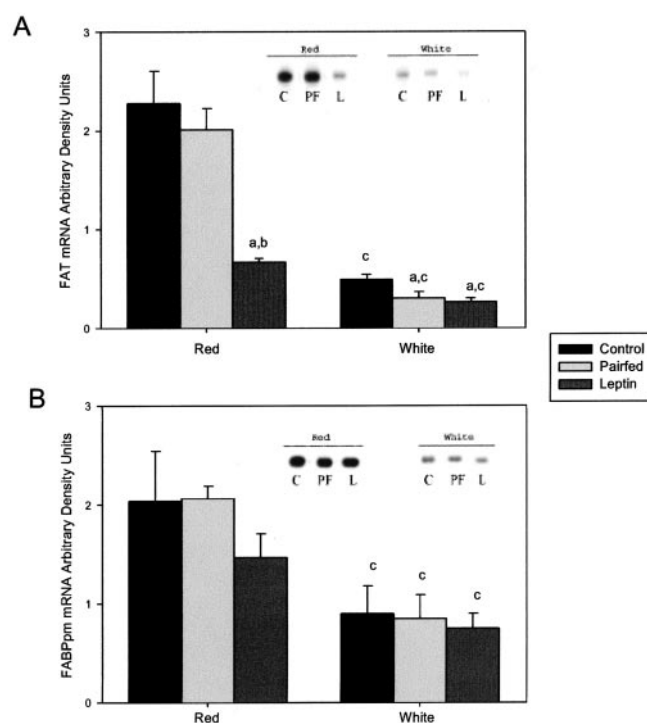


FIG. 3. mRNA abundance of FAT (A) and FABPpm (B) in red and white vastus muscle following a 2-week treatment period. Control, *ad libitum* fed, sedentary animals; Pairfed, pair-fed sedentary animals (pair feeding occurred with leptin-treated animals); Leptin, continuous leptin infusion for 2 weeks. a, significantly different from control; b, significantly different from pair-fed; c, significantly different from red.

*Comparison of Fatty Acid Transport and Plasma Membrane FAT/CD36 and FABPpm*—We compared the rates of fatty acid transport with the plasma membrane FAT/CD36 and FABPpm. For these purposes, the data from all of the experimental groups and red and white muscles were used. These comparisons showed that palmitate uptake by giant sarcolemmal vesicles was highly correlated with the plasma membrane FAT/CD36 protein ( $r = 0.88$ ,  $p < 0.01$ ; Fig. 7A) and plasma membrane FABPpm protein ( $r = 0.94$ ,  $p < 0.01$ ; Fig. 7B).

#### DISCUSSION

The movement of fatty acids across the sarcolemma involves the fatty acid transporters FAT/CD36 (25, 26) and FABPpm (27) and is the first step in the regulation of fatty acid metabolism. Recent studies in our laboratory (12, 23, 24) and others (28, 29) have demonstrated that fatty acid transporter expression is regulated by the metabolic demand of skeletal muscle (23), obesity (28, 29), and diabetes (29). These latter studies (28, 29) suggest that there may be hormonal regulation of fatty acid transporter expression, resulting in altered rates of plasma membrane fatty acid transport. Leptin may be one of the endocrine signals regulating fatty acid transporter expression, and skeletal muscle may be an important target for leptin. This tissue is important for regulating fatty acid homeostasis because of its mass (40% of body weight) and highly variable metabolic rate.

In isolated muscles, the acute ( $\leq 60$  min) effects of leptin include an increased rate of fatty acid oxidation and a concomitantly reduced rate of esterification (5–7). Prolonged hyperleptinemia (6–14 days) reduces muscle triacylglycerol depots (30), an effect that may be achieved, in part, by reducing the protein mediated uptake of fatty acids into the myocyte. Therefore, we have investigated the effects of chronically (14 days) elevated circulating leptin levels on fatty acid transporter expression

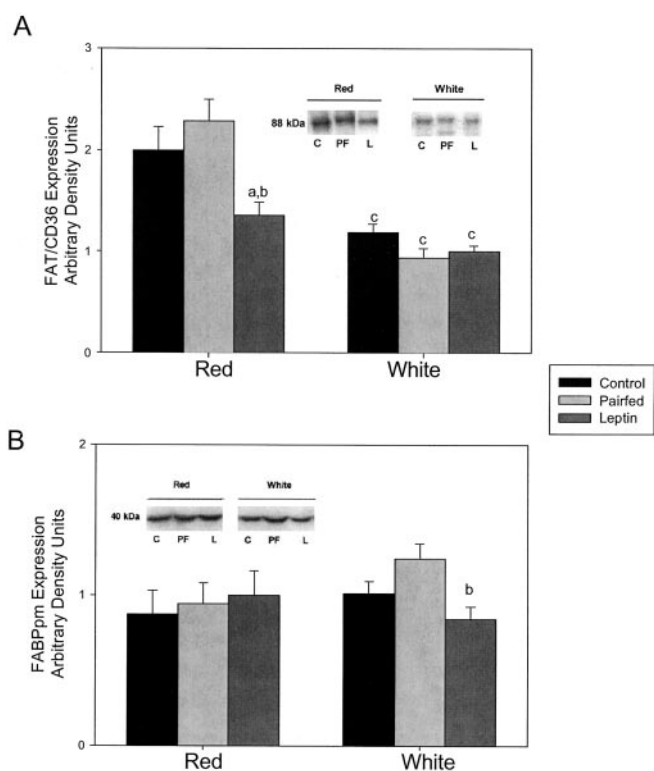


FIG. 4. Muscle homogenate protein expression of FAT/CD36 (A) and FABPpm (B) in red and white gastrocnemius muscle following a 2-week treatment period. *Control*, ad libitum fed, sedentary animals; *Pairfed*, pair-fed sedentary animals (pair feeding occurred with leptin-treated animals); *Leptin*, continuous leptin infusion for 2 weeks; *a*, significantly different from control; *b*, significantly different from pair-fed; *c*, significantly different from red.

and localization in red and white rat skeletal muscle as well as on fatty acid transport into giant sarcolemmal vesicles derived from these two types of muscle. Several novel findings are reported in this study. Leptin treatment reduced FAT mRNA abundance and the expression of FAT/CD36 protein, while FABPpm mRNA and protein expression were not altered; however, both of the fatty acid transport proteins, FAT/CD36 and FABPpm, located at the plasma membrane were reduced, which resulted in a reduced rate of fatty acid transport into red and white skeletal muscle giant sarcolemmal vesicles. These effects were not observed in pair-fed animals that lost the same body weight as the leptin-treated animals.

Importantly, the chronic (2 weeks) effects of leptin on fatty acid uptake and transporters are not comparable with studies in which isolated muscles have been acutely ( $\leq 60$  min) exposed to leptin (5–7). In those studies, leptin did not alter fatty acid uptake; rather, leptin repartitioned the fatty acids taken up toward oxidation and away from esterification (5–7).

In this study, we induced moderate levels of hyperleptinemia (~4-fold increase), a level that is similar to that obtained following 2 weeks of high fat feeding in rodents (5). This physiologic increase in leptin reduced intramuscular triacylglycerol depots and circulating insulin and fatty acids, while not altering circulating glucose concentrations. These results parallel studies in which pharmacological levels of leptin have been administered (8, 30–33). Since the serum insulin concentrations were already quite low in the control animals, it seems unlikely that a retarded rate of insulin-stimulated fatty acid esterification, rather than the increased leptin concentrations, accounted for the reduction in the intramuscular triacylglycerol depots. The reduction in circulating fatty acids is probably due to a selective depletion of the labile visceral adipose stores

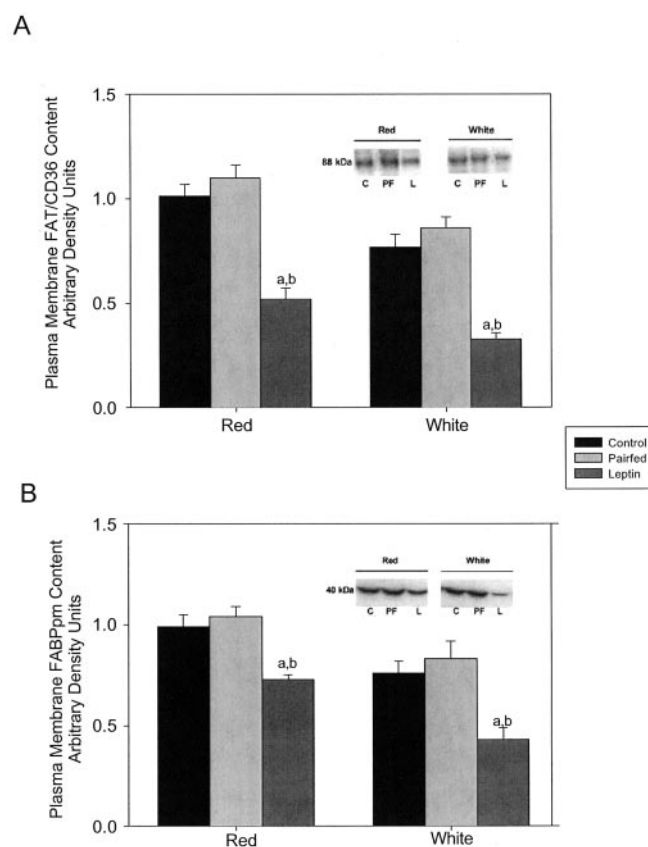


FIG. 5. Plasma membrane protein expression of FAT/CD36 (A) and FABPpm (B) in red and white gastrocnemius muscle following a two-week treatment period. *Control*, ad libitum fed, sedentary animals; *Pairfed*, pair-fed sedentary animals (pair feeding occurred with leptin-treated animals); *Leptin*, continuous leptin infusion for 2 weeks. *a*, significantly different from control; *b*, significantly different from pair-fed; *c*, significantly different from red.

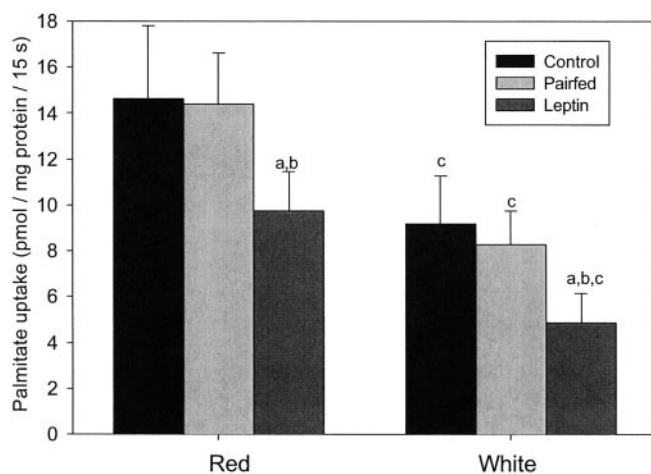


FIG. 6. Fatty acid transport into giant sarcolemmal vesicles derived from red and white gastrocnemius muscle following a 2-week treatment period. *Control*, ad libitum fed, sedentary animals; *Pairfed*, pair-fed sedentary animals (pair feeding occurred with leptin-treated animals); *Leptin*, continuous leptin infusion for 2 weeks. *a*, significantly different from control; *b*, significantly different from pair-fed; *c*, significantly different from red.

(19). Therefore, in the short term (a time point not measured in this study), it would be expected that serum FA levels would be elevated, but as the fat mass decreases with prolonged leptin treatment, FA levels may become normalized (9, 19, 32) or decrease below normal levels (8).

The repression of FAT/CD36, after 2 weeks of leptin treat-

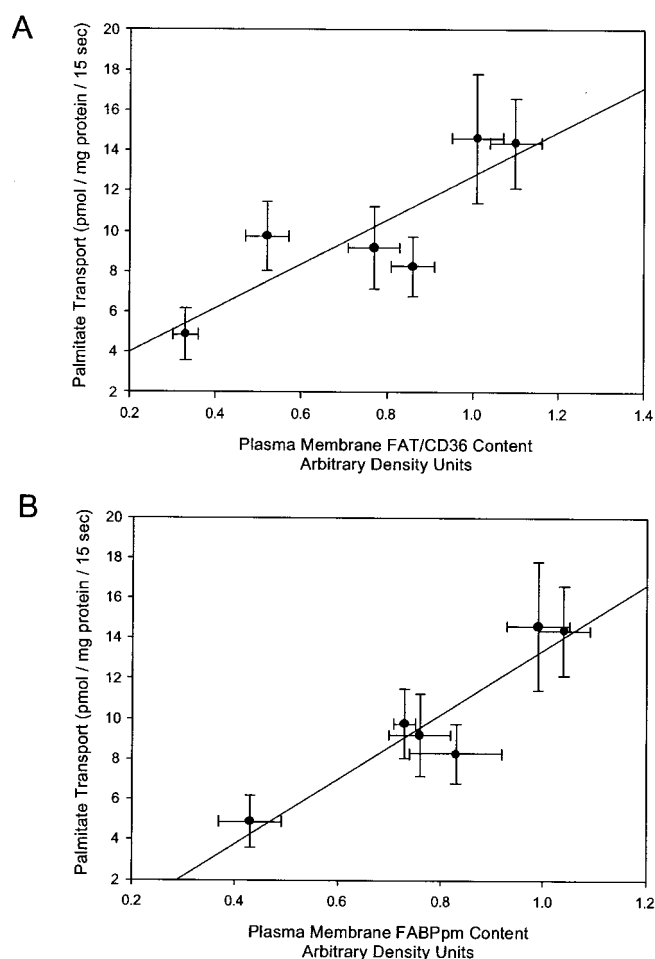


FIG. 7. Relationship between plasma membrane FAT/CD36 (A) and FABPpm (B) and palmitate transport into giant sarcolemmal vesicles. Data are from Figs. 5 and 6.

ment, is probably not attributable to the change in circulating glucose, insulin, or fatty acids. Glucose concentrations were not changed in the leptin-treated animals, and based on evidence from other studies in our laboratory, there is also no relationship between the expression of fatty acid transporters and the circulating levels of either insulin or fatty acids. For example, reductions in circulating insulin, induced by leptin (present study) or severe diabetes (34),<sup>2</sup> are associated with either a decrease (leptin) or an increase (diabetes) in fatty acid transporters. An increase in circulating insulin, such as observed in obese Zucker rats, does not alter skeletal muscle fatty acid transporter expression (35). A recent report has shown that increasing circulating fatty acids, 6-fold in excess of the normal physiological range, reduces total FAT/CD36 expression (36). But, in studies in our laboratory we do not observe a relationship between circulating fatty acids and FAT/CD36 or FABPpm expression. When circulating fatty acids are increased (severe diabetes<sup>2</sup>; obese Zucker rats (35)), there is either no change in fatty acid transporters (obese Zucker rats (35)) or there is an increase (severe diabetes) (34).<sup>2</sup> With mild diabetes, circulating fatty acids are not altered, yet fatty acid transporter expression is increased.<sup>2</sup> Thus, the available evidence suggests that neither glucose, insulin, nor fatty acids are associated with changes in the expression of FAT/CD36. Therefore, it is appropriate to conclude that the present results are attributable to leptin.

The reduced serum insulin and unaltered glucose concentrations indicated that insulin sensitivity was improved, an observation that has previously been observed following chronic leptin treatment (3, 8, 19, 31, 32). This improved insulin sensitivity is probably due to the reductions in muscle triacylglycerol depots, since the relationship between elevated intramuscular triacylglycerol depots and impaired insulin sensitivity is well established in rodents (3, 11) and humans (1, 2), although the underlying mechanism(s) are unknown. It has been suggested that intramuscular triacylglycerol depots may reduce insulin sensitivity by impairing the insulin-signaling pathway (37, 38). Since fatty acid transport in the leptin-treated animals was reduced, this may also contribute to reducing the intramuscular triacylglycerol depots and in this manner contribute to the improved insulin sensitivity observed in leptin-treated animals.

The giant sarcolemmal vesicle preparation used in our studies allows for a true measurement of FA transport, independent of metabolism (12). Unlike other preparations that have been used to measure FA transport, such as hepatocytes, cardiomyocytes, and adipocytes (28, 29), giant sarcolemmal vesicles provide many advantages. We (12) have shown that, in giant sarcolemmal vesicles, (a) initial rates of FA uptake can be determined; (b) giant vesicles contain FABPc in excess, which provides for a large intravesicular fatty acid sink; (c) all of the palmitate taken up by the vesicles is fully recovered as unesterified palmitate (*i.e.* none of the palmitate taken up is esterified, oxidized, or associated with the plasma membrane); and (d) vesicles are 100% oriented right side out. Therefore, the giant sarcolemmal vesicle preparation used in the present study provides an appropriate model with which to examine leptin's effects on FA transport in skeletal muscle.

In the present study, we observed that chronic leptin treatment reduces palmitate transport into giant sarcolemmal vesicles. This reduction was associated with concomitant reductions in plasma membrane FAT/CD36 and FABPpm proteins. Previously, we have shown that fatty acid uptake in heart and skeletal muscle is highly correlated with the fatty acid transporters, FAT/CD36 and FABPpm, but not FATP1, located at the plasma membrane (12, 39). This correlation between these plasmalemmal fatty acid transporters and fatty transport was confirmed in the present studies (Fig. 7). It is believed that FABPpm and FAT/CD36 may interact with each other to facilitate fatty acid uptake across the sarcolemma (12), but the specific role of each transporter has not been completely elucidated. It is known that both proteins are critical for mediating fatty acid transport in skeletal muscle, because blocking of either transporter results in significantly reduced rates of fatty acid uptake (39). While chronic leptin treatment led to significant reductions in both FAT/CD36 and FABPpm protein in the plasma membrane of skeletal muscle, the mechanisms by which these reductions occurred were different for the two transport proteins.

The regulation of expression of FAT/CD36 and FABPpm has been examined in only a few studies. At the level of their mRNAs, one or both of these transporters are altered in some but not all models of genetic obesity and diabetes, and this seems to depend also on the tissue being examined (26–29). Altering the metabolic demands of the muscle by chronic muscle contraction for 7 days (23) has been demonstrated to increase the expression of FAT/CD36 and fatty acid transport rates in skeletal muscle. In the present experiments, leptin decreased both the FAT mRNA abundance and the expression of FAT/CD36 protein in red and white skeletal muscles, suggesting that prolonged exposure to leptin reduced the transcription of FAT. Contrary to the effects on FAT/CD36, leptin

<sup>2</sup> J. J. F. P. Luiken, unpublished data.

did not alter the FABPm mRNA abundance or its protein product. Thus, in muscle, leptin alters the expression of FAT/CD36, but not FABPm.

Our studies demonstrate clearly that whether or not the expression of the fatty acid transport proteins are altered, fatty acid transport can be lowered due to a reduction in plasmalemmal FAT/CD36 and FABPm. We (17) have recently shown that FAT/CD36 is located both at the plasma membrane and in an intracellular (endosomal) depot. Muscle contraction causes a translocation of the FAT/CD36 transporter from endosomal compartments to the plasma membrane within 5 min of the onset of stimulation, leading to an increase in fatty acid transport rates (17). Thus, the plasmalemmal localization of FAT/CD36 can be regulated independently of the total available pool, analogous to the regulation of GLUT-4. However, in the present study, the leptin-induced reductions in plasmalemmal FAT/CD36 would seem to be attributable to the reduced expression of this protein and not its intracellular redistribution.

In contrast, the leptin-induced reductions in plasmalemmal FABPm cannot be explained by reductions in the total pool of this transporter, since the total FABPm availability was not affected by leptin treatment. This suggests that the localization of FABPm in the plasma membrane is also an important means to regulate fatty acid uptake. The selective reduction in plasma membrane FABPm in the face of unaltered total quantities of muscle FABPm protein content suggests that there may therefore also be an intracellular pool of FABPm. Indeed, we now have preliminary evidence for this suggestion.<sup>3</sup>

Dietary and genetic models of rodent obesity and diabetes are characterized by either a lack of leptin (ob/ob mice) (40), or leptin receptor defects (db/db mice and obese Zucker (fa/fa) rat) (41, 42). In skeletal muscles of obese Zucker (fa/fa) rat, FAT/CD36 and FABPm expression are not altered, but there is an increased rate of fatty acid transport, due to an increase in plasmalemmal FAT/CD36 (35). In other studies, it has been shown that transcripts of FATCD36, FABPm, and FATP1 are increased in liver and adipose tissue of ob/ob and db/db mice and obese Zucker fa/fa rats (27–29). Based on these foregoing studies, it seems plausible that the lack of leptin action leads to the overexpression of FAT/CD36 and FABPm (27–29) and/or the subcellular redistribution of FAT/CD36 (35). These effects may be associated with increased rates of fatty acid uptake, with a resultant accumulation of intracellular triacylglycerol depots in muscle, liver, and adipose tissue in these models (27–29).

We have clearly demonstrated that chronic leptin exposure represses FAT/CD36. This may however not occur in obese humans, who characteristically exhibit elevated circulating leptin levels, since in such individuals skeletal muscle FABPm is increased (43). It may be possible that during the development of human obesity, skeletal muscle becomes resistant to leptin, resulting in the overexpression of the fatty acid transporters, leading to the accumulation of intramuscular triacylglycerol depots. We (5) have previously demonstrated that rodent skeletal muscle becomes resistant to leptin following the consumption of high fat diets. The apparently different responses to leptin in rodents and humans suggest that there can be species differences in the molecular responses to leptin.

A number of studies have shown that leptin reduces body weight, due to a large reduction in fat mass (19, 33). Concomitantly, skeletal muscle intramuscular triacylglycerol depots are also reduced (3, 32). Presumably, the loss of fat mass indicates an enhanced rate of fatty acid metabolism. Indeed, a

number of reports have shown that acute (<60-min) exposure to leptin augments fatty acid oxidation in isolated skeletal muscle (5–7). Therefore, it seems somewhat anomalous that with chronic leptin treatment fatty acid transport is reduced. However, a primary function of leptin may be to limit the accumulation of intramuscular triacylglycerol depots, as has been speculated by others (44). Thus, leptin-stimulated increases in the rates of fatty acid oxidation and triacylglycerol hydrolysis, along with reduced rates of esterification (5–7), can be seen as effective mechanisms to lower intramuscular triacylglycerol depots. Hence, limiting fatty acid entry into the myocyte over the long term may also be part of the strategy to reduce the intramuscular triacylglycerol depots. These foregoing suggestions require further study.

The leptin effects on fatty acid transport and transporters were more pronounced in oxidative types of muscle when compared with the more glycolytic types of muscle. Similarly, in CD36 null mice, a greater reduction in fatty acid uptake occurred in oxidative muscles than in glycolytic muscles (45). When we examined the effects of reduced muscle activity (denervation) on the changes in glucose transport and transporters (46, 47) and lactate transport (48) and monocarboxylate transporters (49), the greatest effects were also observed in the more oxidative types of muscles. Thus, it appears that oxidative types of skeletal muscle are more susceptible to alterations in their substrate transport capacities and transporter expression than glycolytic muscles. The basis for this susceptibility is not known.

In conclusion, the present study has demonstrated that chronic leptin treatment reduces circulating insulin and fatty acid levels and decreases the storage of intramuscular triacylglycerol depots in skeletal muscle. In addition, leptin reduces the content of FAT/CD36 and FABPm in the plasma membrane of both red and white skeletal muscle, leading to a reduction in fatty acid transport. These plasma membrane reductions in FABPm occurred in the face of unaltered levels of FABPm mRNA and FABPm protein expression, while reduced plasma membrane FAT/CD36 is due to reductions in FAT mRNA and FAT/CD36 protein. Thus, chronic leptin treatment limits the uptake of fatty acids by skeletal muscle.

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