THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 44, Issue of November 2, pp. 40567–40573, 2001
Printed in U.S.A.

Increased Rates of Fatty Acid Uptake and Plasmalemmal Fatty Acid Transporters in Obese Zucker Rats*

Received for publication, January 3, 2001, and in revised form, August 13, 2001
Published, JBC Papers in Press, August 14, 2001, DOI 10.1074/jbc.M100052200

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Giant vesicles were used to study the rates of uptake of long-chain fatty acids by heart, skeletal muscle, and adipose tissue of obese and lean Zucker rats. With obesity there was an increase in vesicular fatty acid uptake of 1.8-fold in heart, muscle and adipose tissue. In some tissues only fatty acid translocase (FAT) mRNA (heart, +37%; adipose, +80%) and fatty acid-binding protein (FABPpm) mRNA (heart, +148%; adipose, +196%) were increased. At the protein level FABPpm expression was not changed in any tissues except muscle (+14%), and FAT/CD36 protein content was altered slightly in adipose tissue (+26%). In marked contrast, the plasma membrane FAT/CD36 protein was increased in heart (+60%), muscle (+80%), and adipose tissue (+50%). The plasma membrane FABPpm was altered only in heart (+50%) and adipose tissues (+70%). Thus, in obesity, alterations in fatty acid transport in metabolically important tissues are not associated with changes in fatty acid transporter mRNAs or altered fatty acid transport protein expression but with their increased abundance at the plasma membrane. We speculate that in obesity fatty acid transporters are relocated from an intracellular pool to the plasma membrane in heart, muscle, and adipose tissues.

Fatty acids (FA)[1] are important substrates for most mammalian tissues. Based on their hydrophobic structure it has been postulated that FA are sequestered by cells through passive diffusion across the plasma membrane (*cf.* Ref. 1). However, other evidence has shown that FA also traverse the plasma membrane via a protein-mediated mechanism (*cf.* Refs. 2 and 3). Indeed, this latter system is quantitatively more important than passive diffusion, as FA uptake can be reduced markedly by inhibitors of protein-mediated membrane transport (4–6) and by a reactive ester of oleate (4). Thus, a number of groups began to search for FA transport proteins.

Several putative fatty acid transport proteins have been identified that promote the cellular uptake of FA. These are a 43-kDa plasma membrane fatty acid-binding protein (FABPpm) (7), identical to mitochondrial aspartate aminotransferase (7–9), and an 88-kDa heavily glycosylated fatty acid translocase (FAT/CD36), the rat homologue of human CD36 (10). In addition, to these membrane-associated proteins, a soluble cytoplasmic fatty acid-binding protein (FABPc) is also important for cellular FA uptake, because in FABPc null mice there is a marked decrease of FA influx into cardiac myocytes (11). FATP1, another putative fatty acid transport protein (12, 13), correlates inversely with fatty acid transport in muscle and heart (4), and this protein appears to be a very long-chain acyl-CoA synthetase (14, 15). These observations suggest that FATP1 is unlikely to be involved directly in fatty acid translocation across the plasma membrane.

To examine the regulation of transmembrane fatty acid transport, we have characterized giant vesicles (4, 16, 17), which can be prepared from metabolically important tissue such as heart (4) and skeletal muscle (16, 17) as well as adipose tissue (present study). These giant vesicles are oriented right-side-out, and they contain the cytosolic fatty acid-binding protein, FABPc, which acts as a FA sink (4, 16) Importantly, in giant vesicles, FA uptake across the plasma membrane can be examined in the absence of any confounding effects of FA metabolism (4, 16, 17), which has been a problem in studies using respiring cells such as adipocytes (18, 19), hepatocytes (20, 21), and cardiac myocytes (6, 22). With giant vesicles we have shown that fatty acid transport can be increased (*a*) when the number of FAT/CD36 transport proteins are increased over the course of several days (23) or (*b*) when the FAT/CD36 protein is translocated, within minutes, from an intracellular pool to the plasma membrane (17).

It is becoming increasingly evident that disorders in lipid metabolism are an important factor contributing to the etiology of a number of common human diseases such as diabetes and obesity (*cf.* Ref. 24). Our evidence that protein-mediated FA uptake is a rate-limiting step in lipid metabolism allows the attractive speculation that malfunctioning of the FA uptake

* These studies were supported by grants from the Canadian Institutes of Health Research (to A. B.), the Heart and Stroke Foundation of Ontario (to A. B.), The Netherlands Heart Foundation (to J. J. F. P. L. and J. F. C. G.), the National Institutes of Health (AR45168 to L. P. T.), and the Natural Sciences and Engineering Research Council of Canada (to A. B., D. J. D., and R. C. B). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: FA, fatty acid; FABPpm, fatty acid-binding protein; FAT, fatty acid translocase; FABPc, cytoplasmic fatty acid-binding protein; H-FABPc, heart-type cytoplasmic fatty acid-binding protein; MOPS, 4-morpholinepropanesulfonic acid.

process could be a common critical factor in the pathogenesis of these diseases. The obese Zucker rat, a widely used rodent model for obesity and insulin resistance, has a mutation in the gene encoding the receptor for leptin. Homozygosity for this mutation is responsible for alterations in body lipid homeostasis resulting in the accumulation of body fat mass (25). Recent studies using obese Zucker rats (19) and other rodent models of obesity (26, 27) have shown tissue-specific changes in FA transporters at the mRNA and FA utilization levels (*i.e.* fatty acid uptake + metabolism). Up-regulation of FA utilization and transporter mRNAs in adipocytes of obese Zucker rats occur early in life preceding peripheral insulin resistance and obesity, suggesting that accumulation of FA transporters in the plasma membrane of adipocytes may be a critical step leading to obesity (19).

However, these changes in the accumulation of transporters measured at the mRNA level only (19, 26, 27) do not provide evidence that similar changes are occurring either at the protein level or in FA uptake across the plasma membrane (vesicular FA transport). Measurements of FA uptake in isolated cell systems are confounded by the subsequent rapid activation of FA to their CoA esters (19, 26). Importantly, our discovery of an intracellular storage pool of FAT/CD36 (17) suggests that in addition to determining the total amount of FA transporters, their subcellular localization also needs to be considered so as to link properly the involvement of FA transporters with FA uptake rates. Therefore, to examine tissue-specific changes in vesicular fatty acid transport and the subcellular location of FA transporters in obese Zucker rats: (*a*) we examined the levels of the two functionally important FA transporters (FAT/CD36 and FABPpm), both at the mRNA and protein level, as well as FABPc in metabolically important tissues (heart, muscle, adipose tissue); (*b*) we measured FA uptake rates in these tissues using giant vesicles in which FA uptake can be measured in the complete absence of FA metabolism; and (*c*) because giant vesicles have been proven suitable representatives of the plasma membrane (17, 28), we used these vesicles to provide information on the plasmalemmal localization of FA transporters. Our studies demonstrate that in obesity vesicular FA uptake rates are markedly increased, but this is generally not associated with concomitant changes in the expression of fatty acid transporters. Instead, the increases in fatty acid transport are associated with changes in plasmalemmal fatty acid transporters.

EXPERIMENTAL PROCEDURES

Materials—[9,10-^{3}H]palmitate (American RadioChemicals, St. Louis, MO) and [^{14}C]mannitol (ICN, Oakville, Ontario, Canada) were purchased from commercial sources. Collagenase type II was from Worthington, and collagenase 2a and collagenase VII were from Sigma-Aldrich. Fat-free bovine serum albumin was obtained from Roche Diagnostics (Laval, Quebec, Canada).

Animals—Female obese (fa/fa) and lean (fa/+) Zucker rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN). At 3 months of age, they were anesthetized with an intraperitoneal injection of Somnotol. Subsequently, hind limb muscle, epididymal adipose tissue, and heart were removed for FA uptake studies and for determination of FA transporters.

Metabolite Assays—Blood samples were collected into NaF/heparinized microcentrifuge tubes and centrifuged in a microcentrifuge. Plasma was then separated from red blood cells and stored at −80 °C until use. Glucose was determined by a spectrophotometric method (Sigma-Aldrich). Insulin was determined by radioimmunoassay using a rat-specific antibody (Linco, St. Charles, MO). Plasma fatty acids and triacylglycerols were determined using spectrophotometric procedures (Wako Chemicals, Richmond, VA, and Sigma-Aldrich, respectively).

Isolation of Giant Vesicles—Giant vesicles from skeletal muscle and heart were isolated as described previously (4, 17, 29). Minor modifications were introduced to prepare giant vesicles from adipose tissue.

TABLE I
Characteristics of lean and obese Zucker rats

Parameter	Lean (fa/+)[a]	Obese (fa/fa)[a]
Body weight (g)	188.2 ± 17.9 (17)	323.8 ± 16.1[b] (17)
Heart weight (g)	0.55 ± 0.04 (7)	0.70 ± 0.06[b] (9)
Hind limb, lower leg muscle used (g)	1.84 ± 0.27 (10)	1.73 ± 0.23 (10)
Epididymal fat pad (g)	4.48 ± 1.17 (10)	>20[b] (10)
Free fatty acids (mM)	0.73 ± 0.10 (8)	1.39 ± 0.18[b] (10)
Triacylglycerols (mg/100 ml)	64.5 ± 17.0 (8)	426.5 ± 12.9 [b] (10)
Insulin (ng/ml)	1.20 ± 0.18 (8)	4.92 ± 0.56[b] (10)
Glucose (mM)	9.48 ± 0.86 (8)	9.98 ± 0.61 (10)

[a] Data are mean ± S.E. The number for each parameter is indicated in parentheses.
[b] $P < 0.05$, obese *versus* lean.

Briefly, all of the tissues were cut into thin layers (1–3 mm thick) and incubated for 1 h at 34 °C in 140 mM KCl, 10 mM MOPS (pH 7.4), aprotinin (10 mg/ml), and collagenase in a shaking water bath. Collagenase type VII (150 units/ml) was used for muscle tissue, collagenase type II (0.3%, w/v) was used for heart, and collagenase type 2a (0.05%, w/v) was used for adipose tissue. At the end of the incubation, the supernatant was collected, and the remaining tissue was washed with KCl/MOPS and 10 mM EDTA, which resulted in a second supernatant. Both supernatant fractions were pooled, and Percoll and aprotinin were added to final concentrations of 16% (v/v) and 10 mg/ml, respectively. The resulting suspension was placed at the bottom of a density gradient consisting of a 3-ml middle layer of 4% Nycodenz (w/v) and a 1-ml KCl/MOPS upper layer. This sample was centrifuged at 60 × *g* for 45 min at room temperature. Subsequently, the vesicles were harvested from the interface of the upper and middle layers, diluted in KCl/MOPS, and recentrifuged at 900 × *g* for 10 min. In case of skeletal muscle, the pellet was resuspended in KCl/MOPS to a protein concentration of 2–3 mg/ml, in case of the other tissues, the pellet was resuspended to a protein concentration of 0.4–0.8 mg/ml.

Palmitate Uptake by Giant Vesicles—Palmitate uptake studies were performed as we have described previously (4, 16, 23). Briefly, 40 μl of 0.1% bovine serum albumin in KCl/MOPS containing unlabeled (15 μM) and radiolabeled 0.3 μCi of [^{3}H]palmitate and 0.06 μCi of [^{14}C]mannitol were added to 40 μl of vesicle suspension. The incubation was carried out for 15 s. Palmitate uptake was terminated by the addition of 1.4 ml of ice-cold KCl/MOPS, 2.5 mM $HgCl_2$, and 0.1% bovine serum albumin. The sample was then quickly centrifuged in a microcentrifuge at 12,000 rpm for 1 min. The supernatant was discarded, and radioactivity was determined in the tip of the tube. Nonspecific uptake was measured by adding the stop solution prior to addition of the radiolabeled palmitate solution.

Northern and Western Blotting—mRNA abundance of FAT and FABPpm were determined as described previously (30). The cDNA For FAT/CD36 (10) was kindly provided by Dr. N. A. Abumrad (State University of New York, Stony Brook, NY), and the cDNA for mitochondrial aspartate aminotransferase/FABPpm (31) was a gift from Dr. A. Iriarte (University of Missouri). FAT/CD36 and FABPpm protein content were determined in both homogenates and giant vesicles prepared from heart and muscle as we have described previously (4, 16, 17, 23, 32) and from adipose tissue using essentially the same procedures. For detection of FAT/CD36 and FABPpm, we used a monoclonal antibody (MO25) against human CD36 (33) and a rabbit polyclonal anti-FABPpm antiserum, respectively, as described previously (4, 17, 34). For detection of monocarboxylate transporter 1 (MCT1), we used a polyclonal antibody (32).

Cytoplasmic H-FABP Determinations—The contents of heart-type cytoplasmic FABP (H-FABPc) in homogenates and giant vesicles from heart and skeletal muscles were determined by a sandwich-type enzyme-linked immunosorbent assay as previously described (35, 36). Because of the unavailability of the appropriate antibody we were not able to determine the adipocyte lipid binding protein (A-LBP), the predominant FABPc in adipose tissue.

Vesicle Sizing—Giant vesicles were prepared from heart, muscle and adipose tissue from lean rats and obese Zucker rats. These were overlain with a scaling grid and were then photographed through a phase contrast microscope. Vesicle diameter sizes were determined from these photographs.

TABLE II
Cytosolic H-FABP content in homogenates and vesicles from tissues of lean and obese Zucker rats

FABP type	Homogenate[a]		Vesicles[a]	
	Lean (fa/+)	Obese (fa/fa)	Lean (fa/+)	Obese (fa/fa)
H-FABP (mg/g) heart	2.83 ± 0.57 (6)	2.34 ± 0.33 (8)	16.3 ± 2.7 (5)	16.0 ± 1.7 (6)
H-FABP (mg/g) muscle	0.47 ± 0.03 (6)	0.55 ± 0.08 (5)	1.14 ± 0.43 (3)	1.57 ± 0.33 (4)

[a] Data are mean ± S.E. The number of samples is indicated in parentheses.

TABLE III
Sizes of giant vesicles prepared from different tissues of lean and obese Zucker rats

Comparisons in heart and muscle are based on 125 vesicles measured in each tissue from five lean and five obese Zucker rats. Vesicles from epididymal fat pad were measured in three lean (75 vesicles) and three obese rats (63 vesicles). Data are mean ± S.E.

Tissue	Vesicle diameter	
	Lean (fa/+)	Obese (fa/fa)
	μm	
Heart	12.6 ± 0.5	12.8 ± 0.4
Leg muscle	14.3 ± 0.7	14.1 ± 0.6
Epididymal fat pad	14.8 ± 0.5	14.4 ± 0.6

Statistics—The data were analyzed using analyses of variance. All data are reported as mean ± S.E.

RESULTS

Characterization of Zucker Rats

Zucker obese rats had a markedly greater (1.7-fold) body weight than their lean counterparts (Table I). Their hearts were slightly (1.3-fold) but significantly enlarged. This increase in mass is likely due to cellular hypertrophy rather than to hyperplasia for the heart (37). Adipose tissue exhibited the most prominent increase in mass as exemplified by the more than 4-fold increase in the amount of epididymal fat. This mass increase is known to be caused by increases in both cell size and cell number (38). There was no difference between obese and lean animals in the quantity of skeletal muscle mass (*i.e.* hind limb muscles of the lower leg) used in the study (Table I).

The obese Zucker rats exhibited chronically increased blood lipid levels. The circulating FA and triacylglycerols concentrations were 1.9-fold and 6.6-fold higher in the obese Zucker rats. They were also insulin resistant, as indicated by the 4.1-fold higher insulin concentrations but unaltered plasma glucose concentration (Table I). All these results (Table I), are consistent with previous reports concerning this rat model of obesity (39, 40).

Giant Vesicle Characterizations in Lean and Obese Zucker Rats

The ratio of plasma membrane protein/total protein in lean and obese animals did not differ in either heart, muscle or adipose tissue (data not shown). The presence of FABPc in the intravesicular lumen is a prerequisite for uptake of FA by giant vesicles, because these proteins act as a FA sink. In heart and muscle, the predominant FABPc isoform is the heart (H)-type FABPc (4). As we have observed previously (4), H-FABPc is more abundant in giant vesicles from heart than from muscle (Table II). In Zucker obese rats the concentrations of heart and muscle H-FABPc in homogenates and giant vesicles were not significantly different from that found in lean heart and muscle.

The diameters of the giant vesicles obtained from heart, skeletal muscle and adipose tissue did not differ in obese and lean animals (Table III). Giant vesicles diameters in heart and muscle correspond closely to those reported previously for Sprague-Dawley rats (4, 41).

Fatty Acid Transport by Metabolically Important Tissues in Lean and Obese Zucker Rats

FA uptake was measured in heart, muscle and adipose tissue from obese and lean rats using giant vesicles, to divorce the uptake of FAs from their subsequent metabolism. Vesicular FA uptake rates were increased in heart (1.8-fold, $p < 0.05$, Fig. 1*A*), skeletal muscle (1.8-fold, $p < 0.05$, Fig. 1*D*) and adipose tissue (1.8-fold, $p < 0.05$, Fig. 1*G*) of the obese Zucker rats compared with their lean littermates.

Content and Localization of FAT/CD36 and FABPpm in Metabolically Important Tissues in Lean and Obese Zucker Rats

In Northern blots from all tissues studied (Fig. 2), the FABPpm transcript was found at 2.4 Kb, which corresponds to observations in rat heart and skeletal muscles in other studies (43). FAT mRNA (Fig. 2) was found at two sizes (2.9 and 2.0 Kb) in all tissues examined. This is also in agreement with other reports (27, 42). Changes in both sizes were similar and therefore only the results for the major transcript size are shown (Figs. 2 and 3).

FA Transporters in Heart—FAT mRNA was significantly up-regulated in obese hearts (1.4-fold, $p < 0.05$), whereas FABPpm mRNA was somewhat more up-regulated (2.5-fold, $p < 0.05$) (Fig. 3). In contrast, there was no difference in the amount of the total available FAT/CD36 and FABPpm transport proteins in lean and obese hearts ($p > 0.05$) (Figs. 1*B* and 4). However, the sarcolemmal content of FAT/CD36 was 1.6-fold higher ($p < 0.05$) and that of FABPpm 1.5-fold higher ($p < 0.05$) in hearts from obese Zucker rats compared with lean rats (Figs. 1*C* and 4).

FA Transporters in Skeletal Muscle—When compared with muscle of lean rats, there were no significant difference in mRNA levels of FAT and of FABPpm in muscles from obese rats ($p > 0.05$, Fig. 3). At the protein level, the total available pool of FAT/CD36 was not changed whereas there was only a small increase (+14%) in FABPpm in the obese rats ($p < 0.05$, Figs. 1*E* and 4). In contrast, the muscle sarcolemmal content of FAT/CD36 was markedly increased (1.8-fold, $p < 0.05$, Figs. 1*F* and 4) in obese rats, whereas there was no significant difference in the sarcolemmal presence of FABPpm in muscles from obese and lean rats ($p > 0.05$, Figs. 1*F* and 4).

FA Transporters in Adipose Tissue—A marked increase in FAT/CD36 mRNA (1.8-fold, $p < 0.05$) and in FABPpm mRNA (3-fold, $p < 0.05$) was observed in epididymal fat pads of obese Zucker rats (Fig. 3). The amount of total available FAT/CD36 protein was modestly increased (1.3-fold, $p < 0.05$, Figs. 1*H* and 4) in adipose tissue in obese animals. There was no significant difference in the total available FABPpm protein in lean and obese animals ($p > 0.05$, Figs. 1*H* and 4). Plasma membrane contents of FAT/CD36 and FABPpm were increased 1.5-fold ($p < 0.05$, Figs. 1*I* and 4) and 1.7-fold ($p < 0.05$, Figs. 1*I* and 4), respectively.

MCT1 in Plasma Membranes—To determine whether the increases in plasma membrane fatty acid transporters were specific, we also measured the MCT1 in plasma membranes of all tissues examined (Fig. 5). Except for adipose tissue plasma

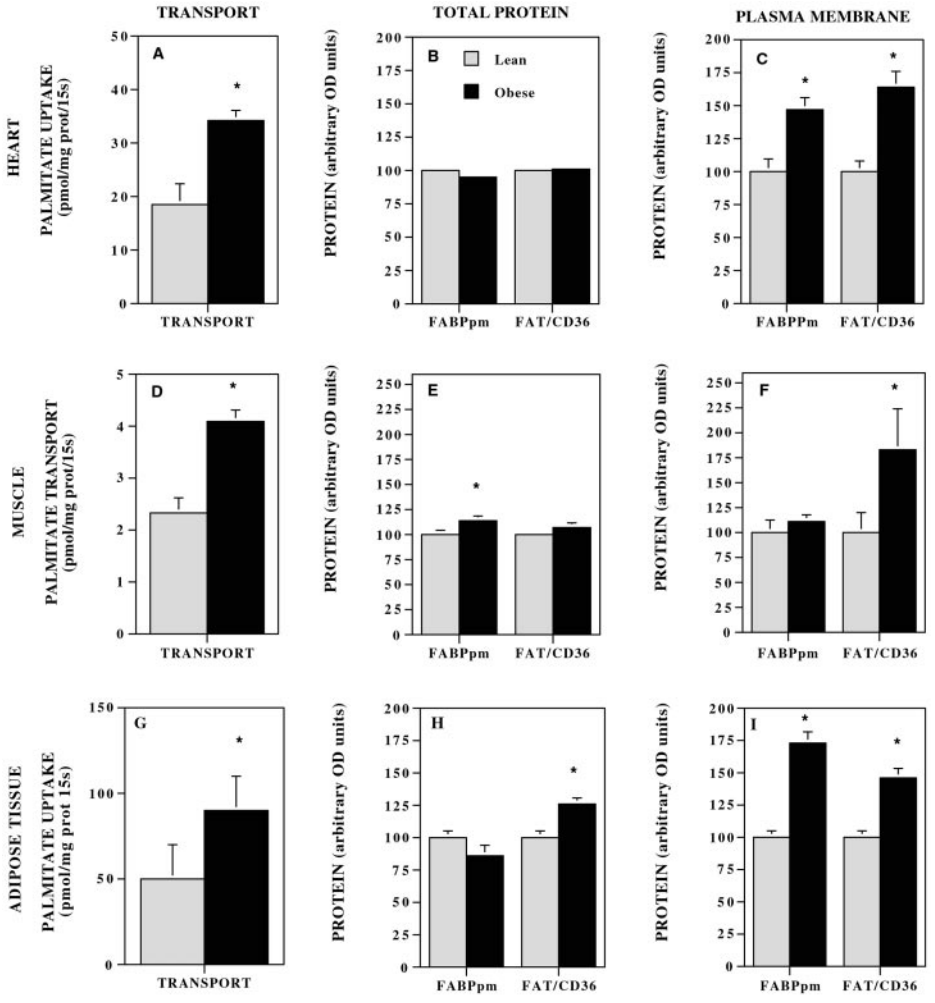

FIG. 1. **Palmitate uptake by giant vesicles and fatty acid transport proteins (FAT/CD36 and FABPpm) in tissue homogenates (total protein) and plasma membranes of giant vesicles in heart muscle and adipose tissue from obese Zucker rats (fa/fa) and their lean littermates (fa/+).** Transport measurements are based on 4–8 vesicle preparations in each group, total FAT and FABPpm measurements are based on 7–11 preparations in each group except in adipose tissue where there were 4 preparations in each group, and finally, plasma membrane FAT/CD36 and FABPpm determinations were made on 4–7 preparations in each group. Procedures are described under "Experimental Procedures." Data are mean ± S.E. *, $p < 0.05$, obese *versus* lean.

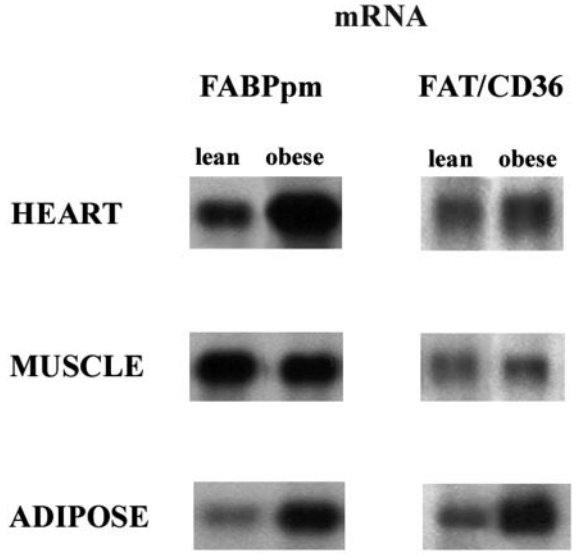

FIG. 2. **Representative Northern blots of FABPpm and FAT/CD36 in heart, muscle, and adipose tissue from obese Zucker rats (fa/fa) and their lean littermates (fa/+).** Northern blotting was performed as described under "Experimental Procedures."

membranes, the MCT1 content in the plasma membranes obtained from heart and muscle did not differ between lean and obese animals.

DISCUSSION

We investigated the vesicular uptake of FA by metabolically important tissues (heart, skeletal muscle, and adipose tissue) in obese Zucker rats and their lean littermates, using the novel model of giant vesicles in which FA uptake rates are divorced from cellular FA metabolism. We report here for the first time the changes in fatty acid uptake in these metabolically important tissues in obese Zucker rats in relation to the FAT and FABPpm transporters at the mRNA and at the protein levels. Importantly, we examined the total available protein levels of FAT/CD36 and FABPpm, as well as their presence on the plasma membrane.

Our studies demonstrate that, generally, neither the FAT mRNA and FABPpm mRNA abundance nor the total available pool of FAT/CD36 and FABPpm proteins show any consistent relation with vesicular FA transport in a number of tissues. Instead, the increased abundance of FA transporters at the plasma membrane, in heart, muscle, and adipose tissue is most closely associated with changes in vesicular FA uptake in metabolically important tissues of obese Zucker rats. Of the two transport proteins, plasma membrane FAT/CD36 always paralleled the changes in vesicular FA uptake in all tissues, whereas this was observed only for plasma membrane FABPpm in heart and adipose tissue.

Our studies are the first in which FA uptake has been measured in giant vesicles from heart, muscle, and adipose tissue of obese Zucker rats. The differences in FA uptake by heart and muscle in lean and obese animals cannot be explained by differences in cytoplasmic FABPs, because these protein levels

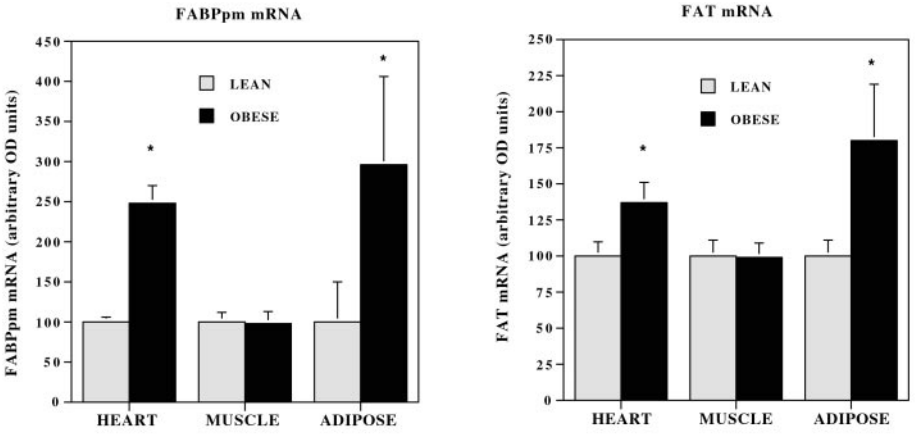

FIG. 3. **FAT mRNA and FABPpm mRNA in heart, muscle, and adipose tissue from obese Zucker rats (fa/fa) and their lean littermates (fa/+).** Measurements were based on three animals in each group. The data were normalized for loading using the 28-S RNA signal. Procedures are described under "Experimental Procedures." Data are mean ± S.E. *, $p < 0.05$, obese *versus* lean.

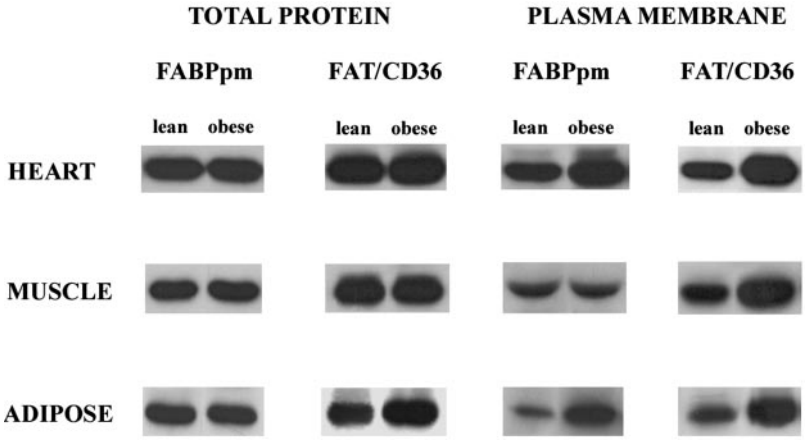

FIG. 4. **Representative Western blots of FABPpm and FAT/CD36 proteins in heart, muscle and adipose tissue from obese Zucker rats (fa/fa) and their lean littermates (fa/+).** *Total Protein* refers to the total available FABPpm and FAT/CD36 proteins in tissue homogenates, whereas *Plasma Membrane* refers to the FABPpm and FAT/CD36 proteins located on the plasma membrane of giant vesicles of the various tissues. Western blotting was performed as described under "Experimental Procedures."

were similar in these tissues from obese and lean animals. Similarly, giant vesicle sizes were also similar in these tissues in the lean and obese animals. Thus, in none of the tissues examined can we explain differences in vesicular FA transport in obese and lean animals on the basis of differences in cytoplasmic FABPs or vesicle sizes.

The increased FA transport in obese skeletal muscle is a novel observation, as there are no other studies that have examined FA transport in obese animals in this tissue. The increase in vesicular FA transport in the obese heart parallels the increase in FA utilization observed by Berk *et al.* (19) in obese Zucker rats. However, they used cardiac myocytes, in which FA uptake and metabolism occur concomitantly, and thus, this preparation does not necessarily reflect solely FA uptake across the plasma membrane. In the same study Berk *et al.* (19) also observed an increase in adipocyte FA uptake (*i.e.* FA transport + metabolism) in obese Zucker rats; *i.e.* a 6.6-fold increase/fat cell or an ~3-fold increase when increased cell surface area is taken into account. This 3-fold increase in FA uptake (*i.e.* FA transport + metabolism) compares favorably with the 2-fold increase in vesicular fatty acid uptake in the present study. Thus, the increases in FA uptake in heart and adipose tissues of obese Zucker rats in our studies using giant vesicles and in the studies of Berk *et al.* (19) with respiring cardiac myocytes and adipocytes are qualitatively similar.

Although changes in transporter mRNA levels could be associated with the changes in FA uptake by the different tissues, as has been argued by others (19, 26, 27), it must be stressed that there was not always a clear relationship between FABPpm mRNA or FAT mRNA and the changes in the total available FA transport proteins, their content in the plasma membrane, or FA transport. Except for the small change in skeletal muscle total available FABPpm protein (+14%), there were no changes in the total available FABPpm in any of the other tissues in the obese Zucker rats. There were small changes in total available FAT/CD36 protein in adipose tissue (+30%), but no changes in total FAT/CD36 protein in muscle or heart. We can only speculate about the consistent lack of correlation between mRNA levels and protein levels of FA transporters in obese tissues. It may be that the change in messengers predisposes the obese tissues to react more quickly to changes in nutritional states via a second level of regulation at the translational level. Alternatively, both transcription and translation may be coordinately regulated, whereas there is additional regulation at the level of protein degradation. In any case, the total available protein levels of the FA transporters in the various tissues examined do not offer an adequate explanation for the differences in vesicular FA uptake between lean and obese tissues.

A clear advantage in our studies was the ability to examine in giant vesicles, from a number of tissues, both the FA uptake and the presence of the FA transporters in the plasma membrane of these vesicles. Obesity induced marked increases in FAT/CD36 protein in the sarcolemma of both heart and muscle. Because the total tissue amount of the FAT/CD36 transporter did not change significantly in the heart and muscle, it is possible that in heart and muscle a portion of the intracellular FAT/CD36 pool is permanently relocated to the sarcolemma during obesity. A similar inference may be drawn for adipose tissue, in which the plasma membrane FAT/CD36 was increased considerably more (+46%) than the increase (+26%) in total available FAT/CD36. Alternatively, one or more of the putative the regulatory steps involved in the endocytosis of the fatty acid transporters is impaired.

The novel observation in the present study is that increases in vesicular FA uptake by heart, skeletal muscle, and adipose

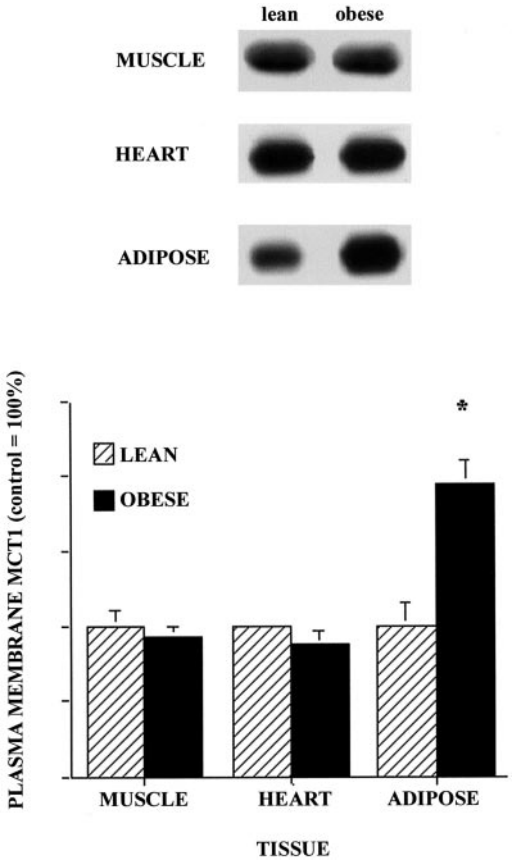

FIG. 5. **MCT1 proteins in the plasma membrane of giant vesicles obtained from heart, muscle, and adipose tissue from obese Zucker rats (fa/fa) and their lean littermates (fa/+).** Representative Western blots are shown *above* the *bar graphs*. Western blotting was performed as described under "Experimental Procedures." MCT1 measurements are based on four preparations in each group. Data are mean ± S.E. *, $p < 0.05$, obese *versus* lean.

tissue are due to the increased localization of FAT/CD36 to the plasma membrane. Because the total amount of FAT/CD36 within the cell did not change, it appears that there is an intracellular pool of FAT/CD36 in these tissues, although this suggestion needs to be confirmed in heart and adipose tissue. However, in muscle we have recently found evidence for the existence of an intracellular pool of FAT/CD36 (17). A portion of this pool was recruited to the sarcolemma during muscle contraction (<30 min), and the relative FAT/CD36 quantity (%) translocated corresponded to a parallel increase (%) in vesicular FA uptake (17). Thus, in muscle the relative contents of sarcolemmal and intracellular FAT/CD36 can be altered, and the translocation mechanism is sensitive to the acute (<30 min) metabolic needs of the muscle. In obesity the mechanism regulating cellular distribution of FAT/CD36 in muscle is likely to have undergone alterations. Our data suggest that a larger portion of the total FAT/CD36 is maintained at the cell surface at the expense of the intracellular storage compartment. Alterations in transporter redistribution associated with obesity have also been observed for GLUT4 in both muscle (43) and heart (44). In healthy muscles (43) and heart (45) GLUT4 is mobilized by insulin from intracellular stores to the sarcolemma, whereas in obese heart and muscles intracellular GLUT4 translocation by insulin is inhibited (43, 45). In contrast, our data show that in obese rats more FAT/CD36 is located at the plasma membrane when compared with lean animals. Taken together, we speculate that the patterns of FAT/CD36 and GLUT4 at the plasma membrane and the intracellular compartments appear to be diametrically opposed in obesity.

Another unexpected observation in our study is the difference in the surface content of FABPpm in obese heart and adipose tissue, whereas the total content of total FABPpm in these tissues was not changed. This may indicate that in the heart and adipocytes there are also intracellular pools of FABPpm that can be relocalized to the plasma membrane. In mitochondria-free muscle fractions we have found an intracellular pool of FABPpm,[2] and we are determining whether or not this intracellular FABPpm pool can be translocated to the plasma membrane. Stump *et al.* (9) have shown that FABPpm is identical to mitochondrial aspartate aminotransferase. Mitochondria are not considered to be part of short term recycling compartments, making it unlikely that this mitochondrial counterpart of FABPpm is involved in the short term translocation of FABPpm to the plasma membrane. However, this mitochondrial FABPpm pool could possibly be involved in a long term relocalization of FABPpm to the plasma membrane. This putative FABPpm relocalization is not observed in muscle in the present study. In this respect it should be noted that tissue-specific differences in transporter recycling are also observed for the glucose transporter family; whereas in the heart both GLUT1 and GLUT4 can be recruited to the sarcolemma by insulin, in skeletal muscle only GLUT4 is translocated by insulin (46).

We cannot determine from the present studies the mechanisms by which FAT/CD36 and FABPpm take fatty acids across the plasma membrane. There has been some speculation (3) and indirect evidence (4) that these transporters may act cooperatively, although definitive evidence for this hypothesis is lacking presently. Increases in vesicular FA uptake in heart, muscle, and adipose tissue reflect qualitatively similar changes in plasma membrane FAT/CD36 in all these tissues. In contrast, plasma membrane FABPpm was increased only in the heart and adipose tissue but was not altered in muscle despite the changes in vesicular FA uptake in all these tissues. Thus, exactly how FAT/CD36 and FABPpm function to facilitate the uptake of FA across the plasma membrane remains unknown. Moreover, whether they are both required is also not known.

A number of parameters were also investigated to ensure that we were examining similar giant vesicles in lean and obese animals and that the data obtained were specific to the fatty acid transport system. The giant vesicle protein recoveries and the giant vesicle diameters were similar in lean and obese animals in the three tissues examined. Also, although the plasma membrane fatty acid transporters were increased in muscle and heart, no increase was observed in the plasma membrane monocarboxylate transporter, MCT1. The increase in adipose tissue plasma membranes MCT1 would therefore seem to be a tissue-specific response to obesity, because protein recoveries did not differ between lean and obese animals in this tissue. Thus, the increases in plasma membrane fatty acid transporters in heart, muscle, and adipose tissue are not to attributable to differences in the giant vesicle protein recoveries, their diameters, or some generalized obesity-induced effect on plasma membrane proteins.

In summary, we have shown that in obese Zucker rats vesicular FA uptake is increased in heart, muscle, and adipose tissue. These alterations in vesicular FA uptake are not attributable to differences in vesicle size or to alterations in the amounts of FA transporters, notably FAT/CD36 and FABPpm, but rather to an increased abundance of these transporters at the plasma membrane, which suggests that the fatty acid

[2] J. J. F. P. Luiken and A. Bonen, unpublished data.

transporters may be relocated from an intracellular membrane pool to the plasma membrane; or alternatively, their rates of endocytosis are impaired. The use of the giant vesicle preparation, in which we can determine both FA uptake and plasma membrane protein content, was critical to uncovering the mechanism of increased rates of FA transport. The data also establish the importance of FA transporter recruitment to the plasma membrane, first observed in contracting muscle and which now appears to be important in a pathologic state in which there are metabolic changes. Therefore, other pathologic states should also be examined for possible alteration in recruitment of FA transporters. Indeed, because it is increasingly evident that alterations in lipid homeostasis precede the development of insulin resistance, a feature of type 2 diabetes, our new evidence for altered localization of FA transporters might lead to the hypothesis that the cellular machinery regulating subcellular localization of FA transporters might play a pivotal role in the development of insulin resistance.

Acknowledgments—We are indebted to Lisa Code and Dawn McCutcheon (University of Waterloo) for valuable technical assistance. We thank Dr. N. A. Abumrad (State University of New York, Stony Brook) for providing FAT cDNA and Dr. A. Iriarte (University of Missouri) for providing mitochondrial aspartate aminotransferase/FABPpm cDNA.

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Increased Rates of Fatty Acid Uptake and Plasmalemmal Fatty Acid Transporters in Obese Zucker Rats

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J. Biol. Chem. 2001, 276:40567-40573.
doi: 10.1074/jbc.M100052200 originally published online August 14, 2001
