Changes in fatty acid transport and transporters are related to the severity of insulin deficiency

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Luiken, Joost J. F. P., Yoga Arumugam, Rhonda C. Bell, Jorge Calles-Escanon, Narendra N. Tandon, Jan F. C. Glatz, and Arend Bonen. Changes in fatty acid transport and transporters are related to the severity of insulin deficiency. Am J Physiol Endocrinol Metab 283: E612–E621, 2002.—We have examined the effects of streptozotocin (STZ)-induced diabetes (moderate and severe) on fatty acid transport and fatty acid transporter (FAT/CD36) and plasma membrane-bound fatty acid binding protein (FABPpm) expression, at the mRNA and protein level, as well as their plasmalemmal localization. These studies have shown that, with STZ-induced diabetes, 1) fatty acid transport across the plasma membrane is increased in heart, skeletal muscle, and adipose tissue and is reduced in liver; 2) changes in fatty acid transport are generally not associated with changes in fatty acid transporter mRNAs, except in the heart; 3) increases in fatty acid transport in heart and skeletal muscle occurred with concomitant increases in plasma membrane FAT/CD36, whereas in contrast, the increase and decrease in fatty acid transport in adipose tissue and liver, respectively, were accompanied by concomitant increments and reductions in plasma membrane FABPpm; and finally, 4) the increases in plasma membrane transporters (FAT/CD36 in heart and skeletal muscle; FABPpm in adipose tissue) were attributable to their increased expression, whereas in liver, the reduced plasma membrane FABPpm appeared to be due to its relocation within the cell in the face of slightly increased expression. Taken together, STZ-induced changes in fatty acid uptake demonstrate a complex and tissue-specific pattern, involving different fatty acid transporters in different tissues, in combination with different underlying mechanisms to alter their surface abundance.

Fatty acid transporter CD6; plasma membrane-bound fatty acid binding protein; muscle; heart; liver; adipose tissue

FATTY ACID UPTAKE by a number of tissues most likely occurs via both diffusion and a protein-mediated mechanism involving a number a proteins (2, 19, 22). Three fatty acid transport proteins have been identified, including fatty acid translocase, the rat homolog of human CD36 (FAT/CD36), plasma membrane-bound fatty acid binding protein (FABPpm), and fatty acid transport protein 1 (FATP1). Each of these proteins increases fatty acid uptake when overexpressed in cell lines (24, 25, 36), although more recently it has been thought that FATP1 may not function as a long-chain fatty acid (LCFA) transporter (15, 29, 42). Another important component of the fatty acid transport system is the 15-kDa cytosolic fatty acid binding protein (FABPc). This protein acts as a metabolic sink for fatty acids that have entered the cell, because fatty acid uptake in heart type FABPc-null mice is markedly reduced (35).

In the past few years, it has been shown that fatty acid transport and/or transporter expression may be altered in animal models of insulin resistance. In ob/ob mice, liver and adipose tissue FAT/CD36 mRNA and FABPpm mRNA are increased (32). In two studies with rodent models of insulin resistance, genetic obesity, and type 2 diabetes, Berk and colleagues (4, 5) found that only adipocyte, but not cardiac myocyte or hepatocyte, fatty acid uptake was increased. In contrast, we (27) found that fatty acid transport was upregulated in heart, skeletal muscle, and adipose tissue of obese Zucker rats. Importantly, several of these studies have shown that the changes in adipocyte fatty acid transporter mRNAs or proteins were not systematically altered in relation to fatty acid uptake (4, 5, 27). Rather, the plasmalemmal localization of FAT/CD36 paralleled the changes in fatty acid transport (27). Because FAT/CD36 can traffic between the plasma membrane and an intracellular compartment (8, 28), changes in the expression of the fatty acid transporters, either at the
mRNA or protein level, may not always be necessary to alter the rate of fatty acid transport.

It is well known that fatty acid metabolism is increased in streptozotocin (STZ)-induced diabetes, a model of insulin deficiency. This may be related, in part, to an increase in fatty acid uptake, because FAT/CD36 and FABPc mRNAs and proteins are increased in skeletal muscle and heart in this model (16, 20, 21, 33, 38). However, whether fatty acid uptake is increased in this model of type 1 diabetes is not known, since the expression of the LCFA transporters does not always correlate well with changes in LCFA transport (4, 5, 27). Therefore, we examined 1) the changes in fatty acid transport across the plasma membrane of selected tissues in STZ-induced diabetic rats and 2) the mechanisms that account for the altered rates of fatty acid transport. Specifically, in the present study we have examined in skeletal muscle, heart, liver, and adipose tissue of insulin-deficient rats 1) fatty acid transport into giant vesicles, 2) FAT/CD36 mRNA and FABPpm RNA abundances, 3) the total fatty acid transporter protein pools (FAT/CD36 and FABPpm) and FABPc, and 4) the presence of FAT/CD36 and FABPpm proteins in the plasma membrane. These parameters were examined in both moderately and severely insulin-deficient animals to ascertain whether the changes in fatty acid transport and transporters in heart, skeletal muscle, and adipose tissue are related to the severity of the insulin deficiency. Our results demonstrate that insulin deficiency increases fatty acid transport in heart, skeletal muscle, and adipose tissue and decreases fatty acid transport in liver. These changes in fatty acid transport are associated with changes in the plasmalemmal FAT/CD36 in heart and skeletal muscle and with plasmalemmal FABPpm in adipose tissue and liver. In addition to these tissue-specific responses, the observed effects also depended on the severity of the insulin deficiency.

METHODS

Materials

[9,10-3H]palmitate (American Radiochemicals, St. Louis, MO) and [14C]mannitol (ICN, Oakville, ON, Canada) were purchased from commercial sources. Collagenase type II was kindly provided by Dr. N. A. Abumrad (SUNY, Stony Brook, NY), and the cDNA for mitochondrial aspartate aminotransferase/FABPpm (31) was a gift from Dr. A. Iriarte (University of Missouri, Columbia, MO). STZ was obtained from Sigma-Aldrich.

Animals

All experimental procedures were approved by the committee on animal care at the University of Waterloo. Male Sprague-Dawley rats weighing ~280 g were randomly divided into three groups: control, moderate type 1 diabetes, and severe type 1 diabetes. Tail vein injections of 55 and 55 mg/kg STZ in citrate buffer (pH 4.5) were used to induce moderate and severe type 1 diabetes. Control animals received a tail vein injection of vehicle. The animals were left untreated for 12–13 wk. On the day before the end of the experiment, a tail vein blood sample was collected for determination of plasma glucose, insulin, triacylglycerol, and free fatty acid concentrations (Table 1). On the next day, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (Somnotol, 50–60 mg/kg). Hindlimb skeletal muscle, liver, epididymal adipose tissue, and heart were removed for fatty acid uptake studies and for the determination of fatty acid transporters.

Plasma Metabolite Assays

Tail vein blood samples were collected into NaF-heparinized centrifuge tubes. Plasma was separated from red cells and stored at −80°C until use. Glucose was determined by a spectrophotometric method (Sigma-Aldrich). Insulin was determined by RIA by use of a rat-specific antibody (Linco, St. Charles, MO). Plasma fatty acids and triacylglycerols were determined using spectrophotometric procedures (Wako Chemicals, Richmond, VA, and Sigma, respectively).

Preparation of Giant Vesicles

Giant vesicles from heart, skeletal muscle, liver, and adipose tissues were generated as previously described (7, 8, 29). Briefly, all 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 U/ml) was used for skeletal muscle and liver tissues; collagenase type II (0.3%, wt/vol) was used for heart, and collagenase type IIA (0.05%, wt/vol) 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

Table 1. Body weights before and after, and glucose, insulin, TG, and FA concentrations after 12 wk of streptozotocin-induced diabetes

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Change</th>
<th>Glucose, mM</th>
<th>Insulin, ng/ml</th>
<th>TG, mg/100 ml</th>
<th>FFA, mM</th>
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<tbody>
<tr>
<td>Control</td>
<td>273 ± 1</td>
<td>450 ± 7.4</td>
<td>+176 ± 13</td>
<td>7.1 ± 0.4</td>
<td>1.09 ± 0.2</td>
<td>102.8 ± 13.0</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>Moderate diabetes</td>
<td>276 ± 13</td>
<td>430 ± 12</td>
<td>+157 ± 17</td>
<td>10.9 ± 0.9</td>
<td>0.55 ± 0.12</td>
<td>130.1 ± 8.8</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>Severe diabetes</td>
<td>285 ± 12</td>
<td>253 ± 6.2</td>
<td>−31 ± 13</td>
<td>31.5 ± 0.5</td>
<td>0.10 ± 0.01</td>
<td>347.0 ± 40</td>
<td>0.64 ± 0.09</td>
</tr>
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Values are means ± SE; n = 20–22/group for body weight and glucose; n = 9–11/group for insulin and triacylglycerol (TG) determinations; n = 6–10 for free fatty acid (FFA) determinations. *P < 0.05, moderate diabetes vs. control, and severe diabetes vs. control. †P < 0.05, severe diabetes vs. moderate diabetes.
concentrations of 16% (vol/vol) 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 (wt/vol) 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 the case of skeletal muscle, the pellet was resuspended in KCl-MOPS to a protein concentration of 2–3 mg/ml; in the 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 previously described (6, 8, 9, 29). Briefly, 40 μl of 0.1% BSA in KCl-MOPS, containing unlabeled (15 μM) and radiolabeled (0.3 μCi [3H]palmitate and 0.06 μCi [14C]mannitol, were added to 40 μl of vesicle suspension. The incubation was carried out for 15 s. Palmitate uptake was terminated by addition of 1.4 ml of ice-cold KCl-MOPS, 2.5 mM HgCl₂, and 0.1% BSA. The sample was then quickly centrifuged in a microfuge 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 before addition of the radiolabeled palmitate solution.

**Northern and Western Blotting**

mRNA abundance of FAT/CD36 and FABPpm was determined as previously described (10). FAT/CD36 and FABPpm protein content was determined in both homogenates and giant sarcolemmal vesicles prepared from heart, skeletal muscle, adipose tissue, and liver (Fig. 1), as we have described previously (6, 8, 9, 11, 29). For detection of FAT/CD36 and FABPpm, we used MO-25 (30) and a rabbit polyclonal anti-FABPpm antiserum (13), respectively. The contents of FABPc (H-FABP) in homogenates and giant vesicles from heart and skeletal muscles were determined by a sandwich-type ELISA, as previously described (40). We were not able to determine cytoplasmic L-FABP or A-FABP proteins in liver and adipose tissue, respectively, because antibodies to these two proteins were not available.
Statistics

The data were analyzed using three-factor (control, moderate, severe) analyses of variance for each parameter under consideration. A Fisher’s least squares difference test was used as a post hoc test. Significance was accepted at \( P < 0.05 \). All data are reported as means ± SE.

RESULTS

Over the course of the 12-13 wk of the study, body weights were increased to a similar extent in control and moderately diabetic animals (\( P > 0.05 \); Table 1), whereas the severely diabetic animals lost weight over the course of the study (\( P > 0.05 \); Table 1).

Circulating glucose concentrations were increased by +53% in the moderately diabetic animals (\( P < 0.05 \); Table 1) and +343% in the severely diabetic animals (\( P < 0.05 \); Table 1). Concurrently, circulating insulin concentrations were reduced by 50 and 91% in the moderately and severely diabetic animals, respectively (\( P < 0.05 \); Table 1). Circulating triacylglycerols (+238%) and fatty acids (+77%) were increased only in the severely diabetic animals (\( P < 0.05 \); Table 1). As in our previous studies (16, 20, 33, 38), cytosolic heart type FABP was increased in heart and skeletal muscle tissues and in giant vesicles in animals with type 1 diabetes (data not shown).

FAT/CD36 and FABPpm mRNAs in Heart, Skeletal Muscle, and Liver

There was an increase in heart FABPpm mRNA (+34%, \( P < 0.05 \); Fig. 2) only in the severely diabetic group. In liver and adipose tissues, FABPpm mRNA was not increased by type 1 diabetes (\( P > 0.05 \); Fig. 2).

Insulin deficiency affected FAT/CD36 mRNA differently in different tissues. In liver, the FAT/CD36 mRNA was increased (+23%) with moderate (\( P = 0.06 \)) and severe insulin deficiency (+30%, \( P < 0.05 \); Fig. 2), although there was no significant difference between severe and moderate insulin deficiency (\( P > 0.05 \)). In the other tissues (skeletal muscles, liver, adipose tissue), type 1 diabetes had no discernible effect on FAT/CD36 mRNA abundance (\( P > 0.05 \); Fig. 2).

Fatty Acid Transport and Transporter Proteins in Selected Tissues

Our primary goal was to examine fatty acid transport and transporters (protein expression and plasmalemmal localization) in four metabolically important tissues. Therefore, the complete results are presented for each tissue, rather than by each parameter in four tissues.

Heart. In the heart, fatty acid transport was increased with moderate insulin deficiency (+71%, \( P < 0.05 \)) and was further increased with severe insulin deficiency (+143%, \( P < 0.05 \); Fig. 3A). The total pool of FABPpm increased with both moderate (+17%, \( P < 0.05 \)) and severe insulin deficiency (+22%, \( P < 0.05 \)), although differences between moderate and severe type 1 diabetes did not differ (Fig. 3B). In contrast, the total pool of FAT/CD36 increased progressively from control to moderate (+13%, \( P < 0.05 \)) to severe (+31%, \( P < 0.05 \)) type 1 diabetes (Fig. 3B). The plasma membrane increase in FABPpm occurred only in the severely diabetic animals (+20%, \( P < 0.05 \); Fig. 3C) despite the fact that fatty acid transport was already increased (+71%) with moderate insulin deficiency (Fig. 3A). In contrast, the plasma membrane changes in FAT/CD36 (moderate +25%, \( P < 0.05 \); severe +50%, \( P < 0.05 \); severe > moderate > control, \( P < 0.05 \); Fig. 3C) paralleled the changes in fatty acid transport (Fig. 3A).

Skeletal muscle. In skeletal muscle, fatty acid transport was increased similarly in the moderately (+37%, \( P < 0.05 \)) and severely diabetic animals (+28%, \( P < 0.05 \); Fig. 3D). The total FABPpm pool was increased in moderate (+25%, \( P < 0.05 \)) and severe (+34%, \( P < 0.05 \)) type 1 diabetes (Fig. 3E), but the difference between the moderately and severely diabetic animals was not significant (\( P > 0.05 \)). There was a progressive increase in the total pool of FAT/CD36 with increasing severity of insulin deficiency (moderate +43%; severe +113%, control < moderate < severe, \( P < 0.05 \); Fig.
There was no change in plasma membrane FABPpm with either moderate or severe insulin deficiency \( (P > 0.05; \text{Fig. 3}F) \). In contrast, the plasma membrane FAT/CD36 was progressively increased with increasing severity of type 1 diabetes (severe +33%, moderate +9%; severe > moderate > control, \( P < 0.05; \text{Fig. 3}F \)).

Liver. In liver, fatty acid transport was reduced with severe \( (-35\%, P < 0.05; \text{Fig. 4}A) \) but not moderate insulin deficiency \( (P > 0.05; \text{Fig. 4}A) \). There was an increase in total FABPpm pool only in the severely type 1 diabetic group \( (+16\%, P < 0.05; \text{Fig. 4}B) \). In contrast, there was a progressive increase in the total pool of FAT/CD36 (severe +144%, moderate +52%; severe > moderate > control, \( P < 0.05; \text{Fig. 4}B \)). Changes in plasma membrane FABPpm was decreased \( (-30\%, P < 0.05; \text{Fig. 4}C) \) in parallel with the decrease in fatty acid transport, whereas plasma membrane FAT/CD36 was increased \( (+158\%, P < 0.05; \text{Fig. 4}C) \).

Adipose tissue. In adipose tissue, there was an increase in fatty acid transport with severe insulin deficiency \( (+171\%, P < 0.05; \text{Fig. 4}D) \). The total pool of FABPpm was increased in the severely type 1 diabetic animals \( (+191\%, P < 0.05; \text{Fig. 4}E) \), but no change in total FAT/CD36 was observed \( (P > 0.05; \text{Fig. 4}E) \). The plasma membrane FABPpm was increased in both the moderately \( (+52\%, P < 0.05) \) and severely type 1 diabetic animals \( (+52\%, P < 0.05; \text{Fig. 4}F) \), whereas concomitantly the plasma membrane FAT/CD36 was decreased in the severely type 1 diabetic animals \( (-23\%, P < 0.05; \text{Fig. 4}F) \).

**DISCUSSION**

We have shown for the first time that STZ-induced diabetes 1) alters fatty acid transport in metabolically important tissues and 2) that these changes are tissue specific and 3) are dependent on the severity of insulin deficiency. Examination of the underlying mechanisms showed 4) that fatty acid transporter expression at the mRNA level is, in general, not a good indicator of FA uptake except in the heart. It appeared 5) that in skeletal muscle the increase in fatty acid transport occurred with moderate insulin deficiency when only
the plasmalemmal FAT/CD36 was increased, whereas an increase in plasmalemmal FABPpm occurred with severe insulin deficiency, but only in the heart, not in skeletal muscle. In contrast, FABPpm in adipose tissue and liver, the changes in fatty acid transport occurred concomitantly with changes in plasmalemmal FABPpm, not plasmalemmal FAT/CD36. Thus these studies have shown that fatty acid transport and transporters respond in a tissue-specific and fatty acid transporter-specific manner to insulin deficiency induced by STZ treatment.

Effects of Type 1 Diabetes on Abundance of FAT/CD36 mRNA and FABPpm mRNA

Among all the tissues examined, FABPpm mRNA abundances were increased only in the heart. In this tissue, FABPpm mRNA was increased only in the severely type 1 diabetic animals, whereas FAT/CD36 mRNA was increased to a similar extent in both moderately and severely diabetic rats. Thus, in the heart, transcriptional activation may account, in part, for the increases observed in FAT/CD36 protein expression in moderate and severe type 1 diabetes and in FABPpm protein expression in the severely diabetic animals. In the other tissues (skeletal muscle, liver, and adipose tissue), the changes observed in fatty acid transporter expression at the protein level would seem to be due to posttranscriptional mechanisms, as no changes were observed in FABPpm mRNA or FAT/CD36 mRNA.

The lack of consistent changes in fatty acid transporter mRNAs among different tissues in the present study has also been observed in rodent models of obesity and type 2 diabetes (4, 5, 27, 32). Thus, generally, inferences about altered tissue content of fatty acid transporters and/or rates of fatty acid transport cannot be drawn from measurements of fatty acid transporter mRNA abundances.

Effects of Type 1 Diabetes on Fatty Acid Transport Proteins: FAT/CD36 and FABPpm

In the present study, the increased rates of fatty acid transport in heart and skeletal muscle were observed when there was also an increase in total FAT/CD36 and a concomitant increase in plasma membrane FAT/CD36 and FABPpm measurements were based on 7–8 preparations in each tissue in each group, and plasma membrane FAT/CD36 and FABPpm measurements were made on 5–8 preparations in each tissue in each group. Procedures are described in METHODS. Data are means ± SE.

Fig. 4. Palmitate transport by giant vesicles and fatty acid transport proteins (FAT/CD36 and FABPpm) in tissue homogenates (total protein) and plasma membranes of giant vesicles in liver and adipose tissue. For transport measurements, n = 7–8/group for liver and n = 4–5/group for adipose tissue. Total FAT/CD36 and FABPpm measurements are based on 7–8 preparations in each tissue in each group, and plasma membrane FAT/CD36 and FABPpm measurements were made on 5–8 preparations in each tissue in each group. Procedures are described in METHODS. Data are means ± SE.
duced both fatty acid transport and plasmalemmal FABPpm. In this tissue, severe insulin deficiency appears to be different. We have shown recently that FAT/CD36 is present at the plasma membrane and in an intracellular depot, from which it can be relocated to the plasma membrane (8, 28). In the STZ-induced diabetic rats, there is an increase in the total FAT/CD36 pool, which results in an increase in plasmalemmal FAT/CD36, whereas in obese Zucker rats, the total pool of FAT/CD36 is unchanged, but there is a larger portion permanently located at the plasma membrane. This central role of FAT/CD36 in promoting fatty acid transport in skeletal muscle and heart in obese (27) and diabetic animals (present study) is consistent with reductions in fatty acid uptake observed in FAT/CD36-null rodents (14, 17). Importantly, our studies demonstrate that plasmalemmal FAT/CD36 can be increased, either when its expression is upregulated (present study) or when FAT/CD36 is relocated to the plasma membrane in the absence of altered levels of expression (28).

In heart and skeletal muscle, the changes in plasmalemmal FABPpm were not observed, in some instances, despite an increase in vesicular fatty acid uptake (i.e., heart in moderate type 1 diabetes; skeletal muscle in moderate and severe type 1 diabetes). Similarly, in skeletal muscle of obese Zucker rats, there was also no change in plasmalemmal FABPpm, whereas fatty acid transport was increased (27). Thus, our present study and others (27) suggest that, in skeletal muscle, and perhaps in the heart, plasmalemmal FABPpm is not the primary fatty acid transporter that regulates fatty acid transport. Plasmalemmal FABPpm may already be present in excess, and only when large increases in fatty acid transport are observed is there also an increase in plasmalemmal FABPpm such as we found in hearts of obese Zucker rats (27) and severely diabetic animals (present study).

In adipose tissue, we have observed previously that the increase in fatty acid transport occurred when there was an increase in both plasmalemmal FAT/CD36 and FABPpm [i.e., in obese Zucker rats (27)]. In other studies, a reduction in adipose tissue fatty acid efflux (3) and uptake (14) has been attributed to parallel changes in FAT/CD36, but FABPpm levels were not measured. In contrast, in the STZ-induced diabetic rats (present study), we observed that the increase in adipose tissue fatty acid transport occurred when only plasmalemmal FABPpm was increased while, concomitantly, plasmalemmal FAT/CD36 was reduced. Thus, in adipose tissue of diabetic animals, in contrast to heart and skeletal muscle from the same animals, it appears that an increase in plasmalemmal FAT/CD36 is not required to increase fatty acid transport. Instead, an increase in plasmalemmal FABPpm appears to be sufficient to increase fatty acid transport in adipose tissue.

On the other hand, the situation in liver is more complex. In this tissue, severe insulin deficiency reduced both fatty acid transport and plasmalemmal FABPpm, whereas plasmalemmal FAT/CD36 was increased. The observation that the plasma membrane abundance of FABPpm in liver is reduced but total FABPpm expression is not altered points toward a relocation of this protein to an intracellular depot. The lack of concordance between fatty acid transport and plasma membrane FAT/CD36 in liver implies that this protein is fundamentally less important for transporting fatty acids in liver. Indeed, in some studies it has been difficult to detect FAT/CD36 in liver (1, 39). Moreover, the expression of FAT/CD36 in liver is quite low, because sulfo-N-succinimidyl oleate (SSO), a specific inhibitor of FAT/CD36, fails to inhibit vesicular fatty acid uptake in liver, whereas this inhibitor markedly impairs fatty acid transport in heart and skeletal muscle (29). Furthermore, because the magnitude of fatty acid transport inhibition by SSO is correlated with the quantity of plasma membrane FAT/CD36 in heart and skeletal muscle (29), the failure of SSO to inhibit hepatic fatty acid transport also suggests strongly that FAT/CD36 levels in liver are low, and/or that this protein has another function in this tissue. It is known that this protein is involved in many cellular processes (for review see Ref. 18).

**Mechanisms Promoting Fatty acid Uptake**

A number of studies have shown that overexpression of either FAT/CD36 or FABPpm in heterologous cells can increase the uptake of fatty acids (24, 25). However, how these proteins function to facilitate fatty acid transport in biological tissues in which FAT/CD36 and FABPpm are co-expressed is not entirely clear. It has been proposed that FAT/CD36 and FABPpm interact, in an unknown manner, to promote fatty acid uptake (19). We (29) have provided some indirect evidence for this suggestion. Fatty acid uptake can be inhibited in giant vesicles obtained from heart and skeletal muscle by blocking either FAT/CD36 or FABPpm independently. When both transporters are inhibited simultaneously, no further reduction in fatty acid uptake occurs (29). These results suggest that FAT/CD36 and FABPpm may interact to promote fatty acid uptake. In addition, we have preliminary evidence that the phosphorylation state of FAT/CD36 at the plasma membrane can alter fatty acid uptake (J. J. F. P. Luiken, J. F. C. Glatz, and A. Bonen, unpublished data). Thus the inability to observe a linear correlation between fatty transport and the plasmalemmal content of either FAT/CD36 or FABPpm in the present study may reflect the complexity of regulating fatty acid transport.

In the present study and others from our laboratory (6, 8, 27), we have now identified a number of mechanisms that contribute to acute and chronic changes in fatty acid uptake across the plasma membrane. With respect to FAT/CD36, these include 1) an increase in the total quantity of the FAT/CD36 protein along with an increase in plasma membrane FAT/CD36 (6), as was observed with STZ-induced diabetes in the present study; 2) the rapid and reversible translocation of FAT/CD36, within minutes, from an intracellular pool to the...
plasma membrane, either by skeletal muscle contraction, when the demand for fatty acid oxidation is increased (i.e., within minutes) (8), or by insulin when fatty acid esterification is increased (28); and 3) a more permanent relocation of the FAT/CD36 from an intracellular pool to the plasma membrane, without any alteration in the total pool of FAT/CD36 in obese Zucker rats (27).

Interestingly, with respect to FABPpm, our studies (27) suggest that this membrane protein may also be translocated to adjust fatty acid flux across the plasma membrane. In a number of studies, it has now been observed that the plasma membrane localization of FABPpm can also be altered, independent of changes in FABPpm expression. For example, plasma membrane FABPpm was increased in heart and adipose tissue of obese Zucker rats (27) and in adipose tissue of moderately type 1 diabetic rats (present study) without concomitant changes in FABPpm expression. Also, in type 1 diabetic animals, tissue-specific relocation of FABPpm might explain altered fatty acid fluxes. For example, in severely type 1 diabetic liver, the reduced fatty acid transport occurred when there was a reduction in plasmalemmal FABPpm in the face of an increased FABPpm expression. Collectively, these studies suggest that cellular redistribution of FABPpm may also be involved in the regulation of fatty acid uptake. Furthermore, obesity and/or diabetes (type 1 and/or 2) could induce changes in the cellular machinery regulating the subcellular FABPpm distribution, resulting in a relocation of this transporter.

Comparison of Glucose Transport and Fatty Acid Transport

It has long been realized that changes in glucose and fatty acid metabolism occur in obesity and in type 1 and type 2 diabetes, in animals and in humans. At the level of the glucose and fatty acid transport systems in skeletal muscle, there appear to be reciprocal changes in glucose transport and fatty acid transport in a number of experimental models. For example, in chronically leptin-treated animals, skeletal muscle glucose transport is increased (41, 43) whereas fatty acid transport is decreased (37). Conversely, in heart and skeletal muscle of STZ-induced diabetic animals, insulin-stimulated glucose transport is reduced due to a reduction in the total quantity of GLUT4 that is available for translocation (23, 34), whereas fatty acid transport is increased due to an increase in fatty acid transporter expression (present study). And similarly, in obese animals, insulin-stimulated glucose transport is reduced due to an impaired GLUT4 translocation, not a reduction in GLUT4 protein expression (12, 26), while fatty acid transport is increased because of the redistribution of fatty acid transporters to the plasma membrane, in the absence of altered fatty acid transporter protein expression (27). These studies indicate that, in skeletal muscle, glucose transport and fatty acid transport are regulated in a reciprocal manner.

Conclusions

In summary, our studies have shown that STZ-induced diabetes increased fatty acid transport in skeletal muscle, heart, and adipose tissue and reduced hepatic fatty acid transport. In some of these tissues (heart), the increase in fatty acid transport was altered in proportion to the severity of diabetes, whereas in other tissues the changes were similar in moderate and severe diabetes (skeletal muscle) or occurred only with severe diabetes (liver). Our study also strengthens the case for a pivotal role of the membrane fatty acid transporters FAT/CD36 and FABPpm in the regulation of fatty acid uptake in metabolically important tissues. The data also suggest that FAT/CD36 is the primary fatty acid transporter in heart and skeletal muscle, although this function appears to be delegated to FABPpm in adipose tissue and liver. Apart from the total protein expression of these transporters, their subcellular localization is an important determinant, because their plasmalemmal abundance determines the cellular capacity for fatty acid uptake. Future studies should be directed at unraveling the signal transduction pathways involved in the regulation of both the expression and the localization (intracellular trafficking) of the transporters. Finally, given their malfunctioning in metabolic diseases such as obesity and diabetes, these membrane proteins may represent suitable targets for therapeutic interventions aimed at restoring the changes in substrate uptake seen in these disease states.

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J. J. F. P. Luiken is a Dekker postdoctoral fellow of the Netherlands Heart Foundation.

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