Fatty acid transport and FAT/CD36 are increased in red but not in white skeletal muscle of ZDF rats

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Chabowski, Adrian, John C. Chatham, Narendra N. Tandon, Jorge Calles-Escandon, Jan F. C. Glatz, Joost J. F. P. Luiken, and Arend Bonen. Fatty acid transport and FAT/CD36 are increased in red but not in white skeletal muscle of ZDF rats. Am J Physiol Endocrinol Metab 291: E675–E682, 2006. First published May 9, 2006; doi:10.1152/ajpendo.00096.2006.—An increased rate of fatty acid transport into skeletal muscle has been shown to be associated with the accumulation of intramuscular lipids and insulin resistance, and red muscles are more susceptible than white muscles in developing fatty acid-mediated insulin resistance. Therefore, we examined in Zucker diabetic fatty (ZDF) rats, relative to lean rats, whether changes in fatty acid transport rates were observed in the white muscles of lean and ZDF rats at any time (weeks 1 – 24), whether such changes occurred mainly in red skeletal muscle, and whether changes in FAT/CD36 and GLUT4 were correlated. In red muscles of ZDF compared with lean rats, the rates of fatty acid transport were upregulated (+66%) early in life (week 6). Compared with the increase in fatty acid transport in lean red muscle from weeks 12–24 (+57%), the increase in fatty acid transport rate in ZDF red muscle was 50% greater during this same period. In contrast, no differences in fatty acid transport rates were observed in the white muscles of lean and ZDF rats at any time (weeks 6 – 24). In red muscle only, there was an inverse relationship between FAT/CD36 and GLUT4 protein expression as well as their plasmalemmal content. These studies have shown that, 1) before the onset of diabetes, as well as during diabetes, fatty acid transport and FAT/CD36 expression and plasmalemmal content are upregulated in ZDF rats, but importantly, 2) these changes occurred only in red, not white, muscles of ZDF rats. The Zucker diabetic fatty (ZDF) rat transitions from being insulin resistant at 6 wk of age to being diabetic by week 12 and beyond (11, 15). Previously, an increased rate of LCFA transport was observed in adipocytes but not in cardiac myocytes or in hepatocytes of 8- to 14-wk-old ZDF rats (3). Whether LCFA transport and transporters were upregulated in skeletal muscle was not examined. However, there may well be an upregulation in LCFA transport and transporters in muscle of ZDF rats, because the severity of insulin resistance progresses over a 6- to 24-wk period. It is also possible that such changes are associated with the reductions in GLUT4 observed in skeletal muscles of ZDF rats (16).

Mature muscle fiber composition may be an important consideration when examining skeletal muscle insulin resistance and the underlying mechanisms, since different types of skeletal muscle have different metabolic capacities and are not equally susceptible to developing insulin resistance. For example, GLUT4 protein is reduced in red muscles, but not in white muscles, of ZDF rats (16). Moreover, red muscles may be more susceptible to developing fatty acid-mediated insulin resistance, since these muscles have a much greater capacity for fatty acid transport and metabolism than white muscles (6, 14). Accordingly, impairment in insulin-stimulated glucose trans-

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The transport of long-chain fatty acids (LCFAs) into skeletal muscle is a highly regulated process (for review, see Refs. 4, 24, and 29) involving LCFA transporters, including plasma membrane-associated fatty acid-binding protein (FABPpm; 40 kDa), a family of fatty acid transport protein [FATP (1–6) 63 kDa], and fatty acid translocase (FAT/CD36), the homolog of human CD36 (4, 24, 29). The rate of LCFA transport into skeletal muscle is markedly increased in obese Zucker rats, an animal model of insulin resistance (28), and in muscle of obese human individuals and type 2 diabetes (7). This increase in fatty acid transport rates in these studies (7, 28) has been associated with an increase in plasmalemmal FAT/CD36 but not plasmalemmal FABPpm (7, 28). Moreover, the increased rate of LCFA transport contributed to the increase in triacylglycerol accumulation in obese human muscle (7). Thus the upregulation of plasmalemmal FAT/CD36 appears to be a key mechanism contributing to fatty acid-associated insulin resistance in skeletal muscle.

A reduced capacity for fatty acid oxidation in insulin-resistant muscle has been linked to the accumulation of intramuscular lipids (22, 23). However, recent work (7, 21) has shown that lipids can also accumulate in obese muscle when their oxidation is not impaired. Therefore, other mechanisms contribute to intramuscular lipid accumulation. One such mechanism appears to be the increased rate of fatty acid transport into skeletal muscle (7, 28).

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rates of fatty acid transport and hypothesized that, with the development of types 2 diabetes, animals evolved from insulin resistance to type 2 diabetes. We upregulated fatty acid transport rates and FAT/CD36 in a muscle fiber-specific manner.

We examined whether there was a muscle fiber type-specific upregulation of fatty acid transport and fatty acid transport proteins in ZDF rats during the 6- to 24-wk period when these animals evolved from insulin resistance to type 2 diabetes. We hypothesized that, with the development of types 2 diabetes, 1) rates of fatty acid transport and 2) expression and 3) plasmalemmal content of FAT/CD36, but not FABPpm, are increased primarily in red, but not in white, types of skeletal muscle. Finally, we also hypothesized that there is 4) an inverse relationship between FAT/CD36 and GLUT4 protein expression and their plasmalemmal content in red muscle. For these purposes, we examined in red and white skeletal muscles in lean and ZDF rats at 6, 12, and 24 wk of age 1) the rates of LCFA transport into giant sarcosomial vesicles; 2) the mRNA and 3) protein expression of FAT/CD36, FABPpm, and GLUT4; and 4) the plasmalemmal content of these proteins.

Methods

Animals

All experimental procedures were approved by the Committee on Animal Care at the University of Guelph. Male lean (n = 29) and ZDF rats (n = 29) were purchased (Charles River, Wilmington, MA). They were maintained on the recommended diet (Purina Labdiet, Formulab 5008; Ralston Purina, Richmond, IN). Animals were used in the experiments when they were 6, 12, or 24 wk of age. After induction of anesthesia (Somnotol, 6 mg/100 g body wt), a tail vein blood sample was collected for determination of glucose, insulin, and fatty acid concentrations in nonfasted animals, as others (16, 26, 35, 41, 42) have also done in nonfasted lean and ZDF rats, to characterize them at different ages. Thereafter, red and white muscles were removed from both hindlimbs. To obtain sufficient muscle tissue for our studies, it was necessary to pool several red and white muscles from a number of animals. Specifically, the red gastrocnemius and red vastus lateralis were pooled to represent red muscles, whereas for white muscle the white gastrocnemius and white vastus lateralis were pooled. In addition, in week 6 we pooled three animals to obtain sufficient quantities of red and white muscle, whereas in weeks 12 and 24 we pooled two animals to obtain sufficient quantities of red and white muscle. Small portions of the pooled red and white muscles were immediately frozen in liquid nitrogen and stored at −80°C until they were analyzed for FABPpm and FAT/CD36 mRNA and protein and GLUT4 protein. The remaining oxidative and glycolytic muscles were processed and stored at −80°C until they were analyzed for plasmalemmal FABPpm, FAT/CD36, and GLUT4.

Plasma Metabolite Assays

Serum samples were analyzed for glucose with the use of a spectrophotometric method (Sigma, St. Louis, MO), and insulin was determined by RIA using a rat-specific antibody (Linco, St. Charles, MO). Fatty acid concentrations were determined using spectrophotometric procedures (Wako Chemicals, Richmond, VA).

Preparation of Giant Vesicles

Giant vesicles from red and white skeletal muscle were generated as previously described (5–7, 25, 27, 28, 37). Briefly, 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 (1.0 mg/ml), and collagenase (type VII, 150 units/ml) in a shaking water bath. At the end of the incubation, the supernatant fraction was collected and the remaining tissue washed with KCl-MOPS and 10 mM EDTA, which resulted in a second supernatant fraction. Both supernatant fractions were pooled, and Percoll and aprotinin were added to final concentrations of 16% (vol/vol) and 0.1 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. The pellet was resuspended in KCl-MOPS.

Palmitate Uptake by Giant Vesicles

Palmitate uptake studies were performed, as we have previously described (5–7, 25, 27, 28, 37). Fat-free BSA (Roche Diagnostics, Laval, QC, Canada), [9,10-3H]palmitate (ICN, Oakville, ON, Canada), and [14C]mannitol (ICN) were purchased from commercial sources. For palmitate transport measurements, 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 the 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 prior to addition of the radiolabeled palmitate solution.

Northern and Western Blotting

mRNA abundance of FAT/CD36 and FABPpm were determined as we have previously described (10, 27, 28). The cDNA For FAT/CD36 was kindly provided by Dr. N. A. Abumrad (Washington University School of Medicine, St. Louis, MO), and the cDNA for mitochondrial aspartate aminotransferase/FABPpm was a gift from Dr. A. Iriarte (University of Missouri, Kansas City, MO). FAT/CD36 and FABPpm protein content were determined in both homogenates and giant sarcosomial vesicles, as we have previously described (5–7, 27, 28, 37). For detection of FAT/CD36 and FABPpm, we used MO-25 (32) and a rabbit polyclonal anti-FABPpm antiserum (9), respectively. GLUT4 was detected using a commercially available antibody (Chemicon International, Temecula, CA).

Statistics

The data were analyzed with analyses of variance (ANOVA; group × time × muscle), using a commercially available statistical package (Statview; Abacus Concepts, Berkeley, CA). Post hoc tests (Fisher’s protected least squares difference), available with this statistical package, were performed only when statistical significance was found with the ANOVA. Comparisons between FAT/CD36 and GLUT4 were performed with regression analysis. Throughout, statistical significance was set at P < 0.05. All data are reported as means ± SE.
RESULTS

In both lean and ZDF rats, circulating glucose concentrations increased progressively from week 6 to week 24 (P < 0.05; Table 1), although the concentrations were far lower in the lean animals than in ZDF rats during weeks 6–24 (P < 0.05). Insulin concentrations were greater in ZDF than in lean rats at week 6 (P < 0.05) and decreased thereafter, being lower at week 24 (P < 0.05; Table 1). Circulating fatty acid concentrations were two- to threefold greater in ZDF than in lean rats during weeks 6 and 12 (P < 0.05), with a further increase occurring by week 24 (P < 0.05; Table 1).

LCFA Transport into Giant Sarcolemmal Vesicles

Red muscle. The rates of palmitate transport into giant sarcolemmal vesicles were determined. In red muscle, the rates of palmitate transport were greater in ZDF animals than in the lean animals throughout the 6-to-24-wk observation period (P < 0.01; Fig. 1). At week 6, palmitate transport rates in ZDF muscles (+66%) were greater than in lean muscles (P < 0.01; Fig. 1). From week 6 to week 12, no changes in the rates LCFA transport occurred in either the lean or ZDF red muscles (P > 0.05; Fig. 1). In both groups, an increase in fatty acid transport occurred from week 12 to week 24 (P < 0.05); in lean red muscle the increase was 57% (P < 0.05; Fig. 1), whereas in the red ZDF muscles the net increase was 50% greater than in the lean red muscles (P < 0.05; Fig. 1).

White muscle. Rates of palmitate transport were lower in white than in red muscle in both lean and ZDF animals (P < 0.05; Fig. 1). In marked contrast to the observation in red muscle, there were no differences in the rates of palmitate transport between lean and ZDF animals in the white muscle during weeks 6–24 (P > 0.05; Fig. 1). Also, the rates of LCFA transport did not change in either group during this period (P > 0.05; Fig. 1).

Skeletal Muscle FABPppm mRNA and Homogenate and Plasmalemmal Protein

In general, no relationships were apparent between the progression of type 2 diabetes and FABPppm mRNA, protein expression, or plasmalemmal content. White muscle FABPppm mRNA declined in ZDF rats by week 12 (P < 0.05; Table 2) and in lean rats by week 24 (P < 0.05; Table 2), although no changes were observed in lean or ZDF red muscles from week 6 to week 24. Red muscle FABPppm protein expression and plasmalemmal content were lower in ZDF animals at week 24 (P < 0.05; Table 2), but in lean or ZDF white muscles, no changes were observed in these parameters (P > 0.05; Table 2).

Table 1. Circulating glucose, insulin, and fatty acids at selected ages in lean and ZDF rats

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>Glucose, mM</th>
<th>Glucose, ZDF/Lean</th>
<th>Insulin, ng/ml</th>
<th>Insulin, ZDF/Lean</th>
<th>Fatty Acids, mM</th>
<th>Fatty Acids, ZDF/Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>8.4±0.5</td>
<td>17.4±1.2</td>
<td>1.2±0.2</td>
<td>4.8±0.8</td>
<td>0.20±0.03</td>
<td>0.66±0.06</td>
</tr>
<tr>
<td>12</td>
<td>9.7±0.7</td>
<td>33.4±1.9*</td>
<td>2.2±0.8</td>
<td>3.1±0.4*</td>
<td>0.23±0.05</td>
<td>0.63±0.06</td>
</tr>
<tr>
<td>24</td>
<td>14.2±0.8*</td>
<td>36.7±0.5*</td>
<td>3.6±0.8*</td>
<td>1.5±0.4*</td>
<td>0.27±0.05</td>
<td>0.88±0.15*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3 at week 6 and n = 5 at weeks 12 and 24. ZDF, Zucker diabetic fatty. Analyses were based on pooled samples from 3 animals in week 6 and from 2 animals in weeks 12 and 24. *P < 0.05, week 12 vs. week 6; **P < 0.05, week 24 vs. week 12; ***P < 0.05, week 24 vs. week 6; *P = 0.06, week 24 vs. week 12; **P < 0.05, ZDF vs. lean at all ages; ***P < 0.05, ZDF vs. lean, different effects during weeks 6–24.
2). In contrast, in ZDF rats, white muscle FAT/CD36 mRNA did not differ from red muscle FAT/CD36 mRNA at weeks 6 and 12 (P > 0.05; Fig. 2). White muscle FAT/CD36 mRNA in ZDF rats was markedly more abundant than in white muscle of lean rats at weeks 6 and 12 (P < 0.05; Fig. 2). As in red muscle, ZDF white muscle FAT/CD36 mRNA increased from week 6 to week 12 (+80%, P < 0.05; Fig. 2). However, from week 12 to week 24 there was a marked reduction in ZDF white muscle FAT/CD36 mRNA (−76%, P < 0.05; Fig. 2).

**Skeletal Muscle FAT/CD36 Protein Expression and Plasmaleminal Content**

*Red muscle.* In lean red muscle, FAT/CD36 protein expression did not change over the 6- to 24-wk period (P > 0.05; Fig. 3A). Plasmaleminal FAT/CD36 in lean red muscle also remained stable from week 6 to week 12 (P > 0.05; Fig. 3B), but by week 24 it had declined (−24%) relative to week 6 (P < 0.05; Fig. 3B). In contrast, in ZDF red muscle at week 6, FAT/CD36 protein expression (+30%; Fig. 3A) and plasmaleminal content (+42%; Fig. 3B) were already greater than in lean red muscle (P < 0.05). From week 6 to week 12, there was a marked increase in red ZDF muscle FAT/CD36 protein expression (+53%, P < 0.05; Fig. 3A) and plasmaleminal content (+27%, P < 0.05; Fig. 3B). These remained upregulated at these levels until week 24 (Fig. 3, A and B).

*White muscle.* Throughout weeks 6–24, white muscle FAT/CD36 protein expression (Fig. 3A) and plasmaleminal content (Fig. 3B) were lower than in red muscles of lean and ZDF animals (P < 0.05; Fig. 3, A and B). There were no differences in FAT/CD36 expression or its plasmaleminal content in white muscles of lean and ZDF rats at any time (weeks 6–24, P > 0.05; Fig. 3, A and B), and no changes occurred in these parameters from week 6 to week 24 in either lean or ZDF white muscles (P > 0.05; Fig. 3, A and B).

**Skeletal Muscle GLUT4 Expression and Plasmaleminal Content**

*Red muscle.* From week 6 to week 24, GLUT4 protein expression in lean red muscle was unaltered and was greater than in all other muscles examined (P < 0.05; Fig. 4A). In the red ZDF muscles, GLUT4 protein expression was already 20% lower at week 6 than lean red muscle (P < 0.05; Fig. 4A). In weeks 12–24, red ZDF muscle GLUT4 protein

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**Table 2. FABPpm mRNA and protein expression and plasmaleminal FABPpm at selected ages in lean and ZDF rats**

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>Muscle Type</th>
<th>FABPpm mRNA Lean</th>
<th>FABPpm mRNA ZDF</th>
<th>FABPpm Protein Expression Lean</th>
<th>FABPpm Protein Expression ZDF</th>
<th>FABPpm PM Protein Lean</th>
<th>FABPpm PM Protein ZDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Red</td>
<td>100±25</td>
<td>118±3</td>
<td>100±14</td>
<td>108±9</td>
<td>100±16</td>
<td>126±14</td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>145±20</td>
<td>90±9</td>
<td>80±4</td>
<td>89±5</td>
<td>80±7</td>
<td>80±7</td>
</tr>
<tr>
<td>12</td>
<td>Red</td>
<td>105±2.6</td>
<td>127±18</td>
<td>97±10</td>
<td>124±9</td>
<td>105±10</td>
<td>126±13</td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>95±15</td>
<td>64±13</td>
<td>90±3</td>
<td>90±6</td>
<td>81±7</td>
<td>91±6</td>
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<tr>
<td>24</td>
<td>Red</td>
<td>100±14</td>
<td>95±3</td>
<td>99±6</td>
<td>56±10</td>
<td>97±9</td>
<td>63±10</td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>50±1†</td>
<td>53±14†</td>
<td>98±5</td>
<td>114±7</td>
<td>79±8</td>
<td>79±6</td>
</tr>
</tbody>
</table>

Values are means ± SE and in arbitrary units; n = 3 independent experiments at each time point based on pooled muscle samples from 3 animals in week 6 and from 2 animals in weeks 12 and 24. FABPpm, plasma membrane–associated fatty acid-binding protein; PM, plasma membrane. Quantification is based on 3 μg of RNA, normalized for loading by 18S ribosomal RNA. Homogenate and plasma membrane protein quantifications were based on loading 20 and 10 μg of sample for all samples, respectively; n = 3 independent experiments at week 6 and n = 5 independent experiments each at weeks 12 and 24. Independent experiments in week 6 were based on pooled muscle samples from 3 animals and from 2 animals in weeks 12 and 24. *P < 0.05, week 24 vs. week 12; †P < 0.05, week 12 or 24 vs. week 6; ‡P < 0.05, ZDF vs. lean in the same week.
expression was 50–60% lower than in lean red muscles (P < 0.05; Fig. 4A). At this time (weeks 12 and 24), the GLUT4 expression in red ZDF muscle did not differ from the levels observed in white muscles of either lean or ZDF rats (P > 0.05; Fig. 4A).

Red muscle plasmalemmal GLUT4 (Fig. 4B) differed slightly from its expression patterns in weeks 6–24. At week 6, red muscle plasmalemmal GLUT4 did not differ in red lean and red ZDF muscles (P > 0.05; Fig. 4B). Thereafter, from week 6 to week 24, the lean red muscle plasmalemmal GLUT4 increased by 27% (P < 0.05; Fig. 4B). In contrast, during the same time (weeks 6–24), ZDF red muscle plasmalemmal GLUT4 decreased by 35% (P < 0.05; Fig. 4B).

White muscle. The GLUT4 protein expression in lean white muscle was 40–50% lower than in lean red muscle (P < 0.05; Fig. 4A). In lean white muscle, GLUT4 protein expression was unaltered from week 6 to week 12 (P > 0.05; Fig. 4A) and decreased only slightly (−15%) from week 12 to week 24 (P > 0.05; Fig. 4A). In ZDF white muscle, GLUT4 protein expression was reduced from week 6 to week 12 (−31%, P < 0.05; Fig. 4A), with an additional reduction of 19% from week 12 to week 24 (P < 0.05; Fig. 4A).
Plasmalemmal GLUT4 at week 6 did not differ in lean and ZDF white muscles (P > 0.05; Fig. 4B). In lean white muscle, plasmalemmal GLUT4 was not altered from week 6 to week 12 but decreased slightly thereafter (−16%) from week 12 to week 24 (P < 0.05; Fig. 4B). In ZDF white muscles, plasmalemmal GLUT4 was reduced by week 12 (−31%, P < 0.05; Fig. 4B) and declined somewhat further (−18%) by week 24 (P < 0.05; Fig. 4B).

Relationships between FAT/CD36 and GLUT4 in Red Muscle

Because there were changes in both FAT/CD36 and GLUT4 in red muscles, but not in white muscles, we compared protein expression and plasmalemmal content of these transporters in red muscle only (Fig. 5). There was a strong inverse relationship between the protein expression of FAT/CD36 and GLUT4 (Fig. 5A) as well as between the plasmalemmal FAT/CD36 and plasmalemmal GLUT4 (Fig. 5B).

DISCUSSION

As ZDF rats age, they progress from an insulin-resistant stage (week 6) to a newly diabetic (week 12) and then a late diabetic stage (week 24). This progression is evident from the changes in circulating glucose, insulin, and fatty acids (present study and Refs. 11, 15, 41, and 42) as well as a reduction in skeletal muscle GLUT4 (present study and Refs. 16, 35, and 36).

These studies are the first to examine in ZDF rats whether rates of fatty acid transport and fatty acid transporters (FAT/CD36 and FABPpm) were upregulated in ZDF rat muscles when these animals transitioned from insulin resistance to type 2 diabetes, and whether such changes occurred primarily in red, but not in white, skeletal muscle. There were a number of novel observations, which were as follows. In ZDF rats, 1) rates of fatty acid transport were upregulated early in life (week 6), when the ZDF animals were already insulin resistant, and these rates were further increased in severely diabetic animals (week 24); 2) the expression and plasmalemmal content of FAT/CD36, but not FABPpm, were upregulated throughout the 6-to-24-wk observation period; 3) there was an inverse relationship between FAT/CD36 and GLUT4 expression as well as their plasmalemmal content; and 4) remarkably, all these foregoing differences between lean and ZDF animals were observed in red skeletal muscle only; because 5) there were no differences in these parameters in white muscle of lean and ZDF rats.

We have previously shown that LCFA transport rates in muscle are increased in insulin-resistant obese Zucker rats (28), in obese humans (7), and in type 2 diabetics (7). However, these studies did not examine whether these changes were muscle fiber type specific. The present study is the first to demonstrate that the rate of LCFA transport is markedly upregulated in red, but not white, muscles of ZDF rats, when the animals are insulin resistant (week 6), and these transport rates are further increased in red muscle when the animals exhibit severe type 2 diabetes (week 24). In high-fat-fed rats, a model of insulin resistance, Hegarty et al. (19) also observed that LCFA influx was increased in red muscle but not in white muscle. However, they did not attempt to identify the underlying mechanisms, other than to note that red muscle FAT/CD36 mRNA abundance was upregulated in high-fat-fed rats (19). Whether white muscle FAT/CD36 mRNA was also altered was not reported in that study. Nevertheless, in different models of insulin resistance [present study, week 6, and high-fat feeding (19)] and in type 2 diabetes (present study, weeks 12 and 24), it is in the red muscle, rather than in the white muscle, where the rate of LCFA transport is upregulated. Presumably, insulin signaling would be expected to be much more impaired in red muscle than in white muscle as a result of the increased intramuscular lipids that accumulated when LCFA transport into muscle is increased. Indirect evidence suggests this to be the case, because insulin-stimulated glucose transport is impaired more in red muscle than in white muscle of high-fat-fed rats (43) and ZDF rats (20).

The mechanisms contributing to the differences in LCFA transport in red and white muscle were also examined. At the protein level, it may be difficult to distinguish between FABPpm and mitochondrial aspartate amino transferase, because these proteins are known to be identical (8, 38). However, FABPpm is present at the plasma membrane (2, 38, 44), where it is involved in transporting fatty acids into the muscle cell (12, 30, 39). In the present study, there were no changes in

![Image](http://ajpendo.physiology.org/DownloadedFrom)
FABPpm at any level (transcript, protein expression, plasmalemmal content) that could be associated with insulin resistance and type 2 diabetes in ZDF rats. These results are in accord with our previous studies where there was no association between FABPpm and insulin resistance in skeletal muscle in either animal (28) or human obesity or in type 2 diabetic patients (7).

The increase in LCFA transport in red ZDF muscle was associated with an increase in FAT/CD36 protein in this muscle but not with changes in FAT/CD36 mRNA. This is not too surprising, because changes in mRNA abundance by themselves provide only a limited explanatory basis for altered protein expression (for review, see Refs. 34 and 40), and generally, FAT/CD36 mRNA does not correspond well with protein expression in adipocytes or in muscle tissue (3, 27, 28). We have previously shown in skeletal muscle of obese animals and obese humans that FAT/CD36 protein expression is not altered (7, 28). Instead, the increase in LCFA transport in skeletal muscle in animal and human obesity, as well as in human type 2 diabetes, was associated with the permanent relocation of FAT/CD36 from an intracellular depot to the plasma membrane (7, 13, 28). However, in the present study, the upregulated plasmalemmal FAT/CD36 in red muscle of ZDF rats appeared to be a result of its increased protein expression, because the changes in red muscle FAT/CD36 expression and plasmalemmal content paralleled each other closely.

The increase in LCFA transport and plasmalemmal FAT/CD36 did not change in a completely coordinate manner in red ZDF muscle. It is quite possible that other transporters are also involved in transporting LCFA. With high-fat feeding, FATP-1 was upregulated in red muscle (soleus) but not in a largely white muscle (gastrocnemius) (31). Whether FATPs are altered in diabetic muscle is not known. There has been speculation that different LCFA transporters collaborate to transport LCFA into muscle cells (30). In the heart, FAT/CD36 is colocalized with FATP6 (17) and with FABPpm (Chabowski A and Bonen A, unpublished data). Thus, whether it is the changes in other LCFA transporters and/or changes in their interaction with FAT/CD36 that prevent fully coordinated changes in FAT/CD36 and LCFA transport remains to be established. Nevertheless, the present study and others (7, 28) demonstrate that FAT/CD36 contributes to the increased rate of LCFA transport into insulin-resistant skeletal muscle, particularly red muscle.

A novel observation in the present study is the complete lack of any change in the white muscle LCFA transport and LCFA transporters in the ZDF rats over the 6- to 24-wk period when these animals become diabetic. Indeed, in white muscle there were no differences between lean and ZDF rats in either the rate of LCFA transport or the FAT/CD36 protein expression or its plasmalemmal content. The reasons for these muscle fiber specific differences are speculative. Because white muscles accumulate much less fatty acid than red muscles (1) and exhibit about a twofold lower rate of insulin-stimulated glucose transport (33), it could be argued that these types of muscle fibers are perhaps much less susceptible to alterations in the substrate-endocrine environment. This may be related to the physiological role of white muscle, which is to support rapid, short bursts of movements that rely heavily on the hydrolysis of intramuscular glycogen, rather than on blood borne substrates. There is a need to develop a better understanding as to why red muscle, rather than white muscle, is apparently so much more susceptible to increasing its uptake of LCFA and becoming more insulin resistant (18, 19, 43).

Previously, it has been shown that GLUT4 mRNA and protein are lower in ZDF rats (15, 16, 20, 35). In agreement with previous observations (16), we found that GLUT4 protein expression was relatively more reduced in red muscle than in white muscle of ZDF rats, and this was paralleled by similar differences in plasmalemmal GLUT4 in ZDF rats. The much greater fatty acid uptake in red muscle compared with white muscle in ZDF rats may contribute, over time, to the greater repression of GLUT4 protein observed in red muscle compared with white muscle. This may also be the reason that FAT/CD36 and GLUT4 protein expression were inversely related in red muscle. These relationships are, however, speculative and require experimental verification.

In summary, we have shown that LCFA transport and the LCFA transport protein FAT/CD36 are increased in ZDF rats. This however, was observed only in red skeletal muscle, not in white skeletal muscle. The present study and others (7, 28) have failed to observe a role for FABPpm in insulin resistance and in type 2 diabetes in either red or white skeletal muscle. There was, however, a strong inverse correlation between FAT/CD36 and GLUT4 in red muscle, further suggesting that there is a link between increased rates of LCFA transport and insulin resistance in this type of muscle. At this point it is not clear whether increased rates of LCFA transport and the increased FAT/CD36 expression and plasmalemmal content in muscle, precede the development of insulin resistance or are a consequence of insulin resistance. It appears that such studies need to focus on red skeletal muscle.

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