Cardiac and skeletal muscle fatty acid transport and transporters and triacylglycerol and fatty acid oxidation in lean and Zucker diabetic fatty rats

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Bonen A, Holloway GP, Tandon NN, Han X, McFarlan J, Glatz JF, Luiken JJ. Cardiac and skeletal muscle fatty acid transport and transporters and triacylglycerol and fatty acid oxidation in lean and Zucker diabetic fatty rats. Am J Physiol Regul Integr Comp Physiol 297: R1202–R1212, 2009. First published August 12, 2009; doi:10.1152/ajpregu.90820.2008.—We examined fatty acid transporters, transport, and metabolism in hearts and red and white muscles of lean and insulin-resistant (week 6) and type 2 diabetic (week 24) Zucker diabetic fatty (ZDF) rats. Cardiac fatty acid transport was similar in lean and ZDF hearts at week 6 but was reduced at week 24 (−40%) in lean but not ZDF hearts. Red muscle of ZDF rats exhibited an early susceptibility to upregulation (+66%) of fatty acid transport at week 6 that was increased by 50% in lean and ZDF rats at week 24 but remained 44% greater in red muscle of ZDF rats. In white muscle, no differences were observed in fatty acid transport between groups or from week 6 to week 24. In all tissues (heart and red and white muscle), FAT/CD36 protein and plasmalemmal content paralleled the changes in fatty acid transport. Triacylglycerol content in red and white muscles, but not heart, in lean and ZDF rats correlated with fatty acid transport (r = 0.91) and sarcomemmal content (FAT/CD36) r = 0.98). Red and white muscle fatty acid oxidation by isolated mitochondria was not impaired in ZDF rats but was reduced by 18–24% in red muscle of lean rats at week 24. Thus, in red, but not white, muscle of insulin-resistant and type 2 diabetic animals, a marked upregulation in fatty acid transport and intramuscular triacylglycerol was associated with increased levels of FAT/CD36 expression and plasmalemmal content. In heart, greater rates of fatty acid transport and FAT/CD36 in ZDF rats (week 24) were attributable to the inhibition of age-related reductions in these parameters. However, intramuscular triacylglycerol did not accumulate in hearts of ZDF rats. Thus insulin resistance and type 2 diabetes are accompanied by tissue-specific differences in FAT/CD36 and fatty acid transport and metabolism. Upregulation of fatty acid transport increased red muscle, but not cardiac, triacylglycerol accumulation. White muscle lipid metabolism dysregulation was not observed.

Fatty acid oxidation is not consistently supported by recent studies in insulin-resistant skeletal muscle (22, 32, 46), whereas the excess influx of fatty acids is gaining more credence (9, 19, 38).

Fatty acid entry into many tissues occurs via a protein-mediated mechanism (6, 34, 35). The high rate of fatty acid transporters include FAT/CD36 and plasma membrane-associated fatty acid-binding protein (FABPpm), which can be induced to translocate by muscle contraction (7, 23, 33, 42, 56) and by stimulation with insulin (23, 33, 43, 44). In addition, FAT/CD36 is involved in upregulation of skeletal muscle fatty acid transport in animal models of insulin resistance, including high-fat feeding (25, 48) and obesity (19, 23, 41), as well as in human obesity and type 2 diabetes (9). In these studies, the increased fatty acid transport rate was not attributable to the increased protein expression of FAT/CD36; rather, this protein was permanently relocated to the plasma membrane (9, 19, 23, 29, 41, 48). In this regard, the trafficking of FAT/CD36 is juxtaposed with that of GLUT4, the translocation of which from intracellular depots is reduced in insulin-resistant tissue (49).

With other models of insulin resistance, such as type 2 diabetic db/db mice (12) and high-fat-fed female Zucker diabetic fatty (ZDF) rats (52), FAT/CD36 expression and plasmalemmal content are increased in heart (12) and skeletal muscle (52). Presumably, these changes would have increased the rate of fatty acid transport. However, this is not entirely certain, since others observed an increased rate of fatty acid transport in adipocytes, but not in cardiac myocytes or hepatocytes, of ZDF rats (5). Tissue-specific responses in upregulation of fatty acid transport and FAT/CD36 have been observed in animal models of type 1 (40) or type 2 (15) diabetes. Muscle tissues with a high capacity for fatty acid metabolism, such as red skeletal muscle, appear to be highly susceptible to alteration of fatty acid transport and FAT/CD36 protein expression and/or plasmalemmal content, whereas tissues with a low capacity for fatty acid metabolism appear not to be affected (15, 25). There is also evidence that the impairment of glucose transport in insulin-resistant animals is considerably greater in red (−43%) than in white (−17%) muscle (59) and that GLUT4 protein is reduced in red, but not white, muscle of ZDF rats (21). These alterations in insulin-stimulated glucose disposal are presumably associated with greatly increased rates of fatty acid uptake into red, but not white, muscle of ZDF (15), obese (29), and fat-fed rats (25) and can result in excess accumulation of intramuscular triacylglycerol in red muscle compared with white muscle (24). Such intramuscular triacylglycerol accumulation serves as an index of bioactive lipids
that are known to interfere with insulin signaling in skeletal muscle (27).

In the hearts of mice with type 2 diabetes (db/db mice), rates of fatty acid oxidation and fatty acid esterification into cellular triacylglycerols are increased (for review see Ref. 13). However, far less is known about fatty acid transport in the diabetic heart and whether rates of fatty acid transport are increased, as has recently been suggested (12, 13). It is known that plasmalemmal FAT/CD36 is increased in hearts of high-fat-fed (48) and obese Zucker (41) rats. It is, however, not known whether fatty acid transport in the heart is further increased in animals with type 2 diabetes. The ZDF rat is insulin resistant (i.e., hyperglycemic and hyperinsulinemic) at 6 wk of age; by 24 wk of age, they are severely diabetic (i.e., hyperglycemic and hypoinsulinemic) (16, 20, 57).

We examined the emerging hypothesis that upregulation of fatty acid transport and transporters and intramuscular triacylglycerol accumulation in type 2 diabetes are largely confined to tissues with a high capacity for fatty acid metabolism, namely, heart and red skeletal muscle, whereas tissue with a low capacity for fatty acid metabolism, such as white muscle, is minimally affected. Therefore, we compared the changes in fatty acid transport and metabolism and fatty acid transporter expression and sarcolemmal content (FAT/CD36 and FABPpm) in heart and red and white muscles of lean and ZDF rats at week 6, when ZDF animals are known to be insulin resistant (57), and at week 24 in type 2 diabetic ZDF rats (57). GLUT4 expression and plasmalemmal content were also examined in these tissues.

METHODS

Animals

All experimental procedures were approved by the Committee on Animal Care at the University of Guelph. Male lean and ZDF rats (Charles River, Wilmington, MA) were maintained on the diet that is recommended for ZDF rats (Purina Labdiet, Formulab 5008, Richmond, IN).

Animals were euthanized [pentobarbital sodium (Somnotol), 6 mg/100 g body wt] at 6 and 24 wk of age (weeks 6 and 24). Thereafter, blood samples were collected from nonfasted animals, as has been done by others in nonfasted rats (21, 39, 51, 57, 58), using cardiac puncture immediately before excision of the heart (left ventricle) and red (red gastrocnemius and red vastus lateralis) and white (white gastrocnemius and white vastus lateralis) muscle from both hindlimbs. Blood was placed in 1.5-ml Eppendorf tubes and centrifuged at 9,300 g for 5 min at 4°C. The plasma was removed and stored at −80°C for later analyses of glucose, insulin, and nonesterified fatty acids. Small blood samples were collected from nonfasted animals, as has been done by others in nonfasted rats (21, 39, 51, 57, 58), using cardiac puncture immediately before excision of the heart (left ventricle) and red (red gastrocnemius and red vastus lateralis) and white (white gastrocnemius and white vastus lateralis) muscle from both hindlimbs. Blood was placed in 1.5-ml Eppendorf tubes and centrifuged at 9,300 g for 5 min at 4°C. The plasma was removed and stored at −80°C for later analyses of glucose, insulin, and nonesterified fatty acids. Small portions of the pooled heart and red and white muscles were frozen (liquid nitrogen) and stored at −80°C. The remaining portions of the heart and red and white muscles were used for preparation of giant sarcoclemmal vesicles. For these experiments, tissues from two to three animals were pooled to yield sufficient tissues for the preparation of giant vesicles from the heart. Cardiac and intramuscular triacylglycerol was determined and fatty acid oxidation was measured in isolated red and white muscle mitochondria in separate groups of lean and ZDF animals at weeks 6 and 24.

Preparation of Giant Vesicles

Giant vesicles from heart and red and white skeletal muscle were generated from fresh tissue and used immediately thereafter for palmitate uptake studies, as we previously described (7, 40). Briefly, the tissues were cut into thin (1- to 3-mm-thick) layers 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 II (heart) and type VII (muscle), 150 U/ml]. Thereafter, supernatant fractions and a Percoll [16% (vol/vol)]-aprotinin (0.1 mg/ml) suspension were placed at the bottom of a density gradient consisting of a 3-mL middle layer of 4% Nycodenz (wt/vol) and a 1-mL top layer of KCl-MOPS and centrifuged at 60 g for 45 min at room temperature. Subsequently, the vesicles were harvested from the interface of the top and middle layers, diluted in KCl-MOPS, and centrifuged at 900 g for 10 min. The pellet was resuspended in KCI-MOPS.

Palmitate Uptake

Palmitate uptake was determined as we previously described (7, 40). Fat-free BSA (Roche Diagnostics, Laval, PQ, 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 KCI-MOPS, both unlabeled (15 μM) and radiolabeled with 0.3 μCi of [3H]palmitate and 0.06 μCi of [14C]mannitol, were added to 40 μl of vesicle suspension, and the sample was incubated for 15 s. Palmitate uptake was terminated by addition of 1.4 ml of ice-cold KCI-MOPS, 2.5 mM HgCl2, and 0.1% BSA. The sample was then quickly centrifuged at maximal speed in a microfuge for 1 min, the supernatant was discarded, and radioactivity was determined in the tip of the tube. Nonspecific uptake was measured by addition of the stop solution before the radiolabeled palmitate solution.

Triacylglycerol

Cardiac and intramuscular triacylglycerol content was determined as we previously reported (3, 30). Briefly, tissues (40–50 mg) were homogenized (Polytron, Kinematica, Brinkmann, Littau-Lucerne, Switzerland) for 15 s in 2 ml of 1:1 chloroform-methanol on ice at a speed setting of 8, allowed to rest for 15 s, and homogenized again under the same conditions. Solvent solution was recovered by centrifugation at 6,000 g for 10 min at 4°C and transferred to a new tube. This solution was washed with 2 ml of H2O, and the lipid-containing chloroform phase was separated from the aqueous phase by centrifugation. Then, 500 μl of the chloroform phase were dried under nitrogen, and samples were reconstituted with 100 μl of 2:1 chloroform-methanol (vol/vol). Samples (50 μl) were spotted onto 250-mm silica gel plates and resolved (60:40:3 heptane-isopropyl ether-acetic acid) for 50 min. Then the plate was air dried and sprayed with chloroform:fluorescein dye [0.02% (wt/vol) in ethanol]. The triacylglycerol lipid band was visualized under long-wave UV light and quantified against known standards.

Mitochondrial Isolation

Mitochondria from red and white muscle were isolated as we described previously (11, 60). Briefly, fresh tissue was placed in ice-cold buffer (100 mM KCl, 50 mM Tris-HCl, 5 mM MgSO4, and 5 mM EDTA, pH 7.4) and minced on ice to remove connective tissue and fat. Muscles were weighed, diluted 10-fold, and homogenized (Teflon pestle). Thereafter, the samples were centrifuged (800 g, 10 min) to yield mitochondria, which were suspended in 500 μl of buffer (220 mM sucrose, 70 mM mannitol, 10 mM Tris-HCl, and 1 mM EDTA) and used immediately for determinations of palmitate oxidation.

Mitochondrial Palmitate Oxidation

Palmitate oxidation was measured in a sealed system, as we described previously (3, 30). Briefly, mitochondria were added to a pregressed modified Krebs-Ringer buffer at 37°C. The reaction (30 min) was initiated by addition of palmitate-BSA complex (75 μM, 1-[14C]palmitate (0.5 μCi/vial, 30 min, 37°C, pH 7.4)). Oxidation was determined from trapping of the 14CO2 produced and the 14C
label released from acidified buffer, as we reported previously (3, 11, 30, 60).

**Plasma Metabolite Assays**

Serum samples were analyzed for glucose using a spectrophotometric method (Sigma, St. Louis, MO), while 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).

**Citrate Synthase**

Activities of citrate synthase (CS) were determined in heart and muscle homogenates using standard enzymatic procedures (53), as we reported previously (3, 30).

**Western Blotting**

FAT/CD36, FABPpm, and GLUT4 proteins were determined in homogenates and giant sarcolemmal vesicles, as we previously described (7, 40), using standard procedures.

**Statistics**

The data were analyzed using a two-way analysis of variance. When significance was detected, Fisher’s least significant difference post hoc test was used to ascertain the points where statistical differences occurred. Values are means ± SE.

**RESULTS**

At week 6, the animals’ body weights differed (P < 0.05): 146.2 ± 3.0 g (lean) and 214.2 ± 3.1 g (ZDF). Both groups gained weight, but at week 24 their body weights did not differ (P > 0.05): 401.0 ± 5.0 g (lean) and 396.2 ± 9.5 g (ZDF).

**Circulating Concentrations of Glucose, Insulin, and Fatty Acids**

At week 6, glucose and insulin concentrations were two- and fourfold greater, respectively, in ZDF rats (P < 0.05; Fig. 1, A and B). By week 24, these concentrations were still markedly different between the two groups: in lean rats, glucose (+69%) and insulin (+200%) concentrations increased (P < 0.05; Fig. 1, A and B), and in ZDF rats, glucose increased (+111%) and insulin decreased (∼70%, P < 0.05; Fig. 1, A and B). Thus, at week 24, glucose and insulin concentrations were 158% higher and 58% lower, respectively, in ZDF than in lean rats (P < 0.05; Fig. 1, A and B).

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**Fig. 1.** Circulating concentrations of glucose (A), insulin (B), and fatty acids (C) and citrate synthase activity in heart (D), red muscle (E), and white muscle (F) in lean and Zucker diabetic fatty (ZDF) rats at 6 and 24 wk of age (weeks 6 and 24). Values are means ± SE; n = 9–10 (A–C) and 5 (D–F) animals. *P < 0.05 vs. lean. **P < 0.05 vs. week 6.
The circulating fatty acid concentrations were 3.3-fold greater at week 6 in ZDF than in lean rats. From week 6 to week 24, there were no changes in circulating fatty acids in lean animals, whereas in ZDF rats there was a further 33% increase (P < 0.05; Fig. 1C). Collectively, these data (Fig. 1, A–C) establish that, in the ZDF rats, insulin resistance at week 6 progressed to severe type 2 diabetes at week 24. This compares well with previous studies (16, 20, 21, 57).

CS Activity

As expected, CS activity, an index of mitochondrial content (22, 28), was greater in the heart than in red or white muscle (Fig. 1D; P < 0.05) and greater in red than in white muscle (P < 0.05; Fig. 1, E and F). No differences in cardiac CS activities were observed between lean and ZDF rats at week 6 or week 24, nor were there changes in either group from week 6 to week 24 (Fig. 1D).

At week 6, CS activity was 17% greater in red muscle of ZDF than lean rats (P < 0.05; Fig. 1E). At week 24, there were no such differences in red muscle between lean and ZDF rats. Nevertheless, compared with week 6, comparable relative reductions in CS activity were observed at week 24 in red muscle of lean (–12%) and ZDF (–20%) rats (P < 0.05; Fig. 1E). In contrast to the observations in red muscle, there were no differences in white muscle CS activity between lean and ZDF rats at week 6 or week 24, and there were no changes in CS activity from week 6 to week 24 (P > 0.05; Fig. 1F).

Fatty Acid Transport and Transporters

Fatty acid transport into heart and red and white muscle. LEAN ANIMALS. At week 6, the rate of palmitate transport was sixfold greater into heart than into red muscle of lean rats, which in turn was twofold greater than into white muscle of lean rats (P < 0.05; Fig. 2A, A, F, and K). These different rates of palmitate transport among heart and red and white muscle have also been observed in nondiabetic animals (8, 40, 45).

LEAN VS. ZDF HEART. At week 6, palmitate transport rates did not differ in lean and ZDF hearts. However, at week 24, the rates of palmitate transport were reduced by 40% in lean hearts (P < 0.05; Fig. 2A), whereas in ZDF hearts, no such reductions were observed, inasmuch as palmitate transport rates were comparable at weeks 6 and 24 (P > 0.05; Fig. 2A). Because of these different changes from week 6 to week 24 in lean and ZDF hearts, the rate of fatty acid transport was 66% greater in ZDF rats at week 24.

LEAN VS. ZDF RED MUSCLE. At week 6, palmitate transport rates were greater in ZDF than in lean muscles (P < 0.01; Fig. 2F). In lean and ZDF rats, palmitate transport rates were increased by ~50% from week 6 to week 24 (P < 0.05). Nevertheless, at both time points, the rates of palmitate transport were greater in ZDF than in lean rats (+66% at week 6 and +47% at week 24).

LEAN VS. ZDF WHITE MUSCLE. In contrast to red muscle, palmitate transport rates did not differ in white muscle of lean and ZDF animals at week 6 or week 24. Moreover, no changes in these rates were observed from week 6 to week 24 in either of the two groups (P > 0.05; Fig. 2K).

Fatty acid transport proteins in heart and red and white muscle. The protein expression of FAT/CD36 and FABPpm was determined in muscle homogenates and at the plasma membrane in heart and muscle tissues.

FAT/CD36 protein expression and plasmalemmal content. LEAN VS. ZDF HEART. At week 6, FAT/CD36 protein expression (+13%) and plasmalemmal content (+12%) were slightly greater in ZDF than in lean hearts (P < 0.05; Fig. 2, B and C). Reductions in FAT/CD36 protein expression (–20%) and plasmalemmal content (–19%) were comparable in lean hearts at week 24 (P < 0.05; Fig. 2, B and C). In contrast, in ZDF hearts, neither FAT/CD36 protein expression nor plasmalemmal content was altered from week 6 to week 24 (P > 0.05; Fig. 2, B and C). Therefore, at week 24, FAT/CD36 protein expression (+40%) and plasmalemmal content (+41%) were considerably greater in the hearts of ZDF than lean rats.

LEAN VS. ZDF RED MUSCLE. At week 6, FAT/CD36 protein expression (+30%) and plasma membrane content (+42%) were greater in the ZDF rats (P < 0.05; Fig. 2, G and H). In the lean rats at week 24, FAT/CD36 protein expression was not altered, but plasmalemmal FAT/CD36 content was reduced (–24%, P < 0.05; Fig. 2H). In contrast, in ZDF rats at week 24, FAT/CD36 protein expression (+54%) and plasma membrane content (+43%) were increased over levels observed at week 6 (P < 0.05; Fig. 2, G and H). Thus, at week 24, FAT/CD36 protein expression (+137%) and plasmalemmal content (+176%) were markedly greater in ZDF than in lean rats.

LEAN VS. ZDF WHITE MUSCLE. Neither FAT/CD36 protein expression nor plasmalemmal content differed between lean and ZDF rats at week 6 or week 24. Moreover, no changes were observed in either group from week 6 to week 24 (P > 0.05; Fig. 2, L and M).

FABPpm protein expression and plasmalemmal content. LEAN VS. ZDF HEART. FABPpm protein expression and plasmalemmal content did not differ between lean and ZDF rats at week 6 or week 24. From week 6 to week 24, no changes were observed in FABPpm expression in either group (P > 0.05; Fig. 2D), whereas there was only a very small reduction (–10%) in plasmalemmal FABPpm in both groups (P < 0.05; Fig. 2F).

LEAN VS. ZDF RED MUSCLE. Protein expression of FABPpm did not differ between groups at week 6 (P > 0.05). At week 24, FABPpm expression and plasmalemmal content were unchanged in the lean rats. In contrast, in ZDF rats, FABPpm protein expression (–44%) and plasmalemmal content (–37%) were reduced at week 24 (P < 0.05; Fig. 2, I and J).

LEAN VS. ZDF WHITE MUSCLE. At week 6, FABPpm protein expression did not differ in lean and ZDF rats (P > 0.05). At week 26, protein expression was increased in lean (+23%) and ZDF (28%) rats (P < 0.05; Fig. 2M). However, no changes in plasmalemmal FABPpm were observed at any time (P < 0.05; Fig. 2O).

Intramuscular and Cardiac Triacylglycerol

LEAN VS. ZDF HEART. Cardiac triacylglycerol content was greater in ZDF than in lean rats at week 6 (Fig. 3A). From week 6 to week 24, there was a reduction and an increase in triacylglycerol content in ZDF and lean hearts, respectively, but within each of the two groups these changes from week 6 to week 24 were not statistically significant (P > 0.05), nor were differences between the groups at week 24 statistically significant (P > 0.05; Fig. 3A).
Lean vs. ZDF red muscle. Large differences were observed in triacylglycerol content between ZDF and lean red muscles. At week 6, triacylglycerol content was 153% greater in ZDF than in lean rats. Triacylglycerol content increased from week 6 to week 24 in lean (31%, P = 0.075) and ZDF (40%, P < 0.05) rats, although the net absolute increase was considerably greater in ZDF (232%) than in lean rats (Fig. 3B).

Lean vs. ZDF white muscle. As expected, intramuscular triacylglycerol content was lower in white than in red muscle in lean and ZDF rats (P < 0.05; Fig. 3C). However, no differences in white muscle intramuscular triacylglycerol content were observed between lean and ZDF rats at week 6 or week 24, and no changes occurred in either group from week 6 to week 24 (P > 0.05; Fig. 3C).

Fatty Acid Oxidation in Isolated Mitochondria

Palmitate oxidation was determined in red and white muscle mitochondria only. Tissue limitations prevented similar measurements in cardiac mitochondria.

Lean vs. ZDF red muscle. Palmitate oxidation in isolated mitochondria was greater in ZDF than in lean rats at week 6 (+19%) and week 24 (+42%, P < 0.05; Fig. 4A). This greater difference at week 24 was attributable to the reduction in fatty acid oxidation in lean rats from week 6 to week 24 (−18%, P < 0.05), whereas in the ZDF rats palmitate oxidation did not differ at weeks 6 and 24 (P > 0.05; Fig. 4A).

Lean vs. ZDF white muscle. Palmitate oxidation rates were consistently 20–30% lower in white than in red muscle mitochondria in lean and ZDF rats at weeks 6 and 24 (P < 0.05;
Although there were no differences in palmitate oxidation between lean and ZDF rats at week 6, palmitate oxidation in white muscle mitochondria was 42% greater in ZDF than in lean rats at week 24 (*P < 0.05; Fig. 4B). This was attributable, just as in red muscle, to a reduction in lean white muscle palmitate oxidation from week 6 to week 24 (*P < 0.05), inasmuch as palmitate oxidation was not altered from week 6 to week 24 in white muscle mitochondria in ZDF rats (**P < 0.05; Fig. 4B).

**Relationship Between FAT/CD36, Fatty Acid Transport, and Triacylglycerol**

In red and white muscle of lean and ZDF rats, there was a strong relationship between plasma membrane FAT/CD36 and rates of fatty acid transport (Fig. 5A). This was also observed in the heart (Fig. 5A, inset), although the data were clustered, and this tended to bias the correlation in this tissue. Rates of palmitate transport (Fig. 5B) and plasma membrane FAT/CD36 (Fig. 5C) were also strongly related to intramuscular triacylglycerol content in red and white muscle of lean and ZDF rats. No such relationships with triacylglycerol content were evident in the heart (data not shown).

**GLUT4 Protein Expression and Plasma Membrane Content**

**Lean vs. ZDF heart.** At week 6, GLUT4 protein expression and plasmalemmal content did not differ in hearts of lean and ZDF rats (Fig. 6, A and B; *P > 0.05*). In the transition from week 6 to week 24, GLUT4 protein expression was not altered in lean heart, but plasma membrane GLUT4 was increased (+28%, *P < 0.05; Fig. 6B). In contrast to the lean animals, GLUT4 protein expression (~33%) and plasmalemmal content (~25%) were reduced at week 24 in ZDF hearts (~33%). Thus, at week 24, GLUT4 protein expression (~40%) and plasmalemmal content (~32%) were markedly lower in hearts of ZDF rats.

**Lean vs. ZDF red muscle.** At week 6, GLUT4 protein expression level was somewhat lower in ZDF red muscle (~18%, *P < 0.05; Fig. 6C), but there were no differences in plasma membrane GLUT4 between the two groups at week 6 (*P > 0.05; Fig. 6D). From week 6 to week 24, GLUT4 protein expression was not altered in lean red muscle, but plasma membrane GLUT4 was increased (+26%, *P < 0.05; Fig. 6D). In contrast, from week 6 to week 24, GLUT4 protein expression (~44%) and plasmalemmal GLUT4 (~35%) were reduced in ZDF red muscle.
ZDF rats (P < 0.05; Fig. 6, C and D). As a result of these different changes, at week 24, GLUT4 protein expression (−47%) and plasmalemmal content (−48%) were considerably lower in ZDF red muscle.

**Lean vs. ZDF white muscle.** At week 6, neither GLUT4 protein expression nor plasmalemmal contents differed in white muscles of lean and ZDF rats (P > 0.05; Fig. 6, E and F). In the transition from week 6 to week 24, lean white muscle GLUT4 protein expression was reduced (−24%, P < 0.05; Fig. 6E), but its plasma membrane GLUT4 was not altered (P > 0.05; Fig. 6F). In contrast, in ZDF rats from week 6 to week 24, there were similar reductions in white muscle GLUT4 expression and plasma membrane GLUT4 (−44%). At week 24, GLUT4 protein expression (−30%) and plasmalemmal content (−28%) were lower in white muscle of ZDF than lean rats.

**FAT/CD36 vs. GLUT4**

To examine whether there was a relationship between fatty acid transporters and GLUT4, we compared their levels at weeks 6 and 24. There was no relationship between FABPppm and GLUT4 (data not shown). However, in heart and red muscle, there was an inverse relationship between FAT/CD36 and GLUT4 expression (r = −0.84, P < 0.05; Fig. 7A) and their plasma membrane content (r = −0.80, P < 0.05; Fig. 6B), whereas no such relationship was found for white muscle (Fig. 7, A and B, insets).

**DISCUSSION**

We examined the changes in fatty acid transport and transporters, mitochondrial density, and fatty acid metabolism in tissues with high (heart and red muscle) and low (white muscle) capacities for fatty acid utilization (19, 23) in lean and ZDF insulin-resistant (week 6) and type 2 diabetic (week 24) rats. There are a number of novel findings. 1) In white muscle, there were virtually no changes in any aspects of lipid metabolism at week 6 or week 24. 2) In contrast, ZDF red muscle exhibited an early susceptibility (week 6) to upregulation of fatty acid transport compared with ZDF heart. 3) In type 2 diabetic rats (week 24), fatty acid transport and FAT/CD36 expression and plasmalemmal content were greatly upregulated in red muscle, but only somewhat in heart. 4) In red muscle, but not heart, rates of fatty acid transport and intramuscular triacylglycerol content were increased. 5) Mitochondrial fatty acid oxidation was greater in red muscle of ZDF than lean rats at weeks 6 and 24. 6) FAT/CD36 and GLUT4 were inversely related in heart and red muscle. Therefore, muscle tissues with a high capacity for fatty acid metabolism (heart and red muscle) exhibit an increase in fatty acid transport compared with muscle tissues with a low capacity for fatty acid metabolism (white muscle), but this only results in intramuscular triacylglycerol accumulation in red muscle, inasmuch as, presumably, excess fatty acids taken up into the heart are oxidized.

**Fatty Acid Transport and Triacylglycerol Accumulation in Muscle and Heart**

The capacity for fatty acid transport and the expression and plasmalemmal content of fatty acid transporters are much greater in the heart than in oxidative or glycolytic muscle (37, 40, 41). In addition, some studies have begun to suggest that oxidative muscles, which have a high capacity for fatty acid utilization, are particularly susceptible to dysregulation of fatty acid transport (15, 25). Therefore, we speculated that the highly oxidative heart would perhaps upregulate fatty acid transport to a much greater extent in type 2 diabetes than in skeletal muscle (15). This was indeed the case: at week 24, the difference in fatty acid transport between lean and ZDF rats followed the oxidative capacity gradients of the different tissues (i.e., heart > red > white muscle). On the other hand, at
week 6, the rates of fatty acid transport were upregulated in ZDF red muscle, but not in ZDF heart or white muscle. These observations suggest that 1) the most oxidative tissue (heart) maintains the largest rate of fatty acid transport in type 2 diabetes, whereas it declines in lean animals, and 2) red muscle is most susceptible to upregulation of fatty acid transport. These increased rates of fatty acid transport are not always directly associated with intramuscular lipid accumulation in all tissues. Red skeletal muscle readily develops insulin resistance, when intracellular lipids accumulate (1). In the present study, there was a high correlation between rates of fatty acid transport and intramuscular triacylglycerol content, consistent with reports that have linked increased rates of fatty acid transport with intramuscular lipid accumulation in human obesity and type 2 diabetes (9, 24) and in oxidative muscle tissues in animal models of type 2 diabetes and insulin resistance (25, 29). Such intramuscular lipid accumulation may be expected to interfere with insulin signaling (for review see Ref. 27). The heart, on the other hand, may not develop insulin resistance quite as readily as red muscle. We found that, despite a greater rate of fatty acid transport in the diabetic heart (week 24), cardiac triacylglycerols did not accumulate compared with lean animals, in which fatty acid transport rates were markedly reduced (week 24). This differs from observations in type 2 diabetic db/db mice, in which cardiac triacylglycerol did accumulate, although much less than the concomitant increase in fatty acid oxidation (12). Presumably, the heart’s continuous contractile activity and its ability to increase fatty acid oxidation in type 2 diabetes (4-fold) (12, 13) allow much of the additional fatty acids to be taken up metabolically. Red muscle, on the other hand, seems unable to increase its fatty acid oxidation sufficiently to dispose of the additional fatty acids that are transported into this tissue, resulting in the accumulation of intramuscular triacylglycerol. This is consistent with a recent study in obese Zucker rats, in which the increase in fatty acid transport resulted in an eightfold greater rate of increase in triacylglycerol accumulation than fatty acid oxidation (29).

Fatty Acid Transporters

FAT/CD36. A number of studies have shown that FAT/CD36 is a key fatty acid transport protein in muscle and heart (for review see Ref. 6). This is also suggested in the present study by the correlation between plasma membrane FAT/CD36 and fatty acid transport rates. In agreement with another model of type 2 diabetes, the db/db mouse (12), cardiac triacylglycerol content was increased in the ZDF animals (week 24), although the mechanisms involved in muscle and heart differed. Although FAT/CD36 expression and plasmalemmal content were increased in red muscle of ZDF rats compared with lean rats. Thus the mechanisms that produce differences in FAT/CD36 at any one point in time are heart and red muscle specific, as further shown in white muscle of ZDF rats, in which changes in FAT/CD36 were nonexistent. Differences in transcriptional control of FAT/CD36 among tissues might explain these tissue-specific responses, but a number of studies, including models of type 2 diabetes (12, 15), have shown no relationship between the changes in fatty acid transporter mRNA abundance and the changes in their protein product in heart and muscle (40, 41). Thus explanations for different susceptibilities among muscle tissues in upregulation of FAT/CD36 remain unknown.

FABPpm. As in other studies with insulin-resistant animals (41) and humans (9) and diabetic animals (12, 15), changes in FABPpm were essentially nonexistent. Protein expression of FABPpm might reflect not only this protein but also mitochondrial aspartate amino transferase (4, 10, 14, 31, 55). The best
indication of a fatty acid transport role for FABPpm is its location at the plasma membrane (31). At this site, FABPpm was reduced in red muscle in ZDF rats at week 24. This, however, was unrelated to the upregulation of fatty acid transport, possibly as a result of the increase in plasmalemmal FAT/CD36, since the transport capacity of FAT/CD36 is considerably greater than that of FABPpm (47).

Mitochondria and Mitochondrial Fatty Acid Oxidation

We used CS activity as an index of mitochondrial content in heart and muscle, as has been commonly done for many years (3, 28, 29). Neither in heart nor in muscle did we observe a reduced mitochondrial content in insulin resistance (week 6) or type 2 diabetes (week 24) compared with age-matched lean animals. There was no evidence that fatty acid oxidation by isolated mitochondria from red or white muscle was impaired in insulin resistance or type 2 diabetes. This is consistent with recent observations in human insulin resistance (32) and type 2 diabetes (2, 46). Indeed, in the present study, mitochondrial fatty acid oxidation was usually greater in muscle from ZDF than lean rats at weeks 6 and 24, as has also been found in human type 2 diabetes (2).

GLUT4 and FAT/CD36

GLUT4 expression and plasmalemmal content were reduced in all tissues from week 6 to week 24. The magnitude of these reductions in heart and muscle corresponds to that reported earlier (51). However, the relative decreases tended to be greater in more oxidative tissues, namely, heart and red muscle, than in white muscle. Since more GLUT4 is harbored in heart and red muscle than in white muscle, the absolute decrease in GLUT4 expression and plasmalemmal content is considerably greater in these tissues than in white muscle. The smaller changes in GLUT4 in white muscle than in red muscle from ZDF rats have also been observed by others (21). Since it is well known that rates of plasmalemmal GLUT4 are highly correlated with rates of glucose transport, it is safe to assume that glucose transport was reduced at week 24 in all tissues examined, but this was quantitatively greater in the oxidative muscle tissues.

We found an inverse correlation between FAT/CD36 and GLUT4 expression in oxidative tissues (heart and muscle), but not in glycolytic tissue (white muscle). Thus a hallmark of full-blown insulin resistance in muscle tissues is a juxtaposed, subcellular localization of FAT/CD36 and GLUT4, with FAT/CD36 being permanently located at the cell surface and GLUT4 being retained within an intracellular compartment. This would seem to link impaired GLUT4 trafficking to the plasma membrane with increased plasmalemmal FAT/CD36 and increased rates of fatty acid transport and intramuscular lipid accumulation, especially in red muscle, although not necessarily in heart. Support for this link in red muscle is evident in a recent study in which we found that reducing plasmalemmal FAT/CD36 increased plasmalemmal GLUT4 and improved insulin-stimulated glucose transport in red muscle of fat-fed ZDF animals (52).

Perspectives and Significance

It is clear from our studies that alterations in lipid metabolism, whether in insulin-resistant or type 2 diabetic animals, are highly specific to given tissues, such that it is difficult to extrapolate observations from one type of tissue to another (heart vs. muscle) or even within a tissue type (red vs. white muscle). The implication is that there are tissue-specific and within-muscle-type-specific regulatory mechanisms to the altered substrate and endocrine milieu in insulin resistance and type 2 diabetes. Given this complexity in mammalian tissues, considerable caution is required to extrapolate regulatory...
mechanisms of substrate metabolism from nonmammalian cells or other tissues (e.g., adipocytes or hepatocytes) to heart and skeletal muscles, both red and white. The dogma that fatty acid oxidation is compromised in mitochondria of insulin-resistant and diabetic muscle is in considerable doubt and will likely be abandoned, particularly inasmuch as other mechanisms (e.g., increased fatty acid transport via FAT/CD36) provide a better explanation for increased intramuscular lipid accumulation in muscle. Finally, it has not been widely appreciated that it is red muscle that is highly susceptible to developing insulin resistance, while determinations as to why white muscles tend to be so much less susceptible have not been explored.

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