Increased Fatty Acid Uptake and Altered Fatty Acid Metabolism in Insulin-Resistant Muscle of Obese Zucker Rats

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Altered muscle fatty acid (FA) metabolism may contribute to the presence of muscle insulin resistance in the genetically obese Zucker rat. To determine whether FA uptake and disposal are altered in insulin-resistant muscle, we measured palmitate uptake, oxidation, and incorporation into di- and triglycerides in isolated rat hindquarters, as well as muscle plasma membrane fatty acid–binding protein (FABPPM) content of lean (n = 16, fa/+) and obese (n = 15, fa/fa) Zucker rats (12 weeks of age). Hindquarters were perfused with 7 mmol/l glucose, 1,000 μmol/l albumin-bound palmitate, and albumin-bound [1-14C]palmitate at rest (no insulin). Glucose uptake was 42% lower in the obese than in the lean rats and indicated the presence of muscle insulin resistance. Fractional and total rates of palmitate uptake were 42 and 74% higher in the obese than in the lean rats and were associated with higher muscle FABPPM content (r² = 0.69, P < 0.05). The percentage of palmitate oxidized was not significantly different between groups. FA disposal to storage was altered according to fiber type. When compared with lean rats, the rate of triglyceride synthesis in red muscle was 158% higher in obese rats, and the rate of palmitate incorporation into triglycerides in white muscle was 93% higher in obese rats. Pre- and postperfusion muscle triglyceride levels were higher in both red and white muscles of the obese rats. These results show that increased FA uptake and altered FA disposal to storage may contribute to the development of muscle insulin resistance in obese Zucker rats. Diabetes 50:1389–1396, 2001

Insulin-resistant states, such as obesity, are characterized by hyperlipidemia and elevated triglyceride stores (1,2). Whereas hyperlipidemia and elevated triglyceride stores may be caused in part by an oversupply of fatty acids (FAs) released from adipose tissue (3), it could also be caused by an alteration in the disposal of FA, especially in muscle tissue. As suggested by the presence of an inverse relationship between insulin sensitivity and triglyceride content in muscle, an alteration in the inherent capacity of the muscle to take up and dispose of an FA load could be critical to the development of muscle insulin resistance (4,5). However, studies investigating the direct effects of insulin resistance on FA metabolism in muscle are scarce, and results are equivocal.

The genetically obese Zucker rat has been used extensively to study the effects of insulin resistance on muscle metabolism because it exhibits many of the pathophysiological alterations observed in obese humans, namely severe obesity, hypertriglyceridemia, hyperinsulinemia, and chronic muscle insulin resistance (1–3). Although defects in glucose metabolism have been well documented in this model of insulin resistance, changes in FA metabolism have only been inferred from changes in carbohydrate metabolism, thus leading to equivocal conclusions (2,6,7). Some researchers have suggested that muscle FA oxidation was increased (2,7) in obese Zucker rats, whereas others have suggested that muscle FA oxidation was decreased (6). Therefore, it is unclear whether muscle FA oxidation is altered with the presence of muscle insulin resistance. Furthermore, because FA kinetics were not measured directly in those studies, it was not possible to determine whether the measured changes in FA utilization were caused by alterations in FA uptake, cellular FA disposal, or both.

It has recently become evident that alterations in FA uptake could be of primary importance in the regulation of FA utilization in muscle (8–10). Indeed, evidence suggests that at least part of the uptake of FA in muscle may be carrier-mediated and that fatty acid transporter proteins located in the plasma membranes are an integral component of this transport system (11,12). Thus, with this system, control of FA uptake would be possible at the transport step, and the content of FA-transporter proteins at the plasma membrane would be critical. Fatty acid–binding protein (FABPPM) is among the several proteins that have been identified as putative FA-transport proteins (11–13). Whereas the specific role of FABPPM in a putative trans-sarcolemmal transport process has not been clearly identified, the protein has been shown to be present in muscle, and its expression in muscle has been shown to be modified by exposure to physiological stimuli associated with changes in FA utilization (13–17). Therefore, if muscle FA uptake is altered by the presence of muscle insulin resistance, this could be associated with concomitant changes in the content of FABPPM. Conversely, the presence of insulin resistance could be caused in part by

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CM, crude membrane; DGAT, diacylglycerol acyltransferase; FA, fatty acid; FABPPM, fatty acid–binding protein; FAT, fatty acid transporter; FFA, free fatty acid; GPAT, glycerol-3-phosphate acyltransferase; PM, plasma membrane; TBST, Tris-buffered saline with Tween.
metabolic impairments in the biosynthetic and oxidative pathways of FA disposal.

Thus, the purpose of this study was to determine whether basal FA metabolism is impaired in insulin-resistant muscle by measuring palmitate uptake and disposal in the perfused hindlimbs of obese and lean Zucker rats. The ability of the muscle to take up FA was also assessed by measuring FABPPm content. FA disposal was assessed by the measurement of palmitate oxidation and incorporation into muscle di- and triglycerides.

**RESEARCH DESIGN AND METHODS**

**Animal preparation.** Lean (fa+/+, n = 16) and obese (fa/ra, n = 15) female Zucker rats (12 weeks of age) were housed in pairs, were maintained on a 12:12-h light-dark cycle, and received regular rat diet and water ad libitum. The obese rats were significantly heavier than the lean rats (407.5 ± 7.2 vs. 207.5 ± 5.5 g, respectively, P < 0.05).

**Hindquarter perfusion.** A total of 10 lean and 9 obese rats were anesthetized with ketamine/Rompun (90 and 12 mg/kg body wt, respectively) and prepared surgically for hindquarter perfusion as previously described (16,18). Before insertion of the perfusion catheters, heparin (150 IU) was administered into the inferior vena cava. The rats were killed with an intracardial injection of ketamine/Rompun immediately before the catheters were inserted, and the preparation was placed in a perfusion apparatus, essentially as described by Ruderman and colleagues (16,18).

The initial perfusate (200 ml) consisted of Krebs-Henseleit solution, 1- to 2-day-old washed bovine erythrocytes (30% hematocrit), 5% bovine serum albumin (Cohn fraction V; Sigma, St. Louis, MO), 7 mmol/l glucose, 1,000 μmol/l albumin-bound palmitate, and 5 μCi of albumin-bound [1-14C]palmitate (ICN Pharmaceuticals, Costa Mesa, CA). This concentration of palmitate was chosen because it is within the physiological range for Zucker rats (3). To minimize the influence of confounding factors related to the presence of insulin and to allow us to make conclusions about inherent alterations caused by the presence of muscle insulin resistance, we chose to perfuse the hindquarters without insulin. The perfusate (37°C) was continuously gassed with 95% O2 and 5% CO2 to maintain arterial pH at 7.35 ± 0.05 (n = 5) and to maintain arterial PO2 at 135 ± 20 mm Hg (n = 5). The arterial blood samples for the analysis of [14C]–free fatty acid (FFA) and [14C]CO2 radioactivities were taken after 20, 30, and 40 min of perfusion. Arterial and venous perfusate samples were analyzed for glucose, lactate, glycerol, and FFA concentrations as well as for [14C]-FFA and [14C]CO2 radioactivities (16). Samples for glucose and lactate were kept on ice and analyzed using YSI glucose and lactate analyzers (Yellow Springs Instruments, Yellow Springs, OH). Samples for FFA and glycerol were put in 200 μl of ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (pH 7) and centrifuged, and the supernatant was frozen until analyzed spectrophotometrically using the WAKO NEFA-C test (Biochemical Diagnostics, Downers Grove, IL) and enzymatic glycerol kinase assay (Sigma), respectively. Because the FFA concentration was low in the absence of added palmitate (<50 μmol/l) and because palmitate was the only FA added, measured FFA concentrations were taken to equal palmitate concentrations.

To determine plasma palmitate radioactivity, duplicate 100-μl aliquots of the perfusate plasma were mixed with liquid scintillation fluid (BudgitSolve, Research Product International, Monticello, IL) and counted in a Tri-car label scintillation counter (model 4000 CA; United Technologies Packard, Downers Grove, IL), as previously described (16). The liberation and collection of [14C]CO2 from the blood were performed within 4–5 min of anaerobic collection (2 ml) as previously described (16). Perfusate samples for the determination of PCO2, PO2, pH, and hemoglobin were collected anaerobically, placed on ice, and measured within 5 min of collection by an ABL5 acid-base laboratory (Radiometer America, Westlake, OH) and spectrophotometrically with Drabkin’s reagent (Sigma, St. Louis, MO), respectively.

Muscle triglyceride concentration was determined as glycerol residues after extraction and separation of the muscle samples as previously described (16). Briefly, lipids were extracted from powdered muscle samples by centrifugation at 1,000g in 2:l chloroform:methanol solution and 4 mmol/l magnesium chloride. The organic extract was evaporated and reconstituted in chloroform, and the phospholipids by centrifugation. The resulting supernatant was evaporated, saponified in ethanolic potassium hydroxide for 30 min at 70°C, and centrifuged with 0.15 mol/l magnesium sulfate. The final supernatant was analyzed for glycerol spectrophotometrically by the enzymatic glycerol kinase method (Sigma). To measure the incorporation of [14C]palmitate into muscle di- and triglycerides, lipids from the extracted organic layer were separated by liquid chromatography as previously described (16).

For Western blot analysis, solubilized PM proteins (30 μg) or CM proteins (10 μg) were separated by SDS-PAGE on a 12% resolving gel and transferred electrophoretically to a polyvinylidene difluoride membrane (15,16). The membrane was blocked in 1% bovine serum albumin in Tris-buffered saline (pH 8.0) and 5% nonfat dry milk for 1 h. The membranes were incubated with Drabkin’s reagent (Sigma, St. Louis, MO), respectively.

**FABPPm content.** A total of six lean and six obese rats were anesthetized with ketamine/Rompun, decapitated, and the white and red portions of the gastrocnemius muscles of both legs immediately removed and trimmed of fat and connective tissues. Plasma membrane fractions were prepared fresh as previously described (15,16). Briefly, the muscles were minced thoroughly with scissors, diluted fourfold in Tris-150 sucrose buffer with 0.1 mmol/l phenylmethylsulfonyl fluoride, 10 mmol/l ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, and 10 mg/ml trypsin inhibitor made fresh daily (pH 7.5), and homogenized by one 10-s burst with a Polytron homoge-
palmitate oxidation was calculated by dividing the total amount of radioactivity recovered as $^{14}$C$_{2}$CO$_{3}$ by the total amount of radioactivity that was taken up by the muscles (16). Total palmitate oxidation was calculated by multiplying palmitate uptake by the percent oxidation. Both percent and total palmitate oxidation were corrected for label fixation by using the acetate correction factor of 1.9. This correction factor was estimated from hindquarter perfusions with $[1^{14}C]$acetate ($n = 4$) and found to be similar to the correction factors estimated by our study and others (16,22). Uptake and release of substrates and uptake of oxygen across the hindquarter were calculated by multiplying perfusate flow by the arteriovenous difference in concentration and were expressed per gram of perfused muscle, which was measured to be 8.7 ± 0.8 and 4.2 ± 0.7% (18.3 ± 0.5 vs. 16.7 ± 0.4 g, respectively, $P > 0.05$) of body weight for unilateral hindquarter perfusion in lean and obese rats, respectively. Palmitate accumulation into muscle di- or triglycerides was calculated as the radioactivity accumulated in each lipid and were 42 and 74% higher ($P < 0.05$) in 10 lean and 9 obese Zucker rats. Because there were no significant changes in values measured after 20, 30, and 40 min of perfusion, average values were used for each rat. *,$P < 0.05$ compared with the lean group.

RESULTS

Palmitate metabolism. As dictated by the protocol, perfusate palmitate concentration and delivery to the hindquarter did not vary over time and were not significantly different between the lean and obese groups (1,031.9 ± 43.4 nmol/l and 186.9 ± 10.9 nmol·min$^{-1}$·g$^{-1}$ vs. 1,103.7 ± 27.3 μmol/l and 211.2 ± 5.9 nmol·min$^{-1}$·g$^{-1}$, respectively, $P > 0.05$). The fractional and total uptake of palmitate did not vary during 40 min of perfusion and were 42 and 74% higher ($P < 0.05$) in the obese (0.067 ± 0.005 and 14.7 ± 1.1 nmol·min$^{-1}$·g$^{-1}$, respectively) than in the lean (0.047 ± 0.004 and 8.5 ± 0.6 nmol·min$^{-1}$·g$^{-1}$, respectively) group, respectively (Fig. 1). Whereas the percentage of palmitate oxidized was not significantly different between groups (28.1 ± 3.9 and 32.6 ± 6.0% for the lean and obese groups, respectively, $P > 0.05$), the total rate of palmitate oxidized was 65% higher in the obese than in the lean group (4.3 ± 0.8 vs. 2.6 ± 0.5 nmol·min$^{-1}$·g$^{-1}$, respectively, $P < 0.05$) (Fig. 1).

Substrate exchange across the hindquarter. Resting oxygen uptake did not vary over time and was not significantly different between the lean and obese groups (18.5 ± 1.4 and 23.9 ± 1.9 μmol·g$^{-1}$·h$^{-1}$, respectively, $P > 0.05$). Arterial perfusate glucose concentrations did not vary significantly over time and were not significantly different between the lean and obese groups (7.0 ± 0.2 and 7.0 ± 0.1 mmol/l, respectively, $P > 0.05$). Glucose uptake did not change significantly over time but was found to be 40% lower in the obese than in the lean group (6.2 ± 0.7 vs. 10.3 ± 0.9 μmol·g$^{-1}$·h$^{-1}$, respectively, $P < 0.05$) (Fig. 2A). Arterial perfusate lactate concentration was on average 23–28% higher in the obese than in the lean group and increased by 32–37% during 40 min of perfusion in both the lean (1.09 ± 0.04 to 1.49 ± 0.08 mmol/l, $P < 0.05$) and obese (1.39 ± 0.12 to 1.83 ± 0.14 mmol/l, $P < 0.05$) groups. Lactate release decreased by 26–31% during 40 min of perfusion in both the lean (5.7 ± 1.3 to 4.2 ± 0.8 μmol·g$^{-1}$·h$^{-1}$) and obese (8.8 ± 0.9 to 6.1 ± 0.8 μmol·g$^{-1}$·h$^{-1}$) groups and was 38–60% higher in the obese than in the lean group (Fig. 2B). Arterial perfusate glycerol concentration was 71–92% higher in the obese than in the lean group and increased by 37–43% over time in both the lean (94.0 ± 23.1 to 129.3 ± 26.2 μmol/l, $P < 0.05$) and obese (171.5 ± 42.1 to 244.4 ± 29.2 μmol/l, $P < 0.05$) groups. Glycerol release did not change significantly over time in either group but was 129–358% higher in the obese than in the lean group (0.99 ± 0.14 vs. 0.30 ± 0.09 μmol·g$^{-1}$·h$^{-1}$, respectively, $P < 0.05$) (Fig. 2C).

Western blot analysis. As previously shown, the PM fractions isolated from the red and white skeletal muscles in both the lean and obese rats were enriched by 10- to 12-fold in the specific activity of 5′-nucleotidase relative to their respective CM fractions (10,20) (Table 1). For each muscle group, protein yield and 5′-nucleotidase activity were not significantly different between the obese and lean groups. 5′-Nucleotidase activity was found to be higher in the red than in the white muscle in both the lean and obese rats. However, this did not affect the more important comparisons between the lean and obese groups. Succinate dehydrogenase activity in the PM fractions was not detectable, thus indicating that contamination from mitochondrial membrane proteins was negligible. These results are consistent with those of previous reports using similar PM-isolation procedures (3,38) and demonstrate...
the integrity of our PM preparation. Scanning densitometry of multiple gels revealed that compared with the lean group, FABP<sub>PM</sub> content in the obese group was significantly higher by 61 ± 5% in red skeletal muscle but not different (17 ± 3%, P > 0.05) in white skeletal muscle (Table 1).

**Muscle metabolites.** Pre- and postperfusion triglyceride concentrations were found to be higher in the obese than in the lean group in both the red and white gastrocnemius muscles (Fig. 3B). In both groups of rats, there were no significant decreases in the triglyceride concentration over time in both the red and white gastrocnemius muscles. Both fiber type and obesity affected the triglyceride synthesis rate (Fig. 3A). In the obese group, the rate of triglyceride synthesis was 2.6-fold higher in the red than in the white gastrocnemius muscle, whereas no difference was found between fiber types in the lean group (Fig. 3A). In the red gastrocnemius muscle, the rate of triglyceride synthesis was 158% higher in the obese than in the lean group. In the white gastrocnemius muscle, the rate of palmitate incorporation into diglycerides was 93% higher in the obese than in the lean group (2.7 ± 0.3 vs. 1.4 ± 0.3 dpm/mg, P < 0.05), whereas no difference was found between fiber types.

FABP<sub>PM</sub> content in plantaris muscle correlated positively with fractional (y = 938.4x + 14.2; r² = 0.69, P < 0.05) (Fig. 4A) and total (y = 3.7x + 24.8; r² = 0.69, P < 0.05) palmitate uptake. The relationships between hindquarter glucose uptake and preperfusion muscle triglyceride concentration in both red (y = 12.9x<sup>-0.34</sup>; r² = 0.45, P < 0.05) and white (y = 10.5x<sup>-0.34</sup>; r² = 0.34, P < 0.05) gastrocnemius muscles as well as total preperfusion triglyceride concentration (y = 13.0x<sup>-0.36</sup>; r² = 0.51, P < 0.05) (Fig. 4B) were exponential and found to be significant. Linear correlations between these variables were also found to be significant but generally lower (r² = 0.43, 0.37, and 0.49, respectively). Glycerol release was positively correlated with total preperfusion triglyceride content (y = 7.2x + 1.6; r² = 0.41, P < 0.05).

**DISCUSSION**

Our results show that the presence of muscle insulin resistance in obese Zucker rats was associated with alterations in muscle FA metabolism as evidenced by an increase in FA uptake and a change in cellular FA disposal. Muscle insulin resistance was associated with an increase in fractional and total palmitate uptake, and this was associated with an increase in the content of muscle FABP<sub>PM</sub>. In the absence of insulin, the relative distribution of FA disposal to oxidation was not changed by the presence of muscle insulin resistance. However, under those conditions, the distribution of FA to storage was modified according to fiber type. Thus, the rate of palmitate incorporation into triglycerides was increased in red muscle, and that of diglycerides was increased in white muscle. Muscle insulin resistance was associated with higher preperfusion muscle triglyceride levels in both red and white muscles, and this was associated with an increased rate of triglyceride hydrolysis during the perfusion. These results show that, even in the absence of insulin, insulin-resistant muscle demonstrates an increased ability to take up FA from plasma and an altered disposal of FA that is fiber-type specific.

With the use of the hindlimb-perfusion system, plasma FA availability, blood flow, and capillary density are all factors that could have some impact on changes in muscle FA metabolism. In this experiment, plasma FA availability and blood flow were not different between groups. Thus, the calculated rate of plasma FA delivery to the muscle was not different between groups and was high enough to
not be limiting (25). Furthermore, because fiber-type distribution and capillary density are only minimally affected by the presence of insulin resistance (24), perfusion of the muscle bed would be expected to be similar between groups. This suggests that under the conditions imposed by our protocol, factors inherent to the perfused muscle mass must be predominantly responsible for the measured alterations in FA metabolism.

With our experimental conditions, the elevation in FA uptake associated with muscle insulin resistance could be attributed in part to an increase in muscle FABP<sub>PM</sub> content, as can be shown by the high correlation between FABP<sub>PM</sub> content and fractional and total palmitate uptake.

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<th>Protein yield (mg/g wet wt)</th>
<th>5'-Nucleotidase activity (μmol·min&lt;sup&gt;-1&lt;/sup&gt;·mg&lt;sup&gt;-1&lt;/sup&gt; protein)</th>
<th>FABP&lt;sub&gt;PM&lt;/sub&gt; content (relative density)</th>
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<td>Red</td>
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<td>Obese</td>
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<td>Obese</td>
<td>0.25 ± 0.08</td>
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Data are means ± SE of six independent membrane preparations for each group. *P < 0.05 compared with the red muscle; †P < 0.05 compared with the lean group.

FIG. 3. Muscle triglyceride synthesis rate (A) and content (B) in red and white muscles of lean and obese Zucker rats. Data are means ± SE for 10 lean and 9 obese Zucker rats. Pre- and postsamples for the red and white gastrocnemius muscles were taken immediately before and after the perfusion period. *P < 0.05 compared with the lean group; †P < 0.05 compared with white muscle. □, lean group; ■, obese group.

FIG. 4. Correlation between FABP<sub>PM</sub> protein content and fractional palmitate uptake (A) and between glucose uptake and preperfusion triglyceride (TG) content (B) in lean and obese Zucker rats. A: Total FABP<sub>PM</sub> protein content was measured in plantaris muscle of the perfused hindquarters of 10 lean (○) and 9 obese (□) Zucker rats. The regression equation and correlation coefficient are y = 938.4x + 14.2; r² = 0.69, P < 0.05. B: Total preperfusion muscle triglyceride content was calculated as described in RESEARCH DESIGN AND METHODS. The regression equation and correlation coefficient are y = 13.0x<sup>-0.36</sup>; r² = 0.51, P < 0.05.
Whereas several proteins have been proposed as candidate long-chain FA transporters (11–13), only three of those are known to be present in skeletal muscle; namely, the FA transport protein, FA transporter (FAT), and FABP<sub>PM</sub>. In rats and humans, muscle FABP<sub>PM</sub> and FAT protein levels have been shown to vary after chronic exposure to different physiological conditions associated with alterations in FA metabolism, such as those associated with fasting, endurance training, chronic electrical stimulation, and caloric restriction (9,14–17). Our measured increase in muscle FABP<sub>PM</sub> content is in line with other results showing increases in FABP<sub>PM</sub> mRNA and protein contents with insulin resistance in liver and adipose tissue of rats and mice and in skeletal muscle of humans (26–28).

The presence of muscle insulin resistance did not affect the percentage of FA that was oxidized. However, because muscle FA uptake was higher in obese Zucker rats, total muscle FA oxidation was also found to be higher. These results agree with some but not all previously reported data (2,6). In incubated soleus muscle, basal glucose oxidation was found to be lower in obese Zucker rats compared with their lean counterparts, leading to the conclusion that muscle FA oxidation was reciprocally higher in obese rats (2). Conversely, in perfused hindlimbs, insulin-stimulated glucose oxidation was found to be higher in obese than in lean Zucker rats; this suggests that FA oxidation was lower rather than higher in the muscles of the obese rats (6). However, it is important to remember that our data, like those of Crettaz et al. (2), were collected under basal conditions and thus reflect the inherent ability of the insulin-resistant muscle. Furthermore, in earlier experiments, no albumin-bound FA was presented to the muscle such that the only available source of lipids was intramuscular triglycerides, and this may have affected the results obtained. It has been suggested that malonyl-CoA may play a critical role in the development of insulin resistance (29). Thus, high malonyl-CoA levels through their inhibitory effect on FA oxidation could be linked to an accumulation of triglyceride stores and ultimately to the development of insulin resistance (29). Whereas this may occur chronically with the presence of hyperglycemia, it is doubtful that under the experimental conditions imposed by our protocol, high malonyl-CoA levels would have been measured. Indeed, the lower rate of glucose uptake measured in the insulin-resistant muscle in conjunction with the absence of insulin would have probably been associated with low malonyl-CoA levels (30), allowing the insulin-resistant muscle to oxidize the same percentage of FA taken up as the control muscle. Our results suggest that the inherent ability of the insulin-resistant muscle to oxidize plasma FA is not diminished and that the higher rate of FA oxidation is caused mostly by the increase in FA uptake. This would agree with data on muscle oxidative capacity that show that the activity of oxidative enzymes is not decreased in obese Zucker rats (24,31).

As reported by others, preperfusion muscle triglyceride concentrations were higher in the obese than in the lean rats, were not different between fiber types, and were closely related to the degree of insulin resistance as measured by basal glucose uptake (2,4,5,32). In agreement with other studies, we found that the inverse relationship between glucose uptake and muscle triglyceride content was not necessarily linear but rather hyperbolic (4). Thus, the decrease in glucose uptake slowed down dramatically when triglyceride levels reached a value of ~8–10 μmol/g. Above that muscle triglyceride level, there was very little change in glucose uptake. The cellular mechanisms by which intracellular lipid availability affects glucose metabolism have not been completely elucidated. However, inhibition of hexokinase by long-chain acyl-CoA suggests that high intracellular triglyceride levels may interact with glucose metabolism by decreasing glucose phosphorylation (33). The small insignificant changes in muscle triglyceride levels observed over time were not surprising, considering that the muscle was perfused at rest and that the energy demand was minimal. The lack of change in muscle triglyceride levels suggests that the rates of triglyceride synthesis and hydrolysis were closely matched. Taking into account fiber-type distribution and muscle mass, our estimated rates of triglyceride synthesis and hydrolysis would have resulted in a net loss of ~0.2 and 1.5 μmol triglyceride/g muscle mass in the lean and obese rats, respectively. These calculated values correspond well with the measured data.

Our calculated rates of triglyceride synthesis are similar to those reported by some studies, lower than those reported by others, and yet follow the reported fiber-type differences (23,34,35). The discrepancy in absolute rates may have been caused in part by differences in the experimental conditions and most importantly by the availability of insulin. In incubated soleus muscle, insulin has been shown to be a potent stimulator of triglyceride synthesis possibly through its stimulatory effect on the activity of glycerol-3-phosphate acyltransferase (GPAT), the purported rate-limiting enzyme of triglyceride synthesis (36,37). Thus, the absence of insulin from the perfusate in our experiments would have affected the absolute rate of triglyceride synthesis. Obesity was associated with an increase in triglyceride synthesis in red oxidative muscle but not in white glycolytic muscle. The lack of change in white glycolytic muscle is in line with other data that showed a lack of change in triglyceride synthesis rate with diabetes in incubated flexor digitorum brevis muscle (38). Conversely, as shown by others (39), we found that muscle insulin resistance was associated with an increased incorporation of FA into diglycerides in white glycolytic muscle. This difference in FA incorporation suggests that diacylglycerol acyltransferase (DGAT), the enzyme unique to triglyceride synthesis (40), may be differentially regulated in distinct fiber types. Thus, it would appear that the cellular adaptations induced by the presence of insulin resistance are specific to each fiber type. In the obese rats, the higher rate of triglyceride synthesis in the red oxidative muscle can be explained in part by the higher rate of FA uptake and hence intracellular FA availability (37). Indeed, higher FA availability has been shown to be associated with a higher rate of incorporation of palmitate into triglycerides (35). In line with this, FA availability has been shown to allosterically increase the activity of DGAT, phosphatidate phosphohydrolase, and GPAT in heart muscle, and this was found to be more important than the availability of glucose (37,41,42). However, because the
relative increase in triglyceride synthesis was twice as high as the relative increase in FA uptake, our results suggest that other cellular factors played a role in the increased rate of triglyceride synthesis in obese rats.

The rate of glycerol release was used to estimate the rate of triglyceride breakdown and was found to be higher in obese Zucker rats (43). Whereas adipose tissue may have contributed to the release of glycerol to some extent, without the stimulatory effect of catecholamines, the rate of triglyceride hydrolysis from fat cells would have been minimal (1). In the absence of insulin, a potent antilipolytic agent, the role of other cellular factors in the regulation of muscle triglyceride breakdown would have prevailed. Results obtained in exercising humans suggest that higher initial muscle triglyceride content is associated with an increased rate of hydrolysis of muscle triglycerides (44). In line with this, we observed a significant correlation between the rate of glycerol release and preperfusion muscle triglyceride content ($r^2 = 0.41$, $P < 0.05$).

As shown by others, basal glucose uptake and lactate release were found to be lower and higher, respectively, in obese than in lean rats (2,45). It has been shown that the lower rate of basal glucose uptake in red and white muscles of obese Zucker rats is associated with a decrease in basal glucose transport (31). These results suggest that the glucose transport system is impaired by the presence of insulin resistance possibly via the inhibitory action of long-chain acyl-CoA on hexokinase (33). The increased rate of lactate release in insulin-resistant muscle could have been caused in part by a decreased efficiency of lactate removal (45). Because glycogen synthesis is a significant avenue of lactate removal in muscle (46) and because the regulation of glycogen synthesis is known to be impaired with insulin resistance (47), lactate removal may have been impaired in obese Zucker rats.

In summary, the present study has shown that muscle insulin resistance is associated with an increase in basal palmitate uptake but with no change in the relative contribution of FA to oxidative metabolism. The increase in palmitate uptake in insulin-resistant muscle was associated with an elevated content of muscle FABP$_{pm}$, a putative plasma membrane FA transporter. FA disposal to storage was altered in a fiber type–specific manner. Muscle triglyceride synthesis was found to be higher in red oxidative fibers, whereas palmitate incorporation into diglycerides was found to be higher in white glycolytic fibers. The rate of triglyceride hydrolysis was found to be higher in obese than in lean rats. These results support the notion that basal FA metabolism is altered in insulin-resistant muscle of obese Zucker rats.

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