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 ($FABP_{PM}$) 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-^{14}C]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 $FABP_{PM}$ content ($r^2 = 0.69$, $P < 0.05$). 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 diglycerides 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 ($FABP_{PM}$) is among the several proteins that have been identified as putative FA-transport proteins (11–13). Whereas the specific role of $FABP_{PM}$ 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 $FABP_{PM}$. 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; $FABP_{PM}$, 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 FABP$_{PM}$ 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*/*fa*, $n = 15$) 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 (80 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-^{14}C] 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 a mixture of 95% O_2/5% CO_2, which yielded arterial pH values of 7.3–7.4 and arterial P_{CO_2} and P_{O_2} values of typically 37–39 and 170–180 Torr, respectively, in both the lean and obese rats. Mean perfusion pressures were 121 ± 7 and 129 ± 8 mmHg during unilateral hindquarter perfusion in the lean and obese rats, respectively.

The first 25 ml of perfusate that passed through the hindquarter were discarded, and then the perfusate was recirculated at a flow of 10 ml/min (0.55 and 0.59 $ml \cdot min^{-1} \cdot g^{-1}$ perfused muscle in lean and obese rats, respectively). After an equilibration period of 20 min, the left superficial fast-twitch white (predominantly type IIb) sections and the deep fast-twitch red (predominantly type IIa) sections of the gastrocnemius muscles as well as the plantaris muscle were taken out and freeze-clamped with aluminum clamps precooled in liquid N_2. The left iliac vessels were then tied off, and a clamp was fixed tightly around the proximal part of the leg to prevent bleeding. The right leg was then perfused at rest for 40 min at a perfusate flow of 5 ml/min (0.27 and 0.29 $ml \cdot min^{-1} \cdot g^{-1}$ perfused muscle in lean and obese rats, respectively). Arterial and venous perfusate samples for the analysis of [^{14}C]–free fatty acid (FFA) and $^{14}CO_2$ radioactivities were taken after 20, 30, and 40 min of perfusion. Arterial and venous perfusate samples for P_{CO_2}, P_{O_2}, pH, and hemoglobin determinations were taken after 15 and 30 min of perfusion. At the end of the 40-min perfusion period, muscle samples from the right leg of the animal were taken and treated as previously described. The exact muscle mass perfused was determined by infusing a colored solution of methyl blue into the arterial catheter and weighing the colored muscle mass at the end of the perfusions (16). The red and white gastrocnemius muscles were used for di- and triglyceride analyses, and the plantaris muscle was used for FABP$_{PM}$ content analysis. The analysis of FABP$_{PM}$ content in plantaris muscle could only be performed in crude membrane fractions because of the small muscle sample size. However, this was done so that FABP$_{PM}$ content could be correlated with FA uptake data in muscles from the same hindquarter.

FABP$_{PM}$ 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 (15,16,19). Briefly, the muscles were minced thoroughly with scissors, diluted fourfold in Tris-15% sucrose buffer with 0.1 mmol/l phenylmethylsulfonyl fluoride, 10 mmol/l ethylene glycol-bis(β-aminoethylether)-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 homogenizer on level 6. The homogenates were filtered through a multi-filter system, and the filtered homogenates were centrifuged at 100,000*g* for 1 h. The pellets were resuspended in Tris-15% sucrose buffer and a small aliquot of the resulting suspension, termed the "crude-membrane (CM) fractions," was retained for analysis. The remaining suspension of CM fractions was layered onto continuous sucrose gradients (35–70%), which were centrifuged at 120,000*g* for 2 h using a Beckman SW28 swing-out rotor. The plasma membrane (PM) layers were harvested, washed in Tris buffer, and centrifuged at 100,000*g* for 1 h. The pellets were reconstituted in a small volume of Tris buffer and frozen in liquid nitrogen for analysis. To assess the purity of the PM preparations, and the protein content and activity of the mitochondrial membrane and PM marker enzyme, succinate dehydrogenase (20) and 5′-nucleotidase (21), respectively, were measured and compared among the CM and PM fractions. Protein content was measured with the commercially available Bio-Rad (Richmond, CA) microassay procedure. The PM fractions isolated from the red and white gastrocnemius muscles as well as the CM fractions isolated from the plantaris muscles were analyzed for FABP$_{PM}$ content by Western blotting.

Blood and muscle sample analysis. Arterial and venous perfusate samples were analyzed for glucose, lactate, glycerol, and FFA concentrations as well as for [^{14}C]-FFA and $^{14}CO_2$ 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 μmol/l 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, Edgewood, NY) and the enzymatic glycerol kinase method (Sigma), respectively. Because the FFA concentration was low in the absence of added palmitate (<80 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 (BudgetSolve, Research Product International, Mount Prospect, IL) and counted in a Tri-carb liquid scintillation counter (model 4000 CA; United Technologies Packard, Downers Grove, IL), as previously described (16). The liberation and collection of $^{14}CO_2$ from the blood were performed within 4–5 min of anaerobic collection (2 ml) as previously described (16). Perfusate samples for the determination of P_{CO_2}, P_{O_2}, 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 1,000*g* in 2:1 chloroform:methanol solution and 4 mmol/l magnesium chloride. The organic extract was evaporated and reconstituted in chloroform, and silicic acid was added for the removal of 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 [^{14}C]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 with Tween (TBST) (500 mmol/l NaCl, 20 mmol/l Tris, 0.05% Tween-20, pH 7.5) for 2 h (23°C), rinsed twice with TBST, and incubated at 4°C overnight with a polyclonal antibody to the rat hepatic FABP$_{PM}$ (1:2,000) developed in our lab (8). After further washing with TBST, the membrane was incubated with ^{125}I-goat anti-rabbit IgG for 2 h at 23°C. The membrane was then washed and dried, and the density of the bands were quantified using a phosphorImager (Molecular Dynamics, Sunnyvale, CA). A rat liver CM preparation was used as standard, and results were expressed as relative density units. To determine whether there were any differences in FABP$_{PM}$ protein content between groups, we measured and compared band density from PM fractions of either red or white skeletal muscles isolated from lean and obese rats. In all cases, multiple gels were analyzed.

Calculations and statistics. Fractional uptake was calculated as the difference in radioactivity between the arterial and venous perfusate samples divided by the radioactivity in the arterial sample (16). Palmitate delivery was calculated by multiplying the perfusate plasma flow by the arterial perfusate plasma palmitate concentration. Palmitate uptake was calculated by multiplying the plasma palmitate delivery by the fractional uptake (16). Percent palmitate oxidation was calculated by dividing the total amount of radioactivity recovered as $^{14}CO_2$ 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 fraction and was expressed per milligram of wet muscle weight. Triglyceride synthesis rate was calculated as the amount of [^{14}C]palmitate incorporated into the triglyceride fraction divided by the specific activity of the perfusate plasma [^{14}C]palmitate and corrected for time (23). The arterial- and venous-specific activities for palmitate did not vary over time and were not significantly different between the lean (52.9 ± 6.8 and 50.1 ± 6.1 μCi/mmol, respectively, $P > 0.05$) and obese (50.3 ± 2.8 and 44.9 ± 2.4 μCi/mmol, respectively, $P > 0.05$) rats. Total muscle triglyceride was calculated by estimating the contributions of red and white muscles to the perfused muscle mass (24). Statistical evaluation of the data was done using analysis of variance with Newman-Keul's test for post hoc multiple comparisons, when appropriate. Pearson product-moment correlations were computed when applicable. In all instances, an α of 0.05 was used to determine significance.

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 μmol/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).

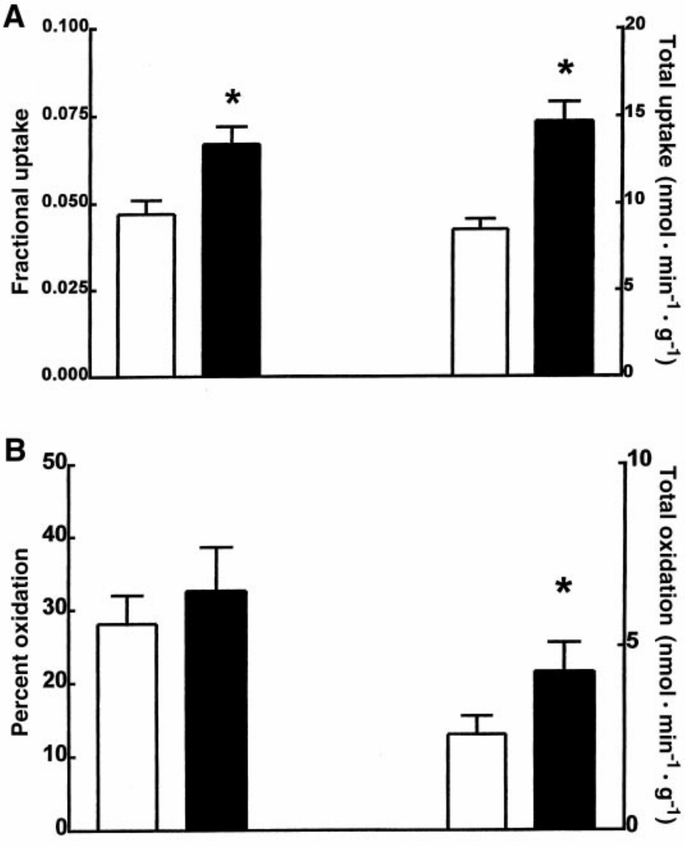

FIG. 1. Palmitate uptake (*A*) and oxidation (*B*) in perfused rat hindquarters of lean and obese Zucker rats. Data are means ± SE of 10 lean and 9 obese Zucker rats. Because there were no significant changes in values measured after 20, 30, and 40 min of perfusion, average values were used for each rat. □, lean group; ■, obese group. *$P < 0.05$ compared with the lean group.

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. 2*A*). 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. 2*B*). 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. 2*C*).

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

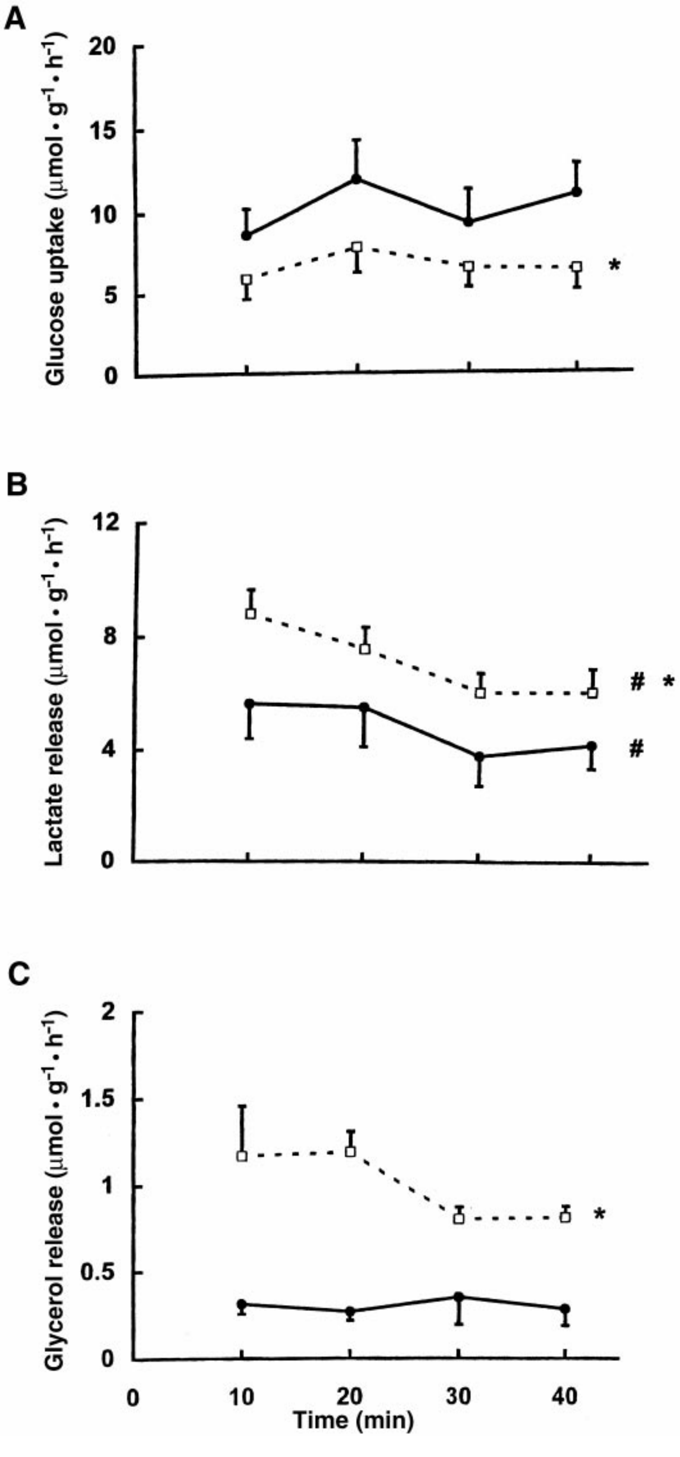

FIG. 2. Glucose uptake (*A*), lactate release (*B*), and glycerol release (*C*) in perfused rat hindquarters of lean and obese Zucker rats. Data are means ± SE of 10 lean and 9 obese Zucker rats. ●, lean group; □, obese group. *$P < 0.05$ compared with the lean group; #$P < 0.05$ represents a time effect.

the integrity of our PM preparation. Scanning densitometry of multiple gels revealed that compared with the lean group, FABP_{PM} 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. 3*B*). 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. 3*A*). 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. 3*A*). 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_{PM} content in plantaris muscle correlated positively with fractional ($y = 938.4x + 14.2$; $r^2 = 0.69$, $P < 0.05$) (Fig. 4*A*) and total ($y = 3.7x + 24.8$; $r^2 = 0.69$, $P < 0.05$) palmitate uptake. The relationships between hindquarter glucose uptake and preperfusion muscle triglyceride concentration in both red ($y = 12.9x^{-0.34}$; $r^2 = 0.45$, $P < 0.05$) and white ($y = 10.5x^{-0.24}$; $r^2 = 0.34$, $P < 0.05$) gastrocnemius muscles as well as total preperfusion triglyceride concentration ($y = 13.0x^{-0.36}$; $r^2 = 0.51$, $P < 0.05$) (Fig. 4*B*) were exponential and found to be significant. Linear correlations between these variables were also found to be significant but generally lower ($r^2 = 0.43$, 0.37, and 0.49, respectively). Glycerol release was positively correlated with total preperfusion triglyceride content ($y = 7.2x + 1.6$; $r^2 = 0.41$, $P < 0.05$).

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_{PM}. 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

TABLE 1
Protein yield, 5′-nucleotidase activity, and $FABP_{PM}$ content of membrane fractions isolated from red and white skeletal muscles of lean and obese rats

	Protein yield (mg/g wet wt)		5′-Nucleotidase activity ($nmol \cdot min^{-1} \cdot mg^{-1}$ protein)		$FABP_{PM}$ content (relative density)	
	Red	White	Red	White	Red	White
Crude membranes						
Lean	207.3 ± 16.8	198.4 ± 31.0	5.3 ± 0.5	3.9 ± 0.4*	—	—
Obese	157.7 ± 30.4	171.3 ± 34.3	7.1 ± 1.6	3.9 ± 0.3*	—	—
Plasma membranes						
Lean	0.18 ± 0.04	0.15 ± 0.02	52.2 ± 6.8	45.6 ± 4.9*	44.6 ± 10.2	46.4 ± 5.0
Obese	0.25 ± 0.08	0.21 ± 0.03	58.2 ± 7.8	37.3 ± 4.6*	72.0 ± 11.4†	54.3 ± 4.9

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.

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.

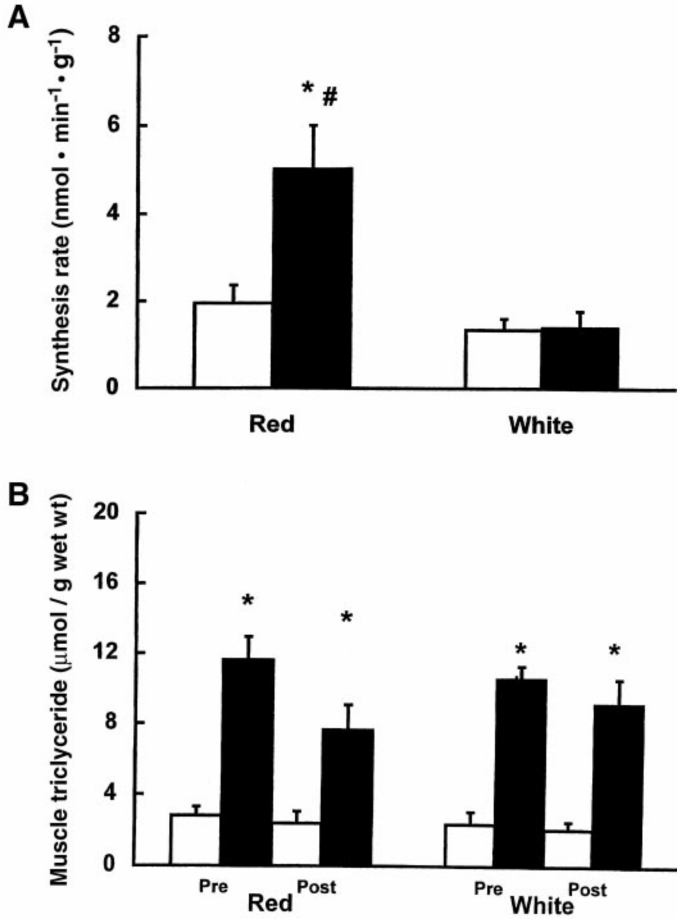

FIG. 3. Muscle triglyceride synthesis rate (*A*) and content (*B*) in red and white muscles of lean and obese Zucker rats. Data are means ± SE for 10 lean and 9 obese Zucker rats. Pre- and postsamples for the red and white gastrocnemius muscles were taken immediately before and after the perfusion period.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

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

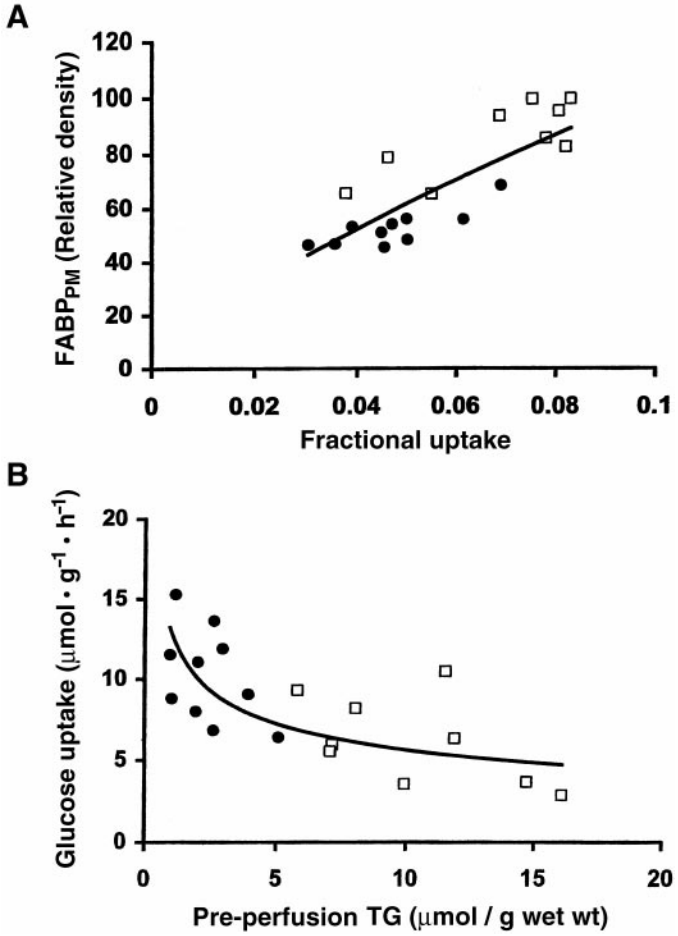

FIG. 4. Correlation between $FABP_{PM}$ protein content and fractional palmitate uptake (*A*) and between glucose uptake and preperfusion triglyceride (TG) content (*B*) in lean and obese Zucker rats. *A*: Total $FABP_{PM}$ protein content was measured in plantaris muscle of the perfused hindquarters of 10 lean (●) and 9 obese (□) Zucker rats. The regression equation and correlation coefficient are $y = 938.4x + 14.2$; $r^2 = 0.69$, $P < 0.05$. *B*: Total preperfusion muscle triglyceride content was calculated as described in RESEARCH DESIGN AND METHODS. The regression equation and correlation coefficient are $y = 13.0x^{-0.36}$; $r^2 = 0.51$, $P < 0.05$.

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 $\mathrm{FABP_{PM}}$. In rats and humans, muscle $\mathrm{FABP_{PM}}$ 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 $\mathrm{FABP_{PM}}$ content is in line with other results showing increases in $\mathrm{FABP_{PM}}$ 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 $\mathrm{FABP_{PM}}$, 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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