Brief food restriction increases FA oxidation and glycogen synthesis under insulin-stimulated conditions

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Tucker, Michelle Z., and Lorraine P. Turcotte. Brief food restriction increases FA oxidation and glycogen synthesis under insulin-stimulated conditions. Am J Physiol Regulatory Integrative Comp Physiol 282: R1210–R1218, 2002; 10.1152/ajpregu.00248.2001.—To determine the effects of brief food restriction on fatty acid (FA) metabolism, hind-limbs of F344/BN rats fed either ad libitum (AL) or food restricted (FR) to 60% of baseline food intake for 28 days were perfused under hyperglycemic-hyperinsulinemic conditions (20 mM glucose, 1 mM palmitate, 1,000 μU/ml insulin, [3-3H]glucose, and [1-14C]palmitate). Basal glucose and insulin levels were significantly lower (P < 0.05) in FR vs. AL rats. Palmitate uptake (34.3 ± 2.7 vs. 24.5 ± 3.1 nmol/g/min) and oxidation (3.8 ± 0.2 vs. 2.7 ± 0.3 nmol·g⁻¹·min⁻¹) were significantly higher (P < 0.05) in FR vs. AL rats, respectively. Glucose uptake was increased in FR rats and was accompanied by significant increases in red and white gastrocnemius glycogen synthesis, indicating an improvement in insulin sensitivity. Although muscle triglyceride (TG) levels were not significantly different between groups, glucose uptake and total preperfusion TG concentration were negatively correlated (r² = 0.27, P < 0.05). In conclusion, our results show that under hyperglycemic-hyperinsulinemic conditions, brief FR resulted in an increase in FA oxidative disposal that may contribute to the improvement in insulin sensitivity.

Food restriction; fatty acid uptake; intramuscular triglycerides; insulin sensitivity; skeletal muscle

FOOD RESTRICTION (FR) has been shown to affect skeletal muscle carbohydrate metabolism by improving insulin sensitivity (7, 8, 25). Furthermore, improvements in insulin-stimulated glucose transport have been observed after only 5 days of FR (8). As suggested by the presence of an inverse relationship between insulin sensitivity and muscle triglyceride (TG) content (35), an inherent capacity of muscle to take up and dispose of a fatty acid (FA) load could be critical to the development of metabolic abnormalities associated with insulin resistance. Thus improvements observed in carbohydrate metabolism after FR may be linked to dietary-induced alterations in FA metabolism. Further support for a relationship between carbohydrate and FA metabolism with FR comes from a recent study by Man and coworkers (24). They observed that 4 mo of FR decreased TG levels by 50% in the soleus muscles of young adult Otsuka Long-Evans Tokushima Fatty rats (24). The decrease in TG was paralleled by significant improvements in insulin-stimulated glucose uptake measured during a euglycemic-hyperinsulinemic clamp (24). Thus it appears that alterations in FA metabolism with FR may affect insulin sensitivity in muscle. Alterations in muscle FA disposal could be due to changes in rates of FA uptake, oxidation, or storage by muscle. It has recently become evident that alterations in FA uptake could be of primary importance in the regulation of FA utilization in muscle (4, 21, 40). Indeed, evidence suggests that at least part of the uptake of FA in muscle may be carrier mediated and that FA transporter proteins located in the plasma membrane are an integral component of this transport system (1, 3). Fatty acid binding protein (FABP<sub>PM</sub>) is among several proteins that have been identified as putative FA transport proteins (1, 3, 37). FABP<sub>PM</sub> content was found to decrease in the flexor digitorum brevis (FDB) muscle in response to 20 days of food restriction (15), indicating that parameters of FA metabolism are influenced by short-term FR. Although rates of FA uptake and oxidation have not been determined after FR, either variable could be responsible for a decrease in TG with FR. Thus adaptations in FA uptake and disposal in response to FR could be connected to improvements in insulin resistance via effects on TG accumulation.

Therefore, the purpose of this study was to determine whether FA metabolism in muscle under hyperglycemic-hyperinsulinemic conditions is altered by FR by measuring palmitate uptake and disposal in perfused hindlimbs of Fischer 344/Brown Norway rats. FA disposal was assessed by the measurement of palmitate oxidation and incorporation into muscle TG. The ability of the muscle to take up FA and to hydrolyze muscle TG was also evaluated by measuring FABP<sub>PM</sub> and hormone-sensitive lipase (HSL) content.

MATERIALS AND METHODS

Animals. Male Fischer 344/Brown Norway rats aged 3–4 mo were obtained from NIA (Bethesda, MD), housed singly,
and maintained on a 12-h light (0600–1800), 12-h dark (1800–0600) cycle. Animals were fed a Harlan Teklad 8604 rodent diet (W) (Teklad, Madison, WI), which has an average composition of 24% protein, 4% fat, 4% fiber, 8% ash, and 4.5% minerals. Daily food intake was measured during a 10-day baseline period as well as the experimental feeding period. Animals were randomly assigned to either an ad libitum (AL, n = 10) or 28-day FR (n = 12) group. FR rats received a daily food allotment at 1800 equal to 60% of the baseline food intake rate, and the feeding period lasted for ~6–8 h. The rats were ~5 mo old at the time of the experiment.

**Hindquarter perfusion.** Animals were fasted overnight and anesthetized intraperitoneally with ketamine-xylazine (40 mg and 6 mg/kg body wt, respectively) between 1000 and 1300. A basal blood sample was taken via a tail vein. Then, the animals were prepared for hindquarter perfusion as previously described (29, 39). Before the perfusion catheters were inserted, heparin (150 IU) was administered into the inferior vena cava. The rats were killed with an intracardial injection of ketamine-xylazine (100 mg/kg body wt). The catheters were inserted, and the preparation was placed in a perfusion apparatus, essentially as described (29).

The initial perfusate (300 ml) consisted of Krebs-Henselot solution, 1 to 2-day-old washed bovine erythrocytes (hematocrit, 29%), 3.5% bovine serum albumin (Cohn fraction V; Sigma, St. Louis, MO), 20 mM glucose, 0.15 mM pyruvate, 1 mM palmitate, 1,000 μU/ml insulin, 8 μCi [1-14C]palmitate (ICN Pharmaceuticals, Costa Mesa, CA), and 10 μCi of [3-3H]glucose (NEN Life Science Products, Boston, MA). Both labeled and unlabeled palmitate were added to the perfusate before addition to the perfusate as previously described (32). The perfusate (37°C) was continuously gassed with a mixture of 95% O2-5% CO2, which yielded arterial pH values of 7.2–7.3 and arterial PCO2 and PO2 values that were typically 39–41 and 116–167 Torr, respectively, in both dietary groups. Mean perfusion pressures were 83 ± 7 and 110 ± 6 mmHg during unilateral hindquarter perfusion in the AL and FR animals, respectively.

The first 25 ml of perfusate that passed through the hindquarter were discarded, whereupon the perfusate was recirculated at a flow of 7 ml/min. Immediately on beginning the perfusion, the left superficial fast-twitch white (predominantly type IIb) and the deep fast-twitch red (predominantly type IIa) sections of the gastrocnemius muscles, as well as the plantaris muscle (mixed fiber types), were taken out and freeze clamped with aluminum clamps precooled in liquid N2. The exact muscle mass perfused was determined by infusion of a black ink solution into the arterial catheter and weighing the colored muscle mass at the end of the perfusions.

To correct for carbon loss, additional experiments were conducted to determine the acetate correction factor under our experimental conditions (30, 39). Thus in subsamples of hindquarters (n = 4 each for AL and FR animals), hindquarters were perfused under identical perfusate conditions except that 5 μCi of [1-14C]acetate (ICN Pharmaceuticals) was added rather than [1-14C]palmitate and [3-3H]glucose. Arterial and venous perfusate samples were taken as described above and analyzed for [14C]acetate and [14C]CO2 radioactivities.

**Blood sample analysis.** Basal venous blood samples were analyzed for glucose, FA, and insulin concentrations. Arterial and venous perfusate samples were analyzed for glucose, lactate, and FA concentrations as well as for [14C]FA, [14C]CO2, and [3-3H]glucose radioactivities. Arterial perfusate samples were also analyzed for insulin concentration. Samples for glucose and lactate were put into 200 μM EGTA (pH 7) and immediately analyzed using the YSI SPORT lactate and glucose analyzers (Yellow Springs Instruments, Yellow Springs, OH). Samples for FA and insulin were put into 200 μM EGTA (pH 7) and centrifuged. The supernatant was collected and frozen until analyzed. FA concentration was determined using the WAKO NEFA-C test (WAKO Chemicals, Richmond, VA). Briefly, FA are acylated by acyl-CoA-synthetase to form acyl-CoA and are subsequently oxidized by acyl-CoA oxidase to produce hydrogen peroxide, which can then be reacted in the presence of peroxidase with 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline to form a purple color that can be measured colorimetrically. Insulin was determined by radioimmunoassay (Linco, St. Charles, MO). Because the FA concentration was low in the absence of added palmitate (<80 μM) and because palmitate was the only FA added, measured FA concentrations were taken to equal palmitate concentration.

To determine plasma palmitate radioactivity, duplicate 100-μl aliquots of the perfusate plasma were mixed with liquid scintillation fluid (BudgetSource, Research Product International, Mount Prospect, IL) and counted in a Tri-carb liquid scintillation analyzer (model 2100TR; Packard, Meriden, CT) using a dual tracer program. The liberation and collection of [14C]CO2 from the blood were performed within 2–3 min of anaerobic collection (2 ml) as previously described (38, 39). Perfusate samples for the determination of Pco2, Po2, pH, and hemoglobin were collected anaerobically, placed on ice, and measured within 5 min of collection with an ABL-5 acid-base laboratory (Radiometer America, Westlake, OH) and spectrophotometrically (Sigma), respectively.

**Muscle sample analysis.** TG concentration was determined as glycerol residues after extraction and separation of the muscle samples, as previously described (33, 39). Briefly, lipids were extracted from powdered muscle samples by vortexing in chloroform-methanol, 2:1 (vol:vol) and 4 mM magnesium chloride, followed by centrifugation at 1,000 g. The organic extract was evaporated and reconstituted in chloroform, and silicic acid was added for 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 M magnesium sulfate. The final supernatant was analyzed spectrophotometrically for glycerol by the enzymatic glycerol kinase method (Sigma). To measure the incorporation of [14C]palmitate into muscle TG, lipids from the extracted organic layer were separated by liquid chromatography as previously described (39).
Muscle glycogen concentration was determined as glucose residues after hydrolysis of the muscle samples as previously described (14, 17). Briefly, each muscle sample was pulverized under liquid nitrogen and subjected to alkaline hydrolysis with 30% potassium hydroxide. The homogenate was precipitated with 95% ethanol and centrifuged at 840,000 x g for 20 min. The pellet was further hydrolyzed with a 2.5-h incubation in 0.6 N HCl at 90°C. The hydrolysate was diluted with 0.6 N HCl and combined with 5% phenol and concentrated sulfuric acid (1:1:5 ratio) to measure the sugar residues spectrophotometrically against a set of known glycogen standards. To measure the incorporation of [3H]glucose into glycogen, an aliquot of the undiluted hydrolysate was mixed with liquid scintillation fluid (Research Product International) and counted in a Tri-carb liquid scintillation counter.

Citrated synthase activity was measured as previously described (22). Briefly, muscle homogenates were added to a cuvette containing 100 μM 5,5'-dithio-bis(2-nitrobenzoic acid) and 250 μM acetyl-CoA, and the reaction was initiated by the addition of 500 μM of oxaloacetate. The reaction was monitored for 5 min, and the specific activity was calculated as the absorbance rate per minute divided by the mercaptide extinction coefficient and expressed per muscle weight.

Western blot analysis. FABPm and HSL protein contents were determined by Western blotting. For FABPm content analysis, the planaris was used as it has a mixed fiber-type composition approximating that of the overall hindquarter preparation (11). Muscle homogenates were prepared by pulverizing tissue under liquid N2 followed by homogenization with liquid scintillation fluid (Research Product International) and counted in a Tri-carb liquid scintillation counter.

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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 (39). Palmitate delivery was calculated by multiplying perfusate plasma flow by the arterial perfusate plasma palmitate concentration. Palmitate uptake was calculated by multiplying plasma delivery by the fractional uptake (39). Percent palmitate oxidation was calculated by dividing the total amount of radioactivity recovered as 14CO2 by the total amount of radioactivity that was taken up by the muscles (39). Total palmitate oxidation was calculated by multiplying palmitate uptake by total palmitate oxidation. Both percent and total palmitate oxidation were corrected for label fixation by using acetate correction factors of 1.311 and 1.008 for AL and FR animals, respectively. Oxygen and glucose uptake and lactate release 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 5.5 and 6.0% of body weight for unilateral hindquarter perfusion in AL and FR animals, respectively. Muscle TG fractional synthesis rate was calculated as muscle TG specific activity divided by the arterial FA specific activity (19). The rate of muscle TG synthesis was calculated as the product of muscle TG fractional synthesis rate and postperfusion muscle TG concentration (19). Muscle glycogen synthesis rate was calculated as the 3H radioactivity recovered in glycogen divided by the arterial glucose specific activity (28). For these calculations, the glycogen synthesis rate was weighted for fiber type composition (2). The calculation was derived with the assumption that type I and type IIa glycoprotein synthesis rates are approximately equal and are distinctly different from type IIb rates. Therefore, the glycogen synthesis rate for type IIa, IIb, and total hindlimb were calculated as follows:

\[ \text{Syn}_{\text{W}} = 0.161\text{IIa} + 0.84\text{IIb} \]
\[ \text{Syn}_{\text{R}} = 0.92\text{IIa} + 0.08\text{IIb} \]
\[ \Rightarrow \text{IIa} = 0.08\text{Syn}_{\text{W}} - 0.84\text{Syn}_{\text{R}} / (0.08(0.16) - 0.84(0.92)) \]
\[ \Rightarrow \text{IIb} = 0.16\text{Syn}_{\text{R}} - 0.92\text{Syn}_{\text{W}} / (0.08(0.16) - 0.84(0.92)) \]
\[ \text{Syn}_{\text{H}} = 0.238\text{IIa} + 0.762\text{IIb} \]

SynW = white gastrocnemius glycogen synthesis rate
SynR = red gastrocnemius glycogen synthesis rate
IIa = type IIa glycogen synthesis rate
IIb = type IIb glycogen synthesis rate
SynH = hindlimb muscle glycogen synthesis rate

The arterial and venous specific activities for palmitate and glucose did not vary over time and were not significantly different between groups. The arterial and venous specific activities averaged 35.5 ± 0.9 and 33.9 ± 0.9 μ Ci/mmol for palmitate and 2.0 ± 0.01 and 2.0 ± 0.1 μ Ci/mmol for glucose. Because the calculated substrate utilization rates did not change significantly during the last 30 min of perfusion, the averages of the values were used to make comparisons between groups. Total muscle TG was calculated by estimating the contributions of red and white muscles to the perfused muscle mass (2).

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FOOD RESTRICTION AND FATTY ACID METABOLISM
Statistical evaluation of the muscle TG and glyogen data was performed using a two-way ANOVA (Statistica, Tulsa, OK). Glucose concentration, glucose uptake, lactate release, and lactate concentration statistical analysis was done using an ANOVA with repeated measures. All other data were analyzed by a one-way ANOVA. Correlation coefficients were computed when applicable. In all instances, an α of 0.05 was used to determine significance.

RESULTS

**Basal metabolic parameters.** Baseline food intake was similar between AL and FR animals (18.0 ± 0.4 and 17.4 ± 0.2 g/day, respectively). Food intake during the experimental feeding period averaged 94.8 ± 4.0% and 60.4 ± 0.4% of baseline intake in AL and FR animals, respectively. In FR animals, basal venous blood glucose and plasma insulin concentrations were significantly decreased by 28% and 50%, respectively, vs. AL animals (Table 1). Basal plasma FA concentration was not significantly different between groups (Table 1). Body weight and hindlimb muscle mass were 26 and 22% lower in FR compared with AL animals, respectively (Table 1). Resting oxygen uptake was not significantly different between diet groups (954 ± 49 vs. AL animals and no difference in percent palmitate oxidation was observed. However, when expressed per gram of perfused muscle, significant differences were noted between groups. Due to the decrease in hindlimb muscle mass with food restriction, palmitate delivery was significantly higher in FR than AL animals (360.8 ± 16.8 and 268.6 ± 12.1 nmol·g⁻¹·min⁻¹, respectively, P < 0.05). Total palmitate uptake was increased by 40% in FR vs. AL animals, but there was no difference in fractional palmitate uptake between groups (Fig. 1, A and B). Correspondingly, total palmitate oxidation was increased by 43% in FR vs. AL animals, and no difference in percent palmitate oxidation was observed between groups (Fig. 2, A and B).

**Substrate exchange across the hindquarter.** Resting oxygen uptake was not significantly different between AL and FR animals (42.2 ± 4.2 and 52.2 ± 4.2 μmol·g⁻¹·h⁻¹, respectively, P > 0.05). As dictated by the protocol, arterial perfusate glucose and insulin concentrations did not vary over time and were not significantly different between AL and FR animals at 10 min (33.9 ± 5.2 and 51.6 ± 2.1 μmol·g⁻¹·h⁻¹ for AL and FR rats, respectively, P < 0.05) but increased over time for FR animals at 40 min (from 33.9 ± 5.2 to 46.3 ± 3.1 μmol·g⁻¹·h⁻¹). The rate of glucose uptake did not change significantly over time in FR animals (from 51.6 ± 2.1 to 46.3 ± 3.1 μmol·g⁻¹·h⁻¹ at 10 and 40 min, respectively, P > 0.05) but increased over time for AL animals and was significantly increased at 40 min vs. 10 min (from 33.9 ± 5.2 to 48.5 ± 5.3 μmol·g⁻¹·h⁻¹), respectively, P < 0.05) (Fig. 3A). In addition, the relationship between glucose uptake and total preperfusion triglyceride concentration (y = 129.29x⁻¹·⁰⁴, r² = 0.27, P < 0.05, Fig. 3B) was exponential and found to be significant. Linear correlation

Table 1. Effects of food restriction on basal metabolic parameters, body weight, and muscle weight

<table>
<thead>
<tr>
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<th>Ad Libitum</th>
<th>Food Restricted</th>
</tr>
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<tr>
<td>Fatty acid concentration, μM</td>
<td>621 ± 77</td>
<td>817 ± 93</td>
</tr>
<tr>
<td>Glucose concentration, mM</td>
<td>7.3 ± 0.5</td>
<td>5.3 ± 0.4*</td>
</tr>
<tr>
<td>Insulin concentration, μU/ml</td>
<td>9.3 ± 1.9</td>
<td>4.6 ± 0.9*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>319 ± 11</td>
<td>235 ± 6*</td>
</tr>
<tr>
<td>Muscle weight, g</td>
<td>18.3 ± 0.5</td>
<td>14.2 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE for ad libitum (AL, n = 10) and food-restricted (FR, n = 12) rats. *P < 0.05 compared with AL animals.
between these variables was also found to be significant but lower ($r^2 = 0.22$).

Perfusate lactate concentration and lactate release rates were not significantly different between groups at any time point. Arterial perfusate lactate concentration significantly increased ($P < 0.05$) over the perfusion period in both groups. After 40 min, arterial perfusate lactate concentration had increased from $1.7 \pm 0.2$ to $2.2 \pm 0.2$ mM in AL animals and from $1.7 \pm 0.1$ to $2.1 \pm 0.1$ mM in FR animals. Lactate release remained stable throughout the perfusion period averaging $7.6 \pm 1.9$ and $8.4 \pm 1.4$ µmol·g$^{-1}$·h$^{-1}$ for AL and FR rats, respectively.

Muscle metabolites. There were no significant differences in pre- or postperfusion TG concentration in either the red or white gastrocnemius muscles between groups (Table 2). In addition, there were no significant changes in TG content from pre- to postperfusion in either group. Red and white gastrocnemius TG synthesis rates were calculated using the rate of palmitate incorporation in TG, as described in MATERIALS AND METHODS.

Table 2. Effects of food restriction on muscle TG concentration and synthesis rates in red and white gastrocnemius muscles

<table>
<thead>
<tr>
<th></th>
<th>Ad Libitum</th>
<th>Food Restricted</th>
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<tbody>
<tr>
<td><strong>Red gastrocnemius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG-pre, µmol/g wet wt</td>
<td>2.9 ± 0.4</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>TG-post, µmol/g wet wt</td>
<td>2.9 ± 0.3</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>TG Δ post-pre</td>
<td>0.1 ± 0.6</td>
<td>−0.2 ± 0.5</td>
</tr>
<tr>
<td>TG synthesis, nmol·min$^{-1}$·g$^{-1}$</td>
<td>1.8 ± 0.3</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td><strong>White gastrocnemius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG-pre, µmol/g wet wt</td>
<td>2.9 ± 0.7</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>TG-post, µmol/g wet wt</td>
<td>3.4 ± 0.6</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>TG Δ post-pre</td>
<td>0.4 ± 0.9</td>
<td>−0.3 ± 0.5</td>
</tr>
<tr>
<td>TG synthesis, nmol·min$^{-1}$·g$^{-1}$</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE for AL ($n = 10$) and FR ($n = 12$). TG, triglyceride; pre, preperfusion; post, postperfusion; Δ post-pre, change in TG level from pre to post. TG synthesis rate was calculated utilizing the rate of palmitate incorporation in TG, as described in MATERIALS AND METHODS.
sis rates were not significantly different between groups (Table 2). There were no significant differences in pre- or postperfusion glycogen concentration in either the red or white gastrocnemius muscles between groups (Table 3). In addition, there were no significant changes in muscle glycogen content from pre- to postperfusion in either group. The rate of glycogen synthesis in FR animals was significantly increased by 73 and 114% in the red and white gastrocnemius, respectively, vs. AL animals (P < 0.05, Table 3).

Western blot analysis and enzyme activity. FABP<sub>PM</sub> and HSL protein contents were not significantly different between groups (P > 0.05, Fig. 4). Citrate synthase activity was not significantly different between groups and averaged 28.5 ± 1.2 and 26.2 ± 0.7 μmol·min<sup>−1</sup>·g<sup>−1</sup> for AL and FR animals, respectively (P > 0.05).

**DISCUSSION**

Our results show that under hyperglycemic-hyperinsulinemic conditions, short-term FR was associated with alterations in FA and carbohydrate metabolism as evidenced by changes in cellular FA and glucose disposal. FR significantly increased total rates of FA uptake and oxidation. Although FR did not affect muscle TG concentration, TG utilization during the perfusion was increased. In addition, there were significant increases in glucose uptake and glycogen synthesis over the perfusion period in FR animals. These results show that short-term FR is associated with improved insulin sensitivity that may be linked to alterations in muscle lipid metabolism.

With the use of the hindlimb perfusion system, plasma FA availability and blood flow are factors that could impact FA metabolism. In this experiment, blood flow was not different between groups. However, because of a slight (22%) decrease in muscle weight in the FR group, plasma FA delivery was 34% higher in that group. In a previous perfusion study in resting hindquarters, we showed that changes in palmitate delivery within this range were accompanied by reciprocal modifications in fractional uptake resulting in equivalent rates of total palmitate uptake (38). In the present study, fractional uptake did not change significantly and actually increased slightly by 9%. The lack of change in palmitate fractional uptake with FR suggests that the capacity of the muscle to take up FA was enhanced independently of the change in FA delivery.

The increase in FA uptake occurred without a corresponding increase in FABP<sub>PM</sub> content. Along with other putative FA transporter proteins, it has been hypothesized that FABP<sub>PM</sub> facilitates the transport of FA across the sarcolemma of muscle cells and that it may be a factor in the regulation of FA metabolism (1, 3, 21). A 20-day FR study found a decrease in FABP<sub>PM</sub> levels in the FDB muscle (15). However, this decrease was observed in combination with a significant decrease in basal FA levels in contrast to our study, which found a slight 32% increase (P = 0.13) in basal FA levels, indicating potentially different metabolic conditions.
states. In addition, the FDB is a highly oxidative muscle composed of 92% type IIa fibers (6) and may adapt differently than the mixed plantaris muscle. Conversely, FABP<sub>PM</sub> content may have been adequate to meet the demands for FA uptake or the activity of the FA transporters could have been modified. It has been suggested that fatty acid translocase, a collateral FA transport protein with the ability to translocate from an intracellular pool to the plasma membrane, may be a factor in acutely regulating FA uptake (5). In this scenario, FABP<sub>PM</sub> would play a permissive role in the regulation of FA uptake, and this might explain its lack of change in content with FR. Alternatively, the results may suggest that intracellular factors contribute to the increase in FA uptake.

Muscle from FR animals appears to have an increased capacity to utilize and dispose of an FA load. Although the increase in FA oxidation with FR may have been due in large part to the increase in FA uptake, alterations in intracellular factors may have also contributed. It is doubtful that muscle oxidative capacity was significantly increased because previous studies have shown that FR is associated with no change (9) or a decline (12) in mitochondrial respiratory rate and oxidative enzyme capacity. In line with this, we showed no change in citrate synthase activity, an overall indicator of oxidative capacity, with FR. It has been suggested that FA transport across the mitochondrial membrane might be an important factor in the regulation of FA oxidation (31). Mitochondrial FA transport capacity is determined by a number of factors, which include the activity of carnitine palmitoyltransferase 1 (CPT 1), the level of malonyl-CoA, and the sensitivity of CPT 1 for malonyl-CoA (34, 43). With similar oxidative capacities, no differences in CPT1 activity levels would be expected between groups, and malonyl-CoA levels should be high in both groups due to the high perfusate glucose and insulin concentrations. However, with equal malonyl-CoA levels, a decrease in CPT 1 sensitivity for malonyl-CoA could explain an increase in FA oxidation. Changes in CPT 1 sensitivity for malonyl-CoA have been measured after both endurance training (34) and high fat diets (27).

In agreement with a previous study (16), muscle TG content was not significantly affected by short-term FR. However, there was a trend (P = 0.14–0.21) for muscle TG to be lower in FR animals that may not have reached significance due to the relatively low TG levels in both groups and the relatively short period of FR. However, FR may prove to be more effective at decreasing TG levels in animals that have higher initial TG levels, such as obese or old animals, or may be effective in young animals if the FR period is increased in duration (24). Recent studies have found muscle TG content to be an important indicator of insulin sensitivity (35). In support of the relationship between TG and insulin sensitivity, our study also found an inverse relationship between glucose uptake and preperfusion muscle TG concentration. Although not statistically significant, TG stores were reduced by 8–11% in FR animals, whereas TG stores were increased by 3–14% in AL animals during the perfusion period. Because TG synthesis rates were nearly identical, this would indicate that FA derived from TG hydrolysis may have contributed more to oxidative metabolism in FR animals. Although HSL content was not changed by FR, a decrease in the concentration of long-chain acyl CoA (LC-CoA) could have relieved the inhibition of HSL activity (23), thus promoting TG hydrolysis. Indeed, higher rates of FA oxidation and TG utilization in FR animals would be expected to reduce muscle LC-CoA levels. Several lines of evidence support the idea of a decrease in LC-CoA concentrations in the FR animals. It has been shown that chronic manipulations of FA oxidation result in reciprocal changes in LC-CoA levels (13). Under our experimental conditions, the chronic increase in FA oxidation may result in significantly decreased LC-CoA concentrations. In line with this, a decrease in LC-CoA concentration has been observed in heart muscle with fasting (41), and both fasting (18) and FR are dietary interventions resulting in increased FA utilization. Conversely, the lack of change in total TG content may be a reflection of increased turnover of intramuscular palmitate in muscle of FR rats.

FR has been shown to increase both submaximal and maximal insulin-stimulated glucose uptake by skeletal muscle in as few as 20 days after reduction of food intake (7, 8, 10, 15). Glucose transport capacity was enhanced in these animals as evidenced by an increase in GLUT4 recruitment to the plasma membrane after a maximal insulin stimulus with no difference in total GLUT4 content (10). In agreement with a previous FR study, we observed significant increases in glucose uptake and glycogen synthesis that resulted in a nearly 50% greater accumulation of glycogen over the perfusion period in FR animals (20). However, in agreement with several other studies (8, 10, 20), there was no significant net effect on glycogen concentration and this may suggest that muscle glycogen turnover was increased with FR. It is of interest to examine the relationship between an increase in glucose uptake and glycogen synthesis. Further support for an increase in HK activity comes from a study that found a greater percentage of phosphorylated 2-deoxyglucose in skeletal muscle of FR compared with AL animals (42).

Perspectives

FR has been shown to improve insulin sensitivity quickly and consistently. Although improvements in insulin action have traditionally been linked to enhanced glucose utilization, the present study has expanded this view to include increased FA utilization. This is an important finding as several metabolic disorders, including obesity, insulin resistance,
noninsulin-dependent diabetes mellitus, and aging, are associated with disturbances in glucose and FA metabolism. Because FR has now been shown to influence both areas of metabolism, elucidation of the mechanisms by which FR exerts its influence may reveal new targets for therapeutic intervention. One possible mechanism may be through reduction of LC-CoA levels, as LC-CoA concentration influences glucose and FA metabolic pathways. However, LC-CoA concentration has not yet been measured after FR. Further study of the interaction between glucose and FA metabolic pathways after FR may provide interesting information that could be used to alter fuel utilization in disease states.

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