Impaired fatty acid oxidation in muscle of aging rats perfused under basal conditions

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Tucker, Michelle Z., and Lorraine P. Turcotte. Impaired fatty acid oxidation in muscle of aging rats perfused under basal conditions. Am J Physiol Endocrinol Metab 282:E1102–E1109, 2002.First published January 8, 2002; 10.1152/ajpendo.00175.2001.—The purpose of the present study was to examine the utilization of fatty acids (FA) and muscle substrates by skeletal muscle in young, middle-aged, and old adult rats under conditions of euglycemia with low insulin levels. Male Fischer 344 × Brown Norway rats aged 5, 15, or 24 mo underwent hindlimb perfusion with a medium of 8 mM glucose, 1 mM palmitate, 25 μU/ml insulin, [1-14C]palmitate, and [3-3H]glucose. Glucose and palmitate uptake were similar among age groups. The percent and total palmitate oxidized (nmol·min⁻¹·g⁻¹) were 30–36 and 41–49% lower (P < 0.05) in 15-mo- and 24-mo-old than in 5-mo-old animals. Compared with 5-mo- and 15-mo-old animals, pre- and postperfusion muscle triglyceride (TG) levels were significantly (P < 0.05) elevated 91–305% in red and 118–219% in white muscles of 24-mo-old animals. Fatty acid-binding protein content was 40–64% higher (P < 0.05) in 24-mo- than in 5-mo- or 15-mo-old animals. In red muscle, hormone-sensitive lipase (HSL) content was 28% lower (P < 0.05) in 24-mo- than in 5-mo- or 15-mo-old animals. These results indicate that, under euglycemic conditions in the presence of low insulin levels, the reduction in FA disposal to oxidation and the decrease in HSL content may contribute to the accumulation of TG in muscle of old animals.

fatty acid metabolism; fatty acid-binding protein; Fischer 344 × Brown Norway rats; glycogen; intramuscular triglycerides

STUDIES ON THE EFFECTS OF AGING on fatty acid (FA) metabolism are scarce and in general indicate that cellular FA disposal is altered with aging (1, 8). In humans, aging has been shown to be associated with either a decrease (8) or an increase (4) in whole body FA oxidation at rest (8). When FA oxidation was blunted, the decrease could not be completely explained by a decrease in FA availability, because the rate of lipolysis has been shown to be higher in elderly subjects (9). This suggests that an age-related decline in FA oxidation would occur, in part, at the muscle level (8). These suggestions agree well with data collected in perfused working hearts showing that the rate of FA oxidation was lower in old than in young muscle (1). Furthermore, if cellular FA disposal to oxidation is decreased with aging, then this may be associated with an increase in muscle triglyceride (TG) levels. As suggested by the presence of an inverse relationship between insulin sensitivity and TG content in muscle (32), an alteration in the inherent capacity of the muscle to take up and dispose of a FA load could be critical to the development of metabolic abnormalities with aging. When it is considered that skeletal muscle can account for >50% of whole body FA disposal, it is critical to determine whether muscle FA oxidation per se is decreased with aging.

Alterations in muscle FA disposal could also be due, in part, to changes in the capacity of the muscle to take up FA. It has recently become evident that alterations in FA uptake could be of primary importance in the regulation of FA utilization in muscle (5, 19, 36). 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 (3). Thus, with this system, control of FA uptake would be possible at the transport step and the content of fatty acid transporter proteins at the plasma membrane would be critical. Plasma membrane fatty acid-binding protein (FABPpm) is among several proteins that have been identified as putative FA transport proteins (3, 33). Although the specific role of FABPpm in a putative transsarcolemmal transport process has not been clearly identified, the protein has been shown to be present in muscle, and its expression has been shown to be modified by exposure to physiological stimuli associated with changes in FA utilization (33, 35). Thus, if muscle FA uptake is altered with aging, this could be associated with concomitant changes in the content of FABPpm.

Thus the purpose of this study was to determine, by measuring palmitate uptake and disposal in the hindlimbs of young, middle-aged, and old Fischer 344 × Brown Norway adult rats perfused under basal conditions, whether FA metabolism is impaired in aged muscle. 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

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hydrolyze muscle TG was also assessed by measuring FABP<sub>PM</sub> and hormone-sensitive lipase (HSL) content.

**MATERIALS AND METHODS**

**Animals.** Male Fischer 344 × Brown Norway adult rats aged 5 (5-mo-), 15 (15-mo-), and 24 (24-mo-) mo were obtained from the National Institute on Aging (Bethesda, MD), housed singly, and maintained on a 12:12-h light-dark cycle. They received regular rat chow and water ad libitum.

**Hindquarter perfusion.** Animals were fasted overnight and anesthetized intraperitoneally with ketamine-xylazine (40 mg and 6 mg/kg body wt, respectively). A basal blood sample was taken via a tail vein. Then, the animals were prepared for hindquarter perfusion as previously described (25, 35). Before the perfusion, catheters were inserted and heparin (150 IU) was administered into the inferior vena cava. The rats were killed with an intracardial injection of ketamine-xylazine immediately before the catheters were inserted, and the preparation was placed in a perfusion apparatus essentially as described (25).

The initial perfusate (300 ml) consisted of Krebs-Henseleit solution, 1- to 2-day-old washed bovine erythrocytes (hematocrit, 29%), 3.5% bovine serum albumin (Cohn fraction V; Sigma Chemical, St. Louis, MO), 8 mM glucose, 0.15 mM pyruvate, 1 mM albumin-bound palmitate, 25 μU/ml insulin, 8 μCi of albumin-bound [1-14C]palmitate (ICN pharmaceuticals, Costa Mesa, CA), and 10 μCi of [3-3H]glucose (NEN Life Science Products, Boston, MA). The perfusate (37°C) was continuously gassed with a mixture of 95% O<sub>2</sub>-5% CO<sub>2</sub>, which yielded arterial pH values of 7.2–7.3 and arterial PCO<sub>2</sub> and PO<sub>2</sub> values that were typically 34–41 and 107–136 Torr, respectively, in all age groups. Mean perfusion pressures were 91 ± 11, 82 ± 11, and 82 ± 3 mmHg during hindquarter perfusion in the 5-mo-, 15-mo-, and 24-mo-old 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 after the perfusion was begun, the left superficial fast-twitch white (predominantly type IIB) and the deep fast-twitch red (predominantly type IIb) 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 N<sub>2</sub>. The exact muscle mass perfused was calculated at a quarter were discarded, whereupon the perfusate was recirculated. The liberation and collection of 14CO<sub>2</sub> from the blood were performed within 2–3 min of anaerobic collection (2 ml) as previously described (37, 38). Perfusion samples for the determination of P<sub>CO</sub><sub>2</sub>, P<sub>O</sub><sub>2</sub>, 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 Chemical), respectively.

Muscle TG concentration was determined as glycerol residues after extraction and separation of the muscle samples, as previously described (29, 35). Briefly, lipids were extracted from powdered muscle samples by centrifugation at 1,000 g in a 2:1 chloroform-methanol solution and 4 nM magnesium chloride. The organic extract was evaporated and reconstituted in chloroform, and silicic acid was added for removal of phospholipids by centrifugation. The resulting supernatant was washed, 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 Chemical). To measure the incorporation of [1-14C]palmitate into muscle TG, lipids from the extracted organic layer were separated by liquid chromatography as previously described (35).

Muscle glycogen concentration was determined as glucose residues after hydrolysis of the muscle samples, as previously described (13, 15). Briefly, each muscle sample was pulverized under liquid N<sub>2</sub> and subjected to alkaline hydrolysis with 30% potassium hydroxide. The homogenate was cooled in liquid N<sub>2</sub>. The exact muscle mass perfused was determined by infusion of a black ink solution into the arterial catheter and weighing of 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 (26, 35). Thus, in subsamples of rats (n = 4 each for 5-mo-, 15-mo-, and 24-mo-old animals), hindquarters were perfused under identical perfusate conditions except that 5 μCi of [1-14C]acetate (ICN pharmaceuticals) were added rather than [1-14C]palmitate and [3-3H]glucose. Arterial and venous perfusate samples were taken as described and analyzed for [14C]acetate and 14CO<sub>2</sub> radioactivities.

**Blood and muscle sample analyses.** 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]-labeled FA, 14CO<sub>2</sub>, and [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 spectrophotometrically by using the WAKO NEFA-C test (WAKO Chemicals, Richmond, VA), and 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 (BudgetSolve, 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 14CO<sub>2</sub> from the blood were performed within 2–3 min of anaerobic collection (2 ml) as previously described (37, 38). Perfusion samples for the determination of P<sub>CO</sub><sub>2</sub>, P<sub>O</sub><sub>2</sub>, 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 Chemical), respectively.
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precipitated with 95% ethanol and centrifuged at 840 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) 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.

Citrate synthase activity was measured in the plantaris as previously described (20). 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 oxaloacetate. The reaction was monitored for 5 min, and the specific activity was calculated as the absorbance rate per minute divided by the mercaptoethanol extinction coefficient and expressed per muscle weight. FABPm and HSL protein contents were determined by Western blotting. For FABPm content analysis, the plantaris was used because it has a mixed fiber type composition approximating that of the overall hindquarter preparation (12). Homogenates were prepared by pulverizing tissue under liquid N2 followed by homogenization with a glass douncer in buffer containing 10 mM Tris, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 20 μM EGTA, 0.1 mg/ml trypsin inhibitor, and 0.02% sodium azide (pH 7.4, solution made fresh daily). Solubilized muscle homogenate proteins (50 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 12% running gel and transferred electrophoretically to a nitrocellulose membrane (Bio-Rad, Hercules, CA). The membrane was blocked, rinsed, and then incubated with a purified polyclonal rabbit anti-FABPm (1:3,000) (36). For HSL protein content analysis, muscle samples from the red and white quadriceps were homogenized with a polytron in a buffer containing 210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 30 mM HEPES, and 5 mM EDTA, as well as freshly made 0.5 M KCl, 25 mM tetrasodium pyrophosphate, and 2 mM PMSF and centrifuged at 175,000 g for 75 min (22). Solubilized muscle homogenate proteins (100 μg) were separated by SDS-PAGE on a 10% running gel and transferred electrophoretically to an Immobilon-P polyvinylidene difluoride membrane (Bio-Rad). The membrane was blocked, rinsed, and then incubated with a polyclonal rabbit anti-HSL (1:5,000, kindly donated by Dr. F. B. Kraemer, Stanford University Medical Center, Palo Alto, CA). For both assays, the secondary incubation was performed with goat anti-rabbit IgG (H+L)-horseradish peroxidase (Pierce, Rockford, IL) followed by detection with enhanced chemiluminescence (Super Signal West Pico; Pierce, Rockford, IL) and exposure to film (CL-Xposure, Pierce). Films were scanned using an HP ScanJet 6200C and quantitated using Scion Image (Scion, Frederick, MD). Rat liver plasma membrane and rat soleus crude membrane preparations were used as standards for the FABPm and HSL content analyses, respectively, and results were expressed as relative density units. In all cases, multiple gels were analyzed.

Calculations and statistics. Fractional uptake was calculated as the difference in radioactivity between the arterial and venous perfuse samples divided by the radioactivity in the arterial sample (35). Palmitate delivery was calculated by multiplying perfuse plasma flow by the arterial perfuse plasma palmitate concentration. Palmitate uptake was calculated by multiplying plasma palmitate delivery by fractional palmitate uptake (35). 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 (35). Total palmitate oxidation was calculated by multiplying palmitate uptake by percent oxidation. Both percent oxidation and total palmitate oxidation were corrected for label fixation by using acetate correction factors of 1.311, 1.152, and 1.224 for 5-mo-, 15-mo-, and 24-mo-old animals, respectively. Glucose uptake and lactate release were calculated by multiplying perfuse flow by the arteriovenous difference in concentration and were expressed per gram of perfused muscle, which was measured to be 5.5, 5.0, and 4.3% of body weight for hindquarter perfusion in 5-mo-, 15-mo-, and 24-mo-old animals, respectively. Muscle TG fractional synthesis rate was calculated as muscle TG specific activity divided by arterial FA specific activity. The rate of muscle TG synthesis was calculated as the product of muscle TG fractional synthesis rate and postperfusion muscle TG concentration (17). The glycogen synthesis rate was calculated as the 3H radioactivity recovered in glycogen divided by arterial glucose specific activity (23). 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 glycogen 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

\[
\text{Syn}_{w} = 0.16\text{IIa} + 0.84\text{IIB}
\]
\[
\text{Syn}_{IIa} = 0.92\text{IIa} + 0.08\text{IIB}
\]
\[
\Rightarrow \text{IIa} = \frac{0.08\text{Syn}_{w} - 0.84\text{Syn}_{IIb}}{0.08(0.16) - 0.84(0.92)}
\]
\[
\Rightarrow \text{IIB} = \frac{0.16\text{Syn}_{w} - 0.92\text{Syn}_{IIb}}{0.08(0.16) - 0.84(0.92)}
\]
\[
\text{Syn}_{IIb} = 0.238\text{IIa} + 0.762\text{IIB}
\]

where Syn w is white gastrocnemius glycogen synthesis rate, Syn IIa is red gastrocnemius glycogen synthesis rate, IIA is type IIA glycogen synthesis rate, IIB is type IIB glycogen synthesis rate, and Syn IIb is 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 among groups. The arterial and venous specific activities averaged 37.1 ± 0.9 and 34.1 ± 0.9 μCi/mmol for palmitate and 4.9 ± 0.1 and 4.9 ± 0.1 μCi/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.

Statistical evaluation of the muscle TG and glycogen 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. Tukey’s honestly significant difference test for post hoc multiple comparisons was performed when appropriate. In all instances, an α of 0.05 was used to determine significance.

RESULTS

Basal metabolic parameters. Basal venous blood glucose, plasma FA, and insulin concentrations were not significantly different among age groups (Table 1). Animal body weight increased with advancing age and was significantly higher in both the 15-mo- and 24-mo-

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old animals compared with the 5-mo-old animals. Hindlimb muscle mass in the 15-mo-old animals was not different from that of the 24-mo-old animals but was 23% higher than that of the 5-mo-old animals.

Palmitate metabolism. As dictated by the protocol, perfusate palmitate concentration did not vary over time and was not significantly different among age groups (1,030 ± 49, 1,018 ± 21, and 1,058 ± 46 μM for 5-mo-, 15-mo-, and 24-mo-old animals, respectively, P > 0.05). However, due to the increase in hindlimb muscle mass in the 15-mo-old group, palmitate delivery was 25% lower in the 15-mo- than in the 5-mo-old animals (222.8 ± 6.4 and 297.4 ± 26.2 nmol·min⁻¹·g⁻¹, respectively, P < 0.05). The fractional and total uptakes of palmitate did not vary over time and were not significantly different among age groups (Fig. 1, A and B). The percentage of palmitate oxidized was 42 and 49% lower in the 15-mo- and 24-mo-old animals compared with the 5-mo-old animals (Fig. 2A). Similarly, total palmitate oxidation was 31 and 38% lower in the 15-mo- and 24-mo-old animals compared with the 5-mo-old animals (Fig. 2B).

Substrate exchange across the hindquarter. Resting oxygen uptake did not vary over time and was not significantly different among the 5-mo-, 15-mo-, and 24-mo-old animals (42.1 ± 2.1, 41.2 ± 1.6, and 35.8 ± 2.2 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 among the 5-mo-, 15-mo- and 24-mo-old animals (8.0 ± 0.2, 8.0 ± 0.3, and 8.0 ± 0.2 mM for glucose, respectively, and 24.3 ± 2.0, 29.2 ± 5.0, and 31.5 ± 3.6 μU/ml for insulin, respectively, P > 0.05). Similarly, glucose uptake did not change significantly over time and was not significantly different among age groups (8.0 ± 1.3, 6.5 ± 1.1, and 7.6 ± 1.2 μmol·g⁻¹·h⁻¹ for the 5-mo-, 15-mo-, and 24-mo-old rats, respectively, P > 0.05). Perfusion lactate concentration and lactate release were not significantly different among age groups. Although arterial perfusate lactate concentration remained stable over the perfusion period in the 5-mo-old animals (from 1.9 ± 0.3 to 2.0 ± 0.3 mM, P > 0.05), it increased by 27% in the 15-mo- and 24-mo-old animals during the first 30 min of perfusion (from 1.5 ± 0.1 to 1.9 ± 0.1 mM in both the 15-mo- and 24-mo-old animals, P < 0.05) and remained stable thereafter (Fig. 3A). Lactate release did not change significantly over time in the 5-mo- and 15-mo-old animals (from 3.9 ± 1.6 to 6.7 ± 2.7 and from 10.0 ± 1.4 to 7.3 ± 1.6 μmol·g⁻¹·h⁻¹, respectively, P > 0.05). In the 24-mo-old animals, lactate release decreased by 38% during the first 20 min of perfusion (from 14.9 ± 4.1 to 9.3 ± 2.2 μmol·g⁻¹·h⁻¹, P < 0.05) and remained stable thereafter (Fig. 3B).

Muscle metabolites. In the red gastrocnemius, preperfusion TG concentration was 166 and 88% higher in the 24-mo-old animals than in the 5-mo- and 15-mo-old animals, respectively (P < 0.05, Table 2). Because there were no significant changes in TG concentration over the perfusion period, postperfusion TG concentration remained significantly (P < 0.05) higher in the 24-mo-old animals than in the 5-mo- and 15-mo-old animals. Similarly, postperfusion TG concentration was 95% higher in the red gastrocnemius of 15-mo-old than of 5-mo-old animals (P < 0.05). In accord with the red gastrocnemius, TG concentration in the white gastrocnemius did not change over the perfusion period and remained significantly (P <
0.05) higher in the 24-mo-old animals than in the 5-mo- and 15-mo-old animals. Furthermore, the rate of red or white gastrocnemius TG synthesis was not significantly different among age groups. In the red and white gastrocnemius, there were no significant differences in pre- or postperfusion glycogen concentrations among age groups (Table 3). In addition, there were no significant age-associated differences in glycogen synthesis rate or change in glycogen content during the perfusion period in the red or white gastrocnemius.

**DISCUSSION**

Our results show that, in muscle perfused at equivalent rates of basal glucose uptake, advancing age was associated with alterations in FA metabolism, as evidenced by a change in cellular FA disposal. Thus, although total palmitate uptake was not changed with aging, the relative distribution of FA to oxidation was decreased in both middle-aged and old animals. Aging was associated with higher preperfusion muscle TG levels in both red and white muscles, and this was associated with an increase in muscle FABP<sub>PM</sub> content and a decrease in muscle HSL content. These results show that, under basal perfusion conditions, muscle from old animals demonstrates a decreased ability to oxidize FA that could, in part, explain the accumulation of muscle TG over time.

With the use of the hindlimb perfusion system, plasma FA availability, blood flow, and muscle composition are all factors that could impact changes in muscle FA metabolism. In this experiment, blood flow and plasma FA concentration were not different among groups. To minimize the possible effects of heparin added during surgery on plasma FA availability due to
Effects of age on muscle glycogen concentration and synthesis rate in red and white gastrocnemius muscles

<table>
<thead>
<tr>
<th>Age of Rats, mo</th>
<th>TG-pre, μmol/g wet wt</th>
<th>TG-post, μmol/g wet wt</th>
<th>TG Δ post-pre</th>
<th>TG synthesis, nmol-min⁻¹·g⁻¹</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>3.2 ± 0.7</td>
<td>4.5 ± 0.4</td>
<td>1.3 ± 0.7</td>
<td>2.4 ± 0.5</td>
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<tr>
<td>15</td>
<td>4.2 ± 0.8</td>
<td>5.0 ± 0.4</td>
<td>0.8 ± 0.2</td>
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<tr>
<td>24</td>
<td>5.6 ± 0.9</td>
<td>6.3 ± 0.7</td>
<td>0.7 ± 0.3</td>
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</table>

Values are means ± SE; n = 8, n = 8, and n = 7 for 5-mo-, 15-mo-, and 24-mo-old animals, respectively. TG, triglyceride; pre, preperfusion; post, postperfusion; Δ post-pre, change in TG level from pre to post. *Significantly different compared with the 5-mo-old animals, P < 0.05; †significantly different compared with the 15-mo-old animals, P < 0.05.

Because perfusion of a muscle bed is partially dependent on muscle fiber type distribution, changes in distribution with aging could have led to different muscle perfusion among the groups (21). The reported changes in fiber type distribution associated with aging have been shown to involve a small loss of type IIa fibers accompanied by a reciprocal increase in type I fibers (18). Because blood flow to types I and IIa fibers has been shown to be similar (21), the reported change in fiber type distribution would be associated with limited changes in muscle perfusion. A shift in muscle composition toward increasing intramuscular adiposity with a corresponding loss of muscle protein could also adversely affect FA metabolism. Although there is no direct evidence regarding adipocyte infiltration into muscle tissue with advancing age, neither differences in peak tetanic tension per total fiber area nor an increase in histological observation of interstitial or intramuscular fat or connective tissue was observed in animals over the age range in our study (6). Further...
FABPPM facilitates the transport of FA across the transporter proteins, it has been hypothesized that of the 24-mo-old animals. Along with other putative FA suggesting that increased expression of FABPPM in muscle and, as reported by others in young rats, were not per se or by chronic exposure to a different metabolic environment.

Despite the presence of similar rates of FA uptake, the total rate of FA oxidation was lower in the 15-mo- and 24-mo-old animals. These results agree with previous data collected in resting humans (8) or in perfused working hearts (1) and reinforce the notion that the age-related decline in FA oxidation must occur, in part, at the muscle level. The decrease in FA oxidative capacity could be explained, in part, by a lower mitochondrial (mt) oxidative enzyme capacity and by a lower capacity for mtFA transport. Age-related decreases in mt-oxidative enzyme capacity have been measured in some but not all muscles (18). Under our experimental conditions, citrate synthase was not affected by aging, suggesting that mt-oxidative capacity does not completely account for the age-related changes in intramuscular FA disposal. Conversely, regulation of FA oxidation at the mt-transport step has been shown to be of primary importance under certain acute and chronic physiological conditions (27, 28). mtFA transport capacity is determined by a number of factors, which include, among others, the activity of carnitine palmitoyltransferase 1 (CPT1), the level of malonyl-CoA, and the sensitivity of CPT1 for malonyl-CoA (31, 37, 38). Although it is not known at this time which factor is more critical in the regulation of FA oxidation under the conditions imposed by our protocol, our results suggest that the inherent ability of the aged muscle to oxidize plasma FA is diminished.

Muscle TG levels were markedly elevated with aging and, as reported by others in young rats, were not different among fiber types (29). Muscle TG accumulation over time could be attributed to an increase in TG synthesis, a decrease in TG utilization, or both. Under the basal conditions imposed by our protocol, the rate of TG synthesis was not altered by aging. However, an age-associated increase in TG synthesis rate might have been detected if the hindquarter had been perfused under hyperglycemic-hyperinsulinemic conditions, because glucose and insulin have been shown to increase the TG synthesis rate (30), and their acute effects on lipogenic enzyme activities might be modulated by aging. Furthermore, although not significantly higher, basal glucose and FA levels were 12–14% higher in the old animals. Because FA levels have also been shown to stimulate TG synthesis (30), these results suggest that chronically elevated basal levels of glucose and FA might play a role in the accumulation of muscle TG in the old animals over time.

Conversely, the age-associated decrease in HSL protein content indicates that muscle of old animals may have a reduced ability to hydrolyze and thus utilize muscle TG. Furthermore, in adipose tissue, translocation of HSL to lipid droplets has been shown to decrease with age, leading to a reduced rate of lipolysis (10). The small, insignificant changes in muscle TG levels observed over the perfusion time were not surprising when it is considered that the muscle was perfused at rest with its associated minimal energy demand and indicate that the rates of TG synthesis and hydrolysis were closely matched under those conditions.

As designed by our protocol, arterial perfusate insulin and glucose as well as glucose uptake were not different among age groups. Given that intracellular glucose disposal is affected by glucose uptake per se and because it has been shown that aging is associated with an increase in insulin resistance (14, 24), we matched glucose uptake rates to eliminate differences in glucose uptake as a confounding variable. Under basal conditions, we found that rates of glycogen synthesis and lactate release were not different among groups. This is in line with results obtained in non-obese humans studied under similar basal rates of glucose uptake (16). The lack of change in glycogen synthesis with aging agrees well with data showing that glycogen synthase activity in mixed fiber types was not different between 12-mo- and 24-mo-old animals (11). Our measured rates of glycogen synthesis are similar to those reported by others under similar experimental conditions (11), and the lack of change in glycogen levels during the 40-min perfusion period indicates that the rate of synthesis was matched by the rate of hydrolysis.

In summary, the present study has shown that aging is associated with changes in cellular FA disposal in muscle perfused at equivalent rates of glucose uptake. Aging was associated with a decrease in plasma FA oxidation, and this was accompanied by a corresponding increase in the utilization of muscle TG. TG levels were severely elevated in both red and white muscle fibers in old animals. Higher FABPPM content and lower HSL content in muscle may contribute to the age-related increase in muscle TG levels.

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