Aerobic power declines with aging in rat skeletal muscles perfused at matched convective O₂ delivery

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Hepple, Russell T., Jason L. Hagen, Daniel J. Krause, and Cory C. Jackson. Aerobic power declines with aging in rat skeletal muscles perfused at matched convective O₂ delivery. J Appl Physiol 94: 744–751, 2003. First published October 4, 2002; 10.1152/japplphysiol.00737.2002.—Although it is well established that maximal O₂ uptake (V₀₂ max) declines from adulthood to old age, the role played by alterations in skeletal muscle is unclear. Specifically, because during whole body exercise reductions in convective O₂ delivery to the working muscles from adulthood to old age compromise aerobic performance, this obscures the influence of alterations within the skeletal muscles. We sought to overcome this limitation by using an in situ pump-perfused hindlimb preparation to permit matching of muscle convective O₂ delivery in young adult (8 mo; muscle convective O₂ delivery = 569 ± 42 µmol O₂·min⁻¹·100 g⁻¹) and late middle-aged (28–30 mo; 539 ± 62 µmol O₂·min⁻¹·100 g⁻¹) Fischer 344 × Brown Norway F1 hybrid rats. The distal hindlimb muscles were electrically stimulated for 4 min (60 tetani/min), and V₀₂ max was determined. V₀₂ max normalized to the contracting muscle mass was 22% lower in the 28–30-mo-old (344 ± 17 µmol O₂·min⁻¹·100 g⁻¹) than the 8-mo-old (441 ± 20 µmol O₂·min⁻¹·100 g⁻¹; P < 0.05) rats. The flux through the electron transport chain complexes I–III was 45% lower in homogenates prepared from the plantaris muscles of the older animals. Coincident with these alterations, the tension (P < 0.05) rats. The flux through the electron transport chain complexes I–III was 45% lower in homogenates prepared from the plantaris muscles of the older animals. Coincident with these alterations, the tension was greater in the 28–30-mo-old vs. 8-mo-old animals. Collectively, these results demonstrate that alterations within the skeletal muscles, such as a reduced mitochondrial oxidative capacity, contribute to the reduction in V₀₂ max with aging.

A REDUCTION IN MAXIMAL O₂ UPTAKE (V₀₂ max) is one of the hallmark features of aging (3, 9, 48) and is intimately tied to the decline in exercise capacity with aging because a reduced aerobic power increases the reliance on the more fatigable nonaerobic energy pathways. The physiological basis for the decline in V₀₂ max is unclear, particularly the roles played by reductions in convective O₂ delivery vs. an intrinsic reduction in skeletal muscle aerobic function. In this respect, it is well known that reductions in V₀₂ max can occur secondary to experimentally induced reductions in convective O₂ delivery [convective O₂ delivery = arterial O₂ content × blood flow (19, 44)]. Indeed, a reduced central circulatory function leading to a reduced muscle convective O₂ delivery has often been cited as the primary cause of reduced V₀₂ max with aging (16, 36). However, aging is also associated with significant alterations in skeletal muscle, such as reduced mitochondrial enzyme activities due to both sedentary lifestyle (8, 54) and accumulation of mitochondrial DNA deletions that ultimately compromise electron transport chain function (4, 28, 29). In this respect, we recently showed that V₀₂ max is a function of an interaction between O₂ supply and mitochondrial oxidative capacity at rates of mitochondrial respiration that are below the maximum attainable (18). In other words, even though mitochondrial respiration per se may not be maximal during whole body maximal exercise in vivo (41), it is highly likely that the aforementioned alterations in mitochondria with aging contribute to the reduction of V₀₂ max.

To help address this problem, we employed an in situ pump-perfused rat hindlimb preparation to permit study of skeletal muscle V₀₂ max in young adult (8 mo old) and late middle-aged (28–30 mo old) Fischer 344 × Brown Norway rats at similar rates of muscle convective O₂ delivery. We hypothesized that the older animals would demonstrate a significant reduction in skeletal muscle V₀₂ max independent of convective O₂ delivery. Although this result would not preclude differences in O₂ delivery occurring at the microvascular level, e.g., due to reduced anatomical capillary surface area and/or impaired microcirculatory erythrocyte distribution, it would assist us in identifying the extent that factors distal to central circulatory function contribute to reducing V₀₂ max with advancing age.

MATERIALS AND METHODS

Animals. All procedures were carried out with the approval of the University of Calgary Animal Care Committee. Eight young adult (8 mo old) and seven late middle-aged (28–30 mo old) Fischer 344 × Brown Norway F1 hybrid (F344BN) rats were obtained from the National Institute on Aging. They were housed two per cage in specific pathogen-free conditions.

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free conditions at 22°C in the Health Science Centre vivarium at the University of Calgary for at least 1 wk before experiments and were provided with rat chow and water ad libitum. Necropsies were performed postexperimenter to detect any abnormalities or lesions within the animal (35). Each necropsy involved an external and internal examination of each animal. The internal assessment involved an examination of the internal organs and tissue looking for tissue lesions. In addition to this generalized approach, because it has been determined that the only lesion that is more prevalent in the F344BN than in either of the Fischer 344 or Brown Norway parental strains was lymphoid nodules on the kidney (32), we placed additional emphasis on identifying these types of lesions. To prevent contamination of our data set by the presence of disease, animals demonstrating tissue lesions were excluded as per National Institute on Aging guidelines (35).

Perfusion medium. Bovine blood was collected each week from a local abattoir and centrifuged at 5,000 g for 10 min three times in Krebs-Henseleit buffer containing sodium bicarbonate to separate the erythrocytes. Theuffy coat was aspirated after each centrifugation. Bovine blood contained 5 mM glucose, 100 mM glucose, 1,000 mM insulin, 0.15 mM pyruvate, and bovine erythrocytes to a hematocrit of 43% (18, 21). On the day of experiments, the perfusion medium was prepared, consisting of a Krebs-Henseleit bicarbonate buffer containing 4% bovine serum albumin, 5 mM glucose, and bovine erythrocytes to a hematocrit of 43% (18, 21).

Surgical procedures. The rat hindlimb was prepared for perfusion as described previously (18, 21). Animals were anesthetized with pentobarbital sodium (65 mg/kg ip). Before isolation of the left hindlimb vasculature, the right iliac artery and vein were ligated and the gastrocnemius-plantaris-soleus muscle group was removed, with individual muscles separated from one another, trimmed of fat and connective tissue, and weighed. The right plantaris muscle was then frozen in liquid nitrogen and stored at −70°C until processed for biochemistry (see Biochemistry). The skin was removed from the entire left hindlimb, and the sciatic nerve was isolated and cut proximally for electrical stimulation, with the gluteal nerve cut to permit stimulation of only the distal hindlimb musculature (i.e., gastrocnemius-plantaris-soleus muscle group, tibialis anterior muscle, and deep tibial muscles; Ref. 15). The Achilles tendon was severed from the calcaneus, and the gastrocnemius-plantaris-soleus muscle group was secured by noncompliant 2.0 silk thread to a force transducer (FT-10, Grass Instruments). The abdominal aorta, inferior vena cava, and femoral artery and vein were isolated by blunt dissection in preparation for catheterization. After surgery, the animal was placed on a heating pad, and a metal clamp was secured to the proximal femur and connected to a base plate to immobilize the hindlimb during force measurements. Catheters (22 gauge in the artery, 20 gauge in the vein) were inserted into the iliac artery and vein and advanced into the respective femoral artery and vein to initiate flow to the hindlimb. After catheterization, the animal was eutanasized with an intracardiac injection of 25 mg pentobarbital. The experimental hindlimb was wrapped in saline-soaked gauze, Saran wrap (encompassing a thermistor probe connected to a heat lamp), and aluminum foil to maintain muscle temperature at 37°C.

Perfusion procedures. Before entering the hindlimb, the perfusion medium was gassed with 95% O2-5% CO2 as it passed through 7 m of Silastic tubing encased in a 4-liter flask and warmed to 37°C. A pressure transducer (PT-300 Grass Instruments) was placed at the height of the hindlimb for determination of total perfusion pressure. Perfusion was controlled by a peristaltic pump (Gilmor minipuls 3) with the flow verified after each experiment by timed blood collection through the arterial catheter. The difference in blood flow through the arterial catheter vs. that collected from the venous catheter during hindlimb perfusion experiments is ~10% in our laboratory (R. T. Hepple, unpublished observations). Note that venous blood obtained from the femoral vein in this preparation comes from both contracting muscle (~24% of total perfused mass) and noncontracting muscle, bone, and fat (~76% of total perfused mass) (15). The desired blood flow for each animal was estimated on the basis of the mass of the contralateral gastrocnemius-plantaris-soleus muscle group (weighed before initiating perfusion, see Surgical procedures) and prior results showing that 14–17% of total hindlimb blood flow is distributed to the gastrocnemius-plantaris-soleus muscle group (15, 18). Once perfusion was initiated, flow was incrementally increased (allowing pressure to stabilize before further increases in flow) until the desired level was achieved (~30 min). Before contractions, resting arterial and venous blood samples were taken and arteriovenous O2 differences were calculated (arterial O2 content (AtO2) − venous O2 content (VtO2)). Arterial and venous blood samples were collected after each experiment by timed blood collection through the arterial catheter vs. that collected from the femoral vein for determination of total perfusion pressure. Blood flow distribution. As described previously (18), after the contraction bout, ~275,000 colored microspheres (15.5-μm diameter, Dye Tak, Triton Technology) were injected slowly (while reducing the flow on the perfusion pump to minimize changes in perfusion pressure) into a side-arm port situated proximal to the arterial catheter. Two milliliters of saline were slowly injected immediately after the microspheres were introduced to ensure that all microspheres entered the hindlimb. The gastrocnemius, plantaris, and soleus muscles

V̇O₂max declines with aging independent of O2 delivery

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were excised, trimmed of fat and connective tissue, and heated in a water bath (60°C) in centrifuge tubes containing 4 M KOH until each muscle was digested. The content of each centrifuge tube was filtered through 8-μm membranes (Whatman Nucleopore) to trap the microspheres. The membranes were then placed in a microcentrifuge tube containing 1 ml of N,N-dimethylformamide to release the color of the microspheres. Absorbance of the contents was measured by using a spectrophotometer (Biochrom Ultrospec 2100 Pro) at a wavelength of 448 nm (wavelength for yellow microspheres). The number of microspheres trapped in each muscle sample was calculated from the regression equation provided by the manufacturer. The blood flow to the gastrocnemius-plantaris-soleus muscle group was determined as the product of total hindlimb blood flow and the proportion of microspheres found in the soleus-plantaris-soleus muscle group. Similarly, muscle O2 delivery was calculated as the product of blood flow to the soleus-plantaris-soleus muscle group and the arterial O2 content. Note that blood flow and convective O2 delivery in the soleus-plantaris-soleus muscle group are representative of the total contracting muscle mass (15, 42; R. T. Hepple and C. C. Jackson, unpublished results). Thus O2 extraction across the contractile muscle mass (15, 42; R. T. Hepple and C. C. Jackson, unpublished observations), all samples were processed after one or more freeze-thaws (R. T. Hepple, unpublished results). Thus data are based on six animals in the 28- to 30-mo-old group. Despite a greater body mass, muscle mass was reduced in the 28- to 30-mo-old animals vs. the 8-mo-old animals. This was reflected in both a reduced mass of the gastrocnemius-plantaris-soleus muscle group (2,641 ± 51 vs. 2,056 ± 32 mg) and total contracting muscle mass (4,590 ± 59 vs. 3,810 ± 47 mg for 8-mo-old and 28- to 30-mo-old animals, respectively). As a result, the proportion of the total contracting muscle mass relative to the whole body mass in the 28- to 30-mo-old group (0.82 ± 0.03%) was significantly lower than in their younger counterparts (1.10 ± 0.01%). The severity of muscle atrophy in the older animals was proportional to the fraction of fast-twitch muscle fibers (2) such that atrophy was greatest in the gastrocnemius (24%), intermediate in plantaris (18%), and least in soleus (10%; Table 1).

Muscle blood flow and O2 delivery. The net perfusion pressure (difference between total perfusion pressure and the pressure within the lines of the perfusion system) was not significantly different between the two groups (Table 2). Arterial O2 content averaged 21.3 ± 0.3 ml/dl for both groups. The estimated total perfused muscle tissue mass was higher in 8-mo-old (19.1 ± 0.2 g) than in the 28- to 30-mo-old (15.9 ± 0.2 g) animals. Blood flow to the whole hindlimb was less in the 28- to 30-mo-old than in the 8-mo-old animals (Table 2). As a result, convective O2 delivery to the whole hindlimb was less in the 28- to 30-mo-old vs. 8-mo-old group. However, because muscle mass was less in the older animals (see Animal characteristics) and the fraction of hindlimb blood flow going to the gastrocnemius-plantaris-soleus muscle group was similar in both age groups, mass-specific blood flow to the gastrocnemius-plantaris-soleus muscle group was not different between the 8-mo-old and 28- to 30-mo-old animals (Table 2). Similarly, there was no difference in mass-specific convective O2 deliv-

### Table 1. Animal characteristics

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Body Mass, g</th>
<th>Gastrocnemius Muscle, mg</th>
<th>Plantaris Muscle, mg</th>
<th>Soleus Muscle, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>417 ± 6</td>
<td>2,091 ± 39</td>
<td>387 ± 9</td>
<td>164 ± 5</td>
</tr>
<tr>
<td>28–30</td>
<td>468 ± 19</td>
<td>1,590 ± 30</td>
<td>318 ± 6</td>
<td>147 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05.

### Table 2. Perfusion conditions

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Perfusion Pressure, Torr</th>
<th>System Pressure, Torr</th>
<th>Net Pressure, Torr</th>
<th>Total Hindlimb Blood Flow, ml/min</th>
<th>GPS Blood Flow/Total Hindlimb Blood Flow, %</th>
<th>GPS Blood Flow, ml/min·100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>144 ± 6</td>
<td>53 ± 4</td>
<td>91 ± 5</td>
<td>11.5 ± 0.4</td>
<td>15 ± 1</td>
<td>66 ± 5</td>
</tr>
<tr>
<td>28–30</td>
<td>125 ± 5</td>
<td>41 ± 3*</td>
<td>84 ± 5</td>
<td>9.8 ± 0.2*</td>
<td>14 ± 1</td>
<td>67 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Perfusion pressure, total perfusion pressure; system pressure, pressure through the tubing; net pressure, difference between perfusion pressure and system pressure; GPS, gastrocnemius-plantaris-soleus muscle group. *P < 0.05.
tery to the gastrocnemius-plantaris-soleus muscle group between age groups (569 ± 42 vs. 539 ± 62 μmol O$_2$·min$^{-1}$·100 g muscle$^{-1}$ for the 8 mo and 28- to 30-mo-old animals, respectively; Fig. 1, black bars), demonstrating that muscle convective O$_2$ delivery was well matched between groups.

**Contractile and metabolic responses.** There was no significant difference in total hindlimb resting V$_{O2}$ between the 8-mo-old and 28- to 30-mo-old animals whether expressed in absolute terms or relative to the estimated total perfused muscle mass (Table 3). Peak tension development was significantly lower in the 28- to 30-mo-old animals (Fig. 2), although this difference was abolished after normalizing the tensions to the contracting muscle mass in each group. Absolute tension (N) at V$_{O2 max}$ demonstrated a trend to being lower in the 28- to 30-mo-old group (Fig. 2), whereas the percent decline in tension during the 4 min contraction bout was greater in the 28- to 30-mo-old animals than in the 8-mo-old animals (Table 3). The V$_{O2 max}$ of the 28- to 30-mo-old group, whether expressed in absolute terms (Table 3) or normalized to the contracting hindlimb muscles (441 ± 20 vs. 344 ± 17 μmol·min$^{-1}$·100 g$^{-1}$, for the 8 mo and 28- to 30-mo-old animals, respectively; Fig. 1, gray bars), was significantly lower in the 28- to 30-mo-old animals than in the 8-mo-old group. The time at which V$_{O2 max}$ occurred was not different between groups, with maximal values attained most frequently at 2 min. Peak O$_2$ extraction across the whole hindlimb during contractions was not different between groups (Table 3). Similarly, although a numerical trend is apparent, the estimated O$_2$ extraction across the contracting muscles at V$_{O2 max}$ was not significantly different between the 28- to 30-mo-old and 8-mo-old group (P = 0.1). Examination of the lactate efflux during the contraction bout revealed a significantly reduced lactate release in the older animals (Fig. 3).

**Table 3. Contractile and metabolic responses**

<table>
<thead>
<tr>
<th></th>
<th>8 Mo Old</th>
<th>28–30 Mo Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak tension N</td>
<td>26.1 ± 4.0</td>
<td>18.2 ± 2.8*</td>
</tr>
<tr>
<td>N/g</td>
<td>9.8 ± 1.4</td>
<td>8.9 ± 1.7</td>
</tr>
<tr>
<td>Tension at V$_{O2 max}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/g</td>
<td>12.6 ± 1.5</td>
<td>8.2 ± 1.2†</td>
</tr>
<tr>
<td>Decline in tension %</td>
<td>61 ± 1</td>
<td>66 ± 1*</td>
</tr>
<tr>
<td>Hindlimb V$_{O2}$ rest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmol/min</td>
<td>6.6 ± 0.5</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>μmol·min$^{-1}$·100 g$^{-1}$</td>
<td>34 ± 3</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>V$_{O2 max}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmol/min</td>
<td>100 ± 4</td>
<td>78 ± 2*</td>
</tr>
<tr>
<td>μmol·min$^{-1}$·100 g$^{-1}$</td>
<td>569 ± 42</td>
<td>539 ± 62</td>
</tr>
<tr>
<td>V$_{O2}$ nonstimulated tissue, μmol/min</td>
<td>5.0 ± 0.4</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>Peak O$_2$ extraction Hindlimb, %</td>
<td>25 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>Contracting muscle, %</td>
<td>81 ± 8</td>
<td>87 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Decline in tension, decrease in tension during the 4-min contraction bout; Hindlimb V$_{O2}$ rest, the resting O$_2$ uptake (V$_{O2}$) calculated for the whole perfused hindlimb before contractions; Q$_{O2}$, convective O$_2$ delivery (arterial O$_2$ content × blood flow); O$_2$ cost of contractions, μmol·min$^{-1}$·100 g$^{-1}$; V$_{O2}$ nonstimulated tissue, O$_2$ contributed to V$_{O2 max}$ by nonstimulated tissues. *P < 0.05; †P = 0.061.

**Muscle oxidative capacity.** Before we completed our analyses, the ~70°C freezer broke down, resulting in the loss of four samples from the 28- to 30-mo-old group and two samples from the 8-mo-old group. As such, the biochemical results from the plantaris muscles in a separate group of four 28- to 30-mo-old F344BN rats were combined with the remaining data set in this group to yield n = 6 in both groups. The flux through

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Fig. 1. Muscle convective O$_2$ delivery (Q$_{O2}$) and maximal O$_2$ uptake (V$_{O2 max}$) in young adult (8 mo old) and late middle-aged (28–30 mo old) rats. Values are means ± SE; *P < 0.05.

Fig. 2. Tension development of the gastrocnemius-plantaris-soleus muscle group during the 4-min contraction bout in young adult (8 mo old) and late middle-aged (28–30 mo old) rats. Values are means ± SE.
DISCUSSION

Although a reduced \( \dot{V}_{O_2\text{max}} \) with aging is well documented, the role played by alterations within the skeletal muscles has remained unclear because reductions in convective \( O_2 \) delivery with aging tend to obscure the influence of factors intrinsic to the skeletal muscles. As such, we utilized an in situ pump-perfused rat hindlimb preparation to permit examination of young adult (8 mo old) and late middle-aged (28–30 mo old) rats at similar rates of muscle convective \( O_2 \) delivery and thereby reveal the influence of alterations within the skeletal muscles on the decline in \( \dot{V}_{O_2\text{max}} \) with aging. Consistent with our hypothesis, \( \dot{V}_{O_2\text{max}} \) was reduced independent of muscle convective \( O_2 \) delivery in the late middle-aged animals, demonstrating that alterations within the skeletal muscles contribute significantly to the decline in \( \dot{V}_{O_2\text{max}} \) with aging.

For our studies, we have utilized a rat model of aging, the F344BN hybrid rat. This model, developed by the National Institute on Aging, is more robust than homozygous strains (35) and better mirrors known changes in human skeletal muscles with aging. For example, the F344BN lives considerably longer (50) and demonstrates fewer age-related pathologies compared with homozygous rat strains such as the Fischer 344 (32). Furthermore, in contrast to homozygous strains of rat such as the Fischer 344 (10, 31), the F344BN demonstrates significant muscle atrophy with aging (5, 47, 53; as seen in humans, Ref. 13). In the present experiments, the mass of each of the gastrocnemius, plantaris, and soleus muscles was significantly lower in the 28- to 30-mo-old animals than in the 8-mo-old animals, showing that muscle atrophy was indeed present in the older animals. These were significant points when considering using this model to examine the potential role that age-associated muscle atrophy plays in the reduction of \( \dot{V}_{O_2\text{max}} \) with aging.

Experimental model. To permit us to examine skeletal muscle performance in young adult and older animals perfused at similar rates of muscle convective \( O_2 \) delivery, we have employed an in situ pump-perfused rat hindlimb model (15, 18). Hindlimb perfusion began at resting levels (~1 ml/min), and the rate of perfusion was then increased in a stepwise manner via a pump-driven extracorporeal circuit to evoke a flow-induced vasodilatory response. Over a period of ~30 min, we were able to achieve a similar rate of blood flow and convective \( O_2 \) delivery to the distal hindlimb muscles in the young adult and older animals at similar perfusion pressures before initiation of contractions. As noted previously (18, 42), this flow-induced vasodilation alters the normal autoregulation of blood flow such that muscle contractions no longer consistently cause a reduction in pressure (flow is held constant with pump perfusion). \( \dot{V}_{O_2\text{max}} \) was assessed during a 4-min maximal tetanic contraction bout at a frequency of 60 tetani/min, on the basis of prior results showing that this contraction frequency yields the highest \( \dot{V}_{O_2} \) (i.e., \( \dot{V}_{O_2\text{max}} \)) for the contracting distal hindlimb muscles in this preparation (21, 34, 42). The relatively large decrease in tension development seen during the first 2 min of contractions (i.e., coinciding with the period when the highest \( \dot{V}_{O_2} \) is attained) is most likely due to fatigue of fast-twitch motor units (21), which comprise a significant proportion of the total motor unit pool in the distal hindlimb muscles of the rat (2). Note that this fatigue does not adversely affect the aerobic metabolic response because we have found \( \dot{V}_{O_2} \) does not attain a higher level when the degree of fatigue is reduced by employing either a milder contraction frequency (30 tetani/min) or a sequential increase in contraction frequency every 60 s in the following order: 7.5, 15, 30, 60, and 90 tetani/min (R. T. Hepple, D. J. Krause, J. L. Hagen, and C. C. Jackson, unpublished observations).

In contrast to other pump-perfused preparations, such as the canine gastrocnemius model (20), only a fraction of the total hindlimb blood flow is distributed to the contracting muscles in the perfused rat hindlimb model (15). Specifically, ~14% of the total hindlimb blood flow was taken up by the gastrocnemius-plantaris-soleus muscle group, irrespective of the age of the animal. Gorski et al. (15) have previously shown that the stimulated distal hindlimb musculature accounts for ~24% of the total hindlimb blood flow, with the remaining 76% distributed to nonstimulated tissues. The calculation of \( \dot{V}_{O_2} \) in this preparation is, therefore, associated with much lower \( O_2 \) extraction across the total perfused mass than seen in the canine gastrocnemius model (20). However, when blood flow distribution is accounted for, \( O_2 \) extraction across the contracting muscles is similar (~80% in young adult rats, present results) to that seen in pump-perfused canine-gastrocnemius muscle (e.g., Ref. 20).
O₂ delivery and the decline in \( \dot{V}_{O_2} \) with aging. Since the first reports by Dehn and Bruce (9), subsequent studies have demonstrated that a reduced \( \dot{V}_{O_2} \) is one of the hallmark features of aging (22, 48). Models of human aging, such as rat (7) and dog (17), have also demonstrated a reduced \( \dot{V}_{O_2} \) in the older animals. In humans, the decline in \( \dot{V}_{O_2} \) is estimated to be 8–10% per decade beyond the age of 30 yr, of which approximately half can be attributed to a more sedentary lifestyle (9, 48, 49). From a physiological standpoint, the cause of this decline is multifactorial and includes alterations in central and peripheral function.

It is well known that changing O₂ delivery to the contracting skeletal muscles in adult humans (25, 40) and animal models (19), including rat hindlimb (18), yields proportional changes in \( \dot{V}_{O_2} \). It is also well established that blood flow to skeletal muscle during maximal exercise (38, 45, 46, 52), and hence O₂ delivery, declines with age in human subjects. In this respect, Irian et al. (23) have previously observed reduced blood flow to the gastrocnemius-plantaris-soleus muscle group of 24-mo-old vs. 12-mo-old male Fischer 344 rats during high-intensity contractions (120 tetani/min) in a self-perfused anesthetized rat hindlimb preparation in which local autoregulation of blood flow was intact. In contrast, our observations of similar blood flow and vascular conductance responses between age groups in pump-perfused gastrocnemius-plantaris-soleus muscles of F344BN rats suggests that the capacity for flow-induced vasodilation is not adversely affected by aging.

Because the age-associated decline in convective O₂ delivery seen in vivo and with self-perfused muscles in situ noted above likely contributes to reduce \( \dot{V}_{O_2} \) with aging, a conclusion reached in several studies previously (7, 39, 45), this effect obscures our understanding of the role that changes within the contracting muscles play in this response. Indeed, this effect has often led to the view that qualitative alterations within the skeletal muscles are relatively unimportant to the decline in \( \dot{V}_{O_2} \) with aging (16, 36). As described above, the use of an in situ pump-perfused rat hindlimb preparation minimizes the confounding effects of the age-associated reduction in convective O₂ delivery by permitting the examination of young adult and older rats at similar rates of muscle convective O₂ delivery. Under these conditions, \( \dot{V}_{O_2} \) in the older animals was significantly reduced compared with the young adult animals. Therefore, the important implications of this investigation is that skeletal muscle factors distal to convective O₂ delivery contribute to the reduction in \( \dot{V}_{O_2} \) with advancing age.

Skeletal muscle and the decline in \( \dot{V}_{O_2} \) with aging. Reduced muscle mass (i.e., sarcopenia; Ref. 43) due to reduced muscle fiber number (30) and, to a more variable extent, fiber size (8, 13, 30) is a well-described feature of human aging. Although the influence of sarcopenia on reduced muscle strength with aging is well documented (1, 14, 27), the implications of sarcopenia for muscle aerobic performance are less clear. Previously, Fleg and Lakatta (12) reported that the decline in \( \dot{V}_{O_2} \) between the ages of 22 and 87 yr in healthy men and women was reduced approximately by half when \( \dot{V}_{O_2} \) was normalized to an estimate of skeletal muscle mass. This suggests that quantitative changes in skeletal muscle mass contribute to the decline in \( \dot{V}_{O_2} \) with aging. Proctor and Joyner (39) later reached a similar conclusion using dual-energy X-ray absorptiometry. The novelty of the present results is that by demonstrating a reduction in mass-specific \( \dot{V}_{O_2} \) that is independent of muscle convective O₂ delivery, they now reveal the influence of qualitative changes within the skeletal muscles on the decline in \( \dot{V}_{O_2} \).

In our study, the 8-mo-old and 28- to 30-mo-old rats correspond to young adult and late middle-aged animals, on the basis of their respective mortality rates (50). The age-associated decrement in absolute \( \dot{V}_{O_2} \) was 29%, and although normalization to the contracting muscle mass (i.e., mass-specific \( \dot{V}_{O_2} \)) reduced this difference, a 22% lower aerobic power prevailed in the older animals. Note that the reduction in absolute \( \dot{V}_{O_2} \) between these age groups in rats is similar to what would be expected between the ages of 20 and 60 yr in humans, on the basis of a decline of 8–10% per decade after the age of 30 yr (9, 48, 49). Because the lower \( \dot{V}_{O_2} \) in the 28- to 30-mo-old animals was associated with a proportionally lower tension development such that \( \frac{\dot{V}_{O_2}}{\text{tension}} \) at \( \dot{V}_{O_2} \) was not different between groups, alterations in the energetic cost of muscle contractions apparently did not affect our results. Furthermore, lactate efflux during the contraction bout was significantly lower in the older animals, suggesting either that a reduced aerobic generation of ATP was not offset by a greater anaerobic glycolytic flux or that lactate release from the contracting muscles was impaired in the older animals. Although we do not have direct measures of muscle lactate concentration, it is likely that the lower lactate efflux reflects lower lactate production in the older animals. Fitts et al. (11) have previously shown an increased lactate concentration in soleus muscles of 28-mo-old Long-Evans rats after an intense (110 tetani/min) contraction protocol; however, previous findings by Campbell et al. (6) showing that lactate concentration was reduced in the white region of gastrocnemius muscle in 25-mo-old vs. 11-mo-old Fischer 344 rats after 1 min of 1-Hz tetanic contractions under occluded blood flow conditions are consistent with the lower lactate efflux observed in the present study.

One should also consider that denervation of muscle fibers due to the motor unit remodeling that occurs in aging skeletal muscle (26, 33) could adversely affect our conclusions. Specifically, the presence of a significant number of denervated fibers in the older animals would result in our overestimating the contracting muscle mass and thus underestimating mass-specific \( \dot{V}_{O_2} \) in the older animals. However, this does not appear likely because studies in both humans (24, 37) and rats (51) indicate that there are minimal differences in the degree of muscle fiber activation with
aging. As such, the lower \( \dot{V}_\text{O}_2\text{MAX} \) normalized to the contracting muscle mass in the older animals of the present study most likely represents the influence of factors distal to muscle convective \( \dot{O}_2 \) delivery. This could include altered mitochondrial biochemistry with aging, in addition to microvascular changes that determine \( \dot{O}_2 \) diffusion from blood to tissue at the individual myocyte level. In this latter respect, although the microscope technique permits one to determine the volume of blood flow delivered to the contracting muscles, it does not reveal the spatial distribution or flux of erythrocytes within the microvasculature, nor does it provide quantitative information about the anatomical volume of the capillary bed. Notwithstanding potential alterations at the microvascular level, we found that the flux through electron transport chain complexes I–III was reduced by \( \sim 45\% \) in homogenates prepared from the plantaris muscles of 28- to 30-mo-old animals, supporting a role for a reduced oxidative capacity in the decreased muscle \( \dot{V}_\text{O}_2\text{MAX} \) seen in the older animals.

In summary, the findings of this study show that \( \dot{V}_\text{O}_2\text{MAX} \) was reduced independent of muscle convective \( \dot{O}_2 \) delivery in late middle-aged animals, demonstrating that alterations within the skeletal muscles contribute significantly to the decline in \( \dot{V}_\text{O}_2\text{MAX} \) with aging. Notably, a reduction in \( \dot{V}_\text{O}_2\text{MAX} \) prevailed after the smaller muscles in the older animals were taken into account, showing that qualitative impairments in aged muscles contribute to reduce \( \dot{V}_\text{O}_2\text{MAX} \) with aging. In particular, the 45% lower flux through electron transport chain complexes I–III suggests that alterations in mitochondrial oxidative capacity play an important role in this decline in muscle \( \dot{V}_\text{O}_2\text{MAX} \) with aging.

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REFERENCES