Aging potentiates the effect of congestive heart failure on muscle microvascular oxygenation

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B e h n k e B J, D e l p M D, P o o l e D C, M u s c h T I. Aging potentiates the effect of congestive heart failure on muscle microvascular oxygenation. J Appl Physiol 103: 1757–1763, 2007. First published August 30, 2007; doi:10.1152/japplphysiol.00487.2007.—Congestive heart failure (CHF) is most prevalent in aged individuals and elicits a spectrum of cardiovascular and muscular perturbations that impairs the ability to deliver (\(Q_\text{O}_2\)) and utilize (\(V_\text{O}_2\)) oxygen in skeletal muscle. Whether aging potentiates the CHF-induced alterations in the \(Q_\text{O}_2\)-to-\(V_\text{O}_2\) relationship (which determines microvascular \(\text{PO}_2 (\text{PmvO}_2)\)) in resting and contracting skeletal muscle is unclear. We tested the hypothesis that old rats with CHF would demonstrate a greater impairment of skeletal muscle \(\text{PmvO}_2\) than observed in young rats with CHF. Phosphorescence quenching was utilized to measure spinotrapezius \(\text{PmvO}_2\) at rest and across the rest-to-contractions (1-Hz, 4–6 V) transition in young (Y) and old (O) male Fischer 344 Brown-Norway rats with CHF induced by myocardial infarction (mean left ventricular end-diastolic pressure \(>20\ \text{mmHg}\) for Y\(\text{CHF}\) and O\(\text{CHF}\)). In CHF muscle, aging significantly reduced resting \(\text{PmvO}_2\) (32.3 ± 3.4 Torr for Y\(\text{CHF}\) and 21.3 ± 3.3 Torr for O\(\text{CHF}\); \(P < 0.05\)) and in both Y\(\text{CHF}\) and O\(\text{CHF}\) compared with their aged-matched counterparts, CHF reduced the rate of the \(\text{PmvO}_2\) fall at the onset of contractions. Moreover, across the on-transient and in the subsequent steady state, \(\text{PmvO}_2\) values in O\(\text{CHF}\) vs. Y\(\text{CHF}\) were substantially lower (for steady-state, 20.4 ± 1.7 Torr for Y\(\text{CHF}\) and 16.4 ± 2.0 Torr for O\(\text{CHF}\); \(P < 0.05\)). At rest and during contractions in CHF, the pressure driving blood-muscle \(\text{O}_2\) diffusion (\(\text{PmvO}_2\)) is substantially decreased in old animals. This finding suggests that muscle dysfunction and exercise intolerance in aged CHF patients might be due, in part, to the failure to maintain a sufficiently high \(\text{PmvO}_2\) to facilitate blood-muscle \(\text{O}_2\) exchange and support mitochondrial ATP production.

THE PATHOLOGICAL CONDITION of congestive heart failure (CHF) is characterized by exercise intolerance, reductions in exercising muscle blood flow (Qm) (17, 38, 54, 60), and slower pulmonary oxygen uptake (\(V_\text{O}_2\)) dynamics (23, 33, 49). Aging may also be associated with multiple cardiovascular structural alterations [e.g., resistance artery rarefaction (4)], decreased muscle capillary-to-fiber ratio (12, 45), but unchanged or increased capillary density (24, 30) and functional alterations [e.g., impaired myogenic response and endothelial function in resistance arterioles (12, 16, 34, 36, 52)], which compromise the ability of structures to deliver (\(Q_\text{O}_2\)) and off-load \(\text{O}_2\) (\(V_\text{O}_2\)) within skeletal muscle. By measuring the pressure of \(\text{O}_2\) within the microvasculature (\(\text{PmvO}_2\)), the dynamic balance between \(V_\text{O}_2\) and \(Q_\text{O}_2\) at the site of capillary-myocyte \(\text{O}_2\) exchange can be investigated (5, 7, 44). We have recently demonstrated that aged skeletal muscle exhibits a lower resting \(\text{PmvO}_2\) as well as \(\text{PmvO}_2\) dynamics characteristic of compromised convective and diffusive \(\text{O}_2\) delivery across the rest-to-contractions transition (6). It is presently unknown whether, or to what extent, aging potentiates the CHF-related perturbation in \(\text{PmvO}_2\) dynamics. However, given the aforementioned age-related decrements in skeletal muscle vascular function, it is likely that CHF concomitant with senescence could dramatically impair the matching of \(Q_\text{O}_2\) to \(V_\text{O}_2\) in skeletal muscle across the rest-exercise transition.

Measurements of \(\text{PmvO}_2\) via phosphorescence quenching (50) provides a real-time assessment of the relationship between muscle \(Q_\text{O}_2\) and \(V_\text{O}_2\) at rest and across exercise transitions (5, 7), as well as detecting perturbations of this relationship (i.e., altered \(\text{PmvO}_2\) indicative of changed fractional \(\text{O}_2\) extraction) in major disease conditions (e.g., CHF) (15, 20). Specifically, \(\text{PmvO}_2\) provides an index of extraction at the microvascular level (32), demonstrated mathematically as follows:

\[
\text{PmvO}_2 = \frac{mV_\text{O}_2 \text{rest} + \Delta mV_\text{O}_2 [1 - e^{-\phi V_\text{O}_2}]}{Qm \text{rest} + \Delta Qm [1 - e^{-\phi Qm}]} \tag{1}
\]

Therefore, the kinetic profile of \(\text{PmvO}_2\) is representative of the dynamic \(Q_\text{O}_2\)-to-\(V_\text{O}_2\) ratio.

In this investigation, we utilized the Fischer 344 Brown-Norway (F344xBN) rat model of aging (27) to test the general hypothesis that CHF concurrent with old age will elicit changes in microvascular oxygenation at rest and across the rest-contractions transition in the spinotrapezius muscle. Specifically, because of impaired skeletal muscle arteriolar endothelium-dependent vasodilation manifest in old rats (35), rats with CHF (14), and elderly individuals with CHF (34), we hypothesized that aging will exacerbate the degree of \(Q_\text{O}_2\)-to-\(V_\text{O}_2\) mismatching (evidenced by a lower \(\text{PmvO}_2\)) induced by the CHF condition in skeletal muscle both at rest and across the rest-contractions transition. Knowledge of these microcirculatory \(\text{O}_2\) exchange impairments in aging and pathological conditions is fundamental to understanding the mechanisms of skeletal muscle dysfunction and exercise intolerance in affected individuals.

METHODS

Animals. All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

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Young (Y) (4–6 mo; n = 20) and old (O) (≥26 mo; n = 20) male F344xBN rats were used in this study. These rats were specifically selected for this investigation because they represent young and late middle age-old rats according to the lifespan for the F344xBN strain (27). In addition, the F344xBN rat has the distinct advantage over the F344 rat because, unlike the F344, it does not develop many of the age-related pathologies that proliferate in their highly inbred cousins (9). Rats were housed individually at 23°C and were maintained on a 12:12-h light-dark cycle. All rats were fed rat chow and water ad libitum.

Myocardial infarction procedures. Rats were assigned randomly to undergo either sham or myocardial infarction (MI) procedures, as described previously (37). Briefly, rats were anesthetized with a 5% isoflurane-O2 mixture, intubated, connected to a rodent respirator (Harvard model 680), and maintained on a 2% isoflurane-O2 mixture. A left thoracotomy was performed between the fifth and sixth ribs (~1.5 cm in length) to expose the heart. The pericardial sac was opened, and the heart was exteriorized. In rats receiving a MI, a 6-0 suture was used to encircle and ligate the left main coronary artery (~2–4 mm distal to its origin). Sham operations were completed by using the same surgical procedures with the exception that the coronary artery was not ligated. The lungs were hyperinflated, and the ribs approximated with 3-0 gut. The muscles of the thorax were sewn together with 4-0 gut, and the skin incision was closed with 3-0 silk. Lidocaine (1.5 mg/kg every 2 h for 8 h) and buprenorphine (0.03 mg/kg every 12 h for 24 h) were administered subcutaneously for postoperative pain alleviation, and ampicillin (50 mg/kg every 24 h for 10 days) was injected subcutaneously to minimize the chance for infection. After surgery, anesthesia was withdrawn and the rats were extubated and monitored for 8–12 h postoperation.

Experimental protocol. Ten weeks after MI or sham procedures, the rats were anesthetized with pentobarbital sodium (30 mg/kg ip, supplemented as needed). The right carotid artery was isolated and, by means of an introducer, cannulated with a 2 Fr catheter-tip pressure manometer (Millar Instruments). The manometer was advanced into the left ventricle (LV) in a retrograde fashion to measure LV end-diastolic pressure (LVEDP) and the rate of pressure change within the LV (LV dp/dt). Subsequently, the manometer was replaced with a fluid-filled catheter (PE-50) to monitor arterial blood pressure and heart rate for the duration of the experiment (Digi-Med BPA model 200). This fluid-filled catheter was used for the administration of additional anesthesia and for the infusion of phosphorescent probe. Rectal temperature was monitored and maintained at 37°C with a heating pad.

The left spinotrapezius was exposed as described previously (2, 15). Briefly, the skin and fascia were carefully removed from the caudal portion of the dorsal aspect of the muscle. Vascular and neural tissues branch primarily from the scapular origin of the spinotrapezius and were left undisturbed. Stainless steel electrodes were used to stimulate the muscle. The cathode was placed in close proximity to the motor point (0.5–1.0 cm caudal to the scapula), whereas the anode was sutured in place at the caudal edge of the muscle, near the fourth thoracic vertebrae. Stimulator settings were 100 Hz in the initial 10 min of each experiment.

The muscle was kept moist with a Krebs-Henseleit bicarbonate-buffered solution equilibrated with 5% CO2-95% N2 at 37°C during a 10-min stabilization period after exposure and throughout the subsequent experiment. The muscle was stimulated to contract at 1 Hz (~4–6 V, 2.0-msec pulse duration, twitch contractions) for 5 min with a Grass S88 stimulator. PmvO2 measurements were recorded every 2 s throughout rest and exercise.

On completion of the experiment, each rat was euthanized with an overdose of anesthesia (50 mg/kg ip pentobarbital sodium). The thorax was opened, and the lungs and heart were excised. The right ventricle (RV) was separated from the LV, and all tissues were weighed and normalized to the body weight of each animal.

PmvO2 measurements and calculations. The probe of a PMOD 1000 frequency domain phosphorimeter (Oxygen Enterprises, Philadelphia, PA) was positioned ~2 mm above the spinotrapezius, as described by Bailey et al. (2). A light guide contained within the probe focused excitation light (524 nm) on the medial region of the exposed spinotrapezius (~2.0 mm diameter, to ~500 μm deep). The PMOD 1000 uses a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows phosphorescence lifetime measurements from 10 μs to ~2.5 ms. In the single-frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm) and repeated every 2 s (for review, see Ref. 56). The phosphorescence lifetime was obtained computationally based on the decomposition of data vectors to a linearly independent set of exponentials (57).

The Stern-Volmer relationship allows the calculation of PmvO2 from a measured phosphorescence lifetime using the following equation (50):

\[
P_{\text{mvO}_2} = \left[ \frac{1}{T} \right] - 1 \left[ k_0 \times T \right]
\]

where \( k_0 \) is the quenching constant (Torr/s) and \( T \) and \( t \) are the phosphorescence lifetimes in the absence of \( O_2 \) and at the ambient \( O_2 \) concentration, respectively. For R2, in in vitro conditions similar to those found in the blood, \( k_0 \) is 409 Torr/s and \( t^\ast \) is 601 μs (29). Because the R2 is tightly bound to albumin in the plasma and is negatively charged, in combination with the extremely high albumin reflection coefficients in skeletal muscle (for review, see Ref. 47), the \( P_{\text{O}_2} \) measurements are ensured to result from signals within the microvasculature, rather than the surrounding muscle tissue (43). The phosphorescence lifetime is insensitive to probe concentration, excitation light intensity, and absorbance by other chromophores in the tissue (50). The effects of pH and temperature are negligible within the normal physiological range, which was maintained herein (29, 41).

Citrate synthase activity. Citrate synthase activity (CSa), a mitochondrial enzyme and marker of muscle oxidative potential, was measured in duplicate from spinotrapezius muscle homogenates according to the method of Sorensen (53). CSa, expressed as micromoles per minute per gram wet weight, was measured spectrophotometrically with a Spectramax 190 microplate (Molecular Devices, Sunnyvale, CA) in 300-μl aliquots at 30°C.

Data analyses. From anatomic dissection and morphological measurements, MI rats were categorized based on lung congestion [lung weight-to-body weight ratio (LW/BW)] and RV hypertrophy [RV to body weight ratio (RV/BW)]. Rats with both LW/BW and RV/BW greater than 4 standard deviations above the mean for the age-matched sham animals were considered to be in CHF (YCHF and OCHF groups). Rats receiving a sham operation comprised the sham (Y sham and O sham) groups.

KaleidaGraph software (Kaleidagraph 3.5) was used to describe the time course of each PmvO2 response using an exponential function, following a time delay (TD):

\[
P_{\text{O}_2}(t) = P_{\text{O}_2(\text{rest})} - \Delta P_{\text{O}_2(\text{rest})} \left[ 1 - e^{-\frac{t-TD}{\tau}} \right]
\]

where \( \tau \) is the time constant of the response, and \( \Delta P_{\text{O}_2(\text{rest})} \) (SS) is the difference between rest and the steady-state value.

When a marked undershoot occurred in the PmvO2 response before the attainment of a steady state (i.e., PmvO2 falling transiently below the steady-state value), a second exponential term was included in the model to reduce the residual sum of squares:

\[
P_{\text{O}_2}(t) = P_{\text{O}_2(\text{rest})} - A_1 \left[ 1 - e^{-\frac{t-TD_{1}(\text{rest})}{\tau_1}} \right]
\]

\[+ A_2 \left[ 1 - e^{-\frac{t-TD_{2}(\text{rest})}{\tau_2}} \right]
\]

where \( A_1 \) and \( A_2 \) are the amplitudes of the two components of the response, respectively. For Y responses (both sham and CHF groups),
the single exponential with TD provided an excellent fit to the PmvO2 data at the onset of contractions as judged from $r^2$, 2 sum of the squared residuals, and 3) visual inspection of the data and the fit of the residual error to a linear model (7). The PmvO2 responses from old animals required a more complex model with two exponentials (as described above), each with independent delays, to fit the PmvO2 response (6, 15). A two-way ANOVA among groups was performed on mean arterial pressure (MAP), heart rate (HR), LV/BW, CSa, citrate synthase activity. *P < 0.05 vs. age-matched sham values; †P < 0.05 vs. corresponding young group; ‡P < 0.1 vs. YCHF.

Table 1. Hemodynamic measures, LV mass normalized to body mass, and spinotrapezius CSa for sham control and CHF rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ysham (n = 8)</th>
<th>YCHF (n = 5)</th>
<th>Osham (n = 9)</th>
<th>OCHF (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>403 ± 5</td>
<td>389 ± 10</td>
<td>530 ± 21†</td>
<td>523 ± 16†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>114 ± 4</td>
<td>96 ± 4‡</td>
<td>94 ± 3§</td>
<td>81 ± 7*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>417 ± 8</td>
<td>328 ± 19</td>
<td>360 ± 12‡</td>
<td>296 ± 7*</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>1.82 ± 0.02</td>
<td>1.94 ± 0.02</td>
<td>1.92 ± 0.09</td>
<td>1.73 ± 0.14</td>
</tr>
<tr>
<td>CSa, μmol·g$^{-1}$·min$^{-1}$</td>
<td>15.2 ± 0.6</td>
<td>13.6 ± 0.4*</td>
<td>14.9 ± 0.6</td>
<td>12.6 ± 0.6‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. Ysham and Osham, young and old rats without heart failure; YCHF and OCHF, young and old rats with congestive heart failure; MAP, mean arterial pressure; HR, heart rate; LV/BW, left ventricle weight-to-body weight ratio; CSa, citrate synthase activity. *P < 0.05 vs. age-matched sham values; †P < 0.05 vs. corresponding young group; ‡P < 0.1 vs. YCHF.

Successful experiments were performed on eight Ysham and nine Osham rats and five rats for each of the CHF groups. The remaining animals were either euthanized for humane reasons during the 10-wk period after receiving a MI, did not fit the criteria for CHF as detailed in METHODS, or demonstrated unstable blood pressure responses during anesthesia and were not included in data analyses. CSa was reduced significantly in the spinotrapezius of YCHF and OCHF groups vs. values for age-matched sham animals (Table 1). In addition, there was a strong tendency (P = 0.08) for CSa values to be lower in OCHF than in YCHF spinotrapezius muscles (Table 1).

Indexes of heart failure and hemodynamic variables. As illustrated in Fig. 1, there was clear evidence of lung congestion and RV hypertrophy in the CHF groups (i.e., greatly increased LW/BW and RV/BW; for further clarification, see METHODS). In addition, LVEDP was increased and LV dP/dt was depressed to a similar degree in YCHF and OCHF animals (Fig. 1). By these criteria, therefore, both YCHF and OCHF animals demonstrated an equivalent severity of heart failure. MAP and HR were lower in CHF animals than in age-matched sham animals (Table 1).

PmvO2. In Figs. 2 and 3 for Y and O spinotrapezius, respectively. In Osham vs. OCHF groups, the precontracting baseline PmvO2 was depressed significantly vs. condition-matched Y values (Table 2). Furthermore, the PmvO2 baseline was 20% lower in OCHF than in Osham. In response to contractions, the change in steady-state (precontracting baseline – end-contracting) PmvO2 was lower in Osham than in Ysham group (8.4 ± 0.9 vs. 12.5 ± 1.9 Torr; P < 0.05) and in OCHF than in YCHF group (5.8 ± 0.5 vs. 12.6 ± 2.8 Torr; P < 0.05). In Osham vs. Ysham, there were no significant differences in the initial TD and time constant (Table 2). However, the Osham and OCHF responses required the more complex two-exponential model fit because of the presence of a PmvO2 undershoot (end-contracting PmvO2 minus absolute nadir value at the end of the exponential fall; see Fig. 3 and Table 2), which was not apparent in the Ysham response (Fig. 2). In addition, the PmvO2 undershoot was greater in Osham vs. Ysham (2.5 ± 0.9 and 0.9 ± 0.6 Torr, respectively;
P < 0.05) as well as in O CHF vs. Y CHF (Table 2). Neither the Y sham nor Y CHF Pmvo2 undershoots were significantly different from zero, and the two-exponential model did not provide a better fit (r value P > 0.05). For both O sham and O CHF animals, the secondary rise of Pmvo2 began ~2 min after the onset of contractions (Table 2).

**Discussion**

The present investigation demonstrates that the driving pressure of oxygen in the microcirculation (i.e., Pmvo2), which facilitates transcapillary O2 flux, is significantly lower in skeletal muscle from O CHF than in muscle from Y CHF rats at rest and at any given time point across the rest-contractions transition (Fig. 4). These findings demonstrate that CHF induces a blunting of the dynamic Pmvo2 response (i.e., altered QO2-to-Vo2 ratio), which is exacerbated by aging. The lower Pmvo2 exhibited in O CHF rats could potentially increase phosphocreatine (PCr) degradation and glycogen utilization in the contracting muscle (58), ultimately contributing to premature fatigue. In addition, because the slowing of Pmvo2 kinetics with CHF (Figs. 2 and 3) may reflect a reduced net muscle O2 exchange, the altered Pmvo2 profiles demonstrated herein provide a mechanistic link to the slowed pulmonary V˙O2 kinetics (3) as a result of the low capillary O2 driving pressure.

**Table 2. Static and dynamic results for the primary and secondary components of the Pmvo2 response**

<table>
<thead>
<tr>
<th></th>
<th>Y sham</th>
<th>Y CHF</th>
<th>O sham</th>
<th>O CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Pmvo2, Torr</td>
<td>34.1±1.7</td>
<td>32.0±3.4</td>
<td>26.9±2.1†</td>
<td>21.3±3.3‡</td>
</tr>
<tr>
<td>Δmvo2 Pmvo2, Torr</td>
<td>13.6±1.7</td>
<td>12.9±2.5</td>
<td>11.0±1.2†</td>
<td>6.4±1.1‡</td>
</tr>
<tr>
<td>Initial time delay, s</td>
<td>9.9±1.3</td>
<td>11.2±2.4</td>
<td>8.5±1.8</td>
<td>18.1±11.4</td>
</tr>
<tr>
<td>Initial time constant, s</td>
<td>23.2±5.9</td>
<td>34.2±12.8*</td>
<td>19.3±4.4</td>
<td>40.3±17.5*</td>
</tr>
<tr>
<td>k Pmvo2, Torr/s</td>
<td>0.79±0.16</td>
<td>0.58±0.21*</td>
<td>0.80±0.16</td>
<td>0.27±0.09†</td>
</tr>
<tr>
<td>MRT, s</td>
<td>34.2±5.2</td>
<td>45.3±13.9†</td>
<td>29.6±3.2</td>
<td>58.4±28*</td>
</tr>
<tr>
<td>Undershoot, Torr</td>
<td>0.9±0.6</td>
<td>0.2</td>
<td>2.5±0.9†</td>
<td>1.5±0.8†</td>
</tr>
<tr>
<td>Secondary time delay, s</td>
<td>114±37†</td>
<td>93±30‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary time constant, s</td>
<td>63±36†</td>
<td>92±5.4‡</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Baseline Pmvo2, precontracting microvascular Pmvo2; Δmvo2 Pmvo2, lowest Pmvo2 value during contractions; k Pmvo2, delta mvo2/Δmvo2 primary time constant (i.e., rate of Pmvo2 fall). Initial time delay and initial time constant are the initial delay after the onset of contractions and the exponential fall to the nadir Pmvo2 value. MRT, time delay + time constant. Undershoot is the steady-state contracting Pmvo2 - nadir value at the end of exponential decline. Undershoot observed in Y sham (n = 2 of 8) and Y CHF (n = 1 of 5) was not significantly different from zero. Pmvo2 undershoot in O sham (n = 8 of 9) and O CHF (n = 4 of 5) was significantly greater than zero. †P < 0.05 vs. age-matched sham values. ‡P < 0.05 vs. corresponding young group value or “0” where no secondary response was detectable. †P = 0.12 vs. age-matched sham values.

Plasticity of QO2 and Vo2 with CHF: impact of aging. In the present investigation, we sought to compare the effects of CHF with aging on the potential for skeletal muscle O2 exchange. Indeed, Y and O rats that underwent the MI procedure demonstrated the presence of an undershoot in Y CHF (Table 2) and not in Y sham (Fig. 2). Dashed line represents model fit to the actual Pmvo2 data (solid line). The 1-Hz contractions were initiated at time 0.

Fig. 2. Representative microvascular PO2 (PmvO2) profiles from Y sham (A) and Y CHF (B) animals. Dashed line represents model fit to the actual PmvO2 data (solid line). The 1-Hz contractions were initiated at time 0.

Fig. 3. Representative PmvO2 profiles from O sham (A) and O CHF (B) animals. Note the PmvO2 undershoot (i.e., steady-state contracting minus absolute nadir value) in both O sham and O CHF animals, whereas no undershoot was present in young animals (Fig. 2). Dashed line represents model fit to the actual PmvO2 data (solid line). The 1-Hz contractions were initiated at time 0.

Fig. 4. These findings demonstrate that CHF induces a blunting of the dynamic Pmvo2 response (i.e., altered QO2-to-Vo2 ratio), which is exacerbated by aging. The lower Pmvo2 exhibited in O CHF rats could potentially increase phosphocreatine (PCr) degradation and glycogen utilization in the contracting muscle (58), ultimately contributing to premature fatigue. In addition, because the slowing of Pmvo2 kinetics with CHF (Figs. 2 and 3) may reflect a reduced net muscle O2 exchange, the altered Pmvo2 profiles demonstrated herein provide a mechanistic link to the slowed pulmonary V˙O2 kinetics (3) as a result of the low capillary O2 driving pressure.
At rest and during the transition to muscular contractions, the ability to deliver O$_2$ (i.e., Q$_{O2}$) is impaired in the old rat (O$_{sham}$, Fig. 3) (6). Furthermore, in spinotrapezius muscle from O$_{CHF}$ rats, the effects of venous congestion (i.e., elevated LVEDP, Fig. 1) and a lower perfusion pressure (MAP, Table 1) would act to reduce the blood pressure differential across the capillary bed (31). This reduced arteriovenous pressure difference might retard RBC flow in perfused capillaries and consequently prolong the interaction between the RBC and myocyte, allowing the PO$_2$ in the RBC to more closely approximate extracellular PO$_2$ values (i.e., lower PmvO$_2$).

Mathematically, the reduced PmvO$_2$ in O$_{sham}$ and O$_{CHF}$ animals could be the consequence of either a reduced Q$_{O2}$ (as discussed previously) and/or an elevated V$_{O2}$. The latter option does not appear to be valid in this instance because the lower PmvO$_2$, at least in the O$_{sham}$, occurs in the presence of an unchanged resting V$_{O2}$ in spinotrapezius from O vs. Y adult animals (51). In contrast, in CHF, there is evidence that resting metabolic rate (RMR) is elevated (42, 55) and that the extent of the elevation is correlated with the severity of heart failure (40). Several mechanisms may contribute to an increased RMR in CHF, including 1) a reduced bioavailability of nitric oxide [reduced nitric oxide would relieve the inhibition of cytochrome-c oxidase (10, 61) and possibly increase V$_{O2}$], 2) increased catecholamines [e.g., epinephrine-stimulated increase in RMR (46)], and 3) potential alterations in mitochondrial uncoupling proteins. An increased resting V$_{O2}$ of O$_{CHF}$ spinotrapezius muscle coupled with a reduced Q$_{O2}$ provides one plausible mechanism for the substantially reduced PmvO$_2$ observed. One factor that does mitigate against an increased RMR in CHF is the oxidative potential (CSa), which showed a tendency ($P = 0.08$) to be reduced in O$_{CHF}$ vs. Y$_{CHF}$ (Table 1). Nonetheless, the Q$_{O2}$-to-V$_{O2}$ ratio is clearly attenuated in O$_{CHF}$, which, as illustrated in Fig. 4, results in a lower PmvO$_2$ at any point from rest to the contracting steady state. This reduced capillary O$_2$ driving pressure may, in part, be responsible for the greater reliance on nonaerobic energy sources (e.g., PCr) after exercise onset manifest in skeletal muscle (31). This reduced arteriovenous pressure difference might retard RBC flow in perfused capillaries and consequently prolong the interaction between the RBC and myocyte, allowing the PO$_2$ in the RBC to more closely approximate extracellular PO$_2$ values (i.e., lower PmvO$_2$).

CHF and PmvO$_2$ dynamics. The primary aim of this investigation was to explore the effects of aging on the PmvO$_2$ dynamics at rest and in the steady state of contractions in animals with CHF. However, it is insightful to consider briefly the alterations in PmvO$_2$ dynamics observed in Y$_{CHF}$ and O$_{CHF}$ animals. In the present investigation, severe heart failure was present in both Y and O animals, which resulted in a reduced CSa in both groups. Indeed, the blunted PmvO$_2$ dynamics in the Y$_{CHF}$ animals in the present study is similar to that found previously for Y animals with severe, but not moderate, indexes of heart failure (15). This would suggest that the reduced oxidative capacity of the Y$_{CHF}$ and O$_{CHF}$ muscle may have a greater effect on the PmvO$_2$ profile than altered Q$_{O2}$ kinetics per se. The delay before PmvO$_2$ declined across the first few seconds of contractions, i.e., primary TD was not different between Y$_{CHF}$ and O$_{CHF}$ (Table 2), indicating that the initial increase (i.e., what has been termed phase I) of the blood flow response might not be different across age groups in the CHF.

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**Fig. 4.** Microvascular PO$_2$ (PmvO$_2$) across the rest-contractions transition in healthy Y$_{sham}$ and O$_{sham}$ rats (A) and in Y$_{CHF}$ and O$_{CHF}$ rats (B). Solid lines represent the model fit to actual data from Figs. 2 and 3. Shaded region reflects the magnitude of the difference in PmvO$_2$ from the onset of contractions (time 0) throughout the entire 1-Hz contractions period. Note the PmvO$_2$ undershoot in the old groups, which was absent in the young groups.

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onstrated similar degrees of severe LV dysfunction and CHF based on predetermined hemodynamic and morphometric criteria (Fig. 1). In Y rats, CHF elicits several structural (e.g., reduced capillary-to-fiber ratio) (59) and functional (e.g., reduced endothelium-dependent vasodilation) (14) modifications within the microcirculation, which impact the ability to deliver and distribute O$_2$ within skeletal muscle. In spinotrapezius muscle of Y$_{CHF}$ rats, for example, there is a reduced percentage of capillaries supporting continuous red blood cell (RBC) flow at rest (65% in CHF vs. 87% in Sham) (26) and during the contracting steady state (48), thus diminishing the functional O$_2$ diffusing capacity of the muscle (D$_{mO2}$) (19). Even with the reduced D$_{mO2}$ in Y$_{CHF}$ rats, blood flow increases adequately with contractions to maintain fairly high PmvO$_2$ values that are commensurate with their age-matched sham groups. Thus any reduction in blood-muscle O$_2$ transport would not be caused by a reduced O$_2$ driving pressure (PmvO$_2$), as measured herein, but rather might be caused by some combination of the impaired microcirculatory perfusion (26, 48) and reduced muscle oxidative capacity (Table 1).

In contrast, in O$_{CHF}$, there is expected to be a cumulative reduction in functional D$_{mO2}$ due to a reduced percentage of RBC-perfused capillaries as seen in Y$_{CHF}$ animals (26, 48), which would be compounded by the effects of aging (i.e., reduced lineal density of RBC-perfused capillaries) (51). Both
condition. However, the presence of the PmvO₂ “undershoot” in O₂CHF (Table 2) suggests that the secondary increase in blood flow (i.e., phase II) could be sluggish relative to that of VO₂ (5, 21). Therefore, it appears that the effects of CHF on muscle hemodynamics (QO₂) and metabolic responses (VO₂) is compounded by the aging process. The mechanistic basis for this observation may well relate to the impaired endothelium-dependent vasodilation present in old muscle (35) and in CHF (14). It is plausible that CHF, concomitant with aging, results in a severe reduction in the ability of resistance vessels to vasodilate in response to endothelium-mediated challenges (e.g., flow-induced) and consequently that QO₂ dynamics across the rest-contractions transition might be slowed, forcing PmvO₂ to very low values.

Experimental considerations. To control for the strength of contractions in this preparation, we held the relative intensity of contractions (i.e., voltage range 4–6 V) as well as electrode placement on the motor point constant for all animals. Therefore, it was assumed that a similar recruitment pattern occurred with respect to motor unit activation and the number of fibers stimulated among groups. Although technical considerations precluded measurement of arterial blood gases in the present investigation, we have previously published evidence that neither CHF nor aging in the rodent model alter arterial blood gases (15, 51).

MAP was lower in the CHF groups vs. sham animals (Table 1). In healthy animals, reductions in MAP to ~70 mmHg do not discernibly affect PmvO₂ kinetics (8). In contrast, the lower MAP values, coupled with venous congestion (i.e., elevated LVEDP; Fig. 1), may have constrained blood flow dynamics and thus PmvO₂ dynamics in response to muscular contractions. However, despite the lower MAP values, there was no significant correlation (P > 0.1) between either the PmvO₂ primary TD or time constant and MAP in any of the CHF or sham responses.

Conclusions. There is significant experimental evidence suggesting that CHF alters the relationship between O₂ delivery (QO₂) and O₂ consumption (VO₂) within skeletal muscle. This study demonstrates for the first time that aging significantly reduces PmvO₂ in the spinotrapezius muscle of CHF rats at rest along with producing alterations in PmvO₂ dynamics during the transition from rest to muscle contractions, thereby suggesting that important age-related changes in the QO₂-/VO₂ relationship occur in the CHF condition. The lower PmvO₂ values in old animals with and without CHF would act to reduce VO₂ in accordance with Fick’s law, as well as elicit greater perturbations to the intracellular milieu (i.e., increased H⁺ production and greater PCR degradation) (58). These findings, therefore, provide a number of potential mechanisms that could contribute to the extreme slowing of the pulmonary VO₂ kinetics found in elderly CHF patients. Whether the slowing of pulmonary VO₂ kinetics found in elderly CHF patients is due to impairment of QO₂ relative to VO₂ within the muscle will require further investigation.

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