Dynamics of microvascular oxygen partial pressure in contracting skeletal muscle of rats with chronic heart failure

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Abstract

Objective: This investigation tested the hypothesis that the dynamics of muscle microvascular $O_2$ pressure ($PO_{2,m}$, which reflects the ratio of $O_2$ utilization [$VO_2$] to $O_2$ delivery [$QO_2$]) following the onset of contractions would be altered in chronic heart failure (CHF).

Methods: Female Sprague–Dawley rats were subjected to a myocardial infarction (MI) or a sham operation (Sham). Six to 10 weeks post Sham ($n=6$) or MI ($n=17$), phosphorescence quenching techniques were utilized to determine $PO_{2,m}$ dynamics at the onset of spinotrapezius muscle contractions (1 Hz).

Results: MI rats were separated into groups with Moderate ($n=10$) and Severe ($n=7$) CHF based upon the degree of left ventricular (LV) dysfunction as indicated by structural abnormalities (increased right ventricle weight and lung weight normalized to body weight). LV end-diastolic pressure was elevated significantly in both CHF groups compared with Sham (Sham, 3±1; Moderate CHF, 9±2; Severe CHF, 27±4 mmHg, $P<0.05$). The $PO_{2,m}$ response was modeled using time delay and exponential components to fit the $PO_{2,m}$ response to the steady-state. Compared with Shams, the time constant ($\tau$) of the primary $PO_{2,m}$ response was significantly speeded in Moderate CHF ($\tau$, Sham, 19.0±1.5; Moderate CHF, 13.2±1.9 s, $P<0.05$) and slowed in Severe CHF ($\tau$, 28.2±3.4 s, $P<0.05$). Within the Severe CHF group, $\tau$ increased linearly with the product of right ventricular and lung weight ($r=0.83$, $P<0.05$).

Conclusions: These results suggest that CHF alters the dynamic matching of muscle $VO_2$-to-$QO_2$ across the transition from rest to contractions and that the nature of that perturbation is dependent upon the severity of cardiac dysfunction.

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1. Introduction

Chronic heart failure (CHF) is characterized by an impaired exercise tolerance. In CHF patients at exercise onset, the dynamics of pulmonary oxygen uptake ($VO_2$) are slowed substantially [1–5] resulting in an elevated oxygen ($O_2$) deficit and greater intracellular perturbations of high energy phosphates and hydrogen ions. One common presumption is that these slowed pulmonary $VO_2$ kinetics result from impaired cardiorespiratory dynamics which limits muscle $O_2$ delivery ($QO_2$) at the onset of exercise. Whereas there is certainly a muscle blood flow deficit during exercise in CHF patients [6], there is also clear evidence in heart transplant patients that an elevated cardiac output prior to exercise onset does not always speed pulmonary $VO_2$ kinetics [5]. Such observations suggest that skeletal muscle dysfunction per se may play an important mechanistic role in the slowed pulmonary $VO_2$ kinetics in CHF. However, to date there are no measurements of microvascular $O_2$ pressures ($PO_{2,m}$) within muscle in CHF that could help elucidate this issue.

Moderate-to-severe left ventricular (LV) dysfunction causes profound structural and functional alterations within skeletal muscle that reduce the ability to distribute and utilize $O_2$ [7–10]. For example, arteriolar vasodilation is impaired (spinothrapezius [11]) and capillary involution (plantaris [12]) occurs concomitant with a substantial increase in the number and proportion of non-flowing
capillaries (spinotrapezius, [13]). As well as decreasing 
QO₂, CHF reduces muscle oxidative enzyme capacity but 
only in response to severe LV dysfunction [i.e., LV end-
diastolic pressures (LVEDP) above 20 mmHg (hindlimb 
muscles [8,14])]. Thus in moderate LV dysfunction (LVEDP~10 mmHg) the ability to deliver and distribute 
O₂ within skeletal muscle is impaired but muscle oxidative 
capacity remains normal. In contrast, in severe LV 
dysfunction (LVD) both QO₂ and its distribution as well as 
oxidative capacity are dysfunctional [8,11,14,15]. Based 
upon these observations, it is likely that the temporal 
profile of the muscle VO₂-to-QO₂ relationship across the 
transition to contractions is altered profoundly in CHF and 
in a manner that is determined by the severity of LVD (i.e., 
Moderate vs. Severe CHF).

Phosphorescence quenching measurement of PO₄₃₋ provides a rapid, high precision assessment of the VO₂-to- 
QO₂ relationship within muscle [16,17]. Moreover, it 
provides an index of the upstream O₂ diffusion pressure 
that drives blood–muscle O₂ exchange. The purpose of the 
present investigation was to determine the effect of CHF 
(Moderate and Severe) on PO₄₃₋ within skeletal muscle of 
rats at rest and following the onset of contractions. Based 
upon the evidence presented above and the responses 
observed by Behnke et al. [17], the following hypotheses 
were tested: (1) Moderate CHF (induced by MI and 
indicated by moderate LVD) will accelerate the PO₄₃₋ kinetics of the contracting spinotrapezius muscle. We also 
anticipate that PO₄₃₋ may undershoot the steady-state value early in contractions consequent to slowing of QO₂ 
dynamics coupled with preserved muscle oxidative func-
tion. (2) Severe CHF (induced by MI and indicated by 
severe LVD) will produce very slow PO₄₃₋ kinetics in the 
contracting spinotrapezius. We anticipate that this response 
is the consequence of an impairment of both VO₂ and QO₂ 
dynamics found in the Severe CHF condition.

2. Methods

2.1. Animals

Twenty-three female Sprague–Dawley rats (initial body 
weight=235–270 g) were used in this study. All pro-
cedures were approved by the Institutional Animal Care 
and Use Committee at Kansas State University. 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.

2.2. Myocardial infarction procedures

Rats were assigned randomly to undergo either sham or 
MI procedures, as described previously [18]. Briefly, rats 
were anesthetized with a 5% halothane/O₂ mixture. They 
were intubated and connected to a rodent respirator 
(Harvard Model 680) and maintained on a 2% halothane/ 
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 
ceircle and ligate the left main coronary artery approxi-
ately 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 closed with 3-0 silk. 
The opportunity for infection was reduced by the adminis-
tration of antibiotics (Ampicillan, 200 mg/kg). Anesthesia 
was withdrawn and the rats were extubated and monitored 
for 8–12 h post-operation.

2.3. Experimental protocol

Six to 10 weeks after MI or sham procedures, the rats 
were anesthetized with pentobarbital sodium (30 mg/kg 
i.p., supplemented as needed). A 2-French catheter-tip 
pressure manometer (Millar Instruments) was used to 
cannulate the right carotid artery. The manometer was 
advanced into the left ventricle in a retrograde fashion to 
measure LV end-diastolic pressure (LVEDP) and the rate of 
pressure change within the chamber (LV dP/dt). Sub-
sequently, 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 sam-
ping of arterial blood. Rectal temperature was monitored 
and maintained at 37 °C with a heating pad.

The left spinotrapezius was exposed as previously 
described [19]. Briefly, the skin and fascia were carefully 
removed from the caudal portion of the dorsal region 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 stimu-
late the muscle. The cathode was placed in close proximity 
to the motor point (0.5–1.0 cm caudal to the scapula), 
while the anode was sutured in place at the caudal edge of 
the muscle, near the fourth thoracic vertebrae. Moreover, 
stimulation parameters (i.e., voltage and placement of 
electrodes) were held constant between all animals. The 
phosphor, palladium meso-tetra-(4-carboxyphenyl)-por-
phine dendrimer (R2), was infused at a dose of 15 mg/kg 
through the arterial cannula ~15 min prior to each experi-
ment.

The muscle was kept moist using a Krebs–Henseleit 
bicarbonate-buffered solution equilibrated with 5% CO₂/ 
95% N₂ at 37 °C during a 10-min stabilization period 
following surgical exposure and throughout the subsequent 
experiment. The muscle was stimulated to contract at 1 Hz 
(∼5 V, 2.0 ms pulse duration, twitch contractions) for 3 min
with a Grass S88 stimulator. PO\textsubscript{2m} measurements were
recorded every 2 s throughout rest and exercise. Arterial
blood samples were drawn from the arterial cannula during
the final 15 s of stimulation for the determination of blood
gases (PaCO\textsubscript{2} and PaO\textsubscript{2}) and lactate concentrations.

Upon completion of the experiment, each rat was killed
with an overdose of anesthesia (pentobarbital sodium, \(\approx 50\)
mg/kg, i.a.). 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. The right spinotra-
pezius was excised, frozen in liquid N\textsubscript{2}, and saved for
citrate synthase activity determination.

2.4. PO\textsubscript{2m} measurements

The probe of a PMOD 1000 Frequency Domain Phos-
phorimeter (Oxygen Enterprises Ltd, Philadelphia, PA) was
positioned \(\sim 2\) mm above the spinotrapezius, as described
by Bailey et al. [19]. A light guide contained within the
probe focuses on the medial region of the exposed
spinotrapezius (\(\sim 2.0\) mm diameter to \(\sim 500\) \(\mu\)m 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 \(\mu\)s to \(\sim 2.5\) 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.
The phosphorescence lifetime was obtained by taking the
logarithm of the intensity values at each time-point and
fitting the linearized decay to a straight line by the least-
squares method [20].

The Stern–Volmer relationship allows the calculation of
PO\textsubscript{2m} responsible for a measured phosphorescence life-
time using the following equation [16]:

\[
PO_{2m} = \frac{(\tau^e/\tau - 1) / (k_Q*\tau^e)}{2}
\]

where \(k_Q\) is the quenching constant (Torr\textsuperscript{-1}s\textsuperscript{-1}) and \(\tau^e\)
and \(\tau\) are the phosphorescence lifetimes in the absence of
O\textsubscript{2} and at the ambient O\textsubscript{2} concentration, respectively. For
R2, in in vitro conditions similar to those found in the
blood, \(k_Q\) is 409 Torr\textsuperscript{-1}s\textsuperscript{-1} and \(\tau^e\) is 601 \(\mu\)s [21,22].
Since 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 [23],
the PO\textsubscript{2} measurements are ensured to result from signals
from the microvasculature, rather than the surrounding
muscle tissue. In addition, the sampled volume under
which PO\textsubscript{2m} values were measured may include some
small arterioles and venules, however, within muscle the
majority of blood volume is contained within the capillary
space. The phosphorescence lifetime is insensitive to probe
concentration, excitation light intensity, and absorbance by
other chromophores in the tissue [16]. The effects of pH
and temperature are negligible within the normal physio-
logical range which was maintained herein [21,22].

2.5. Citrate synthase activity

The citrate synthase activity for the right spinotrapezius
was determined spectrophotometrically at 23°C as de-
scribed by Srere [24].

2.6. Data analysis

Based on anatomical dissection and morphological
measurements MI rats were further divided into two
groups prior to analysis of PO\textsubscript{2m} profiles. The degree of
LVD and the severity of CHF was based on the presence of
lung congestion (lung weight to body weight ratio: LW/BW)
and right ventricular hypertrophy (RV weight to body
weight ratio: RV/BW). Rats with a LW/BW and RV/BW
greater than 4 standard deviations (S.D.’s) above the mean
for Sham were placed in the Severe CHF group, while the
remaining MI rats remained in the Moderate CHF group.
Rats receiving a sham operation comprised the Sham
group.

KaleidaGraph software (Kaleidagraph 3.5) was used to
describe the time-course of each PO\textsubscript{2m} response using an
exponential function, following a time delay (TD):

\[
PO_{2m}(t) = PO_{2m,rest} - A_1(1 - e^{-(t-TD1)/\tau_1})
+ A_2(1 - e^{-(t-TD2)/\tau_2})
\]

where \(\tau\) is the time constant of the response, TD is the
time delay, and \(\Delta PO_{2m}\) is the difference between rest and
the steady-state or end-contraction value.

When a marked undershoot occurred in the PO\textsubscript{2m}
response prior to the attainment of a steady-state, a second
exponential term was included in the model in order to
reduce the residual sum of squares:

\[
PO_{2m}(t) = PO_{2m,rest} - A_1(1 - e^{-(t-TD1)/\tau_1})
+ A_2(1 - e^{-(t-TD2)/\tau_2})
\]

where \(A_1\) and \(A_2\) are the amplitudes of the two com-
ponents of the response, respectively. For control (Sham)
responses, the single exponential with TD provided an
excellent fit to the PO\textsubscript{2m} data at the onset of contractions
as judged from: (1) coefficient of determination (\(r^2\)), (2)
sum of the squared residuals (\(\chi^2\)) and (3) visual inspection
of the raw data and the fit of the residual error to a linear
model [17]. This was also true for the MI rats with Severe
CHF but not for the MI rats with Moderate CHF in which
the more complex model with two exponentials (as de-
scribed above), each with independent delays was required
to fit the PO\textsubscript{2m} response [25].

A one-way analysis of variance among groups was
performed on PaO\textsubscript{2}, PaCO\textsubscript{2}, LVEDP, LV dP/dt, LW/BW,
RV/BW, and TD and Tau from the mathematical modeling
results. A Student–Newman–Keuls test was used for post-
hoc analysis. Spinotrapezius CS activity was also mea-
Table 1
Rat body weight, mean arterial pressure, arterial blood gases and lactate concentrations

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Moderate CHF</th>
<th>Severe CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>300±7</td>
<td>301±9</td>
<td>288±8</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>98±3</td>
<td>99±3</td>
<td>89±3</td>
</tr>
<tr>
<td>P O₂ (mmHg)</td>
<td>90±5</td>
<td>90±3</td>
<td>87±3</td>
</tr>
<tr>
<td>P CO₂ (mmHg)</td>
<td>44±1</td>
<td>43±3</td>
<td>42±3</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>1.7±0.2</td>
<td>2.1±0.3</td>
<td>1.8±0.3</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; P O₂, PO₂ of arterial blood; P CO₂, PO₂ of arterial blood. Values are mean±S.E.M. Sham, n=6; Moderate CHF, n=10; Severe CHF, n=7.

3. Results

Upon completion of the study, of the 17 animals that received the MI surgery, seven were categorized as possessing Severe CHF based upon the predetermined criteria (i.e., LW/ BW and RV/ BW greater than 4 S.D.’s from mean of Sham). Thus, data are presented from six Sham, 10 MI rats with Moderate CHF and seven MI rats with Severe CHF. The groupings were distinct with respect to both criteria for all rats. Tables 1 and 2 show anatomical, blood gas status, hematological and muscle citrate synthase data for the three groups.

The Moderate CHF group showed significant MI on the anterior lateral wall of the LV, but the animals showed no differences in LW/ BW or RV/ BW compared to the Sham group (P>0.05). In comparison, the Severe CHF group demonstrated a significant elevation in both indices compared to the Moderate CHF and Sham groups (P<0.05, Table 2). LVEDP was elevated significantly in both CHF groups (Table 2).

The PO₂ response to electrical stimulation of the spinotrapezius muscle differed substantially between the three groups of rats both qualitatively (as demonstrated in Fig. 1) and quantitatively (Table 3). In all instances, the Sham PO₂ response to stimulation could be fit adequately with a single component plus delay, whereas the Moderate CHF response consistently demonstrated an

Table 2
Heart morphometrics and spinotrapezius citrate synthase activity

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Moderate CHF</th>
<th>Severe CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV/BW (mg/g)</td>
<td>0.61±0.03</td>
<td>0.72±0.03</td>
<td>1.31±0.12² ³</td>
</tr>
<tr>
<td>LW/BW (mg/g)</td>
<td>3.9±0.1</td>
<td>4.2±0.2</td>
<td>11.3±1.6²</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>2.9±0.6</td>
<td>8.8±1.9³</td>
<td>26.6±4.2²</td>
</tr>
<tr>
<td>LV dP/dt (mmHg/s)</td>
<td>7410±580</td>
<td>5870±413⁴</td>
<td>4417±397⁴</td>
</tr>
<tr>
<td>CS (μmol/min/g wet weight)</td>
<td>13.2±1.0</td>
<td>13.8±1.0</td>
<td>10.6±0.5⁴</td>
</tr>
</tbody>
</table>

RV/BW, right ventricle wt/body wt; LW/BW, lung weight/body wt; LVEDP, left ventricular end diastolic pressure; LV dP/dt, left ventricular rate of pressure change; CS, citrate synthase activity. Values are mean±S.E.M.

² P<0.05 vs. Sham.
³ P<0.05 vs. Moderate CHF. Sham, n=6; Moderate CHF, n=10; Severe CHF; n=7.
Table 3
Spinotrapezius microvascular PO\textsubscript{2m} response to electrical stimulation

<table>
<thead>
<tr>
<th></th>
<th>Baseline PO\textsubscript{2m} (mmHg)</th>
<th>Δ PO\textsubscript{2m} (mmHg)</th>
<th>Steady-state PO\textsubscript{2m} (mmHg)</th>
<th>Time delay (s)</th>
<th>(\tau) (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>26.8±1.7</td>
<td>11.3±1.6</td>
<td>16.0±2.0</td>
<td>10.5±0.6</td>
<td>19.0±1.5</td>
</tr>
<tr>
<td>Moderate CHF(^c)</td>
<td>27.1±1.9</td>
<td>10.4±1.2</td>
<td>18.6±1.7(^b)</td>
<td>10.1±1.2</td>
<td>13.2±1.9(^d)</td>
</tr>
<tr>
<td>Severe CHF</td>
<td>22.1±1.9</td>
<td>8.4±0.9</td>
<td>14.2±2.0</td>
<td>6.5±2.1</td>
<td>28.2±3.4(^d)</td>
</tr>
</tbody>
</table>

PO\textsubscript{2m}, microvascular partial pressure of oxygen.

\(^a\) Note that for the Moderate CHF group, a double exponential model with independent time delays was required to fit the data (see text for details). The time delay for the secondary rise in PO\textsubscript{2m} was 95.2±11.3 s with a time constant (\(\tau\)) of 53.2±13.9 s. The undershoot (Moderate CHF) averaged 1.9±0.3 mmHg.

\(^b\) Steady-state PO\textsubscript{2m} for Moderate CHF reflects the end-contraction value.

\(^c\) \(P < 0.05\) vs. Sham.

\(^d\) \(P < 0.05\) vs. Moderate CHF. Values are mean±S.E.M.

undershoot with the PO\textsubscript{2m} response falling transiently below the steady-state or end-contraction value (Fig. 1, Table 3). Therefore, for the Moderate CHF response the more complex two-component model was required in order to satisfactorily fit the PO\textsubscript{2m} profile. This was confirmed via analysis of the sum of the squared error terms. In contrast to Moderate CHF, the Severe CHF response resembled grossly (i.e., no overshoot of the steady-state PO\textsubscript{2m}) that of the Sham group albeit with an altered kinetic profile (Fig. 1, Table 3). The speed of the primary PO\textsubscript{2m} component (\(\tau\)) was significantly faster in the Moderate CHF group compared with that observed in Sham rats, and was significantly slower in the Severe CHF group compared to both Moderate CHF and Sham rats (Table 3). Within the Severe CHF group, the primary \(\tau\) also showed a significant correlation with the degree of CHF or cardiopulmonary pathology present (defined here as the product of RV/BW*LW/BW; Fig. 2).

4. Discussion

This investigation tested the hypothesis that CHF rats will exhibit altered muscle PO\textsubscript{2m} kinetics across the transition from rest to electrical stimulation compared with Sham animals. The results demonstrate clearly that CHF causes profound changes in PO\textsubscript{2m} kinetics and moreover that the direction and magnitude of the changes in the PO\textsubscript{2m} profile are dependent upon the severity of the MI sequelae. Specifically, in MI rats with Moderate CHF without either pulmonary congestion or extensive skeletal muscle metabolic abnormalities (CS activity presented herein [10,14]), PO\textsubscript{2m} dynamics were accelerated (biphasically) compared to Shams. On the other hand, MI rats suffering from Severe CHF, pulmonary congestion and metabolic abnormalities (present results; [10,14,15,26]) exhibited slowed PO\textsubscript{2m} kinetics compared with both Sham and MI rats with Moderate CHF. We postulate that the more rapid reduction in PO\textsubscript{2m} and subsequent undershoot seen in the Moderate CHF group likely reflects a reduced QO\textsubscript{2} (relative to VO\textsubscript{2}) and consequent reduction of capillary O\textsubscript{2} driving pressure from blood to muscle. The former process will increase the transit delay from muscle-to-lung. The latter may actually reduce muscle VO\textsubscript{2} according to Fick’s law. Because either a speeding or a slowing of PO\textsubscript{2m} kinetics may reflect a reduced net muscle and pulmonary O\textsubscript{2} exchange, the altered PO\textsubscript{2m} dynamics attendant with both Moderate and Severe CHF are consistent with and mechanistically linked to, the slowed pulmonary VO\textsubscript{2} kinetics present in CHF.

4.1. Interpretation of PO\textsubscript{2m} kinetics

Whereas at present there are no direct measurements of VO\textsubscript{2} within the spinotrapezius muscle preparation at exercise onset in health or disease, it is known that QO\textsubscript{2}
increases following the first contraction with virtually no delay in healthy muscle [27–31]. Accordingly, the constancy of PO₂,m for 10–15 s after the onset of contractions must result from an increased VO₂ that is proportional in magnitude to the elevation of QO₂ [17]. The presence of the ~10 s time delay in Moderate CHF indicates that the proportionality between QO₂ and VO₂ responses found in muscle is preserved at least for the first few seconds of contractions. In Severe CHF, the delay is foreshortened and PO₂,m begins to decrease after only ~6 s. This is likely to be the consequence of an extreme impairment of the QO₂ response at the onset of contractions. The dichotomous subsequent PO₂,m response (following the delay) in Moderate (speeding of tPO₂,m) versus Severe (slowing of tPO₂,m) CHF is consistent with the observation that arteriolar function and muscle blood flow are impaired progressively in Moderate and Severe CHF whereas muscle oxidative capacity is reduced in Severe but not Moderate CHF (presents results; [14]). A low muscle oxidative capacity is associated mechanistically with slowed VO₂ [32,33] and PO₂,m [34] kinetics.

As discussed previously, PO₂,m serves as an index of the relationship between VO₂ and QO₂ such that:

\[ PO₂,m \propto k[CaO₂ - \frac{[CaO₂][\dot{V}O₂/QO₂]}{[QO₂][\dot{V}O₂}] }\]  

(1)

where \( k \) accounts for the position and shape of the O₂ dissociation curve, and CaO₂ is arterial O₂ content. Eq. (1) describes altered PO₂,m under steady-state conditions of either rest or contractions. To resolve the temporal PO₂,m profile across the rest–contractions transition, the respective amplitudes (\( A \)), delays (TD), and \( \tau \)'s of the VO₂ and QO₂ responses must be considered:

\[ PO₂,m \propto \frac{[\dot{V}O₂]}{[QO₂]} - \left[ \frac{[\dot{V}O₂]}{[QO₂]} + A[\dot{V}O₂(1-e^{-(t-TD)/\tau})] \right] \]  

(2)

Characterization of the PO₂,m dynamics across the rest to stimulation transition allows inferences to be made regarding the relative dynamics of VO₂ and QO₂. The technology needed to simultaneously measure VO₂ and QO₂ is not presently available for the type of in situ preparations used in this study. Furthermore, Laughlin and Shrage [35] have cautioned against direct muscle venous sampling on the grounds that it affects muscle vascular control and hemodynamics. However, mathematical modeling may serve as a valuable tool for evaluating the impact of the kinetic profiles of both VO₂ and QO₂ on that of PO₂,m (Figs. 3a and b). Eq. (2) was formulated to resolve a continuous PO₂,m profile across the rest–contractions transition. The values for TD, \( \tau \), as well as the baseline and amplitude (\( A \)) of the VO₂ and QO₂ response can be manipulated independently to characterize the ensuing effects on the PO₂,m profile. Representative depictions of the model output that replicate most closely the measured responses of PO₂,m for the MI rats with Moderate and Severe CHF are given in Figs. 3a and b, respectively. The effects of the dynamic relationship between VO₂ and QO₂ kinetics on PO₂,m are illustrated clearly in these figures. For the same rate of change of VO₂, the PO₂,m at any point after the time delay is lower than the Sham response when QO₂ is slower (Fig. 3a). However, consistent with the finding of an unchanged steady-state contracting spinotrapezius QO₂ between Sham and Moderate CHF (Behnke, Poole, and Musch, unpublished observations), the end-contracting PO₂,m was not different between Sham and Moderate CHF values. This means that the rate of decrease of the PO₂,m profile will be relatively steep compared to muscles in which QO₂ increases rapidly (i.e., Sham) [29]. This is consistent with the notion presented above that in the muscles of rats with Moderate CHF, \( \dot{V}O₂ \) may be normal but \( rQO₂ \) is slowed [36]. Fig. 3a further demonstrates that when \( \dot{V}O₂ \) increases more
rapidly relative to QO2, across the initial transition PO2,m will undershoot the steady-state value. Fig. 3b illustrates that when the kinetic profile of VO2 is slowed in concert with that of QO2, PO2,m kinetics will also be slowed without exhibiting an undershoot. This profile is consistent with that found in the group of rats with Severe CHF where the progressive slowing of PO2,m kinetics is correlated with the degree of LVD (Fig. 2). Note that the PO2,m profile in the CHF groups of rats departs markedly from that observed in the Sham.

4.2. Inferences from modeling

The τ for the PO2,m response to electrical stimulation was significantly faster in the group of rats with Moderate CHF than in the Sham animals. According to the previous discussion, this change suggests that the response of QO2 is slow compared to that of VO2. While LVD is present in MI rats categorized as Moderate CHF, it would appear that the amount of LVD present is not enough to elicit significant decrements in the metabolic properties of the spinotrapezius muscle (i.e., oxidative capacity is thought to be a primary determinant of tV̇O2; Ref. [33] and Table 2).

As discussed previously, there is a dichotomous effect of CHF on muscle hemodynamic (QO2) versus metabolic (oxidative enzyme capacity, VO2) responses. Specifically, several studies suggest that metabolic abnormalities are present only in Severe CHF (e.g., class III and IV CHF patients) in contrast to QO2 deficits, which may be found in more moderate cases [9,10,13,14]. For example, Drexler and colleagues [9] reported that only patients with severe CHF (VO2 max < 16 ml/min/kg) had a reduction in the volume density of mitochondria. Similarly, Delp et al. [14] reported a decrease in oxidative enzymes across seven hindlimb muscles (containing type I, IIA, and IIB fibers) in MI rats with Severe CHF, while only a single muscle (being predominately type IIB) showed a significant decrease in the MI rats with Moderate CHF. Brunotte and colleagues [10] used sciatic nerve stimulation in an in situ hindlimb preparation to demonstrate that at the same work rate, there was a greater fall in PCr/Pi+PCr in muscles of rats with Severe CHF when compared with Sham and MI rats with Moderate CHF, which were not different from one another.

4.3. Model considerations

1. The spinotrapezius is a postural muscle used to stabilize the scapula in the rat. It is possible that the amount of LVD and CHF found in the MI rats used in this study may have different effects on muscles used primarily for locomotion.

2. Left main coronary artery ligation has been an effective tool for inducing CHF in rats for decades. However, it is not possible to precisely control the severity of LVD and CHF that will develop within a given rat. The young, female Sprague–Dawley rats used in this study proved to be a very robust population. Despite an apparently large portion of necrotic myocardium produced in the LV, most of the rats were able to avoid developing the signs of severe LVD and ‘congestive’ heart failure (i.e. stable pulmonary edema as indicated by congestive lungs (increases in LW/BW) and hypertrophied right ventricle (increases in RV/BW)).

3. Regarding measurement of QO2, technical difficulties including the extended stability of the preparation have so far precluded simultaneous measurement of PO2,m and capillary red blood cell hemodynamics across the rest–exercise transition. However, we do have measurements of spinotrapezius bulk blood flow (via radio-labeled microspheres) in moderate CHF rats at rest and in the steady-state of contractions (Behnke, Poole, Musch, unpublished observations). These results demonstrated that during steady-state contractions, the spinotrapezius QO2 is similar in Sham and Moderate CHF rats. These data are similar to those found for selected hindlimb muscles of rats with Moderate CHF during locomotion [15] and they are consistent with the concept that the PO2,m in the steady-state is not different than that found for the Sham rats (see Fig. 3a).

5. Conclusion

The present results support the hypothesis that CHF alters the dynamic matching of VO2 to QO2 (as determined from PO2,m measurements) across the rest–contractions transition in a manner that is dependent upon the severity of LVD that develops in the animal. Specifically, in muscles from MI rats with Moderate CHF, muscle oxidative enzyme activity is unchanged (Table 2) and thus muscle VO2 kinetics are expected to be normal. However, there is substantial evidence that MI rats with Moderate LVD suffer from impaired muscle capillary blood flow [13] which will slow QO2 kinetics. The significantly faster fall of PO2,m found at the onset of contractions in the muscles of rats with Moderate CHF followed by an undershoot of the steady-state PO2,m is characteristic of a slower QO2 response compared with VO2 (see modeling Fig. 3a) [36]. In contrast, substantial decrements in muscle oxidative capacity occur within muscles of MI rats with Severe CHF (Table 2) and the slow PO2,m kinetics found in this group may be explained by slow VO2 kinetics in combination with slow QO2 kinetics (Fig. 3b). The PO2,m profiles evident in both groups of MI rats are consistent with a reduced net blood–muscle O2 flux across the rest–exercise transition. Thus, the behavior of PO2,m determined in the present investigation may provide important information regarding the mechanistic explanation for the slow pulmonary VO2 dynamics (i.e., large O2 deficit) found in CHF patients.
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