Experimental Physiology

Effects of chronic heart failure in rats on the recovery of microvascular $P_{O_2}$ after contractions in muscles of opposing fibre type

Paul McDonough, Brad J. Behnke, Timothy I. Musch and David C. Poole

Departments of Anatomy, Physiology and Kinesiology, Kansas State University, Manhattan, KS 66506-5802, USA

Chronic heart failure (CHF) impairs muscle O$_2$ delivery ($Q_{O_2}$) and, at a given O$_2$ uptake ($V_{O_2}$), lowers microvascular O$_2$ pressures ($P_{mvO_2}$; determined by the $Q_{O_2}$-to-$V_{O_2}$ ratio), which may impair recovery of high-energy phosphates following exercise. Because CHF preferentially decreases $Q_{O_2}$ to slow-twitch muscles, we hypothesized that recovery $P_{mvO_2}$ kinetics would be slowed to a greater extent in soleus (SOL: $\sim$84% type I fibres) than in peroneal (PER: $\sim$14% type I) muscles of CHF rats. $P_{mvO_2}$ dynamics were determined in SOL and PER muscles of control (CON: $n=6$; left ventricular end-diastolic pressure, LVEDP: $\sim$3 mmHg), moderate CHF (MOD: $n=7$; LVEDP: $\sim$11 mmHg) and severe CHF (SEV: $n=4$; LVEDP: $\sim$25 mmHg) following cessation of electrical stimulation (180 s; 1 Hz). In PER, neither the recovery $P_{mvO_2}$ values nor the mean response time (MRT; a weighted average of the time to 63% of the overall response) were altered by CHF (CON: 66.8 ± 8.0, MOD: 72.4 ± 11.8, SEV: 69.1 ± 9.5 s). In marked contrast, SOL $P_{mvO_2}$, at recovery onset, was reduced significantly in the SEV group ($\sim$6 Torr) and $P_{mvO_2}$ MRT was slowed with increased severity of CHF (CON: 45.1 ± 5.3, MOD: 63.2 ± 9.4, SEV: 82.6 ± 12.3 s; $P < 0.05$ CON vs. MOD and SEV). These data indicate that CHF slows $P_{mvO_2}$ recovery following contractions and lowers capillary O$_2$ driving pressure in slow-twitch SOL, but not in fast-twitch PER muscle. These results may explain, in part, the slowed recovery kinetics (phosphocreatine and $V_{O_2}$) and pronounced fatigue following muscular work in CHF patients.

(Received 27 January 2004; accepted after revision 30 April 2004; first published online 30 April 2004)

Corresponding author

D. C. Poole: Departments of Anatomy, Physiology and Kinesiology, 129 Coles Hall, Kansas State University, Manhattan, KS 66506-5802, USA. Email: poole@vet.ksu.edu

The time course with which pre-exercise cellular energetic status is re-established following muscular work is markedly prolonged in CHF patients (Sietsema et al. 1994; Thompson et al. 1995a,b; Kemp et al. 1996; Belardinelli et al. 1997; Tanabe et al. 2000). Specifically, in CHF patients after cessation of muscular work, the recovery kinetics of phosphocreatine (PCr; Thompson et al. 1995a,b; Kemp et al. 1996) and $V_{O_2}$ (Sietsema et al. 1994; Belardinelli et al. 1997; Koike et al. 1998; Tanabe et al. 2000; Myers et al. 2001) are demonstrably slowed compared with control subjects. These findings are of obvious importance to CHF patients, because it suggests that repetitive daily activities will precipitate marked fatigue (Thompson et al. 1995a; Mitchell et al. 2003) and exercise intolerance (Zelis et al. 1988; Simonini et al. 1996b; Musch et al. 2002) in this population.

In healthy individuals, the recovery of pre-exercise muscle PCr concentration ([PCr]) is acutely dependent upon muscle $Q_{O_2}$ (Idstrom et al. 1985), vascular O$_2$ pressures (Bylund-Fellenius et al. 1981; Haseler et al. 1999), oxidative capacity (Paganini et al. 1997) and pH$_i$ (Arnold et al. 1984). Moreover, the recovery of intramuscular $P_{O_2}$ (Bylund-Fellenius et al. 1981), $P_{mvO_2}$ (McDonough et al. 2004) and pH$_i$ (which is modulated by O$_2$ pressures via their effects upon glycolysis; Wilson et al. 1977) are largely dependent upon $Q_{O_2}$. Therefore, it is likely that the $Q_{O_2}$ at a given level of metabolism (i.e. the $Q_{O_2}$-to-$V_{O_2}$ ratio; McDonough et al. 2001) plays

© The Physiological Society 2004

DOI: 10.1113/expphysiol.2004.027367
a deterministic role in the recovery of muscle energetic status following contractions. Indeed, we recently noted that $P_{\text{mvO}_2}$ recovery following contractions was markedly slowed in the fast-twitch PER muscle (comprised predominately of type II fibres) compared to the slow-twitch SOL (predominately type I fibres), a finding that was attributed to the much lower recovery $Q_{\text{O}_2}$ in PER (Armstrong & Laughlin, 1983; McDonough et al. 2004).

Bulk $Q_{\text{O}_2}$ to the working limbs is reduced in CHF; but this effect is highly variable between individual muscles (Musch & Terrell, 1992). In particular, $Q_{\text{O}_2}$ is reduced to the greatest degree in those muscles with the highest percentage of oxidative fibres, such that these muscles (i.e. SOL; predominately oxidative fibres) exhibit marked decrements, whereas others (i.e. PER; primarily glycolytic) exhibit essentially no deficits in $Q_{\text{O}_2}$ in CHF (Musch & Terrell, 1992). One explanation for the marked variability in CHF-induced reductions in $Q_{\text{O}_2}$ is differences in the reactivity of the arterioles feeding these muscles (Didion & Mayhan, 1997), an effect that appears to be endothelium-dependent (Kubo et al. 1991). Specifically, Hirai et al. (1995) noted that the response to nitric oxide synthase inhibition was severely blunted in the SOL muscle of rats with CHF, yet essentially unaltered in the PER, suggesting that the endothelial defects reside primarily in those individual muscles with the highest percentage of oxidative fibres (i.e. types I and Iia). Therefore, if CHF reduces $Q_{\text{O}_2}$ during recovery from exercise preferentially in oxidative fibres, this would provide a putative mechanism for the exercise intolerance in individuals with CHF because type I and Iia fibres are recruited predominately during low-to-moderate intensity exercise.

Given the above, the most direct way to determine if fibre type, per se, plays a deterministic role in the recovery of cellular energetic status following contractions in CHF is to examine muscles that are polar opposites with respect to their fibre type composition. Two muscles that fit the above criteria are the SOL (84% type I, 7% type Iia, and 9% type Iib and d/x) and PER (14% type I, 19% type Iia, and 67% type Iib and d/x), which demonstrate similar oxidative capacity, yet exhibit a fibre type composition that is essentially the opposite of one another (Delp & Duan, 1996). To help elucidate the mechanisms through which the re-establishment of muscle energetic status is impaired following muscular work in CHF, we tested the following hypotheses: (1) that $P_{\text{mvO}_2}$ recovery kinetics would be slowed by CHF to a greater degree in SOL than in PER; (2) that $P_{\text{mvO}_2}$ (and thus capillary $O_2$ driving pressure) would be reduced in CHF animals during recovery (i.e. lower absolute value during the majority of the contractions off-transient) in SOL, but not in PER; and (3) that $P_{\text{mvO}_2}$ recovery kinetics would be prolonged in SOL in relation to the severity of CHF.

**Methods**

**Myocardial infarction**

Female Sprague–Dawley rats (291 ± 4 g; $n = 11$) were given a myocardial infarction (MI) as previously described (Musch et al. 1986; Musch & Terrell, 1992). Briefly, rats were anaesthetized (5% isoflurane/O$_2$ mixture), intubated and connected to a rodent respirator (Harvard Model 680). The anaesthetic plane was maintained on a 2% isoflurane/O$_2$ mixture. A left thoracotomy was performed (fifth intercostal space; ~1.5 cm in length) to expose the heart. The pericardial sac was opened and the heart exteriorized. A 6-O suture was then used to ligate the left main coronary artery (~2–4 mm distal to its origin). Following this procedure, the lungs were hyperinflated and the ribs approximated (3-O gut), the muscles of the thorax sewn together (4-O gut) and the skin incision closed (3-O silk). To reduce the chance of infection, antibiotics were administered (Ampicillan, 200 mg kg$^{-1}$). Anaesthesia was then withdrawn, and the rat was extubated and monitored for 8–12 h after surgery. Because we had previously noted no significant haemodynamic differences between Control and Sham operated animals (Symons et al. 1999), we chose to use non-infarcted control animals (CON; $n = 6$) to reduce the number of animals undergoing survival recovery procedures. All procedures were conducted according to National Institutes of Health guidelines and approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC).

**Cardiac haemodynamics**

Six to 10 weeks following MI procedures, the rats were anaesthetized (pentobarbital sodium; 30 mg kg$^{-1}$ i.p., supplemented as needed) and a 2-French catheter-tip pressure manometer (Millar Instruments) was introduced into the right carotid artery. The catheter was then advanced into the left ventricle in a retrograde fashion to measure LVEDP and the rate of pressure change within the chamber ($dP/dt$). Following these measurements, the manometer was replaced with a fluid-filled catheter (PE-50) to monitor arterial blood pressure for the duration of the experiment (Digi-Medical BPA Model 200). In addition, the fluid-filled catheter was used for the administration of additional anaesthesia, sampling of arterial blood and to provide a route of access for infusion of the phosphorescent probe [R2; palladium meso-tetra(4-carboxyphenyl)porphine dendrimer; 15 mg kg$^{-1}$].

© The Physiological Society 2004
**Surgical preparation for experiments**

Following measurement of cardiac haemodynamics, the SOL and PER were exposed in the manner detailed previously (Behnke et al. 2003). The exposed tissue was superfused with a Krebs–Henseleit bicarbonate-buffered solution (38°C, equilibrated with 5% CO₂, N₂ balance) and body temperature (rectal thermostatter) was maintained at ∼37°C using a heating pad.

**Contraction protocol**

Prior to beginning the experimental protocol the R2 probe was infused (via the arterial catheter). Approximately 15 min later, the SOL or PER (random order) was stimulated (stainless-steel electrodes attached to the distal and proximal ends of the muscle) at 1 Hz for 3 min (2–4 V, 2 ms pulse duration) using a Grass S88 stimulator (Quincy, MA, USA; for further detail, see Behnke et al. 2004). Following stimulation, recovery data were gathered for at least 3 min or until baseline values (i.e. a clear plateau) were reached. This contraction protocol has been shown in our laboratory to increase muscle blood flow significantly, while not changing arterial acid–base status or elevating plasma lactate concentrations (Behnke et al. 2003). Thus, in this regard, it resembles moderate-intensity exercise and as such interpretation of the recovery data should not be complicated by acidemia (McDonough et al. 2004). All animals were killed with an overdose of pentobarbitol sodium (> 80 mg kg⁻¹, i.a.) following the conclusion of the experimental protocol.

**Principle and measurement of phosphorescence quenching**

The basic principles of phosphorescence quenching have been detailed previously (Poole et al. 1995; McDonough et al. 2001; Behnke et al. 2003); however, a concise outline follows. The Stern–Volmer relationship (Rumsey et al. 1988) describes the relationship between probe phosphorescence and \( P_{mvO_2} \):

\[
t^0/t = 1 + k_Q \times t^0 \times P_{mvO_2}
\]

which rearranged to solve for \( P_{mvO_2} \) gives:

\[
P_{mvO_2} (\text{mmHg}) = \left( (t^0/t - 1) / (k_Q \times t^0) \right)
\]

where \( t = \) the lifetime of the phosphorescence at the prevailing \( O_2 \) tension; \( t^0 = \) the lifetime at a \( P_{mvO_2} \) of ‘zero’ and \( k_Q \) is the quenching constant of the probe (Torr s⁻¹).

For the R2 probe \( t^0 \) is 601 μs and \( k_Q \) 409 Torr s⁻¹ (Lo et al. 1997) and as the phosphorescent characteristics of R2 do not change over the normal range of temperatures and pH extant in the rat, the sole variable that can alter the phosphorescence lifetime is molecular \( O_2 \) pressure (Rumsey et al. 1988; Lo et al. 1997). Therefore, \( P_{mvO_2} \) is wholly dependent upon the lifetime of the phosphorescence decay, which is inversely proportional to the prevailing \( P_{mvO_2} \).

Importantly, the R2 phosphorescent probe is restricted to the intravascular space within the muscle, which allows measurement of \( P_{mvO_2} \) (Poole et al. 2003). To measure \( P_{mvO_2} \), a PMOD 1000 Frequency Domain Phosphorimeter (Oxygen Enterprises, Ltd, Philadelphia, PA, USA) was employed, with the light guide placed 2–4 mm above the medial portion of the muscle and focused on a circular area of exposed muscle of ∼2 mm diameter. Within this area (principally composed of capillary blood, as this compartment constitutes the majority of intramuscular blood volume; Poole et al. 1995), a sample is obtained up to ∼500 μm deep. The PMOD 1000 modulates the excitation frequencies between 100 Hz and 20 kHz, which can measure \( P_{mvO_2} \) values from 0 to 240 Torr. \( P_{mvO_2} \) was measured continuously with data reported at 2 s intervals throughout recovery.

**Curve fitting and statistical analysis**

For the \( P_{mvO_2} \) data, curve fitting was accomplished using KaleidaGraph software (version 3.5; Synergy Software, Reading, PA, USA) and was performed on the off-transient using a one-component model:

\[
P_{mvO_2(t)} \quad = \quad P_{mvO_2(\text{end-ex})} \quad + \quad \Delta P_{mvO_2} \quad \times \quad [1 \quad - \quad e^{-(t\quad -\quad \tau_1)/\tau_2}]
\]

and a more complex two-component model:

\[
P_{mvO_2(t)} \quad = \quad P_{mvO_2(\text{end-ex})} \quad + \quad \Delta_1 \quad \times \quad [1 \quad - \quad e^{-(t\quad -\quad \tau_1)/\tau_1}] \\
\quad \quad \quad + \quad \Delta_2 \quad \times \quad [1 \quad - \quad e^{-(t\quad -\quad \tau_2)/\tau_2}]
\]

where, \( P_{mvO_2(t)} \) is the \( P_{mvO_2} \) at any time, \( P_{mvO_2(\text{end-ex})} \) is the \( P_{mvO_2} \) at the end of the stimulation protocol, \( \Delta_1 \) and \( \Delta_2 \) are the amplitudes of the fast and slow recovery components, \( \tau_1 \) and \( \tau_2 \) are the time constants for each component. Goodness of fit was determined by three criteria: (1) the coefficient of determination (i.e. \( r^2 \)), (2) the sum of the squared residuals and (3) visual inspection and analysis of the residual fit to a linear model. MRT was calculated using the formula of MacDonald et al. (1997):

\[
\text{MRT} = (\Delta_1 / \Delta_{\text{tot}}) \times (\tau_1 + \tau_1) + (\Delta_2 / \Delta_{\text{tot}}) \times (\tau_2 + \tau_2)
\]

where \( \Delta_1 \) and \( \Delta_2 \), \( \tau_1 \) and \( \tau_2 \) are as defined above. In addition, to normalize the rate of recovery to
the amplitude of the response, the relative rate of $P_{mvO_2}$ recovery ($dP_{mvO_2}/dt$) was calculated by dividing the delta $P_{mvO_2}$ by the time constant of the response for both the fast and the slow components of $P_{mvO_2}$ recovery (McDonough et al. 2004).

Rats were separated into groups based on the severity of lung congestion (lung weight to body weight ratio: LW/BW) and right ventricular (RV) hypertrophy (RV to body weight ratio: RV/BW). Using cardiac indices derived from both anatomical dissection and morphology, MI rats were divided into two groups prior to analysis of $P_{mvO_2}$ values during resting and steady-state contractions (e.g. baseline and end contraction) as well as modelling dependent (e.g. TD, $\tau$, MRT) results were analysed using a two-way ANOVA to test for the effects of disease severity (CON, MOD, SEV) and muscle type (SOL and PER). As no interactions were found between muscle type and disease severity, a one-way ANOVA was employed to investigate the effect of disease severity, and an unpaired $t$ test was utilised to study between-muscle effects. When a significant $F$ value was demonstrated by the ANOVA, a Student–Newman–Keuls (SNK) procedure was performed to determine differences among mean values. Pearson product-moment correlations were performed upon select variables. Statistical significance was accepted at $P < 0.05$.

## Results

All comparisons with on-transient responses were performed using the on-transient responses from the study of Behnke et al. (2004) that correspond to the off-transient responses evaluated herein.

### Evidence of heart failure

The criteria for inclusion in the CON or MOD and SEV CHF groups were previously reported for these animals (Behnke et al. 2004). Briefly, based on LW/BW and RV/BW ratios, the rats were separated into MOD and SEV CHF. Those animals with a LW/BW and RV/BW greater than $4\,\text{s.d.}$ above the mean for CON were placed in the SEV group, and the other infarcted animals were placed in the MOD group. RV/BW ($0.61 \pm 0.03, 0.74 \pm 0.02$ and $1.44 \pm 0.12; \text{CON} < \text{MOD} < \text{SEV}, P < 0.05$) and LVEDP ($2.9 \pm 0.6, 11.0 \pm 3.6$ and $24.5 \pm 6.8; \text{CON} < \text{MOD} < \text{SEV}, P < 0.05$) were significantly different between experimental groups, whereas LV $dP/dt$ ($7410 \pm 580, 5217 \pm 444$ and $5100 \pm 513$; CON > MOD = SEV, $P < 0.05$) was reduced in both CHF groups (no difference between CHF groups) compared with CON. LW/BW ($3.9 \pm 0.1, 4.1 \pm 0.2$ and $10.3 \pm 2.0; \text{CON} = \text{MOD} < \text{SEV}, P < 0.05$) was increased for SEV CHF only compared with MOD (which was not different from CON). Furthermore, citrate synthase activity (CSa) was not different between muscles for CON ($25.5 \pm 1.8 \, \mu\text{mol g}^{-1} \text{min}^{-1}$; SOL vs. PER) or MOD ($22.4 \pm 2.4 \, \mu\text{mol g}^{-1} \text{min}^{-1}$; SOL vs. PER) and no difference was noted for either muscle between CON and MOD CHF. However, CSa was significantly different between SOL ($19.6 \pm 1.5 \, \mu\text{mol g}^{-1} \text{min}^{-1}$) and PER ($13.0 \pm 0.8 \, \mu\text{mol g}^{-1} \text{min}^{-1}$; $P < 0.05$) for the SEV CHF group and in both SOL and PER, CSa was significantly reduced in SEV CHF compared with MOD CHF and CON ($P < 0.05$).

### Microvascular $P_{O_2}$ during recovery in CHF

Following contractions, $P_{mvO_2}$ rose, following a short delay (Table 1), in all conditions for both muscles (Figs 1 and 2). However, specific differences were noted for the $P_{mvO_2}$ recovery profiles both within and between muscles.

Moreover, as we have noted previously (McDonough et al. 2004) the data for both SOL and PER were better fit by the more complex two-component (2-comp) model [based on $r^2$ and chi-squared residual term ($\chi^2$)] than the one-component (1-comp) model (SOL: $r^2$: 1-comp: $0.989 \pm 0.003$ and 2-comp: $0.993 \pm 0.002$, $\chi^2$: 1-comp: $8.27 \pm 2.1$ and 2-comp: $7.76 \pm 2.1$; PER: $r^2$: 1-comp: $0.988 \pm 0.007$ and 2-comp: $0.990 \pm 0.007$, $\chi^2$: 1-comp: $8.47 \pm 1.7$ and 2-comp: $6.3 \pm 1.5$; all $P < 0.05$).

#### Within-muscle effects.

In PER, the end-contraction $P_{mvO_2}$ was not significantly different between CON, MOD and SEV (Table 2). This contrasts with SOL, where end-contraction $P_{mvO_2}$ was significantly lower in SEV compared with CON (Table 2). In addition, the primary component time delay (TD$_1$; Table 1) was significantly longer in the SEV CHF group for both muscles vs. CON. This led to a significantly longer MRT (Table 1, Fig. 3) for SOL (as $\tau_1$ generally increased with CHF severity in SOL), but not for PER (as $\tau_1$ generally decreased with CHF in PER) in CHF rats vs. CON. Finally, the initial rate of recovery ($dP_{mvO_2}/dt$ fast) was significantly slowed for the CHF rats vs. CON for SOL (Table 1), such that the relative speed of recovery for $P_{mvO_2}$ was significantly slowed in SOL, but remained unchanged in PER for CHF animals compared to CON (Figs 1 and 2).
severely diseased CHF compared to SOL (Fig. 3). In other words, the off-transient MRT was significantly longer than the corresponding on-transient value for all treatment conditions in PER. However, for SOL, a completely different pattern was noted. Specifically, whereas an on–off symmetry was noted for CON, a significant on–off asymmetry that increased with disease severity was noted for CHF animals (Fig. 3).

Relationship between end-exercise \( P_{\text{mvO}_2} \) and recovery MRT

The overall time course (i.e. MRT) of the off-transient was significantly and inversely related to the end-exercise \( P_{\text{mvO}_2} \) (Fig. 4) for both SOL \( (r = -0.82; P < 0.05) \) and PER \( (r = -0.65; P < 0.05) \).

### Table 1. Microvascular \( P_{\text{O}_2} \) kinetics during the off-transient from stimulation

<table>
<thead>
<tr>
<th>Condition</th>
<th>SOL</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary (fast) component</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>delta ( P_{\text{mvO}_2} ) (( \Delta_1 ): Torr)</td>
<td>CON 5.7 ± 0.7</td>
<td>9.7 ± 1.6‡</td>
</tr>
<tr>
<td></td>
<td>MOD 3.8 ± 0.8</td>
<td>7.1 ± 1.1‡</td>
</tr>
<tr>
<td></td>
<td>SEV 6.2 ± 0.7</td>
<td>6.7 ± 2.2</td>
</tr>
<tr>
<td>TD1 (s)</td>
<td>CON 7.8 ± 2.6</td>
<td>2.6 ± 0.5§</td>
</tr>
<tr>
<td></td>
<td>MOD 7.4 ± 1.5</td>
<td>7.7 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>SEV 19.8 ± 1.4†</td>
<td>16.8 ± 2.5†</td>
</tr>
<tr>
<td>( \tau_1 ) (s)</td>
<td>CON 28.6 ± 5.2</td>
<td>58.1 ± 8.6†</td>
</tr>
<tr>
<td></td>
<td>MOD 33.1 ± 5.8</td>
<td>40.6 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>SEV 39.5 ± 6.9</td>
<td>39.4 ± 9.6</td>
</tr>
<tr>
<td>( dP_{\text{O}_2}/dt ) (fast: Torr s(^{-1}))</td>
<td>CON 0.22 ± 0.03</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>MOD 0.14 ± 0.03*</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>SEV 0.17 ± 0.03*</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td><strong>Secondary (slow) component</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>delta ( P_{\text{mvO}_2} ) (( \Delta_2 ): Torr)</td>
<td>CON 1.7 ± 0.6</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>MOD 2.6 ± 1.0</td>
<td>4.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>SEV 2.6 ± 0.9</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>TD2 (s)</td>
<td>CON 53.0 ± 5.9</td>
<td>62.8 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>MOD 54.6 ± 8.0</td>
<td>46.0 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>SEV 79.7 ± 5.8†</td>
<td>75.8 ± 17.8</td>
</tr>
<tr>
<td>( \tau_2 ) (s)</td>
<td>CON 19.3 ± 4.0</td>
<td>37.2 ± 12.6</td>
</tr>
<tr>
<td></td>
<td>MOD 35.1 ± 10.3</td>
<td>53.2 ± 13.0</td>
</tr>
<tr>
<td></td>
<td>SEV 47.4 ± 14.3*</td>
<td>50.7 ± 14.7</td>
</tr>
<tr>
<td>( dP_{\text{O}_2}/dt ) (slow; Torr s(^{-1}))</td>
<td>CON 0.09 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>MOD 0.08 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>SEV 0.06 ± 0.01</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>MRT (s)</td>
<td>CON 45.1 ± 5.3</td>
<td>66.8 ± 8.0†</td>
</tr>
<tr>
<td></td>
<td>MOD 62.3 ± 9.4*</td>
<td>72.4 ± 11.8</td>
</tr>
<tr>
<td></td>
<td>SEV 82.6 ± 12.3*</td>
<td>69.1 ± 9.5</td>
</tr>
</tbody>
</table>

Values are presented as mean ± s.e.m. *Significant difference from CON; ‡significant difference from MOD; §significant difference between SOL and PER. \( \Delta_1 \) and \( \Delta_2 \) are the amplitudes, TD1 and TD2 are the time delays and \( \tau_1 \) and \( \tau_2 \) are the time constants of the fast and slow component responses, respectively. \( dP_{\text{O}_2}/dt \) fast and slow denote the rate of change in \( P_{\text{mvO}_2} \) for the fast (\( \Delta_1/\tau_1 \)) and slow (\( \Delta_2/\tau_2 \)) component response, respectively, and MRT is the mean response time for the entire response (MRT = \( [\Delta_1/\Delta_{\text{tot}} + (TD_1 + \tau_1)] + [\Delta_2/\Delta_{\text{tot}} + (TD_2 + \tau_2)] \)). \( \Delta_{\text{tot}} \) is the amplitude of the entire recovery response. CON \( (n = 6) \), MOD \( (n = 7) \), SEV \( (n = 4) \).

**Between-muscle effects.** The end-contraction \( P_{\text{mvO}_2} \) was significantly lower in PER compared with SOL for both CON and MOD, but not SEV CHF (Table 2). In addition, \( P_{\text{mvO}_2} \) was significantly lower in PER compared with SOL for CON and MOD at 30 s and 1 min of recovery (Table 2), whereas no differences between muscles were noted at these time points for SEV CHF. Also, the primary time constant (\( \tau_1 \)) and MRT were significantly longer in PER vs. SOL for CON, but not for animals with either MOD or SEV CHF.

**Asymmetry between on- and off-transient**

For PER, a marked asymmetry was noted between the on- and off-transient for all conditions (CON, MOD and SEV; Fig. 3). In other words, the off-transient MRT was significantly longer than the corresponding on-transient value for all treatment conditions in PER. However, for SOL, a completely different pattern was noted. Specifically, whereas an on–off symmetry was noted for CON, a significant on–off asymmetry that increased with disease severity was noted for CHF animals (Fig. 3).
Discussion

In the current study, recovery $P_{mvO_2}$ kinetics were progressively slowed with CHF severity in SOL (i.e. CON < MOD < SEV; Fig. 1). This behaviour resulted from a slowing of the fast component rate (i.e. $dP_{O_2}/dt$) and a delayed onset of (i.e. TD$_2$) and slowing of the kinetics of (i.e. $\tau_2$) the slow component (Table 1). In marked contrast, in PER, recovery $P_{mvO_2}$ kinetics were not appreciably altered by CHF (cf. Figs 1 and 2; Table 1). The effect of CHF was such that whereas the MRT for $P_{mvO_2}$ recovery was much slower in PER vs. SOL for CON, it was not different between PER and SOL for either MOD or SEV conditions. In addition, whereas $P_{mvO_2}$ was significantly higher in SOL vs. PER throughout the majority of recovery in CON and MOD (Table 2), $P_{mvO_2}$ was not different between PER and SOL throughout recovery for SEV. This is an important finding because end-exercise $P_{mvO_2}$ was found to be significantly and inversely correlated to the MRT of $P_{mvO_2}$ recovery in both muscles (Fig. 4). Thus, with respect to $P_{mvO_2}$ and its kinetics, CHF induces changes within the slow-twitch SOL that make it respond both during contractions (Behnke et al. 2004) and during recovery (current study) in a similar fashion to the fast-twitch PER.

![Figure 1. SOL $P_{mvO_2}$ profiles for representative CON, MOD CHF and SEV CHF animals during the exercise off-transient](image)

A, characteristic profiles for each condition; B, the relative time course of recovery [i.e. normalized to delta $P_{mvO_2}$; calculated as $(\Delta_{tot} - \Delta_t)/\Delta_{tot}$ for each condition]. Note the slowing of $P_{mvO_2}$ recovery in MOD and SEV compared with CON for SOL. $\Delta_{tot} = $ delta $P_{mvO_2}$ for the whole response; $\Delta_t = $ delta $P_{mvO_2}$ at any given time $t$. Time ‘zero’ reflects the end of the contraction period.
Recovery of cellular energetic status following contractions: putative mechanisms

The speed with which precontraction muscle cellular energetic state (note: for this study we will consider \([\text{PCr}]\) as a direct marker (Meyer, 1988) and, through its effect on \([\text{PCr}], P_{\text{mvO}_2}\) as an indirect marker (Bylund-Fellenius et al. 1981; Haseler et al. 1999)) is re-established is thought to be determined (both directly and indirectly) by several factors that include oxidative capacity, \(pH_i\), muscle fibre type and oxygen delivery (Idstrom et al. 1985; Iotti et al. 1993; Simonini et al. 1996b; Paganini et al. 1997). Irrespective of the exact controlling mechanism(s), it is clear that recovery of cellular energetic status is markedly slowed in CHF patients (Sietsema et al. 1994; Belardinelli et al. 1997). As CHF has been shown to induce changes in all of the prospective controllers listed above, it is likely that one or more of these factors is responsible for the slowing of the recovery process in these patients. However, it is not known which factor(s) predominate.

**Oxidative capacity.** Oxidative capacity has been posited (Paganini et al. 1997) as a key controller of \([\text{PCr}]\) recovery. Indeed, Paganini et al. (1997) noted that in the rat gastrocnemius–plantaris complex, the rate constant for \([\text{PCr}]\) recovery was strongly correlated \((r = 0.84)\) with muscle oxidative capacity (CSa) in endurance-trained, control and diseased (chemical thyroidectomy) animals. This relationship appears to be preserved in rats with

---

**Figure 2.** PER \(P_{\text{mvO}_2}\) profiles for representative CON, MOD CHF and SEV CHF animals during the exercise off-transient

**A.** Characteristic profiles for each condition; **B.** the relative time course of recovery [again, normalized to delta \(P_{\text{mvO}_2}\); calculated as \((\Delta_{\text{tot}} - \Delta_t)/\Delta_{\text{tot}}\) for each condition]. Note the constancy of \(P_{\text{mvO}_2}\) recovery time independent of disease state in PER, which contrasts with that for SOL shown in Fig. 1. \(\Delta_{\text{tot}} = \text{delta } P_{\text{mvO}_2}\) for the whole response; \(\Delta_t = \text{delta } P_{\text{mvO}_2}\) at any given time \(t\). Time ‘zero’ reflects the end of the contraction period.

© The Physiological Society 2004
Table 2. Mean microvascular $P_{O_2}$ values during the off-transient from stimulation

<table>
<thead>
<tr>
<th>Condition</th>
<th>SOL</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-contraction (Torr)</td>
<td>CON 20.2 ± 0.9</td>
<td>13.3 ± 2.1‡</td>
</tr>
<tr>
<td></td>
<td>MOD 19.1 ± 2.7</td>
<td>11.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>SEV 14.3 ± 1.7*</td>
<td>10.4 ± 1.6</td>
</tr>
<tr>
<td>30 s (Torr)</td>
<td>CON 23.2 ± 1.2</td>
<td>16.7 ± 2.2‡</td>
</tr>
<tr>
<td></td>
<td>MOD 20.9 ± 2.8</td>
<td>14.4 ± 1.6*d</td>
</tr>
<tr>
<td></td>
<td>SEV 15.5 ± 1.8*</td>
<td>13.9 ± 2.7</td>
</tr>
<tr>
<td>1 min (Torr)</td>
<td>CON 25.3 ± 1.1</td>
<td>19.5 ± 2.4‡</td>
</tr>
<tr>
<td></td>
<td>MOD 22.8 ± 3.0</td>
<td>17.6 ± 1.7†</td>
</tr>
<tr>
<td></td>
<td>SEV 18.3 ± 2.1*</td>
<td>14.8 ± 2.1</td>
</tr>
<tr>
<td>End-recovery (Torr)</td>
<td>CON 27.1 ± 1.1</td>
<td>23.7 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>MOD 25.3 ± 2.7</td>
<td>22.1 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>SEV 22.6 ± 1.6*</td>
<td>18.4 ± 2.7</td>
</tr>
</tbody>
</table>

Values are presented as mean ± S.E.M. *Significant difference from CON; ‡Significant difference between SOL and PER. CON (n = 6), MOD (n = 7), SEV (n = 4).

CHF, as Thompson et al. (1995a,b) noted reductions in oxidative capacity and the maximal rate of ATP resynthesis concomitant with reductions in the rate of [PCr] recovery. However, the range of values used in the Paganini study (~85% reduction in CSA from trained to diseased) is much greater than that typically found in CHF (~20–30%; Thompson et al. 1995a; Simonini et al. 1996a; Delp et al. 1997; Pfeifer et al. 2001; Diederich et al. 2002; Behnke et al. 2003). In addition, we recently demonstrated that $P_{mvO_2}$ recovery was significantly prolonged in healthy PER compared with SOL (McDonough et al. 2004), two muscles of near-identical oxidative capacity (as measured by CSA). In the current study, CSA was reduced significantly with SEV CHF in both muscles, but $P_{mvO_2}$ recovery was slowed only in SOL. Thus, whereas oxidative capacity can impact the speed of the recovery of cellular energy state following contractions, it is unlikely to be responsible for the results noted herein.

Intracellular acidaemia. $pH_i$ has also been significantly correlated with the recovery of [PCr] (Paganini et al. 1997). However, whereas low $pH_i$ slows [PCr] recovery (Arnold et al. 1984; Iotti et al. 1993; Thompson et al. 1995a; Kemp et al. 1996), there is evidence in the human gastrocnemius that this effect is negligible for $pH_i > 6.95$ (Iotti et al. 1993). Thus, although $pH_i$ can appreciably affect [PCr] recovery (Kushmerick, 1983), the fact that the contraction protocol employed in the current investigation is of moderate intensity and does not appreciably alter acid/base status (Behnke et al. 2003; McDonough et al. 2004) suggests that changes in $pH_i$ are not the major factor responsible for the results noted herein.

Muscle fibre type

Our previous work suggested a relationship between muscle fibre type and $P_{mvO_2}$ recovery, as $P_{mvO_2}$ recovery was markedly slower in control PER (primarily type II fibres) than in control SOL (primarily type I fibres; McDonough et al. 2004). These findings, in combination with the results of the current study, suggest that fibre type shifts (particularly in SOL) may be responsible for the findings reported herein. However, the literature is equivocal on whether CHF actually causes fibre type shifts (cf. Simonini et al. 1996b; Delp et al. 1997) and when the do occur, they are typically small in scale (Simonini et al. 1996b; Delp et al. 1997; Spangenburg et al. 2002). It is unlikely that small changes in muscle fibre type populations, per se, are responsible for the slowed recovery noted in the current study. Notwithstanding the above, it is important to note the work of several investigators (Crow & Kushnerick, 1982; Kuznetsov et al. 1996; Burelle & Hochachka, 2002), which suggest that intrinsically different differences in mitochondrial function may exist between muscles of differing fibre type and similar oxidative capacities. In particular, Kuznetsov et al. (1996) noted that the $K_{m}$ for ADP-stimulated respiration was much lower in both the presence and the absence of creatine in fast-twitch vs. slow-twitch muscle. Thus, in the face of reduced oxidative capacity (as in CHF), slow-twitch muscle will probably be less sensitive to large changes in cellular energy state (as occur in CHF) than fast-twitch muscle, a scenario that may have contributed to the observed results. Whether this is the case remains to be determined.

Oxygen delivery. Several researchers (Bylund-Fellenius et al. 1981; Idstrom et al. 1985; Haseler et al. 1999, 1998) have noted that [PCr] and the recovery of both vascular and intracellular $O_2$ pressures are intimately dependent upon $O_2$ availability (Bylund-Fellenius et al. 1981; Hogan et al. 1992; Haseler et al. 1998, 1999). This agrees with our previous work (i.e. $Q_{O_2}$ differences are largely responsible for the differences in $P_{mvO_2}$ recovery; McDonough et al. 2004) and agrees nicely with the known differences in blood flow regulation between fibre types (Hirai et al. 1994; Thomas et al. 1994; Wunsch et al. 2000; Woodman et al. 2001; Aaker & Laughlin, 2002; McAllister, 2003). Specifically, a greater reliance upon endothelium-dependent vasodilation in muscles with a high proportion of oxidative (i.e. type I and IIa) fibres has been noted.
(Hirai et al. 1994; Woodman et al. 2001; McAllister, 2003), whereas a greater reliance upon sympatholysis is noted in those muscles with a high percentage of glycolytic (i.e. type IIb; Thomas et al. 1994) fibres. As CHF-induced decrements in endothelial function occur primarily in muscles with a high percentage of oxidative fibres (Hirai et al. 1995), the results of the current study and those of Behnke et al. (2004) suggest strongly a greater reduction in \(\dot{Q}_O_2\) in those muscles with the highest percentage of oxidative fibres, which is corroborated by the data of Musch & Terrell (1992). The finding that CHF progressively slows \(P_{mvO_2}\) recovery in SOL towards that seen in PER is consistent with a selectively (i.e. fibre-type-dependent) blunted blood flow response to exercise in CHF (Hirai et al. 1994, 1995) in SOL but not in PER (Musch & Terrell, 1992; McAllister et al. 1993).

**How CHF alters the recovery of cellular energy state**

CHF causes myriad peripheral (e.g. altered arteriolar vasoreactivity and reduced oxidative capacity; Kubo et al. 1991; Musch & Terrell, 1992; McAllister et al. 1993; Simonini et al. 1996a,b; Delp et al. 1997; Didion & Mayhan, 1997; Kindig et al. 1999; Richardson et al. 2003) and central (e.g. reduced cardiac output and stroke volume; Musch et al. 1986; Musch & Terrell, 1992; Drexler & Coats, 1996) adaptations that become more marked as CHF severity increases (Musch & Terrell, 1992). In general, these adaptations serve to reduce \(\dot{Q}_O_2\) both to (Musch & Terrell, 1992; McAllister et al. 1993; Hirai et al. 1995) and within (Kindig et al. 1999) the working muscles, as well as reducing the muscle’s ability to use that \(O_2\) (Simonini et al. 1996a; Kindig et al. 1999; Nusz et al. 2003; Richardson et al. 2003; Behnke et al. 2004). Germane to

---

**Figure 3.** On–off asymmetry (i.e. difference between MRT-on and MRT-off)  
A, SOL; B, PER. Note the progressively developing asymmetry with increasing severity of disease state in SOL and contrast that with the invariant asymmetry (independent of disease state) in PER.  
MRT = [(\(\Delta_1/\Delta_{100}\) * (TD1 + \(\tau_1\))] + [(\(\Delta_2/\Delta_{100}\) * (TD2 + \(\tau_2\))), where \(\Delta_1\) and \(\Delta_2\) are the amplitudes, TD1 and TD2 are the time delays and \(\tau_1\) and \(\tau_2\) are the time constants of the fast and slow component responses, respectively. \(\ast P \leq 0.05\) between on- and off-transient; \(\dagger P \leq 0.05\) between CON and CHF groups. On-transient data adapted from Behnke et al. (2004). CON (\(n = 6\)); MOD (\(n = 7\)); SEV (\(n = 4\)).
the current investigation, CHF has been shown to induce reductions in skeletal muscle blood flow (and $\dot{Q}_O_2$) that are far greater in SOL than in PER. Indeed, Musch & Terrell (1992) noted that blood flow was markedly reduced to the SOL with CHF (~30%), whereas BF to the PER was unaltered during moderate intensity exercise, a finding that appears to be due to a reduction in endothelium-dependent vasodilation in SOL (Behnke et al. 2004).

In addition to reductions in $\dot{Q}_O_2$, evidence exists that $O_2$ demand is altered inequitably in CHF (Simonini et al. 1996a,b). Indeed, the reduction in oxidative capacity from CON was greater in PER than in SOL for SEV CHF ($\downarrow$36 vs. $\downarrow$23%, respectively), such that CSa was significantly lower for PER than for SOL in SEV CHF animals. Thus, in CHF, the relative under-perfusion during contractions in SOL (i.e. large $\downarrow$ in $\dot{Q}_O_2$) coupled with a modest decrease in oxidative demand (i.e. moderate $\downarrow$CSa) will cause the $Q_{O_2}/V_O_2$ ratio (due to sluggish $\dot{Q}_O_2$ dynamics relative to those of $V_O_2$; Behnke et al. 2002) and $P_{mvO_2}$ and the blood-tissue $O_2$ pressure gradient to fall to a much greater degree in SOL of CHF animals (Table 2). The effects of a reduction in the blood-tissue $O_2$ gradient will probably be exacerbated by the reduction in diffusing capacity that attends CHF (Kindig et al. 1999; Nusz et al. 2003; Richardson et al. 2003). Furthermore, the correlation between end-contraction $P_{mvO_2}$ and MRT is much stronger for SOL than for PER (Fig. 4), which suggests that the

![Graph A](image_url)

$Y = -0.18x + 29.2$

$r^2 = 0.67$

SOL

![Graph B](image_url)

$Y = -0.11x + 19.6$

$r^2 = 0.42$

PER

Figure 4. Relationship between end-contraction $P_{mvO_2}$ and off-transient mean response time (MRT-off)

A, SOL; B, PER for all ($n = 17$) animals. Note that the relationship is stronger and the slope far steeper for SOL, suggesting that the effect of CHF on $P_{mvO_2}$ is more tightly linked to alterations in MRT in SOL than in PER. On-transient data adapted from Behnke et al. (2004).
combined sequelae of CHF (which cause a greater fall in $P_{mvO_2}$ during the on-transient; Behnke et al. 2004) conspire to prolong the recovery of $P_{mvO_2}$ in SOL (and by association [PCr]; Bylund-Fellenius et al. 1981) following contractions.

Summary and conclusions

CHF induces a reduced whole body and exercising limb $\dot{Q}_{O_2}$ (Musch & Terrell, 1992; Tanabe et al. 2000) and $V_{O_2}$ during peak exercise (Musch et al. 1986; Belardinelli et al. 1997) and a prolongation of PCr and $V_{O_2}$ recovery time (Thompson et al. 1995b; Belardinelli et al. 1997). The results of the current study (recovery) and those of Behnke et al. (2004) suggest that much of this maladaptive response to CHF is located within the slow-twitch, oxidative fibres. This is in agreement with previous work showing that much of the $\dot{Q}_{O_2}$ deficits noted during exercise were located in such fibres (Musch & Terrell, 1992; Hirai et al. 1995). In the context of the current study, CHF results in vascular and metabolic adaptations that reduce the $\dot{Q}_{O_2}/V_{O_2}$ ratio and thus $P_{mvO_2}$ following contractions in slow-twitch, oxidative fibres. As slow-twitch fibres (i.e. SOL) are chiefly responsible for sustaining most activities of daily living and moderate exercise in humans, the slowing of recovery $P_{mvO_2}$ kinetics in SOL, but not in PER, in those individuals with SEV CHF supports a schema wherein reductions in $P_{mvO_2}$ will lead to slowed $P_{mvO_2}$ recovery kinetics and consequently to slowed $V_{O_2}$ and PCr recovery kinetics in these patients. This in turn will probably contribute to the fatigue incurred by CHF patients while performing the repetitive activities of daily living or the exercise component of a cardiac rehabilitation programme.

References


© The Physiological Society 2004

P. McDonough and others


**Acknowledgements**

We would like to acknowledge K. Sue Hageman for her expert technical assistance. Support was provided by NIH HL-67619 and 50306, and AG-19228 and a Grant-in-Aid from the American Heart Association (Heartland Affiliate).

**Author’s present address**

Paul McDonough: University of Texas Southwestern Medical Center, Pulmonary & Critical Care Medicine, Department of Internal Medicine, 5323 Harry Hines Boulevard, Dallas, TX 75390-9034, USA.