Myoglobin concentration in skeletal muscle fibers of chronic heart failure patients

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Reduced exercise tolerance is a hallmark of chronic heart failure (CHF). It is not exclusively caused by reduced pump function of the heart because exercise tolerance correlates poorly with central hemodynamic characteristics, like ejection fraction (29, 35). Indeed it has been demonstrated that reduced exercise tolerance in CHF is also related to ventilatory efficiency and skeletal muscle characteristics (51). A shift in muscle fiber type from slow to fast, and a reduction of oxidative capacity in both fiber types, quantified as reduced mitochondrial volume fraction or mitochondrial enzyme activity, have been reported (11, 12, 26, 27, 29, 42). Because oxygen extraction from the blood by skeletal muscle in CHF patients is increased (23), the reduction of the oxidative capacity of skeletal muscle may be an adaptation to reduced oxygen supply, preventing the occurrence of hypoxic cores in skeletal muscle fibers. In theory, prevention of hypoxia is also possible by increasing the myoglobin concentration, because myoglobin facilitates oxygen transport in muscle fibers and reduces the extracellular oxygen tension preventing hypoxic cores (PO2crit; Ref. 30, 32, 50), which improves oxygen extraction from capillaries. Myoglobin also buffers oxygen and can supply oxygen to mitochondria at the onset of exercise while blood flow is increasing or during contraction when the flow of blood may be impaired. It has been shown that myoglobin can increase in humans during exposure to high-altitude hypoxia (38).

Myoglobin is also scavenger of nitric oxide and oxygen radicals. A reduction of the bioavailability of NO reduces the inhibition of cytochrome-c oxidase (6), and NO scavenging by overexpression of myoglobin inhibits angiogenesis (17). In addition, myoglobin may also function as an iron store (40). It follows from these diverse functions of myoglobin that a reduced concentration can cause hypoxia or metabolic inhibition in skeletal muscle fibers and, therefore, that it can be a determinant of exercise intolerance in patients with chronic heart failure. To the best of our knowledge the myoglobin concentration in skeletal muscle fibers of chronic heart failure (CHF) patients is not known.

In normal muscle, the myoglobin concentration correlates with the oxidative capacity of the muscle fiber (for review, see Ref. 21), suggesting common regulatory mechanisms. Both are under control of thyroid hormone (10). The promoters of the myoglobin (22) and peroxisome proliferator-activated receptor-γ coactivator-1α genes (which integrates stimulators of mitochondrial proliferation; for review, see Ref. 19) share the transcription factors nuclear factor of activated T cells and myocyte enhancer factor 2. The myoglobin promoter is also regulated via an unknown signaling cascade by vascular endothelial growth factor (VEGF; 49). VEGF expression is under the control of hypoxia inducible factor-1α, which also activates genes of anaerobic energy production (15), reducing the importance of oxidative phosphorylation. Because the regulatory mechanisms of myoglobin concentration and oxidative capacity are different, the relationship between the two is not necessarily similar in all muscle types (45, 48) and can vary depending on the energy charge of the muscle fiber and the intracellular oxygen tension. Both are expected to decrease in chronic heart failure.

Determining the myoglobin concentration in skeletal muscle fibers is complicated because the myoglobin concentration differs in individual human muscle fibers (34), type I (slow) having a higher concentration than type II (fast). Furthermore, a fiber type shift from type I to type II in skeletal muscle of CHF patients has been reported (11, 12, 26, 27, 29, 42), which
can mask changes in myoglobin concentration determined in homogenates. This complication requires determination of myoglobin concentration in individual muscle fibers. We previously developed a vapor-fixation technique preventing the loss of myoglobin from cryostat sections that allows the histochemical determination of the myoglobin concentration in large numbers of individual muscle fibers (45) and the use of serial sections for other assays. Succinate dehydrogenase (SDH) activity was determined to calculate the oxidative capacity ($V_O^{2\text{max}}$) of the muscle fibers (9, 46).

The purpose of this study was to determine the myoglobin concentration in skeletal muscle fibers of CHF patients and to calculate the effect of myoglobin on oxygen buffering and facilitated diffusion.

**METHODS**

*Patients and controls.* Five controls, all Caucasian, one woman and four men, participated in the study. Nine patients with a history of stable CHF of more than 6 mo were recruited from the Department of Cardiology from the VU University Medical Center in Amsterdam. Symptoms were classified as New York Heart Association class I in one Caucasian man, class II in three Caucasian and one Asian man, and class III in three Caucasian men. Six patients had ischemic cardiomyopathy, and three had idiopathic dilated cardiomyopathy. All subjects with CHF were on a stable medical regimen for at least 3 mo prior to the tests: two were on digoxin, five were taking diuretics, seven took angiotensin-converting enzyme inhibitors, two angiotensin II antagonists, two beta-blockers, and one was taking long-acting nitrates. The study was approved by the institutional ethical committee and in accordance with the Helsinki Declaration of 1975, as revised in 2001. Patients were excluded if they had diabetes mellitus, clinically significant chronic obstructive pulmonary disease, or peripheral vascular disease.

*Ejection fraction, peak $V_O^2$, peak power output.* Left ventricular ejection fraction was determined with equilibrium radionuclide angiograms in all patients. Each subject performed two cycle ergometer tests on different days. On the first day the subject’s maximal workload was estimated from a submaximal cycle ergometer test without measuring ventilatory parameters. From the estimated maximal workload we determined the ramp rates for the maximal cycle ergometer test (33). The second day all subjects underwent an individual incremental upright maximal cycle exercise test to a symptom-limited maximum (caused by dyspnea and/or muscle fatigue; respiratory quotients $>1.1$) on a cycle ergometer (Rehcor 2.25, Lode BV, Groningen, The Netherlands) with electrocardiographic monitoring for $\sim 8$ min (14). The pedal rate was kept at 60–70 revolutions per minute. Expired gas was analyzed continuously using the Sensormedics 2600 unit. Peak $V_O^2$ and peak power output were determined.

*Skeletal muscle biopsy.* Biopsy samples of $\sim 30$ mg wet wt were obtained from the vastus lateralis muscle using the Bergstrom needle technique (5) $\sim 10$ min after the exercise test. Biopsy sites were anesthetized with a $2\%$ lidocaine solution, and an incision of $\sim 1$ cm was made through the skin and fascia lata, 10 cm above the patella. The needle was inserted to a depth of $\sim 4$ cm. Samples were carefully removed from the needle, and the muscle fiber arrangement was determined using a magnifying glass. The biopsy was pinned to a small piece of Sylgard (Dow Corning, Wiesbaden, Germany) and frozen in liquid nitrogen. After freezing, the biopsy was transferred to a cryostat and 10-$\mu$m-thick sections were cut at $\sim 20^\circ$C. The sections were collected on slides coated with Vectabond (Vector Laboratories, Burlingame, CA).

*Fiber typing and fiber cross-sectional area.* Muscle fibers were typed on the basis of myofibrillar (M) ATPase histochemistry (48) as type I (low ATPase activity) or type II (high ATPase activity) as described (4, 43, 45). All type I fibers and type II fibers of one section per subject were counted, and the percentage of type I and type II fibers was calculated. The fiber cross-sectional area was determined in 10 type I fibers and 10 type II fibers in three cross sections in areas where the fibers were cut perpendicularly to their longitudinal axes in sections incubated for SDH activity. Muscle fibers in two biopsies from CHF patients were not cut perpendicularly to their longitudinal axes as indicated by elliptically shaped cross sections. In these biopsies the cross-sectional areas were calculated from the smaller diameters, assuming a cylindrical shape.

*Skeletal muscle fiber $V_O^{2\text{max}}$.* The calculation of skeletal muscle fiber $V_O^{2\text{max}}$ of individual skeletal muscle fibers was based on a method on paired determinations of skeletal muscle fiber $V_O^{2\text{max}}$ under hypoxic conditions in vitro and SDH activity reported previously (9, 46). Sections were incubated at $37^\circ$C under a nitrogen atmosphere in a medium consisting of (final concentrations in mM) 0.4 tetratinobule tetrazolium (Sigma, St. Louis, MO), 75 sodium succinate, 5 sodium azide, and 37.5 sodium phosphate buffer, pH 7.6 (37). We showed previously that SDH activity determined by quantitative histochemistry is proportional to the maximum steady rate of oxygen consumption under hypoxic conditions in vitro (9, 46) and that spatially averaged SDH activity in biopsies of the vastus lateralis muscle is proportional to peak $V_O^2$ in CHF patients and controls during incremental bicycle exercise (3). The relationship between peak SDH and SDH activity in control humans and CHF class I-II patients is similar to the relationship between muscle fiber $V_O^{2\text{max}}$ and SDH activity determined in vitro (3).

*Myoglobin concentration.* The myoglobin concentration was determined in individual muscle fibers by means of a vapor-fixation technique and a quantitative histochemical technique as described in detail previously (24, 45). Sections cut from gelatin blocks containing horse myoglobin were used for calibration.

*Microdensitometry.* The absorbance of the final precipitates in the histochemical procedures were determined using a microdensitometer as described in detail previously (24) at 660 nm for SDH and at 436 nm for myoglobin. The absorbance measured in sections incubated for myoglobin was converted to concentration using an extinction coefficient of $363 \text{mM}^{-1}\text{cm}^{-1}$ (45). Skeletal muscle fiber $V_O^{2\text{max}}$ (nmol $\cdot$ mm$^{-2}$ $\cdot$ s$^{-1}$) was calculated from the SDH staining rate (in absorbance units at 660 nm $\cdot$ mm section thickness$^{-1}$ $\cdot$ s incubation time$^{-1}$) $\times 6,000$ as described (9, 45).

*Calculation of $P_{O_2^{crit}}$.* $P_{O_2^{crit}}$ was calculated using a Hill-type diffusion equation taking myoglobin facilitated oxygen diffusion into account (30, 32):

$$P_{O_2^{crit}} = \frac{\text{skeletal muscle fiber } V_O^{2\text{max}} \cdot \text{CSA} - 4\pi D_{Mb} M_{Mb} \text{(}P_{O_2^{crit}} + P_{50}\text{)}}{4\pi K_O}$$

where skeletal muscle fiber $V_O^{2\text{max}}$ is the maximum rate of oxygen consumption calculated from SDH activity (see above), CSA is the muscle fiber cross-sectional area ($\text{mm}^2$), $D_{Mb}$ is the diffusion coefficient of myoglobin: $2.7 \times 10^{-5} \text{mm}^2 \text{s}^{-1}$ (2, 36), $K_O$ is Krogh’s diffusion coefficient for oxygen: $1.36 \text{nM} \cdot \text{mm}^{-1} \cdot \text{Tor}^{-1} \cdot \text{mm}^{-3} \cdot \text{s}^{-1}$ (16, 20), and $M_{Mb}$ is the concentration of oxygenated myoglobin at the sarcolemma, calculated as:

$$M_{Mb} = P_{2c} M_{Mb} (P_{O_2^{crit}} + P_{50})$$

where $M_{Mb}$ is the myoglobin concentration in the muscle fiber determined as described above, and $P_{50}$ is the oxygen tension at which $50\%$ of myoglobin is oxygenated: $P_{50} = 2.75 \text{ Torr}$ (41). Substitution of $Eq. 2$ into $Eq. 1$ allows the calculation of $P_{O_2^{crit}}$.

Furthermore, rearranging after substitution of $Eq. 2$ into $Eq. 1$ yields:

$$V_O^{2\text{max}} = \frac{4\pi K_O P_{2c}}{\text{CSA} (P_{O_2^{crit}} + P_{50})}$$

The first term represents the flux of oxygen across the sarcolemma by simple diffusion ($V_O^{2\text{diff}}$). The second term equals the flux of myo-
globin facilitated diffusion into the fiber ($V_{\text{O}_2}\text{Mb}$). It follows that the ratio of the fluxes can be calculated as:

$$
\frac{V_{\text{O}_2}\text{Mb}}{V_{\text{O}_2}\text{diff}} = \frac{(D_{\text{Mb}}/K_{\text{O}_2})\text{Mb tot}}{(P_{\text{O}_2}\text{crit} / H_11001)}
$$

where $V_{\text{O}_2}\text{Mb}$ and $V_{\text{O}_2}\text{diff}$ denote myoglobin facilitated diffusion, but also indirectly because it lowers $P_{\text{O}_2}\text{crit}$.

Statistics. Parts of the biopsies used in this study were also used in previous reports (3, 4, 43, 45) dealing with other aspects of skeletal muscle function in CHF. Comparisons between CHF patients and controls were performed using the two-tailed Student's $t$-test. A $P$ value of $<0.05$ was considered significant. Values are means $\pm$ SD.

RESULTS

Table 1 shows that CHF patients compared with control subjects were similar with respect to age, body weight, and body mass index. Peak $V_{\text{O}_2}$ and peak power output were significantly lower in CHF. Ejection fraction and hematocrit were determined for CHF patients only. Ejection fraction was lower than normal. Hematocrit was normal except for the female patient with hematocrit 0.37.

Figure 1 shows cross sections of muscle fibers of the vastus lateralis muscle incubated for myoglobin concentration and SDH activity. Muscle fibers with relatively high SDH activity (type I) had a relatively high myoglobin concentration.

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Table 1. Clinical variables

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>CHF Patients</th>
<th>n</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>51.4 $\pm$ 11.2</td>
<td>63.7 $\pm$ 10.3</td>
<td>5/9</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>81.2 $\pm$ 11.6</td>
<td>80.3 $\pm$ 8.9</td>
<td>5/9</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>25.7 $\pm$ 3.1</td>
<td>26.7 $\pm$ 2.4</td>
<td>5/9</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>n.d.</td>
<td>39 $\pm$ 8</td>
<td>0/9</td>
</tr>
<tr>
<td>Peak $V_{\text{O}_2}$, l/min</td>
<td>2.4 $\pm$ 0.5</td>
<td>1.6 $\pm$ 0.5*</td>
<td>5/9</td>
</tr>
<tr>
<td>Peak power output, W</td>
<td>225 $\pm$ 64</td>
<td>122 $\pm$ 36†</td>
<td>5/9</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>nd</td>
<td>0.43 $\pm$ 0.04</td>
<td>0/7</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD. CHF, chronic heart failure; nd, not determined. Significantly different from control: *$P < 0.05$, †$P < 0.01$.

Figure 2 shows paired data for myoglobin concentration and skeletal muscle fiber $V_{O_{2,\text{max}}}$ of muscle fiber types. The range of myoglobin concentrations among CHF patients (from 0.43 to 0.92 nM) was similar to control (0.44 to 0.83 nM). The female patient with low hematocrit had normal myoglobin concentrations in skeletal muscle fibers. Skeletal muscle fiber $V_{O_{2,\text{max}}}$ was lower in CHF in both fiber types, as reported previously (3, 4).

Table 2 gives mean values of skeletal muscle fiber characteristics of the different fiber types. The cross-sectional area of type I and type II fibers, as well as the myoglobin concentration of type I and type II fibers, was similar in CHF patients and controls. Muscle fiber $V_{O_{2,\text{max}}}$ (calculated from SDH activity) was lower in CHF in both fiber types. Consequently, the
Table 2. Skeletal muscle fiber characteristics

<table>
<thead>
<tr>
<th></th>
<th>5 Controls</th>
<th>9 CHF Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDH activity type I $\times 10^8$, $\Delta A_{560}$ μM·mm$^{-1}$·s$^{-1}$</td>
<td>1.50±0.22</td>
<td>1.02±0.16$^a$</td>
</tr>
<tr>
<td>SDH activity type II $\times 10^8$, $\Delta A_{560}$ μM·mm$^{-1}$·s$^{-1}$</td>
<td>1.10±0.08</td>
<td>0.68±0.18$^{ab}$</td>
</tr>
<tr>
<td>CSA type I fibers, μm$^2$</td>
<td>4326±892</td>
<td>5721±1330</td>
</tr>
<tr>
<td>CSA type II fibers, μm$^2$</td>
<td>5824±1846</td>
<td>5362±1740</td>
</tr>
<tr>
<td>Myoglobin type I fibers, mM</td>
<td>0.70±0.09</td>
<td>0.69±0.11</td>
</tr>
<tr>
<td>Myoglobin type II fibers, mM</td>
<td>0.49±0.07</td>
<td>0.52±0.07$^c$</td>
</tr>
<tr>
<td>Myoglobin/V$\tilde{O_2}$max type I fibers, s</td>
<td>7.8±0.9</td>
<td>11.4±2.1$^{a,b}$</td>
</tr>
<tr>
<td>Myoglobin/V$\tilde{O_2}$max type II fibers, s</td>
<td>7.5±1.0</td>
<td>13.6±3.9$^b$</td>
</tr>
<tr>
<td>P$O_{2 crit}$ type I fibers, Torr</td>
<td>11.5±3.3</td>
<td>10.3±4.7</td>
</tr>
<tr>
<td>P$O_{2 crit}$ type II fibers, Torr</td>
<td>14.7±6.2</td>
<td>6.1±2.8$^b$</td>
</tr>
<tr>
<td>P$O_{2 crit}$ type I fibers without myoglobin, Torr</td>
<td>22.4±3.7</td>
<td>22.5±7.1</td>
</tr>
<tr>
<td>P$O_{2 crit}$ type II fibers without myoglobin, Torr</td>
<td>20.4±5.8</td>
<td>12.7±4.2$^d$</td>
</tr>
<tr>
<td>$V_{O_2MB}/V_{O_2diff}$ type I fibers</td>
<td>1.0±0.3</td>
<td>1.2±0.5</td>
</tr>
<tr>
<td>$V_{O_2MB}/V_{O_2diff}$ type II fibers</td>
<td>0.7±0.5</td>
<td>1.3±0.5$^a$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Muscle fiber $V_{O_2}$max was calculated from SDH activity. *Different from control $P < 0.001$, $^a$different from type I $P < 0.001$, $^c$different from type I $P < 0.01$, $^d$different from control $P < 0.05$.

buffering capacity of myoglobin, i.e., myoglobin concentration divided by skeletal muscle fiber $V_{O_2}$max, which is the period during which myoglobin can supply oxygen to mitochondria operating at $V_{O_2}$max, was increased in CHF. $P_{O_{2 crit}}$ values calculated with and without myoglobin were similar in type I fibers in controls and CHF patients. $P_{O_{2 crit}}$ values of type II fibers were reduced in CHF patients compared with control. Myoglobin reduced $P_{O_{2 crit}}$ by 49% in type I fibers of controls, by 28% in type II fibers of controls, by 52% in type I fibers of CHF, and by 52% in type II fibers of CHF patients. Myoglobin-facilitated diffusion increases in type II fibers of CHF patients compared with control.

DISCUSSION

This study showed that myoglobin concentrations were similar in type I and II skeletal muscle fibers when CHF patients were compared with controls. The normal myoglobin concentrations in combination with a reduced skeletal muscle fiber $V_{O_2}$max, as calculated from SDH activity, implies an increased buffering capacity for oxygen in skeletal muscle of CHF patients. Such increased buffering capacity could compensate for or contribute to impaired peripheral vasodilatation at the onset of exercise in CHF patients (18). Because the $P_{50}$ of human myoglobin is only 2.75 Torr (41), considerably smaller than peripheral venous oxygen tension at rest, it seems likely that myoglobin is almost completely saturated with oxygen at rest. Using proton nuclear resonance spectroscopy, Richardson et al. (39) found that 9% of myoglobin was deoxygenated in human muscle at rest in normoxia. This amount increased slightly to 13% when arterial saturation decreased to 76% under hypoxic conditions, suggesting that oxygen delivery to the resting muscle is a minor determinant of myoglobin saturation when the metabolic rate is low. If so, the time myoglobin can supply oxygen to the mitochondria is at least 0.9 $\times$ 7.5 = 6.8 s in controls (Table 2, assuming 10% deoxymyoglobin at rest). When the rate of oxygen consumption is lower than the estimated muscle fiber $V_{O_2}$max, this time will increase correspondingly, e.g., at 50% of muscle fiber $V_{O_2}$max the buffering capacity without supply from the blood would be 13.5 s in controls and $\sim$20.5 s in CHF patients. The time constant of oxygen uptake measured by respirometry after an instantaneous increase from rest to 50% of maximum workload in CHF patients (65 W, similar to 50% peak power given in Table 1) without manipulation of blood flow is 42 s (18). Hepple et al. (18) showed that the oxygen deficit at the onset this type of submaximal exercise in CHF patients is $\sim$520 mL, corresponding to 23.2 mmol O2. Assuming muscle mass is 40% of body mass, muscle density is 1.05 kg/l, body mass is 80 kg (Table 1), and the myoglobin concentration is 0.6 mM (Table 2), complete desaturation of myoglobin would release 16.5 mmol O2, which is a little below the oxygen deficit. These calculations indicate that the buffering capacity of myoglobin is sufficient to compensate for delayed oxygen uptake kinetics in CHF patients and may reduce net phospho-creatin splitting.

The myoglobin concentrations we observed are higher than most values in human biopsies reported before, ranging from 0.25 to 0.45 mM (8, 45). As discussed previously (45), this is partly due to the fact that determinations in homogenates of biopsies do not usually correct for interstitial space. In addition, myoglobin concentration may increase with age (31), and our subjects were relatively old. Technical difficulties in the myoglobin determination can also be involved, e.g., extracting myoglobin from muscle fibers may be incomplete or alter the molecule, possibly affecting affinity for antibodies used in the assays (unpublished result). The present histochemical method is based on peroxidase activity of myoglobin fixed in situ, using horse myoglobin in gelatin sections as standards fixed and incubated exactly the same way as the test sections. It is not expected that a difference between horse and human myoglobin is the reason for the relatively high myoglobin concentrations found in the present study because the staining reaction continues until all iron is converted via autoreduction of the ferryl intermediate (1) until the peroxidase activity is lost (24).

In controls, muscle fiber $P_{O_{2 crit}}$ without myoglobin would be 20 to 22 Torr, which is close to $P_{50}$ of human blood (27 Torr), leaving a 5–7 Torr pressure difference across the capillary endothelium to drive oxygen from the capillary into the interstitial space at peak $V_{O_2}$. This pressure difference may increase substantially during exercise because $P_{50}$ of human blood can increase to $\sim$38 mmHg during exercise, similar to the mean capillary $P_{O_{2}}$ (7, 39). $P_{O_{2 crit}}$ without myoglobin is higher than end venous $P_{O_{2}}$ during maximum exercise, 14–18.5 Torr (7, 23), indicating that muscle fibers must be hypoxic during maximum exercise when myoglobin does not lower $P_{O_{2 crit}}$. These results also indicate that calculation of tissue $P_{O_{2}}$ using near infrared spectroscopy should take the higher affinity for oxygen of myoglobin compared with hemoglobin into account (for discussion, see Refs. 13, 44).

The calculated reduction of $P_{O_{2 crit}}$ by myoglobin is substantial: 10.9 Torr in type I fibers of controls and 5.7 Torr in type II fibers of controls (Table 2). Thus, in normal humans, facilitated diffusion by myoglobin will reduce hypoxic cores at peak power output. This reduction becomes even larger when the most recent value of the diffusion coefficient of myoglobin, determined using pulsed field magnetic resonance, is the real value (25). This diffusion coefficient is about four times larger than we used in the present calculations, which would imply that nearly all oxygen is transported by myoglobin at $V_{O_2}$max (see, for example, Eq. 4). The role played by myoglobin in
CHF patients is more important than in controls, since oxygen extraction is larger, resulting in a venous oxygen tension of ~10 Torr (23), less than one-half of PO$_{2crit}$ without myoglobin in type I fibers of CHF patients. Myoglobin in these patients reduces muscle fiber PO$_{2crit}$ to 10.3 and 6.1 Torr in type I and II fibers, respectively. This indicates that myoglobin can play an important role in oxygen transport from capillaries to the sarcolemma. Since venous PO$_2$ in CHF patients is similar to PO$_{2crit}$ of type I fibers, it can be hypothesized that an increase of the myoglobin concentration can improve oxygen extraction even more, which may improve exercise tolerance. PO$_{2crit}$ is the lowest in type II fibers in CHF patients, indicating muscle fiber type-specific metabolic changes in CHF. It has been shown recently that NO can protect high oxidative fibers against cacthectic stimuli and that type II muscle fibers are more sensitive to cacthectic stimuli (52).

The calculations suggest that the role played by myoglobin in facilitating oxygen transport in skeletal muscle is more important than previous estimates (e.g., 20, 25). This is partly due to the fact that we found a higher myoglobin concentration than reported thus far. It is also due to the fact that it is assumed that the mitochondria operate at the maximum rate. When the metabolic rate is lower, the contribution to oxygen transport of myoglobin facilitated diffusion is reduced because myoglobin in the core of the fiber may remain nearly completely oxygenated, resulting in a small radial concentration gradient. It should be realized that the relative importance of myoglobin facilitated diffusion depends to a large extent on the values of the intracellular diffusion coefficients for myoglobin and oxygen, and the P$_{50}$ of myoglobin in situ, which have not been measured in human muscle. At exercise levels well below the anaerobic threshold, the oxygen buffering capacity of myoglobin may be of greater functional importance than the reduction of PO$_{2crit}$.

We conclude that exercise intolerance of CHF patients with normal hematocrit is not due to myoglobin deficiency in locomotor muscle fibers.

GRANTS

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REFERENCES


