Alterations of Skeletal Muscle in Chronic Heart Failure

Helmut Drexler, MD; Urs Riede, MD; Thomas Münzel, MD; Helga König, BS; Elisabeth Funke, BS; and Hanjörg Just, MD

Background. The present study was designed to define the prevalence and characteristics of skeletal muscle alterations in patients with chronic heart failure (CHF) and their relation to exercise capacity. Methods and Results. The ultrastructure of skeletal muscle was analyzed by ultrastructural morphometry in 57 patients with CHF and 18 healthy controls. The volume density of mitochondria (Vvm) and the surface density (Svmc) of mitochondrial cristae were evaluated as a structural correlate of oxidative capacity of skeletal muscle. Vvm and Svmc were reduced by approximately 20% in patients with severe CHF irrespective of age and etiology. The cytochrome oxidase activity in mitochondria as determined by cytochemistry and subsequent morphometry in a subset of patients (n=10) was significantly decreased in heart failure (p<0.01). The capillary length density of skeletal muscle was reduced in CHF (n=12, p<0.05), and the fiber type distribution was shifted to type II fibers (n=15, p<0.05). Vvm and Svmc were significantly related to peak exercise VO2 (r=0.56, p<0.001, n=60) and to VO2 at anaerobic threshold (r=0.53, p<0.001, n=60). In 16 patients with severe heart failure, Vvm was inversely related to the duration of heart failure (r=0.545, p<0.03). In 11 patients who underwent repeat biopsies after 4 months, a correlation was observed between the change in Vvm and the change in peak exercise VO2 (r=0.89, p<0.001). Conclusions. These findings indicate that patients with CHF develop significant ultrastructural abnormalities of skeletal muscle reflecting a depressed oxidative capacity of working muscle. It appears that these alterations of skeletal muscle contribute to the decreased exercise capacity of these patients but are, in principle, reversible by an effective treatment regimen. (Circulation 1992;85:1751-1759)

KEY WORDS • heart failure, chronic • muscle, skeletal • blood flow • mitochondria • ultrastructural morphometric analysis

There is now ample evidence that the clinical stage of chronic congestive heart failure is not closely related to the extent of left ventricular dysfunction. Several studies have demonstrated that peripheral alterations, e.g., reduced peripheral perfusion, contribute substantially to the functional state and exercise capacity of patients with chronic heart failure. Compared with normal patients at identical work load, early anaerobic metabolism in skeletal muscle emerges in patients with chronic heart failure. However, increased cardiac output during exercise exerted by vasodilators cannot be translated immediately into increased exercise capacity and peak oxygen consumption. Even when oxygen delivery to skeletal muscle is improved by pharmacological intervention, the oxygen utilization is not augmented acutely. These observations have prompted the hypothesis that intrinsic abnormalities of skeletal muscle emerge in chronic heart failure. Recent studies using 31P nuclear magnetic resonance (NMR) spectroscopy have demonstrated abnormal skeletal muscle metabolism during exercise even in the absence of reduced flow or under ischemic conditions. This issue has recently been addressed more directly by taking biopsies from skeletal muscle in a limited number of heterogeneous patient populations, yielding a variety of different and, in part, conflicting abnormalities. To define the prevalence and characteristics of skeletal muscle alterations and their relation to exercise capacity, we performed exercise testing and an extensive ultrastructural analysis of a large patient population with chronic heart failure of various functional impairment.

Methods

Patient Population

Fifty-seven patients with chronic heart failure (New York Heart Association functional class II–IV) and 18 sedentary normal individuals were studied. The normal subjects had a normal physical examination, ECG, chest x-ray, M-mode and two-dimensional echocardiographic evaluation, and a normal peak oxygen consumption (peak VO2 <25 ml/min/kg). All patients had clinical, radiological, or echocardiographic signs of cardiomegaly and congestive heart failure of various duration (5–72 months). Patients with chronic lung disease, primary valve disease, diabetes mellitus, history or signs of renal failure, hereditary or acquired neuromuscular disor-
ders, or recent myocardial infarction (less than 8 weeks) were excluded from this investigation. In addition, we did not study patients with excessive alcohol intake because chronic alcoholism is associated with myopathy.\(^1^9\) The etiology of heart failure was coronary artery disease in 18 patients, based on documented myocardial infarction, cardiac catheterization, or both. In 39 patients, idiopathic dilated cardiomyopathy was present based on cardiac catheterization or echocardiographic evidence of an enlarged left ventricle with global hypokinesis, normal coronary angiogram or thallium stress test, an ECG without signs of remote infarction, and the absence of angina. Thirty-two patients were receiving digitalis, 27 were receiving diuretics, and 17 were receiving oral nitrates; 10 patients were treated with calcium antagonists and 15 with an ACE inhibitor. All cardiovascular medications were withheld for at least 24 hours before exercise testing. All subjects underwent exercise testing including respiratory gas analysis, M-mode, and two-dimensional echocardiography (Toshiba SSH-65 or SSH-160A) within 2 weeks of percutaneous skeletal muscle biopsy.

The protocol was approved by the ethical committee of the University of Freiburg, and written informed consent was obtained from all subjects.

**Exercise Testing**

All individuals were first subjected to a baseline exercise test to exclude ischemia (in particular, angina), significant arrhythmias, or respiratory problems as limiting factors for exercise and to make the subjects familiar with exercise testing. Within 2 weeks, the subjects exercised on an upright bicycle ergometer (Jaeger, Würzburg, FRG) starting at a work load of 25 W with increases of 25 W every 3 minutes. Maximal oxygen consumption and anaerobic threshold were measured by a spirometric system with on-line processing and respiratory gas analysis (EOS Spring, Jaeger). Anaerobic threshold was determined by criteria described recently and was expressed as the corresponding \( V_O_2 \) (milliliters per minute per kilogram).\(^2^0,2^1\) The VE/\( V_O_2 \) coplootted with VE/VO\(_2\) was the best criterion and applicable in most cases (in 50 of 57 patients with heart failure and in all normal subjects). Determination by serial arterial lactate levels was closely related to this method\(^2^2\) and was used when the respiratory assessment of anaerobic threshold was indeterminant.

**Skeletal Muscle Biopsy**

Percutaneous needle biopsies were obtained from the middle part of the vastus lateralis muscle within 2 weeks of exercise testing but were separated by at least 24 hours from any exercise (usually 48–72 hours after exercise testing) under local anesthesia using the technique of Bergström,\(^2^3\) which was previously used in this setting.\(^1^5,1^6\) All subjects were instructed not to do any physical exercise for 24 hours before biopsy. With each biopsy, we obtained 30–50 mg of muscle tissue. Eleven patients had a second skeletal muscle biopsy (1 cm distal to the first biopsy) and exercise testing after 4 months of therapy. For consecutive duplicate biopsies, the variation regarding fiber type distribution has been shown to be on the order of 5–6%.\(^2^4\)

**Ultrastructural Morphometry**

Tissue was immediately dropped into fixation medium (2.5% glutaraldehyde in phosphate buffer), in which all edges were removed, and the remaining tissue was cut into blocks with a razor blade, each piece measuring \( 1 \times 1 \times 1 \) mm. This was followed by fixation with OsO\(_2\) (2% in phosphate buffer) and embedding in Epon–araldite. Previous studies have shown that tissue sampling by needle biopsy yields similar results compared with conventional surgical techniques.\(^2^5\) It should be noted that when tissue is fixed immediately on excision, tissue fine structure remains intact.\(^2^6\) The specimens were examined with an EM 200 Philips electron microscope. From each biopsy, we randomly cut four blocks and randomly took 15 micrographs from each block. Oblique sections were used regarding the longitudinally directed structures of the muscle fibers according to Mattfeld et al.\(^2^7\) The samples were photographed at a primary magnification of \( 15,500 \) and analyzed at a final magnification of \( 60,000 \) with the aid of a 1,089-point and 121-testline multipurpose test grid superimposed over each micrograph. The volume density of mitochondria (Vvm) and the surface density (Svmc) of cristae mitochondria (in the center of muscle fibers) were analyzed; that is, Vvm=counted points/total points of grid; Svmc=intersections per test line/total length of test line of grid, as reported previously.\(^2^8\) Using standard stereological principles,\(^2^9\) we obtained the Vvm (i.e., the mitochondrial volume fraction per unit volume muscle tissue, expressed as volume percent) and the Svmc (i.e., the surface fraction of mitochondrial cristae per unit volume of muscle, expressed as square meters per cubic centimeter). The morphometric analysis also included determination of volume density of glycogen and lipid, myofibrils, and sarcoplasmatic reticulum. In addition, the triads (triadic junctions of two L tubule cisternes and one terminal T tubule cisterne) were analyzed as an ultrastructural correlate of intracellular and extracellular calcium transport. All specimens for ultrastructural morphometry were coded and analyzed by investigators without knowledge of the clinical data. The photographs of the first 14 biopsies were analyzed independently by two investigators unaware of the results of each other, yielding excellent interobserver variability (\( r=0.96, p<0.0001 \) for the determination of Vvm). The intraobserver variability was \( r=0.97, p<0.001, n=8 \).

**Histochemistry of Cytochrome c Oxidase in Mitochondria**

In a subset of patients, the dianminobenzidine cytochrome oxidase reaction was performed in mitochondria by using a modification of the technique reported previously by Perotti et al\(^2^9\) to provide a reliable criterion of cytochrome oxidase activity. In those patients, skeletal muscle tissue was immediately fixed in a solution containing 1% paraformaldehyde–2% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.2, at 4°C for 30 minutes. The tissue was washed for 8 hours in cold cacodylate buffer (pH 7.20), with several changes to ensure that the fixative had been removed. The tissue slices were then cut into 50-\( \mu \)m-thick sections with a vibratome tissue sectioner, and the sections were incubated in dianminobenzidine (DAB) medium containing...
30 mg 3,3′-diaminobenzidine–tetra HCl in 10 ml Tris–HCl buffer at pH 7.2. The sections were incubated at 37°C in a water bath for 180 minutes. After incubation, the sections were rinsed in Tris–HCl buffer, postfixed for 2 hours in 2% osmium tetroxide in distilled water, dehydrated in a graded alcohol series, and embedded in Epon. From each patient, 60 micrographs were analyzed to determine the Vvm as described above. The mitochondria were classified as cytochrome oxidase–positive when DAB staining was visible within the mitochondria. DAB-negative mitochondria (invisible by inspection of micrographs with a final magnification of ×60,000) may reflect a low cytochrome oxidase activity in facultatively anaerobic type II fibers, which possess fewer mitochondria, although DAB staining does not identify the fiber types as classified by actomyosin ATPase (light microscopy). A qualitative assessment of the mitochondrial cytochrome oxidase activity in skeletal muscle was obtained with this approach.

**Determination of Fiber Types**

One part of the skeletal muscle tissue was immediately frozen in liquid nitrogen for analysis of fiber types (type I, slow twitch; type II, fast twitch), which were determined by the myofibrillar ATPase staining technique resulting from preincubation at pH 9.4.

**Analysis of Capillary Length Density**

In 21 patients, sufficient additional tissue was obtained for light microscopy. After embedding in paraffin, the capillaries were selectively marked with α-L-fucose binding UEA-I-lectin (APAP method). These histological preparations were then subjected to morphometric analysis as described by Mattfeld and Mall, providing an assessment of capillary length density per unit volume muscle fiber. This measure (capillary length density) reflects the amount of blood capillaries per unit volume of muscle tissue (millimeters per cubic millimeter).

**Statistical Analysis**

Data are expressed as mean±SD. Single comparisons (normal versus heart failure) were performed by unpaired Student’s t test. Multiple comparisons were made by ANOVA with a commercially available statistical package (RS 1/Bolt Beranek and Newman, Inc., Cambridge, Mass.). Individual comparisons after ANOVA were performed by Student-Newman-Keuls test. The relations between variables were evaluated by linear regression analysis. A probability value of less than 0.05 was considered significant.

**Results**

Clinical characteristics of the patient population are depicted in Table 1, demonstrating reduced exercise capacity and left ventricular (LV) dysfunction in both groups of heart failure.

**Determination of Skeletal Muscle Fiber Types**

In eight normal, male individuals (age, 51.1±6 years) and 18 male patients with chronic heart failure (age, 56.2±7 years), the fiber types were analyzed (peak VO2, 16.2±4.0 ml/min/kg; 10 patients with coronary artery disease and eight with idiopathic cardiomyopathy; LV diastolic diameter, 67±4 mm). The percentage of fibers that were classified as slow-twitch type I averaged 54.5±14% (type II, 45.5%) in normal patients and 40.5±12% (type II, 59.5%) in patients with chronic heart failure (p<0.05 versus normal individuals). When only the patients with coronary artery disease were compared with normal subjects, the differences in the fiber type distribution were still present (p<0.05).

**Skeletal Muscle Mitochondria**

The Vvm and Svmc of skeletal muscle were significantly reduced in patients with chronic heart failure compared with normal individuals (Vvm, 5.99±1.3 versus 4.94±1.2 ml/min/kg, p<0.005; Svmc, 1.01±0.2 versus 0.84±0.25 ml/min/kg, p<0.05). The analysis of subgroups based on peak exercise VO2 revealed that this reduction in Vvm and Svmc occurred primarily in patients with severely impaired functional capacity (12.6±2.5 ml/min/kg) (Figure 1). Vvm was not significantly decreased in patients with moderate heart failure (peak VO2, 16–25 ml/min/kg). In patients with dilated cardiomyopathy and normal peak exercise VO2 (n=6, >25 ml/min/kg), the analysis of skeletal muscle revealed completely normal values for Vvm and Svmc (Figure 1). These patients were clinically identified by routine chest x-ray or after the onset of paroxysmal atrial fibrillation (LV ejection fraction, 45±8%); LV diastolic diameter, 64±5 mm). Similarly, Svmc was reduced only in patients with severe heart failure (peak VO2 <16 ml/min/kg) (Figure 1). The cristae content of the mitochondria, calculated by the cristae surface per unit volume of mitochondria, was not significantly different for normal subjects and patients with heart failure. However, Vvm and Svmc per unit volume of myofibrils were significantly reduced in severe heart failure (Svmc: normal patients, 1.19±0.28, all patients, 1.02±0.33, NS; patients with peak exercise VO2 <16 ml/min/kg, 0.88±0.23, p<0.002 versus normal).

**Cytochemistry of Cytochrome Oxidase in Mitochondria**

Cytochrome oxidase activity has been used extensively to identify changes in aerobic enzyme activity, (30,31) (See Table 2.) In support of the use of Vvm and Svmc as a measure of oxidative capacity, a cytochemical analysis of cytochrome oxidase activity was performed in a subset of patients. The positive cytochrome oxidase reaction was present in 62±15% of mitochondria in normal subjects but only in 17.5±12% of mitochondria in patients with severe heart failure and a reduced total Vvm (see Figure 2). In patients with moderate heart failure, the percentage of cytochrome oxidase–positive mitochondria...
was reduced, although the total Vvm was within the normal range. It should be noted that the absolute values for Vvm are not comparable to those obtained by normal ultrastructural procedures because fixation procedures differ considerably. In seven of the 10 patients, the etiology of heart failure was coronary artery disease (age, 66±8 years, peak VO₂, 14.9±2.8; range, 10–18.3 ml/min/kg). Cytochrome c oxidase–positive Vvm averaged 2.73±1.6 vol% (p<0.01 versus normal subjects); cytochrome c oxidase–negative Vvm was 3.67±1.3 vol%; total Vvm was 6.4±1.7 vol%.

Effect of Etiology and Age on Skeletal Muscle Mitochondria

The patients with severe heart failure (peak exercise VO₂ <16 ml/min/kg) were also stratified according to the etiology of cardiac failure. There was no difference for Vvm (Figure 3) and Svmc (coronary artery disease/idiopathic dilated cardiomyopathy, 0.79±0.22/0.72±0.15) between the patients with heart failure caused by coronary artery disease (coronary artery disease: peak VO₂, 12.3±2.2 ml/min/kg; average age, 61.7±5 years) and patients with dilated cardiomyopathy (peak VO₂, 12.8±2.7 ml/min/kg; age, 55.4±11 years). Because the patients with coronary artery disease were older (see Table 1) than the control subjects (who were younger than 60 years), a subgroup analysis of patients younger than 60 years old with peak exercise VO₂ <16 ml/min/kg (age-matched group) revealed that the abnormalities of mitochondria cannot be attributed to age (48.5±6 years, n=14; Vvm, 4.63±0.62; Svmc, 0.74±0.21; p<0.01 versus normal). In addition, no correlation was found between age and Vvm or Svmc in normal subjects or heart failure patients (normal subjects: Vvm, r=0.12, p=NS; all patients: r=0.24, p=NS).

Relation of Skeletal Muscle Alterations to Exercise Capacity and Duration of Heart Failure

Both Vvm (Figure 4) and Svmc were weakly but significantly related to peak exercise VO₂ in patients with chronic heart failure, although there was considerable variation (Svmc: r=0.46, p<0.001, n=60; for patients with heart failure only: r=0.50, p<0.001, n=47). Similarly, exercise VO₂ at anaerobic threshold was significantly related to Vvm and Svmc in patients with chronic heart failure (VO₂ at anaerobic threshold versus Vvm: r=0.535, p<0.001; VO₂ at anaerobic threshold versus Svmc: r=0.517, p<0.001). In 16 patients with peak VO₂

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Mitochondrial volume density (vol%) (top panel) and surface density of cristae (m²/cm³) (bottom panel) in patients with chronic heart failure (CHF) and normal subjects (n=13). Patients are divided into subgroups according to peak VO₂ >25 ml/min/kg (n=6), 16–25 ml/min/kg (n=19), and <16 ml/min/kg (n=22). Data are mean±SD; probability values by ANOVA. Data of biopsies processed for cytochrome c oxidase staining (CHF, n=10; normal subjects, n=5) are not included in this analysis because the mitochondria volume density is not strictly comparable to those obtained from conventional ultrastructural procedures.

### Table 2. Cytochemistry of Cytochrome Oxidase Activity in Mitochondria of Skeletal Muscle

<table>
<thead>
<tr>
<th>CHF (&lt;16 ml/min/kg)</th>
<th>CHF (16–25 ml/min/kg)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>4</td>
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</tbody>
</table>

| Age (years)        | 59.2±7.3              | 59.0±10 | 53.0±5.5 |
| Peak VO₂ (ml/min/kg) | 13.3±2.2              | 19.8±3  | 33.0±6.3 |
| Vvm cyt-ox positive (vol%) | 1.66±1.01‡ | 3.96±1.25† | 5.97±0.8 |
| Vvm cyt-ox negative (vol%) | 4.00±1.12* | 3.03±1.12 | 1.78±0.87 |
| Vvm total (vol%)    | 5.67±1.32*            | 6.98±1.45 | 7.75±0.48 |
| Svmc (m²/cm³)      | 0.16±0.09*            | 0.37±0.11 | 0.53±0.03 |

CHF, chronic heart failure; <16 and 16–25 ml/min/kg refers to peak VO₂; Vvm, mitochondrial volume density; cyt-ox, cytochrome oxidase reaction; Svmc, surface density of cristae mitochondria.

*p<0.05 vs. control, ‡p<0.01 vs. control, †p<0.01 vs. CHF 16–25 and p<0.001 vs. control by ANOVA. Data are mean±SD.
<16 ml/min/kg, the onset of chronic heart failure could be clearly identified, e.g., by the documentation of the first episode of cardiac decompensation (pulmonary edema) or the emergence of congestive heart failure after the first or second myocardial infarction. There was a significant relation (independent of peak exercise \( V_{O2} \) by multiple linear regression analysis) between the duration of heart failure and \( V_{vm} \) \((r=0.545, p=0.03)\).

**Repeated Skeletal Muscle Biopsy**

Skeletal muscle biopsies were repeated after 4 months of treatment. The \( V_{vm} \) increased substantially in four patients, along with an improved peak exercise \( V_{O2} \) (the percent increase in \( V_{vm} \) ranged from 0.97 to 1.56 vol%; the percent increase in peak \( V_{O2} \) ranged from 3.0 to 5.9 ml/min/kg). In two patients, neither \( V_{vm} \) nor peak exercise \( V_{O2} \) changed, whereas both peak exercise \( V_{O2} \) and \( V_{vm} \) decreased in five patients. The change in peak \( V_{O2} \) was closely correlated with the change in the volume density of skeletal muscle mitochondria (Figure 5). Both the changes in mitochondrial volume and increase in peak \( V_{O2} \) are beyond the usually observed variation of these measurements. The four patients with substantial increase of \( V_{vm} \) and peak \( V_{O2} \) demonstrated substantial clinical improvement by the time of the second biopsy. Conversely, three of five patients whose \( V_{vm} \) and peak \( V_{O2} \) decreased over time deteriorated clinically, and one of these patients underwent cardiac transplantation shortly thereafter.

**Analysis of Capillary Length Density**

The capillary length density was evaluated in nine normal subjects and 12 patients with chronic heart failure caused by idiopathic dilated cardiomyopathy and coronary artery disease with peak \( V_{O2} <16 \) ml/min/kg (idiopathic dilated cardiomyopathy, 12.8±2.7; coronary artery disease, 12.3±2.2 ml/min/kg).

**FIGURE 2.** Electron micrographs of cytochrome c oxidase in a patient with severe heart failure (left panel) and in a normal subject (right panel). Enzyme activity within the mitochondria (black) is reduced in heart failure.

**FIGURE 3.** Volume density of mitochondria \((V_{vm}) of patients with chronic heart failure caused by idiopathic dilated cardiomyopathy and coronary artery disease with peak \( V_{O2} <16 \) ml/min/kg (idiopathic dilated cardiomyopathy, 12.8±2.7; coronary artery disease, 12.3±2.2 ml/min/kg).
failure (nine patients with cardiomyopathy and three with coronary artery disease; LV diastolic diameter, 68.7±6 mm; peak VO₂, 15.1±3.3 ml/min/kg). The capillary length density was significantly reduced in this subset of patients compared with normal patients (654±141/524±156 mm/mm³, p<0.05).

**Morphometric Analysis of Other Cell Organelles**

The quantitative analysis of volume density for glycogen, lipid deposits, myofibrils, and sarcoplasmic reticulum did not reveal significant changes for patients with heart failure compared with normal subjects. (See Table 3.) However, in patients with chronic heart failure caused by dilated cardiomyopathy and severe functional impairment (peak VO₂ <16 ml/min/kg), the volume density of sarcoplasmic triads was increased in a subgroup of patients compared with normal individuals. Eight of 35 patients with dilated cardiomyopathy had volume densities of sarcoplasmic triads 2 SD above normal, yielding a borderline statistical significance for the group of patients with peak VO₂ <16 ml/min/kg (p<0.04 versus normal subjects by nonpaired t test but p>0.05 in ANOVA: normal subjects versus idiopathic dilated cardiomyopathy, 16–25 ml/min/kg and versus idiopathic dilated cardiomyopathy <16 ml/min/kg) (see Table 3).

**Discussion**

The major findings of this study can be divided into two areas. The first is a characterization of the ultrastructural and cytochemical alterations in skeletal muscle in patients with chronic heart failure. The second is an analysis of how these alterations are related to exercise capacity, duration of heart failure, and changes in exercise capacity over time. The present study provides evidence that the volume density, cristae surface density, and cytochrome oxidase activity of skeletal muscle mitochondria are substantially reduced in patients with severe chronic heart failure, indicating a decreased oxidative capacity of working muscle. Our ultrastructural description of skeletal muscle is compatible with data from NMR spectroscopy⁹,¹⁰ or the biochemical analysis of skeletal muscle observed in a small number of severely ill patients.¹⁶ Importantly, the evaluation of a large cohort of patients with a wide range of functional impairment revealed that skeletal muscle alterations emerge late in the course of this disorder, being present only in patients with severe heart failure and related to the duration of heart failure. One unique feature of the present study is the observation that changes in the oxidative capacity of skeletal muscle are closely related to changes in exercise capacity and are potentially reversible by effective therapy.
Numerous experimental and human studies have shown that exercise training induces major adaptations in skeletal muscle. These include increases in mitochondrial content, respiratory capacity of the muscle fibers, and the capillary supply and are accompanied by metabolic consequences such as slow utilization of muscle glycogen, a greater reliance on fat oxidation, and less lactate bound to proteins. 

These adaptations have responded reasonably well with quantitative data on oxidative enzyme activities (e.g., delayed exercise training and whole body endurance training) and mitochondrial membrane 

It is important to recognize that the fiber type distribution of skeletal muscle is determined by both genetic factors and exercise training. Because the type II fast-twitch fibers contain substantially less Vm, both the training state and the genetic makeup (governing the fiber type distribution) contribute to the oxidative capacity of skeletal muscle. This may explain the large interindividual variation observed in normal individuals and patients (Figure 3). The pre–heart failure state of skeletal muscle is unknown in an individual patient, it is difficult to decide whether the actual mitochondrial content is determined by genetic factors and training state or is altered by chronic heart failure, unless the abnormalities are very severe.

The fiber type distribution was shifted to type II fibers in patients with chronic heart failure, consistent with two recent studies that reported a shift to type IIB fibers. Because type IIB fibers possess less oxidative capacity than type IIA or even type I fibers, the reduced overall oxidative capacity of skeletal muscle in our patient population could be attributed to this shift of oxidative capacity in all fiber type distribution. Conversely, a reduction of oxidative capacity in all fiber types may result in a shift in fiber type distribution, e.g., by reducing the concentration of oxidative enzymes within type I and IIA fibers and thereby missing the critical level required to be classified as type I or type IIA fiber. Notably, histochemical staining techniques are qualitative in nature and not appropriate for quantification of enzyme activity; other factors such as impaired blood flow and activated neurohumoral systems may contribute.

## Table 3. Volume Density of Cellular Compartments

<table>
<thead>
<tr>
<th></th>
<th>Glycogen (vol%)</th>
<th>Lipids (vol%)</th>
<th>Myofibrils (vol%)</th>
<th>SR (vol%)</th>
<th>Triads (vol%)</th>
<th>Peak V̇O₂ (ml/min/kg)</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.66±1.0</td>
<td>0.32±0.26</td>
<td>84±3</td>
<td>1.72±0.59</td>
<td>0.68±0.27</td>
<td>32.0±6.1</td>
<td>48.8±7</td>
</tr>
<tr>
<td>CAD &lt;16</td>
<td>8.50±1.4</td>
<td>0.34±0.26</td>
<td>84±3</td>
<td>2.26±0.53</td>
<td>0.74±0.42</td>
<td>12.3±2.2</td>
<td>61.7±8</td>
</tr>
<tr>
<td>IDC &lt;16</td>
<td>6.66±1.4</td>
<td>0.33±0.30</td>
<td>86±3</td>
<td>1.85±0.62</td>
<td>0.94±0.39*</td>
<td>12.8±2.7</td>
<td>55.4±1</td>
</tr>
</tbody>
</table>

SR, sarcoplasmic reticulum; CAD, coronary artery disease; IDC, idiopathic dilated cardiomyopathy.

*p<0.05 vs. normal subjects (unpaired t test but p=0.06 in ANOVA and Student-Newman-Keuls test). Data are mean±SD.
distinction of mitochondria by the cytochrome oxidase reaction does not identify the fiber type distribution as classified by histochemical ATPase staining, this analysis shows that the percentage of mitochondria with high oxidative enzyme activity is reduced in chronic heart failure. Moreover, analysis of the cytochrome c oxidase–positive surface densities revealed that the total cytochrome c oxidase concentration per volume of muscle is reduced, supporting the notion that the oxidative capacity of skeletal muscle is reduced in severe chronic heart failure across all fiber types; however, all fiber types appear to increase their mitochondrial content and cytochrome oxidase activity during exercise training. Therefore, it appears more likely that the reduced oxidative capacity of skeletal muscle in heart failure is attributed to a general adaptation within all fiber types, resulting in an altered histochemical fiber type pattern caused by changes in the oxidative capacity within each fiber type.

The reduction in cytochrome c oxidase–positive mitochondria was more pronounced than that of the mitochondrial volume. No linear relation exists between Vvm and the concentration of oxidative enzymes. This statement is consistent with the findings of Dudley et al., who observed a nonlinear relation between mitochondrial content and oxygen turnover. It appears, therefore, that the semiquantitative determination of the cytochrome c oxidase concentration by cytochemistry is more sensitive in detecting early mitochondrial alterations than morphometric analysis of volume and cristae surface of mitochondria.

The decrease in mitochondrial mass was accompanied by a similar reduction in capillary length density in a subset of our patients with chronic heart failure, suggesting inadequate capillary blood per unit volume of skeletal muscle. Similarly, the capillary length density has been shown to decrease with immobilization, whereas the capillary supply is substantially increased in accordance with the oxidative capacity of skeletal muscle during exercise training. It is conceivable, therefore, that the same mechanisms control mitochondrial content and capillary supply of skeletal muscle.

Recently, the concept has been put forward that a generalized myopathy may occur in some patients with dilated cardiomyopathy, based on qualitative ultrastructural data in a limited number of patients and divergent morphological abnormalities of upper arm skeletal muscle. Although the present investigation focused on the quantitative assessment of mitochondria in weight-bearing muscles (and did not reveal abnormalities noted by these investigators), we cannot exclude the possibility that generalized myogenic process affects both cardiac and skeletal muscle in a subset of patients with dilated cardiomyopathy, a clinical entity with various and very different etiologies. Our finding of an increase in triads (representing the ultrastructural localization of intracellular/extracellular calcium exchange) raises the possibility that an increased calcium turnover and calcium overload may occur in skeletal muscle which, in turn, could contribute to an altered metabolism in skeletal muscle in some patients with dilated cardiomyopathy. Norgaard et al. reported a reduced concentration of the Na/K pump in skeletal muscle of patients with chronic heart failure. They reasoned that this deficiency may lead to a decreased gradient in sodium across the cell membrane, causing intracellular accumulation of calcium and hydrogen. Compromised function of mitochondria by altered handling of calcium within the cell may result in an early fall of intracellular pH, thereby contributing to early fatigue.

**Summary**

The present study demonstrates that patients with severe chronic heart failure develop a reduction in oxidative capacity of skeletal muscle, which, in turn, may play an important role in the clinical syndrome of heart failure by adversely affecting exercise capacity in this condition. Thus, the functional capacity of patients with heart failure is limited not only by the capacity of the oxygen transport system but also by the oxidative capacity of mitochondria in working muscle. The alterations of skeletal muscle are similar to those observed with prolonged deconditioning or immobilization and are related to the duration of heart failure. Thus, our data would support the notion that chronic deconditioning is involved in the development of these potentially reversible skeletal muscle alterations.

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**References**


Drexler et al  Skeletal Muscle in Heart Failure 1759


Alterations of skeletal muscle in chronic heart failure.
H Drexler, U Riede, T Münzel, H König, E Funke and H Just

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