Depression of force production and ATPase activity in different types of human skeletal muscle fibers from patients with chronic heart failure

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isoform distribution has been observed from slow (type I) to fast (type II) fibers, which leads to an increase in shortening velocity and potentially in maximum power output, but to a decrease in endurance. Moreover, from observations in CHF patients (45), it has been suggested that such changes in skeletal muscle composition may play a determining role in depressed exercise tolerance.

The intrinsic alterations in contractile function at the cellular level in patients with CHF have not been studied in detail. In slow fibers, reduced activity or aging results not only in a MHC isoform shift but also in other (posttranslational) modifications of contractile proteins. Both in animal models (e.g., Refs. 6, 9, 19, 28) and in humans (e.g., Refs. 14, 24), it has been observed that senescence is associated with marked, up to 30%, reductions in specific force [force per cross-sectional area (CSA) of the muscle or the muscle fiber]. In patients with heart failure also, a reduction in specific force has been observed (18), but the origin of this decline is currently unknown. Therefore, we decided to make a comparison between the contractile and energetic properties between skeletal muscle fibers of the same type from patients with CHF and controls. We hypothesized that, if the rate of ATP consumption would remain constant, the reduction in force would be associated with a loss in the economy of contraction, most likely due to a slowing in the rate of cross-bridge formation (5).

CHRONIC HEART FAILURE (CHF) is associated with limited exercise capacity, as is reflected by a decrease in the maximum rate of oxygen consumption ($\dot{V}_{\text{O}}_{2\text{ max}}$) (41, 46). Previous studies have shown abnormalities of skeletal muscle, including atrophy, impaired function, changes in fiber-type composition, and altered metabolism (10, 31, 33, 36, 40).

These abnormalities could contribute to the limited exercise capacity and rapid onset of muscle fatigue observed in CHF patients. A prominent limiting factor in muscle performance is represented by local oxygen supply (2, 10, 21, 22, 41). Local muscle hypoxia is known to initiate a number of different adaptive and maladaptive processes and to cause formation of reactive oxygen species (11), which could lead to alterations in muscle function at the cellular level. Moreover, chronic cardiopulmonary diseases are associated with elevated circulating cytokines, such as TNF-α (26), a potential mediator of contractile dysfunction. This role of TNF-α has gained extra support from recent observations indicating that TNF-α-induced muscle weakness can be partially prevented by inhibitors of muscle-derived antioxidants or nitric oxide (NO) production (1) and that addition of TNF-α induces contractile dysfunction (34).

In animal models of CHF (36), a shift in the myosin heavy chain (MHC) isoform distribution has been observed from slow (type I) to fast (type II) fibers, which leads to an increase in shortening velocity and potentially in maximum power output, but to a decrease in endurance. Moreover, from observations in CHF patients (45), it has been suggested that such changes in skeletal muscle composition may play a determining role in depressed exercise tolerance.

The intrinsic alterations in contractile function at the cellular level in patients with CHF have not been studied in detail. In slow fibers, reduced activity or aging results not only in a MHC isoform shift but also in other (posttranslational) modifications of contractile proteins. Both in animal models (e.g., Refs. 6, 9, 19, 28) and in humans (e.g., Refs. 14, 24), it has been observed that senescence is associated with marked, up to 30%, reductions in specific force [force per cross-sectional area (CSA) of the muscle or the muscle fiber]. In patients with heart failure also, a reduction in specific force has been observed (18), but the origin of this decline is currently unknown. Therefore, we decided to make a comparison between the contractile and energetic properties between skeletal muscle fibers of the same type from patients with CHF and controls. We hypothesized that, if the rate of ATP consumption would remain constant, the reduction in force would be associated with a loss in the economy of contraction, most likely due to a slowing in the rate of cross-bridge formation (5).

Isometric force development and the rate of ATP consumption were measured in single muscle fibers isolated from vastus lateralis muscle made permeable by means of Triton X-100. In this way, the maximum force per CSA, the actomyosin (AM) ATPase activity, and their ratio tension cost, a measure of muscle economy, could be obtained. After these measure-
ments, the MHC isoform composition of the fibers was determined by means of polyacrylamide gel electrophoresis to allow a comparison between fibers of the same type.

MATERIALS AND METHODS

The methodology used in this study has been described in detail previously (4, 39, 42). Below, a summary is given of the methods used, and details are provided of the experimental protocols followed.

Subjects. The study was performed on nine patients with a history of stable CHF of >6 mo (New York Heart Association Class II–III) and on five healthy, age-matched volunteers who had no previous history of musculoskeletal or neuromuscular disease. Exclusion criteria were diabetes mellitus, clinically significant chronic obstructive pulmonary disease, or peripheral vascular disease. The experiments conformed with the Declaration of Helsinki. The study was approved by the institutional ethical committee, and the subjects gave informed consent. The average left ventricular ejection fraction (%EF, ejection fraction; ND, not determined; V\(_{\text{O}}\text{2max}, \) maximum rate of oxygen consumption; CSA, cross-sectional area of single fibers) given in Table 1.

Determination of the V\(_{\text{O}}\text{2max}. Each subject performed two exercise tests on different days. On the first day, the maximum workload was determined on a bicycle ergometer (Vmax series, Sensormedics; Yorba Linda, CA) without measuring ventilatory parameters. The rate of oxygen consumption at rest was measured, and all subjects underwent an incremental exercise test to a symptom–limited maximum (dyspnea and/or muscle fatigue; respiratory quotients >1) with electrocardiography of ~8 min (12). The V\(_{\text{O}}\text{2max} \) obtained was normalized to body weight.

Muscle biopsies and permeabilization of fibers. Muscle biopsies were taken under local anesthesia with a 2% lidocaine solution, from the distal portion of vastus lateralis muscle using the needle biopsy technique (3). The needle (outer diameter 4.5 mm) was inserted to a depth of ~4 cm. The biopsies (wet weight ~50 mg) were pinned to a small piece of Sylgard (Dow Corning, Wiesbaden, Germany) and frozen in liquid nitrogen. The biopsy was freeze-dried (4K Modulyo, Edwards High Vacuum, Crawley, UK) in a container kept on dry ice during the initial phase of the drying process and subsequently stored at −80°C, following a method described previously (37).

On the day of an experiment, a freeze-dried bundle of 10–20 fibers was dissected from the biopsy, and the remainder was stored at −80°C for later use. A number of segments of single fibers of ~5 mm in length were carefully isolated from this bundle and slowly rehydrated by leaving the segments floating in a chamber containing storage solution at a temperature of 5–6°C. In this way, trapping of air inside the fibers was avoided. Thereafter, the fibers were permeabilized by incubating them in storage solution containing 1% (vol/vol) Triton X-100 for 30 min. At the end of the incubation period, the solution was replaced by a relaxing solution without Triton X-100. Permeabilized fibers were mounted in the setup for measuring force and ATPase activity by means of aluminum T-clips. Sarcomere length was adjusted to 2.5 μm.

Determination of CSA. CSA of the permeabilized fibers was calculated from the diameters measured inside the experimental chamber in two perpendicular directions and assuming an elliptical cross section. CSA of the fibers was also measured in histological sections from each biopsy. In this case, fibers were identified on the basis of quantitative myofibrillar ATPase histochemistry (44) as type I (low ATPase activity) or type II (high ATPase activity) using the public domain NIH Image 1.61 program. For this purpose, part of the frozen biopsy was transferred to a cryostat, and 10-μm-thick sections were cut at −20°C, collected on slides coated with Vectabond (Vector Laboratories, Burlingame, CA), and stained as described previously (44). CSA was determined as the mean value of 10–20 fibers from each type. The relative distribution of type I and type II fibers was calculated from a total of ~100 fibers in each biopsy.

Experimental setup. The apparatus used to measure the ATPase activity (16, 39) consisted of several temperature-controlled troughs in which the fiber could be immersed. Temperature was maintained at 20 ± 0.5°C. The fiber segments were mounted, by means of aluminum T-clips, between a force transducer (AE801, SENSORON, Horten, Norway) and a fixed hook. Sarcomere length was measured in relaxing solution by means of He-Ne laser diffraction and was adjusted to 2.5 μm. During the actual measurement of ATPase activity, the preparation was kept in a small trough with glass windows, which had a volume of 30 μl. Hydrolysis of ATP inside the fiber was linked to the oxidation of NADH. The decline in NADH concentration was measured photometrically via the absorption at 340 nm of near UV light from a 75-W xenon light source that passed beneath the fiber. The rate of ATP hydrolysis was derived from the

Table 1. Subject data

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr (M/F)</th>
<th>Diagnosis</th>
<th>NYHA Class</th>
<th>EF, %</th>
<th>V(_{\text{O}}\text{2max}, ) m(^3)/min·kg(^{-1})</th>
<th>CSA type I, μm(^2)</th>
<th>CSA type II, μm(^2)</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con 1</td>
<td>47 M</td>
<td>ND</td>
<td>II</td>
<td>35</td>
<td>21.9</td>
<td>6,700</td>
<td>7,200</td>
<td>ACE, ASA, β</td>
</tr>
<tr>
<td>Con 2</td>
<td>38 M</td>
<td>ND</td>
<td>II</td>
<td>36.1</td>
<td>8,200</td>
<td>7,100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con 3</td>
<td>48 M</td>
<td>ND</td>
<td>II</td>
<td>33.3</td>
<td>4,700</td>
<td>6,700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con 4</td>
<td>45 M</td>
<td>ND</td>
<td>II</td>
<td>30.1</td>
<td>5,400</td>
<td>4,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con 5</td>
<td>64 F</td>
<td>ND</td>
<td>II</td>
<td>22.7</td>
<td>4,200</td>
<td>7,400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>48 ± 4</td>
<td></td>
<td></td>
<td>33.6 ± 3.8</td>
<td>5,300 ± 800</td>
<td>5,900 ± 700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF 1</td>
<td>46 M</td>
<td>IHD II</td>
<td>35</td>
<td>21.9</td>
<td>6,700</td>
<td>7,200</td>
<td></td>
<td>ACE, ASA, β</td>
</tr>
<tr>
<td>HF 2</td>
<td>56 M</td>
<td>IHD II</td>
<td>26</td>
<td>18.1</td>
<td>3,600</td>
<td>3,400</td>
<td></td>
<td>ACE, ASA, β, diuretics, statine</td>
</tr>
<tr>
<td>HF 3</td>
<td>67 M</td>
<td>IHD II</td>
<td>43</td>
<td>22.8</td>
<td>4,900</td>
<td>4,600</td>
<td></td>
<td>ACE, ASA</td>
</tr>
<tr>
<td>HF 4</td>
<td>56 M</td>
<td>ICM III</td>
<td>38</td>
<td>17.9</td>
<td>4,700</td>
<td>3,600</td>
<td></td>
<td>ACE, Ac, diuretics</td>
</tr>
<tr>
<td>HF 5</td>
<td>66 M</td>
<td>ICM II</td>
<td>13</td>
<td>16.3</td>
<td>5,700</td>
<td>5,400</td>
<td></td>
<td>ACE, Ac, digoxine, diuretics</td>
</tr>
<tr>
<td>HF 6</td>
<td>69 M</td>
<td>IHD III</td>
<td>33</td>
<td>9.8</td>
<td>6,500</td>
<td>9,100</td>
<td></td>
<td>ACE, Ac, digoxine, diuretics</td>
</tr>
<tr>
<td>HF 7</td>
<td>53 F</td>
<td>MCD II</td>
<td>32</td>
<td>21.2</td>
<td>4,900</td>
<td>4,900</td>
<td></td>
<td>Asa, ATII, diuretics</td>
</tr>
<tr>
<td>HF 8</td>
<td>50 M</td>
<td>IHD II</td>
<td>29</td>
<td>14.6</td>
<td>3,300</td>
<td>2,500</td>
<td></td>
<td>ATII, Ac, β, statine</td>
</tr>
<tr>
<td>HF 9</td>
<td>79 M</td>
<td>IHD III</td>
<td>26</td>
<td>12.8</td>
<td>8,000</td>
<td>7,400</td>
<td></td>
<td>ACE, Ac, diuretics, statine</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>60 ± 4</td>
<td></td>
<td></td>
<td>31 ± 3</td>
<td>17.3 ± 1.4</td>
<td>5,400 ± 500</td>
<td>5,000 ± 700</td>
<td></td>
</tr>
</tbody>
</table>

Con, control; HF, chronic heart failure; M, male; F, female; IHD, ischemic heart disease; ICM, idiopathic cardiomyopathy; MCD, myocarditis; NYHA, New York Heart Association; EF, ejection fraction; ND, not determined; V\(_{\text{O}}\text{2max}, \) maximum rate of oxygen consumption; CSA, cross-sectional area of single fibers measured in sections, rounded to multiples of 100 μm\(^2\); Medication: ASA, acetyl salicylic acid; ACE, angiotensin I-converting enzyme inhibitor; Ac, anticoagulantum; ATII, ATII antagonist; β, β-blocker. *Significantly different from the control group (P < 0.05).
slope of the absorbance signal relative to the baseline found before and after immersion of the fiber in the measuring chamber (see Figs. 2 and 3). Calibration of the absorbance signal was carried out after each recording by injecting a known amount of ADP into the measuring chamber via a stepper motor-controlled pipette.

Solutions. The storage solution contained (in mmol/l) the following: 3 Na₂ATP, 5 magnesium acetate, 5 EGTA, 5 potassium dihydrogen phosphate, 150 potassium propionate, and 1 DTT. Three different bathing solutions were used during the experiments: a relaxing solution, a preactivating solution with low-EGTA concentration, and an activating solution. The composition of these solutions has been described previously (42).

Experimental protocol. During measurements, the fiber segment was incubated in relaxing solution for 3 min, in preactivating solution described previously (42), and after immersion of the fiber in the measuring chamber via a stepper motor-controlled pipette.

Myosin isoform identification. Myosin composition was determined by means of polyacrylamide gel electrophoresis after denaturation in sodium dodecyl sulphate (SDS-PAGE), using a method similar to that described previously (8). After the force measurements, the fiber segment (typically 2 mm long) was placed in a small test tube filled with 10 μl of solution with the following composition: 62.5 mM Tris·HCl, 2.3% (wt/vol) SDS, 10% glycerol, and 5% (vol/vol) mercaptoethanol (23). After solubilization for at least 2 h at room temperature, it was stored at −70°C. A small fraction of the solution, typically 2 μl, was analyzed with SDS-PAGE. Gel dimensions were as follows: width, 18 cm; height, 16 cm (4 cm stacking gel, 12 cm separating gel); and thickness, 0.75 mm. The acrylamide-to-bisacrylamide ratio was 37.5:1. Total acrylamide was 4 and 6% in the stacking and separating gel, respectively. In addition, the gels contained 25% glycerol (vol/vol), 0.2% N,N,N',N'-tetramethylethylenediamine, 0.05% ammoniumpersulphate, and 0.05% thiosulphate. The stacking gel also contained 0.1% (wt/vol) SDS and 0.12 M Tris·HCl (pH 6.8), and the separating gel contained 0.4% SDS and 0.4 M Tris·HCl (pH 8.8). The electrode buffer contained 25 mM Tris, 192 mM glycine, and 0.1% SDS (pH 8.3). Samples were run for 24 h at constant voltage (125 V) at 10°C. The gels were silver stained.

Data and statistical analysis. Force and ATPase activity were recorded with a pen recorder and after analog-to-digital conversion by a personal computer at a sampling rate of 10 Hz. Data are given as means ± SE of n experiments. One-way ANOVA followed by Bonferroni multiple-comparisons test was used to assess statistical significance of the differences between groups. A probability of <0.05 was considered statistically significant.

RESULTS

Force development and ATPase activity during isometric contraction were studied in 97 fiber segments. All fibers were characterized on the basis of MHC composition and divided into four groups: type I (or slow), type IIA, type IIX, and type IIA/X (showing coexistence of fast IIA and IIX). The mean dimensions of the preparations were as follows: segment length 1.9 ± 0.1 mm, width 104 ± 2 μm, and depth 92 ± 2 μm.

Myosin isoform composition. In Fig. 1, an example is shown from the MHC region of a SDS-PAGE gel used to identify fiber type. In general, the relative amounts of IIA and IIX isoforms in type IIA/X fibers were rather similar. Occasionally, hybrid fibers were found, coexpressing one major isoform and small amounts (<5–10%) of one or (incidentally) two of the other isoforms. These fibers were classified according to the predominant MHC isoform. The number of fibers studied for each type in the control and the patient group and the average CSAs are shown in Table 2.

The mean values per subject of the percentage of type I fibers used in the force and ATPase measurements were very similar in controls (31 ± 7%; n = 5) and in CHF patients (31 ± 9%; n = 9). The fiber-type distribution was also determined from a larger number of fibers (~100 per subject) in the histological sections. The mean values of the percentage

Table 2. Overview of the fiber-type dependence of ATPase activity, specific force, and tension cost

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>IIA</td>
</tr>
<tr>
<td>Number of fibers</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>CSA, μm²</td>
<td>8,900±1,200</td>
<td>9,500±1,000</td>
</tr>
<tr>
<td>AM ATPase, μM/s</td>
<td>134±24</td>
<td>248±17</td>
</tr>
<tr>
<td>Basal ATPase, μM/s</td>
<td>23±5</td>
<td>20±3</td>
</tr>
<tr>
<td>Specific force, kN/m²</td>
<td>64±7</td>
<td>86±11</td>
</tr>
<tr>
<td>Tension cost, μM/s·m⁻¹·Km⁻¹·m²</td>
<td>2.2±0.3</td>
<td>3.7±0.6</td>
</tr>
</tbody>
</table>

Average values (±SE) are shown, derived from the individual measurements in each fiber segment. Tension cost was defined as the actomyosin (AM) ATPase activity divided by isometric specific force. *Significant difference between the control and patient group (P < 0.05). Cross-sectional area (CSA) was calculated from the diameter of the fibers measured in 2 perpendicular directions, assuming an elliptical cross section. I, IIA, IIA/X, and IIX indicate fiber type.
of type I fibers obtained were 38 ± 5% in controls (n = 5) and 33 ± 4% in CHF patients (n = 9). These mean values show a slight slow-to-fast transition, which, however, was not significant.

CSA. The mean CSA of the permeabilized fiber segments differed between groups (ANOVA, P < 0.01). Post hoc analysis indicated that CSA of type IIA fibers in patients was significantly reduced by 27 ± 3% compared with controls (P < 0.05). To investigate the differences between controls and patients and to facilitate a comparison with CSAs determined in the histological sections (next paragraph), the mean values for CSA in type I and type II fibers were calculated. CSA in type I was 8,953 ± 1,174 μm² (control; n = 11) and 6,881 ± 261 μm² (patients; n = 16). In type II fibers, CSA amounted to 8,744 ± 716 μm² (control; n = 24) and 7,358 ± 533 μm² (patients; n = 46). The difference in CSA between controls and patients in type I and type II fibers was significant (P < 0.05). The relative reduction in patients amounted to 18 ± 3% in type I and to 11 ± 1% of the value found in controls.

CSAs of type I and type II fibers were also determined in histological sections (Table 1). It was found that the CSAs varied between subjects, but the differences between patients and controls were not significant (ANOVA). In addition, to test whether fibers from patients reacted differently on the permeabilization protocol, a comparison was made between mean CSA of type I and type II fibers in the sections and after mounting of the individual fiber segments in the setup for measuring force and ATPase activity. It is clear from these data (compare Table 1 with the mean values in the previous paragraph) that CSA of the permeabilized fibers was larger than obtained on fibers in sections. The increases after permeabilization are not surprising, because the fibers swell osmotically after permeabilization, whereas mitochondria and other membranous vesicles are solubilized and washed out. Moreover, it is important to note that fiber sarcomere lengths in the histological sections were ~1.6 μm, whereas, in the case of permeabilized fibers, sarcomere length was adjusted to 2.5 μm. One-way ANOVA carried out on the mean values of type I and type II fibers from the individual subjects did not reveal significant differences of fiber swelling due to permeabilization between patients and controls.

Measurements of ATP activity, force, and tension cost. The total ATPase activity and force development for a type I and type IIA fiber from a subject in the control and the patient group are shown in Figs. 2 and 3, respectively. It can be seen that, in both fiber types, force and ATPase activity in patients were smaller than in controls. In approximately one-third of the fibers, a slight decline in force was observed during the activation period, irrespective of the origin of the fiber (control or patient). In the others, force remained constant or increased slightly. These slow changes were most likely due to creep and/or a slight increase in inorganic phosphate concentration inside the fiber. The averages of the ATPase activities per liter cell volume and the average maximal force per CSA (specific force) measured in type I, IIA, IIX, and IIA/X fibers are given in the Table 2. The differences in maximal specific force and in AM ATPase activity in fibers of the same type between the control and patient group were significant (P < 0.05). Basal ATPase activity, however, did not differ between groups (ANOVA). Table 2 also shows the average values of tension cost, defined as the ratio between isometric AM ATPase activity and specific force, for each fiber type, calculated from the individual values obtained in each fiber. The differences in tension cost in fibers of the same type between the control and patient group were not significant.

A diagram of the average values for AM ATPase activity, specific force, and tension cost obtained for the different fiber types in the control and the patient group illustrating these findings is given in Fig. 4. It can be seen that specific force and AM ATPase activity in CHF are reduced in proportion and that tension cost remained the same in each fiber type.
Correlation with exercise capacity. To investigate whether the reductions in force, AM ATPase activity, and tension cost correlated with exercise capacity, average values of these parameters for type I and type II fibers were plotted as a function of the $V_O^{2\text{max}}$ normalized to body weight (Fig. 5). It can be seen from the $R^2$ values of the regression lines that the intersubject differences in $V_O^{2\text{max}}$ explain only a small fraction of the variability in force, AM ATPase activity, and tension cost of type I and type II fibers. However, the slopes of the regression lines were significantly different from zero for specific force in type II fibers and for AM ATPase activity in both type I and type II fibers. As expected from the mean values of tension cost in the different experimental groups, the slopes for tension cost in type I and type II fibers were not significantly different from zero.

DISCUSSION

The main observation in this study was that isometric force and the rate of ATP consumption in fibers from patients with CHF were reduced in proportion by ∼35% compared with fibers of control subjects. The force and AM ATPase values from control subjects in this study were ∼30% smaller than those previously obtained in our laboratory (38). It should be noted, however, that the fibers in this study were obtained from biopsies frozen in liquid nitrogen and stored at −80°C, while in the earlier study fibers were stored in glycerol at −20°C. A similar reduction in force output due to storage conditions has been observed previously (25). It seems unlikely that this reduction would be different in fibers from patients than from the controls. Therefore, the decline in force and ATP consumption in fibers from patients with heart failure is very likely to reflect an intrinsic change in contractile properties. Our force and ATPase values in control subjects were also smaller than observed previously by Han et al. (17), whereas the fiber-type dependence was very similar. The difference in magnitude might, in part, also be due to differences in experimental conditions, such as the composition and ionic strength of the solutions.
There are two major classes of explanations for the proportional decline in specific tension and ATP consumption in fibers from CHF patients. First, it is possible that the density of contractile proteins is reduced in CHF patients, as is, for instance, the case in denervated (13, 15) muscle or muscle atrophy (35). Recently, it was found that MHC protein content decreased with increasing disease severity in heart failure patients, albeit that MHC content did not differ significantly between patients and controls (43). In line with this type of reasoning, it can be noted that we observed a significant reduction in CSA in permeabilized type IIA fibers. However, on the other hand, no differences in CSAs between patients and controls were found in the histological sections. The second possibility could be a slowing of cross-bridge kinetics. If this slowing would occur in the apparent rate of cross-bridge attachment and not be accompanied by a slowing in the apparent rate of cross-bridge detachment, the fraction of cross bridges attached during contraction would be reduced (20). This causes a reduction in force output as well as in the rate of ATP consumption, but not in tension cost, as this latter property is governed by the apparent rate of cross-bridge detachment. Our experiments do not distinguish between these two classes of explanation, but it is of interest to note that, recently, evidence supporting the latter possibility was obtained in rat semimembranosus muscle (30). In this animal model, reduced muscle activity resulted in almost parallel decreases in specific tension and the fraction of myosin heads in the strong-binding structural state, determined by electron paramagnetic resonance spectroscopy.

How could these effects be mediated? The myosin head contains reactive sulfhydryl groups that inhibit the AM interaction upon oxidation (7). TNF-α or oxidative stress (due to hypoxia) promotes the production of reactive oxygen species or NO derivatives that have been shown to cause contractile dysfunction (1, 27). Circulating TNF-α levels are elevated in patients with severe heart failure (26). Hence they might form the trigger for a cascade of events, ultimately causing a loss in contractile proteins and/or a reduction in the fraction of cross bridges in the strong-binding state(s).

The number of subjects studied was limited (n = 9 and 5 in the patient and age-matched control group, respectively). This limitation is inherent in the study population and our aim to account for fiber-type dependence while studying the intrinsic alterations in contractile function. This requires measurements in a large number of single fibers from each subject and a posteriori identification of fiber type. The force and AM ATPase values correlated well with the VO₂ max (Fig. 5). This supports the notion that the reduction in force and ATP consumption observed is a general feature of patients with limited exercise tolerance due to (moderate) CHF.

The 35% reductions in force per CSA observed are rather large compared with the reduction (~16%) observed in quadriceps muscle in patients with CHF in vivo (18). However, it should be noted that a number of compensatory factors, e.g., alterations in muscle composition and geometry, the switch in fiber types, and the difficulty to determine maximum force output in vivo, complicate the comparison of our data with those obtained in vivo.

It is of interest to note the difference in the cellular changes observed in aging and CHF. Force, but not AM ATPase activity, was reduced with age in rat muscle fibers (29), whereas our data reveal proportional reductions in both parameters. This indicates that the mechanoenergetic effects of modifications in aging and CHF may be diverse.

In conclusion, our study indicates that ultrastructural alterations or changes in cross-bridge kinetics are responsible for the proportional reductions in force and ATP consumption in fibers of different types in patients with heart failure. These changes may exacerbate the contractile weakness due to muscle atrophy in patients with CHF.
REFERENCES


