Contribution of Intrinsic Skeletal Muscle Changes to ^{31}P NMR Skeletal Muscle Metabolic Abnormalities in Patients With Chronic Heart Failure

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Patients with heart failure frequently exhibit abnormal skeletal muscle metabolic responses to exercise, as assessed with ^{31}P NMR. To investigate whether these metabolic abnormalities are due to intrinsic skeletal muscle changes, we performed gastrocnemius muscle biopsies on 22 patients with heart failure (peak $\dot{V}O_2$, 15.4±4.7 ml/kg/min; ejection fraction, 20±7%) and on eight normal subjects. Biopsies were analyzed for fiber type and area, capillarity, citrate synthase, phosphofructokinase, lactate dehydrogenase, and β-hydroxyacyl CoA dehydrogenase activity. All patients with heart failure also underwent ^{31}P NMR studies of their calf muscle during plantarflexion at three workloads. Muscle pH responses and the relation of the ratio of inorganic phosphate to phosphocreatine (P_i/PCr) to systemic $\dot{V}O_2$ were then evaluated. Compared with normal subjects, patients with heart failure exhibited a shift in fiber distribution with increased percentage of the fast twitch, glycolytic, easily fatigable type IIb fibers (normal subjects, 22.7±10.1; heart failure, 33.1±11.1%; $p<0.05$), atrophy of type IIa (normal subjects, 5,477±1,109; heart failure, 4,239±1,247 μm^2; $p<0.05$) and type IIb fibers (normal subjects, 5,957±1,388; heart failure, 4,144±945 μm^2; $p<0.01$), and decreased activity of β-hydroxyacyl CoA dehydrogenase (normal subjects, 5.17±1.44; heart failure, 3.67±1.68 mol/kg protein/hr; $p<0.05$). No significant linear correlation could be identified between the slope of the P_i/PCr to $\dot{V}O_2$ relation and muscle histochemistry or enzyme activities. Similarly, no linear relation was found between intracellular pH at peak exercise and any muscle variable. These data suggest that patients with heart failure develop intrinsic skeletal muscle changes but that these intrinsic muscle changes do not contribute significantly to the abnormal skeletal muscle ^{31}P NMR metabolic responses observed in such patients. (*Circulation* 1989;80:1338–1346)

Exertional fatigue in patients with heart failure has traditionally been attributed to skeletal muscle underperfusion. However, recent investigations suggest that intrinsic skeletal muscle abnormalities may be operative, as well. Abnormal forearm ^{31}P NMR responses to forearm exercise in patients with heart failure have been demonstrated without substantial decreases in limb blood flow.[1,2] Massie et al[3] have shown that patients exhibit abnormal forearm muscle responses during ischemic exercise. Lipkin et al[4] performed quadriceps muscle biopsies on nine patients with heart failure. A variety of different abnormalities were noted in individual patients, including atrophy of type I and II fibers, increased interstitial cellularity, increased lipid deposition, and increased acid phosphatase staining. However, no characteristic histochemical pattern or enzyme abnormality emerged. In preliminary reports, Drexler et al[5,6] described reduced mitochondrial cristae volume in patients with heart failure, suggesting a decrease in oxidative enzymes.

In the present study, we sought to further investigate whether intrinsic skeletal muscle changes occur in patients with heart failure. In addition, we

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sought to examine the relation of skeletal muscle biopsy results to ^{31}P NMR responses to exercise.

Methods

Subjects

Twenty-two men with chronic New York Heart Association class I–IV heart failure were studied. Average age was 57±14 years (±SD). All patients were receiving treatment with digoxin and diuretics. In addition, 12 patients were also receiving vasodilators. None had peripheral edema, intermittent claudication, diabetes mellitus, angina, or valvular heart disease. All patients were screened for peripheral vascular disease by observation of physical signs (e.g., hair loss, dependent rubor), palpation of peripheral pulses, and determination of rest ankle and brachial pressures. All participants had preserved peripheral pulses and an ankle to brachial pressure ratio of 1 or more.

Etiology of heart failure was coronary artery disease in eight patients based on a documented myocardial infarction, cardiac catheterization, or both. The remaining 14 patients were presumed to have a cardiomyopathy, seven due to excessive alcohol intake. There was no evidence of myocardial infarction on the electrocardiogram, and no history of myocardial infarction in these 14 patients. In addition, four patients had normal coronary arteries documented by coronary angiography, and three had a normal thallium exercise test. Left ventricular ejection fraction averaged 20±7%. Peak exercise oxygen consumption during treadmill exercise averaged 15.4±4.7 ml/min/kg.

Eight sedentary normal male subjects also underwent a calf skeletal muscle biopsy. The age of the subjects averaged 60±9 years. Peak exercise oxygen consumption averaged 28.3±1.4 ml/kg/min.

The protocol was approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania. Written informed consent was obtained from all subjects.

Protocol

All patients and control subjects underwent skeletal muscle biopsy. ^{31}P NMR measurements were performed within 1 week of biopsy in all patients with heart failure.

Skeletal Muscle Biopsy

Percutaneous muscle biopsies were obtained from the gastrocnemius muscle using the biopsy technique described by Bergstrom.[7] Gross blood and connective tissue were removed from the samples. One biopsy (approximately 40 mg) was immediately frozen in liquid nitrogen and stored at −80° C, pending enzymatic analysis. Another sample (approximately 20 mg) was mounted in embedding medium (OCT) with fibers aligned in longitudinal direction. This specimen was then frozen in isopentane, cooled to its freezing point with liquid nitrogen, and stored at −80° C, pending histochemical analysis. Muscle samples for histochemical analysis were coded and then analyzed so that the technician was unaware of the subject's group.

Enzymatic assays were performed on tissue homogenates using fluorometric methods, as generally described by Lowry et al[8] and specifically outlined by Chi et al.[9] For each analysis, a 10–20-mg sample was weighed and homogenized in 50 vol 50% glycerol containing 20 mM NaH_2PO_4 buffer, pH 7.4, 5 mM β-mercaptoethanol, 0.5 mM ethylenediaminetetra-acetic acid (EDTA), and 0.02% bovine serum albumin. All of the homogenate assays for a given enzyme were conducted on the same day at 25° C. Muscle homogenates were assayed for protein concentration by the method of Lowry et al[8] and for myoglobin concentration.[10] Enzymes that were assayed included the mitochondrial oxidative enzyme citrate synthase (CS); a mitochondrial enzyme reflective of fat oxidation, β-hydroxyacyl CoA dehydrogenase (BOAC); and glycolytic enzymes phosphofructokinase (PFK) and lactate dehydrogenase (LDH). Enzymatic activities are expressed relative to protein concentration.

Fiber types were determined by the differential myofibrillar ATPase staining resulting from preincubation at either pH 10.3, 4.6, or 4.3.[11] The first method distinguishes type I (light staining) fibers from type II (dark staining) fibers; the second distinguishes type I (dark staining) from IIa (light staining) and IIb or IIc (medium staining) fibers; and the third distinguishes IIc (medium staining) from IIa or IIb.

Muscle fiber type was determined by simultaneously magnifying three sections of the same fibers stained for myofibrillar ATPase after preincubation at either pH 10.3, 4.6, or 4.3, and then visually counting and determining the percentage of each type. This method of determining the percentage of a given fiber type within a biopsy sample was reproducible to within less than 2%. Approximately 200–500 fibers were contained in each section. The percentage of type I and II fibers was also determined from sections stained for NADH2 tetrazolium reductase as a cross validation.

Muscle sections were fixed, treated with a 1% amylase solution, and stained with periodic acid-Schiff reagent to visualize capillaries.[12] The stained sections were analyzed by magnifying (×296) and projecting approximately six to eight artifact-free 0.25-mm^2 areas onto a screen. In 10 biopsy samples, the mean coefficient of variation for four determinations for capillaries per millimeter squared was ±7.2%.

The mean area of the various muscle fiber types was measured from sections stained for NADH2 tetrazolium reductase by tracing the perimeter of individual fibers using computerized planimetry, after type II fibers were subclassified (i.e., IIa, IIb, IIc) by cross reference with the ATPase-stained section. Area was measured on most of the fibers

within a section, and only those fibers that did not appear cross sectional or intact were excluded.[13] In 10 biopsy samples, the mean coefficient of variation for four determinations of fiber area was ±3.6%.

Skeletal Muscle Metabolic Measurements (NMR Protocol)

Exercise was performed at least 3 hours after eating. The protocol has been described previously.[14] In brief, after arrival in the NMR facility, the patient was seated and his leg was positioned with the limb under study in an 11.5-in. bore, 1.9-T superconducting magnet (Oxford Research Systems, Oxford, England). Because the homogeneous area of the magnetic field was 37 cm from each end, the leg was inserted up to the groin and the other leg widely abducted. Data acquisition was accomplished with the application of radiofrequency pulses (pulse width optimized at 25–35 μsec) applied every 5 seconds. After optimization of field homogeneity, a 3-minute rest scan was recorded. The subject then withdrew his leg from the magnet, as all exercise was performed outside the magnet. A rapidly inflating pneumatic cuff (D.E. Hokanson, Issaquah, Washington) was applied to the study leg. The patient then exercised next to the magnet, as herein described. At the completion of each workload, the pneumatic cuff was inflated to a suprasystolic pressure (250 mm Hg) to occlude blood flow into and out of the leg and, thereby, maintain pH, inorganic phosphate (P_i), and phosphocreatine (PCr) levels at end-exercise levels. After the cuff was inflated, the patient rapidly (<1 minute) repositioned his leg within the magnet. After acquisition of a 3-minute ^{31}P NMR scan, the cuff was deflated. Recovery scans were recorded every minute for 5 minutes. At the end of the study, calf circumference at the site of scanning was measured.

Exercise consisted of two-footed upright plantarflexion (toe ups). Three different workloads were performed in each subject: plantarflexion every 3, 6, and 9 seconds. Contractions were sustained for approximately 1 second. Each workload was performed for 4 minutes with continuous monitoring of heart rate and measurement of cuff blood pressure at the end of each workload. Simultaneous oxygen consumption measurements were performed to provide quantification of each level of exercise. Measurements of mixed expired oxygen, mixed expired carbon dioxide, and expired volume were determined at rest and every 30 seconds throughout exercise using a metabolic measurement cart (SensorMedics, Anaheim, California). After each exercise workload, ^{31}P NMR data was obtained, as previously described. The subject then rested for 10 minutes before performing the next workload.

Previously, we have demonstrated that metabolism can be temporarily "arrested" by inflating a limb cuff to suprasystolic pressures at end exercise.[14] Forearm exercise was performed in the magnet by seven normal subjects followed by cuff inflation to suprasystolic pressure. P_i/PCr ratio and intracellular pH were monitored for 4 minutes after exercise and remained stable.

Harris et al[15] also investigated the effect of pneumatic cuff inflation on PCr resynthesis in the quadriceps muscle of humans after exhaustive exercise. By performing serial muscle biopsies, these investigators demonstrated that arterial occlusion abolished resynthesis of PCr throughout 6 minutes of occlusion. Release of arterial occlusion was followed by restoration of PCr to normal levels.

Spectral Analysis

Quantification of metabolic components was obtained from the Fourier transformed NMR spectra.[16,17] An exponential multiplication equivalent to a line broadening of 15 Hz was used, yielding a width at half height for PCr of less than 1 ppm. Triangulation was used to assess P_i and PCr signal area. Intracellular pH was calculated from the chemical shift difference of P_i from PCr.[18]

Statistical Analysis

Data were compared using unpaired Student's *t* testing or analysis of variance, as appropriate. The relation between variables was examined by linear regression analysis. A p value less than 0.05 was considered significant. All data are expressed as mean±SD.

Results

Skeletal Muscle Histology

When compared with control subjects, patients with heart failure exhibited an increased percentage of type IIb fibers (normal subjects, 22.7±10.1; heart failure, 33.1±11.1%; $p<0.05$) (Table 1 and Figure 1). No significant differences were observed between the two groups in the percentage of type IIa fibers. The percentage of type I fibers tended to be lower in patients with heart failure; however, this difference did not achieve statistical significance. Eleven patients with heart failure exhibited type IIc fibers, averaging 1.8±3.1% (range, 0–11.7%), compared with none of the control subjects.

In patients with heart failure, a reduction in type IIa fiber area (normal subjects, 5,477±1,109; heart failure, 4,239±1,247 μm^2; $p<0.05$) and IIb fiber area (normal subjects, 5,957±1,388; heart failure, 4,144±945; $p<0.01$) was observed. No significant difference was noted in the area of type I fibers.

To determine the relative contribution of each fiber type to a given cross-sectional area of muscle, the total area for 100 fibers was calculated by multiplying the percentage of each muscle fiber type by its respective area and then adding the three products. The area occupied by each fiber type was then divided by this total area. With this approach, no significant difference was observed between normal and heart failure populations. In the normal subjects, the relative percentage contributions to

TABLE 1. Comparison of Skeletal Muscle Biopsy Results in Normal Control Subjects and in Patients With Heart Failure

	Normal	Heart failure
n	8	22
Fiber distribution (%)		
Type I	51.2±16.3	44.6±16.3
Type IIa	26.1±12.7	20.7±10.3
Type IIb	22.7±10.1	33.1±11.1*
Type IIc	0	1.8±3.1†
Fiber area (μm^2)		
Type I	5,369±951	5,067±1,154
Type IIa	5,477±1,109	4,239±1,247*
Type IIb	5,957±1,388	4,144±945†
Capillarity		
Capillarity/fiber	1.67±0.25	1.85±0.35
Capillarity/mm^2	260±46	435±66†
Enzymatic activity (mol/kg protein/hr)		
CS	4.65±1.49	4.49±1.79
BOAC	5.17±1.44	3.67±1.68*
LDH	19.4±9.8	15.9±9.8
PFK	10.2±2.7	13.2±6.5
Protein (mg/g)	216±42	198±46

CS, citrate synthase; BOAC, β-hydroxyacyl CoA dehydrogenase; LDH, lactate dehydrogenase; PFK, phosphofructokinase.
*$p<0.05$, †$p<0.01$, normal subjects versus patients with heart failure.

muscle area for type I, IIa, and IIb fibers were 50±15%, 26±12%, and 25±11% versus, in the patients with heart failure, 50±16%, 19±9%, and 31±10% (p=NS for all).

Microscopic sections stained for myofibrillar ATPase at pH of 4.6 in a normal subject and two representative patients with heart failure are shown in Figure 2. The black fibers are type I; the grey fibers, type IIb; and light fibers, IIa. The shift toward an increased percentage of type IIb fibers is demonstrated in both patients with heart failure. Type II fibers are generally larger than or equal in size to type I fibers. Thus, the marked type II fiber atrophy is readily apparent in the second patient with heart failure.

Capillarity data revealed no difference between the two groups in the number of capillaries surrounding each fiber. However, an increase in capillaries/mm^2 was noted in patients with heart failure (normal subjects, 260±46; heart failure, 435±66 capillaries/mm^2; $p<0.001$).

Skeletal Muscle Enzyme Activity

The protein (normal subjects, 216±42; heart failure, 198±46 mg/g; p=NS) and myoglobin content (normal subjects, 4.04±0.89; heart failure, 4.41±1.54 mg/g; p=NS) of skeletal muscle were not significantly different between the normal subjects and patients with heart failure (Table 1 and Figure 1). Statistical results were comparable when enzyme activity was expressed as moles per kilograms protein per hour as compared with moles per kilogram myoglobin per hour. Therefore, all enzyme activity is so reported.

When compared with the normal subjects, patients with heart failure demonstrated reduced activity of the mitochondrial enzyme BOAC (normal subjects, 5.17±1.44; heart failure, 3.67±1.68 mol/kg protein/hr; $p<0.05$), suggesting a reduced ability for oxidation of fats. The enzymatic activity of the mitochondrial enzyme CS, and glycolytic enzymes PFK and LDH were not significantly different in the two groups (Figure 1).

Relation Between Skeletal Muscle Biopsy Results, Etiology of Heart Failure, and Exercise Capacity

Patients were divided into groups based on the etiology of heart failure. Eight patients had coronary artery disease, seven had idiopathic dilated

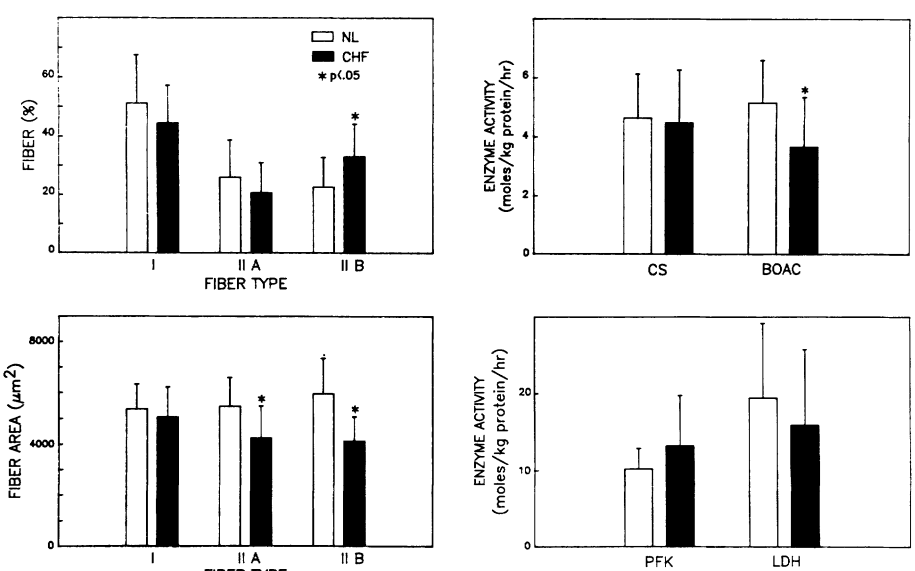

FIGURE 1. *Bar charts of comparison of fiber type distribution, fiber area, and enzymatic activity between normal and heart failure subjects.*

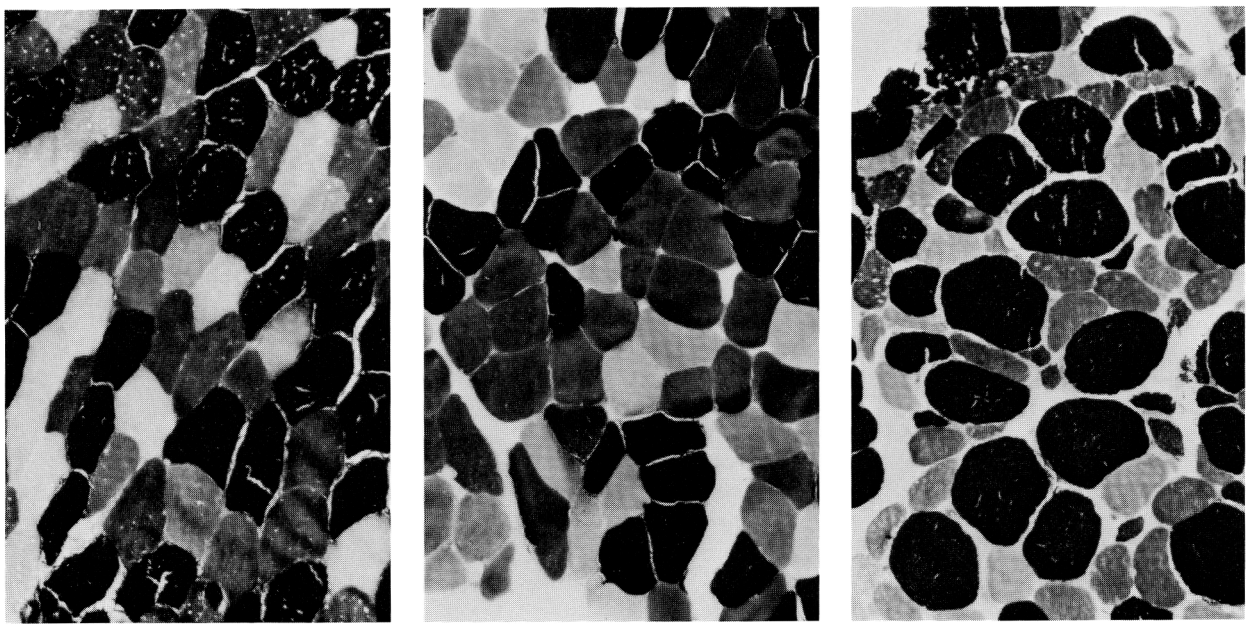

FIGURE 2. *Microphotograph of myofibrillar ATPase stain at pH 4.6 for a normal subject* (left panel) *and two representative patients with heart failure* (middle *and* right panels). *Black-stained fibers are type I; grey fibers, IIb; and light fibers, IIa. In middle panel, predominance of type II fibers in patients with heart failure is clearly demonstrated. In far right panel, atrophy of type II fibers in patients with heart failure is readily apparent because type II fibers are generally equal to or larger than type I fibers.*

cardiomyopathy, and seven had alcoholic cardiomyopathy. These populations were well matched with respect to age, ejection fraction, and exercise intolerance (Table 2). When these groups were compared, no difference was observed among groups in regard to fiber type distribution, fiber area, and enzyme concentration (Figure 3).

Peak exercise $\dot{V}O_2$ range was 9.4–25.8 ml/kg/min in the patients with heart failure. A significant inverse relation was noted between the percentage of type IIb fibers and the peak exercise $\dot{V}O_2$ (Figure 4). In contrast, a significant positive correlation was observed between percentage of type I fibers and peak $\dot{V}O_2$ (Figure 4). No significant correlations were observed between peak $\dot{V}O_2$ and enzyme activities or fiber area.

Only four patients exhibited skeletal muscle biopsies that were normal in regard to every parameter measured. These four subjects were young (age, 36±6.0 years) and were New York Heart Association class I or II (peak $\dot{V}O_2$, 20.7±5.4 ml/kg/min).

TABLE 2. Characteristics of Normal Subjects and Patients With Heart Failure

	n	Age (yr)	EF (%)	$\dot{V}O_2$ (ml/kg/min)
Normal	8	60±9	. . .	28.3±1.4
CAD	8	59±14	17±5	14.5±4.2*
IDC	7	56±17	22±10	17.1±6.0*
ETOH	7	57±13	19±5	15.0±4.1*

CAD, coronary artery disease; IDC, idiopathic cardiomyopathy; ETOH, alcoholic cardiomyopathy.
*$p<0.05$ versus normal subjects.

Relation Between Skeletal Muscle Biopsy Results and ^{31}P NMR Responses to Exercise

To investigate the contribution of skeletal muscle intrinsic changes to muscle metabolic responses to exercise, all patients underwent ^{31}P NMR studies to determine their calf metabolic responses to exercise. Upright plantar flexion resulted in a progressive rise in the ratio of P_i to PCr and decline in intracellular pH (Table 3).

To assess patients' metabolic response to exercise, $\dot{V}O_2$ was correlated with the P_i/PCr ratio, as described previously.[14] The P_i/PCr ratio provides an estimate of ADP concentration. ADP level is closely linked to mitochondrial respiration. Thus, the P_i/PCr ratio provides an index of oxidative metabolism. As described in the transfer function of Chance et al,[16,17] the relation during low-level exercise between power output (oxygen consumption) and the P_i/PCr ratio is linear. Therefore, calculation of the slope of this relation affords a simple way of comparing oxidative metabolism between subjects. The average slope of the work–P_i/PCr relation of all patients was 0.0064±0.0046, significantly more than the mean slope previously noted by us in normal subjects (normal subjects, 0.0014±0.0005 min/ml; $p<0.05$).[14]

Linear regression analysis was performed using the work–P_i/PCr relation versus each biopsy variable. No statistically significant relation was demonstrated. Similarly, linear regression analysis using the pH noted at peak exercise versus each biopsy

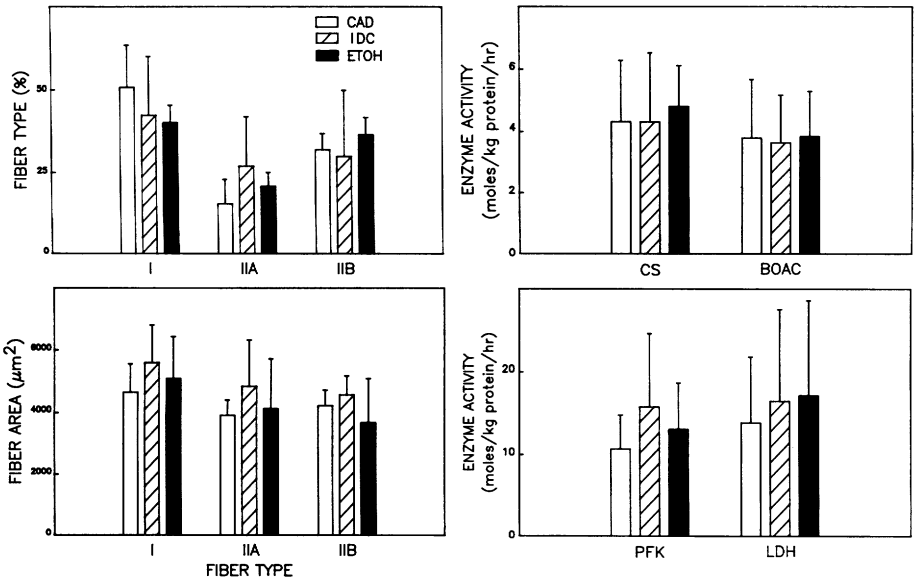

FIGURE 3. *Bar charts of comparison of fiber type distribution, fiber area, and enzyme activity in patients subgrouped by etiology of heart failure (CAD, coronary artery disease; IDC, idiopathic dilated cardiomyopathy; ETOH, alcoholic cardiomyopathy).*

variable was performed. Again, no statistically significant relation was noted.

Discussion

Patients with heart failure are frequently limited by muscular fatigue. Recent ^{31}P NMR studies suggest that this fatigue may be due, in part, to intrinsic skeletal muscle changes.[1-3] In the present study, we sought to establish whether patients with heart failure exhibit such intrinsic skeletal muscle changes and to determine if these changes might contribute to ^{31}P NMR metabolic abnormalities.

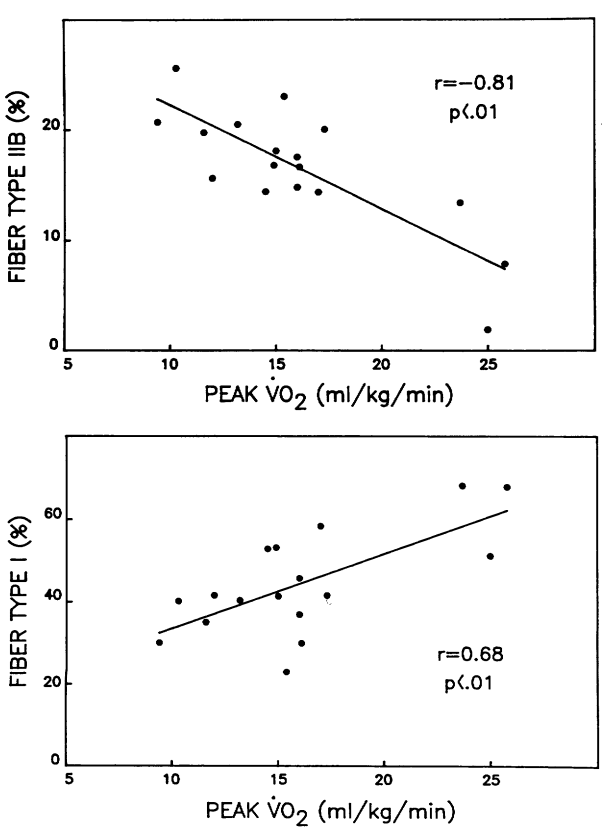

FIGURE 4. *Scatter plots of linear correlations between percentage type I and IIb fibers versus peak oxygen consumption ($\dot{V}O_2$).*

Skeletal Muscle Biopsies

Results of this study demonstrate that patients with heart failure exhibit several skeletal muscle abnormalities. These include a shift in fiber type distribution with a significant increase in the proportion of type IIb fibers, type II fiber atrophy, the presence of type IIc fibers, and reduced activity of the enzyme BOAC. Although an increase in capillaries per millimeter squared was also noted, this was probably due to fiber atrophy because no difference in the number of capillaries surrounding each fiber was observed.

Type IIb fibers represent fibers that are fast twitch, have a low aerobic potential, and are easily fatigued. An increase in the percentage of such fibers might be expected to reduce muscle performance during strenuous exercise; slowly contracting motor units are primarily recruited during low-level exercise. The inverse relation noted between percentage of type IIb fiber and peak exercise $\dot{V}O_2$ is consistent with this possibility. However, the area of type IIb fibers was reduced, so that the contribution of type IIb fibers per unit muscle area was not significantly different from the normal subjects, making it difficult to predict the overall impact of the changes in type IIb fibers.

The significance of the increased percentage of type IIc fibers is also difficult to predict. Type IIc fibers are extremely rare in normal subjects except in the neonatal period and during intense physical training.[19] Type IIc fibers may represent a population of transitional fibers shifting from type I or IIa to IIb. Alternatively, some investigators have spec-

TABLE 3. Skeletal Muscle Metabolic Measurements During Upright Plantarflexion in Patients With Heart Failure

Workload	$\dot{V}O_2$ (ml/min)	P_i/PCr	pH
Rest	295±47	0.20±0.07	7.07±0.18
PF every 9 seconds	414±54*	0.89±0.31*	7.07±0.12
PF every 6 seconds	462±61*	1.07±0.51*	6.94±0.14*
PF every 3 seconds	538±85*	1.68±0.88*	6.86±0.20*

PF, plantarflexion; P_i/PCr, ratio of inorganic phosphorus to phosphocreatine; $\dot{V}O_2$, oxygen consumption.
*$p<0.01$, rest versus PF.

ulated that they represent fibers in the earliest stages of cell death.

BOAC is a mitochondrial-based enzyme involved in β-oxidation of fatty acids. During rest and light exercise, skeletal muscle uses fat and carbohydrates in approximately equal proportions. With prolonged heavier exercise, fatty acid oxidation contributes significantly more to the energy supply than do carbohydrates. A reduction in enzymes involved in β-oxidation, therefore, potentially could force higher than normal use of carbohydrates during prolonged exercise. This could, in turn, augment lactate accumulation by a mass-action effect and reduce endurance because carbohydrate stores are much smaller than fat stores.

The reduction in BOAC activity also could reflect an overall decrease in mitochondrial-based enzymes. However, we found no reduction in CS activity, a mitochondrial-based enzyme involved in the citric acid cycle.

Our finding of type II fiber atrophy is consistent with prior observations by Dunnigan et al[20] in patients with idiopathic cardiomyopathy. These investigators examined skeletal muscle histochemistry and electron microscopy in 22 young patients (age, 4–39) with idiopathic cardiomyopathy, presenting either as ventricular arrhythmias or cardiac failure. Type II fiber atrophy was found almost exclusively in patients with symptoms of heart failure, occurring in seven of the 11 patients in this group. In contrast, patients with and without symptoms of heart failure exhibited lipid deposition (15 of 22 patients) and endomysial fibrosis (14 of 22 patients). Dunnigan et al[20] speculated that the skeletal muscle changes in their patients may reflect a generalized myopathic process affecting both the heart and skeletal muscle. This seems unlikely, at least for the type II fiber–atrophy changes, because this abnormality was found primarily in patients with symptoms of heart failure. If such atrophy was due to a generalized myopathic process, it should also have been observed in patients presenting with ventricular arrhythmias.

Lipkin et al[4] performed quadriceps muscle biopsies on nine patients with heart failure. Marked generalized fiber atrophy was found in two patients, marked type II fiber atrophy in one patient, increased acid phosphatase staining in six patients, and increased lipid deposition in four patients. The explanation for the more consistent abnormalities noted in our patients compared with the findings of Lipkin et al[4] is uncertain; differences in patient selection and population size may play a role.

Several investigators have also reported preliminary muscle biopsy findings in patients with heart failure. Drexler et al[5,6] described reduced mitochondrial cristae volume in 21 patients with heart failure, suggesting a reduction in mitochondrial enzyme activity. Sullivan et al[21] reported marked reduction in CS and succinate dehydrogenase activity in 11 patients with heart failure but normal glycolytic enzyme activity. Yancy et al[22] reported reduced skeletal muscle capillarization and succinate dehydrogenase activity in six patients. Although these investigators appear to be observing a different pattern of changes than in the present study, final comparison of our results with these preliminary observations should await completion of the studies.

Mechanism of Skeletal Muscle Changes

The present study does not indicate the mechanism responsible for the observed skeletal muscle abnormalities. Nevertheless, the most likely mechanism is muscle deconditioning due to inactivity. Previous studies have clearly demonstrated that inactivity can produce fiber atrophy.[23–27] In studies of totally immobilized limbs, atrophy of both type I and II fibers has usually been noted.[24–27] However, our population maintained a constant low activity level. Weight analyses of immobilized muscles have demonstrated that immobilization in a stretched position will limit disuse atrophy, whereas, in a shortened position, atrophy is accelerated.[28] Tonic stretch is especially important for the maintenance of type I fibers. For example, when the anterior tibial muscle of the rat is immobilized in a slightly stretched position, a transient hypertrophy of type I fibers with concomitant atrophy of type II fibers occurs.[23] Interestingly, during rat hindlimb suspension, changes in fiber composition of the soleus muscle also occur with transformation of type I to II fibers.[29] Training increases BOAC and CS activity,[30,31] and inactivity results in decreased enzyme activity.[19] Thus, the particular pattern of atrophy we observed is probably consistent with inactivity.

Malnutrition may also contribute to the abnormalities observed in our patients. Skeletal muscle biopsies of severely malnourished patients have demonstrated extensive necrosis of muscle fibers, neurogeniclike grouping of atrophic type II fibers, and predominant type II fiber atrophy.[32,33]

Another potential mechanism for the muscle changes observed in our cardiomyopathic outpatients is a generalized myopathic problem involving both the muscle and the heart. This seems unlikely given the absence of any significant difference in histochemical skeletal muscle profile when patients were divided into groups on the basis of the etiology of heart failure. Despite this, we cannot exclude the possibility that some of our patients had a component of chronic alcoholic myopathy. Many of our alcoholic patients continued to abuse alcohol. Ethanol has both direct effects on skeletal muscle, as well as indirect effects from its metabolites, acetaldehyde and acetate, which are oxidized by skeletal muscle.[34] Even a low level of alcohol use can expose the skeletal muscle to an altered pattern of substrate availability and, thus, an altered muscle intermediary metabolism. Nutritional deprivation may lead to an imbalance between protein catabolism and synthesis. These factors may contribute to the type II fiber atrophy frequently observed in chronic alcoholic myopathy. Unlike previous reports on patients with chronic alcoholic myopathy, our patients did not exhibit reduced glycolytic enzyme activity.[34]

Other possible explanations for intrinsic skeletal muscle changes include an effect of low muscle flow, alterations in muscle innervation, or hormonal effects. Steroid myopathy, for example, produces type IIb fiber atrophy.[32] Conceivably, stress-related elevations of cortisol by heart failure could influence skeletal muscle.

Relation Between Muscle Biopsies and ^{31}P NMR Observations

^{31}P NMR provides a noninvasive method of assessing intracellular metabolic behavior during exercise. In particular, this technique allows assessment of intracellular pH, an index of glycolytic activity, and of mitochondrial respiratory control. The relation between P_i/PCr ratio and work rate provides a noninvasive index of mitochondrial respiratory control that is sensitive to both muscle mitochondrial content and muscle oxygen delivery.[35,36]

During calf exercise, the patients in the present study exhibited higher slopes of the P_i/PCr ratio to $\dot{V}O_2$ relation and greater decreases in muscle pH than observed previously in normal subjects; ^{31}P NMR studies were not performed on the normal subjects in this study for logistic reasons. This is consistent with previous observations that both forearm and calf metabolic responses to exercise are abnormal in patients with heart failure.[1–3,14,37]

To test the hypothesis that the abnormal metabolic responses observed in our patients were due to intrinsic muscle changes, we correlated each muscle variable with the work–P_i/PCr slope and with the pH level noted at peak exercise. No significant relation was noted for any of the variables. Such a finding suggests that the intrinsic muscle changes do not contribute in a major way to the abnormal metabolic responses.

If the changes observed on muscle biopsy do not produce the abnormal metabolic responses, what then is responsible for these responses? One potential factor is muscle underperfusion. However, the levels of systemic $\dot{V}O_2$ achieved during exercise were modest and unlikely to exceed the capacity of the circulation to deliver blood to working muscle. A more likely explanation for the altered metabolic responses is a reduction in muscle mass. In a previous study of calf exercise, we reported a significant decrease in calf circumference in patients with heart failure.[14] This suggests a reduction in total calf muscle. Lipkin et al[4] have also reported evidence consistent with a reduced muscle mass. Such a reduction in muscle mass would subject each fiber to an increased load, in turn producing a greater change in the P_i/PCr ratio and in muscle pH.

It should be emphasized, however, that our failure to find a relation between metabolic responses to exercise and muscle biopsy characteristics does not totally exclude such a relation. Within a given individual, there is considerable variability of muscle biopsy results when repeated biopsies are taken from the same muscle.[38,39] Therefore, it is possible that the single-biopsy results obtained in our patients do not precisely reflect their muscle characteristics. Such inaccuracies might obscure a weak but significant relation between muscle biopsy results and muscle metabolism.

Clinical Implications

Our findings establish the presence of intrinsic skeletal muscle changes in patients with heart failure. To what extent these muscle changes influence the exercise capacity of patients remains to be determined. If these muscle changes do impair exercise performance, it may be possible to improve the exercise capacity of patients by reversing the process directly responsible for the changes. For example, if these changes are due to inactivity, exercise training may reverse the abnormalities and improve the exercise performance of patients. If these changes are due to malnutrition, protein supplementation may be beneficial. Indeed, in several recent nonrandomized studies, patients with heart failure have demonstrated improved exercise capacity with exercise training.[40,41] An effect of deconditioning on exercise performance may also partially account for the frequent clinical observation of a delay in improvement in maximal exercise performance with therapeutic interventions. These interventions may improve submaximal exercise capacity, permitting patients to increase their activity levels and consequently reverse muscle changes due to inactivity. Alternatively, circulatory dysfunction may be the primary factor limiting the exercise capacity of patients, with intrinsic muscle changes representing an epiphenomenon that does not add appreciably to the exercise intolerance of patients.

References

1. Weiner DH, Fink LI, Maris J, Jones RA, Chance B, Wilson JR: Abnormal skeletal muscle bioenergetics during exercise in patients with heart failure: Role of reduced muscle blood flow. *Circulation* 1986;73:1127–1136
2. Massie B, Conway M, Yonge R, Frostick S, Ledingham J, Sleight P, Radda G, Rajagopalan B: Skeletal muscle metabolism in patients with congestive heart failure: Relation to clinical severity and blood flow. *Circulation* 1988;78:320–326
3. Massie B, Conway M, Rajagopalan B, Yonge R, Frostick S, Ledingham J, Sleight P, Radda G: Skeletal muscle metabolism during exercise under ischemic conditions in congestive heart failure: Evidence for abnormalities unrelated to blood flow. *Circulation* 1988;78:320–326
4. Lipkin D, Jones D, Round J, Poole-Wilson P: Abnormalities of skeletal muscle in patients with chronic heart failure. *Int J Cardiol* 1988;18:187–195
5. Drexler H, Riede U, Schäfer H: Reduced oxidative capacity of skeletal muscle in patients with severe heart failure (abstract). *Circulation* 1987;76(suppl IV):IV-178
6. Drexler H, Riede U, Hiroi M, Münzel T, Holubarsch C, Meinertz T: Ultrastructural analysis of skeletal muscle in chronic heart failure: Relation to exercise capacity and indices of LV dysfunction (abstract). *Circulation* 1988; 78(suppl II):II-107
7. Bergstrom J: Muscle electrolytes in man. *Scand J Clin Lab Invest* 1962;68(suppl):1–110
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–275
9. Chi M, Hintz C, Coyle E, Martin W III, Ivy J, Nemeth P, Holloszy J, Lowry O: Effects of detraining on enzymes of energy metabolism in individual human muscle fibers. *Am J Physiol* 1983;244(*Cell Physiol* 13):C276–C287
10. Moller P, Sylven C: Myoglobin in human skeletal muscle. *Scand J Clin Lab Invest* 1981;41:479–482
11. Brooke MH, Kaiser KK: Three "myosin ATPase" systems, the nature of their pH lability and sulfhydryl dependence. *J Histochem Cytochem* 1970;18:670–672
12. Anderson P, Henriksson J: Capillary supply of the quadriceps femoris muscle of man, adaptive response to exercise. *J Physiol* (Lond) 1977;270:677–690
13. Novikoff AB, Shin W, Drucker J: Mitochondrial localization of oxidative enzymes: Staining results with two tetrazolium salts. *J Biophys Biochem Cytol* 1961;9:47–61
14. Mancini DM, Ferraro N, Tuchler M, Chance B, Wilson JR: Calf muscle metabolism during leg exercise in patients with heart failure: A ^{31}P NMR study. *Am J Cardiol* 1988; 62:1234–1240
15. Harris R, Edwards R, Hultman E, Nordesjo L, Nylind B, Sahlin K: The time course of phosphorylcreatine resynthesis during recovery of the quadriceps muscle in man. *Eur J Physiol* 1976;367:137–142
16. Chance B, Eleff S, Leigh JS Jr, Sokolow D, Sapega A: Mitochondrial regulation of phosphocreatine/inorganic phosphate ratios in exercising human muscle: A gated ^{31}P NMR study. *Proc Natl Acad Sci USA* 1981;78:6714–6718
17. Chance B, Eleff S, Leigh J: Noninvasive, nondestructive approaches to cell bioenergetics. *Proc Natl Acad Sci USA* 1980;77:7430–7434
18. Moon RB, Richards JR: Determination of intracellular pH by ^{31}P NMR. *J Biol Chem* 1973;248:7276–7278
19. Saltin B, Gollnick P: Skeletal muscle adaptability: Significance for metabolism and performance, in Peachey L (ed): *Handbook of Physiology.* Bethesda, Md, American Physiological Society, 1983, pp 555–631
20. Dunnigan A, Staley N, Smith S, Pierpont M, Judd D, Benditt D, Benson D: Cardiac and skeletal muscle abnormalities in cardiomyopathy: Comparison of patients with ventricular tachycardia or congestive heart failure. *JACC* 1987; 10:608–618
21. Sullivan MJ, Higginbotham MB, Green HJ, Cobb FR: Decreased aerobic oxidative capacity in skeletal muscle in chronic heart failure. *J Am Coll Cardiol* 1989;13:39A
22. Yancy CW, Parsons D, Lane L, Carry M, Firth BG, Blomqvist G: Capillary density, fiber type and enzyme composition of skeletal muscle in congestive heart failure. *J Am Coll Cardiol* 1989;13:38A
23. Lindboe C, Presthus J: Effects of denervation, immobilization, and cachexia on fibre size in the anterior tibial muscle of the rat. *Acta Neuropathol* 1985;66:42–51
24. Sargeant A, Davies C, Edwards R, Maunder C, Young A: Functional and structural changes after disuse of human muscle. *Clin Sci Mol Med* 1977;52:337–342
25. Patel A, Razzak Z, Dastur D: Disuse atrophy of human skeletal muscles. *Arch Neurol* 1969;20:413–421
26. MacDougall J, Elder G, Sale D, Moroz J, Sutton J: Effects of strength training and immobilization on human muscle fibres. *Eur J Applied Physiol* 1980;43:25–34
27. Fitts R, Metzger J, Riley D, Unsworth B: Models of disuse: A comparison of hindlimb suspension and immobilization. *J Appl Physiol* 1986;60(6):1946–1953
28. Booth FW: Time course of muscular atrophy during immobilization of hindlimbs in rats. *J Appl Physiol* 1977;43:656–661
29. Templeton G, Sweeney H, Timson B, Padalino M, Dudenhoeffer G: Changes in fiber composition of soleus muscle during rat hindlimb suspension. *J Appl Physiol* 1988; 65:1191–1195
30. Holloszy JO, Booth FW: Biochemical adaptations to endurance exercise in muscle. *Annu Rev Physiol* 1976;18:273–291
31. Holloszy J, Coyle E: Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol* 1984;56:831–838
32. Dubrowitz V: *Muscle Biopsy: A Practical Approach*, ed 2. Philadelphia, WB Saunders, 1985
33. Goldspink G, Ward PS: Changes in rodent muscle fiber types during post-natal growth, undernutrition, and exercise. *J Physiol* 1979;296:453–469
34. Haller R, Knochel J: Skeletal muscle disease in alcoholism. *Med Clin North Am* 1984;68:91–103
35. Ibstrom JP, Subramanian VH, Chance B, Schersten T, Bylund-Fellenius AC: Oxygen dependence of energy metabolism in contracting and recovering rat skeletal muscle. *Am J Physiol* 1985;248:H40–H48
36. Dudley GA, Tullson PC, Terjung RL: Influence of mitochondrial content on the sensitivity of respiratory control. *J Biol Chem* 1987;262:9109–9114
37. Arnolda L, Conway M, Dolecki M, Sharif H, Sleight P, Rajagopalan B, Ledingham J, Radda G: Phosphorus magnetic resonance spectroscopy of leg muscle in heart failure. *Circulation* 1988;78(suppl II):II-341
38. Lexell J, Henriksson-Larsen K, Sjostrom M: Distribution of different fibre types in human skeletal muscle. *Acta Physiol Scand* 1983;117:115–122
39. Blomstrand E, Ekblom B: The needle biopsy technique for fiber type determination in human skeletal muscle—A methodological study. *Acta Physiol Scand* 1982;116:437–442
40. Maskin C, Reddy H, Gulanick M, Perez L: Exercise training in chronic heart failure: Improvements in cardiac performance and maximum oxygen uptake (abstract). *Circulation* 1986;74:310
41. Sullivan M, Higginbotham M, Cobb F: Exercise training in patients with severe left ventricular dysfunction: Hemodynamic and metabolic effects. *Circulation* 1988;78:506–515

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