Skeletal Muscle Biochemistry and Histology in Ambulatory Patients With Long-term Heart Failure

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Recent studies in patients with long-term heart failure have suggested that intrinsic abnormalities in skeletal muscle can contribute to the development of early lactic acidosis and fatigue during exercise. The present study provides an analysis of substrate and enzyme content, fiber typing, and capillarization in skeletal muscle biopsy samples obtained at rest from the vastus lateralis in 11 patients with long-term heart failure (left ventricular ejection fraction, 21±8%) and nine normal subjects. Patients demonstrated a reduced peak exercise oxygen consumption (13.0±3.3 ml/kg/min) when compared with normals (30.2±8.6 ml/kg/min, $p<0.001$) and had an accelerated rise in blood lactate levels during exercise. In mixed fiber skeletal muscle, total phosphorylase and glycolytic enzyme activities were not different in the two groups, whereas mitochondrial enzymes involved in terminal oxidation were decreased in patients as compared with normal subjects as indicated by reductions in succinate dehydrogenase (51±15 vs. 81±17 μM/g protein/min, $p<0.001$) and citrate synthetase (26±7 vs. 43±20 μM/g protein/min, $p<0.05$). 3-Hydroxyacyl-CoA-dehydrogenase, an important enzyme mediating β-oxidation of fatty acids, was also reduced in patients as compared with normals (18±7 vs. 27±10 μM/g protein/min, $p<0.05$). There was no difference in high-energy phosphagens or lactate concentration of mixed muscle in the two groups, whereas glycogen content was decreased in patients (262±29 vs. 298±35 μM glucosyl units/kg dry wt, $p=0.01$). Patients demonstrated a reduced percentage of slow twitch type I fibers (36±7% vs. 52±22%, $p<0.05$) and had a higher percentage of type IIb fast twitch fibers (24±9% vs. 11±12%, $p=0.02$), which were smaller than the type IIb fibers seen in normal subjects ($p<0.05$). In patients, the number of capillaries per fiber was decreased for type I and type IIa fibers (both, $p<0.03$), but the ratio of capillaries to cross-sectional fiber area was not different for the two groups. These data demonstrate major alterations in skeletal muscle histology and biochemistry in patients with long-term heart failure, including fiber atrophy, a decrease in percentage of composition of type I fibers, and an increase in type IIb fibers accompanied by a decrease in oxidative enzyme capacity. These skeletal muscle adaptations to the heart failure state represent a potentially important mechanism underlying the early onset of anaerobic metabolism and fatigue seen in this disorder and might play a role in determining both day-to-day exercise tolerance and the response to therapeutic interventions in patients with long-term heart failure. (*Circulation* 1990;81:518–527)

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Several studies have demonstrated early anaerobic metabolism in skeletal muscle during exercise in patients with long-term heart failure[1–3] and have suggested that this is an important mechanism underlying exercise intolerance in ambulatory patients with this disorder.[1,2,4,5] Studies by Weiner et al[6] and Massie et al[7] of skeletal muscle metabolism and limb blood flow during arm exercise in normal subjects and in patients with long-term heart failure using ^{31}P-NMR spectroscopy have demonstrated an acceleration in the development of acidosis in relation to workload in patients that was not accompanied by a reduction in arm blood flow.

These studies suggest that skeletal muscle metabolic abnormalities in patients might account for early anaerobic metabolism during exercise in this disorder. A large number of studies in humans[8–12] and animals[8,13] indicate that the biochemical and histologic characteristics of skeletal muscle have important effects on patterns of substrate use and the development of fatigue during exercise. Previous studies in our laboratory[14] have demonstrated a delay in muscle lactate production during submaximal exercise after 4–6 months of exercise training in patients with long-term heart failure, which was not accompanied by improved hemodynamics, suggesting that skeletal muscle biochemical adaptations were responsible for the improved metabolic response to exercise. Although decreased muscle blood flow[2,3] and increased adrenergic activation[15] might be important in determining the metabolic response to exercise in this disorder, recent studies suggest that intrinsic abnormalities in skeletal muscle can also contribute to early anaerobic metabolism.

The present study was designed to provide an analysis of skeletal muscle biochemistry and histology from biopsy specimens of the vastus lateralis in patients with long-term heart failure and to compare these results with data obtained from normal volunteers. The biochemical studies focused on analysis of enzymes involved in the major metabolic pathways including glycogenolysis, glycolysis, the citric acid cycle, and β-oxidation of fatty acids and, also, determination of phosphagen and substrate content. Histologic and histochemical studies examined factors that have important effects on the metabolic response to exercise[8] including fiber typing, capillarization, and fiber size.

Methods

Patient Group

Eleven males with long-term heart failure because of left ventricular systolic dysfunction and nine normal males participated in the study. All patients had coronary artery disease. The New York Heart Association functional classification of the patients was as follows: one was functional class I, three were class II, six were class III, and one was class IV. All patients were taking digoxin (0.25 mg daily) and diuretics. Three patients were taking vasodilators; that is, patients 6 and 7 (see Table 1) were taking captopril 25 mg t.i.d., and patient 11 was taking isordil 10 mg t.i.d. Patient 9 was taking a phosphodiesterase inhibitor, enoximone 50 mg t.i.d. Vasodilators and oral inotropes were withheld for 48 hours before exercise testing. Patients did not have rales or peripheral bruits and were limited during exercise primarily by leg fatigue. Six patients were not involved in any exercise activities, four regularly walked 1–3 hr/wk, and one patient was regularly engaged in aerobic exercise for 3 hr/wk.

Normal Subjects

Nine normal males volunteered to participate in the study. All had normal medical history, physical examinations, and electrocardiographic and radionuclide angiographic responses to screening maximal bicycle exercise test. Four subjects did not engage in any exercise activities, three walked or jogged 1–3 hr/wk, and two were engaged in regular aerobic exercise for 3–4 hr/wk.

Exercise Testing

After a familiarization exercise study, all subjects underwent graded upright bicycle exercise to a symptom-limited maximum. The exercise workload began at 150 kpm/min and advanced in 3-minute stages of 150 kpm/min. Two hours before exercise, a catheter was inserted into the femoral vein at the inguinal ligament. Expired gases were analyzed continuously using a Sensormedics 4400 unit (Anaheim, California) as previously described in our laboratory.[14,16] Femoral venous blood was obtained at upright rest and at each workload and analyzed for lactate concentration (Calbiochem Behring, San Diego, California), oxygen content, and saturation using an Instrument Laboratories 282 Co-oximeter (Lexington, Massachusetts).

Skeletal Muscle Biopsy

Within 2 weeks of the exercise study, subjects underwent biopsy of the vastus lateralis muscle using the needle technique of Bergstrom.[17] Two biopsy samples were obtained; one was immediately plunged into liquid nitrogen and was used for analysis of phosphagens and substrates. The second needle sample was divided into two pieces, placed in liquid nitrogen, and transferred to a freezer at $-70°$ C. One piece was used for analysis of enzymes, and the second was mounted in embedding media for histologic analyses before storage in liquid nitrogen.

Biochemical Muscle Analysis

The first biopsy samples were weighed, freeze-dried, and extracted according to the methods of Harris et al.[18] Adenine nucleotides and inosine monophosphate were determined by high-performance liquid chromatography.[19] Creatine, phosphocreatine, glycogen, glucose, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, lactate, and pyruvate were analyzed fluorometrically using the methods of Lowery and Passonneau[20] as previously described for skeletal muscle by Green et al.[21,22] Analysis of skeletal muscle phosphagen and substrate content was performed with values expressed as both mM/kg dry weight of muscle and mM/mM total creatinine with results presented as mM/kg dry weight. Creatine kinase, total phosphorylase, phosphofructokinase, pyruvate kinase, lactate dehydrogenase, succinate dehydrogenase, citrate synthetase, 3-hydroxyacyl-CoA dehydrogenase, and hexokinase were assayed in the second muscle sample by fluoro-

TABLE 1. Demographics, Resting Left Ventricular Ejection Fraction, and Peak Oxygen Consumption in 11 Patients and Nine Normal Subjects

	Age (yr)	WT (kg)	BSA (m^2)	LVEF (%)	Peak $\dot{V}O_2$ (ml/kg/min)
Patients with heart failure					
1	59	70	1.82	21	15.4
2	41	60	1.70	25	14.8
3	66	62	1.68	16	12.2
4	42	86	2.06	13	8.1
5	71	62	1.72	32	10.4
6	70	93	2.09	16	14.2
7	62	72	1.82	27	20.5
8	65	60	1.66	36	13.1
9	35	52	1.61	10	12.4
10	65	72	1.83	16	10.7
11	69	70	1.73	22	10.7
Mean	58	69	1.79	21	13.0
SD	13	12	0.16	8	3.3
Normal subjects					
1	34	67	1.80	73	43.2
2	30	75	1.96	77	32.9
3	42	73	1.79	59	25.8
4	48	86	2.10	69	40.3
5	55	72	1.91	...	36.8
6	54	82	1.88	59	18.7
7	51	82	1.94	55	27.8
8	55	73	1.81	55	21.0
9	70	81	1.95	67	25.1
Mean	49	76	1.92	64	30.2
SD	12	6	0.19	8	8.6
p	0.10	0.10	0.09	<0.001	<0.001

WT, body weight; BSA, body surface area; LVEF, radionuclide left ventricular ejection fraction; Peak $\dot{V}O_2$, peak exercise oxygen consumption.

metric methods as previously described.[20-24] Protein content was determined by the method of Polachek et al.[25] Enzyme content is reported as both μM/mg protein/min and as mM/min/kg wet wt of muscle.

Histology and Histochemistry

Samples were cut in a cryostat at −20° C and stained for myofibrillar ATPase for classification of fibers into type I, IIa, IIb, and IIc fibers as previously described in this laboratory.[22,23] Fiber size was determined on sections stained for nicotinamide adenine dinucleotide (NADH) tetrazolium reductase in at least 20 fibers measured from projections on a calibrated Numonics digitizer (Numonics Corp., Montgomeryville, Pennsylvania).[23,24] The number of capillaries adjacent to each fiber and the ratio of capillaries to fiber area were determined in sections using the periodic acid Schiff stain as previously described.[22-24] Data on capillarization is presented for type I and type IIa fibers; type IIb fibers were

TABLE 2. Exercise Testing Results in Patients and Normal Subjects

	Upright rest		Submaximal exercise (300 kpm/min)		Peak exercise	
Variable	Patients	Normal subjects	Patients	Normal subjects	Patients	Normal subjects
HR (beats/min)	83±16	74±10	108±19	99±14	121±25*	168±14
RER	0.90±0.07	0.89±0.07	1.22±0.11*	0.90±0.07	1.37±0.14	1.31±0.09
FVO_2ST (%)	38±15*	54±12	24±8*	37±4	21±9	27±6
FVO_2CT (g/dl)	8.0±3.8†	11.8±2.4	4.7±2.5†	7.9±1.3	4.4±2.3	5.7±1.5
FLAC (mM/l)	1.1±0.6	0.9±0.5	4.2±1.4‡	1.7±0.7	7.6±3.1†	11.5±3.7

HR, heart rate; RER, respiratory exchange ratio; FVO_2ST, femoral venous oxygen saturation; FVO_2CT, femoral venous oxygen content; FLAC, femoral venous lactate concentration.

*$p<0.05$, †$p<0.01$, ‡$p<0.001$; patients vs normals.

TABLE 3. Bioenergetics and Substrate Content of Mixed Fiber Skeletal Muscle at Rest in Patients and Normal Subjects

	Patients	Normal subjects
Total creatine	114±13	114±11
Creatine phosphate	64.5±22.6	64.0±14.9
Adenosine triphosphate	21.6±2.8	21.6±1.1
Adenosine diphosphate	4.30±0.49	4.02±0.42
Inosine monophosphate	0.86±0.86	0.59±0.24
Glycogen (glucosyl units)	262±29*	298±35
Glucose	4.82±2.79	3.50±2.13
Glucose-6-PO_4	3.86±3.66	4.61±4.08
Glucose-1-PO_4	0.37±0.25	0.45±0.21
Fructose-6-PO_4	0.54±0.53	0.53±0.40
Lactate	11.6±12.1	6.4±5.1
Pyruvate	0.85±0.53*	0.24±0.25

All values are expressed in mM/kg dry wt.
*$p<0.05$ patients vs. normal subjects by the unpaired Student's *t* test.

present in inadequate numbers in normal subjects for intergroup comparison.

Statistical Analysis

Intergroup comparisons were performed using the unpaired Student's *t* test. A p value less than 0.05 was considered statistically significant. All data are displayed as mean±SD.

Results

Table 1 contains the baseline demographics, left ventricular ejection fraction, and peak oxygen consumption ($\dot{V}O_2$) values in the two groups. Although patients tended to be older and demonstrate reduced weight and surface area as compared with normal subjects, these differences were not statistically significant. The rest left ventricular ejection fraction was severely reduced in patients, as was peak $\dot{V}O_2$ (13.0±3.3 ml/kg/min vs. 30.2±8.6 ml/kg/min, $p<0.001$). The exercise testing results for the two groups are illustrated in Table 2. Femoral venous oxygen content and saturation were

TABLE 4. Individual Values for Glycolytic Enzymes in 11 Patients and Nine Normal Subjects

	Total phosphorylase	Phosphofructokinase	Pyruvate kinase	Lactate dehydrogenase
Patients				
1	45	148	1,526	584
2	47	196	1,159	805
3	58	173	1,759	1,662
4	49	274	1,394	1,056
5	55	195	1,683	1,322
6	55	233	1,235	1,316
7	54	188	1,966	1,253
8	57	252	1,501	1,094
9	62	258	1,601	1,161
10	43	267	1,692	1,071
11	51	255	1,783	895
Mean	53	222	1,573	1,111
SD	6	43	241	289
Normal subjects				
1	56	112	1,238	573
2	83	236	2,567	1,486
3	40	290	1,574	1,649
4	63	173	1,372	648
5	51	158	1,101	699
6	44	217	806	548
7	46	156	1,449	660
8	35	110	1,124	526
9	56	277	1,975	1,735
Mean	53	192	1,471	947
SD	14	66	527	513
p	0.96	0.24	0.57	0.38

Values are expressed as μM/g protein/min.
Subject numbers correspond to Table 1.

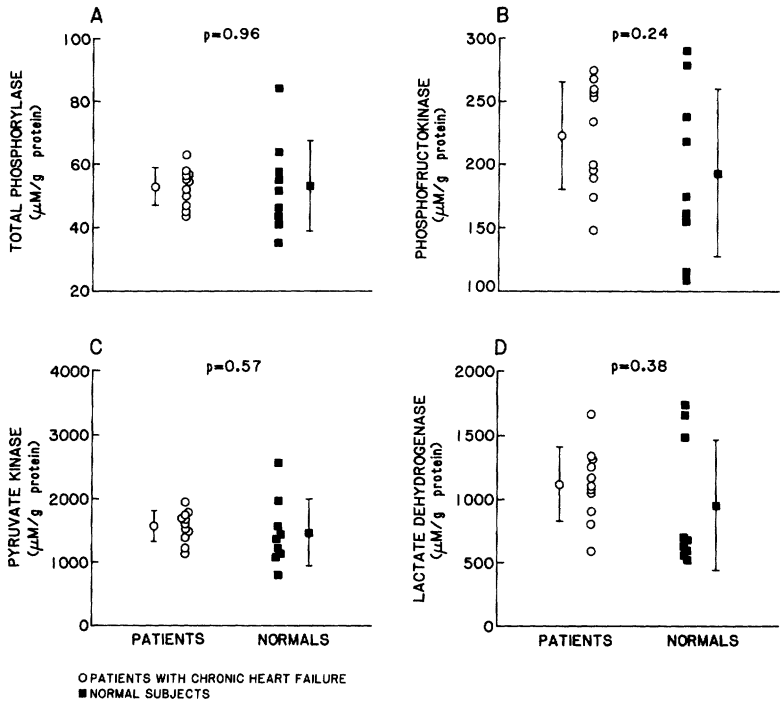

FIGURE 1. *Graphic plotting of total phosphorylase, phosphofructokinase, pyruvate kinase, and lactate dehydrogenase in mixed skeletal muscle samples in patients with long-term heart failure and in normal subjects.*

decreased in patients at rest and during submaximal exercise but were not different in the two groups at peak exercise. Femoral venous lactate was not different in the two groups at rest but increased at an accelerated rate in patients during submaximal-exercise. Peak respiratory exchange ratio was 1.37±0.14 in patients and 1.31±0.09 in normal subjects (p=0.25), demonstrating comparable maximal or near maximal exercise efforts in both groups.

Biochemical Analysis

Analysis of rest-mixed fiber muscle samples revealed no difference in total creatine, creatine phosphate, adenosine triphosphate, adenosine diphosphate, or inosine monophosphate between the two groups (Table 3). Glycogen content was decreased in patients as compared with normals, whereas there was no difference in glycolytic intermediates or lactate in the two groups (Table 3).

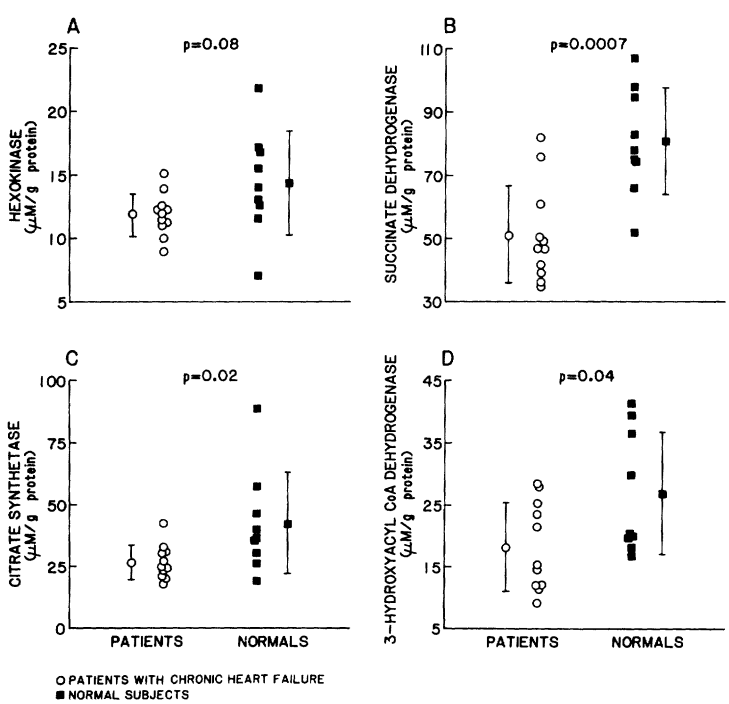

FIGURE 2. *Graphic plotting of hexokinase, succinate dehydrogenase, citrate synthetase, and 3-hydroxyacyl-CoA dehydrogenase in mixed skeletal muscle samples in patients with long-term heart failure and in normal subjects.*

TABLE 5. Hexokinase and Oxidative Enzyme Content of Mixed Skeletal Muscle in 11 Patients With Long-term Heart Failure and Nine Normal Subjects

	Hexokinase	Succinate dehydrogenase	Citrate synthetase	3-Hydroxyacyl-CoA-dehydrogenase
Patients				
1	12.0	35.0	20.5	15.0
2	12.2	36.2	17.4	11.7
3	8.8	47.0	27.1	11.7
4	15.0	40.2	23.1	11.4
5	9.9	47.0	23.6	9.1
6	12.5	81.7	28.8	21.4
7	13.8	76.2	42.0	28.3
8	12.3	60.8	31.3	27.9
9	11.2	49.5	24.1	14.5
10	11.0	41.0	20.8	23.4
11	11.1	49.6	30.7	25.2
Mean	11.8	51.3	26.3	18.2
SD	1.7	15.5	6.8	7.2
Normal subjects				
1	11.4	74.8	30.4	17.9
2	14.2	65.8	46.4	19.8
3	16.9	75.3	39.3	29.5
4	21.8	106.8	88.7	41.1
5	16.8	97.8	57.1	39.4
6	6.9	51.9	18.8	17.1
7	15.5	83.1	37.8	19.6
8	12.6	94.8	25.9	19.6
9	13.1	78.1	37.7	36.3
Mean	14.4	80.9	42.5	26.7
SD	4.2	16.9	20.6	9.9
p	0.08	0.0007	0.02	0.04

Values are expressed as μM/g protein/min.
Subject numbers correspond to Table 1.

Pyruvate, however, was increased in patients as compared with normal subjects, suggesting a possible increase in glycolytic rate in patients. There was no difference in the results of the above intergroup comparisons when substrates were expressed as mM/mM total creatine.

TABLE 6. Enzyme Content of Muscle in Mixed Skeletal Muscle in 11 Patients with Long-term Heart Failure and Nine Normal Subjects

	Patients	Normal subjects	*p*
Total phosphorylase	5.2±1.1	5.3±1.3	0.81
Phosphofructokinase	22±6	19±7	0.38
Pyruvate kinase	156±42	147±51	0.68
Lactate dehydrogenase	108±27	94±51	0.43
Hexokinase	1.2±0.2	1.5±0.4	0.07
Succinate dehydrogenase	5.0±1.7	8.3±2.5	0.002
Citrate synthetase	2.6±0.9	4.3±2.0	0.02
3-Hydroxyacyl-CoA-dehydrogenase	1.8±0.9	2.7±1.2	0.06

Values are expressed as mM/kg wet wt.

There was no difference in creatine kinase in patients versus normal subjects (14,268±1,724 vs. 14,515±2,556 μM/g protein/min, $p=0.80$). The mixed fiber skeletal muscle content of enzymes mediating

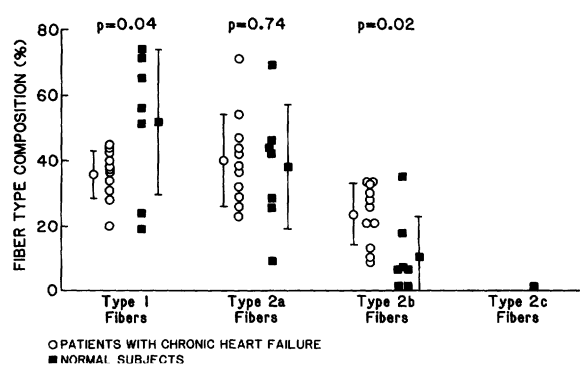

FIGURE 3. *Graphic plotting of relative fiber type composition of the vastus lateralis in patients with long-term heart failure and in normal subjects, in mixed skeletal muscle samples in patients with long-term heart failure and in normal subjects.*

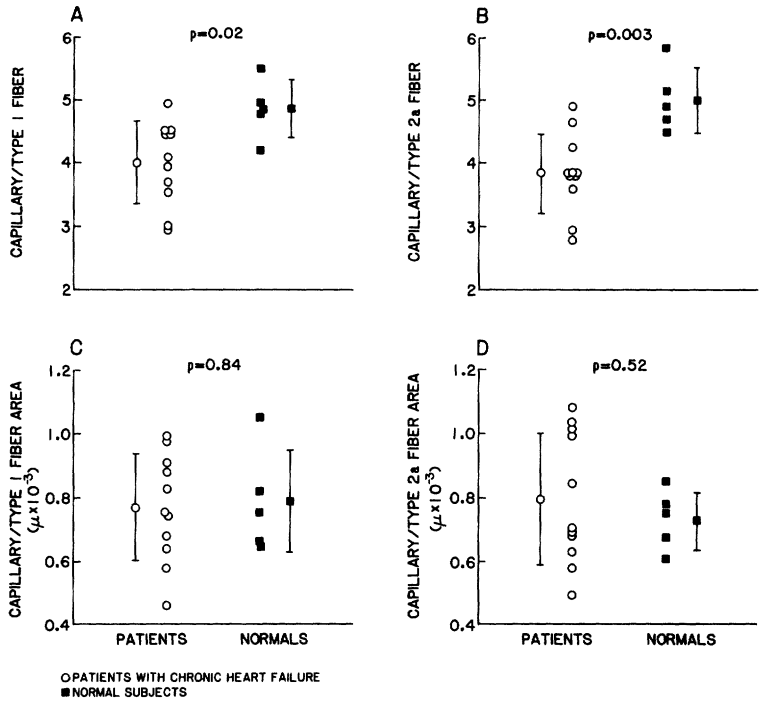

FIGURE 4. *Graphic plotting of ratio of capillaries to individual fibers* (Panels A and B) *and ratio of capillaries to fiber area* (Panels C and D) *for type I and type IIa fibers in patients and in normal subjects.*

glycogenolysis and glycolysis, including total phosphorylase, phosphofructokinase, pyruvate kinase, and lactate dehydrogenase, was no different in the two groups (Figure 1). Table 4 contains glycolytic enzyme content in individual patients and normal subjects. Hexokinase, an enzyme that mediates both glucose entry into the cell and glycolysis, tended to be decreased in patients (Figure 2A), but this difference did not reach statistical significance ($p=0.08$). Succinate dehydrogenase and citrate synthetase, representative oxidative enzymes of the citric acid cycle, were decreased in patients as compared with normals (Figures 2B and 2C). 3-Hydroxyacyl-CoA dehydrogenase, an enzyme involved in β-oxidation of fatty acids, was also decreased in patients (Figure 2D). Table 5 lists hexokinase and oxidative enzyme data in individual patients and normal subjects. Similar intergroup differences in enzyme activity were seen when the results were expressed in mM/min/kg of wet weight of muscle (Table 6).

Histologic Analysis

Biopsy samples for histology were not adequate for analysis in two normal subjects. The percentage of fibers that were classified as slow twitch type I was reduced in patients, whereas the percentage of fast twitch type IIb fibers was increased (Figure 3). There was no difference in the relative composition of fast twitch type IIa or type IIc fibers in the two groups (Figure 3). There was no statistical difference in the size of type I fibers (5.4±1.4 vs. 6.0±1.1 $\mu m^2 \times 10^3$, $p=0.45$) or type IIa fibers (5.2±1.6 vs. 6.4±1.2 $\mu m^2 \times 10^3$, $p=0.11$) in patients as compared with normal subjects, whereas type IIb fibers were smaller in patients (4.1±1.4 vs. 5.7±0.8 $\mu m^2 \times 10^3$, $p=0.03$). Capillary staining was technically inadequate in two normal subjects. The number of capillaries adjacent to individual fibers was reduced in type I and type IIa fibers in patients (Figures 4A and 4B). Because muscle fibers tended to be smaller in patients, however, there were no differences in the ratio of capillaries to muscle fiber area in the two groups (Figures 4C and 4D).

Discussion

The results of the present study demonstrate important alterations in skeletal muscle histology and biochemistry in patients with long-term heart failure. When compared with normal subjects, our patients demonstrated a decrease in slow twitch type I fibers, which have a high potential for aerobic oxidation, and an increase in fast twitch type IIb fibers, which was accompanied by reductions in aerobic oxidative enzyme activity. Previous studies[8–12,26,27] have demonstrated that skeletal muscle enzymatic and histologic changes similar to those seen in our patients provide a potent stimulus for the early onset of anaerobic metabolism and, consequently, contribute to fatigue during exercise. The present study provides evidence that supports the concept that skeletal muscle biochemistry and histology can play a role in the pathophysiology of exertional fatigue in patients with long-term heart failure.

Previous studies demonstrated that exercise training in normal subjects delays lactate production during exercise and increases mitochondrial enzymes involved in aerobic oxidation in the citric acid cycle, cytochromes, and fatty acid pathways.[8–11] Exercise training also increases glycogen stores, hexokinase, triglyceride stores, capillary density, fiber size, and,

possibly, the percentage of slow twitch fibers in normal subjects usually without altering enzymes involved in glycolysis or glycogenolysis.[8,11] In contrast, exercise deconditioning has the opposite effects on skeletal muscle[8,11,26,27] and is accompanied by an increase in lactate production during submaximal exercise. Many of the biochemical and histologic alterations seen in our patients are consistent with the effects of exercise deconditioning; that is, glycolytic enzymes were unchanged, aerobic oxidative enzymes and glycogen content were reduced, and fiber size was decreased. Some of the histologic changes seen in our patients, including alterations in fiber type composition and a decrease in the number of capillaries around each fiber, however, were not demonstrated in several studies in normal subjects[8,26,28] after a period of reduced physical activity. Because both fiber type composition and aerobic enzyme levels were altered in patients, it is possible that reduced type I fiber composition can be largely responsible for the reduced aerobic enzyme activity seen in this group. Evidence from cross-sectional population studies suggests that alteration in fiber type composition might occur as the result of years of physical inactivity.[8] Thus, the pattern of skeletal muscle changes seen in our patients is consistent with the effects of long-term exercise deconditioning, but the magnitude of these changes exceeds what has previously occurred after exercise deconditioning in normal subjects.[8,26,28]

The finding that femoral venous oxygen saturation was decreased at rest and during submaximal exercise suggests that skeletal muscle hypoxia because of hypoperfusion was present in our patients.[2,3] Long-term exposure to hypobaric hypoxia causes reductions in aerobic enzyme content[22] and fiber size in skeletal muscle but does not seem to change the ratio of capillaries to fibers.[8] Our patients demonstrated a decrease in the number of capillaries adjacent to individual fibers although the fiber area served by each capillary was not changed.

Some studies have shown that patients with long-term peripheral arterial insufficiency and claudication during exercise can develop a compensatory increase in skeletal muscle oxidative enzyme content.[8] These changes serve to delay anaerobic metabolism during exercise in the setting of hypoperfusion.[29,30] Previous studies by Walker et al[29] indicate that during ischemic exercise, type I fibers, which are rich in oxidative enzymes, are more resistant to fatigue than type II fibers. In contrast to the adaptations seen in some patients with claudication, the skeletal muscle adaptations in patients with long-term heart failure would be expected to accelerate the onset of anaerobic metabolism in the setting of decreased skeletal muscle blood flow. Thus, despite the finding that skeletal muscle blood flow during exercise is reduced in both patients with claudication[31] and long-term heart failure,[2,3] it is possible that the stimuli inducing skeletal muscle adaptations might be different in these two disorders.

Dunnigan et al[32] examined skeletal muscle histology in a group of young patients with idiopathic cardiomyopathy and in a group of young patients with ventricular tachyarrythmias. Both groups demonstrated increased lipid deposits and fibrosis in skeletal muscle, suggesting that a generalized myopathy might have been responsible for both the cardiac and skeletal muscle abnormalities. All of our patients had heart failure on the basis of left ventricular dysfunction because of coronary artery disease. Therefore, it is unlikely that the skeletal muscle changes demonstrated in the present study were caused by a generalized myopathic process affecting both skeletal and cardiac muscle. Lipkin et al[33] have reported a variable decrease in type II fiber size and an increase in lipid content in skeletal muscle in patients with long-term heart failure. In preliminary reports, Mancini et al[34] and Yancey et al[35] have also reported decreased aerobic enzyme content in patients with long-term heart failure. Drexler et al[36] have described alterations in mitochondrial morphology that suggest reduced oxidative enzyme capacity. Thus, it seems that a number of laboratories have also recently identified alterations in skeletal muscle in patients with long-term heart failure.

The aerobic enzyme levels and percentage of fibers classified as type I reported in our normal subjects were typical for sedentary males as reported in previous studies.[8,22,24] Elite athletes can demonstrate levels of citrate synthetase and succinate dehydrogenase that are 200% higher than the values seen in our normal subjects and can demonstrate more than 75% type I fibers.[8] Thus, although our normal subjects were probably more physically active than our patients, their skeletal muscle biochemical and histologic characteristics are representative of untrained community-dwelling males of this age.

Study Limitations

We did not study large numbers of patients or normal subjects, and comparison of individual values for aerobic enzyme levels (Figure 2, Table 5) reveals an overlap between the two groups. Patient 7, who was involved in aerobic exercise training and had a peak $\dot{V}O_2$ of 20.6 ml/kg/min, demonstrated oxidative enzyme levels that were consistently in the normal range, whereas normal subject 6, who was completely sedentary (did not climb stairs) and had a peak $\dot{V}O_2$ of 18.7 ml/kg/min, demonstrated reduced aerobic enzyme levels. Our data suggest that reduced activity levels can play an important role in inducing skeletal muscle alterations in patients. Thus, the present study does not provide conclusive evidence that the heart failure state leads to abnormalities in skeletal muscle that are independent of deconditioning effects.

Summary

The present study has demonstrated that important alterations in skeletal muscle histology and biochemistry can be present in ambulatory patients

with long-term heart failure, including a decrease in oxidative enzyme content, 3-hydroxyacyl-CoA-dehydrogenase, glycogen, and hexokinase when compared with age-matched normal subjects. These changes were accompanied by a decrease in the relative composition of highly oxidative, fatigue resistant, type I fibers and an increase in fast twitch type IIb fibers, and a decrease in the number of capillaries surrounding each fiber when compared with normal subjects. Similar biochemical and histologic changes have been previously demonstrated after exposure to hypoxia or exercise deconditioning[8,22] and are associated with the acceleration of lactate production during exercise. Thus, the results of the present study suggest that alterations in skeletal muscle might play a role in both the pathophysiology of exercise intolerance and, possibly, the response to therapy in this disorder.

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