JACC Vol. 29, No. 5
April 1997:1067–73

Effects of Endurance Training on Mitochondrial Ultrastructure and Fiber Type Distribution in Skeletal Muscle of Patients With Stable Chronic Heart Failure

RAINER HAMBRECHT, MD, EDUARD FIEHN, MD, JIANGTAO YU, MD, JOSEF NIEBAUER, MD, CLAUDIA WEIGL, MD, LUTZ HILBRICH, MD, VOLKER ADAMS, PHD, URS RIEDE, MD,* GERHARD SCHULER, MD

Leipzig and Freiburg, Germany

Objectives. **The present study was designed to evaluate the effects of an ambulatory training program in patients with chronic heart failure (CHF) on the ultrastructural morphology of mitochondria and fiber type distribution of skeletal muscle and its relation to peripheral perfusion.**

Background. **Recent studies in patients with CHF have suggested that intrinsic abnormalities in skeletal muscle can contribute to the development of early lactic acidosis and fatigue during exercise.**

Methods. **Patients were prospectively randomized to either a training group (n = 9; mean [±SD] left ventricular ejection fraction [LVEF] 26 ± 10%) participating in an ambulatory training program or to a physically inactive control group (n = 9; LVEF 28 ± 10%). At baseline and after 6 months, patients underwent symptom-limited bicycle exercise testing with measurement of central and peripheral hemodynamic variables as well as percutaneous needle biopsies of the vastus lateralis muscle. The mitochondrial ultrastructure of skeletal muscle was analyzed by ultrastructural morphometry; cytochrome *c* oxidase activity was visualized by histochemistry and subsequently quantitated by morphometry. The fiber type distribution was determined by adenosine triphosphatase staining.**

Results. **After 6 months of exercise training there was a significant increase of 41% in the surface density of cytochrome *c* oxidase–positive mitochondria (SVMOcox+) ($p < 0.05$ vs. control) and of 43% in the surface density of mitochondrial cristae (SVMC) ($p < 0.05$ vs. control). Furthermore, exercise training induced a 92% increase in the surface density of the mitochondrial inner border membrane ($p < 0.05$ vs. control). In contrast, the total number of cytochrome *c* oxidase–positive mitochondria remained essentially unchanged. Exercise-induced improvement in peak oxygen uptake was closely linked to changes in SVMOcox+ ($p < 0.01$, $r = 0.66$). After exercise training, changes in submaximal femoral venous lactate levels were not related to changes in submaximal leg blood flow ($r = -0.4$), but were inversely related to changes in the volume density of mitochondria ($p = 0.01$; $r = -0.6$) as well as to changes in SVMC ($p < 0.05$; $r = -0.5$). After exercise training there was a "reshift" from type II to type I fibers ($p < 0.05$ vs. control).**

Conclusions. **Patients with CHF who engage in regular physical exercise show enhanced oxidative enzyme activity in the working skeletal muscle and a concomitant reshift to type I fibers. These exercise-induced changes in oxidative capacity appear to be unrelated to changes in peripheral perfusion.**

(J Am Coll Cardiol 1997;29:1067–73)

It has been well established that alterations in peripheral hemodynamic variables and intrinsic abnormalities in skeletal muscle structure and metabolism in patients with chronic heart failure (CHF) are responsible for the early onset of anaerobic metabolism during exercise, which contributes substantially to their reduced exercise capacity (1–5). Major alterations in skeletal muscle ultrastructure and biochemistry have been demonstrated in patients with CHF, including fiber atrophy, transformation of slow-twitch type I to fast-twitch type II fibers and a decrease in oxidative enzyme capacity.

Recently published studies in our laboratory (6) and others (1,2,5,7–10) have focused on the effect of exercise training on central and peripheral hemodynamic data as well as on skeletal muscle structure and metabolism. We demonstrated that regular physical exercise increases maximal exercise tolerance and delays anaerobic metabolism during submaximal exercise in patients with CHF. Improved aerobic exercise capacity was closely linked to an exercise-induced increase in oxidative capacity of the working skeletal muscle.

In the present study, we sought to investigate in the same patient group the effects of regular physical exercise on the ultrastructure of mitochondria (i.e., surface density of mito-

From the Herzzentrum der Universität Leipzig, Abteilung Kardiologie, Leipzig; and *Pathologisches Institut, Universität Freiburg, Freiburg, Germany. This study was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) (HA2165/3-2) and by Zeneca Pharmaceutical Company, Plankstadt, Germany and was presented in part at the 69th Scientific Sessions of the American Heart Association, New Orleans, Louisiana, November 1996.

Manuscript received August 5, 1996; revised manuscript received December 11, 1996, accepted December 20, 1996.

Address for correspondence: Dr. Rainer Hambrecht, Heart Center, University of Leipzig, Department of Cardiology, Russenstrasse 19, 04289 Leipzig, Germany.

0735-1097/97/$17.00
PII S0735-1097(97)00015-6

Abbreviations and Acronyms

ATPase	=	adenosine triphosphatase
CHF	=	chronic heart failure
LVEF	=	left ventricular ejection fraction
SVM_{IBM}	=	surface density of mitochondrial inner border membrane
SVMC	=	surface density of mitochondrial cristae
SVMO	=	surface density of mitochondria
SVMOcox+	=	surface density of cytochrome *c* oxidase–positive mitochondria
Vt	=	ventilatory threshold
VVM	=	volume density of mitochondria
VVMcox+	=	volume density of cytochrome *c* oxidase–positive mitochondria

chondrial cristae and inner border membrane) and muscle fiber distribution in skeletal muscle. In addition, we intended to examine the relation between exercise-induced changes in peripheral hemodynamic data and the oxidative capacity of mitochondria in skeletal muscle.

Methods

The patient group, exercise training methods and procedures for incremental bicycle exercise testing were described previously (6). In brief, 22 patients were initially enrolled in the study; three patients from the training group and one patient from the control group did not complete the study and were excluded from analysis. One patient from each group refused follow-up examinations. One patient from the training group had to be excluded because of atrioventricular node reentrant tachycardia, and another patient from the training group died suddenly, unrelated to exercise. The remaining 18 patients (nine patients from the training group and nine patients from the control group) who completed the study were included in the analysis. The central and peripheral hemodynamic changes in response to long-term exercise were reported previously (6).

Patient selection. Male patients with clinical, radiologic and echocardiographic signs of CHF (New York Heart Association functional class II–III) as a result of dilated cardiomyopathy or ischemic heart disease were asked to participate in this study. Inclusion criteria were reduced left ventricular ejection fraction (LVEF <40%), as assessed by radionuclide scintigraphy, or a restricted fractional shortening <30% by echocardiography, willingness to participate in the study for at least 6 months and permanent residence within 25 km of the training facilities. Physical work capacity at baseline had to be >25 W without signs of myocardial ischemia, i.e., angina pectoris or ST segment depression. Patients with exercise-induced myocardial ischemia, ventricular tachyarrhythmias (>Lown 4a), significant valvular heart disease, uncontrolled hypertension, peripheral vascular disease, chronic obstructive pulmonary disease and orthopedic or other conditions precluding regular participation in exercise sessions were excluded from the study.

Study protocol. Written informed consent was obtained from all patients before they entered the study, which was approved by the Local Ethics Committee. Patients had to be clinically stable for at least 3 months before entry into the study. Before baseline measurements, all patients underwent a maximal exercise test and participated in one group training session with 24-h Holter monitoring to get acquainted with the examinations and to detect exercise-induced ventricular tachyarrhythmias.

At baseline and after 6 months, percutaneous needle biopsies of the vastus lateralis muscle were obtained. Two days after exercise testing, patients were instrumented with a 5F thermodilution Swan-Ganz catheter, which was positioned in the right femoral vein as described by Sullivan et al. (10), and with a 7F Swan-Ganz catheter, which was introduced into the right pulmonary artery. Hemodynamic and gas exchange measurements as well as blood samples for determination of oxygen saturation and femoral venous lactate levels were simultaneously obtained at rest and at the end of each work load during bicycle exercise. Exercise testing was performed on a calibrated, electronically braked bicycle in an upright position. Work load was increased progressively every 3 min in steps of 25 W until exhaustion. Exercise testing was repeated after 6 months.

After baseline measurements patients were randomized to either a training group or to an inactive control group. Patients assigned to the training program stayed on an intermediate care ward for the initial 3 weeks of the training program. Patients exercised six times daily for 10 min on a bicycle ergometer under close supervision. They were instructed to warm up before each training session at 25 W for 1 min and then to increase the resistance setting until the heart rate was 70% of the previously determined maximum. On discharge from the hospital patients were lent a bicycle ergometer for use at home. They were asked to exercise close to their target heart rate twice daily for a minimum of 40 min altogether. In addition, they were expected to participate in at least two group training sessions of 60 min each per week. Exercise sessions consisted of walking, calisthenics and ball games. An electrocardiography-based pulse-rate monitor was used during exercise outside the hospital to minimize the risk of exercise-induced arrhythmia. Patients assigned to the control group received their individually tailored cardiac medication and were supervised by their private physicians.

Respiratory gas exchange variables. Patients breathed air through a mouthpiece with a nose clip in place, and continuous on-line ventilation and expiratory gas data were collected using a commercially available system (Jaeger EOS-Sprint). Ventilatory threshold (Vt) was defined as the oxygen uptake before the systematic increase in the ventilatory equivalent for oxygen (V_E/V_{O_2}) without a concomitant increase in the ventilatory equivalent for carbon dioxide (V_E/V_{CO_2}) (11). Ventilatory threshold was evaluated by two independent observers who had no knowledge of the patients' identity and the sequence of exercise tests performed.

Muscle biopsy. At baseline and after 6 months, percutaneous needle biopsies from the middle part of the vastus lateralis muscle were obtained under local anesthesia as described by Bergström (12). The specimens were examined using an EM 200 Philips electron microscope. Each biopsy was cut into four blocks and 15 microphotographs were randomly taken from each block. The samples were photographed at a primary magnification of ×15,500 and analyzed at a final magnification of ×60,000 with the aid of a 1,089-point and 121-testline multipurpose test grid superimposed over each microphotograph. The following mitochondrial structures were analyzed: volume density of mitochondria (VVM), surface density of mitochondria (SVMO), surface density of mitochondrial inner border membrane (SVM_{IBM}) and surface density of mitochondrial cristae (SVMC), that is, VVM = counted points/total points of grid, and SVM = intersections per test line/total length of test line of grid, as reported previously (1,13). Using standard stereologic principles of electron microscopy, we obtained the VVM (i.e., mitochondrial volume fraction per unit volume muscle tissue, expressed as percent volume), the SVMO (i.e., surface fraction of mitochondria per unit volume of muscle, expressed as square meters per cubic centimeters), the SVM_{IBM} (i.e., surface fraction of mitochondrial inner border membrane per unit volume of muscle, expressed as square meters per cubic centimeters) and the SVMC (i.e., surface fraction of mitochondrial cristae per unit volume of muscle, expressed as square meters per cubic centimeters).

Histochemistry of cytochrome c *oxidase in mitochondria.* In all patients, the diaminobenzidine cytochrome oxidase reaction was performed in mitochondria as described elsewhere (1), using a modification of the technique reported by Perotti et al. (14), to provide a reliable criterion of the mitochondrial cytochrome oxidase activity in skeletal muscle. From each patient 60 microphotographs were analyzed to determine the VVM and SVMO as described above. The mitochondria were classified as cytochrome oxidase–positive when diaminobenzidine staining was visible within the mitochondria. Diaminobenzidine-negative mitochondria may reflect a low cytochrome oxidase activity. All specimens for ultrastructural morphometry were coded and analyzed by an independent technician who was unaware of clinical data or group assignment.

The first 10 biopsies were analyzed independently by two investigators. The interobserver variability was $r = 0.95$ for the determination of VVM ($p < 0.0001$) and $r = 0.91$ for the determination of SVMC ($p < 0.001$).

Determination of fiber types. One part of the skeletal muscle tissue was immediately frozen in liquid nitrogen for analysis of fiber types (slow-twitch type I and fast-twitch type II), which were determined by the myofibrillar adenosine triphosphatase (ATPase) staining technique resulting from preincubation at pH 9.4 (15). This method distinguishes type I (light staining) fibers from type II (dark staining) fibers. From each muscle biopsy, we randomly cut four blocks and also randomly took five samples from each block. After preincubation the samples were photographed at a final magnification of ×100. Determination of each muscle fiber type were visually conducted as described elsewhere (1). Approximately 50 fibers were contained on each photograph. This method of determining the percentage of a given fiber type within a muscle biopsy was reproducible to within <5% (16).

Table 1. Clinical Characteristics

	Training Group (n = 9)	Control Group (n = 9)
Age (yr)	50 ± 12	52 ± 8
Etiology of heart failure		
Dilated cardiomyopathy	9	8
Ischemic heart disease	0	1
LVEF (%)	26 ± 10	28 ± 10
LVDD (mm)	69 ± 5	66 ± 8
NYHA status		
II	4	6
III	5	3

*Data are presented as mean value ± SD or number of patients. LVDD = left ventricular diastolic dimension assessed by echocardiography; LVEF = left venticular ejection fraction; NYHA = New York Heart Association.

Statistical analysis. The mean value ± SD was calculated for all variables. For statistical evaluation nonparametric tests (Mann-Whitney *U* test, Wilcoxon signed rank test) were used to avoid potential errors from non-normal distribution of data. Linear regression analysis was used to determine the relation of changes of hemodynamic and morphologic variables to change in maximal oxygen uptake. A p value <0.05 was considered statistically significant.

Results

Study group (Table 1). Nine patients with CHF due to left ventricular dysfunction (LVEF 26 ± 10%) completed a period of 6 months of aerobic exercise conditioning. At baseline, patients in the control group (n = 9) did not differ significantly from those in the training group (n = 9) with respect to age, etiology of heart failure, LVEF and left ventricular end-diastolic diameter or functional class. All patients completing the study were taking angiotensin-converting enzyme inhibitors. Eighty-seven percent of the patients were on diuretics and 82% were on digoxin. Drug treatment was comparable in both groups and was not changed in any subject completing the trial during the last 4 weeks before the study or during the study course.

Mitochondrial ultrastructure of skeletal muscle (Table 2). *Training group.* Comparable to the recently reported exercise-induced increase of volume density of cytochrome *c* oxidase–positive mitochondria (VVMcox+) by 41% ($p < 0.05$ vs. control) (6), surface density of cytochrome *c* oxidase–positive mitochondria (SVMOcox+) increased significantly by 41% ($p < 0.05$ vs. control), whereas SVMOcox− remained unchanged ($p = 0.5$) after 6 months of physical exercise. Surface density of cox+ mitochondrial cristae increased by 43% ($p < 0.05$ vs. control) to the same extent as SVMOcox+ and

Table 2. Ultrastructural Changes in Skeletal Muscle After Exercise Training

Variable	Baseline	6 Months	Change (%)
Training			
$SVMO_{cox+}$ (m^2/cm^3)	0.24 ± 0.12	0.33 ± 0.10*†	0.09 ± 0.09 (41%)†
$SVMO_{cox-}$ (m^2/cm^3)	0.37 ± 0.09	0.31 ± 0.06	−0.06 ± 0.08 (−15%)
SVM_{IBM} (m^2/cm^3)	0.05 ± 0.05	0.10 ± 0.06*†	0.05 ± 0.04 (92%)†
SVMC (m^2/cm^3)	0.34 ± 0.21	0.48 ± 0.20*†	0.14 ± 0.14 (43%)†
Ncox+ (n)	4.3 ± 2.0	5.1 ± 1.8	0.8 ± 1.1 (19%)
Ncox− (n)	9.4 ± 2.3	8.2 ± 2.0	−1.2 ± 2.8 (−13%)
$SVMO_{cox+}/N_{SVMOcox+}$ (m^2/cm^3)	0.005 ± 0.001	0.007 ± 0.001*†	0.002 ± 0.001 (21%)†
Control			
$SVMO_{cox+}$ (m^2/cm^3)	0.22 ± 0.12	0.17 ± 0.10	−0.05 ± 0.08 (−23%)
$SVMO_{cox-}$ (m^2/cm^3)	0.36 ± 0.08	0.36 ± 0.06	0 ± 0.09 (±0%)
SVM_{IBM} (m^2/cm^3)	0.04 ± 0.04	0.04 ± 0.03	0 ± 0.02 (±0%)
SVMC (m^2/cm^3)	0.32 ± 0.22	0.29 ± 0.18	−0.03 ± 0.10 (−10%)
Ncox+ (n)	3.9 ± 2.2	3.4 ± 1.8	−0.5 ± 1.5 (−13%)
Ncox− (n)	9.4 ± 1.5	9.8 ± 1.4	0.4 ± 2.2 (4%)
$SVMO_{cox+}/N_{SVMOcox+}$ (m^2/cm^3)	0.006 ± 0.002	0.006 ± 0.003	0 ± 0.003 (±0%)

Significantly different from baseline: *$p < 0.05$. Significantly different from control: †$p < 0.05$. Data are presented as mean value ± SD. $SVMO_{cox+(cox-)}$ = surface density of cytochrome *c* oxidase–positive (negative) mitochondria; SVM_{IBM} = surface density of mitochondrial inner border membrane; SVMC = surface density of mitochondrial cristae; Ncox+ (cox−) = mean number of cytochrome *c* oxidase–positive (negative) mitochondria per micrograph; $SVMO_{cox+}/N_{SVMOcox+}$ = mean surface density of a single cytochrome *c* oxidase–positive mitochondrium.

VVMcox+. As a consequence, there were no changes in the relations between SVMC and SVMOcox+ (1.34 ± 0.32 vs. 1.42 ± 0.26; $p = 0.38$) as well as SVMC and VVMcox+ (0.14 ± 0.04 vs. 0.15 ± 0.03; $p = 0.6$) after the training period. Surface density of mitochondrial inner border membrane, however, increased after exercise training by 92% ($p < 0.05$ vs. control), resulting in a significant increase in the relations between SVM_{IBM} and SVMOcox+ (0.18 ± 0.12 to 0.27 ± 0.12; $p < 0.05$ vs. control) and SVM_{IBM} and VVMcox+ (0.02 ± 0.01 to 0.03 ± 0.01; $p < 0.05$ vs. control). The total number of cytochrome oxidase–positive mitochondria did not change significantly after training ($p = 0.18$), but the mean surface density of a single cytochrome *c* oxidase–positive mitochondrium increased by 31% ($p < 0.05$ vs. control).

Control group. With regard to the surface density of mitochondria, mitochondrial cristae or mitochondrial inner border membrane, there was no significant change after 6 months.

Skeletal muscle fiber types (Table 3, Fig. 1). At the beginning of the study the percentage of slow-twitch type I fibers was not different between the groups. The percentage of slow-twitch type I fibers in patients of both groups averaged 48 ± 6% (type II, 52%) compared with 55 ± 14% (type II, 45%) in normal patients (1). After 6 months of exercise training, a significant 8% increase in type I fibers ($p < 0.05$ vs. control) was observed, whereas type II fibers decreased significantly ($p < 0.05$ vs. control) in these patients. In the control group, a 6% decrease of type I fibers ($p < 0.05$ vs. begin) was detected, and type II fibers tended to increase after 6 months ($p = 0.1$).

Table 3. Muscle Fiber Distribution

Variable	Baseline	6 Months	Change (%)
Training			
Fiber type I (%)	48 ± 7	52 ± 7*	4 ± 4 (8%)†
Fiber type II (%)	52 ± 7	48 ± 6*	4 ± 5 (−8%)†
Control			
Fiber type I (%)	49 ± 5	46 ± 7*	−3 ± 5 (−6%)
Fiber type II (%)	51 ± 5	54 ± 10	3 ± 6 (6%)

Significantly different from baseline: *$p < 0.05$. Significantly different from control: †$p < 0.05$. Data are presented as mean value ± SD.

Relation between changes in femoral venous lactate levels and changes in leg blood flow. To assess the relation of hemodynamic data and skeletal muscle factors in determining the delayed onset of anaerobic metabolism after exercise training, we performed linear regression analysis of changes in femoral venous lactate at submaximal exercise with changes in skeletal muscle ultrastructure and leg blood flow at the corresponding work rate. At the beginning there was no significant correlation between femoral venous lactate levels and leg blood flow during submaximal exercise ($r = -0.33$, $p = 0.22$). After exercise training changes in femoral venous lactate during submaximal exercise were furthermore unrelated to changes in submaximal leg blood flow ($r = -0.36$, $p = 0.2$), but were inversely correlated with changes in volume density of mitochondria ($r = -0.58$, $p = 0.01$) and with changes in SVMC ($r = -0.48$, $p < 0.05$) (Fig. 2).

Correlation between functional capacity and skeletal muscle morphology. After 6 months of physical exercise, changes in peak oxygen uptake was significantly related to changes in SVMOcox+ ($r = 0.66$, $p < 0.01$), SVM_{IBM} ($r = 0.6$, $p < 0.01$) and SVMC ($r = 0.58$, $p < 0.01$). No correlation was found between the surface density of cytochrome oxidase–positive mitochondria at the beginning of the study or the change in surface density of cytochrome oxidase–positive mitochondria and patient age, duration of heart failure or functional class.

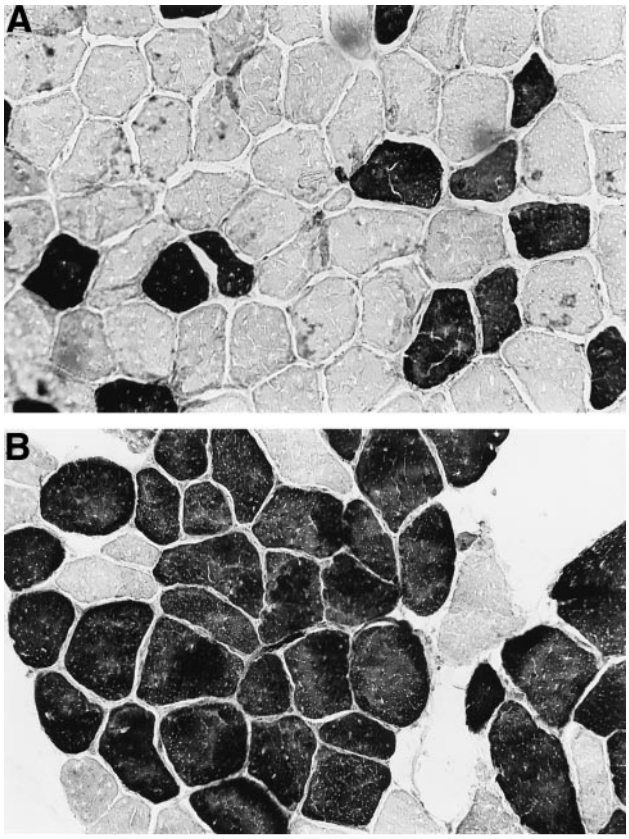

Figure 1. Microphotograph of myofibrillar ATPase stain at pH 9.4 for a normal subject **(A)** and a patient from the control group **(B)**. **Black-stained fibers** are type II and **light fibers** are type I. In the patient with CHF a shift to an increased percentage of type II fibers compared with the normal subject is apparent.

Changes in percentage of fibers being classified as slow-twitch type I fibers were significantly correlated with changes in total volume density of mitochondria ($r = 0.48$, $p < 0.05$), as well as with volume density of cytochrome *c* oxidase–positive mitochondria ($r = 0.52$, $p < 0.05$) (Fig. 3). Furthermore, changes in percentage in type I fibers were also significantly correlated with both changes in oxygen uptake at the Vt ($p < 0.01$, $r = 0.57$) and peak oxygen uptake ($p < 0.01$, $r = 0.62$).

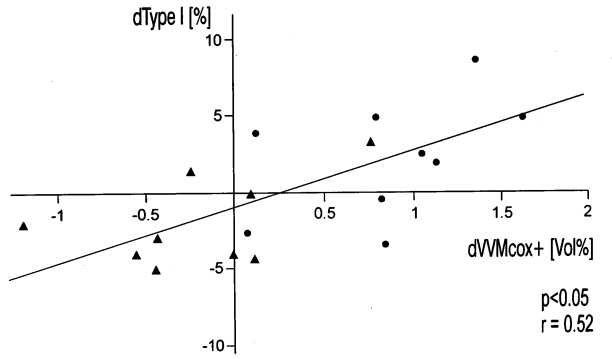

Figure 3. Changes in volume density of cytochrome *c* oxidase–positive mitochondria (dVVMcox+) correlated with changes in muscle fiber type I (dType I). **Circles** = patients from the training group; **triangles** = patients from the control group.

Discussion

The two salient findings derived from this study are 1) aerobic endurance training induces correction of ultrastructural abnormalities and muscle fiber distribution in skeletal muscle in patients with CHF; and 2) the increase of aerobic functional capacity is closely linked to changes in mitochondrial ultrastructure but unrelated to changes in peripheral perfusion. The results of this study highlight the part of skeletal muscle alterations played in the genesis of exercise intolerance in CHF and indicate that persistent regular exercise may at least in part correct some of these changes.

Training effects on mitochondrial ultrastructure. Exercise intolerance and fatigue are hallmarks of CHF, but paradoxically their severity correlates poorly with central hemodynamic variables (17–20). This discrepancy is in part explained by compromised peripheral blood flow, but sufficient evidence has been gleaned from previous studies to suggest a central role of skeletal muscle (5,21–25). These findings include alterations in oxidative metabolism of the skeletal muscle in patients with CHF by ultrastructural, cytochemical and biochemical analysis as well as by nuclear magnetic resonance spectroscopy (1,5,16,26–28). Furthermore, it has been shown that changes in oxidative capacity of skeletal muscles are correlated with

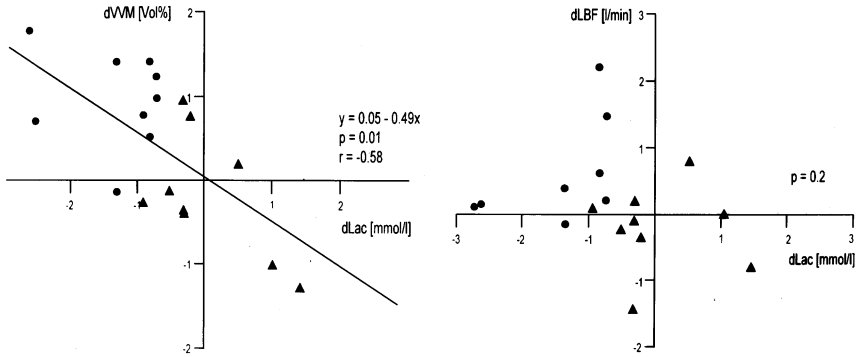

Figure 2. Changes in submaximal femoral venous lactate level (dLac) correlated with changes in submaximal leg blood flow (dLBF) (**right**); changes in submaximal femoral venous lactate level (dLac) correlated with changes in volume density of mitochondria (dVVM) (**left**). **Circles** = patients from the training group; **triangles** = patients from the control group.

changes in exercise capacity (1). In a recently published study (6), we were able to show that in patients with CHF, the volume density of cytochrome *c* oxidase–positive mitochondria could be increased by regular physical exercise. Improvement of functional status was closely linked to changes in oxidative capacity of skeletal muscle. The present study sought to determine the ultrastructural changes of mitochondria in the same patient group. Initially, the surface density of cytochrome *c* oxidase–positive mitochondria was reduced by 45% compared with normal subjects, thereby compromising the oxidative capacity of working skeletal muscle (1). The origin of these changes awaits definition, although several mechanisms have been implicated, such as deconditioning and expression of the inducible form of nitric oxide synthase (1,29,30). This enzyme could be located by immunohistochemical analysis in skeletal muscle myocytes of patients with CHF in our laboratory (unpublished data). The large amounts of nitric oxide produced by nitric oxide synthase is likely to interfere with numerous cellular functions and properties, such as key enzymes of the mitochondrial respiratory chain (31). The results of this study indicate that some of these untoward changes can be ameliorated by regular physical exercise: surface density of mitochondrial inner border membrane increased by 92% and surface density of mitochondrial cristae by 41%. Interestingly, the exercise-induced increase in inner border membranes appears more pronounced than the increase in mitochondrial cristae, suggesting a higher increase in the activity of cytochrome *c* oxidase, a rate limiting enzyme, at the inner border membranes of mitochondria compared with mitochondrial cristae in skeletal muscle cells.

Training effects on muscle fiber distribution. Skeletal muscle abnormalities represent an inherent feature of CHF. These include a shift in fiber type distribution with a significant increase in the proportion of type II fibers and a reduced activity of cytochrome *c* oxidase (1). Fast-twitch fibers (type II) have a low aerobic potential and therefore are easily fatigued. An increase in the percentage of such fibers might be expected to reduce muscle performance during strenuous exercise; slowly contracting, high endurance motor units are primarily recruited during low level exercise (16). In the present study a "reshift" from fast-twitch type II fibers to slow-twitch type I fibers was observed in patients of the training group. These changes were linked to an improvement in functional capacity.

Individual muscle fibers are classified by ATPase staining techniques according to their content of oxidative capacity; fibers falling below a certain critical level are classified as type II, fibers falling above this level as type I. Thus, an overall loss of oxidative capacity, as observed in CHF, may effect fiber type distribution in skeletal muscle by a shift toward type II fibers (1). In this study physical exercise was shown to enhance oxidative capacity across both muscle fiber types and therefore to reverse this trend. In other words, the pattern of fiber types observed at baseline and after exercise therapy reflects overall changes in oxidative capacity.

Metabolic and peripheral hemodynamic variables. In the present study changes in femoral venous lactate accumulation at submaximal exercise are inversely related to changes in aerobic enzyme capacity of skeletal muscle. This finding is consistent with previous studies that identified aerobic enzyme content as an important determinant of lactate production during exercise in humans (32,33) and animals (32,34). Short-term improvements of central hemodynamic data, elicited by administration of inotropic agents, fail to increase peripheral oxygen utilization within the working muscle (35–37), whereas long-term vasodilator therapy may result in correction of peak oxygen uptake after a period of months (35,37). Similarly, skeletal muscle function remains abnormal for up to a year, even after successful heart transplantation associated with nearly instantaneous normalization of central hemodynamic data (38). The previous uncontrolled study by Sullivan et al. (10) has shown that exercise training in this disorder decreases blood lactate levels during submaximal exercise and improves functional capacity without improving cardiac output or leg blood flow during submaximal exercise. In the present study we found an inverse correlation between changes in femoral venous lactate accumulation during exercise and changes in oxidative capacity of skeletal muscle after the training period, but no link to changes in submaximal leg blood flow, suggesting that increases in aerobic enzyme activity in skeletal muscle may play an important role in improving exercise tolerance after training. It is noteworthy that the absent correlation between leg blood flow and femoral venous lactate levels does not necessarily indicate that decreased leg blood flow does not contribute to early anaerobic metabolism in this disorder. However, other factors are likely to be involved in local muscular exercise intolerance of patients with CHF. Such factors may include failure of excitation-contraction coupling (39), inefficiency of chemical to mechanical energy transduction or reflex inhibition of motor unit activation (27). Piepoli et al. (40) demonstrated in a recently published study that physical training not only improves the exercise capacity of the trained forearm but also partially reverses the abnormal responses to exercise through a reduction of the activity of the muscle ergoreflex, either directly or through a reduction of the metabolic signal activating the ergoreceptors. Moreover, CHF is associated with endothelial dysfunction, including impaired endothelium-mediated, flow-dependent dilation. In animal models endothelial function could be partially restored by exercise (41,42), and most recently, Hornig et al. (43) were able to demonstrate that physical exercise restores endothelium-mediated, flow-dependent dilation of the radial artery in patients with CHF after 4 weeks of daily handgrip training, possibly by enhanced endothelial synthesis and release of nitric oxide.

Clinical implications. The results of the present study show that regular physical exercise in patients with CHF induced an improvement in exercise tolerance associated with a reduction in ultrastructural abnormalities of skeletal muscle. Changes in anaerobic metabolism assessed by femoral venous lactate accumulation during submaximal exercise were inversely related to changes in oxidative capacity of skeletal muscle, but unrelated to changes in peripheral perfusion.

Endurance training, carefully and individually tailored to the patient's requirements, seems to be an additional therapeutical option to prevent the deleterious effects of deconditioning in stable patients with CHF.

References

1. Drexler H, Riede U, Münzel T, König H, Funke E, Just H. Alterations of skeletal muscle in chronic heart failure. Circulation 1992;85:1751–9.
2. Wilson JR, Mancini DM, Dunkman WB. Exertional fatigue due to skeletal muscle dysfunction in patients with heart failure. Circulation 1993;87:470–5.
3. Sullivan MJ, Higginbotham MB, Cobb FR. Exercise training in patients with chronic heart failure delays ventilatory anaerobic threshold and improves submaximal exercise performance. Circulation 1989;79:324–9.
4. Mancini DM, Walter G, Reichek N, et al. Contribution of skeletal muscle atrophy to exercise intolerance and altered muscle metabolism in heart failure. Circulation 1992;85:1364–73.
5. Massie BM, Conway M, Yonge R, et al. Skeletal muscle metabolism in patients with congestive heart failure. Circulation 1987;78:1009–19.
6. Hambrecht R, Niebauer J, Fiehn E, et al. Physical exercise in patients with stable chronic heart failure: effects on cardiorespiratory fitness and ultrastructural abnormalities of leg muscles. J Am Coll Cardiol 1995;25:1239–49.
7. Adamopoulos S, Coats AJS, Brunotte F, et al. Physical training improves skeletal muscle metabolism in patients with chronic heart failure. J Am Coll Cardiol 1993;21:1101–6.
8. Minotti JR, Johnson EC, Hudson TL, et al. Skeletal muscle response to exercise training in congestive heart failure. J Clin Invest 1990;86:751–8.
9. Sullivan MJ, Green HJ, Cobb FR. Altered skeletal muscle metabolic response to exercise in chronic heart failure: relation to skeletal muscle aerobic enzyme activity. Circulation 1991;84:1597–1607.
10. Sullivan MJ, Higginbotham MB, Cobb FR. Exercise training in patients with severe left ventricular dysfunction: hemodynamic and metabolic effects. Circulation 1988;78:506–15.
11. Wassermann K, Whipp BJ, Koyal SH, Beaver WL. Anaerobic threshold and respiratory gas exchange during exercise. J Appl Physiol 1973;35:236–43.
12. Bergström J. Muscle electrolytes in man. Scand J Clin Lab Invest 1962;14: 7–110.
13. Hoppeler H, Lüthi P, Claassen H, Weibel ER, Howald H. The ultrastructure of the normal human skeletal muscle: a morphometric analysis on untrained men, women, and well-trained orienteers. Pflugers Arch 1973;344:217–32.
14. Perotti ME, Anderson WA, Swift H. Quantitative cytochemistry of the diaminobenzidine cytochrome oxidase reaction product in mitochondria of cardiac muscle and pancreas. J Histochem Cytochem 1983;31:351–65.
15. Padykula HA, Herman E. The specifity of the histochemical method for adenosine triphosphatase. J Histochem Cytochem 1955;3:170–95.
16. Mancini DM, Coyle E, Coggan A, et al. Contribution of intrinsic skeletal muscle changes to 31-P NMR skeletal muscle metabolic abnormalities in patients with chronic heart failure. Circulation 1989;80:1338–46.
17. Franciosa JA, Park M, Levine TB. Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. Am J Cardiol 1981;47:33–9.
18. Myers J, Froehlicher VF. Hemodynamic determinants of exercise capacity in chronic heart failure. Ann Intern Med 1991;115:377–86.
19. Szlachcic J, Massie BM, Kramer BL, Topic N, Tubau J. Correlates and prognostic implication of exercise capacity in chronic heart failure. Am J Cardiol 1985;55:1037–42.
20. Higginbotham MB, Morris KG, Conn EH, Coleman RE, Cobb FR. Determinants of variable exercise performance among patients with severe left ventricular dysfunction. Am J Cardiol 1983;51:52–60.
21. Zelis R, Nellis SH, Longhurst J, Lee G, Mason DT. Abnormalities in the regional circulations accompanying congestive heart failure. Prog Cardiovasc Dis 1975;72:494–510.
22. Wilson JR, Martin JL, Schwartz D, Ferraro N. Exercise intolerance in patients with chronic heart failure: role of impaired nutritive flow to skeletal muscle. Circulation 1984;69:1079–87.
23. Buller NP, Jones D, Poole-Wilson PA. Direct measurement of skeletal muscle fatigue in patients with chronic heart failure. Br Heart J 1991;65: 20–4.
24. Minotti JR, Christoph I, Oka R, Weiner MW, Wells L, Massie BM. Impaired skeletal muscle function in patients with congestive heart failure: relationship to systemic exercise performance. J Clin Invest 1991;88:2077–82.
25. Wilson JR, Fink L, Maris J, et al. Evaluation of energy metabolism in skeletal muscle in patients with heart failure with gated phosphorus-31 nuclear magnetic resonance. Circulation 1985;71:57–62.
26. Sullivan MJ, Green HJ, Cobb FR. Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. Circulation 1990;81:518–27.
27. Massie BM, Conway M, Rajagopalan B, et al. Skeletal muscle metabolism during exercise under ischemic conditions in congestive heart failure: evidence for abnormalities unrelated to blood flow. Circulation 1988;78: 320–6.
28. Wiener 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;73:1127–36.
29. Bredt DS, Snyder SH. Nitric oxide: a physiological messenger molecule. Ann Rev Biochem 1994;63:175–95.
30. Gross SS, Wolin MS. Nitric oxide: pathophysiological mechanisms. Ann Rev Physiol 1995;57:737–69.
31. Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH. Reversible inhibition of cytochrome *c* oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. FEBS Lett 1994;345:50–4.
32. Dudley GA, Tullson PC, Terjung RL. Influence of mitochondrial content on the sensitivity of respiratory control. J Biol Chem 1987;262:9109–14.
33. Ivy JL, Withers RT, Van Handel PJ, Elger DH, Costill DL. Muscle respiratory capacity and fiber type as determinants of the lactate threshold. J Appl Physiol 1980;48:523–7.
34. Walker PM, Idstrom JP, Schersten T, Bylund-Fellenius AC. Metabolic response in different muscle types to reduced blood flow during exercise in perfused rat hindlimb. Clin Sci 1982;63:293–9.
35. Massie BM. Exercise tolerance in congestive heart failure: role of cardiac function, peripheral blood flow, and muscle metabolism and effect of treatment. Am J Med 1988;84:75–82.
36. Wilson JR, Martin JL, Ferraro N, Weber KT. Effect of hydralazine on perfusion and metabolism in one leg during upright bicycle exercise in patients with heart failure. Circulation 1983;68:425–32.
37. Drexler H, Banhardt U, Meinertz T, et al. Contrasting peripheral short-term and long-term effects of converting enzyme inhibition in patients with congestive heart failure. Circulation 1989;79:491–502.
38. Stratton JR, Kemp GJ, Daly RC, Yacoub M, Rajagopalan B. Effects of cardiac transplantation on bioenergetic abnormalities of skeletal muscle in congestive heart failure. Circulation 1994;89:1624–31.
39. Perreault CL, Gonzalez-Serratos H, Litwin SE, Sun X, Franzini-Amstrong C, Morgan JP. Alterations in contractility and intracellular Ca^{2+} transients in isolated bundles of skeletal muscle fibers from rats with chronic heart failure. Circ Res 1993;73:405–12.
40. Piepoli M, Clark AL, Volterrani M, Adampopoulos V, Sleight P, Coats AJS. Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. Circulation 1996;93:940–52.
41. Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze T. Chronic exercise in dogs increases coronary vascular nitric oxide synthase gene expression. Circ Res 1994;74:349–53.
42. Muller JM, Myers PR, Laughlin MH. Vasodilator responses of coronary resistance arteries of exercise-trained pigs. Circulation 1994;89:2308–14.
43. Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. Circulation 1996;93:210–4.