Anti-Inflammatory Effects of Exercise Training in the Skeletal Muscle of Patients With Chronic Heart Failure

Stephan Gielen, MD,* Volker Adams, Ph.D.,* Sven Mobiüs-Winkler, MD,* Axel Linke, MD,* Sandra Erbs, MD,* Jiangtao Yu, MD,* Werner Kempf, MD,‡ Andreas Schubert, Ph.D,† Gerhard Schuler, MD,* Rainer Hambrecht, MD*
Leipzig, Germany; and Zürich, Switzerland

OBJECTIVES

The aim of this study was to assess the effects of regular physical exercise on local inflammatory parameters in the skeletal muscle of patients with chronic heart failure (CHF).

BACKGROUND

Inflammatory activation with increased serum cytokine levels and expression of inducible nitric oxide synthase (iNOS) in the myocardium and peripheral skeletal muscles has been described in CHF.

METHODS

Twenty male patients with stable CHF (left ventricular ejection fraction 25 ± 2%; age 54 ± 2 years) were randomized to a training group (n = 10) or a control group (n = 10). At baseline and after six months, serum samples and vastus lateralis muscle biopsies were obtained. Serum tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, and IL-1-beta levels were measured by enzyme-linked immunosorbent assay, local cytokine, and iNOS expression by real-time polymerase chain reaction.

RESULTS

Exercise training improved peak oxygen uptake by 29% in the training group (from 20.3 ± 1.0 to 26.1 ± 1.5 ml/kg · min; p < 0.001 vs. control group). While serum levels of TNF-alpha, IL-6, and IL-1-beta remained unaffected by training, local skeletal muscle TNF-alpha decreased from 1.9 ± 0.4 to 1.2 ± 0.3 relative U (p < 0.05 for change vs. control group), IL-6 from 71.3 ± 16.5 to 41.3 ± 8.8 relative U (p < 0.05 vs. begin), and IL-1-beta from 2.7 ± 1.1 to 1.4 ± 0.6 relative U (p = 0.02 vs. control group). Exercise training also reduced local iNOS expression by 52% (from 6.3 ± 1.2 to 3.0 ± 1.0 relative U; p = 0.007 vs. control group).

CONCLUSIONS

Exercise training significantly reduced the local expression of TNF-alpha, IL-1-beta, IL-6, and iNOS in the skeletal muscle of CHF patients. These local anti-inflammatory effects of exercise may attenuate the catabolic wasting process associated with the progression of CHF.

(J Am Coll Cardiol 2003;42:866–8) © 2003 by the American College of Cardiology Foundation

Inflammatory activation with increased serum cytokine levels has recently been described as an important factor for the progression of chronic heart failure (CHF). In multifactorial analyses, elevated levels of tumor necrosis factor (TNF)-alpha and interleukin (IL)-6 were identified as novel prognostic markers (1). The pathophysiologic background was further elucidated by the observation that cytokines act as catabolic factors involved in the pathogenesis of peripheral muscle wasting and cardiac cachexia (2,3). Increased levels of serum TNF-alpha, for example, were found in patients with reduced leg cross-sectional area and peripheral muscle strength (4). Despite the intriguing statistical association between elevated serum cytokine levels (most notably TNF-alpha) and subjective (New York Heart Association [NYHA] functional class) (5) as well as objective (quadriceps muscle strength) (4) parameters of exercise intolerance, the question of whether elevated serum cytokine levels reflect the degree of local inflammation in the skeletal muscle, the target organ for wasting and cachexia, has yet to be addressed.

Cytokines may affect muscle metabolism and strength by direct effects, for example, on the expression of the sarcoplasmic reticulum Ca2+ -adenosine triphosphatase and phospholamban (as observed in cardiomyocytes) (6) or by induction of other pathologic factors, most notably the inducible isof orm of nitric oxide synthase (iNOS), which has been shown to be stimulated by IL-1-beta and TNF-alpha via activation of nuclear factor-kappa B (7). The elevated expression of iNOS was confirmed in skeletal muscle biopsies from patients with CHF (8,9), and it has been proposed that excessive intracellular levels of nitric oxide produced by iNOS may inhibit key aerobic enzymes like cytochrome c oxidase (COX) and reduce peak oxygen uptake (9).

Exercise training in CHF has been consistently documented to improve exercise tolerance, increase maximal oxygen uptake (VO2max), and augment skeletal muscle COX activity and mitochondrial volume density (10,11). It remains a matter of continuing discussion, however, whether exercise training just reverses changes caused by muscle disuse or interferes with the catabolic factors in-
Before discharge from the hospital, maximal symptom-limited ergospirometry was performed to calculate training target heart rate for home exercise training, which was defined as the heart rate reached at 70% of the VO$_2$max uptake during symptom-limited exercise. Upon discharge, patients were provided with bicycle ergometers for daily home exercise training. They were asked to exercise close to their target heart rate daily for 20 min per day. In addition, they were expected to participate in one group training session (walking, calisthenics, and noncompetitive ball games) of 60 min each week.

Patients assigned to the control group continued their sedentary lifestyle and remained on their individually tailored cardiac medication supervised by their private physicians. All examinations including exercise testing were repeated after six months.

Exercise testing and respiratory variables. Exercise testing was performed on a calibrated, electronically braked bicycle in an upright position with work load increasing progressively every 3 min in steps of 25 W beginning at 25 W. Respiratory gas exchange data were determined continuously throughout the exercise test as previously described (12).

Measurement of serum TNF-alpha, IL-6, and IL-1-beta. The concentration of TNF-alpha, IL-6, and IL-1-beta in the serum of patients was determined using commercial available high-sensitive enzyme-linked immunosorbent assay kits (R&D Systems, Wiesbaden, Germany). The sensitivity for the respective assays was 0.18 pg/ml for TNF-alpha, 0.09 pg/ml for IL-6, and 0.10 pg/ml for IL-1-beta.

Skeletal muscle biopsy. Percutaneous needle biopsies were obtained between two and four days before maximal ergospirometry at baseline and again after six months of training. Samples were obtained from the middle part of the vastus lateralis muscle under local anesthesia as described previously (9). The biopsies were either fixed with 4% buffered formaldehyde or snap frozen in liquid nitrogen and stored at −80°C. All investigators involved in the analysis of serum samples and skeletal muscle biopsies were blinded to patient identity, patient assignment (i.e., training or control group), and intervention status (i.e., samples before and after the six month study period).

Quantification of local TNF-alpha, IL-6, IL-1-beta, and iNOS by quantitative reverse transcription-polymerase chain reaction. To quantify local TNF-alpha, IL-1-beta, IL-6, and iNOS messenger ribonucleic acid (mRNA) real time polymerase chain reaction using the Light Cycler System (Roche Diagnostics Inc., Mannheim, Germany) was used. Briefly, aliquots (1 µg) of isolated total ribonucleic acid were reverse transcribed in a volume of 25 µl into complementary deoxyribonucleic acid (cDNA) using random hexamers; 1 µl of cDNA was added to the polymerase chain reaction containing the following specific primers: TNF-alpha-U: 5’-TGT GGT GTC CTT CCT GCA AC-3’, TNF-alpha-L: 5’-CTT GTA GGT GCC CAG GAG AG-3’, IL-1-beta-U: 5’-GGG CCT CAA GGA AAA GAA TC-3’, IL-1-beta-L: 5’-TTC TGC TTG

### Abbreviations and Acronyms

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<th>Abbreviation</th>
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<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<tr>
<td>CHF</td>
<td>chronic heart failure</td>
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<tr>
<td>COX</td>
<td>cytochrome c oxidase</td>
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<tr>
<td>iNOS</td>
<td>inducible nitric oxide synthase</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
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<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>rRNA</td>
<td>ribosomal ribonucleic acid</td>
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<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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<tr>
<td>VO$_2$max</td>
<td>maximal oxygen uptake</td>
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The aim of this prospective randomized clinical trial was, therefore, to quantify serum and local markers of inflammation (TNF-alpha, IL-1-beta, IL-6, and iNOS) in CHF patients in relation to healthy subjects and to assess changes in systemic and local cytokine levels associated with a six month exercise training program in patients with stable CHF.

**METHODS**

**Patient population.** The study group included 20 male patients ≤70 years with CHF (NYHA functional class II to III) as a result of dilated cardiomyopathy or ischemic heart disease as assessed by cardiac catheterization. All patients had clinical, radiologic, and echocardiographic signs of CHF and a left ventricular ejection fraction of <40% as assessed by ventriculography. They had to be in a clinically stable condition for a minimum of three months before enrollment. Patients with CHF received their individually tailored medication including angiotensin-converting enzyme inhibitors, digitalis, diuretics, and beta-blockers when tolerated. All patients were on stable uptitrated CHF medication for three months before randomization. All cardiac medications were held constant throughout the study period, and no additional drugs were introduced after enrollment. Ten age-matched men admitted with atypical chest pain who had normal coronary angiograms and left ventricular function served as healthy controls.

Exclusion criteria were significant valvular heart disease, uncontrolled hypertension, peripheral vascular disease, pulmonary disease, or musculoskeletal abnormalities precluding exercise training. The study protocol was approved by the Ethics Committee of the University of Leipzig, and written informed consent was obtained from all subjects.

**Training intervention.** In order to ensure close supervision, the initial phase of the exercise program was performed on an in-patient basis. During the first two weeks, patients exercised four to six times daily for 10 min on a bicycle ergometer. Workloads were adjusted so that 70% of the symptom-limited maximal oxygen uptake was reached. Before discharge from the hospital, maximal symptom-
AGA GGT GCT GA-3’, IL-6-U: 5’-TAC CCC CAG GAG AAG ATT CC-3’, IL-6-L: 5’-GCC ATC TTT GGA AGG TTC AG-3’, iNOS-U: 5’-CAA GAA CGT GTT CGC CAT GAG GC-3’, iNOS-L: 5’-GGT GAG GCC TTT CCT GAG TGA-3’. The results for mRNA concentrations are expressed as ratio over 18S-ribosomal ribonucleic acid (rRNA), which was amplified as a house keeping gene using the following primers 18S rRNA-U: 5’-TAG AGG GAC AAG TGG CGT TC-3’, 18S rRNA-L: 5’-TGT ACA AAG GGC AGG GAC TT-3’. The variability for triplicate measurements of one biopsy sample was <5%. Between-sample variability was 5.6% for TNF-alpha, 10.0% for IL-1-beta, 6.7% for IL-6, and 9.3% for iNOS expression.

Immunohistochemistry. CD-68–POSITIVE CELLS. To determine the amount of infiltrating macrophages in the muscle biopsies, the sections were stained with a monoclonal anti–CD-68 (Dako GmbH, Hamburg, Germany) using the alkaline phosphatase antialkaline phosphatase (APAAP) technique. For quantification, CD-68–positive cells were counted in four randomly chosen high-power fields and averaged.

CYTOKINES. To identify the cytokine–producing cell-type, IL-1-beta, IL-6, and TNF-alpha were immunohistochemically stained in situ using specific polyclonal antibodies for IL-1-beta (Upstate Biotechnology, Lake Placid, New York), IL-6, and TNF-alpha (Sigma, St. Louis, Missouri). In brief, paraffin sections (3 μm) of the tissue material were dewaxed with xylene and rehydrated. To block the endogenous peroxidase activity the sections were treated with 0.3% hydrogen peroxide in 60% of methanol for 30 min, followed by a 20-min incubation in 0.2% Triton X-100/PBS. The unspecific binding of the antibodies was blocked by 1.5% horse serum diluted in 2% fat free milk/PBS (30 min at room temperature [20°C]). After this incubation, the primary antibody diluted in 2% fat free milk/PBS (IL-1-beta: 1:50; IL-6: 1:50; TNF-alpha: 1:100) was applied to the section and incubated overnight at 4°C. The bound primary antibody was detected by the APAAP method.

iNOS AND NITROTYROSINE. To specifically detect the inducible isoform of nitric oxide synthase in tissue sections, a polyclonal anti–iNOS–specific antibody (Transduction Laboratories, Lexington, Kentucky) was used, and the sensitivity was enhanced by an immunohistochemical protocol using biotinylated tyramid (NEN-DuPont, Dreieich, Germany) as described previously (9). For consecutive duplicate biopsy samples, the variation regarding percent iNOS–positive tissue area was <10%. To confirm the bioactivity of iNOS, nitrotyrosine production was assessed in the skeletal muscle biopsies as recently described by Bachmaier et al. (13).

All immunohistochemical slides for iNOS and nitrotyrosine were prepared in pairs (i.e., one section of a skeletal muscle biopsy before and one section of a biopsy after the intervention) to avoid differences in staining intensity. The positive stained tissue area on each slide was quantified using an automatic image analysis software measuring the area of red staining (KS300, Zeiss Inc., Oberkochem, Germany) by three different researches blinded for patient identity, assignment, and status. The values were then averaged for further statistical analysis.

Statistical analysis. All data are expressed as mean value ± standard error of mean. The data were tested for normal distribution using the Kolmogorov-Smirnov test, and for homogeneity of variances using Levene’s test. Age, baseline left ventricular ejection fraction, and peak oxygen uptake in healthy subjects, control patients, and training patients were compared using the one-way analysis of variance procedure followed by the Tukey post-hoc test. The prevalence of dilated and ischemic cardiomyopathy in each group was compared using Fisher exact test. All intragroup comparisons were made using the Wilcoxon signed rank test, and intergroup comparisons were assessed using the Mann Whitney U test. A p value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics. Twenty patients with stable CHF were randomly assigned to the exercise training group (10 patients) or to the control group (10 patients). Patients in the training group and in the control group showed a significantly reduced left ventricular ejection fraction (training group: 26.1 ± 3.1%, control group: 24.7 ± 2.4%; p = NS) and exercise capacity as determined by peak oxygen uptake (training group: 20.3 ± 1.0 ml/kg min, control group: 17.9 ± 1.6 ml/kg min; p = NS). Age-matched healthy controls, on the other hand, had normal parameters of cardiac function (left ventricular ejection fraction, 72.7 ± 1.5%; VO2max 27.2 ± 0.3 ml/kg min; p < 0.001 vs. training and control groups) (Table 1).

Medical therapy was similar in the training and the control group. Patients were on angiotensin–converting enzyme inhibitors (10 of 10 in the training group and 9 of 10 in the control group), digitals (5 of 10 in the training group and 5 of 10 in the control group), diuretics (4 of 10

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<th>Table 1. Baseline Characteristics</th>
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<tr>
<td>Age (yrs)</td>
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<tr>
<td>NYHA functional class II/III</td>
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<tr>
<td>DCM/ICM</td>
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<tr>
<td>LVEF (%)</td>
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<td>VO2 max (ml/kg min)</td>
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*p < 0.001 versus training CHF and versus control CHF.

CHF = chronic heart failure; DCM = dilated cardiomyopathy; ICM = ischemic cardiomyopathy; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; \( \text{VO}_2 \text{max} \) = peak oxygen uptake.
in the training group and 9 of 10 in the control group), and beta-blockers (4 of 10 in the training group and 4 of 10 in the control group). The medication did not change during the study period.

**Clinical follow-up.** Exercise training improved peak oxygen uptake by 29% in the training group (from 20.3 ± 1.0 to 26.1 ± 1.5 ml/kg/min; p < 0.001 vs. control group), whereas it remained virtually unchanged in the control group (17.9 ± 1.6 vs. 18.1 ± 1.1 ml/kg/min; p = NS). No deaths occurred during the study period. One patient of the training group was admitted to hospital due to symptomatic bradyarrhythmia requiring pacemaker implantation. He continued the study after discharge from hospital.

**Skeletal muscle monocyte/macrophage infiltration.** As quantified by CD-68 staining, the number of inflammatory cells infiltrating the skeletal muscle was not elevated in CHF patients (0.61 ± 0.08 CD-68–positive cells vs. 0.71 ± 0.04 CD-68–positive cells per high power field in healthy subjects; p = NS).

**Skeletal muscle expression of iNOS and local nitrotyrosine concentration.** The increase in local skeletal muscle cytokine mRNA expression in CHF patients was paralleled by a significant increase in iNOS mRNA expression (6.6 ± 0.8 vs. 1.8 ± 1.1 relative U in healthy subjects; p = 0.002) and iNOS protein concentration in CHF patients (2.2 ± 0.4 vs. 1.0 ± 0.2% positive tissue area in healthy subjects; p < 0.05). The functional relevance of increased iNOS levels was confirmed by elevated local nitrotyrosine concentrations (4.1 ± 0.5% vs. 2.0 ± 0.6% positive tissue area in healthy subjects; p = 0.018) (Table 2). In line with the effects on local cytokine concentrations, exercise training reduced local iNOS mRNA expression by 52% (from 6.3 ± 1.2 to 3.0 ± 1.0 relative U, p = 0.017 vs. begin; p = 0.007 vs. control group) and iNOS protein content by 35% (from 2.3 ± 0.5% to 1.5 ± 0.4% positive tissue area; p = 0.017 vs. begin, p = 0.007 vs. control group for change). The reduction in local iNOS expression after the training intervention was associated with a 38% decrease in skeletal muscle nitrotyrosine content (from 4.7 ± 0.8% to 3.1 ± 0.5% positive tissue area; p = 0.028 vs. begin, p = 0.016 vs. control group for change) (Fig. 3).

**DISCUSSION**

Several important findings emerge from this study analyzing the influence of regular physical exercise on skeletal muscle cytokine and iNOS expression.

1. In skeletal muscle biopsies from patients with stable CHF, an increased expression of TNF-alpha, IL-1-beta, and IL-6 was observed in the absence of increased numbers of proinflammatory monocytes/macrophages (as documented by CD-68 staining). Both mRNA quantification and immunohistochemistry indicate a local intramyocyte production of inflammatory cytokines in stable CHF.

### Table 2. Local Skeletal Muscle Cytokine and iNOS Expression

<table>
<thead>
<tr>
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<th>CHF Patients (n = 20)</th>
<th>Healthy Subjects (n = 10)</th>
<th>p Value</th>
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<tbody>
<tr>
<td>TNF-alpha mRNA</td>
<td>1.9 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>&lt;0.001</td>
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<tr>
<td>(relative U)</td>
<td></td>
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<tr>
<td>IL-1beta mRNA</td>
<td>2.8 ± 0.6</td>
<td>0.3 ± 0.1</td>
<td>&lt;0.001</td>
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<tr>
<td>(relative U)</td>
<td></td>
<td></td>
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<tr>
<td>IL-6 mRNA</td>
<td>62.7 ± 10.4</td>
<td>7.0 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(relative U)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iNOS mRNA</td>
<td>6.6 ± 0.8</td>
<td>1.8 ± 1.1</td>
<td>0.002</td>
</tr>
<tr>
<td>(relative U)</td>
<td></td>
<td></td>
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<tr>
<td>iNOS protein (%)</td>
<td>2.2 ± 0.4</td>
<td>1.0 ± 0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(PTA)</td>
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<tr>
<td>Nitrotyrosine (%)</td>
<td>4.1 ± 0.5</td>
<td>2.0 ± 0.6</td>
<td>0.018</td>
</tr>
<tr>
<td>(PTA)</td>
<td></td>
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CHF = chronic heart failure; IL = interleukin; iNOS = inducible nitric oxide synthase; mRNA = messenger ribonucleic acid; PTA = positive tissue area; TNF = tumor necrosis factor.
2. A six-month program of regular physical exercise significantly reduced the local expression of TNF-alpha, IL-1-beta, and IL-6 in the skeletal muscle of patients with stable moderate CHF while serum cytokine levels remained virtually unchanged.

3. Expression of iNOS was confirmed in skeletal muscle biopsies of heart failure patients and was reduced by more than 50% after six months of exercise training. This reduction was paralleled by a significant decrease in local nitrotyrosine levels after the training intervention.

Taken together, these results indicate that long-term aerobic endurance training in CHF patients has anti-inflammatory effects on the skeletal muscle. The reduction of local cytokine expression by exercise may represent a potential anti-catabolic intervention in CHF.

**Effects of exercise training on systemic and local cytokine levels in CHF. Serum cytokine levels.** Because CHF patients included in the present study had to be in a stable clinical condition for a minimum of three months before enrollment, the majority of randomized patients was in NYHA functional class II. It has previously been described that the serum levels of TNF-alpha, IL-1-beta, and IL-6 correlate with the degree of functional impairment as assessed by NYHA functional class (5,14) or 6-min walk test (15). As a consequence, serum cytokine levels were only mildly elevated in CHF patients of the present study with TNF-alpha levels similar to those published by Torre-Amione (5) for Studies Of Left Ventricular Dysfunction (SOLVD) patients in NYHA functional class II. Exercise training did not exert any significant effect on serum cytokine levels in patients with mild CHF. It may well be that the result would have been different in more advanced stages of heart failure.

While no data are available on training effects on systemic cytokine levels Adamopoulos et al. (16) has recently described a reduction in serum levels of granulocyte-macrophage colony-stimulating factor, macrophage chemotactant protein-1, and the soluble adhesion molecules intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1 after training in patients with CHF (16). These endothelial adhesion molecules are regarded as surrogate markers of inflammation because both cytokines and oxygen free radicals have been shown to induce their expression. However, they are cleaved into the circulation by activated endothelial cells. Therefore, the findings by Adamopoulos et al. (16) are important in that they prove a reduction in endothelial cell inflammatory activation after training in CHF.

**Skeletal muscle cytokine levels.** Loss of muscle bulk starts early in the course of CHF (17) as a result of both inactivity and intrinsic alterations associated with the heart failure syndrome (18). Despite reports about correlations between elevated serum cytokines and weight loss (19) or muscle wasting (2), the local expression of cytokines in the skeletal muscle has never been quantified. The present study is the first to show that local expression of TNF-alpha, IL-6, and IL-1-beta is significantly increased in skeletal muscle biopsies of CHF patients with only mildly elevated serum cytokines. The observation that cytokine expression occurs in the absence of infiltrating monocytes or macro-

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**Figure 1.** Local expression of tumor necrosis factor (TNF)-alpha (A), interleukin (IL)-6 (B), and IL-1-beta (C) in skeletal muscle biopsies of patients in the training group (black bars) and the control group (open bars) at study baseline and after six months. *p < 0.05 versus control group at six months; $p < 0.05$ at six months versus respective baseline; $\#p < 0.05$ for change at six months from baseline in training versus control group. mRNA = messenger ribonucleic acid.
phages indicates that skeletal myocytes may produce cytokines in a parakrine/autokrine fashion in quantities large enough to induce iNOS expression. The hypothesis that skeletal myocytes are capable of producing proinflammatory cytokines is consistent with previous observations of Sagizadeh et al. (20), who first described the expression of TNF-alpha by human skeletal muscle, and has been confirmed in the present study by the mRNA quantifications and the immunohistochemical stainings of TNF-alpha, IL-1-beta, and IL-6 in skeletal muscle biopsies.

Up to now, serum cytokine levels in CHF have been regarded as representative and possibly even causative of the peripheral inflammatory activation. In the present study, the expression of TNF-alpha, IL-1-beta, and IL-6 in skeletal muscle biopsies was elevated independently from serum cytokine levels, which were still within normal range in moderate CHF (except for TNF-alpha). High local expression of IL-6 and IL-1-beta in the presence of normal serum levels is consistent with the notion that the local inflammation may precede the systemic cytokine spillover. These findings favor local factors like hypoperfusion and oxidative stress as the initial proinflammatory stimulus.

It has been proposed that the combination of low cardiac output and endothelial dysfunction might precipitate repetitive skeletal muscle ischemia (21). In ischemia-reperfusion situations, reactive oxygen species (ROS) are generated at an increased rate and may play a proinflammatory role at the local level in CHF. Administration of antioxidants abolishes the serum cytokine increase observed after acute exercise (22). Therefore, the well-established effects of exercise on anti-oxidative enzyme expression (23) could contribute to a reduced local proinflammatory cytokine generation. A recently published study Ennezat et al. (24) confirmed that training of CHF patients induces a nearly two-fold increase in Cu/Zn superoxide dismutase and glutathione peroxidase in skeletal muscle biopsies.

Alternatively, improved endothelial function and lower total peripheral resistance after training may reduce skeletal muscle ischemia during exercise (25). The increased expression of endothelial cell nitric oxide synthase could reduce local oxidative stress because nitric oxide itself may act as a free radical scavenger. Even more importantly, endothelial cell nitric oxide synthase–derived nitric oxide directly augments vascular endothelial cell superoxide dismutase expression in vascular smooth muscle cells and modulates the extracellular redox state after exercise training (26). In the present study we did not measure endothelial function and vascular nitric oxide production, so that any definitive answer to which degree better skeletal muscle antioxidative protection or reduced vascular oxidative stress contribute to the lower inflammatory activation must be left for future studies.

Some words of caution remain. As previously reported by Ennezat et al. (24), the tissue samples harvested by skeletal muscle needle biopsy are too small to provide enough material for Western blots. Therefore, the results of the
Effects of exercise on iNOS induction in the skeletal muscle of patients with CHF. Basically, iNOS may be induced by three known pathogenetic factors: cytokines, ROS, and mechanical factors like increased wall-stress in the myocardium (21).

Based on a series of in vitro incubation experiments, we have recently reported that several cytokines are involved in the induction of iNOS expression in L6 skeletal myoblasts (27): after priming with gamma-interferon to elevate the expression of IL-1-beta receptors, IL-1-beta was able to increase iNOS expression in a time- and dose-dependent manner via nuclear factor-kappa-B. While TNF-alpha alone does not affect local iNOS production, it can further increase iNOS levels in the presence of IL-1-beta and gamma-interferon (27). It is consistent with these data that local iNOS expression was reduced in parallel to the reduced local cytokine levels after exercise training.

The role of oxidative stress for iNOS induction has been analyzed in detail in hepatocytes where the iNOS promotor region was found to contain an antioxidative responsive element by which ROS enhances iNOS transcription (28). The previously mentioned antioxidative effects of training in the skeletal muscle may, therefore, directly contribute to the reduction of iNOS expression.

Conclusions. In conclusion, a six-month exercise training program in patients with stable CHF is effective in reducing elevated expression of TNF-alpha, IL-6, and IL-1-beta in the skeletal muscle. The reduction of local inflammatory factors is associated with a reduced iNOS expression and intracellular accumulation of nitric oxide.

The present study confirms that training interventions do not only reverse changes associated with disuse but may, in fact, interfere with the inflammation-induced CHF myopathy, that may—in the long run—result in muscle catabolism, wasting, and cardiac cachexia. Thus, regular exercise in CHF patients should be considered not only as a symptomatic intervention aimed at maintaining exercise capacity but also as a therapeutic strategy with local anti-inflammatory effects.

Reprint requests and correspondence: Prof. Dr. med. R. Hambrecht, Universität Leipzig, Herzzentrum GmbH, Department of Internal Medicine/Cardiology, Strümpellstr. 39, 04289, Leipzig, Germany. E-mail: hamr@medizin.uni-leipzig.de.

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