Antioxidative Effects of Exercise Training in Patients With Chronic Heart Failure

Increase in Radical Scavenger Enzyme Activity in Skeletal Muscle

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Background—In chronic heart failure (CHF), cross-talk between inflammatory activation and oxidative stress has been anticipated in skeletal muscle (SM). The role of the radical scavenger enzymes superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPX), which remove oxygen radicals, has never been assessed in the SM in this context. Moreover, it remains unknown whether exercise training augments the activity of these enzymes in CHF.

Methods and Results—Twenty-three patients with CHF were randomized to either 6 months of exercise training (T) or a sedentary lifestyle (C); 12 age-matched healthy subjects (HS) were studied in parallel. Activity of Cat, SOD, and GPX was assessed in SM biopsies before and after 6 months (6 months). Oxidative stress was determined by measuring nitrotyrosine formation. SOD, Cat, and GPX activity was reduced by 31%, 57%, and 51%, respectively, whereas nitrotyrosine formation was increased by 107% in SM in CHF (P<0.05 versus HS). In CHF, exercise training augmented GPX and Cat activity in SM by 41% (P<0.05 versus before and group C) and 42% (P<0.05 versus before and group C), respectively, and decreased nitrotyrosine production by 35% (from 3.8±0.4% tissue area before to 2.5±0.3% after 6 months; P<0.05 versus before).

Conclusions—The reduced activity of major antioxidative enzymes in the SM of CHF patients is associated with increased local oxidative stress. Exercise training exerts antioxidative effects in the SM in CHF, in particular, due to an augmentation in activity of radical scavenger enzymes. (Circulation. 2005;111:1763-1770.)

Key Words: exercise ■ heart failure ■ muscles ■ free radicals ■ enzymes

In patients with chronic heart failure (CHF), excessive oxidative stress in skeletal muscle (SM) has been linked to peripheral hypoperfusion as a consequence of low cardiac output and peripheral endothelial dysfunction.1–4 On one hand, reactive oxygen species (ROS), produced by a variety of enzymes,1,5–8 are known to induce the expression of inflammatory cytokines, eg, in the SM of patients with CHF.9 On the other hand, cytokines themselves might promote the production of reactive oxygen metabolites.9,10 These findings emphasize the cross-talk between local inflammation and oxidative stress.9–12 Excessive production of free radicals partially contributes to the impairment of muscle function and apoptosis of SM fibers, with the consequent loss in muscle bulk.1,13–16 Nevertheless, SM contains an enzymatic antioxidative system encompassing copper-zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (Mn-SOD), glutathione peroxidase (GPX), and catalase (Cat), which protect the cells from attacks by ROS.17 Currently, it is unknown whether this enzyme system adapts to the enhanced production of ROS or whether the impaired activity of radical scavenger enzymes leads to a further increase in local oxidative stress. Therefore, we aimed to determine whether alteration in the activity of total SOD, GPX, and Cat is associated with enhanced production of free radicals in the SM of patients with CHF. Moreover, we were interested to determine whether significant cross-talk exists between local inflammation and the activity of key radical scavenger enzymes.

Exercise training has been established as adjuvant therapy in CHF, which improves exercise capacity and partially reverses intrinsic alterations of SM.3,4,18,19 Most intriguing was the training-induced reduction of the local expression of tumor necrosis factor (TNF)-α that is known to diminish intracellular levels of reduced glutathione (ie, glutathione containing an SH group [GSH]), which is an essential cofactor of GPX and hence, decrease GPX activity11,20,21; however, it is currently unknown whether the training-mediated downregulation of SM inflammation is associated...
with restoration of the local enzymatic radical scavenger system. Therefore, we aimed to investigate whether aerobic exercise training in stable CHF reconstitutes the activity of SOD, Cat, and GPX, thereby reducing oxidative stress and apoptosis of myocytes in SM.

Methods
A detailed description of the methodology is provided in the online Data Supplement.

Patient Selection
The Ethics Committee of the University of Leipzig approved the protocol of this study, and written, informed consent was obtained from all patients and healthy subjects (HSs). Patients with CHF were randomized to either 6 months of exercise training (training group, T) or a sedentary lifestyle (control group, C). A total of 12 age-matched men, who were admitted for nonspecific chest pain to rule out coronary artery disease, served as HSs. Inclusion and exclusion criteria for CHF patients are described in detail in the online Data Supplement.

Training Protocol
During the first 2 weeks, patients in group T exercised in hospital 4 to 6 times daily for 10 minutes each on a bicycle ergometer adjusted to a workload at which 70% of peak oxygen uptake was reached. The target heart rate for home training was defined as the heart rate reached at 70% of maximum oxygen uptake. Patients were provided with bicycle ergometers for home exercise training. They were encouraged to exercise close to their target heart rate daily for 20 minutes for a period of 6 months and were expected to participate in one group training session, consisting of walking, noncompetitive ball games, and calisthenics, for 60 minutes each week. Patients assigned to group C continued their sedentary lifestyle.

Exercise Testing, Respiratory Variables, and SM Biopsy
Exercise testing was performed on a calibrated, electronically braked bicycle in an upright position. Respiratory gas exchange data were determined continuously throughout the exercise test. Percutaneous needle biopsy samples were obtained from the vastus lateralis muscle as described in the online Data Supplement.

Measurement of mRNA
MnSOD, CuZnSOD, GPX, Cat, interleukin (IL)-1β, and TNF-α mRNAs were quantified by real-time polymerase chain reaction with specific primers (Light Cycler system, Roche Diagnostics Inc.). The mRNA of the radical scavenger enzymes and the cytokines was expressed as a ratio to 18S rRNA, which was amplified as a housekeeping gene.

Quantification of Protein Expression
Protein expression of MnSOD, CuZnSOD, and Cat was determined by Western blot techniques. Expression was quantified by applying a 1-D analysis software package and was normalized to an internal standard that was loaded onto each gel.

Quantification of Enzymatic Activities
The frozen biopsy samples were homogenized, and protein content was determined. Activities of total SOD, GPX, and Cat were measured photometrically according to standard protocols and expressed as units per milligram protein or milliunits per milligram protein.

Measurement of Lipid Peroxidation as a Marker of Oxidative Stress
Homogenates of SM were processed according to the instructions of the manufacture, and lipid peroxide concentrations were determined as a ratio to 18S rRNA, which was amplified as a housekeeping gene.

Results

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics of Patients With CHF and Sedentary HSs</th>
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<tr>
<td></td>
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<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>V0,max, mL·kg⁻¹·min⁻¹</td>
</tr>
<tr>
<td>LVEF, %</td>
</tr>
<tr>
<td>DCM/IHD, n/n</td>
</tr>
<tr>
<td>NYHA, No. in class II/III</td>
</tr>
</tbody>
</table>

V0,max indicates maximal oxygen uptake; LVEF, left ventricular ejection fraction; DCM, dilated cardiomyopathy; IHD, ischemic heart disease; and NYHA, New York Heart Association.

Statistical Analysis
Mean±SE values were calculated for all variables. Intergroup and intragroup comparisons were performed with a 2-way, repeated-measures ANOVA followed by the Tukey post-hoc test, a Mann-Whitney U test, or a Wilcoxon signed-rank test when appropriate. A probability value <0.05 was considered statistically significant.

Baseline Characteristics and Clinical Follow-Up
A total of 23 CHF patients and 12 healthy, age-matched subjects (Table 1) were enrolled in the study. Twelve CHF patients were randomized to the training group, whereas 11 CHF patients remained sedentary as a control group. At baseline, patients in groups T and C did not significantly differ with respect to left ventricular ejection fraction, peak oxygen uptake, and New York Heart Association class. Age-matched HSs had normal parameters of cardiac function (Table 1) and a higher maximal oxygen uptake (V0,max) compared with CHF patients (18.3±0.8 mL·kg⁻¹·min⁻¹ in CHF versus 26.4±1.7 in HSs, P<0.001).

Medical therapy was similar in T and C groups and did not change during the study period. Patients received angioten-
sin-converting enzyme inhibitors (12 patients in group T and 9 patients in group C), digitalis (7 in T and 8 in C), diuretics (8 in T and 8 in C), β-blockers (5 in T and 5 in C), nitrates (1 in T and 2 in C), and statins (0 in T and 2 in C).

One patient with CHF in each group withdrew his consent for study participation. The medication and baseline characteristics of those patients did not differ from those who successfully participated in the entire study. No death or cardiac decompensation occurred, and none of the patients was admitted to the hospital during the study period.

The exercise training program effectively increased peak oxygen uptake in group T by 25%, from 19.0 ± 0.8 before to 23.7 ± 1.4 mL · kg⁻¹ · min⁻¹ at 6 months (P < 0.05 versus before and group C), whereas VO₂max remained unchanged in group C (17.5 ± 1.5 before to 17.8 ± 1.2 mL · kg⁻¹ · min⁻¹ at 6 months).

Expression and Activity of Radical Scavenger Enzymes in SM

**Superoxide Dismutase**
In patients with CHF, SM had a 31% lower total SOD activity compared with that in HSs (5.7 ± 0.4 U/mg in CHF versus 8.3 ± 1.4 in HSs, P < 0.05). MnSOD expression was diminished by 62% at the protein level (P < 0.01 versus HSs) and by 52% at the RNA level (P < 0.05 versus HSs) in comparison with HSs (Table 2). The CuZnSOD protein content was not significantly attenuated (P = NS) in CHF patients, whereas CuZnSOD mRNA was significantly reduced by 48% (P < 0.05 versus HS) compared with HSs (Table 2).

**Catalase**
In CHF, SM was characterized by a 57% reduction in Cat activity (12.7 ± 1.4 U/mg in CHF versus 29.5 ± 4.7 in HSs, P < 0.001), in Cat protein content by 44% (P < 0.001 versus HS), and in Cat mRNA expression by 51% (P < 0.001 versus HSs) in comparison with those of HSs (Table 2).

**Glutathione Peroxidase**
In patients with CHF, the activity of GPX in SM was significantly diminished by 51% (35.5 ± 3.3 μmol/mg in CHF versus 71.9 ± 9.6 g in HSs, P < 0.001) compared with that in HSs. This decrease in GPX activity was accompanied by a 77% attenuation of GPX mRNA expression (P = 0.001 versus HSs) in CHF (Table 2). To elucidate the underlying mechanism, we aimed to determine GPX protein expression. Unfortunately, we (like others) discovered that all of the commercially available antibodies did not reveal signals on Western blots because either cross-reactivity with human proteins was missing or the size of the band was not the expected one for GPX.

Local Oxidative Stress
GPX and Cat are mainly detoxifying hydroxyl radicals that would react with lipids from the SM. Those lipid peroxides can be considered a measure of local oxidative stress and partially represent a readout of GPX as well as Cat activity. Local oxidative stress, measured as lipid peroxidation, in the SM of patients with CHF was significantly elevated (by 151%) compared with HSs (377 ± 70 μmol/mg protein in CHF versus 150 ± 50 in HSs, P < 0.05). In addition, nitrosative production, reflecting formation of the highly reactive peroxynitrite radical, was significantly increased (by 103%) in the SM of patients with CHF in comparison with HSs (3.79 ± 0.34% positive tissue area in CHF versus 1.87 ± 0.45% in HSs, P < 0.05).

Circulating Cytokines, Local Cytokine Expression, and Cross-Talk Between Local Inflammation and Radical Scavenger Enzymes
TNF-α serum levels were significantly elevated (by 135%) in CHF. On the contrary, IL-1β serum levels did not differ between patients with CHF and HSs (Table 3). In CHF, TNF-α and IL-1β mRNA expression in the SM was significantly elevated (6-fold and 9.5-fold, respectively), which was associated with an increase in TNF-α and IL-1β protein levels (by 84% and 63%, respectively) in comparison with HSs (Table 3). Those cytokines were most likely primarily myocyte derived because the majority of the tissue was found to be composed of myocytes (Figure 1). In addition, there was no correlation between serum levels of TNF-α and IL-1β and local expression of the respective cytokines in SM. In contrast, local TNF-α expression was inversely related to GPX (r = −0.63, P < 0.05) and Cat activity (r = −0.55, P < 0.05) in SM.

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**Table 2. mRNA Abundance and Protein Expression of Radical Scavenger Enzymes in SM of Sedentary HSs and Patients With CHF**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sedentary HSs</th>
<th>CHF Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnSOD</td>
<td>2.69 ± 0.73</td>
<td>1.28 ± 0.27*</td>
</tr>
<tr>
<td>CuZnSOD</td>
<td>0.31 ± 0.08</td>
<td>0.16 ± 0.03*</td>
</tr>
<tr>
<td>GPX</td>
<td>0.26 ± 0.06</td>
<td>0.06 ± 0.01†</td>
</tr>
<tr>
<td>Cat</td>
<td>92 ± 13</td>
<td>45 ± 6†</td>
</tr>
</tbody>
</table>

Data are expressed in arbitrary units (mean ± SEM). ND indicates not determined.

*P < 0.05, †P < 0.001 vs sedentary HSs.

**Table 3. mRNA Abundance and Protein Expression of Inflammatory Cytokines in SM of Sedentary HSs and Patients With CHF**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Sedentary HSs</th>
<th>CHF Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, serum</td>
<td>0.91 ± 0.19</td>
<td>2.15 ± 0.17*</td>
</tr>
<tr>
<td>TNF-α, mRNA</td>
<td>0.30 ± 0.09</td>
<td>1.80 ± 0.26†</td>
</tr>
<tr>
<td>TNF-α, protein</td>
<td>0.76 ± 0.10</td>
<td>1.40 ± 0.06†</td>
</tr>
<tr>
<td>IL-1β, serum</td>
<td>0.26 ± 0.14</td>
<td>0.63 ± 0.20</td>
</tr>
<tr>
<td>IL-1β, mRNA</td>
<td>0.29 ± 0.11</td>
<td>2.78 ± 0.40‡</td>
</tr>
<tr>
<td>IL-1β, protein</td>
<td>0.67 ± 0.07</td>
<td>1.09 ± 0.08†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Serum concentration are reported in pg/mL. TNF-α and IL-1β mRNA expression were normalized to the expression of 18S rRNA and were reported in arbitrary units. TNF-α and IL-1β protein expression is reported in percentage of tissue area that stained positive for respective cytokines.

*P < 0.05, †P < 0.01, ‡P < 0.001, vs sedentary HSs.
Association Between Oxidative Stress and Structural Alterations of SM

Apoptosis was found to occur more frequently in SM myocytes of patients with CHF (49±4 apoptotic nuclei/10,000 SM myocyte nuclei) compared with HSs (22±4 apoptotic nuclei/10,000 SM myocyte nuclei, P<0.01). The functional association between the increase in oxidative stress as measured by nitrotyrosine formation and the occurrence of apoptotic cell death is supported by the close correlation between both parameters (r=0.62, P<0.01).

Effects of Exercise Training on Activity of Radical Scavenger Enzymes in Patients With CHF

Superoxide Dismutase

Regular physical exercise training failed to affect total SOD activity (5.6±0.6 U/mg before versus 5.8±0.8 at 6 months, Figure 2A), mRNA content (Table 4), or protein expression (Table 5) of the 2 isoforms. In patients in group C, no changes with respect to SOD mRNA content (Table 4), protein expression (Table 5), and activity were determined (5.9±0.3 U/mg before versus 6.3±0.8 at 6 months).

Catalase

In CHF patients (group T), exercise training resulted in an increase in Cat activity by 42% (from 12.4±2.1 U/mg before to 17.6±2.7 at 6 months, P<0.05 versus before and change versus group C; Figure 2B) without altering mRNA and protein expression in SM (Tables 4 and 5). Despite the improvement, Cat activity still tended to be low after 6 months of exercise training in CHF patients (17.6±2.7 U/mg in CHF versus 29.5±4.7 U/mg in HSs, P=0.051). Cat activity (Figure 2B), mRNA content, and protein expression remained unchanged in group C (Tables 4 and 5).

TABLE 4. mRNA Abundance of Radical Scavenger Enzymes in SM of Patients With CHF

<table>
<thead>
<tr>
<th></th>
<th>CHF Patients, Group T</th>
<th>CHF Patients, Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Begin 6 Mo</td>
<td>Begin 6 Mo</td>
</tr>
<tr>
<td>MnSOD</td>
<td>1.28±0.32</td>
<td>1.47±0.27</td>
</tr>
<tr>
<td>CuZnSOD</td>
<td>0.15±0.05</td>
<td>0.14±0.07</td>
</tr>
<tr>
<td>GPX</td>
<td>0.05±0.02</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>Cat</td>
<td>45±9</td>
<td>49±8</td>
</tr>
</tbody>
</table>

Data are expressed in arbitrary units (mean±SEM).
### GPX Expression

Exercise training augmented GPX activity in SM by 41% (from 35.3±4.3 mU/mg before to 49.9±3.5 at 6 months, P<0.05 versus before and versus group C; Figure 3A) in CHF patients, but the activity of this enzyme still tended to be lower when compared with that in HSs (49.9±3.5 mU/mg in CHF versus 71.9±9.6 in HSs, P=0.052); however, GPX mRNA expression was not altered by exercise training (Table 4). GPX activity (Figure 3A) and GPX mRNA expression did not change during the study in group C (Table 4).

### Local Oxidative Stress

In CHF patients, local oxidative stress in SM was considerably reduced by 6 months of regular physical activity (decrease in lipid peroxidation by 57%, from 368±92 μmol/mg protein before to 166±51 at 6 months, P<0.05 versus before) but remained constantly elevated in the inactive control group (386±100 μmol/mg protein before versus 398±124 at 6 months, Figure 3B). In parallel, nitrotyrosine production decreased by 34% (from 3.76±0.36% positive tissue area before to 2.48±0.3% at 6 months, P<0.05 versus before) but tended to increase in group C (from 3.61±0.61% positive tissue area before to 4.50±0.50% at 6 months, P=0.15 versus before).

### Circulating Cytokines, Local Cytokine Expression, and Cross-Talk Between Local Inflammation and Radical Scavenger Enzymes

During the study period, serum levels of TNF-α as well as IL-1β did not change in either the CHF control or CHF training group (Table 6). In contrast, exercise training resulted in a reduction in local TNF-α and IL-1β mRNA expression (by a respective 46% and 35%) that was linked to a decrease in TNF-α and IL-1β protein levels by 27% and 30%, respectively, in SM in CHF (Table 6). The decrease in local TNF-α protein levels in the CHF training group was associated with an enhancement of GPX (r=−0.68, P<0.05) and Cat (r=−0.60, P<0.05) activity.

### Association Between Oxidative Stress and Structural Alterations of SM

Exercise training led to a reduction in apoptosis by 24% in group T (from 49±6 apoptotic nuclei/10 000 SM myocyte nuclei before to 37.8±8.1000 SM at 6 months, P<0.05 versus before and for the change versus group C), whereas no changes were observed in group C (from 44±7 apoptotic nuclei/10 000 SM myocyte nuclei before to 49±7 1000 at 6 months). The training-induced decrease in oxidative stress (reduction in nitrotyrosine formation) was directly related to the reduction in apoptosis (r=0.69, P<0.01; Figure 4).

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**TABLE 5. Protein Expression of Radical Scavenger Enzymes in SM of Patients With CHF**

<table>
<thead>
<tr>
<th></th>
<th>CHF Patients, Group T</th>
<th>CHF Patients, Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Begin 6 Mo</td>
<td>Begin 6 Mo</td>
</tr>
<tr>
<td>MnSOD</td>
<td>0.88±0.20</td>
<td>0.68±0.16</td>
</tr>
<tr>
<td>CuZnSOD</td>
<td>1.70±0.31</td>
<td>1.69±0.33</td>
</tr>
<tr>
<td>Cat</td>
<td>0.63±0.09</td>
<td>0.73±0.06</td>
</tr>
</tbody>
</table>

Data are expressed in arbitrary units (mean±SEM).

**TABLE 6. Serum Concentration and mRNA Abundance of Inflammatory Cytokines in SM of Patients With CHF**

<table>
<thead>
<tr>
<th></th>
<th>CHF Patients, Group T</th>
<th>CHF Patients, Group C</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Begin 6 Mo</td>
<td>Begin 6 Mo</td>
</tr>
<tr>
<td>TNF-α serum</td>
<td>2.24±0.21</td>
<td>2.05±0.26</td>
</tr>
<tr>
<td>TNF-α mRNA</td>
<td>1.71±0.39</td>
<td>1.86±0.38</td>
</tr>
<tr>
<td>TNF-α protein</td>
<td>1.43±0.09</td>
<td>1.37±0.09</td>
</tr>
<tr>
<td>IL-1β serum</td>
<td>0.59±0.26</td>
<td>0.67±0.32</td>
</tr>
<tr>
<td>IL-1β mRNA</td>
<td>2.79±0.73</td>
<td>2.78±0.37</td>
</tr>
<tr>
<td>IL-1β protein</td>
<td>1.12±0.10</td>
<td>1.06±0.13</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. Serum concentration are reported in pg/mL. TNF-α and IL-1β mRNA expression were normalized to the expression of 18S rRNA and are reported in arbitrary units. TNF-α and IL-1β protein expression is reported in percentage of tissue area that stained positive for the respective cytokines.

*P<0.05 for the change vs CHF group C.
†P<0.01 vs at the beginning of the study.
The enzyme SOD represents the first defense against superoxide anions and converts superoxide radicals to hydrogen peroxide. In our study, we provide evidence that total SOD activity is significantly reduced in SM of patients with CHF, suggesting impaired detoxification of ROS. Unfortunately, because of the small samples obtained from our patients, we were unable to perform additional assays to distinguish the activities of CuZnSOD, which is primarily found in the cytosol, from those of MnSOD, which is mainly located in the mitochondrial matrix, scavenging ROS produced by the respiratory chain. Nevertheless, the reduction in total SOD activity seems to be primarily the result of impaired MnSOD expression, because CuZnSOD protein content was comparable between patients with CHF and HSS.

Hydrogen peroxides, end-products of the detoxification of superoxide radical by SOD, are subjected to further decomposition by GPX. In the present study, GPX activity was determined, because CuZnSOD protein content was comparable between patients with CHF and HSS. GPX17; however, Cat converts hydrogen peroxide to water and shows, therefore, some overlap with the function of GPX. Nevertheless, the reduction in total SOD activity is consistent with the hypothesis of a partial defect at the transcriptional level in CHF. Furthermore, diminished tissue concentrations of the necessary cofactor glutathione, seen in patients with CHF, under the influence of elevated local levels of TNF-α could further aggravate the impaired GPX activity, leading to ROS-mediated cell damage.17,20,21 This notion is strongly supported by the inverse correlation between TNF-α protein levels in SM and GPX activity in the present study. One might argue that the increase in local TNF-α and IL-1β protein levels in CHF is the result of enhanced binding of circulating cytokines on tissue-specific receptor. Indeed, serum concentrations of TNF-α were found to be elevated in patient with CHF, whereas serum IL-1β levels remained unaffected by CHF; however, the missing correlation between circulating and local levels of inflammatory cytokines, in conjunction with the augmented mRNA levels of TNF-α and IL-1β in SM in CHF compared with HSS, strongly supports our hypothesis of local inflammatory activation with increased local expression of the aforementioned cytokines in CHF.

The enzyme Cat primarily decomposes hydrogen peroxide and shows, therefore, some overlap with the function of GPX; however, Cat converts hydrogen peroxide to water and oxygen, independent of cellular glutathione concentrations and might, therefore, be specially important for the elimination of hydrogen peroxide in CHF. In this study, the remarkable decrease in Cat activity in SM of patients with CHF was accompanied by a reduction in mRNA and protein expression, suggesting a defect at the transcriptional level or a reduction in Cat mRNA stability. Moreover, it is also tempting to speculate that the decline in GPX activity results in accumulation of hydrogen peroxide within the cell, finally leading to substrate inhibition of Cat.29 The close inverse correlation between TNF-α protein levels in SM and Cat activity also suggests that this inflammatory cytokine inhibits Cat directly, but the exact mechanism remains to be determined.

In summary, these data are consistent with the hypothesis that inflammatory cytokines that are locally expressed in SM act as dual enhancers of oxidative stress: they induce the expression of radical producers and inhibit the activity of key radical scavengers.9,10,12 In the present study, elevation of local oxidative stress was confirmed by the increase in lipid peroxidation and nitrotyrosine formation in SM. The accumulation of ROS has been shown to feed back on the expression of cytokines and, hence, might contribute to further acceleration of the inflammatory activation in SM.1,9,11,30–32 The concept of ROS-mediated muscle fiber damage in CHF is strongly supported by the correlation between nitrotyrosine formation and the occurrence of apoptotic cell death of SM myocytes in our study. Although we did not assess muscle function in the present clinical trial, data from recent studies suggest that the increases in local oxidative stress and apoptosis are associated with muscle dysfunction and loss of muscle bulk, partially contributing to the typical clinical sign of early muscle fatigue.14,15 Besides other derangements, the aforementioned alterations might at least partially contribute to the development of cardiac cachexia in CHF.1,13–16,32,33
Influence of Exercise Training on Expression of Radical Scavenger Enzymes in SM

Although Cat and GPX activities were significantly augmented after 6 months of exercise training in SM of CHF patients, total SOD activity remained unchanged, suggesting a persistent impairment of superoxide radical detoxification in the SM. Because GPX mRNA expression was not changed by exercise training in CHF, the increase in activity is the result of either enhanced GPX mRNA stability or posttranscriptional modification of GPX protein. Nevertheless, we demonstrated that local TNF-α expression decreased in SM in CHF in response to exercise training. Because TNF-α has been shown to impair intracellular levels of the essential cofactor of GPX (GSH), the attenuated local inflammation after exercise training in CHF might be associated with restoration of local tissue concentrations of GSH, thus contributing to partial restoration of GPX activity.20,21 This hypothesis is supported by the close correlation between the change in local TNF-α protein levels and the change in GPX activity. Because GPX is able to convert hydrogen peroxide to water and oxygen, it is tempting to speculate that the training-induced augmentation of GPX activity results in a reduction in cellular hydrogen peroxide levels and consequently, the elimination of substrate inhibition of Cat.29 This might explain the increase in Cat and GPX activity in CHF patients seen with exercise training, despite the fact that no change occurred at the mRNA or protein level. Nevertheless, because GPX and Cat expression was not normalized, these data suggest that 6 months of exercise training are not sufficient to correct the impaired transcription of GPX and Cat in CHF.

To assess whether the decrease in TNF-α and IL-1β protein content in SM in CHF is secondary to alterations of circulating cytokines, the latter were determined by ELISA. Exercise training did not affect serum concentration of TNF-α and IL-1β in patients with CHF. In contrast, we observed a decrease in TNF-α and IL-1β mRNA expression in SM in response to exercise training, which was associated with a reduction in local TNF-α and IL-1β protein levels; however, we did not find any correlation between circulating TNF-α or IL-1β concentrations and the change in mRNA or protein levels of the respective cytokines in SM in patients with CHF undergoing exercise training. These findings are consistent with the hypothesis of an established self-perpetuating vicious oxidative-inflammatory circle in SM in CHF that is locally regulated and partially independent of the circulating cytokine network.

The concept that exercise training in CHF exerts long-term antioxidative effects because of correction of the enzymatic radical scavenger system is further supported by the convincing normalization of lipid peroxidation and a reduction in nitrotyrosine formation, as measures of local oxidative stress, seen in SM of CHF patients after 6 months of exercise training in the present study. This attenuation in local oxidative stress was closely associated with a decrease in the rate of SM apoptosis in CHF to values normally determined in healthy individuals. Nevertheless, there was no direct correlation between the change in Cat or GPX activity and the change in local oxidative stress in the training group, which is not surprising because of the multifactorial effects of exercise training.

Recently, in an elegant study, Ennezat and coworkers demonstrated an increase in mRNA abundance of GPX and CuZnSOD in SM of patients with CHF after 12 weeks of exercise training. In that study, mRNA transcripts of GPX and CuZnSOD were normalized to those of vWF, a protein that is almost exclusively found in endothelial cells. Because CuZnSOD and GPX are expressed not only in endothelial cells but also in skeletal myocytes and that the majority of RNA in muscle biopsy samples is derived from the largest proportion of cells, which are clearly myocytes, we normalized mRNA expression of the radical scavenger enzymes to 18S rRNA that is known to be ubiquitously expressed in all cell types. Because of different normalization procedures (18S rRNA versus vWF), the results of our study and those of Ennezat et al cannot be directly compared.

Previously, we were able to show that patients with CHF benefit from an aerobic endurance exercise training with respect to endothelial function, central hemodynamics, and reversal of peripheral alterations, in the absence of obvious harmful side effects.3,4,19 Because of those positive experiences, patients with CHF were subjected to such a training program in the present study. It is conceivable that resistance training or a combination of resistance and endurance training has higher antiinflammatory and antioxidative properties; however, further studies are necessary to address this issue.

Limitations

In the present study, we examined the cross-talk between local inflammation and expression as well as the activity of radical scavenger enzymes in crude SM homogenates, which consist of SM myocytes and small vessels. Therefore, we were unable to elucidate the exact source of radical scavenger enzymes and inflammatory cytokines. Moreover, we cannot rule out the possibility that circulating cytokines bind to their specific receptor on the surface of myocytes, thereby partially contributing to the TNF-α and IL-1β signal detected by immunohistochemistry. The missing correlation between circulating and local levels of inflammatory cytokines, however, strongly argues against this hypothesis. Given that most of the cells are of muscular origin (Figure 1), it is highly probable that the measured differences with respect to mRNA, protein content, and activity of radical scavenger enzymes and cytokines are due to alterations in SM myocytes in CHF.

We assessed lipid peroxidation in SM, which can be considered a cumulative marker of radical damage; however, lipid peroxides can also be catalyzed enzymatically, and they do not necessarily reflect the present radical load at the time when the SM biopsy is obtained. Therefore, further studies with spin traps are necessary to exactly elucidate sources and consequences of increased radical production in CHF.

Clinical Implications

In general, these data suggest that exercise training in CHF represents an adjuvant therapy with remarkable antiinflammatory and antioxidative properties: it not only decreases the expression of inflammatory cytokines, but it also enhances the activity of radical scavenger enzymes, leading to a clear
reduction of oxidative stress and an attenuation of SM damage induced by apoptosis.

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