Immune activation is associated with reduced skeletal muscle mass and physical function in chronic heart failure

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Abstract

Background: Chronic heart failure is characterized by immune activation and increased circulating levels of cytokines. Whether humoral factors contribute to the peripheral manifestations of the heart failure syndrome, such as muscle atrophy and reduced physical work capacity, however, is not clear.

Methods: We measured circulating cytokines (tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6)), their soluble receptors (sTNF-α RII, IL-6sR), markers of immune activation (C-reactive protein (CRP)), muscle mass, aerobic capacity and muscle strength in 10 patients with heart failure (mean ± S.E.; 63 ± 3 years) and 11 controls (70 ± 3 years).

Results: Heart failure patients exhibited decreased aerobic capacity (P < 0.01) and leg muscle strength (P < 0.05). Reduced muscle strength persisted in heart failure patients after statistical adjustment for differences in skeletal muscle size. All inflammatory markers were increased in heart failure patients (P ≤ 0.05 to P < 0.01) compared to controls, with the exception of TNF-α. Despite no group differences in TNF-α, higher concentrations of this cytokine were correlated to lower skeletal muscle mass in the combined study population (range of r-values: −0.436 to −0.545; P < 0.01 to P < 0.02), as were IL-6 levels (range of r-values: −0.438 to −0.443; P < 0.05). TNF-α, sTNF-α RII, IL-6 and CRP showed strong negative relationships to aerobic capacity (range of r-values: −0.579 to −0.751; P < 0.01 to P < 0.001). In addition, elevated levels of IL-6 and TNF-α were associated with reduced leg and forearm skeletal muscle strength (range of r-values: −0.440 to −0.674; P < 0.05 to P < 0.01). Finally, correlations between cytokines and functional measures were present when heart failure patients were analyzed separately (range of r-values: −0.646 to −0.673; P < 0.05).

Conclusions: Our results suggest that circulating cytokines are related to both skeletal muscle mass and physical function. These findings provide further evidence to support the hypothesis that immune activation contributes to skeletal muscle atrophy and reduced functional capacity in heart failure patients.

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Keywords: Cytokines; Muscle atrophy; Cardiac cachexia; Exercise intolerance

1. Introduction

Chronic heart failure is characterized by immune activation and increased circulating concentrations of several proinflammatory cytokines [1–3]. These inflammatory mediators may contribute to the pathophysiology and progression of the disease by altering cardiac structure and function [4,5]. In addition to their detrimental effects on cardiac muscle, elevated cytokine levels may contribute to the peripheral manifestations of the heart failure syndrome; most notably, skeletal muscle wasting and reduced muscle function. The catabolic effects of cytokines on skeletal muscle have been well-characterized [6,7]. In addition, recent evidence has shown that, similar to cardiac muscle, cytokines promote contractile dysfunction in skeletal muscle [8,9]. Despite these detrimental effects, few studies have explored the relationship of inflammatory mediators to muscle mass and function. Thus, our goals in the present study were threefold: (1) to measure and compare aerobic
capacity, muscle strength and cytokine levels between heart failure patients and controls; (2) to examine the relationship of circulating cytokines to skeletal muscle mass; and (3) to determine the relationship of cytokine levels to aerobic capacity and skeletal muscle strength measures. To accomplish these objectives, we measured circulating tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), their soluble receptors (sTNF-α RI, IL-6sR) and markers of immune activation (C-reactive protein (CRP)) and related these measures to total and regional skeletal muscle mass, peak aerobic capacity and knee and forearm muscle strength in a cohort of heart failure patients and controls. We hypothesized that heart failure patients would be characterized by decreased aerobic capacity and muscle strength and increased circulating concentrations of inflammatory markers. Moreover, immune activation would be related to reduced muscle mass and physical function.

2. Materials and methods

2.1. Subjects

Ten male volunteers with chronic heart failure due to left ventricular systolic dysfunction were recruited from the Heart Failure Clinic of the Cardiology Unit of the University of Vermont (ejection fraction: 32% by echocardiography). New York Heart Association (NYHA) functional class averaged 2.5±0.2 with six patients in class II, 3 in class III and 1 in class IV. Heart failure was due to coronary artery disease in seven patients, defined by a history of myocardial infarction and/or multi-vessel coronary obstructions at cardiac catheterization, idiopathic dilated cardiomyopathy in two patients and dilated cardiomyopathy secondary to severe hypertension in one patient. Heart failure patients were taking the following medications: diuretics (n=10; 100%), digoxin (n=7; 70%), angiotensin-converting enzyme inhibitors/receptor blockers (n=9; 90%) and β-adrenergic blocking agents (n=8; 80%). Additionally, one patient had insulin-dependent diabetes mellitus and two patients had non-insulin dependent diabetes mellitus (NIDDM) and were treated with oral hypoglycemic drugs. At the time of testing, patients had no signs of peripheral edema.

Eleven male volunteers were recruited to serve as controls. Nine of these volunteers were healthy and free of disease, had no signs or symptoms of heart disease and normal rest and exercise electrocardiograms. The control group also included two individuals with known coronary artery disease, but normal left ventricular contraction patterns and no exertional ischemia, as demonstrated by a normal electrocardiographic stress test to exhaustion without angina. Both of the latter patients were treated with aspirin, one with a Ca2+ channel blocker and the other with an HMG-CoA reductase inhibitor. Because inclusion of these two controls did not impact group differences or associations among variables (see Results), they were retained in the control group. Other medications in the control group were: anti-epileptic (n=1), anti-anxiety (n=1), 5α-reductase inhibitor (n=1), non-steroidal anti-inflammatory (n=1). Controls had no history of diabetes mellitus, normal fasting blood glucose (<6.22 mmol/L) and normal glucose tolerance (glucose<7.77 mmol/L 2 h following 75 g oral glucose load). The nature, purpose and possible risks of the study were explained to each subject before they gave written consent to participate. The experimental protocol was approved by the Committee on Human Research at the University of Vermont. Data from these volunteers regarding the effect of heart failure on skeletal muscle myofibrillar protein metabolism have been published elsewhere [10].

2.2. Protocol

Body composition, exercise capacity and muscle strength were measured on an outpatient basis at least 1 week prior to obtaining blood for cytokine measurements. Blood for cytokine measurements was drawn the morning following an inpatient visit to the research center. For 3 days prior to admission, all subjects were provided a standardized, weight-maintenance diet (60% carbohydrate; 25% fat; 15% protein). The last meal of the standardized diet was consumed by 1900 h the evening of admission and subjects fasted until completion of testing. Volunteers were asked to refrain from exercise the day prior to admission to prevent any effect of physical exercise on inflammatory markers. Medications were maintained for all volunteers per normal dosing regimens.

2.3. Inflammatory markers

CRP was measured by enzyme-linked immunosorbent assay (ELISA; [11]) with an interassay coefficient of variation (CV) in our laboratory ranging from 2% to 4%. TNF-α and IL-6 plasma concentrations and soluble receptors (sTNF-α RI and IL-6 sR, respectively) were measured by ultra-sensitive ELISA assays (R&D Systems; Minneapolis, MN) with interassay CVs of 16% and 6% for TNF-α and IL-6 concentration and 9% and 10% for their respective receptors.

2.4. Body composition

Body mass was measured on a digital scale (Scale-Tronix, Inc.; Wheaton, IL). Fat mass, fat-free mass and bone mass were measured by dual energy X-ray absorptiometry, using a Lunar DPX-L densitometer (Lunar Co, Madison, WI). Bone mass data are not reported. Appendicular skeletal muscle mass was measured using discrete skeletal landmarks, as described by Heymsfield et al. [12]. We have shown previously that there is no effect of heart failure on total body water or the hydration of fat-free tissue in patients
on stable diuretic therapy and no signs of peripheral edema [13]. Thus, we do not believe that alterations in fluid homeostasis impacted group differences in muscle mass or their association with cytokines.

2.5. Exercise capacity

Peak oxygen consumption (VO2) was measured during a graded, treadmill test to volitional fatigue. Briefly, a comfortable initial walking speed was found for each volunteer and was maintained throughout the test. The grade was increased 2.5% every 2 min until volitional fatigue. Peak VO2 was defined as the highest 30 s average VO2 value measured during the last 2 min of the test. One heart failure patient with severe symptoms (NYHA class IV) did not attempt the exercise test.

2.6. Skeletal muscle strength

Isometric and isokinetic knee extensor strength were measured using a multi-joint dynamometer (Lido Active, Loredan Biomedical Inc., Sacramento, CA). The right leg was tested in all subjects. The volunteer was seated and positioned so that the lateral femoral epicondyle was aligned with the central axis of the dynamometer. Extraneous movement was restricted by a velcro strap placed across the abdomen and a padded restraint at the distal point of the thigh. The lever arm of the dynamometer was attached just proximal to the lateral malleolus. Following instructions, volunteers were allowed to perform several practice trials for each condition at moderate intensity to ensure familiarity with the procedure. For isometric measurements, the lever arm was fixed at 55°. Volunteers performed three brief (5 s) maximal voluntary contractions each separated by 1 min of rest. The highest torque (Nm) value for each contraction was recorded. The average from the three trials was calculated. Isokinetic measurements were performed at 90°/s. The range of motion was set from 0° to 90° flexion relative to full knee extension. Volunteers performed 15 consecutive contractions. The average of the three highest torque values from the first five contractions were recorded and averaged. Forearm isometric grip strength was measured using an isometric hand grip dynamometer (Lafayette Instruments Co., Lafayette, IN). Three trials were performed for each hand and the average of all six trials was calculated. For two heart failure patients with carpal tunnel syndrome, forearm grip strength measurements were unilateral (average of three measurements).

2.7. Statistical analysis

Differences between groups were determined by unpaired, Student t-tests or Mann–Whitney U-tests where appropriate. Analysis of covariance was used to examine differences between groups after statistically controlling for relevant covariates (listed in Results). Relationships between variables were assessed using Pearson correlation coefficients. Variables that were not normally distributed (e.g., cytokines) were log10 transformed prior to correlation analysis. Distributional assumptions of log10 transformed variables were confirmed prior to analysis using Shapiro–Wilk test. Partial correlation analysis was used to examine the relationship among variables after statistical adjustment for covariates. For statistical adjustment for NYHA functional class, controls were designated as class 0. All analyses were conducted with SPSS software 9.0 (SPSS Inc.; Chicago, IL). All values are mean±S.E., unless otherwise specified.

3. Results

3.1. Physical characteristics

Physical characteristics are shown in Table 1. No differences in age, body size, body composition or total or regional muscle mass were observed between groups. Exclusion of the two controls with coronary artery disease did not affect group differences.

3.2. Exercise capacity

Differences in peak VO2, expressed on an absolute basis, and relative to either body mass or fat-free mass, are shown in Table 2. Peak VO2 was 36% lower (P<0.01) in heart failure patients compared to controls. This reduction in exercise capacity persisted after peak VO2 data were

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Physical characteristics</td>
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<tr>
<td>n</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Body mass (kg)</td>
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<td>Fat mass (kg)</td>
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<td>Fat-free mass (kg)</td>
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<tr>
<td>Appendicular skeletal muscle mass (kg)</td>
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<td>Arm muscle mass (kg)</td>
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<td>Leg muscle mass (kg)</td>
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Data are mean±S.E.

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td>Peak oxygen consumption</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Peak VO2 (L/min)</td>
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<tr>
<td>Peak VO2 (mL/kg BW min^-1)</td>
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<tr>
<td>Peak VO2 (mL/kg FFM min^-1)</td>
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<td>Adjusted peak VO2 (L/min)</td>
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</table>

Values are mean±S.E. For heart failure patients, n=9. Peak VO2, peak oxygen consumption; BW, body mass; FFM, fat-free mass. Adjusted peak VO2 represents peak VO2 data following statistical adjustment for FFM via analysis of covariance. *P<0.01.
expressed relative to body mass ($P<0.01$) or fat-free mass ($P<0.01$). Similarly, peak VO$_2$ was lower ($P<0.01$) in heart failure patients after statistical adjustment for fat-free mass by analysis of covariance. Exclusion of the two controls with coronary artery disease did not alter these differences between groups.

### 3.3. Skeletal muscle strength

Isokinetic and isometric knee extensor strength (Panel A) and isometric forearm grip strength (Panel B) are shown in Fig. 1. Both isokinetic (HF: $88\pm10$ vs. Control: $121\pm11$ N m) and isometric (HF: $144\pm13$ vs. Control: $190\pm15$ N m) knee extensor strength were reduced (both $P<0.05$) in heart failure patients compared to controls. After statistical adjustment for age and leg muscle mass, both isokinetic failure patients compared to controls. After statistical control for age and muscle mass using analysis of covariance.

#### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Heart failure</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (µg/ml)</td>
<td>5.11±1.26</td>
<td>2.00±0.56*</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.91±0.30</td>
<td>1.52±0.15</td>
</tr>
<tr>
<td>TNF-α RII (pg/ml)</td>
<td>3662±315</td>
<td>2758±147*</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3.96±0.76</td>
<td>1.92±0.38</td>
</tr>
<tr>
<td>IL-6 sR (ng/ml)</td>
<td>38.8±1.16</td>
<td>32.1±1.49</td>
</tr>
</tbody>
</table>

Data are mean±S.E. Group differences in CRP, TNF-α and IL-6 levels were determined by Student $t$-tests. All other group differences were determined by Mann–Whitney $U$-test. *$P<0.05$; $^*$ $P<0.01$.

### 3.4. Inflammatory markers

Table 3 shows group differences in circulating concentrations of cytokines and markers of immune activation. As expected, heart failure patients had increased levels of all inflammatory markers ($P<0.05$ to $P<0.01$), with the exception of TNF-α ($P=0.46$). Exclusion of the two controls with coronary artery disease did not affect group differences, with the exception that CRP levels were no longer different between groups ($P=0.11$).

#### 3.5. Relationship of inflammatory markers to muscle mass

The relationships of inflammatory mediators to skeletal muscle mass measurements are shown in Table 4. TNF-α was consistently related to skeletal muscle mass measurements ($P<0.05$ to $P<0.01$). In addition, IL-6 levels were negatively correlated to leg and appendicular skeletal muscle mass ($P<0.05$) and showed a trend toward being related to arm muscle mass ($P=0.06$). Scatterplots showing the relationship of TNF-α to arm, leg and appendicular skeletal muscle mass are shown in Fig. 2. Exclusion of two controls with coronary artery disease did not alter these relationships (range of $r$-values: $-0.475$ to $-0.545$; $P<0.05$). After statistical adjustment for NYHA functional class, TNF-α remained significantly correlated to leg ($r=−0.520$; $P<0.02$) and appendicular ($r=−0.507$; $P=0.02$) skeletal muscle mass and showed a trend towards being related to arm muscle mass ($r=−0.426$; $P=0.06$). The relationships between IL-6 and leg and appendicular skeletal muscle mass were no longer significant after controlling for NYHA functional class, although trends

#### Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Arm muscle mass</th>
<th>Leg muscle mass</th>
<th>Appendicular muscle mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>$−0.276$</td>
<td>$−0.270$</td>
<td>$−0.279$</td>
</tr>
<tr>
<td>IL-6</td>
<td>$−0.414$</td>
<td>$−0.438^*$</td>
<td>$−0.443^*$</td>
</tr>
<tr>
<td>IL-6 sR</td>
<td>0.230</td>
<td>0.133</td>
<td>0.166</td>
</tr>
<tr>
<td>TNF-α</td>
<td>$−0.436^*$</td>
<td>$−0.545^*$</td>
<td>$−0.528^*$</td>
</tr>
<tr>
<td>TNF-α RII</td>
<td>$−0.170$</td>
<td>$−0.297$</td>
<td>$−0.268$</td>
</tr>
</tbody>
</table>

Values are Pearson correlation coefficients. CRP, IL-6 and TNF-α data were log$_{10}$ transformed prior to correlation analysis. *$P<0.05$; $^*$ $P<0.01$.
were noted ($r = -0.404; P = 0.08$ and $r = -0.422; P = 0.06$, respectively). When heart failure patients were analyzed separately, the strength of the correlation coefficients persisted (range of $r$-values for relationships of TNF and IL-6 to muscle mass measures: $-0.403$ to $-0.558$), but did not reach statistical significance (range of $P$-values: 0.25 to 0.09).

3.6. Relationship of inflammatory markers to exercise capacity and muscle strength

The relationship of inflammatory mediators to aerobic capacity and skeletal muscle strength is shown in Table 5. All inflammatory markers, with the exception of IL-6 sR ($P = 0.06$), were negatively related to peak VO$_2$ ($P = 0.01$ to $P < 0.001$). Scatterplots of significant relationships are shown in Fig. 3. After statistical adjustment for New York Heart Association functional class, the relationships of CRP ($r = -0.707; P < 0.01$), IL-6 ($r = -0.532; P < 0.02$) and TNF-α ($r = -0.467; P < 0.05$) to peak VO$_2$ persisted. IL-6 levels were negatively related to isokinetic and isometric knee extensor strength ($P < 0.05$), as well as isometric forearm grip strength ($P < 0.01$). TNF-α levels were negatively related to isometric forearm grip strength ($P < 0.02$) and sTNF-α RII levels were negatively related to isometric knee strength ($P < 0.02$). Scatterplots showing the relationship of IL-6 to peak and arm muscle strength measures are shown in Fig. 4. After adjustment for NYHA class, the relationship of IL-6 to isometric knee extensor strength ($r = -0.508; P < 0.02$) and isometric forearm grip strength ($r = -0.532; P < 0.02$) persisted. Because cytokines were negatively related to muscle size (see Table 4), the relationships between immune markers and muscle strength may reflect their colinearity to muscle size. This did not appear to be the case entirely since the relationship of IL-6 to isometric knee extensor strength ($r = -0.574; P < 0.01$) and forearm grip strength ($r = -0.470; P < 0.05$) persisted after statistical control for leg and arm muscle mass, respectively. Exclusion of two controls with coronary artery disease did not alter relationships of cytokines to physical performance measures (range of $r$-values: $-0.499$ to $-0.684$; $P < 0.05$ to $P < 0.01$). When heart failure patients were analyzed separately, sTNF-α RII was significantly related to peak VO$_2$ ($r = -0.673; P < 0.05$) and a strong trend was observed toward a relationship between TNF-α and peak VO$_2$ ($r = -0.643; P = 0.06$). For muscle strength measures, IL-6 was correlated to forearm grip strength ($r = -0.646; P < 0.05$) and a trend toward a relationship between IL-6 and isometric knee extensor strength was found ($r = -0.553; P = 0.10$).

### 4. Discussion

Numerous studies suggest a role for immune activation and increased circulating concentrations of proinflammatory cytokines in the pathophysiology and progression of heart failure. Whether inflammatory mediators contribute to the peripheral manifestations of heart failure, such as muscle atrophy and reduced functional capacity, however, is not clear. The present study was undertaken to explore the relationship of circulating inflammatory mediators to skeletal muscle mass and physical function in heart failure patients and controls. Heart failure patients exhibited reduced exercise capacity and leg muscle strength. Differences in muscle strength persisted after statistical control for

<table>
<thead>
<tr>
<th>Variable</th>
<th>Peak VO$_2$</th>
<th>Isokinetic knee extensor strength</th>
<th>Isometric knee extensor strength</th>
<th>Isometric forearm grip strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>$-0.654^*$</td>
<td>$-0.295$</td>
<td>$-0.423$</td>
<td>$-0.136$</td>
</tr>
<tr>
<td>IL-6</td>
<td>$-0.720^*$</td>
<td>$-0.440^*$</td>
<td>$-0.674^*$</td>
<td>$-0.573^*$</td>
</tr>
<tr>
<td>IL-6 sR</td>
<td>$-0.426$</td>
<td>$-0.011$</td>
<td>$0.060$</td>
<td>$0.448^*$</td>
</tr>
<tr>
<td>TNF-α</td>
<td>$-0.579^*$</td>
<td>$-0.433^*$</td>
<td>$-0.509^*$</td>
<td>$-0.401$</td>
</tr>
<tr>
<td>TNF-α RII</td>
<td>$-0.751^*$</td>
<td>$-0.371$</td>
<td>$-0.356$</td>
<td>$-0.512^*$</td>
</tr>
</tbody>
</table>

Values are Pearson correlation coefficients. For peak VO$_2$, $n = 20$. Peak VO$_2$ represents data expressed relative to fat-free mass (i.e., ml/kg FFM min$^{-1}$). CRP, IL-6 and TNF-α data were log$_{10}$ transformed prior to correlation analysis. *$P < 0.05$; †$P < 0.01$. 

![Graph](image-url)
age and leg muscle mass, suggesting that heart failure is characterized by skeletal muscle contractile dysfunction. All of the inflammatory markers measured, with the exception of TNF-α, were higher in heart failure patients compared to controls. Despite a lack of group differences, TNF-α levels were negatively related to indices of muscle mass, as were IL-6 levels. Nearly all of the inflammatory markers showed a strong negative relationship with peak VO₂. Additionally, higher levels of IL-6 and TNF-α were associated with reduced knee extensor and forearm grip strength. Finally, relationships between cytokines and functional measures were present when heart failure patients were analyzed separately. Our results further support the notion that immune activation may contribute to muscle atrophy and reduced functional capacity in heart failure.

Exercise intolerance and muscle weakness in heart failure is well-documented [14]. That our patients showed similar reductions in aerobic capacity and muscle strength suggests that our cohort reflects the pattern of physical disability characteristic of the heart failure syndrome. Of particular note is our finding that decreased knee extensor strength persisted after differences in age and leg muscle mass were taken into account. Our results are similar to those of Harrington et al. [15], who showed reduced knee extensor strength in heart failure patients after adjustment for muscle cross-sectional area, but differ from other studies [16]. Although reasons for divergent results among studies are not apparent, our findings, together with those of Harrington et al. [15], suggest that heart failure is associated with reduced skeletal muscle strength that is not due to muscle atrophy. These data suggest an intrinsic

Fig. 3. Relationship of peak oxygen consumption to CRP, IL-6, TNF-α and TNF-α RII in the combined cohort of heart failure patients (●; n=9) and controls (□; n=11).

Fig. 4. Relationship of circulating IL-6 levels to isokinetic and isometric knee extensor and isometric forearm grip strength in the combined cohort of heart failure patients (●; n=10) and controls (□; n=11).
defect in skeletal muscle contractile function in heart failure patients. Studies in animal models showing alterations in excitation–contraction coupling in skeletal muscle [17–19] similar to those observed in cardiac muscle [20, 21] further suggest a mechanism for skeletal muscle contractile dysfunction. These findings raise the intriguing possibility that heart failure is characterized by a generalized myopathy of striated muscle.

The increased levels of IL-6, IL-6 sR and sTNF-α RII in heart failure patients were similar to those reported previously [22]. In addition, plasma levels of CRP, a general indicator of immune activation, were increased greater than twofold in heart failure patients. In contrast with some reports in the literature [1,2], however, we did not observe group differences in TNF-α. The absence of differences in TNF-α may relate to the fact that the majority of patients in our cohort had relatively mild heart failure (NYHA functional class II; n = 6). Although increased TNF-α levels have been observed in these patients [2], the magnitude of the increase is much less than that in patients with more severe failure (NYHA class III and IV; [2]). We should stress, however, that our results are not unique. Several investigators have failed to observe increased TNF-α in larger samples of heart failure patients with a wider range of disease severity [23–25]. The reasons for differences among studies are uncertain, but may relate to natural variation in TNF-α levels during the course of the disease [22,26]. Regardless of the absence of group differences in TNF-α, the fact that we observed increased levels of other inflammatory markers in heart failure patients, and that group differences in cytokines were similar to prior reports in the literature [22], suggests that our population reflects the characteristic pattern of immune activation observed in heart failure.

Despite the lack of group differences in TNF-α, increased levels of this cytokine were correlated to reduced skeletal muscle size. Our results agree with several reports in the literature [1,27–29] showing increased TNF-α in cachectic heart failure patients compared to non-cachectic patients and controls. To our knowledge, however, only one study has examined directly the relationship between muscle mass and immune markers. Anker et al. [28] observed negative relationships between TNF-α and several indices of muscle mass in a combined cohort of cachectic and non-cachectic heart failure patients and controls. In addition to associations with TNF-α, skeletal muscle mass measures were negatively related to IL-6 levels in the present study. This finding agrees with data from Larsen et al. [30] showing a negative relationship between IL-6 levels and skeletal muscle fiber diameter measured on muscle biopsies. Negative associations between immune markers and muscle mass are not unexpected considering that cytokines promote skeletal muscle catabolism [7,31,32]. Of interest, the relationships between cytokines and muscle mass were observed despite the fact that our cohort of heart failure patients was not characterized by muscle atrophy (Table 1). Prior work in our laboratory has shown that muscle atrophy in both humans [13] and animal models of failure [33] is primarily related to weight loss. Because dietary energy deficiency may alter inflammatory mediators [34], the relationship between cytokines and muscle wasting in cohorts containing cachectic patients may partially reflect the fact that both variables are potentiated by malnutrition. Our results argue against this notion since we observed these relationships in heart failure patients not characterized by muscle atrophy. That is, relationships between cytokines and indices of muscle mass in prior studies are likely not related to concomitant malnutrition [28]. Collectively, these findings provide further evidence, albeit correlative, towards a role for cytokines in promoting muscle atrophy.

All of the markers of immune activation, with the exception of IL-6 sR, were negatively associated to peak VO_{2}. Our results agree with prior studies [24,27,35] showing negative correlations between TNF-α and its receptors and peak aerobic capacity in heart failure patients. We extend these findings to show that other markers of inflammatory status, such as IL-6 and CRP, behave similarly. After statistical adjustment for NYHA class, the relationship of CRP, IL-6 and TNF-α to peak aerobic capacity remained significant in the combined cohort, suggesting that these correlations were not due to the fact that these variables share collinearity with symptom severity. There are several lines of evidence which suggest that elevated levels of cytokines could impair exercise capacity by reducing cardiac function [5], decreasing skeletal muscle blood flow [36,37], altering skeletal muscle contractile function [9] or some combination of these effects. Ultimately, however, it is difficult to discern whether cytokines correlate independently to physical function using peak VO_{2} data since both variables share a strong association to disease severity [2,38].

A better approach to examine the association of cytokines to physical performance is to examine their relationship to skeletal muscle contractile function. To our knowledge, however, only one study has evaluated the relationship of cytokines to skeletal muscle strength in heart failure patients [35]. This study found no differences in skeletal muscle strength among heart failure patients separated into quartiles based on circulating TNF-α levels. In contrast, we found that IL-6, TNF-α and sTNF-α RII were negatively related to leg and forearm skeletal muscle strength measures in the combined cohort and that several of these correlations persisted when heart failure patients were analyzed separately. The relationships of IL-6 and TNF-α to muscle function are not unexpected. Recent studies in aging humans have shown that increased circulating concentrations of both of these cytokines are related to reduced skeletal muscle strength [39] and loss of muscle function over time [40]. Although we are careful not to ascribe cause-and-effect to these correlations, studies in both cardiac and skeletal muscle demonstrate an inhibitory effect of cytokines on striated muscle contraction [9,41,42]. That these features of the inhibitory effects of cytokines on muscle
contraction are shared by both cardiac and skeletal muscle
further supports the hypothesis of a generalized myopathy
of striated muscle in heart failure. Furthermore, the relation-
ships between cytokines and muscle function build on this
hypothesis to include a potential role for immune activation
in this myopathy. Additional studies are needed to identify a
mechanistic link between increased levels of cytokines and
reduced muscle function.

Negative relationships between cytokines and both
muscle mass and strength were not always borne out as
group differences. For instance, if we assume that the negative relationship between muscle mass and IL-6 reflects
the catabolic effect of IL-6 on skeletal muscle, one might
expect reduced muscle mass in heart failure patients in light
of their higher level of IL-6. In contrast to this hypothetical
scenario, we found no difference in muscle mass between
groups. The absence of group differences in muscle size may
be related to the fact that cytokine levels were only modestly
elevated in our population. Prior studies have shown that
cytokine levels increase with disease progression [2].
Because the majority of patients in our study had mild to
moderate heart failure, it is possible that cytokine levels were
not increased enough to promote changes in muscle size and
function. There may be a threshold level at which cytokines
induce muscle atrophy and weakness. The fact that we
observed correlations between cytokines and both muscle
mass and physical function measurements in a cohort of
heart failure patients that are relatively early in the course of
the disease, however, suggests that these relationships are not
limited to patients with severe failure. An alternate inter-
pretation of our findings is that circulating cytokines are not
primary effectors of muscle atrophy and weakness, but are
related to muscle mass and function because of their
colinearity with other factors. For example, Anker et al.
[24] has shown that TNF-α level correlated positively with
the ratio of circulating cortisol-to-dehydroepiandrosterone,
suggesting that increasing cytokines are accompanied by a
more catabolic balance in steroid hormones. Further studies
are needed to discern whether the relationships of cytokines
to muscle mass and function reflect a cause–effect relation-
ship or are an epiphenomenon of the heart failure syndrome.

In summary, our results show that increased circulating
concentrations of several cytokines are associated with
reduced skeletal muscle mass and decreased exercise
capacity. In addition, we report the novel observation that
skeletal muscle contractile function is negatively related to
circulating cytokines and, in particular, IL-6. Taken
together, these findings contribute to a growing body of
literature implicating immune activation in the peripheral
manifestations of heart failure.

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