Reduction of Insulin–Like Growth Factor-I Expression in the Skeletal Muscle of Noncachectic Patients With Chronic Heart Failure

Rainer Hambrecht, MD,* Paul Christian Schulze, MD,* Stephan Gielen, MD,* Axel Linke, MD,* Sven Möbius-Winkler, MD,* Jiangtao Yu, MD,* Jürgen Kratzsch, MD,§ Gerhard Baldauf, MD,‡ Martin W. Busse, MD,|| Andreas Schubert, PtID,† Volker Adams, PtID,*, Gerhard Schuler, MD*

Leipzig, Germany

OBJECTIVES
We sought to assess the role of insulin-like growth factor-I (IGF-I) in muscle wasting in chronic heart failure (CHF), serum concentrations and local muscular IGF-I expression were determined in patients with severe CHF.

BACKGROUND
Chronic heart failure is associated with progressive muscle atrophy, leading to cardiac cachexia. Skeletal muscle disuse and inflammatory activation with elevated cytokine levels have been implicated; however, the pathomechanism involved remains largely unknown.

METHODS
Serum levels of IGF-I were measured by competitive solid phase immunoassay in 47 patients with severe CHF (left ventricular ejection fraction ≤30%) and 15 age-matched healthy subjects (HS). Insulin-like growth factor-I and IGF-I receptor expression were assessed in vastus lateralis biopsies by real-time PCR and Western blot analysis.

RESULTS
Although serum IGF-I was not significantly different (175 ± 10 ng/ml in CHF vs. 170 ± 12 ng/ml in HS, p = NS), local muscle IGF-I mRNA expression was reduced by 52% in CHF (6.7 ± 0.4 vs. 14.0 ± 0.9 arbitrary units in HS, p < 0.001). This was accompanied by an increase in IGF-I receptor mRNA expression (86.8 ± 5.4 in CHF vs. 23.1 ± 1.8 arbitrary units in HS, p < 0.001). Local IGF-I expression was significantly correlated with muscle cross-sectional area (R = 0.75, p = 0.01). Chronic heart failure patients with a body mass index of <25 kg/m² showed signs of peripheral growth hormone (GH) resistance, as indicated by elevated serum GH, and reduced IGF-I levels.

CONCLUSIONS
In CHF patients, muscle IGF-I expression is considerably reduced in the presence of normal serum IGF-I levels, possibly contributing to early loss of muscle mass. These findings are consistent with a potential role of IGF-I for skeletal muscle atrophy in CHF. (J Am Coll Cardiol 2002;39:1175–81) © 2002 by the American College of Cardiology Foundation

In recent years it has become widely accepted that the catabolic syndrome associated with advanced stages of chronic heart failure (CHF) is more than a symptom of disease progression; cardiac cachexia has been shown to be correlated with lower exercise tolerance and reduced survival (1). However, the process leading to progressive loss of muscle mass and exercise capacity, finally resulting in cardiac cachexia, is still not completely understood.

Muscle wasting starts long before the clinical signs of overt cachexia become apparent. It has already been documented that noncachectic patients with CHF show reduced leg lean tissue compared with healthy subjects (HS) (2), suggesting that the factors responsible for the initiation of the catabolic process may be present at early stages of the heart failure syndrome.

Until now a number of different humoral factors have been accused of being responsible for the catabolic process in CHF, among them elevated serum cytokines (3) and catecholamines (4), cortisol/dihydroepiandrosterone imbal-

ance (5) and growth hormone (GH) resistance (5). A possible causal relationship between these factors and the catabolic syndrome was inferred based on the presumption that serum hormone levels are representative of local concentrations in target organs—most notably the skeletal muscle. However, this presumption has not been validated.

In the context of the present study we focused on the GH/IGF-I axis (GH/IGF-I)—a key system involved in regulation of normal cell growth, hypertrophy and atrophy. It has been shown that, in patients with CHF, low levels of systemic IGF-I are associated with a decreased leg muscle cross-sectional area and strength (6). We therefore hypothesized that the GH/IGF-I axis may be involved in the pathogenesis of skeletal muscle catabolism and dysfunction in CHF. Insulin–like growth factor-I exerts its functions via both autocrine and paracrine pathways. In order to assess the relation between local and systemic concentrations of IGF-I and GH the serum IGF-I and GH levels were measured and compared to the local expression of IGF-I and IGF-I receptor in the quadriceps muscle and noncachectic patients with CHF and of HS.

METHODS
Patient selection and study protocol. Forty-seven male patients ≥70 years old with CHF (NYHA functional class...
II to IV) as a result of dilated cardiomyopathy (DCM) or ischemic heart disease (IHD) were included in this study. All 47 patients had clinical, radiological and echocardiographic signs of CHF and a severely reduced left ventricular ejection fraction (LVEF ≤30%) as assessed by left ventriculography.

A total of 15 age-matched male patients (age 58.3 ± 3.2 years), who were admitted for nonspecific chest pain to rule out coronary artery disease, served as HS. They were normal by physical examination, ECG, chest X-ray, two-dimensional echocardiography, coronary angiography and left ventriculogram (LVEF 70 ± 2%). Healthy subjects had no evidence of hypertension or diabetes mellitus and had normal findings on routine hematologic and biochemical blood analyses. They were on no medication before or during the study period.

The protocol of this study was approved by the Ethics Committee of the University of Leipzig, and written informed consent was obtained from all patients and HS before enrollment.

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 (7).

Measurement of parameters of the GH/IGF-I axis and tumor necrosis factor-α. Blood samples were collected from both study groups in the morning, after a fasting period of 12 h. Growth hormone was measured by immunofluorometric assay using the AutoDELFIA System (Wallac, Turku, Finland) with a sensitivity of <0.9 ng/ml. Intraassay and interassay coefficients of variation were below 10%, in the range of 100 to 500 ng/ml. Serum levels of insulin-like growth factor binding protein-3 (IGFBP-3) were determined by a commercially available ELISA (DSL, Sinsheim, Germany). The sensitivity of that assay was found to be lower than 0.8 ng/ml. Intraassay and interassay coefficients of variation were below 10%, in the range of 4 to 40 ng/ml. Serum concentrations of tumor necrosis factor (TNF)-α were measured by a specific high sensitive enzyme-linked immunosorbent assay kit (Quantakine, R&D Systems, Minneapolis, Minnesota) with a sensitivity <0.18 pg/ml. All samples were run in duplicates, and the average value of the two measurements is reported.

Measurement of local skeletal muscle IGF-I expression. Skeletal muscle biopsies. Percutaneous needle biopsies were obtained from the right vastus lateralis muscle at least two days before exercise testing, as described previously (9).

MEASUREMENT OF IGF-I MESSENGER RIBONUCLEIC ACID (mRNA). To quantify IGF-I and IGF-I receptor mRNA, real-time PCR using the Light Cycler System (Roche Diagnostics Inc., Mannheim, Germany) was used. Briefly, aliquots (1 μg) of isolated total RNA was reverse transcribed in a volume of 25 μl into cDNA using random hexamers; 1 μl of cDNA was added to the PCR reaction containing the following specific primers: IGF-I-U: 5’-CCACATGTCCCTCCTGATCT-3’, IGF-I-L: 5’-ATCCACGATGCTCTGAGG-3’, IGF-I receptor-U: 5’-GGAAGCGGAGAGATGTCATG-3’ and IGF-I receptor-L: 5’-ATGGATGCGGTACAATTGTA-3’. The results for IGF-I and IGF-I receptor expression are expressed as ratio over 18S rRNA, which was amplified as house keeping gene using the following primers 18S rRNA-U: 5’-TAGAGGGCAAGTGCGGTT-3’ and 18S rRNA-L: 5’-TGTACACAGGCGGACTT-3’. The variability for triplicate measurements was <5%.

QUANTIFICATION OF IGF-I PROTEIN. Expression of IGF-I protein was analyzed by Western blot using a monoclonal anti-IGF-I antibody (clone Sm1.2, Upstate Biotechnology, Lake Placid, New York) followed by densitometry. Briefly, 30 μg of skeletal muscle proteins were separated on a 15% SDS-polyacrylamide-gel, followed by electrotransfer to a polyvinylidene fluoride membrane (Roth GmbH, Karlsruhe, Germany). The transferred proteins were incubated with a 1:500 dilution of the anti-IGF-I antibody at 4°C overnight. The bound antibody was detected by a peroxidase coupled antimouse antibody followed by a chemiluminescent reaction using luminol (SuperSignal West Pico, Pierce Rockford, Illinois). To quantify IGF-I protein the blots were analyzed by densitometry with a 1-D analysis software package (One-Dscan, Scanalytics, Billerica, Massachusetts). To compensate for blot to blot variations, an internal standard was loaded on each SDS-polyacrylamide-gel, and the densitometry results were expressed as ratio between sample and standard intensity.
Table 1. Baseline Characteristics of Patients with Chronic Heart Failure and Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>CHF (n = 47)</th>
<th>Healthy Subjects (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>58.0 ± 1.4</td>
<td>58.5 ± 3.2</td>
</tr>
<tr>
<td>VO2max, ml/kg^-1·min^-1</td>
<td>14.4 ± 0.5</td>
<td>26.2 ± 1.4</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>23 ± 1</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>No. DCM/IHD, (n)</td>
<td>20/27</td>
<td>—</td>
</tr>
<tr>
<td>NYHA</td>
<td>0/5/30/12</td>
<td>—</td>
</tr>
</tbody>
</table>

CHF = chronic heart failure; CMP = cardiomyopathy; DCM = dilated cardiomyopathy; VO2 max = maximal oxygen uptake.

Determination of lower limb muscle cross-sectional area. In a subgroup of 10 CHF patients and 8 healthy controls, the muscle cross-sectional area was measured by computed tomography at the mid-femur level as an established morphologic parameter for skeletal muscle mass (6). Using a Somatom Plus 4 CT-scanner (Siemens, Erlangen, Germany) sections were made halfway between the major trochanter and the medial condylus of the femur (slice thickness 5 mm, 130 mA). Cross-sectional areas of all upper right limb muscles and the quadriceps muscle were separately determined by manual tracing, using the built-in CT software VB 40 C.

Determination of quadriceps muscle function. Maximal isometric tension development and muscle fatigability were measured in the quadriceps muscles of the same patient subgroup in whom muscle cross-sectional area was determined. Using a calibrated isometric force transducer (Mechanotronic, Hagen, Germany) with a minimal resolution of <1 N, the peak force at maximal voluntary quadriceps contraction for 20 s was quantified five times with 1 min resting intervals. Peak force and decrease of force over the 20-s period were averaged for the five tests.

Statistical analysis. Data were tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variances by Levene’s test. Mean value ± standard error was calculated for all variables. Because all data were normally distributed, intergroup comparisons were made using the two-sided Student’s t test. Linear regression analysis was performed to assess the relationship between local IGF-I expression and quadriceps muscle function/cross-sectional area. A p value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics. A total of 47 CHF patients (58.0 ± 1.4 years, LVEF 23 ± 1%) and 15 healthy age-matched subjects (58.5 ± 3.2 years, LVEF 70 ± 2%) were enrolled in the study (Table 1). The majority of CHF patients were diagnosed with postischemic CHF (n = 27 vs. n = 20 with DCM). Left ventricular ejection fraction was not significantly different between dilated or ischemic CMP (25 ± 2% vs. 22 ± 2%, p = NS). Forty-three CHF patients (91.5%) received angiotensin-converting enzyme inhibitors, 40 (85.1%) were on digitalis, 41 (87.2%) on diuretic medications and 19 (40.4%) on beta receptor blockers. As determined by the criterion of unintentional documented weight loss of at least 7.5% of previous normal weight over the last six months, all patients were noncachectic (5). Maximal oxygen uptake (VO2 max), as determined by bicycle ergospirometry, was significantly reduced in patients with CHF (14.4 ± 0.5 vs. 26.2 ± 1.4 ml/kg-min in HS, p < 0.001).

Systemic levels of GH, IGF-I and IGFBP-3. The 47 CHF patients tended to have higher systemic GH levels (1.14 ± 0.23 ng/ml vs. 0.48 ± 0.25 ng/ml in HS); however, due to the considerable variation of individual values within the groups, this difference was not statistically significant (p = 0.134) (Table 2). Although GH binding protein levels were reduced in CHF patients (685 ± 65 pmol/l in CHF vs. 917 ± 110 pmol/l, p = 0.08), this difference was not significant either. No alterations in both IGF-I and IGFBP-3 were observed between CHF patients and HS. In a subgroup analysis, CHF patients with a body mass index < 25 kg/m2 showed signs of peripheral GH resistance, as indicated by elevated GH and reduced IGF-I levels. The IGF-I/IGF-I ratio was reduced in patients with a body mass index < 25 kg/m2 (Table 2).

Local expression of IGF-I/IGF-I receptor. The expression of IGF-I in skeletal muscle biopsies was assessed by two independent methods: Western blot analysis and real-time PCR (Light Cycler). Insulin-like growth factor-I mRNA expression was significantly lower in patients with CHF (6.7 ± 0.4 in CHF vs. 14.0 ± 0.9 arbitrary units in HS, p = 0.001). Specific Western blot analysis confirmed these results on the IGF-I protein level (0.66 ± 0.06 in CHF vs. 1.29 ± 0.15 arbitrary units in HS, p = 0.001) (Fig. 1). The reduction in local IGF-I expression was accompanied by an increase in local IGF-I receptor mRNA (86.8 ± 5.4 in CHF vs. 23.1 ± 1.8 arbitrary units in HS, p < 0.001) (Fig. 2). As a consequence, the ratio IGF-I/IGF-I receptor...
was significantly reduced in CHF (0.094 ± 0.010 in CHF vs. 0.664 ± 0.064 in HS, p < 0.001).

**Skeletal muscle cross-sectional area and maximal isometric force.** Patients and HS of this subgroup did not differ significantly from the total study group in any relevant baseline parameter. Both total and quadriceps muscle cross-sectional areas were reduced in CHF patients, as compared with HS (Table 3). There was a trend toward a reduced maximal isometric contraction of the quadriceps muscle in CHF patients (p = 0.1). Chronic heart failure patients had a significantly greater muscle fatigability as assessed by percent decrease of maximal force during the 20-s contraction (19 ± 5% vs. 4 ± 2%, p = 0.014).

**Correlation between local IGF-I expression and skeletal muscle cross-sectional area.** In the subgroup of CHF patients and HS, with quantification of both quadriceps muscle cross-sectional area and maximal isometric voluntary contraction, a significant correlation between IGF-I mRNA and cross-sectional area (R = 0.75, p = 0.01) and between IGF-I protein and cross-sectional area (R = 0.75, p = 0.01) was observed (Fig. 3).

**DISCUSSION**

Studies in patients with advanced heart failure and cardiac cachexia have revealed a possible involvement of GH and IGF-I in muscle catabolism. It has been shown that skeletal muscle dysfunction and loss of lean muscle bulk can occur in noncachectic patients with CHF (2,5,6). To determine whether alterations in systemic or local IGF-I levels may contribute to skeletal muscle dysfunction and exercise intolerance even before the development of cachexia, we initiated this first study investigating local IGF-I expression in skeletal muscle biopsies of noncachectic CHF patients. Two key messages emerge from this study:

1. Expression of IGF-I is considerably reduced (by more than 50%) in the skeletal muscle of noncachectic patients with severe CHF as compared with HS, although serum
concentrations of IGF-I, GH and their binding proteins remained unchanged. In a subgroup (body mass index < 25 kg/m²), IGF-I decreased, whereas GH increased significantly, consistent with the development of a peripheral GH-resistance.

2. Local IGF-I expression was closely correlated with muscle cross-sectional area, indicating that local IGF-I deficiency might contribute to loss of muscle bulk in CHF.

Role of systemic versus local levels of IGF-I. Although the clinical picture of cardiac cachexia is well known in patients with advanced heart failure, the factors that determine who is at risk for this progressive catabolic syndrome remain unclear. Different endocrine systems have been accused of being involved in this process:

1. An imbalance between catabolic and anabolic steroids with an elevated cortisol/dihydroepiandrosterone ratio has been observed in CHF patients (3,5).

2. An increased resting metabolic rate due to high levels of circulating catecholamines has been reported in CHF (4).

3. Various cytokines are activated in CHF (i.e., TNF-α, IL-6 and others) and have been shown to contribute to loss of muscle bulk and cachexia (3).

4. As a key regulator of normal growth, hypertrophy and atrophy of tissues, the GH/IGF-I axis has recently received more attention as a potential factor for muscle catabolism and wasting in CHF. Elevated levels of GH with inappropriately normal serum levels of IGF-I have been described in cardiac cachexia (5).

All studies mentioned above shared the same methodological approach, i.e., assessment of serum hormone levels. The underlying hypothesis to justify this approach is that endogenous or exogenous stimuli (i.e. endotoxins) induce changes in endocrine secretion and that, therefore, serum hormone levels reflect the activation state of the endocrine system under investigation. This presumption may be correct for hormones like cortisol or GH. However, a significant proportion of IGF-I is produced locally by skeletal muscle fibers and acts as a paracrine, rather than endocrine, regulator of skeletal muscle hypertrophy/atrophy (10). We therefore measured local skeletal muscle IGF-I expression to analyze its possible contribution to the pro-/anticatabolic signaling in CHF.

Local IGF-I expression in severe CHF. As documented in the present study, a reduction of skeletal muscle cross-sectional area may occur before any changes in systemic IGF-I levels can be detected. Local IGF-I expression was substantially downregulated in the skeletal muscle of non-cachectic patients with CHF despite normal IGF-I serum concentrations. Insulin-like growth factor-I receptor expression, on the other hand, increased, indicating a possible feedback mechanism between local IGF-I concentrations and receptor density.

Local skeletal muscle IGF-I expression responds to stimuli from two different sources: Serum GH has the potential to augment local IGF-I expression. This mechanism is important for normal growth and development of the organism. It has been previously shown that a state of GH resistance may develop in patients with advanced CHF (6). Skeletal muscle IGF-I expression is modulated in response to alterations in muscle use. In animal experiments it has been shown that muscle unloading resulted in growth retardation and decreased skeletal muscle IGF-I expression in neonatal rats (11).

The IGF-I receptor has a central role in normal cellular proliferation, as well as in transformation processes. Activation of the receptor after binding of IGF-I elicits a repertoire of cellular responses including proliferation and protection of cells from programmed cell death. Overexpression of the IGF-I receptor results in the malignant transformation of cultured cells; conversely, downregulation of IGF-I receptor levels can reverse the transformed phenotype of tumor cells and may render them sensitive to apoptosis in vivo (12,13).

Increased prevalence of apoptosis has also been observed

![Figure 2. Local expression of insulin-like growth factor (IGF)-I receptor messenger ribonucleic acid (mRNA). CHF = chronic heart failure. ***p < 0.001 vs. healthy subjects.](image)

### Table 3. Skeletal Muscle Mass and Function

<table>
<thead>
<tr>
<th></th>
<th>CHF (n = 10)</th>
<th>Healthy Subjects (n = 8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total muscle area (cm²)</td>
<td>133 ± 7</td>
<td>172 ± 6</td>
<td>0.001</td>
</tr>
<tr>
<td>Quadriceps muscle area (cm²)</td>
<td>59 ± 4</td>
<td>82 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Peak isometric force (N)</td>
<td>237 ± 31</td>
<td>283 ± 31</td>
<td>0.100</td>
</tr>
<tr>
<td>Force decline 20 s (N/s)</td>
<td>−2.3 ± 0.52</td>
<td>−0.6 ± 0.33</td>
<td>0.022</td>
</tr>
<tr>
<td>Force after 20 s (% of peak)</td>
<td>81.2 ± 4.5</td>
<td>96.1 ± 2.3</td>
<td>0.014</td>
</tr>
</tbody>
</table>
in skeletal muscle biopsies from patients with CHF (14, 15). It is, therefore, conceivable that the apoptotic process in the skeletal muscle might be accelerated in response to a decline in local IGF-I expression. The significant up-regulation of IGF-I receptor expression we observed in skeletal muscle biopsies of CHF patients could be interpreted as a "survival signal." One might speculate that skeletal myocytes respond to a pro-apoptotic environment with increased levels of oxidative stress and inflammatory cytokines, as present in CHF, by augmenting their sensitivity to the anti-apoptotic factor IGF-I. Alternatively, the local IGF-I deficiency, itself, might act as a stimulus for increased IGF-I receptor expression in skeletal myocytes.

The anti-apoptotic effects of IGF-I in the presence of proinflammatory cytokines are supported by previous observations that IGF-I receptor activation prevents TNF-α-induced cell death (16). However, it seems that in our study population the increased expression of the IGF-I receptor was not sufficient to compensate the local IGF-I deficit, with the result that skeletal muscle atrophy (as measured by the decrease of muscle cross-sectional area in CHF patients) was not prevented.

Study limitations. Some limitations apply to our findings. Only male patients <70 years of age were included in the present study. It may well be that females or older patients show different patterns of local/systemic IGF-I alterations in CHF. Another aspect requiring further investigation concerns differences in muscle atrophy between the upper and lower extremities. Anker et al. (2) have shown that in noncachectic patients only leg muscle tissue is reduced. This could be caused by different degrees of muscle disuse or different sensitivities to catabolic stimuli.

Additional animal experiments and intervention studies are necessary to confirm a causal role of IGF-I in skeletal muscle apoptosis and atrophy in CHF for two reasons. First, the differentiation between disuse-induced skeletal muscle changes and intrinsic alterations caused by CHF remains difficult in any clinical trial because it is impossible to subject healthy control subjects to exactly the same level of daily physical activity as observed in CHF patients. Second, the local skeletal muscle IGF-I expression needs to be correlated with the local apoptotic rate in the same biopsy. However, the needle biopsy does not provide enough material to reliably perform multiple methods in the same patient—a limitation also experienced by other groups (17). Further studies in animal models where sample size is not an issue will close this gap.

Role of local IGF-I expression for skeletal muscle mass and dysfunction. In the present study, skeletal muscle fatigability was significantly increased, as documented by the rapid decrease of contractile force over time. This finding cannot be explained by skeletal muscle atrophy alone because muscular properties seem to be differentially affected. Although maximal strength depends on the number of contractile filaments, fatigability is influenced by intra-
cellular energy stores and rapid resynthesis of phospho-
creatine. We and others have previously shown that oxida-
tive phosphorylation is attenuated in CHF patients; energy 
transfer by means of mitochondrial creatine kinase is im-
paired, and overall ATP levels are reduced (18–20). These 
facors could explain why muscle fatigability develops earlier 
and does reduction of maximal contractile force.

Previous studies suggested that reduced serum IGF-I 
levels are associated with reduced quadriceps cross-sectional 
area and muscle strength (6). In the present study, however, 
serum IGF-I levels in CHF patients were not significantly 
different from those in HS, indicating that these patients 
might be in a less advanced stage of CHF. However, in 
these non cachectic patients, local muscular IGF-I expres-
sion and quadriceps cross-sectional area were significantly 
reduced, suggesting that a local IGF-I defcit might be a 
more important facor for muscle atrophy than a change in 
IGF-I serum levels. This hypothesis is supported by the 
correlation between local IGF-I expression and muscle 
cross-sectional area.

Clinical implications. This study provides ﬁrst-time evi-
dence that local IGF-I expression is substantially reduced in 
skeletal muscle biopsies of non cachectic CHF patients and 
may be a contributing facor to early loss of leg muscle mass 
and muscular dysfunction in CHF.

Acknowledgment
We acknowledge the expert technical assistance of Ms. Silke 
Krabbes.

Reprint requests and correspondence: Dr. Rainer Hambrecht, 
Professor of Medicine, Heart Center, University of Leipzig, 
Strümpellstr. 39, 04289 Leipzig, Germany. E-mail: hamr@ 
medizin.uni-leipzig.de.

REFERENCES
1. Anker SD, Ponikowski P, Varney S, et al. Wasting as independent risk 
2. Anker SD, Ponikowski P, Levy F, et al. Cytokines and neurohormo-
ones relating to body composition alterations in the wasting syn-
3. Anker S, Clark AL, Kemp M, et al. Tumor necrosis factor and steroid 
metabolism in chronic heart failure: possible relation to muscle 
metabolic rate in patients with congestive heart failure. Ann Intern 
5. Anker SD, Chua TP, Ponikowski P, et al. Hormonal changes and 
catabolic/anabolic imbalance in chronic heart failure and their impor-
factor I in chronic heart failure predicts altered body composition, 
anabolic deﬁciency, cytokine and neurohormonal activation. J Am Coll 
43.
growth factor I (IGF-I) in normal adults, patients with liver cirrhosis 
and acromegaly: experience with a new competitive enzyme immuno-
with stable chronic heart failure: effects on cardiorespiratory ﬁtness 
and ultrastructural abnormalities of leg muscle. J Am Coll Cardiol 
10. Adams GR. Role of insulin-like growth factor-I in the regulation of 
skeletal muscle adaptation to increased loading. Exerc Sport Sci Rev 
in skeletal muscle of transgenic mice does not prevent unloading-
12. Adams TE, Epa VC, Garrett TP, et al. Structure and function of the 
type 1 insulin-like growth factor receptor. Cell Mol Life Sci 2000;57: 
1050–93.
13. Resnicoff M. Antitumor effects elicited by antisense-mediated down-
regulation of the insulin-like growth factor I receptor. Int J Mol Med 
patients with chronic heart failure is associated with exercise intol-
muscle of patients with heart failure: investigation of clinical and 
factor-I receptor inhibits tumor necrosis factor-induced cell death. 
17. Ennezat PV, Malendowicz LS, Testa M, et al. Physical training in 
patients with chronic heart failure enhances the expression of genes of 
mitochondrial ultrastructure and ﬁber type distribution in skeletal 
muscle of patients with stable chronic heart failure. J Am Coll Cardiol 
1997;29:1067–73.
patients with chronic heart failure and increased expression of induc-
ible nitric oxide synthase in the skeletal muscle. J Am Coll Cardiol 
20. Sousa ED, Veksler V, Bigard X, et al. Heart failure affects mitochon-
drial but not myofibrillar intrinsic properties of skeletal muscle. 