Beneficial effects of GH/IGF-1 on skeletal muscle atrophy and function in experimental heart failure

Luciano Dalla Libera, Barbara Ravara, Maurizio Volutrani, Valerio Gobbo, Mila Della Barbera, Annalisa Angelini, Daniela Danieli Betto, Elena Germinario, and Giorgio Vescovo. Beneficial effects of GH/IGF-1 on skeletal muscle atrophy and function in experimental heart failure. Am J Physiol Cell Physiol 286: C138–C144, 2004. First published September 17, 2003; 10.1152/ajpcell.00114.2003.—Skeletal muscle contributes to the origin of symptoms in exercise capacity in heart failure (CHF) (6, 11, 20, 22). An exaggerated hyperventilation due to altered ergoreflex sensitivity has been proposed as the cause of dyspnoea (6, 27). Fatigue may be the result of skeletal muscle bulk loss and changes in fiber type with preferential synthesis of fast anaerobic myosin heavy chains (MHCs) (9, 29, 30, 31, 32). Skeletal muscle atrophy, which strongly correlates with exercise capacity (22, 33), seems to be the consequence of an inflammatory status with production of proapoptotic cytokines such as tumor necrosis factor-α (TNF-α) (5, 9, 10, 11, 30, 32).

Several attempts to prevent apoptosis by blocking the ATII receptor (9) or the caspases cascade (for instance, by using the sphingomyelinase inhibitor L-carnitine) (30) have led to preservation of muscle mass that may have a positive reflection on patients’ exercise capacity.

Growth hormone (GH) has been proposed as a therapeutic tool for the treatment of CHF (8, 14, 15, 18, 25, 34). It has been used either with the purpose of improving hemodynamics (13, 28, 34, 35) or myocardial mass (17). The results have not been univocal, and the potential side effects have discouraged clinicians from using GH in CHF. Recently, the observation that the activation of the GH/IGF-1 (insulin-like growth factor-1) axis can mediate cells’ survival and proliferation has reawakened interest in this molecule, but with a different target: the skeletal muscle (24). The aim of the present study was to see whether in an experimental model of CHF GH may prevent the apoptosis-dependent muscle waste, therefore forming the potential for improving exercise capacity.

MATERIALS AND METHODS

Experimental Model

Five groups of male 80–100 g Sprague-Dawley rats were studied: 20 controls and 50 with CHF induced by monocrotaline (Sigma, St. Louis, MO). This alkaloid produces severe and irreversible pulmonary hypertension followed by right ventricular (RV) failure that mimics the hemodynamic and neurohumoral pattern of heart failure in humans (11, 32). Monocrotaline was injected intraperitoneally at the dose of 30 mg/kg. After 2 wk, the 50 monocrotaline-treated animals were divided into 3 groups. One group of 20 rats had recombinant human GH (Pharmacia, Uppsala, Sweden) 0.2 mg·kg⁻¹·day⁻¹ subcutaneously. A third group of controls had saline. After 2 additional weeks, rats were killed. Tibialis anterior cross-sectional area, myosin heavy chain (MHC) composition, and a study on myocyte apoptosis and serum levels of TNF-α and SPH were carried out. The number of apoptotic nuclei, muscle atrophy, and serum levels of TNF-α and SPH were decreased with GH at high but not at low doses compared with CHF rats. Bcl-2 was increased, whereas activated caspases and bax were decreased. The MHC pattern in GH-treated animals was similar to that of controls. Monocrotaline slowed down both contraction and relaxation but did not affect specific tetanic force, whereas absolute force was decreased. GH treatment restored contraction and relaxation to control values and brought muscle mass and absolute twitch and tetanic tension to normal levels. These findings may provide an insight into the therapeutic strategy of GH given to patients with CHF to improve exercise capacity.
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Health. Experiments were approved by the University of Padua Biological Ethical Committee.

Right Ventricle Hypertrophy and Failure

Right ventricle mass (RVM), left ventricle mass (LVM), and right ventricular mass/right volume (RVM/vol) were measured as previously reported (32). A computerized planimeter was used on photographic pictures of formaline-fixed transverse sections of the heart taken in the middle portion of the interventricular septum. We used the ratio between RVM and RVM/vol as an index of RV dilatation, which we have shown correlates with the severity of heart decompensation (10). We included in the CHF group only animals that showed at post mortem clear signs of right heart failure, such as pleural, pericardial, and peritoneal effusions, together with marked RV dilatation, expressed by a RVM/vol <2.

MHC Distribution

The percentage of distribution of the MHCs was separated electro- phoretically on a 7% polyacrylamide slab with 37.5% vol/vol glyc- erol. Gels were stained with Coomassie brilliant blue (11). The percent of distribution of the three different isoforms was determined by densitometric scan (Sigma Gel, SPSS, Chicago, IL). A linear response in terms of electrophoretic band area was attained on densitometry when 0.1–2 µg of individual MHC were analyzed.

Single-Fiber Cross-Sectional Area

Fiber cross-sectional area (CSA) was taken as an index of muscle atrophy (32). Cross cryosections were taken for histological examina- tion and stained with hematoxylin-eosin. The fiber CSA was cal- culated with a computerized interactive method (Kontron IBAS) and expressed in µm² by counting at least 400 fibers/specimen.

Apoptosis

In situ DNA nick-end labeling. In situ nick-end labeling (TUNEL) of fragmented DNA was performed on cryosections (In Situ Cell Death Detection Kit, POD Roche, Boehringer Mannheim). The total number of labeled nuclei present in the whole specimen was counted and expressed as the number of TUNEL positive nuclei/mm³ (31).

Mitochondria

A gram of tissue was minced in 10 ml of homog- enizing buffer [250 mM sucrose, 0.1 mM EGTA, 10 mM Tris-HCl, pH 7.4, and 1 tablet per 50 ml of the broad spectrum Complete inhibitors cocktail (Roche, Mannheim, Germany)]. The first pellet obtained at 600 g for 10 min represents the nuclear fraction. Supernatant was then centrifuged at 15,000 g for 10 min to obtain the mitochondrial fraction. The microsomal fraction was, pelleted by centrifuging at 100,000 g for 60 min to obtain cytosolic fraction.

Tibialis anterior Western blot for activated caspases 9, Bcl-2, Bax, and cytochrome c. Western blot was performed with total tissue homogenates in SDS buffer on 12.5% polyacrylamide gels (9). The following antibodies were used: anti-Bcl-2 (1:500) (Oncogene, Darmstadt, Germany), anti-cleaved caspase-9 (1:1,000) (Cell Signaling Technology, Beverly, MA), anti-Bax (1:500), and monoclonal anti- cytochrome c (1:1,000) (Alexis, San Diego, CA). For caspases, Bax, and Bcl-2, 50 µg of protein were loaded; for cytochrome c, 5 µg were loaded. All blots were revealed by chemiluminescent substrate (SuperSignal West Pico, Pierce, IL). All the blots were run under conditions to yield a linear relationship between protein and signal.

ELISA for apoptosis. DNA ladder assay was performed as previ- ously described (30). Briefly, 10 muscle cryosections 20 µm thick were solubilized in 200 µl of lysis buffer (0.1% Triton X-100, 5 mM Tris-HCl, 20 mM EGTA, and 20 mM EDTA). Polyethylene glycol 8000 and NaCl were then added to a final concentration of 2.5% and 1 M, respectively. Samples were centrifuged and protein concentra- tion was adjusted by the method of Bradford to 0.01 µg/µl. Cell death enzyme-linked immunoabsorbent assay (ELISA) analysis was per- formed according to the manufacturers instructions with the Cell Death Detection ELISA PLUS kit (Roche Mannheim, Germany).

Caspase-3 assay. Caspase-3 activity was measured with the CaspACE assay system (Promega, Madison, WI).

TNF-α

TNF-α was measured with a solid-phase sandwich ELISA using a monoclonal antibody specific for rat TNF-α (Euroclone, UK).

ANG II

ANG II was measured by using an enzyme-immunometric assay kit (SPL-BIO, Massy Cedex, France). We used ANG II as an index of the CHF neurohormonal activation. In this model of CHF, decompensated rats have significantly increased levels of this peptide (9).

IGF-1

IGF-1 was measured by using an enzyme-immunometric assay kit (R&D Systems Minneapolis, MN).

SPH

SPH was determined with high-performance liquid chromatogra- phy (HPLC) with the method previously described by Dalla Libera et al. (10). Briefly, sphingolipids were extracted with butanol and de- rivatized with ophthaldialdehyde. Samples were separated on a Bio- Rad hi-Pore reversed phase column on a Bio-Rad HPLC system with a Perkin-Elmer fluorescence detector (excitation 340 nm, emission 455 nm).

Plasma Glucose

Plasma glucose was determined with a colorimetric method.

Mechanical Studies

In a separate set of six control, two monocrotaline, and sixmono- crotaline animals with CHF treated with high doses of GH, the isometric contractile properties of isolated soleus were investigated. Contractility of soleus muscle was studied in vitro at 30 ± 1°C. Muscle bathing, stimulation conditions, and tension recording were performed as previously described (23). Twitches were obtained by applying single supramaximal stimuli (0.5-ms duration). The follow- ing parameters were measured: contraction time (CT), half-relaxation time (HRT), and twitch tension (Pt). The frequency-tension curves were constructed from recordings of the force exerted by soleus muscles during 1-s stimulation at 15, 30, 60, 90, and 120 Hz. From the 120-Hz tetani, the following parameters were obtained: half-relaxa- tion time of the tetanus (HRTT) and maximum tetanic tension (P0). Absolute twitch tension and tetanus tension were normalized to wet weight of the muscles to obtain the specific tension (N g⁻¹). Muscle responses were recorded via an AT-MIO 16 analog-to-digital card and data were analyzed by a LabView-based computer program (National Instruments).

Statistical Analysis

Means ± SD are reported. Student t-test for unpaired data was used. P ≤ 0.05 was considered statistically significant. ANOVA was used for comparing skeletal muscle, apoptotic cascade measurements, and contractile parameters measurements.

RESULTS

Body Weight

As shown in Table 1, body weight (BW) reached 237 ± 10 g in the control rats at the end of the study period. Both low
Table 1. *Body weight and cardiac morphometry*

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>LVM, mm³</th>
<th>RVM, mm³</th>
<th>RVM/Vol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>237±10 b,c,d</td>
<td>43.5±5.4 b,h,k</td>
<td>10.8±3.4 m,a,p</td>
<td>2.6±0.2 u,l,i,n</td>
</tr>
<tr>
<td>GH low</td>
<td>260±20</td>
<td>55.0±6.4 b</td>
<td>19.2±1.0 p</td>
<td>2.2±0.4 b</td>
</tr>
<tr>
<td>GH high</td>
<td>272±10 a</td>
<td>50.5±2.8 g</td>
<td>25.1±0.2 k</td>
<td>2.7±0.2 a</td>
</tr>
<tr>
<td>CHF</td>
<td></td>
<td>31.5±6.1 h</td>
<td>16.3±4.0 m,o</td>
<td>1.7±0.2 m</td>
</tr>
<tr>
<td>CHF+GH (low dose)</td>
<td>206±20 b,e</td>
<td>47.9±6.2</td>
<td>19.2±4.4</td>
<td>1.4±0.2 m</td>
</tr>
<tr>
<td>CHF+GH (high dose)</td>
<td>241±31 b,f</td>
<td>48.2±7.2 j</td>
<td>20.2±6.3 m,o</td>
<td>2.0±0.6 a</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.01; bP < 0.00002; cP < 0.003; dP = NS; eP < 0.0001; fP = NS; gP < 0.0001; hP = NS; iP < 0.01; jP < 0.001; kP < 0.0002; lP < 0.000001; mP < NS; nP = NS; oP < 0.00001; iP < 0.002.

(L-GH) and high (H-GH) doses of GH led to a significant increase in BW (*P < 0.01*).

Animals with overt heart failure and no treatment showed a significantly lower BW compared with controls (*P < 0.0002*).

L-GH given to CHF rats was unable to produce any change in BW, whereas CHF rats treated with H-GH showed a BW identical to that of controls, indicating that high doses of GH were able to produce a restoration of body weight in the monocrotaline-treated rats.

*Occurrence of Heart Failure in the Monocrotaline-Treated Animals*

After 4 wk, all the CHF rats showed a significantly higher RV mass. The right ventricular cavity was markedly dilated as reflected by the RVM/vol index, which was 1.7 ± 0.2 in the CHF rats vs. 2.6 ± 0.2 in the control group.

In control rats, H-GH and L-GH were able to increase both RV and LV mass compared with controls. In CHF animals, H-GH and L-GH showed an increase in mass of both ventricles of similar magnitude compared with control CHF rats, which indicates a probable trophic effect of GH on the myocardium.

*ANG II Levels*

As shown in Table 2, ANG II levels in control rats were the same as those of the GH rats. The CHF rats showed a significant (*P < 0.02*) increase in ANG II, whereas H-GH failed to produce in the monocrotaline-treated rats a significant decrease in ANG II levels, indicating that there were no effects on the neurohormonal activation produced by the RV decompensation.

*IGF-1*

Control rats treated with H-GH showed a significant increase of IGF-1 (*P < 0.02*). CHF rats showed the same IGF-1 levels of controls. In CHF rats treated with H-GH, IGF-1 was significantly increased compared with control CHF animals (*P < 0.02*). There was also a further 30% increase compared with GH-treated controls.

Table 2. *Serum measurements*

<table>
<thead>
<tr>
<th>Group</th>
<th>ANG II, pg/ml</th>
<th>IGF-1, % of control</th>
<th>Glucose, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28±3 b,h,k</td>
<td>100±10 e</td>
<td>132±21 b,k</td>
</tr>
<tr>
<td>GH</td>
<td>26±4</td>
<td>165±25 f</td>
<td>105±9 g</td>
</tr>
<tr>
<td>CHF</td>
<td>50±13 c,e</td>
<td>95±21 d,l</td>
<td>128±18 h</td>
</tr>
<tr>
<td>CHF+GH (high dose)</td>
<td>53±20 b,h</td>
<td>195±30 e</td>
<td>114±28</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.02; bP < 0.005; cP = NS; dP = NS; eP < 0.02; fP = NS; gP = NS; hP = NS; iP = NS.

Blood Glucose

We did not find significant alterations in blood glucose in any group of rats: the values were in the range 105–132 mg/dl.

*Tibialis Anterior MHC Pattern*

As shown in Table 3, we found in the CHF animals a shift from the fast oxidative (MHC2a) to the fast glycolytic isoform (MHC2b), which was 27 ± 1.4% and 73 ± 1.4% in the control vs. 18 ± 3% (*P < 0.00001*) and 82 ± 3% in the CHF, respectively. H-GH produced a reshift toward the MHC2a (26 ± 2.7; *P < 0.009*), whereas MHC2b was 74 ± 2.7%. L-GH gave no reshift in that the values of MHCs were similar to that of CHF. We therefore achieved a complete restoration of the control MHCs pattern only after high GH doses.

*Tibialis Anterior CSA*

The degree of muscle atrophy as measured by fiber CSA was 1,969 ± 257 μm² in the control vs. 1,310 ± 267 in the CHF rats (*P < 0.00007*). The H-GH produced an increase in CSA (1,788 ± 264), which reached statistical significance (*P < 0.001*) compared with CHF. By contrast, L-GH had no effect. Only high GH doses were therefore able to restore the TA muscle mass nearly to the same degree of the control rats.

*TUNEL-Positive Nuclei*

As shown in Table 4, the CHF rats showed an enormous increase in the number of TUNEL-positive nuclei, which was 168 ± 66 nuclei/mm³, as opposed to 7 ± 3 of the control and GH control animals (*P < 0.006*). The treatment with H-GH produced in the monocrotaline-treated animals a significant reduction in the number of TUNEL-positive nuclei (30 ± 17; *P < 0.00001 vs. CHF and P = not significant vs. control). The number of apoptotic nuclei was brought to levels similar to those of the control rats. L-GH did not influence the number of

Table 3. *Skeletal muscle measurements*

<table>
<thead>
<tr>
<th>Group</th>
<th>MHC IIa, % of total</th>
<th>MHC IIb, % of total</th>
<th>CSA, μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27±1.4 b,h,k</td>
<td>73±1.4 b,h</td>
<td>1969±257 b,h</td>
</tr>
<tr>
<td>GH</td>
<td>25±3.3</td>
<td>74.6±2.3</td>
<td>1735±400</td>
</tr>
<tr>
<td>CHF</td>
<td>18±3.3</td>
<td>82±3</td>
<td>1310±267 b,h</td>
</tr>
<tr>
<td>CHF+GH (low dose)</td>
<td>15.6±6.1 b,d</td>
<td>84.3±6.1</td>
<td>1370±300 b</td>
</tr>
<tr>
<td>CHF+GH (high dose)</td>
<td>26±2.7 b,d</td>
<td>74±2.7</td>
<td>1788±264 b</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.00001; bP < 0.008; cP = NS; dP = NS; eP < 0.009; fP < 0.00007; gP < 0.0004; hP = NS; iP = NS; jP < 0.0001.
and inevitably lead to programmed cell death. Bax (Fig. 1, lanes b), which are detectable during the execution phase of apoptosis, is located between inner and outer mitochondrial membranes and is released into the cytosol upon stimulation to bind Apaf-1 and activate caspase-3. In CHF animals, we have shown that the concentration of cytochrome c is absent in cytosolic fraction from control and GH-treated TA muscle.

TUNEL-positive nuclei, which was similar to that of CHF animals.

**Activated Caspase-9, Bcl2, Bax, and Confocal Microscopy for Caspase 3**

We confirmed the occurrence of apoptosis in animals with a high number of TUNEL-positive nuclei by testing for the presence of the proapoptotic Bax and activated caspases 9 and 3, which are detectable during the execution phase of apoptosis and inevitably lead to programmed cell death. Bax (Fig. 1F) and caspase 9 (Fig. 1E) were shown with immunoblotting. Activated caspase 3 was shown with immunofluorescence (Fig. 1C). Higher levels of Bax and caspase 9 were demonstrated at immunoblotting (Fig. 1, lanes b in F and E) in CHF animals, compared with controls (Fig. 1, lanes a). The GH-treated rats (Fig. 1, lanes c–e) showed a reduced expression of these two proapoptotic molecules compared with CHF.

The antiapoptotic Bcl-2 behaved in the opposite manner. It was decreased in CHF (Fig. 1C and lanes b) compared with control (Fig. 1, lanes a). The GH animals showed Bcl-2 expression similar to that of controls. (Fig. 1, lanes c–e). These data parallel and therefore confirm those previously reported for the count of apoptotic nuclei.

**Cytochrome c**

As shown in Fig. 2, cytochrome c, together with Apaf-1 and caspase-9, has been reported to cause caspase-3 activation. Normally, cytochrome c is located between inner and outer mitochondrial membranes and is released into the cytosol upon stimulation to bind Apaf-1 and activate caspase-3. In CHF animals, we have shown that the concentration of cytochrome c in the cytosol is increased. In contrast, GH-treated rats showed no cytochrome c in the cytosolic fraction.

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**Table 4. Apoptotic cascade in skeletal muscle and serum**

<table>
<thead>
<tr>
<th></th>
<th>TUNEL+, nuclei/mm³</th>
<th>TNFα, pg/ml</th>
<th>SPH, pmoles/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7 ± 3,b,c</td>
<td>114±12s,h</td>
<td>850±70k,m</td>
</tr>
<tr>
<td>GH</td>
<td>5 ± 2</td>
<td>118±20</td>
<td>900±80</td>
</tr>
<tr>
<td>CHF</td>
<td>168±66,α,d</td>
<td>282±100,i,j</td>
<td>1300±210,a,n</td>
</tr>
<tr>
<td>CHF+GH (low dose)</td>
<td>140±38,α,d</td>
<td>272±85,α,i</td>
<td>1250±170,a,m</td>
</tr>
<tr>
<td>CHF+GH (high dose)</td>
<td>30±17,α,e</td>
<td>175±39,α</td>
<td>710±110,a,m</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.006; †P < 0.006; ‡P = NS; §P = NS; ¶P < 0.0001; ‐P < 0.04; *P < 0.04; †P < 0.03; ‡P = NS; §P < 0.002; ¶P < 0.04; ‐P < 0.04; ″P = NS; †P = NS; ‡P < 0.00001.

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**Fig. 1.** Skeletal muscle apoptosis in exercise capacity in heart failure (CHF). A: double labeling for TUNEL and laminin. B: double exposure for laminin and Hoechst. Arrows indicate apoptotic nuclei (magnification, ×400). C: immunofluorescence for activated caspase 3 and dystrophin from a CHF animal. Thin, granulated pattern indicates diffuse cytosolic distribution of caspase 3. Positive fibers (red) are surrounded by negative fibers (black). Sarcolemma is stained in green (magnification, ×400). D: Western blot for Bcl-2. Lane a, control; lane b, untreated CHF rat; lanes c–e, growth hormone (GH)-treated rats (high dose). E: Western blot for activated Caspase 9. Lane a, control; lane b, untreated CHF rat; lanes c–e, GH-treated rats (high dose). F: Western blot for Bax. Lane a, control; lane b, untreated CHF rat; lanes c–e, GH-treated rats (high dose).
ELISA for Apoptosis

As shown in Fig. 3, apoptosis in the CHF was also confirmed by detection of fragmented DNA by ELISA. The GH animals showed values similar to those of controls.

Caspase-3 Activity

As shown in Fig. 4, caspase-3 was found to be activated in CHF animals. The GH animals showed values very similar to those of controls, indicating that GH may prevent caspases activation.

TNF-α

As shown in Table 4, Serum levels of TNF-α in the CHF animals were raised compared with controls (282 ± 100 vs. 114 ± 12 pg/ml; \( P < 0.04 \)). H-GH treatment produced a slight decrease in TNF-α levels compared with CHF (\( P < 0.002 \)). L-GH was unable to modify levels of TNF-α that were the same of the CHF rats.

Table 5. Soleus muscle mechanical properties

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CHF</th>
<th>CHF + GH (high dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle weight, mg</td>
<td>128 ± 3*</td>
<td>83 ± 1*</td>
<td>141 ± 5</td>
</tr>
<tr>
<td>( n )</td>
<td>8</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Twitch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute ( P_t ) (N)</td>
<td>0.170 ± 0.03</td>
<td>0.133 ± 0.024</td>
<td>0.161 ± 0.025</td>
</tr>
<tr>
<td>Specific ( P_t ) (N g(^{-1}))</td>
<td>1.38 ± 0.19</td>
<td>1.42 ± 0.20</td>
<td>1.21 ± 0.14</td>
</tr>
<tr>
<td>CT, ms</td>
<td>45.1 ± 5.1</td>
<td>47.7 ± 2.8( b )</td>
<td>43.3 ± 2.1( b )</td>
</tr>
<tr>
<td>HRT, ms</td>
<td>52.5 ± 7.5( c )</td>
<td>61.6 ± 2.9( d, d )</td>
<td>51.1 ± 4.5( d )</td>
</tr>
<tr>
<td><strong>Tetanus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute ( P_0 ) (N)</td>
<td>0.99 ± 0.19( e )</td>
<td>0.66 ± 0.12( d, f )</td>
<td>1.08 ± 0.05( g )</td>
</tr>
<tr>
<td>Specific ( P_0 ) (N g(^{-1}))</td>
<td>7.97 ± 0.97</td>
<td>8.02 ± 1.26</td>
<td>7.77 ± 0.88</td>
</tr>
<tr>
<td>HRTT</td>
<td>99.0 ± 14.0</td>
<td>109.0 ± 8.5( f )</td>
<td>86.2 ± 9.5( f )</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n \) is the number of muscles examined. \( P_t \), maximal twitch tension; CT, contraction time; HRT, half-relaxation time; \( P_0 \), maximal tetanic tension; HRTT, half relaxation tetanic tension. *\( P < 0.0005; \) \( P < 0.0001; \) \( P < 0.005; \) \( P < 0.005; \) \( P < 0.0001; \) \( P < 0.05. \)

SPH

SPH increased in CHF (1,300 ± 210 vs. 850 ± 70 pmol/ml in control; \( P < 0.04 \)). H-GH produced a decrease in SPH levels (710 ± 110) that were significantly different from CHF and similar to control rats. As for TNF-α, L-GH did not change SPH levels.

Soleus Muscle Isometric Contractile Properties

As shown in Table 5, monocrotaline treatment reduced body weight and soleus weight compared with controls (83 ± 1 vs. 128 ± 3; \( P < 0.0005 \)). After high doses of GH, both body weight and soleus weight in the monocrotaline-treated rats increased to values even higher than controls (141 ± 5; \( P < 0.05 \)). Monocrotaline treatment significantly increased twitch time of soleus muscle (47.7 ± 2.8 vs. 45.1 ± 5.1) while not affecting specific tension. This slowing of the soleus muscle is confirmed by the significant shift to the left of the frequency-tension curve compared with controls (\( P < 0.05 \)) (Fig. 5). GH treatment produced a normalization of the contraction (42.3 ± 2.1 vs. controls 45.1 ± 5.1) and half-relaxation times (51.1 ± 4.5 vs. controls 52.5 ± 3.7). Also, the half-relaxation time of the tetanus was increased in CHF rats (109 ± 8.5 vs. 98.6 ± 14.0) and returned to control values after GH treatment (86.2 ± 9.5, \( P < 0.05 \) vs. CHF). In tetanus, absolute but not specific tension significantly decreased in CHF rats as a consequence of the reduced muscle mass (0.66 ± 0.12 vs. 0.99 ± 0.19, \( P < 0.005 \)). Specific and absolute tension of the GH-treated animals were not different from the control values. Differences seen in absolute force are therefore related to the muscle mass, which is decreased in CHF rats. GH-treated animals have an absolute force identical to that of controls because they have similar muscle mass.

DISCUSSION

In this paper, we describe for the first time the effect of GH administration on skeletal muscle of rats with right-sided heart failure. At the cardiac level, both doses of GH produced only a slight degree of hypertrophy in both ventricles, as previously reported (13). By contrast, at the skeletal muscle level, the
The vertical bars indicate means (±SD). The frequency-tension curve of CHF muscle is significantly shifted to the left, thus confirming the slowing of contraction. GH-treated muscle behaved as control.

Fig. 5. Frequency-tension relationships for control (○, n = 7), monocrotaline (□, n = 4), and CHF + GH (□, n = 8) soleus muscles. *p < 0.05 with respect to the control. The vertical bars indicate means ± SD. The frequency-tension curve of CHF muscle is significantly shifted to the left, thus confirming the slowing of contraction. GH-treated muscle behaved as control.
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REFERENCES


