Effects of Growth Hormone Supplementation on Left Ventricular Morphology and Myocyte Function With the Development of Congestive Heart Failure

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Background—Release of growth hormone (GH), putatively through alterations in insulin growth factor-1 (IGF-1) levels, has been implicated to influence left ventricular (LV) myocardial structure and function. The objective of this study was to determine contributory mechanisms by which GH supplementation may influence LV function with the development of congestive heart failure (CHF).

Methods and Results—Pigs were assigned to the following groups: (1) chronic pacing at 240 bpm for 3 weeks (n = 10), (2) chronic pacing and GH supplementation (200 μg · kg⁻¹ · d⁻¹, n = 10), and (3) controls (n = 8). GH treatment increased IGF-1 plasma levels by nearly 2.5-fold throughout the pacing protocol. In the untreated pacing CHF group, LV fractional shortening was reduced and peak wall stress increased. In the pacing CHF and GH groups, LV fractional shortening was higher and LV wall stress lower than untreated CHF values. Steady-state myocyte velocity of shortening was reduced with pacing CHF and was unchanged from CHF values with GH treatment. In the presence of 25 nmol/L isoproterenol, the change in myocyte shortening velocity was reduced in the untreated CHF group and increased in the GH-treated group. LV sarcoplasmic reticulum Ca²⁺-ATPase abundance was reduced with pacing CHF but was normalized with GH treatment.

Conclusions—Short-term GH supplementation improved LV pump function in pacing CHF as a result of favorable effects on LV remodeling and contractile processes. Thus, GH supplementation may serve as a novel therapeutic modality in developing CHF. (Circulation. 1999;100:2003-2009.)

Key Words: ventricles • contractility • hormones • growth substances

A lthough the causes of congestive heart failure (CHF) are diverse, a common event in the progression of this disease process is left ventricular (LV) dilation and subsequent pump dysfunction. Past clinical and experimental studies have demonstrated that growth hormone (GH) can directly influence LV myocardial structure and function.¹⁻⁶ For example, GH supplementation in patients with cardiomyopathic disease resulted in an improvement in LV pump function.⁵ GH supplementation may produce 2 potentially independent effects with developing CHF. First, GH treatment may induce a myocardial growth response that will alter LV geometry and wall thickness and as a consequence, reduce LV wall stress. The reduction in LV wall stress that may be induced by GH treatment with CHF would be expected to yield a favorable effect on LV pump function. Second, GH supplementation with developing CHF could potentially provide a direct beneficial effect on LV myocyte contractile performance, which would be independent of changes in LV geometry and loading conditions. The overall goal of the present study was to measure LV function and geometry, as well as LV myocyte contractile function after GH supplementation with developing CHF.

Methods

Instrumentation and Experimental Design

Twenty-eight Yorkshire pigs (25 kg, male, Hambone Farms, Orangeburg, SC) were chronically instrumented with an aortic catheter (model GPV, 9F, Access Technologies) and a modified atrial pacemaker (8329, Medtronic, Inc) as described previously.⁷⁻¹⁰ After a 14- to 21-day recovery from the surgical procedure, pigs were randomly assigned to the following treatment groups: (1) chronic rapid pacing at 240 bpm for 3 weeks (n = 10), (2) chronic rapid pacing and GH supplementation (n = 10), and (3) sham controls (n = 8). GH treatment by daily subcutaneous injection of 200 μg/kg recombinant porcine GH was started 3 days before the activation of the pacemaker and continued throughout the

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Dr Pan and C. Pirie are employed by Pfizer, which also supported this study financially.

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21-day pacing protocol. In preliminary studies, this dosing regimen caused a 2- to 2.5-fold increase in plasma IGF-1 levels in normal pigs, and this was the therapeutic target used for the present study. All animals were treated and cared for in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC, 1996).

LV Function Measurements
All studies were performed with the pigs in the conscious state, and the pacemaker was deactivated. After a 30-minute stabilization period, 2D and M-mode echocardiographic studies (ATL Ultramark VI, 2.25-MHz transducer) were used to image the LV from a right parasternal approach. LV fractional shortening, LV stroke volume, and peak circumferential wall stress were computed. From the arterial catheter, blood was drawn into chilled tubes containing EDTA and centrifuged. In 5 control pigs, LV fractional shortening was measured after incremental increases in LV myocardial wall stress through a phenylephrine infusion so as to obtain 3 to 6 isochronal LV peak circumferential wall stress versus shortening points. After the completion of the experimental protocols, the animals were anesthetized deeply with 4% isoflurane, and the LV was removed and processed for studies.

Neurohormonal Profiles and IGF-1 Levels
Plasma renin activity was determined by computing angiotensin I production by a radioimmunoassay procedure (ARUP Laboratories), and norepinephrine was measured by high-performance liquid chromatography. IGF-1 plasma levels were measured in acid-methanol–extracted samples by radioimmunoassay (No. 40-2100, Nichols Institute Diagnostics).

LV Myocyte Contractile Function
Isolated LV myocyte contractility was examined by computer-assisted videomicroscopy. After baseline measurements, contractile function was examined after β-adrenergic receptor stimulation with 25 nmol/L isoproterenol (−Isoproterenol, Sigma Chemical Co).

LV Myocardial Structure and Composition
LV myocardial sections cut in the circumferential orientation were examined by light microscopy to evaluate myocyte cross-sectional area by computer-assisted methods described previously. LV myocyte volumes were determined from the cross-sectional area and isolated myocyte resting length. Total myocyte number was then computed from the LV myocardial volume and the morphometrically determined isolated myocyte volume. LV sections were stained with the lectin GSA-B, to identify capillary endothelium and compute capillary density. LV myocardial collagen content was determined by use of a biochemical assay for hydroxyproline. LV crude membrane preparations were used to measure the abundance of sarcoplasmic reticulum Ca2+-ATPase (SR Ca2+-ATPase) by immunoblotting.

Data Analysis
Comparisons between the treatment groups were performed by ANOVA. If the ANOVA revealed significant differences, pairwise tests of individual group means were compared by use of Bonferroni probabilities. Results are presented as mean±SEM. Values of P<0.05 were considered to be statistically significant.

Results
LV Function and Neurohormonal Profiles With Rapid Pacing: Effects of GH Supplementation
Weekly indices of LV function obtained with each week of chronic rapid pacing, with and without GH supplementation, are summarized in Figure 1. LV end-diastolic dimension and peak wall stress increased and LV fractional shortening decreased in a time-dependent manner in the untreated pacing group. After 2 and 3 weeks of GH treatment, LV fractional shortening was significantly higher than untreated rapid pacing values. After 3 weeks of rapid pacing, LV peak wall stress was 35% lower in the GH-treated group than in the untreated group.
In the rapid pacing only group, ambient resting heart rate was increased and resting blood pressure decreased from control values (Table 1). In the GH treatment group, heart rate was reduced from rapid pacing only values, and blood pressure was normalized. With GH treatment, LV stroke volume was increased from untreated pacing values. To more carefully examine the relationship between changes in LV ejection performance and wall stress, the steady-state values for fractional shortening versus peak wall stress were plotted (Figure 2). After chronic pacing, a significant shift was observed, indicating a significant decline in LV ejection performance. With GH treatment, the reduction in LV wall stress accompanied by an improvement in LV pump function resulted in a left-upward shift in this relationship.

After 1 week of rapid pacing, plasma norepinephrine and renin activity increased from control values and appeared to plateau with longer durations of pacing (Figure 3). In the GH-treated group, plasma norepinephrine was lower than untreated values after 1 and 2 weeks of rapid pacing. In the rapid pacing only group, IGF-1 levels increased from control values after 1 and 2 weeks of rapid pacing. In the GH-treated group, plasma norepinephrine was lower than plateaus with longer durations of pacing (Figure 3). In the GH-treated rapid pacing group, LV end-diastolic wall thickness was similar to that of controls and was associated with a 36% increase in the LV mass/body weight ratio. The body weight in the GH-treated group was increased by 33% compared with time-matched control pigs. The average kidney weight at autopsy was unchanged in the rapid pacing group compared with controls (41±2 g vs 41±2 g) but was increased in the GH-treated group (55±4 g, P<0.05). Consistent with hepatomegaly secondary to CHF, liver weight tended to be higher in the rapid pacing group than in controls (667±134 g vs 487±21 g, respectively, P=0.23). In the GH-treated group, liver weight increased from both control and untreated rapid pacing values (726±40 g, P<0.05). It has been demonstrated previously that GH supplementation in normal pigs induces an ~40% increase in liver weight after a 28-day treatment period.16

**Figure 2.** An LV ejection–stress relationship was determined for 5 control pigs through measurements of isochronal points during a phenylephrine infusion to determine LV fractional shortening–peak wall stress relation. Solid lines indicate linear regression (fractional shortening–stress) relation for these isochronal points. Steady-state values obtained after 3 weeks of rapid pacing or with rapid pacing and GH treatment are shown. After chronic pacing, a significant shift was observed, indicating a decline in LV ejection performance. With GH treatment, reduction in LV wall stress accompanied by an improvement in LV pump function resulted in a left-upward shift in this relationship.

<table>
<thead>
<tr>
<th>TABLE 1. Systemic Hemodynamics, LV Mass, and Morphometry With Chronic Rapid Pacing: Effects of GH Administration</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
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<tr>
<td><strong>Systemic hemodynamics</strong></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
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<tr>
<td>Stroke volume, mL</td>
</tr>
<tr>
<td><strong>LV wall thickness and mass</strong></td>
</tr>
<tr>
<td>Wall thickness, mm</td>
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<tr>
<td>LV mass/body weight ratio, g/kg</td>
</tr>
<tr>
<td>LV mass/volume ratio, ×10−2 g·kg−1·mL−1</td>
</tr>
<tr>
<td><strong>LV myocardial structure and composition</strong></td>
</tr>
<tr>
<td>Myocyte length, μm</td>
</tr>
<tr>
<td>Myocyte cross-sectional area, μm²</td>
</tr>
<tr>
<td>Myocyte volume, ×10³ μm³</td>
</tr>
<tr>
<td>Myocyte number, ×10⁶</td>
</tr>
<tr>
<td>Capillary density, n/mm²</td>
</tr>
<tr>
<td>Sample size, n</td>
</tr>
</tbody>
</table>

Values presented as mean±SEM.
*P<0.05 vs control; †P<0.05 vs rapid pacing only.
§Rapid pacing: 3 weeks of chronic rapid pacing at 240 bpm.
||LV mass indexed to body weight and LV end-diastolic volume computed from echocardiography.

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LV Myocyte Contractility With Rapid Pacing: Effects of GH Supplementation

Myocyte contractile function was examined in >800 LV myocytes in each of the 3 groups. The values for LV myocyte resting length are summarized in Table 1. Steady-state myocyte contractile function was significantly reduced in the untreated rapid pacing group compared with normal control values (Table 2). In the GH-treated group, indices of steady-state myocyte contractile function were unchanged from untreated rapid pacing values. β-Receptor stimulation with isoproterenol increased myocyte function from basal values in all 3 groups. In the GH-treated group, plasma norepinephrine was lower than untreated values after 1 week of pacing. In GH-treated group, plasma norepinephrine increased from control values after 3 weeks of rapid pacing. Plasma renin activity was similar in both rapid pacing groups.

LV Myocardial Structure and Composition

LV myocyte cross-sectional area decreased from control values in the rapid pacing group (Table 1). In the GH-treated rapid pacing group, myocyte cross-sectional area was significantly increased from control and rapid pacing only values. The increased LV myocyte length and concomitant reduction in myocyte cross-sectional area resulted in similar computed myocyte volumes in the control and rapid pacing only groups (Table 2). However, computed myocyte volume was greater in the GH-treated rapid pacing group than in the control or untreated pacing values. Compared with control values, myocyte number was reduced in the pacing CHF group but remained unchanged in the GH-treated group. LV capillary density was higher in the GH-treated group than in pacing CHF or control values. LV myocardial hydroxyproline content was reduced with pacing CHF compared with controls (2.26 ± 0.20 versus 2.52 ± 0.17 mg/g dry wt, P = 0.07). With concomitant GH treatment, LV myocardial hydroxyproline values were reduced from control values, but this did not reach statistical significance (2.35 ± 0.25 mg/g dry wt, P = 0.25).

LV membrane preparations with identical protein concentrations were analyzed from each treatment group, and the relative abundance of SR Ca²⁺-ATPase was determined with

Figure 3. Neurohormonal profiles were serially measured with each week of rapid pacing with and without concomitant GH treatment (rapid pacing + GH). Top, In untreated rapid pacing group, plasma norepinephrine increased by ~5-fold from basal levels after 1 week of pacing and plateaued with longer durations of pacing. In GH-treated group, plasma norepinephrine was lower than untreated values after 1 week of pacing. In GH-treated group, plasma norepinephrine increased from control values after 3 weeks of rapid pacing. Bottom, Plasma renin activity increased by ~3-fold in both rapid pacing groups after 1 week of pacing and remained elevated with longer durations of pacing. Plasma renin activity was similar in both rapid pacing groups. *P < 0.05 vs control values, +P < 0.05 vs respective rapid pacing only values.
respect to the control signal (Figure 5). In the rapid pacing group, the relative abundance of SR Ca\(^2^+\)-ATPase was reduced from control levels but was normalized in the GH-treated rapid pacing group.

**Discussion**

Although a clinical study as well as experimental reports suggest that GH supplementation may be beneficial during the development of CHF, contributory mechanisms for these effects are unclear. Accordingly, the present study examined the direct effects of short-term GH supplementation on LV myocyte contractile performance and myocardial structure in an animal model of pacing-induced CHF. The important findings of the present study are based on the underlying mechanisms for improved LV function with GH treatment. First, GH treatment with pacing CHF increased LV wall thickness, which in turn reduced LV peak wall stress. This favorable effect on LV wall stress patterns was further demonstrated by an increased LV mass/volume ratio with GH treatment. Second, GH supplementation with CHF improved the capacity of the LV myocyte to respond to an inotropic stimulus. Third, the structural basis for the effects of GH supplementation included increased LV myocyte volumes, protection from LV myocyte loss, increased capillary density, and a normalization of SR Ca\(^2^+\)-ATPase. Thus, the improved LV pump function that occurred with GH supplementation in this model of CHF was probably due to favorable effects on LV myocardial remodeling and contractile processes.

**LV Myocardial Growth and GH Supplementation With Pacing CHF**

GH release into the systemic circulation causes increased plasma levels of IGF-1, which is an important local mediator for the effects of GH. With developing LV hypertrophy in rats, increased content and expression of IGF-1 have been identified within the myocardium. With pacing CHF, LV dilation and increased wall stress are not accompanied by significant changes in LV mass or steady-state contractile protein content, possibly because of an acceleration of contractile protein degradative rates. In the present study, GH supplementation in pigs undergoing chronic pacing caused an increase in LV mass and was accompanied by a 2.5-fold increase in circulating IGF-1 levels. IGF-1 has been reported to accelerate contractile protein synthesis rates in the myocardium. Thus, an important mechanism for the increased LV mass in this model of pacing CHF was probably a result of the direct effects of IGF-1 on myocardial contractile protein synthesis.

**TABLE 2. Isolated Myocyte Contractile Function With Chronic Rapid Pacing: Effects of GH Administration**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>25 nmol/L Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percent shortening, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.9±0.1</td>
<td>8.8±0.9†</td>
</tr>
<tr>
<td>Rapid pacing§</td>
<td>2.5±0.2*</td>
<td>5.2±0.5†</td>
</tr>
<tr>
<td>Rapid pacing + GH</td>
<td>2.8±0.2*</td>
<td>6.0±0.4†</td>
</tr>
<tr>
<td><strong>Shortening velocity, μm/s</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>54±5</td>
<td>149±18‡</td>
</tr>
<tr>
<td>Rapid pacing§</td>
<td>36±3*</td>
<td>103±11†</td>
</tr>
<tr>
<td>Rapid pacing + GH</td>
<td>39±3*</td>
<td>121±11††</td>
</tr>
<tr>
<td><strong>Relengthening velocity, μm/s</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>57±3</td>
<td>132±18‡</td>
</tr>
<tr>
<td>Rapid pacing§</td>
<td>31±3*</td>
<td>76±9‡</td>
</tr>
<tr>
<td>Rapid pacing + GH</td>
<td>34±2*</td>
<td>98±10††</td>
</tr>
<tr>
<td><strong>Sample size, n¶</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8 (848)</td>
<td>8 (70)</td>
</tr>
<tr>
<td>Rapid pacing§</td>
<td>10 (922)</td>
<td>10 (145)</td>
</tr>
<tr>
<td>Rapid pacing + GH</td>
<td>10 (1445)</td>
<td>10 (273)</td>
</tr>
</tbody>
</table>

Values presented as mean±SEM.

†P<0.05 vs control; ††P<0.05 vs rapid pacing only; ‡P<0.05 vs baseline.
§Rapid pacing: 3 weeks of chronic rapid pacing at 240 bpm.
| Rapid pacing + GH: Rapid pacing with GH treatment (200 μg · kg\(^{-1}\) · d\(^{-1}\)).
| ¶Number of pigs (number of myocytes).
GH supplementation during the progression of rapid pacing resulted in increased myocyte cross-sectional area and volumes. A similar directional increase was observed to occur with GH supplementation during rapid pacing with respect to LV mass (136%) and LV myocyte volume (128%). The LV structural basis for the myocardial remodeling that occurs with the development of pacing CHF appears to be at both the cellular and extracellular levels.7–10,12,14,18,20 Although significant changes in LV myocyte geometry occur with pacing CHF, it has been clearly demonstrated that this process occurs in the absence of reactive fibrosis.7,12 The institution of GH treatment with chronic rapid pacing did not appear to significantly increase the LV fibrillar collagen, as determined by myocardial hydroxyproline content. A significant reduction in the computed total LV myocyte number was observed to occur with the development of pacing CHF. Recent studies have suggested that a potential mechanism for the LV remodeling with CHF is myocyte loss due to apoptosis.20

Although GH supplementation prevented the reduction in LV myocyte number that occurred with pacing CHF, a direct relation between IGF-1 levels and the prevention of LV myocyte apoptosis was not demonstrated and warrants further investigation.

LV Function and Contractility With GH Supplementation

To the best of our knowledge, this is the first study to examine the effects of GH supplementation on LV structure, function, and myocyte contractility in a large-animal model of CHF. GH supplementation and increased IGF-1 levels in rodent models have been demonstrated to induce changes in myocardial protein expression and the emergence of myosin isoforms that do not occur in higher mammals.3,21 Thus, extrapolation of the results obtained with GH or IGF-1 supplementation in these rodent models to the setting of CHF, particularly with respect to contractile performance and protein expression, can be problematic. In the present study, GH supplementation with chronic rapid pacing resulted in improved LV pump function, which was accompanied by increased LV end-diastolic wall thickness and therefore reduced LV wall stress. The LV shortening–stress relationship further demonstrated that the reduction in LV wall stress was a contributory factor for the improved LV pump function that occurred with GH supplementation in this model of CHF. GH supplementation instituted during the progression of pacing CHF increased myocardial capillary density, which may have improved oxygen delivery/consumption with pacing CHF. In the GH-treated group, ambient resting heart rate was reduced. Plasma norepinephrine values were lower in the GH-treated group; therefore, diminished sympathetic stimulation may have contributed to the reduction in heart rate. In addition, animal studies have demonstrated a relationship between IGF-1 levels and chronotropy,22,23 which may be centrally mediated.24 A recent clinical study demonstrated that in GH-deficient patients, sympathetic efferent firing is increased.25 Thus, the GH-mediated increase in IGF-1 levels may have resulted in direct chronotropic effects.

GH treatment with pacing CHF was not accompanied by a significant improvement in steady-state myocyte contractility. However, with GH supplementation, myocyte contractile function was significantly improved from CHF values in the presence of the β-receptor agonist isoproterenol. Stromer and colleagues2 demonstrated that chronic IGF-1 supplementation in rats increased the maximal Ca2+ response in myocardial preparations. In studies of human myocardium with end-stage CHF, abnormalities in Ca2+ homeostatic mechanisms have been identified.15,26 For example, Pieske and colleagues26 demonstrated that Ca2+ uptake by the SR was reduced with CHF and was associated with diminished myocardial force generation. The present study demonstrated that the relative abundance of SR Ca2+-ATPase was reduced with the development of pacing-induced CHF. These changes in SR Ca2+-ATPase with pacing CHF probably contributed to the reduction in myocyte function and inotropic response. GH supplementation with chronic rapid pacing normalized SR Ca2+-ATPase and is a potential contributory mechanism for

Figure 5. Representative immunoblot for SR Ca2+-ATPase in control LV myocardial membrane preparations (5 μg), after chronic rapid pacing, and after rapid pacing with concomitant GH treatment. Positive signal for SR Ca2+-ATPase was consistent for this protein and antisemum.15 Signal was digitized and quantified with respect to controls, and this analysis is summarized in lower portion of figure. Relative abundance of SR Ca2+-ATPase was reduced to 72±3% of control levels with pacing-induced CHF (P<0.05) but was normalized with concomitant GH treatment.
the improved LV myocyte inotropic capacity with GH supplementation.

Although the majority of studies have demonstrated that GH supplementation influences LV pump function, some past reports have demonstrated that this treatment modality has neutral effects.\(^{27,28}\) For example, GH treatment in dogs with pacing CHF failed to elicit a myocardial growth response. In the present study, recombinant porcine GH was used in pigs during chronic rapid pacing and resulted in a significant myocardial hypertrophic response. Conditions of chronic GH excess, such as acromegaly, are associated with severe LV hypertrophy and the development of pump dysfunction.\(^4\) The present study instituted GH supplementation for a 3-week period, and therefore the long-term effects of chronic GH supplementation in the setting of CHF remain to be established. Nevertheless, short-term GH supplementation in a model of developing CHF, at a dose that significantly increased basal levels of IGF-1, increased LV pump function and improved myocyte inotropic responsiveness. These results suggest that GH supplementation may be a useful adjunctive therapy in the setting of developing CHF.

**Acknowledgments**

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**References**

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