Low-Dose Growth Hormone is Cardioprotective in Uremia

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ABSTRACT

Growth hormone (GH) is required to maintain normal cardiac structure and function and has a positive effect on cardiac remodeling in experimental and possibly human disease. Cardiac resistance to GH develops in the uremic state, perhaps predisposing to the characteristic cardiomyopathy associated with uremia. It was hypothesized that administration of low-dosage GH may have a salutary effect on the cardiac remodeling process in uremia, but because high levels of GH have adverse cardiac effects, administration of high-dosage GH may worsen uremic cardiomyopathy. In rats with chronic renal failure, quantitative cardiac morphology revealed a decrease in total capillary length and capillary length density and an increase in mean intercapillary distance and fibroblast volume density evident. Low-dosage GH prevented these changes. Collagen and TGF-β immuno-staining, increased in chronic renal failure, were also reduced by GH, suggesting a mechanism for its salutary action. Low-dosage GH also prevented thickening of the carotid artery but did not affect aortic pathology. In contrast, high-dosage GH worsened several of these variables. These results suggest that low-dosage GH may benefit the heart and possibly the carotid arteries in chronic renal failure.


Cardiovascular disease (CVD) is the most common cause of death in adults with advanced chronic renal failure (CRF) and also is of concern in children. In ESRD, CVD may present as a cardiomyopathy with left ventricular (LV) hypertrophy or dilation or as ischemic heart disease often culminating in congestive heart failure (CHF). Also of concern is decreased tolerance to myocardial ischemia with reduced long-term survival after a myocardial infarct. In addition to the usual forms of heart disease, a characteristic cardiomyopathy develops in uremia. Features include LV hypertrophy, interstitial expansion with fibrosis, and reduced capillary length density, abnormalities that contribute to the increased susceptibility to ischemic injury. These features are reproduced in uremic rats. Uremia also seems to alter cardiac function directly, impairing myocyte relaxation and calcium handling.

Maintenance of cardiac structure and function depends on the interplay of several growth factors and hormones, among which growth hormone (GH) is essential. In GH deficiency, cardiac size diminishes, there is endothelial dysfunction and stiffness of large arteries, and death from CVD increases. In contrast, when GH levels are persistently elevated as in acromegaly, the heart enlarges, interstitial fibrosis develops, and cardiac performance falls. These manifestations of GH deficiency or excess can be reversed with correction of the abnormal GH state. Interestingly, there is evidence that GH may have a favorable effect on cardiac remodeling and cardiac output in pituitary

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intact animals and possibly humans with cardiac disease.14,20,21
In advanced CRF, resistance to GH develops, and this is a cause of
growth retardation in children and muscle wasting in adults.22–24 Fortu-
nately treatment with GH can overcome the resistant state. In animals, we established that GH resistance arises, in part, because of a defec-
t in GH-mediated JAK-STAT5 signaling.23,25,26 This pathway is used by GH to increase production of IGF-1 that in turn mediates most but not all of GH’s actions.27,28 It is thus difficult to separate the actions of these two hormones on cardiac structure and function, although it seems that the hemo-
dynamic response to GH is largely mediated through IGF1.13,29,30
Because GH is required for maintenance of cardiac structure and function and may positively affect cardiac remodel-
ing, we postulate that the cardiac resistance to GH that de-
velops in CRF (26) may predispose to the remodeling characteristic of uremia, and, hence, that GH in low dosages
(LD) will have a salutary effect on the remodeling process. Conversely, because high GH levels have adverse cardiac ef-
effets, we postulated that high-dosage (HD) GH therapy may well
worsen uremic cardiomyopathy. To test this thesis, we studied the
impact of LD, intermediate-dosage (ID), and HD GH treat-
ment on the cardiac changes in chronically uremic rats.

RESULTS
Study 1: Cardiac Morphometrics
Body Weight and Serum Biochemistry.
Serum creatinine and urea nitrogen levels were elevated in the
five-sixths-nephrectomized rats and were not altered signifi-
cantly by GH treatment. Although body weight was similar
before nephrectomy (approximately 245 g), after 10 wk, CRF
vehicle (V)-treated rats weighed less than the sham-operated
(SO)-V controls (409 ± 21 versus 508 ± 55 g; P < 0.05); how-
ever, after 6 weeks of GH, body weight increased in the CRF
groups, and net weight gained was comparable to control
SO-V group gain (66 ± 15 g) and greater than the gain in the
CRF-V group (15 ± 7 g; P < 0.05), confirming the bioefficacy
of the administered GH. On average, the HD group gained
most weight, but this did not reach statistical significance. Se-
rum C-reactive protein (CRP) levels were similar in the SO-V–
and CRF-V–treated rats but increased significantly in all of the
GH-treated CRF groups, likely reflecting the immunoregula-
tory properties of GH (Table 1).31

BP and Heart Weight.
Systolic BP was elevated in CRF rats versus SO-V controls
(149 ± 16 versus 120 ± 5 mmHg; P < 0.05) and was unaltered
by GH. Heart and LV weight corrected for body weight in-
creased significantly in CRF rats, and these parameters were
not altered by GH (Table 2).

Quantitative Cardiac Morphology:
Capillary length density was reduced significantly in CRF. Of
note, GH at an LD, 1.5 mg/kg, prevented this decrease. In contrast,
when given at an ID or HD, 4 or 10 mg/kg, GH had no protective
effect. Mean intercapillary distance increased significantly in CRF, and,
again, LD had a salutary effect preventing this increase. ID
GH had no protective effect, whereas HD GH increased the inter-
capillary distance significantly. Total capillary length was reduced
in CRF, and this decline was prevented by LD and ID GH (P <

Table 1. Body weight and serum biochemistry in study 1a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>1: SO-V</th>
<th>2: CRF-V</th>
<th>3: CRF-GH 1.5</th>
<th>4: CRF-GH 4.0</th>
<th>5: CRF-GH 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td></td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
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<tr>
<td>initial</td>
<td></td>
<td>508 ± 35</td>
<td>409 ± 21</td>
<td>406 ± 50</td>
<td>422 ± 21</td>
<td>407 ± 63</td>
</tr>
<tr>
<td>final</td>
<td></td>
<td>574 ± 33</td>
<td>424 ± 26</td>
<td>482 ± 45</td>
<td>487 ± 57</td>
<td>506 ± 62</td>
</tr>
<tr>
<td>net gain</td>
<td></td>
<td>66 ± 15</td>
<td>15 ± 7</td>
<td>76 ± 23</td>
<td>66 ± 45</td>
<td>99 ± 39</td>
</tr>
<tr>
<td>SCr (mg/dl)</td>
<td></td>
<td>0.33 ± 0.10</td>
<td>0.62 ± 0.11</td>
<td>0.82 ± 0.15</td>
<td>1.02 ± 0.31</td>
<td>0.93 ± 0.22</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td></td>
<td>28 ± 12</td>
<td>132 ± 57</td>
<td>146 ± 31</td>
<td>179 ± 57</td>
<td>149 ± 65</td>
</tr>
<tr>
<td>Serum CRP (µg/ml)</td>
<td></td>
<td>387 ± 60</td>
<td>358 ± 101</td>
<td>585 ± 98</td>
<td>510 ± 105</td>
<td>546 ± 103</td>
</tr>
</tbody>
</table>

aAfter 10 wk of uremia, rats were treated with V or GH 1.5, 4.0, and 10.0 mg/kg, respectively, for 6 wk; SO rats served as controls. In each row, dissimilar numbers above group values (mean ± SD) reflect significant differences between these groups (P < 0.05). Groups with the same numbers do not differ.

Table 2. BP and heart weight in study 1a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>1: SO-V</th>
<th>2: CRF-V</th>
<th>3: CRF-GH 1.5</th>
<th>4: CRF-GH 4.0</th>
<th>5: CRF-GH 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mmHg)</td>
<td></td>
<td>120 ± 5</td>
<td>149 ± 16</td>
<td>141 ± 8</td>
<td>140 ± 17</td>
<td>145 ± 10</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td></td>
<td>1.35 ± 0.12</td>
<td>1.54 ± 0.24</td>
<td>1.60 ± 0.32</td>
<td>1.77 ± 0.37</td>
<td>1.67 ± 0.22</td>
</tr>
<tr>
<td>HW/BW (x10^-3)</td>
<td></td>
<td>2.35 ± 0.14</td>
<td>3.64 ± 0.69</td>
<td>3.31 ± 0.54</td>
<td>3.67 ± 0.84</td>
<td>3.34 ± 0.52</td>
</tr>
<tr>
<td>LVW (g)</td>
<td></td>
<td>1.08 ± 0.11</td>
<td>1.09 ± 0.15</td>
<td>1.19 ± 0.24</td>
<td>1.46 ± 0.35</td>
<td>1.31 ± 0.12</td>
</tr>
<tr>
<td>LVW/BW (x10^-3)</td>
<td></td>
<td>1.88 ± 0.13</td>
<td>2.58 ± 0.39</td>
<td>2.48 ± 0.42</td>
<td>3.03 ± 0.78</td>
<td>2.62 ± 0.39</td>
</tr>
</tbody>
</table>

aIn each row, dissimilar numbers above group values (mean ± SD) reflect significant differences between these groups (P < 0.05). Groups with the same numbers do not differ. HW, heart weight; LVW, LV weight; BW, body weight.
adverse effect, because it caused an increase in intercapillary distance (Table 3).

Quantitative Morphology of the Arteries.
Ascending aorta wall thickness was increased significantly in CRF rats, whereas luminal diameter was unchanged (Table 4). Accordingly wall thickness/lumen diameter ratio was increased in CRF; all of these parameters were unaffected by GH. Similar results were obtained for wall area/lumen area ratio. Carotid artery wall thickness and wall lumen ratio were significantly increased in CRF and reduced to control values with LD GH (Table 5). Intramyocardial artery wall thickness increased on average in CRF but did not reach statistical significance (Table 6). Of note, wall thickness increased significantly in the HD GH group ($P < 0.05$).

Study 2: Hemodynamics and Gene and Protein Expression

**Body Weight, Biochemistry, and Hematocrit.**
As in study 1, net weight gain was reduced in the CRF-V group compared with SO-V group ($30 \pm 20$ versus $48 \pm 18$ g/6 wk; $P < 0.05$). GH treatment, $1.5$ mg/kg per d, stimulated a similar increase in weight in CRF and SO groups ($84 \pm 18$ versus $96 \pm 20$ g). Serum creatinine and urea nitrogen were elevated in CRF rats, and the creatinine level was considerably higher than in study 1. Urinary albumin excretion (albumin/creatinine ratio) was increased, whereas hematocrit levels were reduced in CRF. None of these parameters were affected by GH (Table 7).

### Table 3. Cardiac capillary morphometrics in study 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1: SO-V</th>
<th>2: CRF-V</th>
<th>3: CRF-GH 1.5</th>
<th>4: CRF-GH 4.0</th>
<th>5: CRF-GH 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary length density (mm$^2$/mm$^3$)</td>
<td>$8798 \pm 1492^{2,4,5}$</td>
<td>$6453 \pm 419^{1,3}$</td>
<td>$8500 \pm 1038^{2,5}$</td>
<td>$7194 \pm 1441^{1,5}$</td>
<td>$5402 \pm 1543^{1,3,4}$</td>
</tr>
<tr>
<td>Volume density of capillaries (%)</td>
<td>$11.58 \pm 1.79$</td>
<td>$11.29 \pm 2.22$</td>
<td>$10.43 \pm 2.30$</td>
<td>$12.88 \pm 2.69$</td>
<td>$12.10 \pm 3.79$</td>
</tr>
<tr>
<td>Mean intercapillary distance (µm)</td>
<td>$11.56 \pm 0.94^{2,5}$</td>
<td>$13.39 \pm 0.43^{1,2,3,5}$</td>
<td>$11.71 \pm 0.74^{2,5}$</td>
<td>$12.83 \pm 1.28^{2,5}$</td>
<td>$14.97 \pm 1.94^{1,2,3,4}$</td>
</tr>
<tr>
<td>Total length of capillaries (m)</td>
<td>$9110 \pm 173^{1,2,5}$</td>
<td>$6732 \pm 682^{1,2,3,4}$</td>
<td>$9640 \pm 147^{2,5}$</td>
<td>$9775 \pm 152^{2,5}$</td>
<td>$6780 \pm 1820^{1,3,4}$</td>
</tr>
<tr>
<td>Nonvascular interstitial tissue Vv (%)</td>
<td>$0.72 \pm 0.13$</td>
<td>$0.79 \pm 0.16$</td>
<td>$0.63 \pm 0.17$</td>
<td>$0.79 \pm 0.17$</td>
<td>$0.81 \pm 0.16$</td>
</tr>
<tr>
<td>Fibroblasts Vv (%)</td>
<td>$0.61 \pm 0.09^{2,4,5}$</td>
<td>$1.01 \pm 0.21^{1,3}$</td>
<td>$0.59 \pm 0.10^{2,4,5}$</td>
<td>$0.88 \pm 0.25^{1,3}$</td>
<td>$0.99 \pm 0.16^{1,3}$</td>
</tr>
<tr>
<td>Myocytes Vv (%)</td>
<td>$86.7 \pm 1.8$</td>
<td>$86.6 \pm 2.1$</td>
<td>$87.9 \pm 2.4$</td>
<td>$85.1 \pm 2.9$</td>
<td>$85.8 \pm 3.9$</td>
</tr>
</tbody>
</table>

*In each row, dissimilar numbers above group values (mean ± SD) reflect significant differences between these groups ($P < 0.05$). Groups with the same numbers do not differ.

### Table 4. Aorta morphometrics in study 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1: SO-V</th>
<th>2: CRF-V</th>
<th>3: CRF-GH 1.5</th>
<th>4: CRF-GH 4.0</th>
<th>5: CRF-GH 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall thickness (µm)</td>
<td>$107.40 \pm 11.30^{2,3,4,5}$</td>
<td>$133.10 \pm 9.50^{1}$</td>
<td>$130.90 \pm 13.60^{1}$</td>
<td>$136.70 \pm 17.30^{1}$</td>
<td>$133.30 \pm 18.10^{1}$</td>
</tr>
<tr>
<td>Lumen diameter (µm)</td>
<td>$2160.00 \pm 132.50$</td>
<td>$2055.00 \pm 133.10^{3,4}$</td>
<td>$2242.00 \pm 116.30^{2,5}$</td>
<td>$2249.00 \pm 123.60^{2,5}$</td>
<td>$2050.00 \pm 144.90^{3,4}$</td>
</tr>
<tr>
<td>WLR</td>
<td>$0.05 \pm 0.01^{2,3,4,5}$</td>
<td>$0.07 \pm 0.01$</td>
<td>$0.06 \pm 0.01^{1}$</td>
<td>$0.06 \pm 0.01$</td>
<td>$0.07 \pm 0.01$</td>
</tr>
<tr>
<td>Wall area (mm$^2$)</td>
<td>$0.77 \pm 0.12^{3,4}$</td>
<td>$0.92 \pm 0.06$</td>
<td>$0.98 \pm 0.14^{1}$</td>
<td>$1.03 \pm 0.17^{1}$</td>
<td>$0.92 \pm 0.15$</td>
</tr>
<tr>
<td>Lumen area (mm$^3$)</td>
<td>$3.68 \pm 0.45$</td>
<td>$3.33 \pm 0.44^{1,4}$</td>
<td>$3.96 \pm 0.41^{2,5}$</td>
<td>$3.99 \pm 0.44^{2,5}$</td>
<td>$3.32 \pm 0.47^{1,4}$</td>
</tr>
<tr>
<td>Wall area/lumen area</td>
<td>$0.21 \pm 0.02^{2,3,4,5}$</td>
<td>$0.28 \pm 0.03^{1}$</td>
<td>$0.25 \pm 0.02^{1}$</td>
<td>$0.26 \pm 0.03^{1}$</td>
<td>$0.28 \pm 0.04^{1}$</td>
</tr>
</tbody>
</table>

*In each row, dissimilar numbers above group values (mean ± SD) reflect significant differences between these groups ($P < 0.05$). WLR, wall to lumen ratio.
Cardiac Gene Expression.

There was a small but NS increase in IGF-1 mRNA level in the GH-treated rats. Atrial natriuretic factor mRNA levels increased two-fold in CRF-V rats (P < 0.05) and rose further with GH, although not statistically significantly. Collagen type I and collagen type III mRNA were unaffected by CRF but increased in the SO and CRF GH-treated groups. α-Smooth muscle actin (α-SMA), TGF-β, and GH receptor mRNA levels did not differ between groups (Table 8).

Immunohistochemistry.

Immunohistochemistry revealed a significant increase in collagen type I in CRF-V rats; GH attenuated (P < 0.05) but did not normalize the overexpressed protein in CRF-GH rats (Figure 2). Collagen type IV expression increased significantly in CRF-V rats, and GH reduced the levels to a value that did not differ from the SO-GH rats. TGF-β protein was barely detectable in the SO-V or SO-GH groups but increased significantly in the CRF-V group (Figure 3). GH significantly reduced TGF-β protein expression, which fell to values that did not differ from SO values. Vascular endothelial growth factor expression increased significantly in CRF-V and was not reduced significantly by GH.

Hemodynamics.

Echocardiography findings are shown in Table 9. There was a significant increase in LV mass in CRF that did not change with GH. Values for interventricular septal thickness and posterior wall thickness revealed a concentric hypertrophy consistent with a response to the hypertension in these animals. CRF animals also demonstrated a smaller LV cavity and increased fractional shortening and ejection fraction. Although GH did not change fractional shortening in the SO group, it did increase fractional shortening in CRF animals. In addition, cavity size was modestly increased with GH, although this did not reach statistical significance.

DISCUSSION

The pathogenesis of uremic cardiomyopathy is poorly understood and is under intense investigation. An important area of focus has been the impact of increased levels of circulating factors, including vasoactive hormones, inflammatory cytokines, and cardiac glycosides.1,4,32 Less well studied is the impact of uremia-induced resistance to hormones essential for normal cardiac structure and function, such as GH and IGF-

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Table 5. Carotid artery morphometrics in study 1*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1: SO-V</td>
</tr>
<tr>
<td>Wall thickness (µm)</td>
<td>29.50 ± 6.501,4,5</td>
</tr>
<tr>
<td>Lumen diameter (µm)</td>
<td>540.10 ± 62.10</td>
</tr>
<tr>
<td>WLR</td>
<td>0.054 ± 0.0081,4,5</td>
</tr>
</tbody>
</table>

*Superscript numbers above a group value reflect groups in the same row that differ significantly from the marked groups (P < 0.05).

Table 6. Morphometrics of small intramyocardial arteries in study 1*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1: SO-V</td>
</tr>
<tr>
<td>Wall thickness (µm)</td>
<td>3.90 ± 1.145</td>
</tr>
<tr>
<td>Lumen diameter (µm)</td>
<td>39.60 ± 10.30</td>
</tr>
<tr>
<td>WLR</td>
<td>0.11 ± 0.075</td>
</tr>
</tbody>
</table>

*In each row dissimilar numbers above group values (mean ± SD) reflect significant differences between these groups (P < 0.05).

Table 7. Body weight, serum biochemistry, and BP in study 2*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1: SO-V</td>
</tr>
<tr>
<td>No. of rats</td>
<td>11</td>
</tr>
<tr>
<td>Body weight (g) initial</td>
<td>393 ± 323,4</td>
</tr>
<tr>
<td>final</td>
<td>441 ± 353,4</td>
</tr>
<tr>
<td>net gain</td>
<td>48 ± 183,4,4</td>
</tr>
<tr>
<td>SCr (mg/dl)</td>
<td>0.40 ± 0.073,4</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>15.0 ± 2.91,4</td>
</tr>
<tr>
<td>Urine albumin/creatinine</td>
<td>1.14 ± 0.843,4</td>
</tr>
<tr>
<td>Serum IGF-1 (ng/ml)</td>
<td>828 ± 153,4</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41 ± 43,4</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>123 ± 53,4</td>
</tr>
</tbody>
</table>

*After 10 wk of chronic uremia, rats were treated with V or GH, 1.5 mg/kg, daily for 6 wk; SO rats served as controls. In each row, dissimilar numbers above group values (mean ± SD) reflect significant differences between these groups (P < 0.05).
Table 8. Relative mRNA levels (specific mRNA/18s RNA) in study 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
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</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>1: SO-V</td>
<td>100 ± 42</td>
<td>120 ± 71</td>
<td>93 ± 36</td>
<td>135 ± 61</td>
<td></td>
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</tr>
<tr>
<td>ANF</td>
<td>2: SO-GH</td>
<td>100 ± 87</td>
<td>92 ± 82</td>
<td>191 ± 106</td>
<td>275 ± 84</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Collagen I</td>
<td>3: CRF-V</td>
<td>100 ± 25</td>
<td>138 ± 25</td>
<td>112 ± 29</td>
<td>182 ± 51</td>
<td></td>
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</tr>
<tr>
<td>Collagen III</td>
<td>4: CRF-GH</td>
<td>100 ± 43</td>
<td>142 ± 51</td>
<td>89 ± 33</td>
<td>158 ± 79</td>
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<tr>
<td>α-SMA</td>
<td></td>
<td>100 ± 17</td>
<td>97 ± 20</td>
<td>78 ± 36</td>
<td>76 ± 25</td>
<td></td>
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<tr>
<td>TGF-β</td>
<td></td>
<td>100 ± 45</td>
<td>110 ± 69</td>
<td>84 ± 53</td>
<td>115 ± 44</td>
<td></td>
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<tr>
<td>GH receptor</td>
<td></td>
<td>100 ± 43</td>
<td>96 ± 33</td>
<td>86 ± 27</td>
<td>127 ± 49</td>
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*In each row dissimilar numbers above group values (mean ± SD) reflect significant differences between these groups (P < 0.05). ANF, atrial natriuretic factor.

Figure 2. Immunohistochemistry score. Semiquantitative analysis of the effect of uremia and GH treatment on proteins involved in cardiac remodeling. After 10 wk of chronic uremia, rats were treated with V (group 3) or LD GH (group 4). 1.5 mg/kg, for 6 wk; SO rats treated in the same manner with V (group 1) or GH (group 2) served as controls. Results are expressed as a percentage of the control group values (mean ± SD). The numbers above each bar refer to groups with values that differ significantly from the group value below (P < 0.05, ANOVA or Kruskal-Wallis test).

1,26,33 Accordingly, we tested the hypothesis that GH resistance in uremia predisposes to the development of a cardiomyopathy and that administration of GH in low dosages is sufficient to overcome the resistance will have a salutary effect on the remodeling process. Conversely, because high GH levels adversely affect the heart,13,14 we proposed that HD GH may worsen the cardiomyopathy.

To test this thesis, we studied the impact of 6 wk of LD, ID, or HD recombinant bovine GH on the cardiac changes that develop during 4 mo of CRF in rats. GH increased body weight and any antibodies that might have formed in response administered were sufficient to overcome resistance to the hormone and any antibodies that might have formed in response to the exogenous GH.34 Serum CRP levels were not elevated in the V-treated CRF rats but rose significantly with GH treatment. This was unanticipated, because GH is without effect on CRP levels in patients with ESRD.35 and GH replacement in GH-deficient humans lowers CRP levels.36 Conversely, GH does stimulate cytokine production in human monocytes and in liver of endotoxin-treated rat; therefore, further study in humans with ESRD is indicated. Whereas GH had no impact on serum creatine, urea nitrogen, albuminuria, or elevated BP levels in the CRF rats, it had a notable impact on cardiac morphology. At an LD, GH prevented the decrease in capillary length density evident in the untreated CRF rats, whereas at higher dosages GH had no protective effect. LD GH also had a beneficial effect on mean intercapillary distance, preventing the CRF-induced increase, whereas HD GH caused an even greater increase in intercapillary distance. Total capillary length was reduced in CRF, and this was prevented by LD and ID GH treatment and is consistent with the reported ability of GH to stimulate cardiac capillary growth.39 Capillary volume density, nonvascular interstitial tissue volume density, and myocyte volume density were not altered in CRF and did not change with GH. In contrast, fibroblast volume density increased significantly in CRF, and LD GH prevented this increase. When given in higher dosages GH had no protective effect. The development of CRF was associated with a significant increase in aorta and carotid artery wall thickness. Although LD GH had no impact on aortic pathology, it did prevent carotid artery thickening. There was a modest but not statistically significant increase in intramyocardial artery wall thickness in CRF, and this was worsened by administration of GH in high dosages. Together, these results indicate that treatment with an LD GH regimen has a salutary effect on the course of the cardiac and possibly carotid artery disease of CRF, whereas HD treatment may have an adverse effect. Extrapolating these findings, it is conceivable that the protection against uremia-induced cardiac microcirculatory disease afforded by LD GH therapy may reduce the susceptibility to ischemic events in CRF.
Immunohistochemistry revealed a significant increase in cardiac collagen types I and IV in CRF. GH reduced but did not normalize the overexpressed type I collagen, whereas there was a tendency for type IV collagen expression to be reduced. Consistent with our findings, Grimm et al.46 noted that GH reduced the accumulation of type I collagen that followed a myocardial infarct in rats. We found that cardiac TGF-β protein was barely detectable in the SO-V or SO-GH groups but increased significantly in CRF. LD GH reduced TGF-β protein expression to values similar to normal control values. This suggests that the beneficial effect of GH may be mediated partly by suppression of TGF-β protein levels. In this regard, Imanishi et al.41 reported that GH may also suppress TGF-β-mediated cardiac fibrosis by inhibiting signal transduction. In contrast to the changes in protein expression, collagen type I and type III mRNA expression were unaffected by CRF but increased after GH treatment in the SO and CRF groups. This dissociation between protein and mRNA expression likely reflects a translational event,42 but, taken together, these changes in gene expression indicate that attention be paid to the long-term effects of GH treatment. Note that in all of these experiments, normal pair-fed control animals were not included for logistical reasons and additional experiments to dissect out any impact of reduced food intake will be required.

In a previous report,43 we described the impact of GH on bone and remnant kidneys of the same rats described in this study. GH had a positive effect on bone turnover and increased bone volume that was reduced in the untreated CRF rats. In contrast, GH had a negative impact on the remnant kidney: Tubulointerstitial damage worsened, and glomerular volume increased. A deleterious effect of GH on the rodent kidney has been noted by several investigators.44,45 Fortunately, this noxious effect seems to be limited to rodents, because prolonged treatment of children with uremia has not been associated with adverse renal effects.46

Because of its physiologic role in regulating cardiac structure and function, there has been interest in evaluating whether GH might be beneficial in cardiac disease treatment. Several studies suggested that GH may have a salutary effect on cardiac disease, although this is not a uniform finding. For example, when rats with myocardial infarcts are treated with GH, most21,47 but not all reported studies48 showed that LV remodeling and adaptive fibrosis may be attenuated and cardiac function may increase. GH also improves cardiac function in rodents with CHF49 and reverses age-related cardiac myofibron-ment dysfunction50 while improving regional coronary blood flow and capillary density.51 GH has been studied in relatively small clinical trials of patients with ischemic and nonischemic cardiomyopathy and CHF.52,53 The results have been mixed and thus inconclusive, although a recent meta-analysis suggested that GH does improve several cardiovascular parameters in chronic CHF, suggesting the need for large-scale studies.20

In this study with uremic rats in which LV hypertrophy, a decrease in cavity size, and an increase in fractional LV shortening were present, GH treatment induced a substantial increase in fractional shortening and also tended to cause an increase in cavity size. Although diastolic dysfunction was not directly measured, the increase in fractional shortening and decrease in cavity size seen in uremic rats is highly suggestive of this widely recognized phenomenon. Physiologic effects of a therapy, namely GH, known to cause vasodilation and positive inotropy is less clear in this “compensated” setting. Although peripheral vasodilation would be expected to lead to lower BP and perhaps less hypertrophy, the lack of change in BP is likely explained by a concomitant increase in systolic function. In fact, this is what we observed. The increased cavity size in the GH group (P = 0.055) is an additional indication of improved filling that may be a reflection of this overall more favorable hemodynamic milieu, which exists despite the hyperdynamic overall function. Finally, it is conceivable that more prolonged treatment and/or more frequent administration of GH might affect a more prominent hemodynamic response.

We have shown that when GH is administered in an LD sufficient to overcome the acquired resistance of uremia, significant cardiac protection against the development of microvascular disease and possibly interstitial fibrosis is achieved. In contrast, at HD, this response to GH tends to be lost and cardiac disease may even be worsened. Although caution must be exhibited when extrapolating animal data, this study suggests that the lowest effective dosage of GH should be used when treating children with CRF to promote body growth or adults to correct protein-energy wasting,44 especially because GH may induce cardiac enlargement in patients with ESRD.55

Table 9. Echocardiography parameters in study 2a

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<tr>
<td>IVSd (cm)</td>
<td>0.14 ± 0.04a,4</td>
<td>0.14 ± 0.02a,4</td>
<td>0.24 ± 0.08a,2</td>
<td>0.22 ± 0.05a,2</td>
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<tr>
<td>PWd (cm)</td>
<td>0.16 ± 0.04a,4</td>
<td>0.17 ± 0.043</td>
<td>0.24 ± 0.07a,2</td>
<td>0.21 ± 0.05a</td>
</tr>
<tr>
<td>LVIDd (cm)</td>
<td>0.89 ± 0.05a,4</td>
<td>0.92 ± 0.08a,4</td>
<td>0.76 ± 0.10a,2</td>
<td>0.82 ± 0.06a,2</td>
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<tr>
<td>Fractional shortening (%)</td>
<td>41 ± 7a,4</td>
<td>41 ± 8a,4</td>
<td>50 ± 9a,2,4</td>
<td>57 ± 7a,2,3</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>78 ± 8a,4</td>
<td>79 ± 6a,4</td>
<td>86 ± 8a,2</td>
<td>91 ± 4a,2</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>1.1 ± 0.3a,4</td>
<td>1.2 ± 0.2a,4</td>
<td>1.6 ± 0.6a,2</td>
<td>1.5 ± 0.4a,2</td>
</tr>
<tr>
<td>LV mass/BW (g/kg)</td>
<td>0.24 ± 0.06a,4</td>
<td>0.24 ± 0.02a,4</td>
<td>0.45 ± 0.18a,1,2</td>
<td>0.37 ± 0.10a,1,2</td>
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In each row dissimilar numbers above group values (mean ± SD) reflect significant differences between these groups (P < 0.05). IVSd, interventricular septum at end diastole; PWd, posterior wall thickness at end diastole; LVIDd, LV internal diameter at end diastole.
nally, it will be of great interest to determine whether pro-
longed LD GH therapy has a sustained benefit on the cardio-
myopathy of uremia and whether this leads to a reduction in
susceptibility to ischemic injury.

**CONCISE METHODS**

**Experimental Animals and Protocols**

Male Sprague-Dawley rats weighing approximately 245 g were en-
tered into the study. CRF was created by a two-step five-sixths ne-
phrectomy procedure with ketamine (80 mg/kg) and xylazine (10
mg/kg) anesthesia as described previously. Sham nephrectomy op-
erations were performed on other animals. Free access to food and
water was allowed. In the first series of experiments carried out over 4
mo, the CRF rats were divided into four groups 10 wk after the partial
nephrectomy and treated with V or bovine GH for 6 wk and then
killed, at which time the heart was perfusion-fixed for histology. Re-
nephrectomy and treated with V or bovine GH for 6 wk and then
killed, at which time the heart was perfusion-fixed for histology. Re-
combiant bovine GH (gift of Monsanta Corp., St Louis, MO) was
used because it does not cross-react with the prolactin receptor and
has low immunogenicity in the rat. The SO control group was
-treated with V alone for 6 wk. Thus, five groups of rats were studied:
Group 1, SO control V treated; group 2, CRF V treated; group 3, CRF
GH treated, LD (1.5 mg/kg per d); group 4, CRF GH treated, ID (4
mg/kg per d); and group 5, CRF GH treated, HD (10 mg/kg per d).
Dietary protein content was increased from the standard 20 to 40%
for the last 4 wk of the study.

In the second set of experiments, four groups were studied for 4
mo and were treated with V or GH, 1.5 mg/kg per d for the last 6 wk of
the experiment. Group 1, SO control V treated; group 2, SO control
GH treated; group 3, CRF V treated; and group 4, CRF GH treated.
Echocardiography was performed on these rats. At killing, the heart
was saline-perfused and collected as described next. Animals were
provided a standard diet throughout.

**Perfusion Fixation, Tissue Collection, and Processing**

The abdominal aorta was catheterized under ketamine/xylazine anes-
thesia, and blood was collected. In the first set of experiments, perfu-
sion at a pressure of approximately 100 mmHg was initiated with a
10% dextran solution containing 0.5 g/L procaine-hydrochloride,
and the vena cava was incised to allow drainage of the blood contain-
ing perfusate. After 2 min, the perfusate was changed to 0.2 mol/L
phosphate solution containing 3% glutaraldehyde and continued for
12 min. The heart and aorta were collected and processed according to
the orientator method. Uniformly random sampling of the myocardium was achieved by preparing a set of equidistant slices of the LV
and the interventricular septum with a random start. Two slices were
selected by area weighted sampling and processed accordingly. Eight
pieces of LV muscle, including the septum, were prepared and after-
ward embedded in Epon-Araldite. Semithin sections (0.8 μm) were
stained with methylene blue and basic fuchsin and examined by light
microscopy with oil immersion and phase contrast at a magnification of 1000×1. A 1-mm-thick section of the descending aorta was cut per-
pendicular to the vessel axis and embedded and sectioned as described already.

In the second series of experiments, tissues were perfused as al-
ready described, with PBS containing 0.5 g/L procaine hydrochloride for 2 min at room temperature followed by 2 min of perfusion with
ice-cold saline. The heart was excised, the apex was frozen in liquid
nitrogen for gene analysis, and the remainder was bisected and fixed in
10% formal saline before embedding in paraffin or placed in OCT
and frozen at −70°C. The following antibodies were used using the avidin biotin method: TGF-β1 (sc-146, polyclonal rabbit; Santa Cruz
Biotechnology, Santa Cruz, CA; 1:50), vascular endothelial growth
factor (BP2990, polyclonal rabbit; ID Labs, London, ON, Canada;
1:25), collagen I (BT2150-0160, polyclonal rabbit; Bio Trend, Co-
ologne, Germany; 1:200), collagen IV (BT2150-1470, rabbit polycl-
onal; Bio Trend; 1:100), collagen III (AM167-5M, mouse monoclo-
nal; Bio Genex, San Ramon, CA; 1:100), IGF-I (sc-1422, goat polyclonal; Santa Cruz Biotechnology; 1:100).

**Morphologic Analysis**

All investigations were performed in a blinded manner (i.e., the ob-
server was unaware of the animal’s study group). Stereologic analysis
was performed on eight random samples of differently oriented sec-
tions of the LV myocardium per animal according to the orientator
method. Length density (LV) of capillaries (i.e., the length of capil-
laries per unit tissue volume) and the volume density (Vv) of cardiac
capillaries, defined as the volume of capillaries per unit of myocardial
tissue volume, were measured in eight systematically subsampled ar-
eas per section using a Zeiss eyepiece with 100 points for point count-
ing. The length density of myocardial capillaries (LV) was determined
using the equation

\[
LV = \frac{2Q_A}{Q_X}
\]

where \(Q_X\) is area density, for example, the number of capillary transects per area of myocardial reference tissue. Total capillary length per heart was calculated using the volume of the left ventricle (V) according to the formula

\[
L = \frac{L_v}{V} \times V
\]

Inter-capillary distance (i.e., the distance between the centers of two adja-
cent intramyocardial capillaries) was calculated according to a mod-
ification of the formula of Henquell and Honig. Volume density
\(V_v\) of the capillaries, interstitial tissue, fibroblasts, and myocytes was
obtained using the point-counting method according to the equation

\[
P_p = \frac{V_v}{V}
\]

where \(P_p\) is point density. Reference volume was the total myocardial tissue (exclusive of noncapillary vessels, i.e., arterioles and
veins). Vascular geometry of intramyocardial arteries and of the aorta
(i.e., wall thickness, lumen diameter, media, and lumen area) was
analyzed using a semiautomatic image analysis system (Videoplan;
Kontron Co., Eching, Germany) as described previously.

**Immunohistochemical Evaluation**

Immunohistochemical evaluation was performed using a semiquan-
titative immunoreactive score: Two investigators, blinded with re-
spect to the animal group, used a semiquantitative scoring system for
the analysis (light microscopy; magnification ×200). The intensity of
staining was ranked on an arbitrary scale: Grade 0, no staining; grade
1, faintly positive staining; grade 2, positive staining involving up to
50% of the field of view; grade 3, positive staining involving >50%;
grade 4, positive staining of all structures within the field of view.

**Echocardiography**

Echocardiography was carried out using the GE Vivid 7 (GE Medical
Systems, Milwaukee, WI) and the small animal probe (13 MHz). Rats
were anesthetized with ketamine/xylazine, then placed supine and warmed using a heating pad. Using a gel buffer, parasternal long- and short-axis views were recorded in each animal to allow estimation of structure and function. Indices of structure included LV internal diameter at end diastole (LVIDd), LV internal diameter at end systole (LVIDs), interventricular septum at end diastole (IVSd), posterior wall thickness at end diastole (PWd), and LV mass \(1.05 \times (\text{IVSd} + \text{LVIDd} + \text{PWd})^3 - \text{LVIDd}^3\). LV mass was also corrected for body size. Indices of contractility included fractional shortening \(\left[\frac{\text{LVIDs}}{\text{LVIDd}}\right]\) and ejection fraction \(\left[\frac{\text{LVIDd}^3 - \text{LVIDs}^3}{\text{LVIDd}^3}\right]\). All measurements were made by one operator, who was blinded to group.

### Biochemistry

Serum creatinine and urea nitrogen (mg/dl) were measured with a Beckman LX 20 Analyzer (Beckman Coulter, Fullerton, CA). Serum IGF-1 levels (ng/ml) were measured by using the OCTEIA Rat/Mouse IGF-1 Kit (IDS, Fountain Hills, AZ). Serum CRP levels were measured with a highly sensitive rat CRP ELISA Kit (Alpha Diagnostic International, San Antonio, TX).

### Real-Time Quantitative RT-PCR Assay

Real-time quantitative RT-PCR with SYBR green dye was performed as described previously\(^6\) using the ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) and protocols from the manufacturer. Primers for quantification of IGF-1, atrial natriuretic factor, collagen I, collagen III, \(\alpha\)-SMA, TGF-\(\beta\), and GHR receptor and the internal control gene ribosomal 18S (Table 10) were received from the manufacturer. Primers for quantification of IGF-1, atrial natriuretic factor, collagen I, collagen III, \(\alpha\)-SMA, TGF-\(\beta\), and GHR receptor and the internal control gene ribosomal 18S (Table 10) were received from the manufacturer. Primers for quantification of IGF-1, atrial natriuretic factor, collagen I, collagen III, \(\alpha\)-SMA, TGF-\(\beta\), and GHR receptor and the internal control gene ribosomal 18S (Table 10) were received from the manufacturer.

### Statistical Analysis

All data are means \(\pm SD\). Comparisons between more than two normally distributed groups were made by one-way ANOVA followed by pair-wise multiple comparison with the Duncan test.\(^60\) For more than two non-normally distributed groups, the Kruskal-Wallis statistic was applied followed by the Dunn test to distinguish between groups. \(P < 0.05\) was considered statistically significant.

### DISCLOSURES

None.

### REFERENCES


36. Brue A, Oxhund L, Nyengaard JR: The total length of myocytes and capillaries, and total number of myocyte nuclei in the rat heart are time-dependently increased by growth hormone. Growth Horm IGF Res 15: 256–264, 2005


