Growth hormone receptor deficiency in mice results in reduced systolic blood pressure and plasma renin, increased aortic eNOS expression, and altered cardiovascular structure and function

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Departments of 1Physiology and 2Cardiology, 3Wallenberg Laboratory for Cardiovascular Research, 4Department of Metabolism and Cardiovascular Research, Sahlgrenska Academy, Göteborg University, Gothenburg, Sweden; 5Edison Biotechnology Institute and Department of Biomedical Sciences, Ohio University, Athens, Ohio; 6Department of Physiology and Pharmacology, Institute of Medical Biology, Syddansk University, Odense, Denmark; 7Department of Clinical Physiology, Sahlgrenska Academy, Göteborg University, Gothenburg, Sweden; and 8AstraZeneca R&D, Mölndal, Sweden

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Growth hormone receptor deficiency in mice results in reduced systolic blood pressure and plasma renin, increased aortic eNOS expression, and altered cardiovascular structure and function. Am J Physiol Endocrinol Metab 292: E1418–E1425, 2007. First published January 23, 2007; doi:10.1152/ajpendo.00335.2006.—To study the role of the growth hormone receptor (GHR) in the development of cardiovascular structure and function, female GHR gene-disrupted or knockout (KO) and wild-type (WT) mice at age 18 wk were used. GHR KO mice had lower plasma renin levels (12 ± 2 vs. 20 ± 4 mGU/ml, P < 0.05) and increased aortic endothelial NO synthase (eNOS) expression (146%, P < 0.05) accompanied by a 25% reduction in systolic blood pressure (BP, 110 ± 4 vs. 147 ± 3 mmHg, P < 0.001) compared with WT mice. Aldosterone levels were unchanged, whereas the plasma potassium concentration was elevated by 14% (P < 0.05) in GHR KO. Relative left ventricular weight was 14% lower in GHR KO mice (P < 0.05), and cardiac dimensions as analyzed by echocardiography were similarly reduced. Myograph studies revealed a reduced maximum contractile response in the aorta analyzed by echocardiography were similarly reduced. Myograph P

CONDITIONS LINKED TO REDUCED GROWTH HORMONE (GH) activity, such as GH deficiency or GH insensitivity/resistance, or GH excess as exemplified in acromegaly, are associated with an increase in cardiovascular disease (5, 26). The mechanisms by which abnormally low or high levels of GH signaling cause structural and functional changes of the cardiovascular system are, however, unclear.

GH-deficient patients have a higher prevalence of cardiovascular morbidity and mortality than the general population (16, 20, 26). However, patients with GH insensitivity/resistance (Laron syndrome), which is a much rarer condition, are less well studied in relation to cardiovascular mortality and morbidity. These patients have reduced cardiac dimension, with reductions in left ventricular (LV) volume and mass, as well as in stroke volume and cardiac output (CO) (11). However, systolic and diastolic functions appear intact, and the cardiac response to exercise is normal. Thus, it appears that the heart, despite its smaller size, can meet the metabolic need in GH-resistant patients. To our knowledge, consequences of GH insensitivity on vascular function and structure have not been studied in patients with GH insensitivity/resistance, whereas patients with GH deficiency, conversely, display endothelial dysfunction (10) and decreased systemic formation of nitric oxide (NO) (1).

An animal model resembling GH resistance is the GH receptor (GHR)/GH-binding protein gene disrupted, or knockout (GHR KO), mouse (33). Although these mice secrete GH, it has previously been reported that the resultant mouse phenotype has very low levels of serum insulin-like growth factor I (IGF-I) and insulin, loss of GHR and GH-binding protein, and an ∼50% weight reduction compared with littermates at 12 wk of age (9, 33). The aim of the study was to investigate the importance of an intact GH/IGF-I axis in the development of normal cardiovascular function and morphology, in particular, the importance of the GHR in cardiac and vascular function and structure.

MATERIALS AND METHODS

Animals. The GHR KO mouse was generated as previously described (33). Homozygous GHR KO and wild-type (WT) littermates females (Sv129Ola-Balb/c background) were identified by PCR analysis of DNA from tail biopsy specimens as previously described (33). Animals were housed together with littermate controls during the whole study. The environment of the animal rooms was controlled...
with a 12:12-h light-dark cycle, a relative humidity of 45–55%, and a temperature of 20°C. The mice had free access to tap water and standard pellet chow. The ethics committee of Göteborg University gave prior approval of the animal procedures. Eighteen-week-old mice were used in the study.

**Systolic blood pressure.** Systolic blood pressure (SBP) was measured using a computerized noninvasive tail-cuff system (RTBP Monitor; Harvard Apparatus, South Natick, MA). Conscious animals (n = 8 in each group) were kept in a restraining cylinder with a standard acclimatization time of 10 min, and the tail was gently heated before every recording session. The recordings were performed in the afternoon on four consecutive days with at least 10 measurements at each recording. Final SBP was obtained from the three last days of recording by averaging the mean values.

**Echocardiography.** Mice (GHR KO, n = 8; WT, n = 7) were anesthetized with isoflurane (Baxter Healthcare, Chicago, IL). The chest was shaved and a peeling cream applied, removing remaining hair. Electrocardiography (ECG) leads were placed on the extremities; a warming pad was used to maintain body temperature. Cardiac ultrasound studies were performed using a commercially available ultrasonograph (ATL HDI 5000 SonoCT; Phillips Ultrasound, Bothell, Seattle, WA). Briefly, a 15-MHz linear transducer was used to obtain two-dimensional parasternal short-axis images close to the papillary muscle. This served as a guide for M-mode tracing. Two-dimensional guided, pulsed, Doppler was used to record the estimated peak LV outflow tract velocity and the mitral inflow velocities (E and A wave and their ratio). This was done using minimal sample size and a pulsed frequency of 5 MHz. All tracings were recorded at a sweep of 100 mm/s and were stored on magnetic optical discs for offline measurements. Offline measurements were done by one observer blinded to the type of mice.

M-mode measurements of LV internal diameters and wall thickness in diastole and systole were made using the convention of the American Society of Echocardiography (www.asecho.org). End diastole was taken at the onset of the QRS complex, and end systole was taken at the peak inward of interventricular septum motion. Four or more beats were averaged for each measurement.

LV fractional shortening (FS) was calculated as follows: (LVIDd-LVIDs)/LVIDd × 100, where LVIDd and LVIDs are LV internal diameters in diastole (d) and systole (s), respectively. Relative wall thickness and velocity of circumferential shortening (Vcf) were calculated using formulas described previously (30).

**ECG.** The mice were anesthetized with isoflurane and ECG leads were placed on the extremities (GHR KO n = 7, WT n = 7). A heating pad was used to maintain body temperature. ECG was recorded at 2 kHz using Pharmlab 3.0 (AstraZeneca, Möln达尔, Sweden) for a minimum of 20 min, and the level of anesthesia was adjusted to keep heart rate stable at ~400 beats/min. Time parameters were measured using PharmLab 3.0, and a mean QRS complex was generated as a mean of ~20 beats. A graphic interface of the analysis program allowed visual reviewing and manual editing of the detected events. PQ time was defined as the time from start of the P wave to the start of the Q or R wave, the QRS time as the time from the start of the Q or R wave to the neutralization of the R wave, and the QT time as the start of the Q wave or R wave to the neutralization of the T wave. QT time was also correlated to the RR interval due to heart rate influence on the QT time. RR interval and R amplitude were also calculated.

**Plasma renin, aldosterone, sodium, and potassium measurements.** At 17 wk of age, plasma renin concentration was measured (n = 17 in each group). Following a very short anesthesia (isoflurane) blood was taken from the orbital plexa, and the plasma was frozen for later measurement of plasma renin concentration by radioimmunoassay of angiotensin I by use of the antibody-trapping technique (19). Only results with linearity in serial dilutions (between 50- and 1,000-fold) were accepted. Renin values were standardized with renin standards obtained from the National Institutes for Biological Standards and Control (Potters Bar, Hertfordshire, UK) and are expressed in milli-Goldblatt units per milliliter (mGU/ml).

Plasma aldosterone was determined using a commercial kit (n = 12 in each group, COAT-A-COUNT; Diagnostic Products, Los Angeles, CA).

Sodium and potassium concentrations in plasma were determined using flame photometry (n = 12 in each group, model ILS 943; Instrumentation Laboratory, Lexington, MA).

**Ex vivo vascular function.** GHR KO (n = 8) and WT (n = 8) mice were weighed and anesthetized with pentobarbital sodium (60 mg/kg, Apoteksbolaget, Gothenberg, Sweden) before the aorta, mesenteric arteries, and heart were dissected out. The vessels were placed into physiological salt solution (PSS). The aorta was separated into three parts; one part was fixed in formalin for further morphological analysis, one part was used for ex vivo functional tests, and the third part was trimmed free of surrounding tissues and frozen in liquid nitrogen and saved for further mRNA analysis. The heart was separated into LV including septum and right ventricle (RV) and weighed.

Ring segments of thoracic aorta (~3 mm, GHR KO n = 8, WT n = 8) and mesenteric arteries (GHR KO n = 10, WT n = 13) were mounted in an organ bath (Department of Physiology, Göteborg University, Gothenberg, Sweden) and a Multi myograph 610M (Danish Myo Technology, Aarhus, Denmark), respectively, as described before (15). Aortic rings were stretched to 3 mN and equilibrated for 30 min. Before the pharmacological protocol, they were further stretched to 12 mN and allowed to stabilize. The internal circumferences of the mesenteric arteries (GHR KO n = 10, WT n = 13) were normalized according to the manufacturer’s protocol. All vessels were activated with norepinephrine (NE, 10^-5 mol/l) and KCl (K+, 10^-1 mol/l) with 20-min washout between each procedure. The cumulative concentration-response relationship to NE was then studied (aorta 10^-9 to 10^-5 mol/l, mesenteric arteries 10^-8 to 10^-5 mol/l). The endothelium-dependent relaxation response was studied by cumulative concentration response to acetylcholine (ACh, 10^-9 to 10^-3 mol/l) on vessels precontracted to 50–100% of maximal recorded NE contraction. A second concentration-response to ACh was performed in the presence of the NO synthase (NOS) inhibitor N^6-nitro-L-arginine (L-NNA, 10^-4 mol/l). Sodium nitroprusside (Sigma, St. Louis, MO; 10^-5 and 10^-4 mol/l) was applied after each ACh concentration-response to validate the endothelium-independent relaxation of the smooth muscle cells.

**Morphological aorta analysis.** Aortic sections (n = 6 in each group) were paraffin imbedded, sectioned in a microtome, and mounted on slides for hematoxylin staining. Aortic wall thickness and number of muscle layers were measured on these sections. Wall thickness was measured and muscle layers were counted by a blinded observer on five different sections of the aortic wall at ×40 magnification.

**Real-time PCR analysis.** Total RNA from aorta (n = 8 in each group) was extracted with Tri Reagent (Sigma), and concentrations were determined by Ribo Green (Molecular Probes, Leiden, The Netherlands) following the manufacturer’s protocol. Standard cDNA from pooled aortic RNA was synthesized in parallel with the sample cDNAs. A standard curve, obtained by serial dilution of the standard cDNA (range 0.6 to 80 ng original RNA per well), was included on each plate.

PCR primers for GAPDH, caveolin-1, and extracellular superoxide dismutase (ecSOD) were designed using LightCycler Probe Design Software (version 1.0; Roche Diagnostics, Mannheim, Germany).

**PCR primers for GAPDH, caveolin-1, and extracellular superoxide dismutase (ecSOD).** GAPDH, caveolin-1, and ecSOD were amplified using the LightCycler (Roche Diagnostics) and FastStart Master SYBR Green I (Roche Diagnostics), and expression levels were normalized to GAPDH. Transcript levels of eNOS, IGF-I, and prolactin receptor were analyzed using predesigned TaqMan assay-on-demands (Applied Biosystems, Foster City, CA) and performed on an ABI Prism 7900HT Sequence Detection System
Table 1. Primer sequences, times, and temperatures for denaturizing, annealing, and elongation for the genes GAPDH, PPIA, caveolin-1, and ecSOD used in real-time quantitative PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Elongation</th>
<th>Oligonucleotide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>0 s, 95°C</td>
<td>4 s, 67°C</td>
<td>3 s, 72°C</td>
<td>Forward primer</td>
<td>5'/GTC GTG CAT CGT AGG TGC C-3'</td>
</tr>
<tr>
<td>PPIA</td>
<td>15 s, 95°C</td>
<td>60 s, 60°C</td>
<td></td>
<td>Reverse primer</td>
<td>5'/GTC CTT CGC AAC CAT CTT AGT</td>
</tr>
<tr>
<td>Caveolin-1</td>
<td>15 s, 95°C</td>
<td>4 s, 58°C</td>
<td>8 s, 72°C</td>
<td>Forward primer</td>
<td>5'/GAC GAC GTG CGT AAG ATG C-3'</td>
</tr>
<tr>
<td>ecSOD</td>
<td>15 s, 95°C</td>
<td>4 s, 56°C</td>
<td>9 s, 72°C</td>
<td>Reverse primer</td>
<td>5'/GAG AAG GAG AGA ATG GC-3'</td>
</tr>
</tbody>
</table>

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPIA, cyclophilin A; ecSOD, extracellular superoxide dismutase.

(Submitted by Biotechnology, Inc., a subsidiary of Applied Biosystems). Reagents (TaqMan Universal PCR Mastermix, Applied Biosystems) and reaction conditions were used according to the manufacturer’s instructions. Cyclophilin A (PPIA; CyberGene, Huddinge, Sweden; Table 1) was used as a reference gene for eNOS and prolactin receptor and β-actin for IGF-I. All standards and samples were analyzed in triplicate.

Statistics. Values are expressed as means ± SE. If not stated otherwise, two-tailed t-tests for unpaired data were used for statistical comparisons between groups. Variables without homogeneity in variance or normal distribution were analyzed with a Mann-Whitney U-test. P values <0.05 were considered to be statistically significant.

RESULTS

Systolic blood pressure was reduced by 25% in GHR KO mice compared with WT (KO 110 ± 4 vs. WT 147 ± 3 mmHg, P < 0.001; Fig. 1). Since the tail cuff technique is sensitive to the relation between the cuff and the size of the tail, we apprehended that the smaller tail of the GHR KO animals possibly could affect our measurements. However, when two different sizes of WT mice on a different background (C57/BL6) were studied (18 vs. 28 g), similar systolic blood pressures were recorded in both groups [18 g, n = 5: 112 ± 2 vs. 28 g, n = 5: 110 ± 2 g, not significant (NS); unpublished observations].

The plasma renin concentrations were 40% lower in GHR KO compared with WT mice (KO 12 ± 2 vs. WT 20 ± 4 mGU/ml, P < 0.05 as analyzed with Mann-Whitney U-test; Fig. 2A). No change in aldosterone levels was found in GHR KO compared with WT mice (KO 815 ± 101 vs. WT 812 ± 46 pg/ml, NS; Fig. 2B).

The plasma potassium concentrations were increased by 14% in GHR KO compared with WT mice (KO 74.1 ± 0.28 vs. WT 65.1 ± 0.27 mmol/l, P < 0.05 as analyzed with Student’s t-test; Fig. 2C). Sodium concentrations were unchanged in GHR KO (KO 157 ± 2 vs. WT 155 ± 2 mmol/l; Fig. 2D).

The levels of aortic eNOS mRNA were increased by 146% in GHR KO compared with WT (KO 16.5 ± 3.7 vs. WT 6.7 ± 0.9, P < 0.05; Fig. 3).

GHR KO mice had 53% decreased body weight compared with WT (KO 17 ± 1 vs. WT 36 ± 3 g, P < 0.001; Table 2). The relative LV weights were also lower in the GHR KO mice compared with WT (KO 22.7 ± 1.6 vs. WT 26.5 ± 0.9 mg/10 g body wt, P < 0.05). There was no difference in the relative RV weight between GHR KO and WT mice (KO 5.9 ± 0.3 vs. WT 7.0 ± 0.6 mg/10 g body wt).

In GHR KO mice, posterior wall thickness was decreased by 25% (P < 0.05) and LVdDd was similarly decreased by 28% (P < 0.05) compared with WT (Table 2). The resultant relative wall thickness was similar in both groups. When %FS and Vcf were calculated, GHR KO mice displayed decreased %FS and Vcf by 11 and 24%, respectively (P < 0.05; Table 2), suggesting decreased systolic cardiac function. The diastolic cardiac function was also studied by calculating the E/A quote, but no differences were detected between the groups. CO was decreased by 48% (P < 0.05; Fig. 4A) in GHR KO; but when CO was related to body weight, no differences between the groups were detected (Table 2 and Fig. 4B).

During ECG measurements, heart rate was kept at ~400 beats/min in both groups (KO 412 ± 17 vs. WT 448 ± 8 beats/min). GHR KO mice showed longer QT time compared with WT (KO 27.7 ± 1.0 vs. WT 22.7 ± 1.8 ms). However, when the QT time was corrected for the small difference in heart rate (QTc), no significant difference was detected between the groups (KO 2.26 ± 0.05 vs. WT 1.95 ± 0.06, P = 0.08). PQ time and QRS time were similar in both groups (PQ: KO 37.5 ± 0.3 vs. WT 35.7 ± 1.0; QRS: KO 12.1 ± 0.6 vs. WT 10.9 ± 0.4 ms).

In the mesenteric arteries, no difference in maximum contractile response to K+ and NE could be detected between...
GHR KO and WT mice (K+: KO 1.73 ± 0.09 vs. WT 1.91 ± 0.068 nM/mm, respectively; NE: KO 1.59 ± 0.11 vs. WT 1.58 ± 0.1 nM/mm, respectively; Fig. 5A). GHR KO showed an increased sensitivity to NE compared with WT in the mesenteric arteries (log EC50: KO −5.5 ± 0.1 vs. WT −5.2 ± 0.1 log mol/l, respectively, P < 0.05; Fig. 5C). Sodium nitroprusside was used to test the function of the smooth muscle cells and caused a less profound relaxation in the mesenteric arteries in GHR KO compared with WT (KO 72.0 ± 3.9 vs. WT 83.4 ± 2.6%, respectively, P < 0.05). In the mesenteric arteries, there was no difference in the maximal relaxation response induced by ACh relative to the maximal endothelium-independent relaxation induced by sodium nitroprusside (KO 49.4 ± 6.1 vs. WT 40.9 ± 6.7%) and no difference in the sensitivity to ACh (log EC50: KO 7.6 ± 0.058 vs. WT 6.9 ± 0.18; Fig. 5E). Blockade of NOS by l-NNA reduced ACh-induced dilations significantly more in the mesenteric arteries in GHR KO compared with WT (KO 11.2 ± 5.2 vs. WT 27.5 ± 4.1%, respectively, P < 0.05; Fig. 5E).

In the aorta, the maximum contractile response to K+ and NE was reduced in GHR KO compared with WT (K+: KO 0.7 ± 0.1 vs. WT 1.4 ± 0.1 mN/mm, respectively; NE: KO 1.2 ± 0.07 vs. WT 2.3 ± 0.1 mN/mm, respectively, P < 0.05 for both; Fig. 5B). There were no differences in the sensitivity to NE (log EC50: KO −7.35 ± 0.11 vs. WT −7.44 ± 0.13 log mol/l; Fig. 5D) or in ACh-induced maximal relaxation relative to the maximal endothelium-independent relaxation induced by sodium nitroprusside in aorta (KO 54.2 ± 6.6 vs. WT 44.5 ± 6.7%; Fig. 5F). However, sensitivity to ACh was increased in aorta in GHR KO compared with WT (log EC50: KO −7.6 ± 0.1 vs. WT −7.1 ± 0.2 log mol/l, respectively, P < 0.05). The presence of l-NNA totally abolished ACh-induced relaxations (Fig. 5F), and sodium nitroprusside caused profound relaxation in aorta in both GHR KO and WT (KO 98.5 ± 1.5 vs. WT 100 ± 1.9%, respectively). The thickness of the GHR KO aortic media was significantly reduced by 29% compared with WT (KO 45.83 ± 4.5 vs. WT 64.58 ± 4.78 μm, P < 0.05), whereas no changes in the
number of muscle layers could be found between GHR KO and WT (average: KO 4.4 vs. WT 4.6, respectively).

The levels of aortic caveolin-1 mRNA tended to be lower in the GHR KO animals (KO 0.6 vs. WT 0.89, \(P = 0.1\)). Expression levels of ecSOD (KO 1.55 vs. WT 1.43, \(P = 0.5\)) and IGF-I mRNA (KO 1.43 vs. WT 0.73, \(P = 0.01\)) were unchanged as was that of the prolactin receptor (KO 217.12 vs. WT 270.64). However, variances in the mRNA levels tended to be high in some of the measurements. Therefore, lack of significance in the statistical analysis of differences between groups does not necessarily indicate that there was no biological difference.

**DISCUSSION**

In the present study, we have shown that lack of GHR signaling causes a reduction in systolic blood pressure and plasma renin levels as well as an increase in aortic eNOS expression. Furthermore, cardiac weight and volume were reduced to a larger extent than could be explained by the lower body weight of the GHR KO mouse. However, despite a reduced global systolic function, the GHR KO mice seem to be able to produce a CO that matches their reduction in body weight and metabolic rate but display a lower systolic blood pressure. The aorta of the GHR KO shows a markedly hypotrophic phenotype with a reduction in media thickness and a reduced maximal contractile response. Sensitivity to ACh-mediated dilatation is increased in the aorta with a matching increase in eNOS mRNA expression. The resistance vasculature appears normally developed but with an alteration in the factors responsible for ACh-mediated dilatation. Thus, we conclude that functional GH signaling is important for the maintenance of normal cardiac and large vessel structure and function.

GHR KO mice had reduced systolic blood pressure, which also has been found in hypophysectomized (Hx) rats (13, 24, 31). The mechanisms underlying this reduction remain unclear, but it has been speculated that a generally hypotrophic cardiovascular system could be causative (12). The present study offers several explanations for the reduction in systolic blood pressure seen in GHR KO mice. The observed reduction in systolic cardiac function may reduce systolic blood pressure. The aorta of the GHR KO shows a markedly hypotrophic phenotype with a reduction in media thickness and a reduced maximal contractile response. Sensitivity to ACh-mediated dilatation is increased in the aorta with a matching increase in eNOS mRNA expression. The resistance vasculature appears normally developed but with an alteration in the factors responsible for ACh-mediated dilatation. Thus, we conclude that functional GH signaling is important for the maintenance of normal cardiac and large vessel structure and function.

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may contribute to the maintained aldosterone levels. Systolic and diastolic blood pressure are essentially independent of mammal size (8), diminishing the significance of size differences between the GHR KO and WT. However, even though we had a reasonable number of observations in the aldosterone measurements, the lack of statistical significance between the groups does not exclude a potential biological difference.

ECG and posteuthanasia weights revealed a reduction in cardiac volume and mass that exceeded the observed reduction in body weight in GHR KO mice. This is in agreement with data from Hx rats, which display reductions in relative LV weight (31) and cardiac dimensions, estimated by ECG (Bollano E, personal communication).

Global systolic function, measured as ejection fraction or fractional shortening, was also reduced in GHR KO mice compared with WT. It has previously been shown that GH affects myocardial contractility via regulation of calcium handling during excitation/contraction coupling (4, 28). Thus, the lack of GHR signaling in GHR KO mice may result in a
defective cardiac calcium handling and resultant reduction of myocardial contractility. Furthermore, CO was reduced in GHR KO mice, which is in accord with the situation in Hx rats (17, 27) as well as for patients with GH resistance and/or GH insensitivity (11). The GHR KO mice showed a 48% decrease in CO, which is closely related to a similar reduction in body weight (−43% in this part of the study). This may suggest that the GHR KO mouse is small and has a small heart that, despite systolic dysfunction, appears able to produce a resting CO sufficient to supply the body at rest. The cardiac features of GHR KO mice, in part, agree with those in adults with primary GH resistance, which exhibit reduced cardiac dimensions but normal systolic and diastolic cardiac function at rest (11). The cardiac function of GHR KO mice is also comparable with the situation in GH-deficient patients (22). Taken together, these data suggest that a functional GHR signaling is of importance for both cardiac structural development and cardiac contractile function.

In accord with the situation in the heart, the aorta of the GHR KO mice appeared hypotrophic, evidenced by a morphological reduction in the mean media thickness. This was in agreement with the finding of a marked reduction in the contractile function of the aortic segments in response to both NE and K+. Thus, as for the heart, it appears that intact GH signaling is of importance for development of both functional and structural aspects of large vessels.

The functional studies of GHR KO mesenteric vessels revealed a close to normal function with an intact contractile response. However, GHR KO mice showed an increased sensitivity to NE. Effect of GH on NE sensitivity is in line with findings from studies of Hx rats, which consistently show increased NE sensitivity (12, 13, 24, 25) and also with results from bovine GH transgenic mice, in which overproduction of GH results in decreased sensitivity to NE (2). The mechanisms for the alterations in NE sensitivity caused by GH are not known. One of the best known causes of changes in NE receptor sensitivity is compensatory changes in response to alterations in sympathetic outflow. Another established fact is that a chronic decrease in circulating catecholamines upregulates the number and/or sensitivity of adrenoceptors (6). Decreased release of catecholamines and adrenal catecholamine-synthesizing enzymes have been described after hypophysectomy (23, 32). Thus, a compensatory upregulation of the vascular adrenoceptors due to reduced sympathetic activity may be responsible for the increased NE sensitivity. Reduced sympathetic activity is supported by the low blood pressure and low plasma renin activity in GHR KO mice but would be in contrast to the situation in GH-deficient patients, reported to have increased sympathetic activity (29).

Endothelial function was intact in both aorta and mesenteric artery from GHR KO mice. In fact, the sensitivity toward ACh was increased in the aorta of GHR KO mice compared with WT mice. l-NNA effectively blocked the ACh-mediated dilatory response in the aorta of both WT and GHR KO mice, suggesting that it is principally mediated by NO. In contrast, l-NNA minimally affected the dilatory response to ACh in WT mesenteric arteries, suggesting that other factors, such as prostaglandins, endothelial-derived relaxing factor, or endothelial-derived hyperpolarizing factor, are responsible for the vasodilation in this vascular segment. Interestingly, in GHR KO mesenteric arteries, l-NNA effectively blocked vasodilatation, suggesting that vasodilatation is mediated by NO. The observed change in endothelial function in the direction of a more NO-dependent vasodilatation is hard to explain. It is in line with our observation of increased expression of eNOS mRNA in the aorta of GHR KO mice, but to directly link it to the loss of GH action, further studies are needed.

In contrast to the GHR KO mice, GH-deficient patients display impaired endothelial function (10), which may be linked to a decreased systemic production of NO (1). It is possible that GHR KO mice have developed compensatory systems that maintain NO production and endothelial function. A possible explanation to the increased eNOS expression and NO dependency is the observation of high levels of prolactin in GHR KO mice (18). It has been shown that prolactin activates the NO system (3), and its receptors are located in endothelial cells (21). Thus, increased activity of NO by prolactin might help improve NO-dependent endothelial function in GHR KO mice. Due to these observations, aortic prolactin receptor gene expression in GHR KO and WT mice was measured, but the levels did not differ between the groups.

Loss of GHR causes functional and morphological changes in both heart and vasculature beyond the observed alterations in body size. Despite these changes, the GHR KO mice have an extended longevity (7). Cardiac changes in GHR KO mice have similarities with those observed in GH-resistant and GH-deficient patients, but important differences are observed relating to vascular function. All together, this study suggests an important role for an intact GH/IGF-I axis in the development of a normal cardiovascular system.

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