Vasoprotective Effects of Life Span-Extending Peripubertal GH Replacement in Lewis Dwarf Rats

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In humans, growth hormone deficiency (GHD) and low circulating levels of insulin-like growth factor 1 (IGF-1) significantly increase the risk for cerebrovascular disease. Genetic growth hormone (GH)/IGF-1 deficiency in Lewis dwarf rats significantly increases the incidence of late-life strokes, similar to the effects of GHD in elderly humans. Peripubertal treatment of Lewis dwarf rats with GH delays the occurrence of late-life stroke, which results in a significant extension of life span. The present study was designed to characterize the vascular effects of life span-extending peripubertal GH replacement in Lewis dwarf rats. Here, we report, based on measurements of dihydroethidium fluorescence, tissue isoprostanate, GSH, and ascorbate content, that peripubertal GH/IGF-1 deficiency in Lewis dwarf rats increases vascular oxidative stress, which is prevented by GH replacement. Peripubertal GHD did not alter superoxide dismutase or catalase activities in the aorta nor the expression of Cu-Zn-SOD, Mn-SOD, and catalase in the cerebral arteries of dwarf rats. In contrast, cerebrovascular expression of glutathione peroxidase 1 was significantly decreased in dwarf vessels, and this effect was reversed by GH treatment. Peripubertal GHD significantly decreases expression of the Nrf2 target genes NQO1 and GCLC in the cerebral arteries, whereas it does not affect expression and activity of endothelial nitric oxide synthase and vascular expression of IGF-1, IGF-binding proteins, and inflammatory markers (tumor necrosis factor alpha, interleukin-6, interleukin-1β, inducible nitric oxide synthase, intercellular adhesion molecule 1, and monocyte chemoattractant protein-1). In conclusion, peripubertal GH/IGF-1 deficiency confers pro-oxidative cellular effects, which likely promote an adverse functional and structural phenotype in the vasculature, and results in accelerated vascular impairments later in life.

Key Words: Oxidative stress—GH deficiency—GH replacement—Vasoprotection—IGF-1 deficiency.

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Disruption of the insulin/insulin-like growth factor 1 (IGF-I) pathway increases life span in invertebrates. However, effects of decreased growth hormone (GH)/IGF-I signaling in mammals remain controversial. The cardiovascular system is an important target organ for GH and IGF-I, and it is well documented that in human patients, growth hormone deficiency (GHD) and low circulating levels of IGF-1 significantly increase the risk for cardiovascular and cerebrovascular diseases (1–6). Although there are mouse models extant in which disruption of GH/IGF-1 signaling is associated with lifespan extension (eg, Ames (7) and Snell dwarf mice (8,9) and male mice heterozygous for the deletion of the IGF-1 receptor (10)), other rodent models with compromised GH/IGF-1 signaling do not exhibit a longevity phenotype (eg, female mice heterozygous for the deletion of the IGF-1 receptor (10) and genetically GH/IGF-1-deficient strain of Lewis rats (11)). The Lewis dwarf rat is a useful model of human GHD as Lewis dwarf rats have normal pituitary function except for a selective genetic GH deficiency and they mimic many of the pathophysiological alterations present in human GHD patients (including mild cognitive impairment (12)). Importantly, GH deficiency in Lewis rats significantly increases the incidence of late-life strokes (11), similar to the effects of GHD in elderly humans. It is significant that short-term peripubertal treatment of GH-deficient Lewis dwarf rats with GH decreases the incidence and delays the occurrence of late-life stroke, which results in a significant (~14%) extension of life span (11). These findings suggest that peripubertal levels of GH and IGF-I are important for developing an adequate tissue architecture, maintaining vascular health, and delaying the development of age-related cardiovascular diseases. Although previous studies have established that GH/IGF-1 deficiency in adult patients and laboratory animals results in deleterious effects on vascular endothelial and smooth muscle cells (13–16), there is little or no information available on vascular alterations either in childhood GHD patients or animal models of peripubertal GHD.
The available data demonstrate that in adult patients and laboratory animals, the GH/IGF-1 axis is an important regulator of vascular redox homeostasis (13,17,18), although the specific mechanisms are not well understood. Previously, we demonstrated that in the vasculature of adult GH/IGF-1-deficient Ames dwarf mice, antioxidant enzymes are downregulated, which is associated with increased vascular reactive oxygen species (ROS) levels and impaired endothelial function (13). The present study was designed to characterize the effects of peripubertal GH/IGF-1 deficiency on vascular redox homeostasis, the abundance and activity of antioxidant enzymes in the vascular wall, the local IGF-1 system, and the expression of inflammatory mediators in the cerebrovasculature, using Lewis dwarf rats as a model system. Moreover, we also determined whether life span-extending peripubertal GH replacement exerts vaso-protective effects in Lewis dwarf rats.

**METHODS**

**Animals**

In the present study, we used male Lewis rats that are heterozygous or homozygous for the spontaneous autosomal recessive dw-4 mutation, which causes a decrease in GH secretion from the pituitary gland (19). Lewis dwarf (dw-4/dw-4) rats have chronically low levels of GH and IGF-1 and make an excellent animal model of childhood-onset GHD (19). These rats have a spontaneous mutation that results in decreased GH secretion from the pituitary beginning around postnatal Day 26 (9,19,20). Female heterozygous (dw-4/−) Lewis rats were bred with male homozygous Lewis dwarf rats (dw-4/dw-4) to generate heterozygous (dw-4/−) offspring with a normal phenotype (“control”, n = 6) or homozygous rats (dw-4/dw-4) with a dwarf phenotype (“dwarf”). Classification as control or “dwarf” was based on their body weight as well as the serum IGF-1 levels at 33 days of age. Pubertal status was determined by age and the associated changes in growth and IGF-1 levels. Beginning on Day 35, dwarf rats were divided into two experimental groups: (a) early-onset GHD given saline (n = 6) and (b) GH replete with GH administered beginning at 5 weeks of age and continued throughout the experimental period of 30 days (termed “GH replete,” n = 6). Saline or GH (300 µg recombinant porcine GH; Alpharma, Victoria, Australia) was injected subcutaneously twice daily. The heterozygous rats were used as controls and given saline injections twice daily from 5 weeks of age to the end of the experimental period. Rats had access to food and water ad libitum and were housed in pairs in the barrier facility of the University of Oklahoma Health Sciences Center. Animals were fed standard rodent chow (PicoLab Rodent Diet 20 from Purina Mills [Richmond, IN], containing 20% protein by mass and 24% protein by caloric content). All studies were approved by the Institutional Animal Care and Use Committee.

**Measurement of Serum IGF-1**

Rats were anesthetized with isoflurane, and serum was obtained via tail bleed. Total IGF-1 levels in serum were determined as previously described (12,20–22).

**Measurement of Vascular O$_2^-$ Production**

Production of O$_2^-$ in the aortic wall was determined using dihydroethidium (DHE), an oxidative fluorescent dye, as we previously reported (23,24). In brief, vessels were incubated with DHE (3 × 10^-6 mol/L; at 37°C for 30 minutes). The vessels were then washed three times, embedded in optimal cutting temperature media, and cryosectioned. Red fluorescent images were captured at 20× magnification and analyzed using the Metamorph imaging software as reported (13). Four entire fields per vessel were analyzed with one image per field. The mean fluorescence intensities of DHE-stained nuclei in the endothelium and medial layer were calculated for each vessel. Thereafter, the intensity values for each animal in the group were averaged.

**8-Isoprostane Assay**

Total tissue 8-isoprostane content was measured in aorta homogenates using the 8-isoprostane EIA kit (Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer’s guidelines.

**Determination of Endogenous Glutathione and Ascorbate Using high-performance liquid chromatography Electrochemical Detection**

Concentrations of redox-active GSH and ascorbate were measured in aorta homogenates using a Perkin-Elmer high-performance liquid chromatography equipped with an eight-channel amperometric array detector as described (25). In brief, 10 mg aliquots of tissue samples were washed with ice-cold phosphate-buffered saline and homogenized in 5% (w/v) metaphosphoric acid. Samples were centrifuged at 10,000g for 10 minutes to sediment protein, and the supernatant fraction was stored for analysis of redox-sensitive compounds. Precipitated proteins were dissolved in 0.1 N NaOH and stored for protein determination by a spectrophotometric quantitation method using BCA reagent (Pierce Chemical Co., Rockford, IL). Concentrations of GSH and ascorbic acid in supernatant fractions were determined by injecting 5 µL aliquots onto an Ultrasphere 5 u, 4.6 × 250 mm, C18 column and eluting with mobile phase of 50 mM NaH$_2$PO$_4$, 0.05 mM octane sulfonic acid, and 1.5% acetonitrile (pH 2.62) at a flow rate of 1 mL/min. The detectors were set at 200, 350, 400, 450, 500, 550, 600, and 700 mV, respectively. Peak areas were analyzed using ESA, Inc. (Chelmsford, MA) software, and concentrations of GSH and ascorbate are reported as nanomoles per milligram protein.
Antioxidant Enzyme Activities

Activity of antioxidant enzymes and endothelial nitric oxide synthase (eNOS) in aorta homogenates was measured using the Glutathione Peroxidase Assay Kit (#703102), Catalase Assay Kit (#707002), Superoxide Dismutase Assay Kit (#706002), and NOS Activity Assay Kit (#781001) according to the manufacturer’s guidelines (Cayman Chemical Company).

Quantitative Real-Time RT-PCR

A quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR) technique was used to analyze messenger RNA (mRNA) expression of SOD1, SOD2, glutathione peroxidase 1, catalase, Sirt1, eNOS, the Nrf2/antioxidant response element target genes NQO1 and GCLC, components of the local IGF-1 system (IGF-1, IGF-1R, insulin-like growth factor–binding protein 1, IGF-BP1, IGF-BP2, IGF-BP3, and IGF-BP4), and inflammatory markers (including tumor necrosis factor alpha, interleukin-6, interleukin-1β, inducible nitric oxide synthase, intercellular adhesion molecule 1, and monocyte chemotactic protein-1) in the middle cerebral arteries, as previously reported (26–29). In brief, total RNA was isolated with a Mini RNA Isolation Kit (Zymo Research, Orange, CA) and was reverse transcribed using Superscript III RT (Invitrogen, Carlsbad, CA) as described previously (26,30). A real-time RT-PCR technique was used to analyze mRNA expression using a Stratagen MX3000, as reported (30). Amplification efficiencies were determined using a dilution series of a standard vascular sample. Quantification was performed using the efficiency-corrected ΔΔCq method. The relative quantities of the reference genes GAPDH, hypoxanthine phosphoribosyltransferase 1, and β-actin were determined, and a normalization factor was calculated based on the geometric mean for internal normalization. Oligonucleotides used for quantitative real-time RT-PCR are listed in Table 1. Fidelity of the PCR reaction was determined by melting temperature analysis and visualization of the product on a 2% agarose gel.

Data Analysis

Gene expression data were normalized to the respective control mean values. Statistical analyses of data were performed by Student’s t test or by two-way analysis of variance followed by the Tukey post hoc test, as appropriate. p < .05 was considered statistically significant. Data are

Table 1. Oligonucleotides for Real-Time Reverse Transcriptase–Polymerase Chain Reaction

<table>
<thead>
<tr>
<th>mRNA Targets</th>
<th>Description</th>
<th>Sense</th>
<th>Antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD2</td>
<td>Mn-SOD</td>
<td>AGGCAACGGTGGTTCTGGAGATTTCGTC</td>
<td>CTGGTAGAAGCAGAGGTTGTC</td>
</tr>
<tr>
<td>SOD2</td>
<td>Cu-Zn-SOD</td>
<td>CTCAGGAGGATTCCATCATTTG</td>
<td>AATCAACAGTACAGGCAGAGC</td>
</tr>
<tr>
<td>Gpx-1</td>
<td>Glutathione peroxidase 1</td>
<td>TAGGTTCCAGACGGTGGTC</td>
<td>GATTCAAGGATGCTCTTGGG</td>
</tr>
<tr>
<td>Catalase</td>
<td></td>
<td>GCCCTACAGATTACATCG</td>
<td>ATCCAAAACAGAATCTCAAGC</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
<td>TTCCTGCCACTCTGCTTGGT</td>
<td>AAGAGATCTTCAAATCTGGG</td>
</tr>
<tr>
<td>NQO1</td>
<td>NAD(P)quinone oxidoreductase 1</td>
<td>TGGGATATGGAAGGGAGAGGAG</td>
<td>GAGAGGATCTAAATGCAACAG</td>
</tr>
<tr>
<td>GCLC</td>
<td>Glutamate-cysteine ligase, catalytic subunit 1</td>
<td>GCTTCTTCATCCCTGCTTGGT</td>
<td>TGGCAAGATTCATCGTCCGG</td>
</tr>
<tr>
<td>Sirt1</td>
<td>Sirtuin (silent mating type information regulation 2 homolog 1)</td>
<td>CGCCATATCCTTATCCCTGCTG</td>
<td>GCCTGTGACTGACATCTTCAG</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1 (somatomedin C)</td>
<td>GGTGTTGATGAAATGTTT</td>
<td>GCCTAGACGAGATAATTAGGTT</td>
</tr>
<tr>
<td>IGF1R</td>
<td>Insulin-like growth factor 1 receptor</td>
<td>TGAACATCTATCTACACA</td>
<td>CTATGATGAGCTTGCAATTGGT</td>
</tr>
<tr>
<td>IGF-BP1</td>
<td>Insulin-like growth factor–binding protein 1</td>
<td>TCCACACTTGCTGGAT</td>
<td>GAAGACTGAGAAAGGGTAGATT</td>
</tr>
<tr>
<td>IGF-BP2</td>
<td>Insulin-like growth factor–binding protein 2</td>
<td>TGTTATTATATGGAGAGAGAGAG</td>
<td>TCTTATAGGACAGACAGAAC</td>
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<tr>
<td>IGF-BP3</td>
<td>Insulin-like growth factor–binding protein 3</td>
<td>GAGGAAATACGCTTGGT</td>
<td>GTTACACTACATGACACTG</td>
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<tr>
<td>IGF-BP4</td>
<td>Insulin-like growth factor–binding protein 4</td>
<td>CAAGAGTCTGAGGTGTAAGT</td>
<td>TGATAGTATGTGGTCAATGAT</td>
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<tr>
<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
<td>AACCCAAAGCAGGAGAGGAGG</td>
<td>CTTGATGCGGAGGAGAGAG</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
<td>TACCCCAACCTCACTCAG</td>
<td>GATATCCACTGACAGAG</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin 1 β</td>
<td>CAGCAAGTCTGCGGAGGAC</td>
<td>ATATGAAAGATGTGCTGCT</td>
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<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
<td>TCCCGAAGACTCAGCT</td>
<td>CAATCCCAAACGCTCGT</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule 1</td>
<td>CAACAGCTGAAGCTCCT</td>
<td>CCCCCCTCTAAGGTTTGGAAGAA</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemotactic protein-1</td>
<td>GCATCAACCTTAAAGACTCAG</td>
<td>GGCATCAACCTTTAACAC</td>
</tr>
<tr>
<td>Hmox1</td>
<td>Heme oxygenase (HO-1)</td>
<td>GGGCTGGTAACCTGCTCCT</td>
<td>GGGCTGGTAACCTGCTCCT</td>
</tr>
<tr>
<td>HPRT</td>
<td>Hypoxanthine phosphoribosyltransferase 1</td>
<td>AAGACAGGGCCAGGATGTAAC</td>
<td>AAGGGCAGGACACAGACAC</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>CCAAGGATGAAAGAC</td>
<td>TTGATGATTCGAGAGAAGG</td>
</tr>
<tr>
<td>β-actin</td>
<td>Beta actin (ACTB)</td>
<td>GAACTGTCGACTGGAGGT</td>
<td>ACATCTGTCGAGAGGT</td>
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</table>

Table 2. Changes in Body Mass and Serum IGF-1 Levels of Homozygous Control Rats, GHD Dwarf Rats, and GH-Treated Dwarf Rats

<table>
<thead>
<tr>
<th>Serum IGF-1</th>
<th>After Treatment (ng/mL)</th>
<th>Body Mass, Before Treatment (g)</th>
<th>Body Mass, After Treatment (g)</th>
<th>Gain in Body Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>970 ± 96*</td>
<td>113 ± 8</td>
<td>274 ± 11</td>
<td>161 ± 8</td>
</tr>
<tr>
<td>GHD</td>
<td>464 ± 51*</td>
<td>84 ± 5*</td>
<td>170 ± 7*</td>
<td>86 ± 6*</td>
</tr>
<tr>
<td>GH replete</td>
<td>849 ± 110*</td>
<td>86 ± 5*</td>
<td>238 ± 11*</td>
<td>152 ± 8</td>
</tr>
</tbody>
</table>

Notes: See Methods section for description of experimental groups. Serum level of IGF-1 did not differ between the GHD and GH-replete groups (322 ± 69 and 325 ± 62 ng/mL, respectively) before GH treatment. Data are mean ± standard deviation.

*p < .05 vs respective controls. GH = growth hormone; GHD = growth hormone deficiency; IGF-1 = insulin-like growth factor 1.
expressed as means ± standard error of the mean, unless otherwise indicated.

**RESULTS**

**Serum IGF-1 Levels and Body Weight**

IGF-1 levels were measured in serum obtained from tail blood before and after the 30-day experimental period. Table 2 shows that at the end of the experimental period, control and dwarf GH-replete rats had significantly higher serum IGF-1 levels compared with the untreated dwarf rats (p ≤ .05, each), indicating that twice daily administration of GH to the dwarf rats normalized serum IGF-1.

The control and GH-replete rats expressed the typical phenotype of adequate GH levels, indicated by comparable increases in body weight during the experimental period, whereas untreated dwarf rats gained significantly less weight than the control group (Table 2).

**Vascular Oxidative Stress**

Representative fluorescent images of cross sections of DHE-stained aortas isolated from control, dwarf, and GH-replete rats are shown in Figure 1A–C. Analysis of nuclear
DHE fluorescent intensities showed that, compared with vessels from control rats, $O_2^-$ production was significantly increased in aortas of dwarf rats (Figure 1D). Vascular $O_2^-$ generation was significantly reduced by GH treatment (Figure 1D). Vascular 8-isoprostane content also tended to increase in dwarf rats, yet, the difference did not reach statistical significance (Figure 1E). Consistent with the presence of GHD-related oxidative stress, aortic GSH content and ascorbate concentrations were significantly reduced in dwarf rats (Figure 2E and F). GH treatment normalized GSH content in the aorta of GH-replete dwarf rats (Figure 2E).

**Antioxidant Enzyme Activities**

In dwarf rats and GH-replete animals, we did not detect any significant changes in superoxide dismutase, catalase, and glutathione peroxidase enzyme activities as compared with controls (Figure 3A–C).

**Vascular Expression of Antioxidant Enzymes**

A quantitative real-time RT-PCR technique was used to analyze mRNA expression of major antioxidant enzymes in branches of the middle cerebral artery. We found that expression of Mn-SOD, Cu,Zn-SOD, and catalase (Figure 4A–C) did not differ among the three groups. By contrast, expression of glutathione peroxidase 1 was significantly decreased in the cerebral arteries of dwarf rats and was significantly increased by GH treatment (Figure 4D).

**Vascular Expression of Nrf2 Targets**

Cerebral arteries of dwarf rats exhibited a significantly reduced expression of the known Nrf2 targets GCLC and
NQO1 compared with vessels from control animals. These changes were normalized by GH treatment (Figure 5A and B). Expression of SIRT-1 in dwarf and GH-replete animals showed similar pattern, SIRT1 being downregulated in arteries of dwarf rats and upregulated by GH repletion (Figure 5C).

Vascular eNOS Expression and Activity

Expression of eNOS mRNA in the middle cerebral arteries (Figure 6A) and nitric oxide synthase activity in the aortas (Figure 6B) were not statistically different among the three groups.

Vascular Expression of Components of the Local IGF-1 System

Expression of IGF-1 (Figure 7A) was similar in the middle cerebral arteries of control and dwarf rats, whereas expression of insulin-like growth factor 1 receptor was significantly increased in vessels of dwarf rats (Figure 7B). Expression of IGFBP1, IGFBP2, and IGFBP4 (Figure 7C, D, and F) was unchanged in arteries of dwarf animals. Expression of IGFBP3 tended to be decreased in dwarf arteries as compared with controls, yet the difference did not reach statistical significance (Figure 7E).

Vascular Expression of Inflammatory Markers

We found that mRNA expression of the inflammatory markers tumor necrosis factor alpha, interleukin-6, interleukin-1β, inducible nitric oxide synthase, Intercellular adhesion molecule 1, and monocyte chemotactic protein-1 in the middle cerebral arteries was statistically not different among the three groups (Figure 8).

Discussion

The levels of GH and IGF-1 vary substantially throughout the life span. Postnatal GH and IGF-1 levels are low but increase to higher concentrations immediately before puberty and then progressively decline with increasing age (31–33). Late-life changes in the endocrine milieu, including the age-related decline of GH/IGF-1 axis, have led to the formulation of a number of neuroendocrine theories of aging, which aim to explain peripheral aging on the basis endocrine dysregulation (34). Recent findings provide key evidence that changes in peripubertal GH and IGF-1 levels can also modulate peripheral aging (11), especially in the vascular system.

Deficits of GH in childhood can be due to a congenital deficiency (incidence: 1 in every 3,800 live births) or acquired in childhood due to hypothalamic–pituitary tumors. Previous studies focused on the deleterious effects of GHD on systemic cardiovascular risk factors in adults but provided little information on vascular effects directly affected by peripubertal GHD. Here, we demonstrate for the first time that peripubertal GH/IGF-1 deficiency in Lewis dwarf rats results in significant changes in vascular redox homeostasis. Measurements of DHE fluorescence (Figure 1A–D) as well as measurements of tissue isoprostane (Figure 1E), GSH, and ascorbate content (Figure 2) suggest that peripubertal GH/IGF-1 deficiency increases vascular oxidative stress. Because rats can synthesize ascorbate, we interpret the simultaneous decline in tissue ascorbate (35) and GSH levels as a sign of GHD-induced oxidative stress rather than a sign of dietary vitamin deficiency. Importantly, a relatively brief peripubertal exposure of Lewis dwarf rats to GH prevented or attenuated most of the aforementioned pro-oxidative alterations (Figures 1 and 2). These in vivo findings are in complete agreement with the observations that in...
Vaso protection by GH/IGF-1

In vitro treatment with GH and/or IGF-1 also reduces ROS production in cultured endothelial cells (13,36). It is significant that the same peripubertal treatment of young Lewis dwarf rats with GH was previously reported to significantly postpone the development of age-associated cerebrovascular disease and increase life span (11). Taken together, these observations raise the possibility that levels of GH present in the circulation during a brief transitional period starting around puberty and ending in young adulthood are likely to influence vascular pathology later in life (11) by modulating vascular redox homeostasis. We speculate that GHD-induced increased oxidative stress during development likely modulates the activity of various redox-sensitive pathways (e.g., mitogen activated protein kinases), which affect cerebrovascular architecture, the composition of extracellular matrix, and/or elicit epigenetic changes in vascular endothelial and smooth muscle cells, which will promote the development of cerebrovascular pathologies later in life. Importantly, if GHD persists in adulthood, it will likely exert a progressive deleterious effect on the vasculature (13,37–39). The clinical emphasis on treating childhood-onset GHD currently focuses on the somatic role of GH in increasing body size (mainly height) during puberty (40,41). Continued GH treatment during the important transition period between adolescence and adulthood is important for the normal development of bone and muscle in adulthood (20–30 years old) (40,41). In light of the aforementioned findings, it would be interesting to determine in follow-up studies how peripubertal GH replacement affects late-life cardiovascular morbidity and mortality in humans.

The underlying mechanisms for elevated ROS production in the cardiovascular system of GH/IGF-1–deficient rats are likely multifaceted. Previous studies suggest that GH/IGF-1 deficiency is associated with downregulation of vascular expression of major cellular antioxidant enzymes in adult Ames dwarf mice (13). In the present study, peripubertal GH/IGF-1 deficiency did not alter superoxide dismutase or catalase activities in the aorta nor the expression of Cu,Zn-SOD, Mn-SOD, and catalase in the cerebral arteries of dwarf rats. In contrast, cerebrovascular expression of glutathione peroxidase 1 was significantly decreased in dwarf vessels, and this effect was reversed by GH treatment. Glutathione peroxidase activity also tended to decrease in aortas of dwarf rats, but the difference did not reach statistical significance. The hitherto hypothetical concept, named mitochondrial hormesis, suggests that low levels of oxidative stress may have beneficial effects on the aging process by upregulating antioxidants. It is significant that in the vasculature free radical detoxification pathways driven by the transcription factor NF-E2-related factor 2 (Nrf2) play an important role in maintaining cellular redox homeostasis under conditions of oxidative stress (42). Nrf2 triggers expression of genes, including NAD(P)H:quinone oxidoreductase 1 (NQO1, a key component of the plasma membrane redox system and γ-glutamylcysteine ligase [GCLC]), via the antioxidant response element, which attenuates cellular oxidative stress. The second interesting finding of the present

![Figure 4. Quantitative real-time–polymerase chain reaction data showing messenger RNA (mRNA) expression of Cu,Zn-SOD (A), Mn-SOD (B), catalase (C), and glutathione peroxidase-1 (D) in branches of the middle cerebral arteries of control rats, growth hormone–deficient (GHD) dwarf rats, and growth hormone (GH)–treated dwarf (GH-replete) rats. Data are mean ± standard error of the mean. *p < .05 vs control, #p < .05 vs GHD (n = 5–6 in each group).](https://academic.oup.com/biomedgerontology/article-abstract/65A/11/1145/597083)
study is that peripubertal GH/IGF-1 deficiency significantly decreases expression of both of these Nrf2 target genes in the cerebral arteries, showing that adaptive induction of antioxidant enzymes in dysfunctional in Lewis dwarf rats. Because the plasma membrane redox system is responsible for the maintenance of the redox status of ascorbate, we posit that downregulation of NQO1 may contribute to GHD-induced decreases in ascorbate levels in the vascular wall of dwarf rats (Figure 2F). Because GCLC is the rate-limiting enzyme for GSH synthesis, it is likely that this downregulation contributes to the decline of vascular GSH content in these animals.

Recent studies demonstrate a cross talk between IGF-1 signaling and the prosurvival factor SIRT1 (43,44), both of which regulate cellular redox status, including mitochondrial ROS production in endothelial cells (45). The finding that in dwarf rats, vascular expression of SIRT1 is downregulated, whereas it is induced in GH-replete animals raises the possibility that this pathway also may contribute to the pro-oxidative effects of peripubertal GHD. Previously, we found that mitochondrial ROS generation is increased in arteries of Ames dwarf mice (13); thus, future studies are warranted to determine whether downregulation of SIRT1 is associated with an increased mitochondrial oxidative stress in blood vessels of rats with peripubertal GHD. eNOS is an important downstream target of both SIRT1 (46) and IGF-1 (13) that confers multifaceted vasoprotective effects. Importantly, low IGF-1 levels were shown to be associated with downregulation of eNOS both in the vasculature of adult Ames dwarf mice (13) and arteries of aged rats (26). The findings that cerebrovascular expression of eNOS and nitric oxide synthase activity in the aortas are unaffected by peripubertal GH/IGF-1 deficiency in dwarf rats give support to the view that young organisms have greater ability to adapt to changes in the endocrine milieu than older animals.

In addition to its endocrine function, IGF-1 also exerts its biologic effects via autocrine/paracrine pathways, and recent studies suggest that circulating IGF-1 levels and tissue expression of components of the local IGF-1 system are not

Figure 5. Quantitative real-time reverse transcriptase–polymerase chain reaction data showing messenger RNA (mRNA) expression of GCLC (A), NQO1 (B), and SIRT1 (C) in branches of the middle cerebral arteries of control rats, growth hormone–deficient (GHD) dwarf rats, and growth hormone–treated dwarf (GH-replete) rats. Data are mean ± standard error of the mean. *p < 0.05 vs control, #p < 0.05 vs GHD (n = 5–6 in each group).

Figure 6. Quantitative real-time reverse transcriptase–polymerase chain reaction data (Panel A) showing messenger RNA (mRNA) expression of endothelial nitric oxide synthase (eNOS) in branches of the middle cerebral arteries of control rats, growth hormone–deficient (GHD) dwarf rats, and growth hormone–treated dwarf (GH-replete) rats. Panel B depicts nitric oxide synthase activity in homogenates of aortas isolated from control, GHD, and GH-treated dwarf rats. Data are mean ± standard error of the mean. (n = 5–6 in each group).
always correlated (47–49). We found that peripubertal GH/IGF-1 deficiency did not affect the expression of IGF-1 in the middle cerebral arteries but upregulated insulin-like growth factor 1 receptor, which likely represents an adaptive mechanism resulting from IGF-1 deficiency. Expression of IGFBP1, IGFBP2, and IGFBP4 did not change in the wall of dwarf arteries. As expected, cerebrovascular expression of IGFBP3 tended to decrease in the dwarf rats because this is regulated by circulating GH levels. In that regard, it is significant that in humans, there is a close correlation between peripubertal serum levels of IGF-1 and IGFBP3 (31) and that GH deficiency in childhood is characterized by decreased levels of IGFBP3 (50).

Recent studies suggest that IGF-1 confers anti-inflammatory vascular effects (17) and that short-term GH treatment attenuates age-related inflammation, including upregulation of tumor necrosis factor alpha, in the cardiovascular system of senescence-accelerated mice (51). Human GH/IGF-1-deficient dwarfs at adulthood are known to develop premature atherosclerosis in the cerebral arteries. Importantly, in Lewis dwarf rats, peripubertal GH replacement, sufficient to raise IGF-1 levels to that of normal animals during adolescence, significantly delays cerebrovascular alterations associated with old age and increases life span (11). To determine whether peripubertal GH/IGF-1 deficiency promotes vascular inflammation during adolescence, we assessed the expression of various inflammatory markers in the middle cerebral arteries of Lewis dwarf rats. We found that expression of proinflammatory cytokines, chemokines, and adhesion molecules was unaffected either by peripubertal GH/IGF-1 deficiency or GH treatment (Figure 8). These data provide additional support for the view that young organisms have greater ability to adapt to changes in circulating GH/IGF-1 than older animals.

In conclusion, peripubertal GH/IGF-1 deficiency results in a pro-oxidative cellular environment, which likely promotes...
an adverse functional and structural phenotype in the vasculature that contributes to the development of stroke later in life. Peripubertal GH treatment of Lewis dwarf rats prevents these adverse vascular effects during a critical developmental time window, which contributes to the delay of stroke thereby extending the life span of these animals. Additional studies are warranted to further characterize the effects of peripubertal GH treatment on age-related structural and functional cerebrovascular alteration in Lewis rats. On the basis of the available data, we predict that beneficial effects of peripubertal normalization of GH and IGF-1 levels continue regardless of the presence or absence of these hormones during adulthood.

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