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Early-onset Growth Hormone Deficiency Results in Diastolic Dysfunction in Adult-life and is Prevented by Growth Hormone Supplementation

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Abstract

Objective—The primary goal of growth hormone (GH) replacement is to promote linear growth in children with growth hormone deficiency (GHD). GH and insulin-like growth factor-1 (IGF-1) are also known to have roles in cardiac development and as modulators of myocardial structure and function in the adult heart. However, little is known about cardiac diastolic function in young adults with childhood onset GH deficiency in which GH treatment was discontinued following puberty. The aim of the study was to evaluate the effects of long standing GHD and peri-pubertal or continuous GH replacement therapy on diastolic function in the adult dwarf rat.

Design—The dwarf rat, which possesses a mutation in a transcription factor necessary for development of the somatotroph, does not exhibit the normal peri-pubertal rise in GH around day 28 and was used to model childhood or early-onset GHD (EOGHD). In another group of male dwarfs, GH replacement therapy was initiated at 4 weeks of age when GH pulsatility normally begins. Ten weeks after initiation of injections, GH-treated dwarf rats were divided into 2 groups; continued treatment with GH for 12 weeks (GH-replete) or treatment with saline for 12 weeks. This latter group models GH supplementation during adolescence with GHD beginning in adulthood (adult-onset GHD; AOGHD). Saline-treated heterozygous (HZ) rats were used as age-matched controls. At 26 weeks of age, cardiac function was assessed using invasive or noninvasive (conventional and tissue Doppler) indices of myocardial contractility and lusitropy.

Results—Systolic function, as determined by echocardiography, was similar among groups. Compared with HZ rats and GH-replete dwarfs, the EOGHD group exhibited significant reductions in myocardial relaxation and increases in left ventricular filling pressure, indicative of moderate diastolic dysfunction. This was further associated with a decrease in the cardiac content of sarcoplasmic reticulum Ca²⁺ ATPase (SERCA2), one of the important cardiac calcium regulatory proteins. Dwarfs supplemented with GH during the peri-adolescence stage, but not

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beyond (AOGHD), exhibited a subtle prolongation in the deceleration time to early filling. In contrast, continual GH replacement preserved diastolic function such that the cardiac phenotype of the GH-replete dwarfs resembled that of their age-matched HZ counterpart.

Discussion—Our data indicate that GHD during adolescence leads to overt diastolic dysfunction in early adulthood and this is prevented by continual GH replacement therapy. Since discontinuation of GH replacement following adolescence only mitigated the lusitropic deficits that were observed in untreated dwarfs, GH treatment into adulthood could be beneficial.

Keywords

dwarf; diastolic function; growth hormone; IGF-1, myocardial relaxation; tissue Doppler

INTRODUCTION

Appreciation of the importance of the GH/IGF-1 axis in the development and maintenance of cardiac structure and function in humans has depended substantially on studies in subjects with GH-deficiency (GHD). Adults with GHD have reduced cardiac mass, a decrease in contractility, and subtle abnormalities in diastolic function that contribute, in part, to reductions in exercise capacity and quality of life.¹⁻³ Treatment with recombinant human GH reverses many of the cardiovascular alterations associated with GHD.⁴⁻⁸ However, uncertainty remains regarding the primary actions of GHD in the pathogenesis of diastolic dysfunction in those persons with either long-standing GHD from childhood or adult-onset GHD. Also, the effect of discontinuation of GH replacement therapy on the subsequent development of diastolic function abnormalities in young adults who had received optimal GH treatment for somatic growth (mainly height) during puberty is not known.

Approximately 35,000 adults have GHD, and about 6,000 new adult patients are diagnosed annually.⁹ In addition to a congenital origin, GHD is a frequent consequence of pituitary radiotherapy in children and adults—for non-functioning adenomas, adrenocorticotrophic hormone- or prolactin-secreting adenomas.¹⁰ Epidemiologic data suggest that adults with GHD have reduced life expectancy as a result of premature coronary heart disease presumably because of associated dyslipoproteinemia, hypercholesterolemia, and central adiposity-related cardiometabolic abnormalities.¹¹ Until now, however, data on the prevalence and corresponding morbidity and mortality related to diastolic heart disease among GHD patients are not known. In the general population, the frequency of LV diastolic dysfunction increases with age.^{12,13} In most cases, it remains clinically silent, beginning with relaxation abnormalities accompanying normal systolic function. However, nearly half of patients with isolated diastolic dysfunction develop symptoms of congestive heart failure (CHF) over a follow-up period of 5 years, and a quarter of these patients require frequent hospitalizations for these symptoms.¹⁴ Although the link between GHD and increased incidence of diastolic heart disease remains speculative, studies show that reduced activity of the GH/IGF-I axis in chronic heart failure (CHF) is associated with poor clinical status and outcome.¹⁵⁻¹⁷

The dwarf rodent is a promising model to study specific GH/IGF-1 effects on various organ functions,¹⁸ including the heart, that are independent of age. The dw/dw rat has a spontaneous mutation that results in a decrease in the number of somatotrophs, a decrease in GH secretion from the pituitary and a decline in plasma GH and IGF-1. This model has been used to mimic childhood or early onset GHD.¹⁹ Administering exogenous GH to dwarf rats (dw/dw) starting at postnatal day 28, when the characteristic pulsatile secretion of GH normally begins, provides adequate GH/IGF-I levels during ongoing periods of tissue development and somatic growth.²⁰ Discontinuation of GH, following puberty (14 wks of

age), generates a cohort of animals with the same developmental history as GH-replete dwarfs but which are GH/I-IGF-1-deficient in young adulthood (AOGHD). This is analogous to prepubertal GH intervention in children for the promotion of linear growth. Continuous GH replacement therapy in the dwarf is used to mimic long-term supplementation in clinical practice while littermates that are heterozygous (HZ) for the autosomal recessive dwarf trait serve as control animals resembling normal lifetime fluctuations in GH/IGF-1. This model allows for the investigation of the consequences of GH/IGF-1 deficiency on cardiac morphology and function apart from other physiological and pathologic changes known to occur with age and controls for the amount and duration of GH replacement therapy during the peri-pubertal period.

Accordingly, the aim of the present study was to determine the effect of peri-pubertal GH supplementation on the cardiac phenotype of adult dwarfs. We hypothesize that peri-pubertal GH supplementation is sufficient to retard the lusitropic abnormalities that occur in the GHD adult. Moreover, continual GH supplementation, to achieve physiologic levels of circulating IGF-1, preserves myocardial relaxation such that the diastolic cardiac phenotype of the dwarf resembles that of their age-matched counterpart with an intact GH/IGF-1 axis.

METHODS

As described previously,¹⁹ subjects are 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 and a subsequent decline in IGF-1 production.²¹ Female heterozygous rats were bred with homozygous male rats to produce heterozygous offspring (HZ) of normal size or homozygous rats possessing a “dwarf” phenotype. Identification of rats was based on body weight at 25-28 days of age, the age at which GH pulse amplitude increases in rodents and serum IGF-1 levels.²² Dwarf offspring were subsequently randomized into one of three treatment groups: 1) early-onset GHD (EOGHD) given saline; 2) adult-onset GHD (AOGHD) administered GH for 10 wk followed by saline; or 3) GH-replete with GH administered beginning at 4 weeks of age and continued throughout the experimental period of 26 weeks. Saline or GH (200 µg recombinant porcine GH; Alpharma, Victoria, Australia) was injected subcutaneously twice daily per animal. Heterozygous animals (normal size and GH/IGF-1 levels) were used as controls and given saline injections (Schematic of protocol, Figure 1). This dose and protocol of GH is based on previous publications and it is the minimal effective dose necessary to raise IGF-1 levels to that found in heterozygous, wild-type rats.²² Also, porcine GH is approximately 90% homologous to rat GH and its long-term use in rodent models has not been associated with the development of antibodies or the loss of an effective response.²² At 26 weeks of age, cardiac structure and function were studied in two, independent, experimental cohorts that had undergone identical GH dosing protocols within a two-year period (Figure 1). Rodents in cohort A that had been randomized at 4-5 weeks of age to HZ (n = 8), GH-replete (n = 8), EOGHD (n = 7), AOGHD (n = 8) groups, underwent non-terminal, echocardiographic examinations. Rodents in cohort B, consisting of HZ (n = 6), GH-replete (n = 6), EOGHD (n = 6), AOGHD (n = 4) groups, were instrumented for terminal invasive hemodynamic studies prior to blood and heart retrieval for serum IGF-1 and SERCA2 immunoblot analyses, respectively. Rats had access to food and water *ad lib* and were housed in pairs in the vivarium of the Department of Physiology & Pharmacology at Wake Forest University Medical School. Body weights were measured at the time of cardiovascular assessment. All procedures were in compliance with the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health and reviewed and approved by the Wake Forest University School of Medicine's animal care and use committee before commencement of the study.

Echocardiography (cohort A)

Following 26 weeks of saline or GH treatment, transthoracic echocardiographic (TTE) examinations by an experienced echocardiographer masked to the treatment protocol were obtained from spontaneously breathing rats that were lightly anesthetized with an intramuscular mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg). As previously described,²³ animals were placed in a shallow left lateral decubitus position with electrocardiographic adhesive electrodes applied to the paws. The left hemithorax was shaved and prepped with acoustic coupling gel to increase probe contact. Animals were secured to the surface of a warming table to maintain normothermia. Using two commercially available sector scanners equipped with a 12 MHz phased-array transducer (Philips Envisor; Philips Medical Systems, Andover, MA), images were obtained at 100 mm/s sweep speed, and recorded on a digital storage optical disc for off-line analysis. Left ventricular M-mode images were obtained in the 2-D short-axis view, close to the papillary muscles. Diastolic posterior wall thickness (PWT_{ed}) and LV end-diastolic and end-systolic dimensions (LVDD, LVSD) were measured. The percentage of LV fractional shortening (%FS), an index of global systolic function, was calculated as $((LVDD - LVSD) / LVDD) \times 100$. Left ventricular mass was calculated using a standard cube formula, which assumes a spherical LV geometry according to the formula: $LV\ mass\ (LV_{mass}) = 1.04 \times [(LV_{EDD} + PWT + AWT)^3 - LV_{EDD}^3]$, where 1.04 is the specific gravity of muscle. Relative wall thickness (RWT) was calculated as: $2 \times PWT / LV_{EDD}$. LV mass and RWT were normalized to body weight. Mitral inflow measurements of isovolumic relaxation time (IVRT), early filling velocity (E_{max}), deceleration time of early filling flow velocity ($E_{dec\ time}$), and late filling velocity (A_{max}) were obtained using pulsed Doppler, with the sample volume placed at the tips of mitral leaflets from an apical four-chamber orientation. The following measurements were made from the septal mitral annular velocity by tissue Doppler imaging (DTI): early diastolic (e'), late diastolic (a') and E/e' , as a measure of filling pressure.

Invasive Hemodynamics (cohort B)

A tracheotomy was performed in rats anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (5 mg/kg) and maintained on isoflurane/air-oxygen mixture (1.5%) during positive pressure ventilation. Hemodynamic measurements were obtained using a 2F sensor tip, pressure catheter (SPR-869, Millar Instruments, Houston, TX), inserted into the carotid artery and then passed into the left ventricle to measure left ventricular systolic (LVSP) and end diastolic pressures (LVDP). Derived systolic and diastolic indices included $dp/dt\ max$, $dp/dt\ min$, and τ , respectively. At the completion of the study, rats were killed by exsanguination, blood was collected for IGF-1 and hematocrit, and hearts were rapidly removed, weighed and stored at $-80^{\circ}C$ for immunoblots.

Immunoblot Analysis

Membrane proteins from the left ventricular myocardium were isolated from cohort B hearts as described previously.^{23,24} The frozen hearts were homogenized and centrifuged at 1,000 g for 10 min. The supernatants were centrifuged at 100,000 g for 60 min at $4^{\circ}C$. The 100,000-g pellets were cellular membrane fractions and were used for immunoblotting of SERCA2 with rabbit polyclonal antibodies (Abcam, Cambridge, MA) and peroxidase-conjugated secondary anti-rabbit IgG antibodies. Bands were identified by chemiluminescence. To normalize the variability of protein loading, the antibody to β -actin (Sigma Aldrich Chemical Co., St. Louis, MO) was probed onto the SERCA2-stripped membrane (Recycling kit, Alpha Diagnostic International, Inc., San Antonio, TX). Each SERCA2 was normalized to its own β -actin and expressed in arbitrary units.

IGF-1 Measurements

IGF-1 (Bachem, Torrance, CA) was radiolabeled using the lactoperoxidase, glucose oxidase method, and purified on a Sep-Pak silica cartridge (Waters, Milford, MA). Serum was extracted in acid-ethanol,²⁵ and IGF-1 was measured by radioimmunoassay (RIA).²⁵ Results are reported as ng IGF-1 per milliliter plasma.

Statistical Analysis

All data are presented as mean $\pm$ SEM. When significant effects were identified by one-way analysis of variance (ANOVA) of the single parameters, the Newman-Keuls multiple-sample comparison test was used to localize differences between groups. If homogeneity or normality were not satisfactory, a nonparametric one-way ANOVA (Kruskal-Wallis) was performed on the ranked measurements (e', IGF-1, SERCA2) followed by Dunn's multiple-sample comparisons. A P-value of <0.05 was considered significant.

RESULTS

Physical Characteristics

Physical characteristics from animals in cohort B are summarized in Table 1. At 26 weeks of age, body weight was significantly reduced in EOGHD and AOGHD dwarfs when compared to GH-replete littermates and HZ rats. Also, adult onset GHD dwarfs weighed more than EOGHD dwarfs ($P<0.05$). As expected, tibial length was notably reduced in EOGHD dwarfs versus HZ and GH-replete littermates, and there was no difference in this index of somatic growth among HZ, GH-replete and AOGHD rats. Heart weight was lowest in EOGHD dwarfs, but when normalized to body weight or tibial length, no differences were observed among groups. Serum IGF-1 levels were 45 to 60% lower in AOGHD and EOGHD dwarfs, respectively, compared to age-matched GH-replete littermates and HZ controls (Figure 2). Hematocrit did not show differences among the groups.

Invasive Hemodynamic Studies

Resting hemodynamic data from 26-week-old rats in cohort B are summarized in Table 1. There were no differences in heart rate, left ventricular pressure, or the time constant of relaxation (τ) among groups. However, in the EOGHD dwarfs there was a 33% reduction in maximum rate of pressure rise ($+dP/dt$) ($P<0.05$) and more than a 20% reduction in maximum rate pressure decline ($-dP/dt$) compared to HZ control rats, substantiating the presence of cardiac functional deficits from longstanding GHD.

Echocardiography

M-mode and Doppler measurements of left ventricular structure and function obtained at 26 weeks of age from animals in cohort A are summarized in Table 2. HZ and GH-replete rats had a 1.5- and 2.5-fold greater left ventricular mass than EOGHD littermates. In EOGHD dwarfs, LV mass normalized for body weight was only minimally reduced compared to the other groups ($P=0.07$). The RWT normalized for body weight was significantly greater in EOGHD and AOGHD dwarfs compared to HZ controls and GH-replete dwarfs. Relative wall thickness indexed to body weight was also greater in EOGHD versus AOGHD dwarfs, most likely due to the reduced body weight in EOGHD rather than due to thicker walls in untreated dwarfs compared to AOGHD dwarfs. Despite significantly reduced LVDD in EOGHD dwarfs compared to HZ and GH-replete dwarfs, the calculated index of systolic function, or percent fractional shortening, was similar among groups.

The effect of GH replacement therapy on heart rate and Doppler-derived indices of LV diastolic function are shown in Table 2. Heart rate was similar among the 4 groups.

Although the transmitral Doppler velocity E/A was >1 in all groups, EOGHD dwarfs demonstrated nearly a 20% reduction in mitral annular descent (e') and significantly elevated filling pressures, or E/ e' , when compared to GH-replete and HZ rats (Figure 3). The modest variation in e' among groups was significant (Group F (3, 29) = 3.139, $P=0.04$); however, post-hoc analysis by Newman-Keuls failed to localize the difference. Taken together with the tendency for reduced IVRT, these Doppler data are suggestive of a pseudonormal transmitral flow pattern, or moderate diastolic dysfunction. Nonetheless, an increased early deceleration time in AOGHD dwarfs compared with GH-replete and HZ littermates, indicates a subtle abnormality in myocardial relaxation.

Cardiac SERCA2 Expression

The reduction of $+dP/dt$ max in the EOGHD dwarfs was associated with significant changes in protein levels of SERCA2 (Figure 4). The expression of SERCA2 was decreased by 25% in the ventricles of EOGHD dwarfs ($P<0.001$) when compared with GH-replete and HZ rats. GH supplementation throughout the experimental life of the dwarf, as opposed to only peripubertal GH supplementation, increased and sustained cardiac levels of SERCA2 compared to that of the HZ rats.

DISCUSSION

The results of the current study demonstrate that untreated early onset GHD leads to significant alterations in LV diastolic function and reductions in absolute cardiac size by adulthood in the GH-deficient dwarf rat. In contrast, GH supplementation during the peri-adolescence stage, but not beyond (AOGHD), not only facilitates somatic and cardiac growth but it also limits the onset of overt diastolic dysfunction. Continual GH supplementation, beyond 14 weeks of age (GH-replete), maintains normal cardiac size and preserves diastolic performance in the adult dwarf, as indicated by comparable morphologic and diastolic indices between GH-replete and HZ littermates. GH substitution in the dwarf from youth through early adulthood leads to an increase in IGF-1 that is within the age-related physiologic range of the wild-type rat, and does not have deleterious effects on cardiac structure and function.

Despite clinical evidence linking GHD26 and low IGF-1 to cardiovascular disease,^{11,15-17} little is understood about the mechanisms connecting GH/IGF-1 deficiency to diastolic heart disease and no information is available regarding the underlying basis for differential susceptibility to diastolic dysfunction in patients with childhood-onset and adult-onset GHD. The absence of information in this area is due in part to the complexity of long-term cardiovascular studies in GHD patients are complex. For instance, body composition and metabolism, cardiac structure, and exercise tolerance change with age even in normal subjects,²⁷ making it difficult to differentiate these changes from that of GHD. Furthermore, to assess long-term effects of GH replacement therapy, a suitable model would be the adult patient with congenital isolated GH deficiency. However, finding a sizable group of such untreated patients in developed countries is challenging. The mutant GH-deficient dwarf, on the other hand, is particularly useful for studies of GH and IGF-I-dependent regulation in the adult myocardium since it permits comparison of animals with similar histories but significantly different levels of IGF-I in the plasma.^{18,20} The 55% reduction in serum levels of IGF-1 in AOGHD compared with GH-replete and HZ animals in the present study confirms that the animals that become GH deficient in early adult life are also IGF-1 deficient. Likewise, IGF-1 levels in dwarfs receiving lifelong GH supplementation are comparable to that of their heterozygous counterparts. Although the dwarf animal is not an aging model *per se*, it is a valuable model to test the effects of GH/IGF-I deficiency on the myocardium in the absence of other (GH-independent) aging-related changes.^{18,20} This is important given that the incidence of diastolic dysfunction is age related.¹² Several recent

studies have used the model to assess the effects of GH/ IGF-I deficiency on lifespan, tumor and disease burden, reproductive fitness, and cognitive function.^{19-22,28}

It is important to note that one limitation to using the Lewis dwarf model is that these rats have partial, rather than complete, GH deficiency. However, given that GH and IGF-1 have a broad range of actions on multiple tissues, a total knockout model is not appropriate as it can impair the development of tissues/organs resulting in functional changes throughout the lifespan of the animal.²⁰⁻²² In order to circumvent these concerns, we have used a mutant animal that exhibits partial GH deficiency. A complete analysis of the Lewis dwarf model has been published and, importantly, it shows no alterations in basal glucose and insulin levels or other hormones, and it responds appropriately to treatment with GH.²² Accordingly, in addition to early or childhood onset GHD, this becomes an excellent model of adolescent and/or adult GH/IGF-1 deficiency when GH supplementation is provided only in the peri-pubertal period and, thus, can be used to mimic that which occurs in humans.

Abnormalities in cardiac morphology and function have been shown in other experimental models of GH deficiency, but information is scarce regarding the specificity of GHD on the development of diastolic dysfunction. Longobardi et al.²⁹ observed cardiac atrophy, described by reductions in LV volumes (indexed to tibial length) and myocyte size, and abnormal stress-shortening relationships in 3-month old dwarf females with GH deficiency when compared to age- and sex-matched control rats. Despite the complete restoration of cardiac size and contractile function to normal following three weeks of GH supplementation, no data on diastolic function were presented. In an *in vitro* whole heart preparation, Cittadini et al.³⁰ showed evidence of reduced distensibility, characterized by increased left ventricular end diastolic pressure and a leftward shift in the pressure-volume curve in GH-deficient hearts when compared to hearts from control and GH-replete dwarfs. Other measures of resting diastolic function, including tau, $-dP/dt$, and time to 90% relaxation, were not affected by GH deficiency. Reddy et al.³¹ found a reduction in LV pressure-derived $-dP/dt$ max in 4-month old mice with isolated GH deficiency (*Little* strain) but no difference in the load-independent measure of lusitropy, Tau, when compared to age-matched wild-type animals.

Indeed, to discriminate between inherent lusitropic abnormalities and the effects of altered loading conditions, measures that are relatively independent of preload and afterload are used. While conventional Doppler has been shown to be a useful technique for assessing ventricular diastolic function by measuring atrioventricular valve inflow and pulmonary venous flow velocities in various rodent models and humans, this method is affected by loading conditions and heart rate.³² Tissue Doppler imaging, on the other hand, provides measurements of regional systolic and diastolic function with high spatial and temporal resolution that are relatively independent of left atrial pressure, pseudonormalization, volume status and rate of myocardial relaxation.³³⁻³⁴ For instance, in the EOGHD dwarfs, the transmitral E/A ratio was >1 , suggesting normal diastolic function. However, the lower e velocity and higher E/e' compared to HZ and GH-replete, confirmed the presence of diastolic impairment among EOGHD animals. The ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity (E/e') is a noninvasive surrogate of left ventricular filling pressures in humans and a marker of an advanced stage of diastolic dysfunction.³³⁻³⁵

Even though there was a relative reduction in serum levels of IGF-1 in AOGHD dwarfs compared to HZ and GH-replete littermates, overt diastolic dysfunction was not apparent. Nonetheless, the prolongation of early deceleration time in the AOGHD dwarfs implies that lusitropic abnormalities may be delayed rather than mitigated by the early GH therapy. Our data parallel the clinical findings reported by Valcavi et al.⁵ of an increased early

deceleration time despite a normal E/A ratio and isovolumic relaxation time in 20 AOGHD patients compared with controls. Shahi et al.³⁶ also found that 30% of their AOGHD patients had diastolic dysfunction as defined by an increased isovolumetric relaxation time. However, Climent et al.³⁷ reported normal diastolic and systolic function in a subset of their patients with adult onset GHD even though they had reduced exercise capacity when compared to healthy, age matched individuals. The discrepancies among these clinical studies may be due to differences in age of GH-onset and duration of GH deficiency, or to variations of how peripubertal GH, followed by no replacement therapy, were considered. Also, the use of transmitral Doppler for the evaluation of diastolic function may not have been sensitive enough to detect subclinical alterations in myocardial relaxation. In two recent studies that used tissue Doppler, significant reductions in early myocardial relaxation, or early diastolic annular velocity (e'), were observed among patients with GHD when compared to age matched healthy subjects.^{38,39} Standard Doppler showed only a prolongation of IVRT but no differences in conventional Doppler measures of diastolic function including transmitral inflow velocities and deceleration time. Tissue Doppler imaging of mitral annular descent, or e' , is deemed to be a better discriminator between control subjects and patients with diastolic dysfunction, in part because it reliably differentiates pseudonormal patterns of diastolic dysfunction from normal function.⁴⁰

GH replacement therapy has improved global systolic function in experimental models and in patients with congenital GHD.^{11,18} The morphologic benefit of GH therapy is apparent by normalization of LV mass,⁸ and the addition of new sarcomeres has been confirmed by endomyocardial biopsy in GHD patients.⁴¹ The prevailing findings relating to diastolic function, however, have not been as consistent. In studies involving treatment durations ranging between 4 and 26 months, at least six studies have reported no change in diastolic function,^{37,38,42-45} and only three have shown that it increases after GH treatment in adolescents and adults with GHD.^{5,46,47} Certainly, improvements in exercise tolerance or capacity with GH replacement,^{37,44,48} independent of changes in systolic function at rest, could also be indicative of a lusitropic benefit. Exercise intolerance is often the first clinical manifestation of diastolic dysfunction.⁴⁹ Nevertheless, in the dwarf, uninterrupted GH therapy yielded a cardiac phenotype similar to that of age-matched heterozygous rats, implying that GH replacement therapy, either directly or indirectly through IGF-1, normalizes diastolic function.

Whether the lusitropic benefit of prolonged GH therapy in the dwarf persists beyond 6 months of age is not known. In clinical studies, the beneficial effects on cardiac function seem to be sustained for at least 3 years after restoration of GH therapy,⁶ and possibly after seven years of treatment.⁵⁰ Importantly, long-term replacement in GHD patients has not produced LV hypertrophy or an increase in the prevalence of diabetes;⁵⁰ two risk factors for diastolic dysfunction. Moreover, five months of uninterrupted physiologic GH therapy in dwarf animals did not have negative effects on cardiac structural or functional noninvasive or invasive parameters nor was there evidence of increased malignancies.

Several mechanisms may be responsible for diastolic dysfunction in GHD. Preclinical studies suggest a potential role for GH/IGF-1 in the maintenance of myocardial calcium homeostasis, particularly in models of heart failure.^{51,52} Growth hormone directly stimulates the expression of SERCA2, an important regulator of Ca^{2+} uptake by the sarcoplasmic reticulum. In rats with postinfarction heart failure, SERCA2 is increased by GH treatment.⁵¹ Correspondingly, we found a relative reduction in SERCA2 protein expression in the left ventricle of EOGHD compared to GH-replete dwarfs and this was associated with a reduction in an invasive measure of contractility, $+dP/dt$. Certainly, systolic dysfunction by itself can have a negative impact on diastolic function.⁵³ However, given that fractional shortening was not significantly different among treatment groups in

the cohort of animals that underwent echocardiograms, implies that systolic dysfunction, *per se*, was likely not the principle cause for the reduction in myocardial relaxation in GHD dwarfs. Evidence from various experimental and human models indicates that SERCA2a down-regulation might also contribute to diastolic heart disease.^{54,55} Moreover, we have previously reported its relation to the tissue Doppler index of lusitropy, e' , in an experimental aging model.^{23,24} Indeed, the negative lusitropic effects of GHD could also be the result of GHD-mediated vascular aberrations as recently reported by Ungvari et al.²⁸ Moreover, patients with GHD have reduced aortic distensibility and endothelial dysfunction which are potent stimuli for increasing left ventricular load and impairing left ventricular diastolic relaxation⁵⁶ and endothelial dysfunction.⁵⁷ Even though we did not assess vascular morphology or function in the present study, conscious blood pressures measured by telemetry were similar between dwarf rats and their age-matched heterozygous counterparts.²² Whether long-term GH deficiency directly influences the myocardial determinants of stiffness, including the extracellular matrix, cytoskeleton, or myofilaments, is not clear. All the same, the increase in E/e' in EOGHD dwarfs suggests an upward shift in the diastolic pressure-volume relationship.

Besides GH/IGF-1's endocrine effects, the autocrine/paracrine functions of IGF-1 and its binding proteins may also contribute to the cardiac phenotype. Several reports have shown that the expression of the mRNA and protein levels for IGF-1 are elevated in the myocardium^{58,59} and the circulation⁶⁰ of hypertrophic cardiomyopathy patients. In cardiac surgical patients with aortic and mitral valve disease, pericardial total IGF-1/IGFBP-3 ratio was positively correlated with left ventricular mass index, further suggesting that the local IGF-1 system plays a role in the pathogenesis of LV hypertrophy.⁶¹ An interaction between IGF-1 and IGFBP-3 in the regulation of hypertrophy has also been implied in an experimental study. Ito et al.⁶² showed that that IGF-I has a direct hypertrophic effect on cultured rat cardiomyocytes with concomitant increases in the transcripts of muscle-specific genes (MLC-2, troponin I, and skeletal α -actin) and that IGFBP-3 has an inhibitory effect on the IGF-I-induced cardiac hypertrophy in vitro. Although we did not measure local IGF binding proteins in the present study, Ungvari et al.²⁸ showed that expression of IGFBP1, IGFBP2, and IGFBP4 did not change in the cerebrovascular wall of the dwarf but that there was a tendency for IGFBP3 to be decreased. Thus, future studies are warranted to determine whether cardiac tissue expression of components of the IGF-1 system is related to the diastolic dysfunction phenotype of GHD.

Cardiovascular disease is the leading cause of premature death in adults with GHD. The reasons for this are presumed to be due to an increase in cardiovascular risk factors, including hyperlipidemia, atherosclerosis, endothelial dysfunction, and intimal thickening.¹¹ However, since diastolic abnormalities may be the earliest manifestations of heart disease,⁵⁸ it is important to document their occurrence in GHD individuals. Also, since GH treatment in children with GHD is often discontinued in adolescence after attainment of final height, the clinical concern of premature diastolic dysfunction becomes valid. Using the Lewis Dwarf model, we have found significant impairments in diastolic functional parameters in EOGHD compared with heterozygous and GH-replete dwarfs by means of tissue Doppler imaging. Although overt diastolic dysfunction was not observed in AOGHD dwarfs, the subtle prolongation in early filling implies that the lusitropic effects of peri-pubertal GH may not have lasting benefits. Given that ventricular diastolic dysfunction, whether from aging or from underlying disease, is a strong predictor of mortality,³⁵ these experimental data support the consideration that GH therapy in individuals with childhood onset GHD should be continued into adulthood.

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Figure 1.

Model representing heterozygous, GH-replete, Early-onset GH deficient, and Adult-onset GH deficient rats. Normal-sized females heterozygous for the dwarf trait (dw/+) were mated with homozygous dwarf males to produce heterozygous and homozygous (dw/dw) littermates. Dark areas represent high levels of IGF-1.

Figure 2.

Serum levels of IGF-1 in heterozygous (HZ), GH-replete, Early-onset GH deficient dwarfs (EOGHD), and Adult-onset GH deficient dwarfs (AOGHD) from cohort B. Data presented as mean $\pm$ SEM. * $P < 0.05$ vs. EOGHD.

Figure 3.

Early mitral annular velocity (e') and early transmitral filling velocity-to-mitral annular velocity ratio (E/e') in heterozygous (HZ), GH-replete, Early-onset GH deficient dwarfs (EOGHD), and Adult-onset GH deficient dwarfs (AOGHD). The overall group ANOVA for e' was significant ($P=0.04$), but post-*hoc* multiple comparisons did not localize the difference. Data presented as mean $\pm$ SEM. * $P<0.05$ vs. EOGHD.

Figure 4.

SERCA2 protein normalized to β -actin among GH-replete, Early-onset GH deficient dwarfs (EOGHD), and Adult-onset GH deficient dwarfs (AOGHD) are expressed relative to heterozygous (HZ) band intensity. Data represent mean $\pm$ SEM (n= 4-5 hearts/group). * P<0.05 vs. EOGHD. Representative SERCA2 immunoblot from each group is shown below. GH = GH-replete dwarf; EO = Early-onset GH deficient dwarf; AO = Adult-onset GH deficient dwarf.

TABLE 1

Physical characteristics and hemodynamics (cohort B)

Groups	BW, g	HW, mg	TL, mm	HW/BW, mg/g	HW/TL, mg/mm	Hct, %
HZ	490 ± 19 [*]	1.31 ± 0.08 [†]	52.6 ± 0.8 [‡]	0.27 ± 0.02	0.024 ± 0.002	40.8 ± 0.7
GH-replete	469 ± 16 [*]	1.21 ± 0.09 [†]	52.2 ± 0.9 [‡]	0.26 ± 0.02	0.023 ± 0.002	41.8 ± 1.4
EOGHD	307 ± 15	0.80 ± 0.03	43.5 ± 1.1	0.26 ± 0.005	0.018 ± 0.001	42.3 ± 1.0
AOGHD	374 ± 15 [‡]	0.98 ± 0.11	47.8 ± 1.6	0.26 ± 0.02	0.020 ± 0.002	39.2 ± 1.2
<i>P value</i>	0.008	0.007	0.002	--	0.087	--

	HR, b/min	dP/dtmax, mmHg/s	dP/dtmin, mmHg/s	LVPmax, mmHg	LVPmin, mmHg	Tau, sec
HZ	235 ± 31	14,080 ± 736 [§]	-10,430 ± 776	109 ± 3	-1.5 ± 1.1	4.3 ± 0.2
GH-replete	248 ± 13	13,542 ± 1150	-11,276 ± 1002	117 ± 8	0.10 ± 1.5	5.4 ± 0.6
EOGHD	245 ± 17	9,313 ± 1273	-8182 ± 965	106 ± 7	-0.40 ± 1.4	5.3 ± 0.5
AOGHD	222 ± 23	11,993 ± 1630	-9664 ± 1369	102 ± 6	-0.70 ± 1.9	5.8 ± 1.4
<i>P value</i>	--	0.037	0.196	--	--	--

HZ, heterozygous (n=6); GH-replete, growth hormone-replete (n=6); EOGHD, early-onset GHD (n=6); AOGHD, adult-onset GHD (n=4); BW, body weight; HW, heart weight; TL, tibial length; Hct, hematocrit; SERCA2, cardiac sarcoplasmic reticulum Ca 2+ ATPase protein normalized to p-actin (n = 4-5/group); HR, heart rate; LVPmax, maximum left ventricular pressure, LVPmin, minimum left ventricular pressure

^{*} P<0.01 vs. EOGHD, AOGHD

[†] P<0.01 vs. EOGHD

[‡] P<0.05 vs. EOGHD

[§] P<0.05 vs. EOGHD

Table 2

echocardiographic data (Cohort A)

Groups	BW, g	M-mode LVIDd, cm	LVIDs, cm	LV Mass	LV Mass index	RWT	RWT index	FS%
HZ	422 ± 4 [*]	0.704 ± 0.02 [*]	0.278 ± 0.03	0.504 ± 0.07 [*]	1.19 ± 0.16	0.519 ± 0.03	1.23 ± 0.08 [*]	61 ± 3
GH-replete	402 ± 5 ^{*†}	0.682 ± 0.03 [‡]	0.305 ± 0.02	0.489 ± 0.08 [*]	1.21 ± 0.19	0.571 ± 0.05	1.42 ± 0.13 [‡]	55 ± 2
EOGHD	291 ± 8	0.586 ± 0.01	0.254 ± 0.02	0.199 ± 0.02	0.69 ± 0.07	0.604 ± 0.04	2.08 ± 0.14	57 ± 2
AOGHD	329 ± 4	0.641 ± 0.02	0.277 ± 0.02	0.328 ± 0.06	1.09 ± 0.14	0.556 ± 0.03	1.70 ± 0.09	57 ± 2
<i>P value</i>	<0.0001	0.007	--	0.002	0.079	--	0.001	--

	Doppler HR, beats/min	E max, cm/s	Edec time, s	A max, cm/s	E/A	e', cm/s	E/e'
HZ	236 ± 6	67 ± 2	0.053 ± 0.004 [§]	38 ± 1	1.95 ± 0.17	2.5 ± 0.10	26 ± 2 [‡]
GH-replete	240 ± 8	66 ± 3	0.054 ± 0.007 [§]	36 ± 2	1.87 ± 0.10	2.6 ± 0.27	27 ± 2
EOGHD	239 ± 5	67 ± 3	0.067 ± 0.004	33 ± 2	2.10 ± 0.18	1.9 ± 0.14	38 ± 3
AOGHD	221 ± 6	68 ± 3	0.078 ± 0.006	38 ± 3	1.84 ± 0.11	2.1 ± 0.19	34 ± 2
<i>P value</i>	0.142	--	0.015	--	--	0.035	0.006

HZ, heterozygous (n=8); GH-replete growth hormone-replete (n=8); EOGHD, early-onset GHD (n=7); AOGHD, adult-onset GHD (n=8)

LVIDd, left ventricular internal diameter end diastole; LVIDs, left ventricular internal diameter end-systole; LV Mass index, [left ventricular mass/body weight] x 103; RWT, relative wall thickness; RWT index, [RWT/BW] x 103; FS, fractional shortening; HR, heart rate; E max, early transmitral filling velocity; Edec time, early deceleration time; A max, late transmitral filling velocity; e', early mitral annular velocity; E/e', transmitral early filling velocity normalized to early mitral annular velocity

^{*} P<0.01 vs. EOGHD

[†] P<0.01 vs. AOGHD

[‡] P<0.05 vs. EOGHD

[§] P<0.05 vs. AOGHD