Young *Little* Mice Express a Premature Cardiovascular Aging Phenotype

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To investigate the effect of growth hormone and insulin-like growth factor 1 deficiency on the aging mouse arterial system, we compared the hemodynamics in young (4 months) and old (30 months) growth hormone–releasing hormone receptor null dwarf (*Little*) mice and their wild-type littermates. Young *Little* mice had significantly lower peak and mean aortic velocity and significantly higher aortic impedance than young wild-type mice. However, unlike the wild-type mice, there were no significant changes in arterial function with age in the *Little* mice. Aortic pulse wave velocity estimated using characteristic impedance increased with age in the wild-type mice, but it changed minimally in the *Little* mouse. We therefore conclude that arterial function in *Little* mice expresses a premature aging phenotype at young age and may neither enhance nor reduce their longevity.

Key Words: GH/IGF-1 deficiency—Cardiovascular function—Aortic impedance—Pulse wave velocity—Longevity.

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MECHANISMS that contribute to increased longevity in mouse models of dwarfism are of interest to investigators in the field of gerontology. Certain mouse models of dwarfism (such as Snell dwarf and Ames dwarf mice) have significant anterior pituitary dysfunction including loss of thyroid-stimulating hormone (1) and subsequent hypothyroidism (2) in addition to their somatotrophic lesions. The profound cardiovascular effects of hypothyroidism (3) make the assessment of growth hormone (GH) or insulin-like growth factor 1 (IGF-1) effects difficult in the Snell and Ames dwarf mice especially as they age. One dwarf mouse model, known as the “*Little*” mouse generated by a missense mutation in the hypothalamic GH-releasing hormone receptor (4,5), has an anterior pituitary that retains prolactin-producing cells (1) and normal thyroid function (6). Additionally, the *Little* mouse has normal hematopoiesis (7) making it a reasonable model to study GH and IGF-1 effects on aging.

The *Little* mouse (also symbolized as *Ghrhr^{lit/lit}*) has circulating GH levels at 1% of that in normal mice (6,8) and the levels of serum IGF-1 are greatly reduced (10%–20% of normal mice [6,9]). These deficiencies, in part, cause the *Little* mouse to weigh about a third less (6) and live a third longer than its wild-type (WT) littermates (8).

Both GH and IGF-1 play critical parts in the development and regulation of the cardiovascular system (10). The anabolic effects of GH and IGF-1 are well recognized, but the effects of their deficiency on the mouse arterial hemodynamics as the mice age are not well characterized.

In humans, adult onset of low GH/IGF-1 was shown to be associated with increased blood pressure (11), increased vessel intima-media thickness and accelerated atherosclerotic plaque formation in the carotid artery (12,13), reduced aortic distensibility (14), and endothelial dysfunction (15). In stark contrast, with lifelong GH deficiency (GHD), blood pressures were significantly lower (mean systolic $\pm$ SD/diastolic $\pm$ SD: $69 \pm 14/33 \pm 5$) in a group of humans with familial dwarfism thought to resemble the phenotype of the *Little* mice most closely (16). We previously had found that resting cardiac function is diminished in young *Little* mice compared with their WT littermates, but their diminished cardiac function remains relatively unchanged with age (17). This conclusion did not consider the potential alterations of vascular function with aging; therefore, in this study, we examined vascular hemodynamics and its contribution to the overall cardiovascular function of *Little* dwarf mice at young age.

MATERIALS AND METHODS

Studies of arterial hemodynamics were conducted in 23 young mice (4 months old; 8 *Little* and 15 WT mice) and 15 old mice (30 months old; 6 *Little* and 9 WT mice). All mice (*Little-Ghrhr^{lit/lit}* and WT-C57BL/6J) were obtained from a colony maintained by Dr. Gretchen Darlington at Baylor College of Medicine. Mice were housed in the animal facility at the Neurosensory vivarium of Baylor College of Medicine. This facility is approved by American Association

for Accreditation of Laboratory Animal Care. Animals were kept in rooms at controlled temperature (24°C) and lighting (14:10 hour light-dark cycle) with free access to food and water. The diets of both groups of mice consisted of normal chow. All animal protocols were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHHS Publication No. 85-23, Revised 1996).

Measurement of Aortic Blood Pressure and Aortic Blood Flow Velocity

Mice were anesthetized with pentobarbital sodium (40 mg/kg of body weight) administered intraperitoneally. The anesthetized mouse was placed on an ECG/heater board with the board temperature adjusted to maintain mouse body temperature at $37 \pm 1^\circ\text{C}$. The limbs of the mouse were taped to the four electrodes, the quality of ECG assessed, and the electrode contact optimized as needed. Aortic blood pressures were measured in the mice as described previously (17,18). Briefly, blood pressure near the aortic root was measured using a modified RADI catheter (0.36 mm diameter; PressureWire³, RADI Medical System, Uppsala, Sweden) accessed via the right carotid artery of the mouse. The catheter and pressure amplifier were calibrated for each experiment from 0 to 250 mm Hg using a mercury manometer. The ascending aortic blood flow velocity signal was measured with a 20-MHz Doppler probe placed slightly to the right of the suprasternal notch and oriented toward the heart and aortic root (Figure 1). The probe was positioned such that the angle between the sound beam and the ascending aorta was close to zero, and the range gate depth was adjusted between 3 and 4 mm to obtain maximal

velocity. Care was taken to ensure that the sample volume was placed very close to the location of the pressure sensor such that the foot of the velocity waveform was aligned with the foot of the pressure waveform to avoid potential errors in phase relation between pressure and velocity signals (19) when calculating aortic impedance parameters (pressure–velocity relationship). The Doppler audio signals, the pressure, and the ECG signals were recorded on a computer-based real-time Doppler spectrum analyzer (DSPW, Indus Instruments, Houston, TX). Typically, 2–3 seconds of raw data were acquired and stored for analysis offline. Aortic velocity waveforms and aortic pressure waveforms were extracted and processed to calculate peak and mean velocities; systolic, diastolic, mean, and pulse pressures; and impedance (Z) parameters (peripheral vascular resistance [Z_0], characteristic impedance [Z_c], and impedance at first harmonic [Z_1] as described previously) (18,20).

Determination of Pulse Wave Velocity

Pulse wave velocity (PWV) was determined as described elsewhere (18,21). Briefly, flow velocity signals were measured in anesthetized mice along with ECG signals. The 20-MHz Doppler probe was placed in the second intercostal space to the right of the sternum and positioned to record velocity in the aortic arch. The probe was then moved to another site about 35–40 mm distally on the abdomen. At this site, the Doppler probe was positioned to measure abdominal aortic velocity. The separation distance between the measurement sites was measured as distance between sites on the surface of the mouse. Aortic PWV was calculated by dividing the separation distance by the pulse transit time (difference in arrival times of the velocity pulse timed with respect to ECG). This is the foot-to-foot (at end-diastolic

Figure 1. Experimental setup to measure aortic blood flow velocity, aortic blood pressure, and ECG in mice.

pressure) method of estimation of PWV_{DP} . PWV was also estimated using the characteristic impedance (at mean pressure) as $PWV_{MP} = Z_C/\rho$, where ρ is the density of blood.

Data Analysis

All the parameters are presented in the form of mean $\pm$ SE and comparisons were made using Student's *t* test at a significance level of .05. Comparisons within the group were made using paired Student's *t* tests.

RESULTS

Both young and old *Little* mice weighed significantly lower than their respective *WT* littermates. Aging from 4 to 30 months increased body weight similarly in both groups, by 24% in the *WT* mice and by 34% in the *Little* mice. (young *WT*: 25.3 ± 0.8 g vs old *WT*: 31.3 ± 1.0 g, $p < .0001$ and young *Little*: 14.0 ± 0.3 g vs old *Little*: 18.7 ± 1.4 g, $p < .0001$).

The aortic pressure and aortic velocity data are summarized in Table 1. No significant differences were observed between the heart rates of the four groups. Peak and mean aortic velocities trended lower with age in the *WT* mice. In the *Little* mice, peak and mean aortic velocities did not change with age, but they were significantly lower than their respective *WT* littermates. Systolic and mean pressures trended higher with age in the *WT* mice. Diastolic pressure trended lower with age in the *Little* mice. We confirmed that PWV_{DP} (PWV calculated by foot-to-foot method at end-diastolic pressure using flow velocity waveforms) was significantly higher in the old *WT* mice than that in the young *WT* mice. Surprisingly, the PWV_{DP} in the young *Little* mice was higher than that in the old *Little* mice.

The impedance data (expressed in $\text{dyn}\cdot\text{s}/\text{cm}^3$) are shown in Figure 2. Comparing young *WT* mice with old *WT* mice, impedance parameters of Z_0 (9160 ± 690 vs 11328 ± 574 , $p < .01$), Z_1 (636 ± 29 vs 884 ± 100 , $p < .05$), and Z_C (348 ± 18 vs 541 ± 48 , $p < .01$) were significantly higher with age in the *WT* mice. The peripheral resistance in the young *Little* mice compared with the old *Little* mice tended to be greater in the young mice, Z_0 (12968 ± 1322 vs 11353 ± 930 , $p = \text{NS}$), whereas the pulsatile elements were unchanged with age, Z_1 (902 ± 99 vs 983 ± 75 , $p = \text{NS}$) and Z_C (474 ± 56 vs 524 ± 49 , $p = \text{NS}$). There were no significant functional changes with age in the *Little* mice.

From the impedance data, we calculated the average PWV (PWV_{MP}), reflecting the pulsatile load over the entire cardiac cycle or mean pressure, using $PWV = Z_C/\rho$ ($\rho = 1.06 \text{ g}/\text{cm}^3$ —blood density). The PWV_{MP} in the young *WT* mice was significantly less than that in the old *WT* mice (Table 1). In contrast, there was essentially no change with age in the *Little* mice. $PWVs$ obtained by both methods and the relevant pressures are shown in Figure 3A and the squared $PWVs$ (because PWV^2 is directly proportional to pressure) obtained by both methods are shown in Figure 3B. In these figures, PWV_{DP} is plotted versus diastolic pressure and PWV_{MP} is plotted versus mean pressure. The slopes of the lines connecting PWV_{DP} and PWV_{MP} (calculated as slope = $[PWV_{MP} - PWV_{DP}]/[P_{\text{mean}} - P_{\text{dia}}]$) are chords from the (PWV^2 vs pressure or PWV vs pressure) curvilinear relationship(s). In the young *WT* mice, the slope ($0.3 \pm 2.3 \text{ cm}/(\text{mm Hg}\cdot\text{s})$) was very flat. In contrast, slopes were much greater in the old *WT* mice ($11.0 \pm 3.6 \text{ cm}/(\text{mm Hg}\cdot\text{s})$). In the young *Little* ($6.8 \pm 5.1 \text{ cm}/(\text{mm Hg}\cdot\text{s})$) the slope tended to be lower than that in the old *Little* ($15.7 \pm 4.6 \text{ cm}/(\text{mm Hg}\cdot\text{s})$) mice. The pattern of

Table 1. Baseline Parameters of Aortic Blood Flow Velocity and Aortic Blood Pressure Waveforms, Foot-to-Foot Pulse Wave Velocity, and Characteristic Impedance Pulse Wave Velocity in Young and Old, Wild-Type (*WT*) and *Little* Mice

Parameters	WT Mice		Little Mice	
	Young (15)	Old (9)	Young (8)	Old (6)
Heart rate (beat/min)	401 ± 14	440 ± 17	395 ± 30	429 ± 30
Aortic flow velocity (cm/s)				
Peak aortic velocity	91.9 ± 3.4	86.0 ± 3.0	$70.1 \pm 1.1^*$	$71.5 \pm 2.9^†$
Mean aortic velocity	22.3 ± 1.2	20.1 ± 0.7	$15.0 \pm 0.8^*$	$14.1 \pm 0.4^†$
Aortic pressure (mm Hg)				
Systolic pressure	90.2 ± 3.8	95.7 ± 6.6	87.5 ± 5.3	$76.4 \pm 4.8^‡$
Diastolic pressure	65.6 ± 3.5	66.6 ± 6.6	63.2 ± 5.2	51.3 ± 4.7
Mean pressure	77.7 ± 3.7	79.7 ± 6.3	74.1 ± 5.2	$62.3 \pm 4.6^‡$
Pulse pressure	24.6 ± 1.9	29.1 ± 3.7	24.3 ± 1.5	25.1 ± 1.9
Aortic PWV (cm/s)				
PWV_{DP}	321 ± 15	$373 \pm 14^§$	361 ± 10	307 ± 26
PWV_{MP}	328 ± 17	$510 \pm 45^§$	$447 \pm 53^*$	494 ± 46

Notes: PWV_{DP} = pulse wave velocity at end-diastolic pressure; PWV_{MP} = pulse wave velocity at mean pressure.

All values are presented as means $\pm$ SE.

* $p < .01$ —young *Little* vs young *WT* mice.

† $p < .01$ —old *Little* vs old *WT* mice.

‡ $p < .05$ —old *Little* vs old *WT* mice.

§ $p < .05$ —old *WT* vs young *WT* mice.

slopes of *squared* PWVs in Figure 3B is similar to that of PWVs in Figure 3A.

DISCUSSION

The *Little* mouse lives 30% longer than its WT littermates and has preserved anterior pituitary function (normal thyroid regulation and prolactin-producing cells [1,6,8,9]). In the Snell or Ames dwarf mice, very low thyroid hormone levels are part of the phenotype and lead to altered cardiac function, including changes in heart rate (2). Hence,

alterations in cardiovascular function in the aging Snell or Ames dwarf mice may or may not reflect changes from the GH/IGF-1 axis. In the *Little* mouse, other endocrine axes are minimally affected (6,8,9) and therefore allows us to study the effects of low GH/IGF-1 as the animals age. Additionally, the *Little* mouse may be clinically relevant, due to the resemblance of its phenotype to the human homologue (humans with familial dwarfism) described by Maheshwari and colleagues (16). We hypothesized that vascular function in the *Little* mice with lifelong absence of somatotrophic pathways might be stable and not deteriorate

Figure 2. Plot of impedance (Z) parameters, peripheral vascular resistance (Z_0), impedance at first harmonic (Z_1), and characteristic impedance (Z_C). Impedance values presented as mean $\pm$ SE are expressed in dyn·s/cm² (1333.3 dyn/cm² = 1 mm Hg). ‡ indicates $p < .05$ and † indicates $p < .01$ old wild type (WT) versus young WT; § indicates $p < .05$; and # indicates $p < .01$ young *Little* versus young WT mice.

Figure 3. Plot of (A) pulse wave velocity (PWV) and (B) PWV² determined by using foot-to-foot method (●) plotted against respective diastolic pressures, and using characteristic impedance (■) plotted against respective mean pressures. Except in the young wild-type (WT) mice, PWVs are higher when measured using Z_C , which includes PWVs from all pressure levels in the cardiac cycle. The slopes of lines drawn between PWV_{DP} and PWV_{MP} for each group shows that old *Little* and old WT have high slopes and are similar. The slope in young *Little* mice is lower but not different from any of the older groups. YL = young *Little*; OL = old *Little*; YW = young WT; and OW = old WT.

with age. We examined the vascular hemodynamics in these mice through the simultaneous measurement of aortic blood flow velocity, aortic blood pressure, the pressure–velocity relationship, and PWV to evaluate arterial function compared with their *WT* littermates at adulthood and in old age.

Body Weight

Significant lower body size and weight in animals and humans are the hallmark of GHD. The young and old *Little* mice in this study had significantly decreased body weights (45% and 40% lower, respectively) compared with their respective age-matched *WT* littermates. This is in agreement with the reduction in body weight reported in animals (6,22–25) and in humans (26,27), specifically in humans with familial dwarfism whose body weights were significantly lower than normal participants (16). However, the relative increase in body weight with age in the *Little* mice is similar to their *WT* counterparts much like the age-associated weight gain in humans (28). Although lower than normal body weight, resulting from either IGF-1 deficiency or calorie-restricted diet, leads to longevity in mice (22,29), longevity is not always associated with low body weight or GHD. Thus, for age-associated weight gain, there did not appear to be an effect on the age-related process.

Aortic Blood Pressure

Alterations in blood pressure are observed in animal models and humans deficient in GH/IGF-1. Blood pressure changes in GH-deficient humans seem to depend on the timing of the loss of GH/IGF-1. High blood pressure was reported in normal participants with low IGF-1 levels (10) and in hypopituitary patients with GHD (11), and normal blood pressure was reported in adult GH-deficient patients (10,30). Lower than normal blood pressure was reported in patients with Laron syndrome (31) and in patients with childhood onset of GHD (32). Blood pressure in humans with familial dwarfism (human homologue of *Little* mice) of relatively young age (7–28 years) was significantly lower than that in normal participants (16). Egecioglu and colleagues (33) reported low blood pressures in young GH receptor-deficient mice compared with *WT* mice. A lower than normal blood pressure was reported in low GH adult *rdw* rats (24) and GH-deficient Lewis rats (34), whereas another study reported that blood pressure in young GH-deficient dwarf rats was similar to normal F344 rats (35). Unfortunately, with all these data from young participants, it is unclear what happens to blood pressure as these participants (animals or humans) age.

We found that blood pressure in the young *Little* mice was similar to that of their young *WT* littermates under anesthesia. However, with aging, there was a trend toward increasing systolic and pulse pressure in the *WT* mice due to progressive stiffening of the aorta observed with aging (18).

This may be caused by an age-related increase in inflammation, tissue calcification, or loss of nitric oxide (36). In contrast, the old *Little* mice showed evidence of decreasing blood pressure with aging compared with the young *Little* mice and had significantly lower blood pressures than the old *WT* mice. Lower arterial systolic blood pressure in GH receptor knockout mice was attributed to lower contractile force caused by reduced left ventricular systolic function (33). We previously reported reduced left ventricular systolic function in the young *Little* mice, which did not change with age (17). However, aortic pressure in the young *Little* mice was similar to that in the *WT* mice, and it decreased with age. The drop in aortic pressure in the old *Little* mice may be explained by the mechanism observed by Mitchell and colleagues (37) who reported that as age-related aortic stiffness increases in humans, the impedance mismatch at the periphery decreases. Age-related aortic stiffness causes pressure pulse to travel faster to the periphery and is partially reflected due to impedance mismatch, which occurs at about 60 years of age, diminishing the strength of wave reflections (37). Several issues such as the age at which aortic stiffness and high blood pressure occurs and when and for how long impedance begins to decrease in the *Little* mice need further investigation.

Pressure–Velocity Relationship and Aortic Impedance

Aortic impedance, defined as the ratio of pressure to velocity, provides a more complete description of the afterload experienced by the left ventricle by describing the pulsatile and the steady components of the hydraulic load compared with when pressure and velocity are considered individually (18,20,38). We used velocity instead of volume flow because both velocity and pressure are independent of body weight or size (18). We found that the young *Little* mice had higher peripheral vascular resistance (Z_0) than the young *WT* mice (Figure 2). This is similar to increase in peripheral vascular resistance reported in young mutant dwarf rats (39) and in humans with childhood-onset GHD (15). Increased peripheral vascular resistance in the *Little* mice may be due to decreased levels of peripheral nitric oxide caused by low IGF-1 levels (12,15,40) leading to accelerated aging. Thus, it is not surprising to find that peripheral resistance vessels that contain more smooth muscle may vasoconstrict due to less nitric oxide and increase peripheral vascular resistance in the *Little* mice just as in the mutant dwarf rat (39). Our data showed that there are no further increases in peripheral vascular resistance with age. Increased peripheral vascular resistance increases the load on the heart, but at young age, the heart of the *Little* mouse may adapt better and hypertrophy more physiologically initially and without further changes with age may keep the left ventricular systolic function stable. With age, we observed no significant changes either in peripheral resistance or in the reported left ventricular systolic function of *Little* mice.

In our previous studies (18), *WT* mice experienced about 20% increases in Z_0 , which is in agreement with our data in this study. In contrast, Z_0 was about 14% lower in the old *Little* mice compared with the young *Little* mice, which was due to the drop in the mean pressure.

Impedance at the first harmonic (Z_1) usually correlates with strength of the wave reflections from the periphery. The young *Little* mice had significantly elevated Z_1 compared with the young *WT* mice (Figure 2) indicating strong reflections due to high peripheral vascular resistance. With age, Z_1 increased significantly (20%) in the *WT* mice. In contrast, Z_1 remained relatively unchanged with age in the old *Little* mice indicating no additional increase in wave reflections.

Characteristic impedance (Z_c) represents the pulsatile component of the aortic pressure-flow relationship and is determined by local aortic wall stiffness and diameter. In young mice, Z_c is significantly higher in the *Little* mice than in the *WT* mice (Figure 2) indicating a stiffer aorta as it accepts blood throughout the cardiac cycle. With age, Z_c increased significantly (55%) in the *WT* mice but increased only by 10% in the *Little* mice. Critically, although Z_c is high in the young *Little* mice, it is unchanged with age and there was no evidence of enhanced aortic compliance in the old *Little* mice. This lack of change in Z_c with age in the *Little* mice suggests that there is no age-related decrement in the distensibility of the large conduit in the *Little* mice. The increase in stiffness at early age in the *Little* mice may be due to accelerated aging phenotype (40) but less drastic changes with age may suggest the role of anti-inflammatory, antioxidative, and cellular stress resistance mechanisms that are observed in GH/IGF-1-deficient animals (40–43). The *Little* mice had a diminished cardiovascular function at young age, and the minimal change in afterload with age has perhaps allowed for steady age-related cardiovascular function as previously reported by us (17). Although we do not believe that the diminished, but stable, cardiovascular function contributes to longevity in the *Little* mice, it is not severe enough to decrease their life span in laboratory conditions.

Aortic PWV Measurement—Importance of Evaluation Technique

PWV is a commonly utilized clinical index of arterial stiffness and can be readily calculated noninvasively using the foot-to-foot method (21) or using invasively determined characteristic impedance (18). Because PWV is related to stiffness by $PWV^2 = (V/\rho)dP/dV$, where ρ is the density of blood and dP/dV (P = pressure and V = volume) is stiffness, it is higher at systolic than at diastolic pressure in normal vessels and the relationship is curvilinear. In stiffer vessels, the increase in PWV is greater at a given systolic pressure than at diastolic pressure. Thus, PWV calculated from the difference in arrival times of the upstrokes (foot-to-foot) of

pressure or velocity waveforms at two arterial sites is measured at diastolic pressure, where both its magnitude and the changes with increasing stiffness are minimized.

PWV estimated from impedance (PWV_{MP}) represents the PWV averaged over the entire cardiac cycle, and more likely reflects the aortic stiffness experienced by the left ventricle while it performs work. In the young *WT* mice, PWV determined by the foot-to-foot method (PWV_{DP}) is not much different from the PWV_{MP} as the aorta remains compliant and distensible over that pressure range. In the young and old *Little* mice and the old *WT* mice, PWV_{DP} is significantly lower than PWV_{MP} (Figure 3A) or with PWV^2 (Figure 3B) because in the physiologic state distending pressure modifies the aortic stiffness. Critically, PWV_{DP} in the old *Little* mice tended to be lower than that in the young *Little* mice suggesting that aorta has increased distensibility with aging. However, the diastolic blood pressure in the old *Little* mice is lower than that in the young *Little* mice and at comparable average distending pressures (mean pressure for old *Little* and diastolic pressure for young *Little*, shown with the dotted ellipse in Figure 3), an age-related increase in stiffness is seen. Additionally, PWV_{MP} is higher in the old *Little* mice compared with the young *Little* mice and this is because Z_c accounts for PWVs at all pressure levels within the cardiac cycle. These findings show that PWV_{MP} provides more representative assessment of aortic stiffness experienced by the heart and how PWV measured by the foot-to-foot method may be difficult to interpret if diastolic blood pressures are not similar.

The lines drawn between the PWV_{DP} and PWV_{MP} in Figure 3 represent chords from the curvilinear pressure–stiffness relationship. The slopes of these lines are higher and similar in the young and old *Little* and the old *WT* mice compared with the young *WT* mice indicating that all these mice have stiffer aortas than the young *WT* mice. The pulse wave velocities of the *WT* mice measured using foot-to-foot method in our study are similar to the values in mice previously reported by us (18,21) and to the PWV in young humans (38,44) and other animals (45,46). The results indicate that the aorta of the *Little* mouse gets stiffer at young age and remains in that state throughout its life, and may neither enhance nor reduce longevity in these mice.

Antioxidant Defense Mechanisms

One of the basic mechanisms of aging is the accumulation of oxidative damage resulting in increased risk of cancer among other factors and decline in immune system function (36,43). Thus, reduction in oxidative damage may lead to increased life span. There is general consensus that congenital GH/IGF-1 deficiency, rather than postnatal declines, is associated with reduced inflammation and extended life span (36,40,42,43). The GH/IGF-1-deficient *Little* mice may have increased stress resistance indices as observed through the upregulation of several xenobiotic detoxification

genes, which are associated with potent resistance of various xenobiotics and could have delayed their aging through the reduction in the cumulative effect of tissue oxidative damage (41,47). But, this may not necessarily mean that vascular tissues are protected in the *Little* mice.

Labinskyy and colleagues (48) reported that the long-lived white-footed mouse has increased resistance to pro-oxidant and proinflammatory effects of metabolic stress and lower cellular reactive oxygen species generation resulting in extended life span. The age-dependent decline in aortic relaxation was much slower in the white-footed mouse suggesting a gradual cardiovascular aging (48). However, this does not seem to be the case in the *Little* mice, which had elevated PWV and characteristic impedance at young age indicating that the aorta may have become stiffer at young age but may remain at a steady state for the rest of its life span under relatively stress-free life in laboratory environments. This observation is similar to that in the long-lived naked mole rat, which acquires and tolerates more oxidative damage at 2 years (young) age, but this level of damage is maintained steadily over the next 20 years of the life indicating lack of additional age-related increase in oxidative damage (49). Whether similar mechanisms may be responsible for the maintenance of diminished cardiovascular function at steady state in the *Little* mice needs to be further investigated.

Limitations of the Study

It has been reported that IGF-1 deficiency causes endothelial oxidative stress adversely affecting bioavailability of nitric oxide and endothelium-dependent vasorelaxation (42) resulting in adverse cardiovascular events (40). Our studies have shown that the *Little* mice have a diminished cardiovascular function at young age but remain steady at this diminished level throughout their life span. We do not believe that vascular function promotes longevity in these mice. A main limitation of this study is that we have not done any histological or molecular analysis to determine the mechanistic effects of GH/IGF-1 deficiency on the arterial system of the *Little* mice at young or old ages to conclude the status of the endothelium-related changes in the vascular tissues.

Conclusions

Studies involving GH/IGF-1-deficient dwarf animals reflect the critical developmental impact of these hormones showing diminished arterial function at young age. However, to assess the effects of GH/IGF-1 deficiency on aging, old dwarf animals need to be compared with young dwarf animals. We studied the effect of age on arterial hemodynamics in the *Little* (dwarf) mice and their *WT* littermates. The young *Little* mice indeed had significantly diminished cardiovascular function (lower peak and mean

velocities and significantly higher Z_0 , Z_1 , Z_C , PWV) compared with the young *WT* littermates but remained stable at the initial diminished level with age in the *Little* mice. Similarly, aortic stiffness is increased in the young *Little* mice and the stiffness level remains steady with age. In general, young mice may be able to adapt better to changes in youth and maintain them at steady state with age, but old mice may not be able to adapt to those changes when they occur later. *Little* mice, which live much longer than their *WT* counterparts perhaps due to antioxidant defense mechanisms affording them protection from cancer, have a diminished arterial function over their life span. We also demonstrated that PWV estimated from characteristic impedance Z_C was significantly higher than PWV estimated using the foot-to-foot method. Focusing only on foot-to-foot PWV could lead to misinterpretation of aging changes or other processes, where blood pressure affects vessel stiffness.

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