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Growth Hormone and IGF-1 Deficiency Exacerbate High-Fat Diet–Induced Endothelial Impairment in Obese Lewis Dwarf Rats: Implications for Vascular Aging

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Previous studies suggest that the age-related decline in circulating growth hormone (GH) and insulin-like growth factor-1 (IGF-1) levels significantly contribute to vascular dysfunction in aging by impairing cellular oxidative stress resistance pathways. Obesity in elderly individuals is increasing at alarming rates, and there is evidence suggesting that elderly individuals are more vulnerable to the deleterious cardiovascular effects of obesity than younger individuals. However, the specific mechanisms through which aging, GH/IGF-1 deficiency, and obesity interact to promote the development of cardiovascular disease remain unclear. To test the hypothesis that low circulating GH/IGF-1 levels exacerbate the prooxidant and proinflammatory vascular effects of obesity, GH/IGF-1–deficient Lewis dwarf rats and heterozygous control rats were fed either a standard diet or a high-fat diet (HFD) for 7 months. Feeding an HFD resulted in similar relative weight gains and increases in body fat content in Lewis dwarf rats and control rats. HFD-fed Lewis dwarf rats exhibited a relative increase in blood glucose levels, lower insulin, and impaired glucose tolerance as compared with HFD-fed control rats. Analysis of serum cytokine expression signatures indicated that chronic GH/IGF-1 deficiency exacerbates HFD-induced inflammation. GH/IGF-1 deficiency also exacerbated HFD-induced endothelial dysfunction, oxidative stress, and expression of inflammatory markers (tumor necrosis factor-α, ICAM-1) in aortas of Lewis dwarf rats. Overall, our results are consistent with the available clinical and experimental evidence suggesting that GH/IGF-1 deficiency renders the cardiovascular system more vulnerable to the deleterious effects of obesity.

Key Words: Accelerated aging—Endothelial dysfunction—IGF-1—Obesity—Vascular pathophysiology.

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OBESITY is increasing at alarming rates worldwide, and there is overwhelming evidence indicating that increased body weight is associated with accelerated atherosclerosis and increased rates of cardiovascular mortality (1). Epidemiological studies provide strong evidence that aging exacerbates the deleterious cardiovascular effects of obesity (2,3). Currently, 37.4% of adults aged 65 years and older are obese, yet there are only a few studies addressing the specific mechanisms through which aging and obesity interact to promote the development of vascular pathologies (including myocardial infarction, stroke, vascular dementia; for a recent review, see [4]). Previous studies in laboratory rodents and nonhuman primates provide evidence that increasing age renders the vasculature more prone to oxidative insult elicited by obesity and related metabolic conditions (including diabetes mellitus and the metabolic syndrome) likely by impairing cellular oxidative stress resistance (5–7). However, the mechanisms by which aging impairs vascular resistance to metabolic stress associated with obesity are not well understood.

In recent years, considerable evidence has accumulated that in addition to cell-autonomous changes in the gene expression signature in vascular cells, non-cell autonomous endocrine mechanisms also have an important role in age-related vascular impairment. In particular, circulating levels of growth hormone (GH) and, consequently, hepatic production of insulin-like growth factor-1 (IGF-1) significantly decline with age both in humans and laboratory animals (8–10), and the available evidence suggests that reduced GH and IGF-1 levels are causally linked to vascular impairment in aging (11–13). There is strong clinical and experimental evidence that GH and IGF-1 exert beneficial effects on cardiovascular function and cardiovascular mortality

(12,14–22) and are atheroprotective (23,24) by regulating pathways involved in prevention of macromolecular damage. Importantly, previous studies in IGF-1–deficient Lewis dwarf rats (25) and Ames dwarf mice (26) demonstrated that low circulating IGF-1 levels are associated with impaired expression/activity of antioxidant enzymes in the vasculature, leading to increased vascular oxidative stress and endothelial dysfunction (26). Furthermore, our recent studies show that in mice, adult-onset endocrine IGF-1 deficiency (induced by adeno-associated viral knockdown of IGF-1, specifically in the liver of postpubertal mice using Cre-lox technology [27,28]) impairs the ability of vascular cells to mount an effective NF-E2-related factor 2 (Nrf2)-dependent antioxidant defense in response to oxidative stressors administered ex vivo (27). We demonstrated that the impairment of cellular oxidative stress resistance induced by IGF-1 deficiency renders cultured aorta segments vulnerable to the metabolic stress of high glucose treatment in vitro, resulting in an exacerbation of hyperglycemia-induced endothelial dysfunction, oxidative stress, and an increased rate of endothelial apoptosis (27).

The present study used Lewis dwarf rats as model system to test the hypothesis that low circulating IGF-1 levels impair vascular resistance to metabolic stress in vivo. The advantages of this experimental design are that the decline in plasma levels of GH and IGF-1 in Lewis dwarf rats mimic the endocrine changes associated with aging (25,29–31), and consumption of a high-fat diet (HFD) is known to result in endothelial dysfunction, vascular oxidative stress, and inflammation in young rodents (32–34), which is exacerbated in aged rodents (Z. Ungvari, M.D., Ph.D., A. Csiszar, M.D., Ph.D, manuscript in preparation, 2011). Using isolated aorta preparations, we assessed whether GH/IGF-1 deficiency in Lewis dwarf rats exacerbates endothelial dysfunction, oxidative stress, and vascular inflammation induced by consumption of a HFD.

Methods

Animals

In the present study, we used male Lewis rats that are heterozygous or homozygous for the spontaneous autosomal recessive *dw-4* mutation that results in a decrease in GH secretion from the pituitary gland and consequential low circulating IGF-1 levels (35–37). Because Lewis dwarf (*dw-4/dw-4*) rats exhibit chronically low levels of GH and IGF-1, which mimic age-related endocrine alterations, they are often used as models of accelerated vascular aging (25,36). To generate the cohort used in the present study, female heterozygous (d*w-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") 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. At 6 weeks of age, dwarf and control animals were divided into four groups and placed on either a standard diet (SD) or HFD. The four groups were (a) control animals fed an SD, (b) control animals fed a HFD, (c) dwarf animals fed an SD diet, and (d) dwarf animals fed a HFD. The high-fat chow, commonly used to induce obesity, delivers 60% kcal from fat, whereas the SD provides only 10% kcal from fat (D12492, D12450B, respectively; Research Diets Inc., New Brunswick, NJ). The animals continued on the specified diets (with water and food ad libitum) for 7 months. Animals were housed in pairs in the Rodent Barrier Facility at University of Oklahoma Health Sciences Center on a 12-hour light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee of University of Oklahoma Health Sciences Center.

Serum Biochemical Profile and Analysis of Circulating Levels of Metabolic Hormones and Inflammatory Cytokines

Whole blood was collected at sacrifice and was centrifuged at 2,500*g* for 20 minutes at 4°C; serum was collected, aliquoted, and stored at −80°C. Total IGF-1 levels (nanograms per milliliter) in serum were determined by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) as previously described (30,36,38,39). The serum biochemical profile was assessed by Vance Veterinary Laboratories (Oklahoma City, OK). Circulating levels of metabolic hormones (insulin, adiponectin, resistin, leptin), cytokines, chemokines, and other inflammatory markers relevant for aging research (40,41) (including tumor necrosis factor-alpha [TNF-α], interleukin [IL]-6, IL-7, IL-1α, leukemia inhibitory factor, IL-1β, interferon gamma—induced protein-10 [IP-10], IL-18, eotaxin, interferon gamma, IL-2, IL-17A, IL-3, monocyte chemotactic protein 3 [MCP-3], lymphotactin, vascular endothelial growth factor A [VEGF-A], monocyte chemotactic protein-1 [MCP-1], macrophage inflammatory protein-1 beta [MIP-1β], C-reactive protein, macrophage colony–stimulating factor-1 [M-CSF-1], IL-5, IL-11, oncostatin-M (OSM), macrophage inflammatory protein-2 [MIP-2], monocyte chemotactic protein-5 (MCP-5), fibroblast growth factor (FGF-basic), macrophage inflammatory protein-1 alpha [MIP-1α], macrophage inflammatory protein-3 beta [MIP-3β], macrophage inflammatory protein-1 gamma [MIP-1γ], granulocyte chemotactic protein-2 [GCP-2], stem cell factor, IL-4, RANTES, haptoglobin, IL-10, macrophage-derived chemokine [MDC]) were analyzed using a multiplex protein array system (Rules Based Medicine, Austin, TX) according to the manufacturer's protocol.

Weight Gain and Body Composition

Body mass of each animal was recorded every 4 weeks. Body fat content was determined using magnetic resonance technology (MiniSpec LF90; Bruker Instruments, Billerica, MA) and reported as a percentage of total body mass.

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

Messenger RNA Targets	Description	Sense	Antisense
Nox1	NADPH oxidase homolog 1	TGAATCTTGCTGGTTGACACTTGC	GAGGGACAGGTGGGAGGGAAG
TNF-α	Tumor necrosis factor-alpha	AACCACCAAGCAGAGGAG	CTTGATGGCGGAGAGGAG.
IL-6	Interleukin-6	CTTCCAGCCAGTTGCCTTCTTG	TGGTCTGTTGTGGGTGGTATCC
ICAM-1	Intercellular adhesion molecule-1	CACAGCCTGGAGTCTC	CCCTTCTAAGTGGTTGGAA
HPRT	Hypoxanthine phosphoribosyltransferase 1	AAGACAGCGGCAAGTTGAATC	AAGGGACGCAGCAACAGAC
ActB	Beta-actin	GAAGTGTGACGTTGACAT	ACATCTGCTGGAAGGTG
B2m	Beta-2-microglobin	ATTCACACCCACCGAGAC	GGATCTGGAGTTAAACTGGTC
Ywhaz	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide 1	AACTGCCTACATATTGGT	CACACAGACTACACTCAT

Oral Glucose Tolerance Test

An oral glucose tolerance test was performed after fasting rats for 18 hours. d-Glucose (25% solution in water) was administered orally at a dosage of 1 g/kg body weight. Blood glucose was measured immediately prior to gavage of the solution and every 30 minutes thereafter up to 2 hours. Tail vein blood samples were taken using a sterile lancet (Medipoint, Mineola, NY), and glucose was measured with a OneTouch UltraMini glucose meter (LifeScan, Milpitas, CA).

Vessel Isolation and Functional Studies

The animals were fasted overnight and euthanized by decapitation 7 months after starting the HFD or SD. The aortas were isolated, cleaned, and sectioned. Then endothelial endothelial function was assessed by measuring relaxation of the aortic rings in response to acetylcholine and the calcium ionophore A23187, as previously described (25,27,33). Endothelium-independent vasorelaxation was assessed using *S*-nitroso-*N*-acetylpenicillamine (SNAP), a nitric oxide donor. In brief, an aorta ring segment (2 mm in length) was isolated from each animal and mounted on 40-μm stainless steel wires in myograph chambers (Danish Myo Technology A/S, Inc., Denmark) for measurement of isometric tension. The vessels were superfused with Krebs buffer solution (118 mM NaCl, 4.7 mM KCl, 1.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.1 mM $MgSO_4$, 1.2 mM KH_2PO_4, and 5.6 mM glucose; at 37°C; gassed with 95% air and 5% CO_2). Optimal passive tension (as determined from the vascular length–tension relationship) was applied for 1 hour (equilibration period) and then relaxation of precontracted (by 10^{-6} mol/L phenylephrine) vessels to acetylcholine (from 10^{-9} to 10^{-6} mol/L), A23187 (from 10^{-9} to 10^{-7} mol/L), or *S*-nitroso-*N*-acetylpenicillamine (from 10^{-9} to 10^{-6} mol/L) was obtained.

Measurement of Vascular $O_2^{\bullet-}$ Production

$O_2^{\bullet-}$ content in the aorta was determined using dihydroethidium (DHE), an oxidative fluorescent dye, as previously reported (42,43). Freshly harvested vessels were incubated with DHE (3×10^{-6} mol/L; at 37°C for 30 minutes). After three washes with phosphate-buffered saline, the vessels were embedded in OCT medium and cryosectioned. Images of $O_2^{\bullet-}$-specific red fluorescence were captured at 20× magnification and analyzed using Metamorph software, as previously reported (26,27). Three 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.

Quantitative Real-Time Reverse Transcription–Polymerase Chain Reaction

Quantitative real-time reverse transcription–polymerase chain reaction was used to analyze messenger RNA expression of TNF-α, IL-6, ICAM-1, and Nox1 in the aorta as previously reported (44–47). Total RNA was isolated using a Mini RNA Isolation Kit (Zymo Research, Orange, CA). The messenger RNA was then reverse transcribed using Superscript III RT (Invitrogen), and expression was analyzed using a Strategen MX3000 machine (44,48). To determine the primer efficiencies, a dilution series of a standard vascular sample was quantified for each plate. Quantification was performed using an efficiency-corrected ΔΔCq method. The reference genes Hprt, YWHAZ,B2M, and β-actin levels were quantified, and a normalization factor was calculated based on the geometric mean for internal normalization. The oligonucleotide sequences for quantitative real-time reverse transcription–polymerase chain reaction are listed in Table 1. Fidelity of the polymerase chain 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 one-way analysis of variance or by two-way analysis of variance followed by the Tukey post hoc test, as appropriate (49–58). A $p < .05$ was considered statistically significant. Data are expressed as means ± *SEM*.

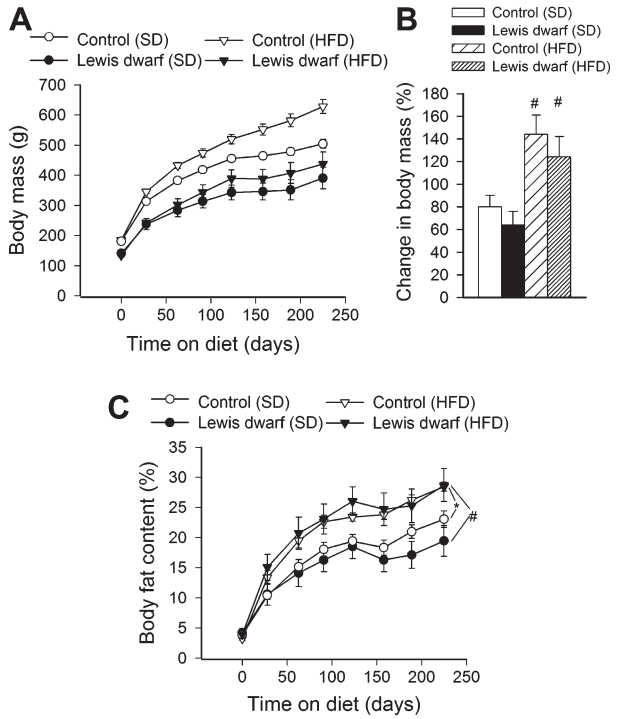

Figure 1. (**A**) Changes in body mass of Lewis dwarf rats and control rats fed a high-fat diet (HFD) or standard diet (SD). Each time point represents the average body mass of each group. (**B**) Shows percentage change in body mass. Data are means ± *SEM* (n = 5–7); #$p < .05$ versus SD. (**C**) Body composition expressed as percent fat mass in Lewis dwarf rats and control rats fed a HFD or SD. HFD-fed rats of each genotype had significantly more body fat than those fed an SD. Data are means ± *SEM* (n = 5–7). *$p < .05$ versus control (SD); #$p < .05$ versus Lewis dwarf (SD).

Results

Effects of Chronic HFD and GH/IGF-1 Deficiency on Body Mass and Body Composition

At the end of the experimental period, both dwarf rats and control rats consuming a HFD showed significantly increased body mass as compared with SD-fed animals (Figure 1A and B). The relative increase in body mass (calculated as a percentage of body mass at the beginning of treatment) of HFD-fed controls and Lewis dwarf rats did not differ significantly (Figure 1B). Feeding a HFD led to a similar increase in relative body fat content in control rats and Lewis dwarf rats (Figure 1C).

Effects of Chronic HFD and GH/IGF-1 Deficiency on Blood Glucose Levels, Glucose Tolerance, and Circulating Levels of Metabolic Hormones

Fasting blood glucose levels were significantly lower in SD-fed Lewis dwarf rats than in SD-fed control rats (Figure 2A). Feeding a HFD did not change blood glucose levels in control rats, whereas HFD resulted in a significant increase in fasting blood glucose levels in Lewis dwarf rats (Figure 2A).

To determine the effect of HFD on glucose tolerance, an oral glucose tolerance test was performed (Figure 2A). During the oral glucose tolerance test, there was a significant increase in plasma glucose levels in each group. Although control rats fed a HFD administration of glucose tended to lead to a more rapid increase of blood glucose levels and a higher peak value than in SD-fed control rats, these differences did not reach statistical significance. Comparison of the time course of relative changes in plasma glucose levels did not reveal a significant difference between SD-fed control rats and SD-fed Lewis dwarf rats. In contrast, in HFD-fed Lewis dwarf rats, administration of glucose led to more sustained increases in plasma glucose levels than in SD-fed Lewis dwarf rats (Figure 2A), indicating impaired glucose tolerance. This was also illustrated by the measurement of the calculated area under the curve (Figure 2B). The area under the curve was significantly reduced in SD-fed Lewis dwarf rats as compared with SD-fed control rats, whereas feeding a HFD resulted in a significant increase in area under the curve in Lewis dwarf rats eliminating the difference between the two genotypes (Figure 2B). In HFD-fed Lewis dwarf rats, glucose intolerance and the relative increase in fasting blood glucose levels were not associated with increases in serum cholesterol or triglyceride levels (Table 2).

Lewis dwarf rats fed an SD had low serum IGF-1 levels, and they maintained low IGF-1 levels when fed a HFD (Figure 2C). Consumption of a HFD tended to increase insulin levels in control rats but not in Lewis dwarf rats (Figure 2D). As a result, serum insulin levels were significantly elevated in HFD-fed control rats as compared with HFD-fed Lewis dwarf rats (Figure 2D), indicating that insufficient insulin secretion was a contributing factor in impaired glucose tolerance. The homeostatic model assessment for insulin resistance (HOMA-IR) was used to assess insulin resistance. It was calculated from fasting insulin and glucose concentration using the formula of Matthews and colleagues (59): HOMA-IR = fasting glucose (mmol/L) ×fasting insulin (mU/L)/22.5. There was no significant difference in HOMA-IR between the SD-fed groups (control [SD]: 1.18 ± 0.34; dwarf [SD]: 0.80 ± 0.24), whereas HOMA-IR was significantly lower in HFD-fed dwarf rats than in HFD-fed control rats (control [HFD]: 1.59 ± 0.21; dwarf [HFD]: 0.73 ± 0.04, $p < .05$). The quantitative insulin sensitivity check index (QUICKI) was calculated as an additional parameter to assess insulin sensitivity using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose: 1/(log [fasting insulin in μU/mL] + log [fasting glucose in mg/dL]). There was no significant difference in QUICKI between the SD-fed groups (control [SD]: 0.38 ± 0.01; dwarf [SD]: 0.41 ± 0.02), whereas QUICKI was significantly greater in HFD-fed dwarf rats than in HFD-fed control rats (control [HFD]: 0.358 ± 0.008; dwarf [HFD]: 0.404 ± 0.004, $p < .05$).

In control rats fed a HFD, there was a significant increase in serum levels of leptin and adiponectin (Figure 2E and F,

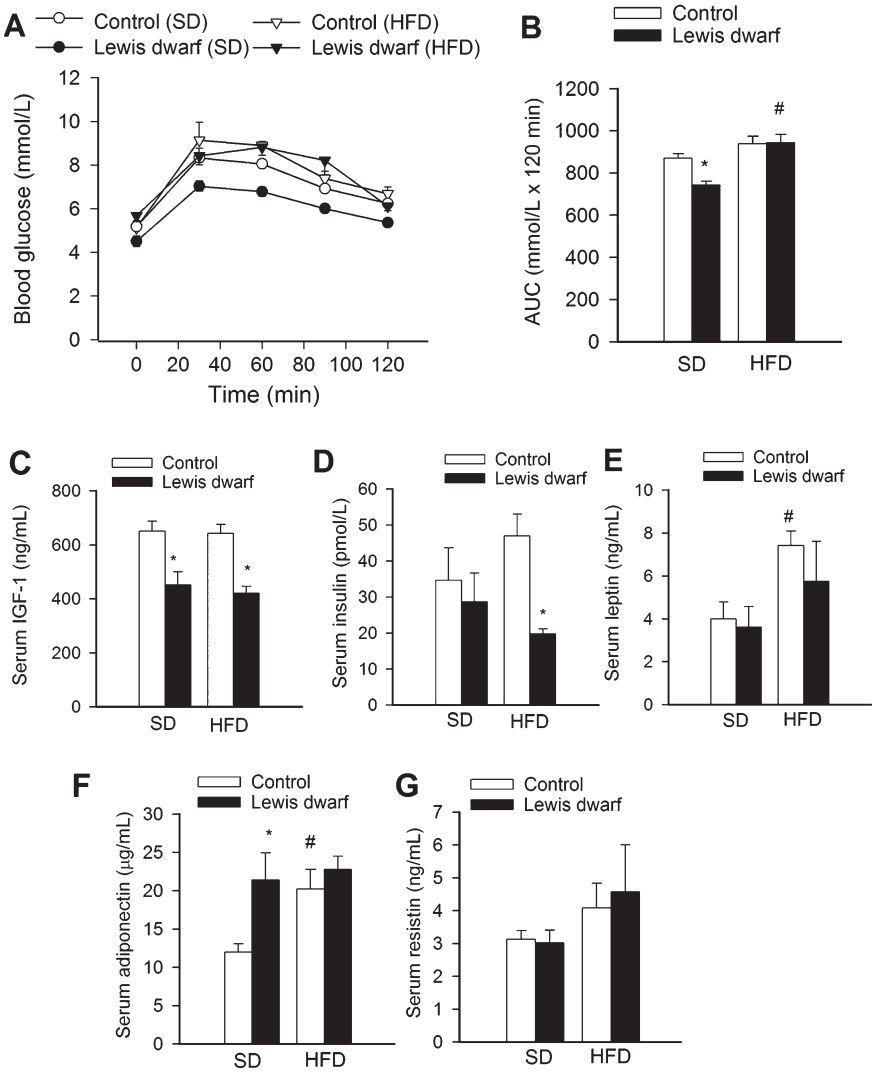

Figure 2. (**A**) High-fat diet (HFD) impairs glucose tolerance in Lewis dwarf rats. Shown are changes in blood glucose levels after 1 g/kg oral glucose dose in Lewis dwarf rats and control rats fed a HFD or standard diet (SD). (B) Areas under the curves (AUC) were significantly reduced in Lewis dwarf rats fed an SD. HFD significantly increased AUC in Lewis dwarf rats eliminating the difference between the two genotypes. Data are means ± *SEM*; **p* < .05 versus control; #*p* < .05 versus SD. (**C**–**G**) Serum levels of IGF-1 (**C**), insulin (**D**), leptin (**E**), adiponectin (**F**), and resistin (**G**). Data are means ± *SEM*; **p* < .05 versus respective control; #*p* < .05 versus SD (*n* = 5–7).

respectively). In contrast, baseline levels of adiponectin were significantly elevated in the Lewis dwarf rats, but the HFD was not associated with significant increases in serum levels of leptin (Figure 2E) or adiponectin (Figure 2F). Serum resistin levels in Lewis dwarf rats fed an SD were not significantly different from the levels measured in SD-fed control animals (Figure 2G). Consumption of a HFD did not increase significantly serum resistin levels in either group (Figure 2G).

Effects of Chronic HFD and GH/IGF-1 Deficiency on Circulating Levels of Inflammatory Cytokines and Chemokines

We examined the effects of GH/IGF-1 deficiency and HFD on inflammatory protein expression signature pattern in rat serum using a multiplex protein array. GH/IGF-1 deficiency in SD-fed Lewis dwarf rats was not associated with marked increases in serum levels of inflammatory markers (Figure 3). In contrast, several inflammatory markers had higher expression in HFD-fed Lewis dwarf rats compared with HFD-fed control rats (Figure 3). These markers included several cytokines (eg, TNF-α, IL-6, IL-1β, interferon gamma) and various chemokines (eg, eotaxin, MIP-3β, leukemia inhibitory factor).

Effects of Chronic HFD and GH/IGF-1 Deficiency on Endothelial Function and Vascular Reactive Oxygen Species Production

Feeding a HFD elicited significant endothelial dysfunction in aortas of control rats, as shown by the impaired relaxation

Table 2. Effects of a High-Fat Diet (HFD) on Various Serum Biomarkers in Control Rats and Insulin-Like Growth Factor-1/Growth Hormone–Deficient Lewis Dwarf Rats

Parameter	Control (SD)	Lewis Dwarf (SD)	Control (HFD)	Lewis Dwarf (HFD)	Units
Fast triglycerides	249.3 ± 55.8	137.16 ± 37.6	88.33 ± 10.6*	73.4 ± 12.1*	mg/dL
Fast cholesterol	122.5 ± 8.7	110.67 ± 11.0	96 ± 3.69	92 ± 1.97	mg/dL
Total protein cc	6.3 ± 0.17	6.1 ± 0.14	5.6 ± 0.06*	5.66 ± 0.09*	g/dL
Albumin	3.8 ± 0.09	3.71 ± 0.08	3.45 ± 0.03*	3.48 ± 0.07*	g/dL
ALP	95 ± 11.24	109 ± 12.20	116.3 ± 5.07	103.8 ± 13.9	U/L
SGOT/AST	129.6 ± 12.5	188.5 ± 26.21	182.16 ± 19.0	230 ± 51.19	U/L
SGPT/ALT	55.6 ± 4.7	69.67 ± 6.7	107.33 ± 17.6	96.6 ± 21.23	U/L
Bilirubin	<0.1	<0.1	<0.1	<0.1	mg/dL
BUN	15.1 ± 0.95	17.67 ± 0.77	12.83 ± 0.48	11.4 ± 0.68†	mg/dL
Creatinine	0.45 ± 0.03	0.45 ± 0.03	0.43 ± 0.02	0.40 ± 0.03	mg/dL
Cl	105.3 ± 1.1	103.3 ± 0.4	105.8 ± 1.2	107.6 ± 1.2†	mmol/L
Na	142 ± 1	140 ± 0.5	141.1 ± 1.4	143.4 ± 1.4	mmol/L
K	6.1 ± 0.7	6.5 ± 0.3	5.9 ± 0.3	6.3 ± 0.6	mmol/L
Ca	9.56 ± 0.2	9.15 ± 0.12	8.91 ± 0.09*	8.9 ± 0.221	mmol/L
PO_4	4.06 ± 0.04	4.1 ± 0.03	3.95 ± 0.04	4.06 ± 0.02	mmol/L
C-reactive protein	377.8 ± 20.3	410.4 ± 32.1	429.4 ± 10.8	392.8 ± 22.8	µg/mL
sVCAM-1	169.4 ± 4.8	195 ± 7.6*	181.4 ± 5.3	183.8 ± 6.2	ng/mL
VEGF	252.0 ± 8.6	255.0 ± 34.5	284.2 ± 10.5	247.5 ± 34.9	pg/mL
von Willebrand factor (vWF)	83.74 ± 20	121.5 ± 21	91.24 ± 17.6	105.7 ± 14.7	ng/mL
Tissue factor	8.92 ± 0.64	6.58 ± 1.21	9.05 ± 1.03	4.22 ± 1.01	ng/mL
Haptoglobin	805.4 ± 80.6	603.6 ± 175	721 ± 58.4	657.8 ± 157.2	µg/mL
ACE	45.1 ± 2.6	51.5 ± 5.4	38.7 ± 3.2	42.6 ± 6.4	ng/mL
Angiotensinogen	232.6 ± 8.2	238.2 ± 6.4	229.4 ± 11.5	237.4 ± 5.2	µg/mL

Notes: Data are mean ± *SEM* (n = 5–7 for each data point). VEGF = vascular endothelial growth factor.
*p < .05 versus SD-fed control; †p < .05 versus SD-fed Lewis dwarf.

responses to acetylcholine (Figure 4A) and the calcium ionophore, A23187 (Figure 4B). HFD-induced endothelial dysfunction was more severe in Lewis dwarf rats, as shown by the significantly diminished acetylcholine-induced (Figure 4A) and A23187-induced (Figure 4B) relaxations of aortas of these animals compared with responses obtained in vessels from HFD-fed control rats. In contrast, aorta relaxations in response to the endothelium-independent vasodilator SNAP were unaffected by either GH/IGF-1 deficiency or HFD (Figure 4C).

Representative fluorescent images of cross sections of DHE-stained aortas isolated from control and Lewis dwarf rats are shown in Figure 5A. Analysis of nuclear DHE fluorescent intensities indicated that the HFD tended to increase $O_2^{\cdot-}$ production more in vessels of Lewis dwarf rats than in vessels of control rats, although the difference did not reach statistical significance (Figure 5B). We also found that messenger RNA expression of the Nox1 subunit of the vascular NADPH oxidase was significantly upregulated in the aorta of HFD-fed Lewis dwarf rats as compared with both SD-fed Lewis dwarf rats and HFD-fed control rats (Figure 5C).

Effects of Chronic HFD and GH/IGF-1 Deficiency on Vascular Inflammatory Gene Expression

Under basal conditions, inflammatory gene expression did not differ significantly between control rats and Lewis dwarf rats (Figure 6). In contrast, GH/IGF-1 deficiency in Lewis dwarf rats exacerbated vascular inflammation induced by consumption of a HFD, as indicated by the significantly increased messenger RNA expression of TNF-α (Figure 6A), IL-6 (Figure 6B), and ICAM-1 (Figure 6C) in aortas of Lewis dwarf rats.

Discussion

In the present study, the effects of HFD-induced obesity on vascular function and metabolic alterations in a rat model of GH/IGF-1 deficiency were investigated. Interestingly, Lewis dwarf rats share many characteristics with aged animals, including a moderate decline in circulating GH and IGF-1 levels, increased incidence of vascular oxidative stress (25), stroke (60), and neurocognitive decline (29,38,61). Our results provide compelling information that GH/IGF-1 deficiency not only impairs metabolic function but also exacerbates vascular dysfunction and increases the inflammatory response in response to a HFD. Our results are consistent with the hypothesis that the decrease in GH and IGF-1 contributes to vascular dysfunction and an increased inflammatory response with age.

We found that both control rats and Lewis dwarf rats gained significant weight and became obese when consuming a HFD. It is well known that GH and IGF-1 regulate fat metabolism and adipocyte function (62). However, contrary to our prediction, GH/IGF-1 deficiency in Lewis dwarf rats did not affect either relative weight gain or changes in body composition in response to the HFD challenge. Both Lewis dwarf rats that have a specific deficiency in GH and IGF-1 and Ames dwarf mice that have low levels of GH, IGF-1, thyroid-stimulating hormone, and prolactin exhibit low plasma

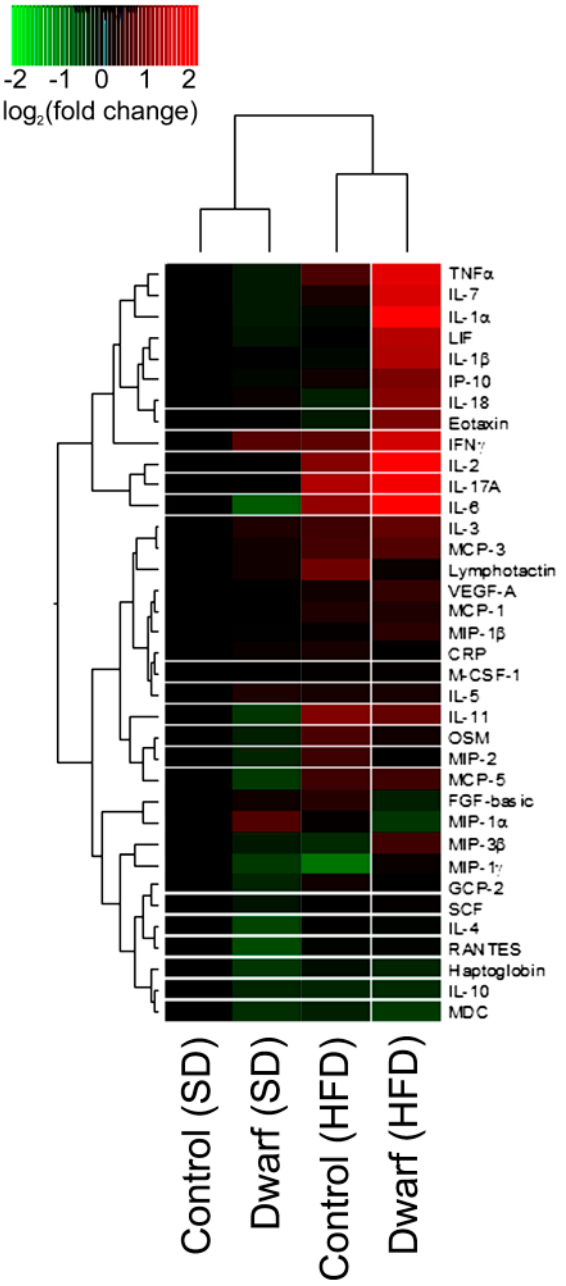

Figure 3. Proteomic profiles of serum inflammatory markers in Lewis dwarf rats and control rats fed a high-fat diet (HFD) or standard diet (SD). The heat map is a graphic representation of normalized cytokine serum concentration values depicted by color intensity, from highest (bright red) to lowest (bright green) expression. Values represent average serum protein expression levels (log_2 [fold change, normalized to the respective control mean value]) of replicate SD-fed control rats (n = 5), HFD-fed control rats (n = 5), SD-fed Lewis dwarf rats (n = 5), and HFD-fed Lewis dwarf rats (n = 5). Lewis dwarf rats on HFD have the highest levels of inflammatory markers.

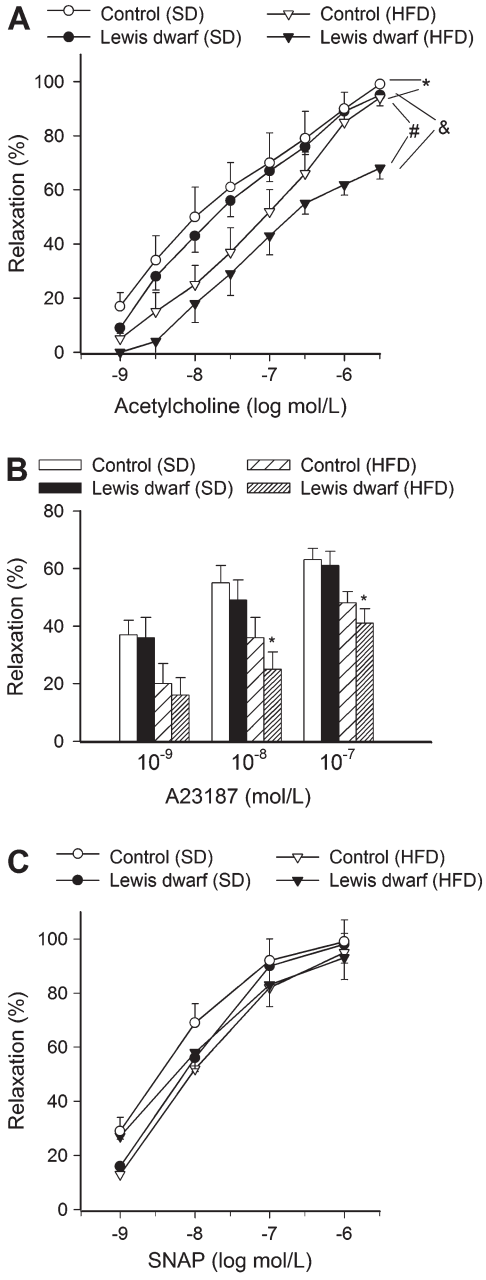

Figure 4. Relaxation of aorta rings isolated from Lewis dwarf rats and control rats fed a high-fat diet (HFD) or standard diet (SD). Vasomotor responses were induced by the endothelium-dependent agents acetylcholine (**A**) and A23187 (**B**) and *S*-nitroso-*N*-acetylpenicillamine (SNAP; **C**), an endothelium-independent vasodilator. Data are means ± *SEM*; **p* < .05 versus SD-fed control; #*p* < .05 versus SD-fed Lewis dwarf; &*p* < .05 versus HFD-fed control (n = 5–7).

insulin levels and maintain lower fasting glucose concentrations compared with controls, a phenomenon that has been attributed to increased insulin sensitivity in GH/IGF-1–deficient animals (63). It is well accepted that GH administration decreases insulin sensitivity (64), which underlies its diabetogenic effects (65). In addition to alterations in GH levels, previous studies in Ames dwarf mice suggest that increases in insulin sensitivity may be due, at least in part, to increased release of adiponectin and/or altered release of other adipokines (66) as well as changes in the insulin signaling pathway (63,67,68). Interestingly, a recent study on Ecuadorian individuals, who carry mutations in the GH receptor (GHR) gene, indicates that dysfunctional GH signaling and severe IGF-1 deficiency in humans are not associated with a relative hypoglycemia (69). Presently, the underlying

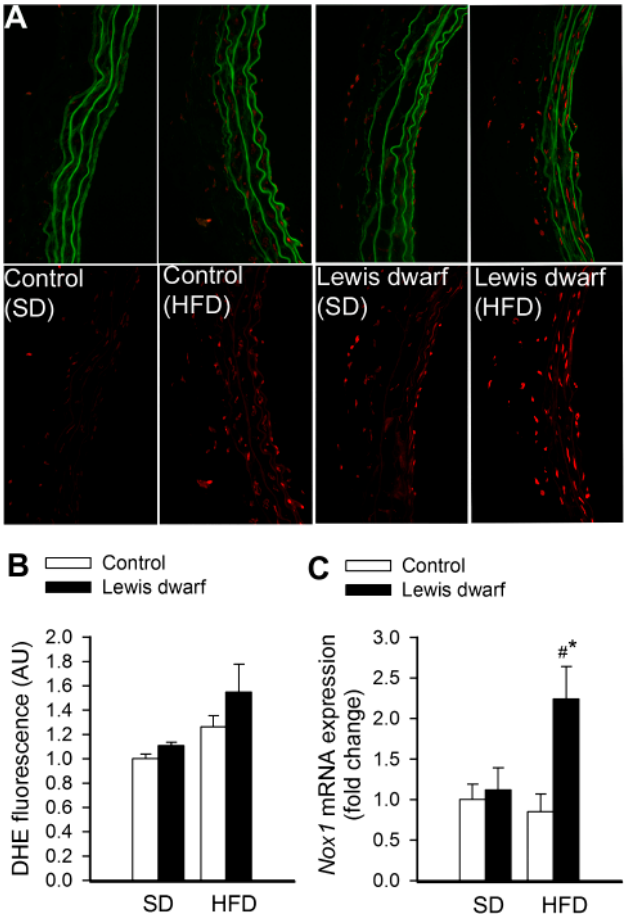

Figure 5. Representative micrographs showing red nuclear dihydroethidium (DHE) fluorescence, representing cellular $O_2^{\cdot-}$ production, in sections of cultured aortas isolated from Lewis dwarf rats and control rats fed a high-fat diet (HFD) or standard diet (SD). For orientation purposes, overlay of DHE signal and green autofluorescence of elastic laminae is also shown (upper panels). Original magnification: 20×. (**B**) Summary data for nuclear DHE fluorescence intensities. Data are mean ± *SEM*. (**C**) Quantitative real-time reverse transcription–polymerase chain reaction data showing messenger RNA expression of Nox1 in the aortas of HFD-fed and SD-fed Lewis dwarf rats and control rats. Data are mean ± *SEM* ($n = 5$–7). *$p < .05$ versus SD-fed Lewis dwarf; #$p < .05$ versus HFD-fed controls.

mechanism responsible for the discrepancy among the metabolic phenotypes of the Lewis dwarf rats and Ames dwarf mice and GHR-deficient human dwarfs is not well understood.

Importantly, we found that when challenged with a HFD, Lewis dwarf rats exhibit a marked intolerance to glucose coupled with a significant decline in insulin levels as compared with the respective controls. Previous studies also showed that GH/IGF-1–deficient Ames dwarf mice have significantly reduced amounts of insulin and exhibit intolerance to glucose (63). Human dwarfs with GHR deficiency also exhibit an ~68% decline in insulin (69). We posit that the lower than normal insulin levels in Ames dwarf mice and HFD-fed Lewis dwarf rats may be due to compromised β-cell numbers or function and impaired β-cell compensation in response to metabolic challenge. Indeed, recent studies demonstrate that GH and IGF-1 have key roles in the regulation of β-cell growth and insulin secretion (70). Importantly, when challenged with a HFD, mice with pancreatic β-cell–specific knockdown of the GH receptor (βGHRKO) exhibit impaired glucose tolerance and blunted glucose-stimulated insulin secretion (70).

Both aging and GH/IGF-1 deficiency were reported to be associated with complex alterations in the secretory phenotype of adipocytes. GH-deficient patients have significantly higher adiponectin and leptin levels than controls (71), and treatment with GH was reported to decrease the levels of both adipokines (72). Similar to findings in GH-deficient humans, we found that GH/IGF-1 deficiency in Lewis dwarf rats was associated with increased adiponectin levels but serum levels of leptin were within the normal range. Interestingly, Ames dwarf mice and other mouse models of GH deficiency also have higher levels of adiponectin but exhibit lower levels of leptin (66,73). We found that in control rats, the HFD increased circulating levels of adiponectin, extending the results of previous studies in mice (74,75) and rats (76) fed a similar diet. In healthy animals, high adiponectin levels may confer cardiovascular protection. Because cells of HFD-fed rodents develop adiponectin resistance (77), the vasoprotective action of adiponectin is likely blunted in these animals. Recent studies using adiponectin-deficient mice suggest that the increases in leptin and insulin in response to a HFD are dependent on increases in adiponectin levels (78). Consequently, the lack of significant changes in leptin and insulin in GH/IGF-1–deficient Lewis dwarfs may be the

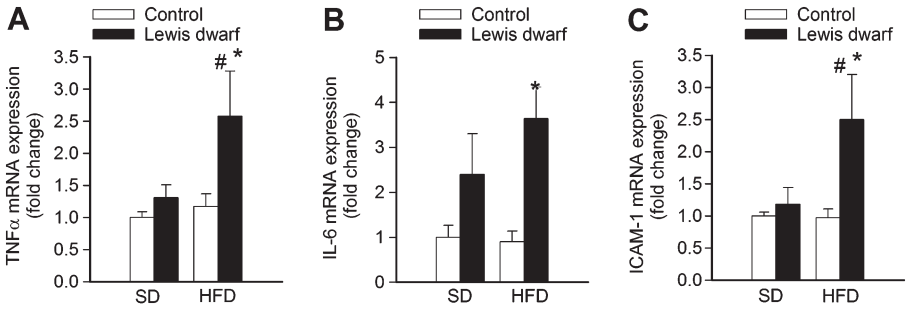

Figure 6. Quantitative real-time reverse transcription–polymerase chain reaction data showing messenger RNA expression of tumor necrosis factor-α (TNF-α; **A**), interleukin-6 (IL-6; **B**), and ICAM-1 (**C**) in the aortas of control rats and Lewis dwarf rats fed a high-fat diet (HFD) or standard diet (SD). Data are mean ± *SEM* ($n = 5$–7). *$p < .05$ versus HFD-fed controls; #$p < .05$ versus SD-fed Lewis dwarf.

result of the absence of a rise in adiponectin when fed a HFD.

In control animals, as expected, the HFD stimulated an inflammatory response (76) increasing circulating levels of multiple inflammatory cytokines, including IL-6 and TNF-α. Importantly, analysis of serum cytokine expression signatures suggests that chronic GH/IGF-1 deficiency leads to a profound increase in HFD-induced inflammation. This effect is likely a contributing factor in the higher incidence of atherosclerosis and perhaps other chronic inflammatory diseases in IGF-1–deficient individuals. There are multiple sources of inflammatory cytokines and chemokines present in the circulation of HFD-fed animals, including the liver, vasculature, and adipose tissue. Deficiency for IGF-1 in mice has previously been shown to exacerbate HFD-induced inflammatory cytokine expression in the liver (79), but further studies are needed to characterize metabolic stress–induced changes in the secretome of adipocytes and other cell types in chronic GH/IGF-1 deficiency.

Changes in circulating factors and cellular metabolism induced by obesity and consumption of a HFD are known to impair endothelial function by upregulating reactive oxygen species (ROS) production in the vascular wall (33,80), an effect that appears to be exacerbated in aging (7,31,33,57). Our results suggest that GH/IGF-1 deficiency in Lewis dwarf rats exacerbates endothelial dysfunction associated with obesity, likely by increasing vascular oxidative stress. Previously, we found that mitochondrial ROS generation is increased and antioxidant defenses are impaired in arteries of GH/IGF-deficient Ames dwarf mice fed an SD (26). Moreover, isolated hepatic IGF-1 deficiency in mice exacerbates hyperglycemia-induced endothelial dysfunction in vitro (27), but further studies are required to determine whether obesity also exacerbates vascular oxidative stress and endothelial dysfunction in IGF-1–deficient mice. Taken together, the available experimental findings support the view that GH/IGF-1 deficiency in rodents mimics aspects of the vascular aging phenotype (33). Accordingly, consumption of a HFD results in a significantly more severe endothelial impairment in arteries of aged mice as compared with that in vessels from young mice (Z. Ungvari, M.D., Ph.D, manuscript in preparation, 2011).

In previous studies, aging was reported to exacerbate high glucose-induced ROS production in cultured arteries (6), likely by impairing cellular Nrf2-dependent pathways. Importantly, we found that low circulating IGF-1 levels in mice also impair the ability of vascular cells to mount an effective Nrf2-driven antioxidant defense necessary to withstand diverse oxidative stress challenges (27). Of note, IGF-1 was shown to increase Nrf2 activity in endothelial cells via the PI3 kinase–Akt pathway (27). Nrf2 dysfunction induced by endocrine IGF-1 deficiency directly mimics the vascular aging phenotype in that isolated arteries, and cultured endothelial and smooth muscle cells derived from aged animals exhibit a dysfunctional Nrf2-driven response under oxidative stress conditions (5,6). Previously, we demonstrated that genetic lack of a functional Nrf2/ARE pathway also results in significant increases in vascular ROS levels and exacerbation of endothelial dysfunction in arteries of HFD-fed $Nrf2^{-/-}$ mice (34). In addition, IGF-1 can also upregulate eNOS in endothelial cells (26). Most human data also support the concept that normal levels of GH and IGF-1 confer endothelial protection in young individuals. For example, in GH-deficient subjects, flow-mediated endothelium-dependent dilation of peripheral arteries is impaired even at young ages (11,81,82). Plasma markers of oxidative stress are also elevated in GH-deficient subjects and are lowered by GH replacement (82). Furthermore, in vitro IGF-1 and GH treatment reduces ROS production in cultured human endothelial cells (26). Thus, further studies are warranted to test the interaction of GH/IGF-1 deficiency and obesity in aged humans as well.

The deleterious effects of GH/IGF-1 deficiency are likely organ and disease specific. For example, both human GH/IGF-1–deficient dwarfs (69) and Ames dwarf mice (83) exhibit a significant decrease in cancer incidence. The incidence of liver disease also appears to be lower in human GH/IGF-1–deficient dwarfs (69). In that regard, it is interesting that the liver of HFD-fed Lewis dwarf rats also appears to be protected from damage inflicted by HFD (Z. Ungvari, M.D., Ph.D, unpublished observation, 2011), although the mechanism for this effect remains unknown.

There is strong evidence demonstrating that cellular oxidative stress associated with metabolic diseases and aging is causally linked to vascular inflammation (4). We found that obese GH/IGF-1–deficient Lewis dwarf rats exhibit an increased expression of inflammatory mediators and markers of endothelial activation as compared with arteries of obese control animals. These findings are consistent with the results of previous studies demonstrating that IGF-1 confers anti-inflammatory vascular effects (24) and that short-term GH treatment attenuates age-related inflammation, including upregulation of TNF-α, in the cardiovascular system of mouse models of accelerated aging (84). GH/IGF-1 deficiency in aging is also associated with upregulation of TNF-α, IL-6, and other proinflammatory cytokines (44,45,85,86). Importantly, incubation of cultured cells with sera from human GH/IGF-1–deficient dwarfs significantly impairs cellular resistance to H_2O_2 and upregulates cellular pathways that regulate inflammatory processes (including regulators of NF-κB and activators of IL-6 production [69]). It is likely that chronic oxidative stress and low-grade vascular inflammation associated with GH/IGF-1 deficiency in obese individuals promote the development of atherosclerosis. Indeed, in ApoE knockout mice which were fed a Western diet, even a 20% decline in circulating IGF-1 was shown to significantly increase atherosclerosis progression (87). In contrast, treatment of ApoE-null mice with IGF-1 significantly attenuates HFD-induced vascular oxidative stress, atherosclerotic plaque progression and vascular inflammation (24). Interestingly, a recent study on human dwarfs with GHR

mutations demonstrated that severe IGF-1 deficiency in humans is associated with a shorter than expected life span (69), and analysis of the known causes of mortality (independent of accidents and death related to substance abuse) in this population reveals that almost half died as a result of stroke and cardiovascular disease (69). Because in the aforementioned human dwarf population IGF-1 deficiency is associated with an ~69% increase in the rate of obesity (a cardiovascular risk factor per se), further studies are warranted to study the interaction among GH/IGF-1 deficiency, metabolic status, and parameters relevant for cardiovascular health in the general elderly population (88).

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