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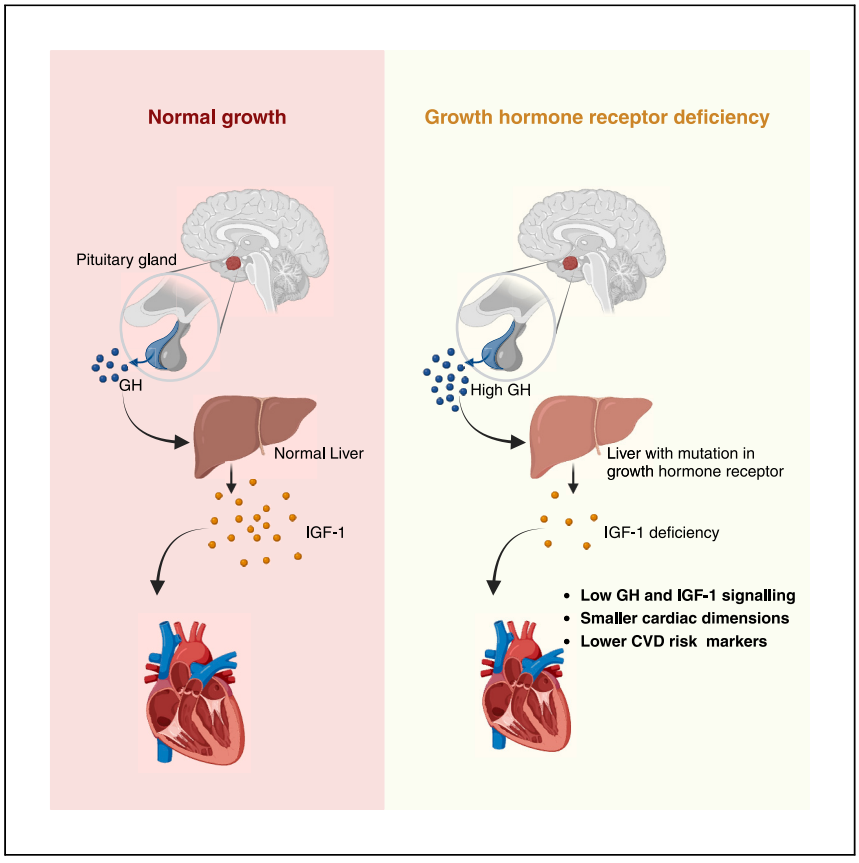
Normal or improved cardiovascular risk factors in IGF-I-deficient adults with growth hormone receptor deficiency



Guevara-Aguirre et al. evaluated the cardiovascular disease risk factors in individuals with Laron syndrome and their relatives to identify the effects of decreased growth hormone signaling on cardiovascular disease in humans. The study reports that decreased GH signaling has neutral to protective effects on cardiovascular disease risk factors.

Jaime Guevara-Aguirre, Amrendra Mishra, Marco Canepa, ..., John J. Kopchick, Priya Balasubramanian, Valter D. Longo

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Highlights

Individuals with Laron syndrome/GHRD have low growth hormone signaling

GHRD individuals have smaller cardiac dimensions

GHRD individuals have normal or improved levels of cardiovascular disease risk factors

Translation to Patients

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Normal or improved cardiovascular risk factors in IGF-I-deficient adults with growth hormone receptor deficiency

Jaime Guevara-Aguirre,[1,2,3,10,*] Amrendra Mishra,[4,5,10] Marco Canepa,[6,7] Carolina Guevara,[1,2] Álvaro Villacres,[1] Alexandra Guevara,[2] Gabriela Peña,[1] Daniela Lescano,[1] John J. Kopchick,[8] Priya Balasubramanian,[4] and Valter D. Longo[4,9,11,*]

SUMMARY

Background: Human subjects with generalized growth hormone (GH) insensitivity due to GH receptor deficiency (GHRD)/Laron syndrome display a very low incidence of insulin resistance, diabetes, and cancer, as well as delayed age-related cognitive decline. However, the risk of cardiovascular disease (CVD) in these subjects is poorly understood. Here, we have assessed cardiovascular function, damage, and risk factors in GHRD subjects and their relatives.

Methods: We measured markers of CVD in two phases: one in a cohort of 30 individuals (GHRD = 16, control relatives = 14) brought to USC (in Los Angeles, CA) and one in a cohort including additional individuals examined in Ecuador (where the subjects live) for a total of 44 individuals (GHRD = 21, control relatives = 23). Data were collected on GHRD and control groups living in similar geographical locations and sharing comparable environmental and socio-economic circumstances.

Results: Compared to controls, GHRD subjects displayed lower serum glucose, insulin, blood pressure, smaller cardiac dimensions, similar pulse wave velocity, lower carotid artery intima-media thickness, lower creatinine, and a non-significant but major reduction in the portion of subjects with carotid atherosclerotic plaques (7% GHRDs vs. 36%, Controls $p = 0.1333$) despite elevated low-density lipoprotein cholesterol levels.

Conclusion: The current study indicates that individuals with GHRD have normal or improved levels of cardiovascular disease risk factors as compared to their relatives.

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CONTEXT AND SIGNIFICANCE

Growth hormone receptor deficiency (GHRD)/Laron syndrome, which results in disrupted GH signaling and lower insulin growth factor-1 (IGF-1) and insulin, is associated with protection against diabetes, cancer, and age-related cognitive decline. However, the impact of decreased GH and IGF-1 signaling on the risk of cardiovascular disease is unclear, with studies suggesting both increased and decreased risk.

Guevara-Aguirre et al. performed state-of-the-art comprehensive evaluation of the cardiovascular function and risk factors in GHRD subjects and their relatives to establish the role of GH signaling in cardiovascular health. They report that individuals with GHRD have normal or improved levels of cardiovascular disease risk factors as compared to their relatives living in the same geographical locations and with similar lifestyles.

INTRODUCTION

Human subjects with generalized growth hormone (GH) insensitivity due to GH receptor deficiency (GHRD)/Laron syndrome (LS) display insulin growth factor-1 (IGF-1) deficiency, a very low incidence of insulin resistance, diabetes, and cancer, as well as delayed age-related cognitive decline.[1–4] IGF-I deficiency has been associated with increased cardiovascular disease (CVD) in humans,[5,6] yet in mice, it is associated with an extended lifespan, raising the possibility that the limited role of atherosclerosis in mouse longevity could hide the detrimental effects of GHR/IGF-I deficiency. Interestingly, it has also been shown that cardiac-specific disruption of the GHR leads to IGF-1 deficiency but does not affect cardiac structure and

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function in adult male mice, although it does affect glucose homeostasis.[7] In general, arteries and the heart are known for their sensitivity to circulating IGF-I, and its deficiency has been associated with vascular derangements leading to atherosclerosis and impairment of cardiac mass and functionality.[8–11] However, subjects with excess levels of circulating GH, i.e., patients diagnosed with acromegaly and hence high IGF-1 serum concentrations, also display arterial endothelial cell abnormalities that eventually lead to CV disorders and death.[12] GHRD/LS subjects do not appear to display the 40% lifespan extension observed in GHR knockout ($GHR^{-/-}$) mice, although they may live longer than relatives[1,13] raising the possibility that increased incidence of CVD may prevent in humans the major longevity extension observed in mice.

Here, we tested many markers of CVD in two phases: one in a cohort of 30 individuals (GHRD = 16, control relatives = 14) brought to USC (in Los Angeles, CA) for assessment and the second including additional individuals tested in Ecuador (where the subjects live) for a total of 44 individuals (GHRD = 21, control relatives = 23) (Figure 1). The aim was to evaluate CV risk factors and markers using state-of-the-art non-invasive techniques.

RESULTS

Los Angeles study

Clinical assessment of blood markers in GHRD subjects compared to control relatives showed higher total cholesterol (196.9 $\pm$ 30.79 vs. 175.3 $\pm$ 26.3, p = 0.0493), higher low-density lipoprotein (LDL) cholesterol (123.5 $\pm$ 24.33 vs. 100.7 $\pm$ 27.18, p = 0.0245), lower glucose (86.81 $\pm$ 11.41 vs. 103.9 $\pm$ 24.52, p = 0.0184), lower insulin (3.51 $\pm$ 1.37 vs. 8.16 $\pm$ 3.13, $p < 0.0001$), and lower homeostatic model assessment for insulin resistance (HOMA-IR; 0.8 $\pm$ 0.3 vs. 2.2 $\pm$ 1.0, $p < 0.0001$) (Table S1). GHRD showed higher counts for white blood cells, absolute neutrophils, and absolute eosinophils (Table S2).

Carotid intima-media thickness (CIMT) is useful to assess the extent of carotid atherosclerotic vascular disease.[14] GHRD subjects had lower CIMT values as compared to their control relatives for both left (0.49 $\pm$ 0.10 vs. 0.60 $\pm$ 0.11, p = 0.0106) and right (0.45 $\pm$ 0.11 vs. 0.62 $\pm$ 0.15, p = 0.0015) common carotid arteries (Table 1).

Flow-mediated dilation (FMD) is a non-invasive measure of CV risk.[15] GHRD subjects had a smaller baseline brachial artery diameter as compared to control relatives (2.98 $\pm$ 0.50 vs. 3.64 $\pm$ 0.75, p = 0.0101) but a similar percentage of FMD (5.66 $\pm$ 3.66 vs. 3.65 $\pm$ 3.14, p = 0.1336) (Table 1).

Electrocardiography showed an increased heart rate (68 $\pm$ 7 vs. 57 $\pm$ 10, p = 0.0023) and shorter intervals (PR segment and QRS segment) in GHRD individuals as compared to those in the control group (Table 2). A much higher percentage of control relatives showed minor symptoms of cardiac risk, mainly sinus bradycardia, as compared to GHRD subjects (85.71% vs. 31.25%, **p = 0.0039, Fisher's exact test), although it was determined to be not clinically relevant by the cardiologist (Table 2).

Ecuador study

A larger follow-up study on an overlapping cohort of individuals (23 out of 30 participants [76.6%] of the participants from the Los Angeles study) was carried out in Ecuador, where a significant portion of the world's GHRD/LS population resides. This study included 21 GHRD subjects and 23 control relatives (44 total) (Figure 1).

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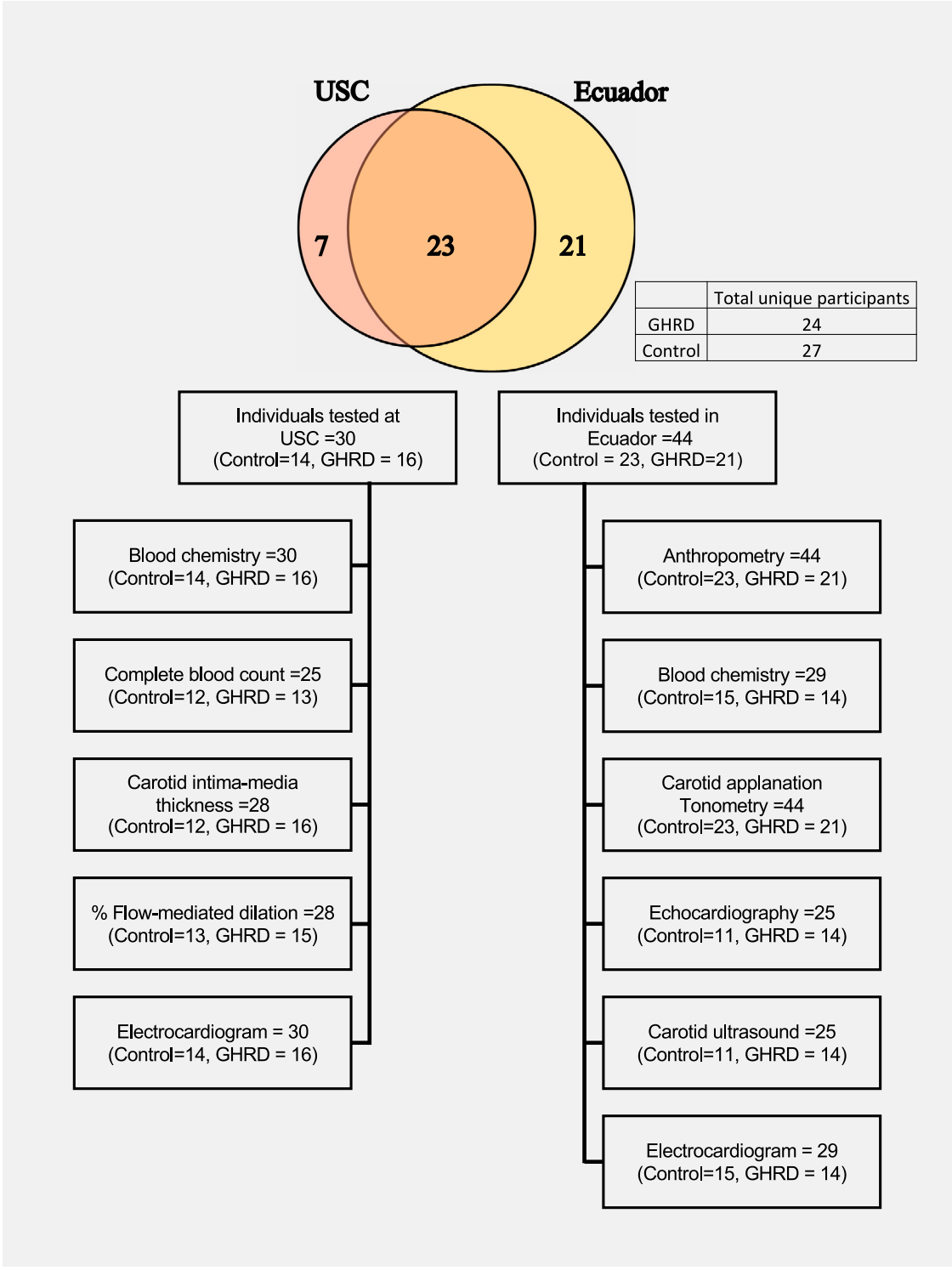

Figure 1. CONSORT-style diagram showing the patient groups and the tests performed

Blood pressure (BP) was significantly lower in the GHRD group as compared to the control group (systolic: 112.1 ± 18.6 vs. 124.5 ± 16.5 mmHg, $p = 0.025$; diastolic: 66.3 ± 7.8 vs. 72.5 ± 12.3 mmHg, $p = 0.057$), with similar findings observed during

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Table 1. Cardiac assessment of patients with GHRD and controls (USC cohort)

	Control (mean ± SD)	*n*	GHRD (mean ± SD)	*n*	*p* value	Significance
Common carotid artery intima-media thickness (CIMT), left (mm)	0.60 ± 0.11	12	0.49 ± 0.10	16	0.0106	*
Common CIMT, right (mm)	0.62 ± 0.15	12	0.45 ± 0.11	16	0.0015	**
Baseline branchial artery diameter (mm)	3.6 ± 0.8	13	3.0 ± 0.5	15	0.0101	*
% Flow-mediated dilation	3.7 ± 3.1	13	5.7 ± 3.7	15	0.1336	ns

ns, not significant.

measurements repeated before applanation tonometry (systolic: 115.8 ± 17.5 vs. 126.3 ± 15.4 mmHg, $p = 0.038$; diastolic: 65.6 ± 6.2 vs. 76.3 ± 8.5 mmHg, $p < 0.0001$) (Table 3).

Pulse wave velocity (PWV) is a measurement of arterial stiffness and a predictor of CVD risk.[16] During applanation tonometry ($n = 21$ LS vs. 23 control relatives), GHRD subjects displayed a shorter estimated traveled distance but had similar carotid-femoral PWV (7.2 ± 1.6 vs. 8.1 ± 2.1 m/s, $p = 0.109$) and central pulse pressure (40.3 ± 14.6 vs. 37.9 ± 11.4 mmHg, $p = 0.529$) as compared to those in control relatives. Also, GHRD subjects had higher augmented pressure (14.1% ± 6.9% vs. 10.6% ± 5.5%, $p = 0.07$) as well as augmentation index (33.6% ± 7% vs. 26.7% ± 8.8%, $p = 0.001$). A higher augmentation index was observed in GHRD subjects even when normalized to a heart rate of 75 (Table 3).

During electrocardiography, both GHRD subjects and the control group displayed normal sinus rhythm, and no subjects displayed major conduction delays (Table 3). During echocardiography ($n = 14$ GHRD vs. 11 control subjects), GHRD subjects were found to have smaller cardiac dimensions than controls even when parameters were corrected for body surface area. These measurements included left ventricular mass (42.6 ± 12.0 vs. 51.6 ± 11.6 g/m^2, $p = 0.07$) and end-diastolic volume (32.9 ± 5.0 vs. 42.5 ± 6.4 mL/m^2, $p = 0.0003$). Neither GHRD nor control subjects reached the international standard threshold for left ventricular hypertrophy.[17] Systolic function parameters were normal in both GHRD subjects and controls, and no participants displayed a left ventricular ejection fraction of less than 55%. However, GHRD subjects showed a higher percentage of ejection fraction as compared to that in the control group (70.3% ± 5.7% vs. 64.5% ± 4.7%, $p = 0.014$). Similar findings were observed in diastolic function parameters. 5 controls, but only 2 GHRD subjects, had abnormal left ventricular relaxation and normal average filling pressure values (E/E′ 8 ± 3 vs. 9 ± 2, $p = 0.823$). No major valve disease was noticed in either group. IMT was lower in the GHRD subjects as compared to that in the control group (0.3 ± 0.1 vs. 0.5 ± 0.2 mm, $p = 0.001$). GHRD subjects displayed a non-significant trend for a major reduction in

Table 2. Cardiac assessment of patients with GHRD and controls (USC cohort) using electrocardiogram

Electrocardiogram	Control (mean ± SD)	*n*	GHRD (mean ± SD)	*n*	*p* value	Significance
ECG	28.57% abnormal	14	18.75% abnormal	16	0.6746	ns
Sinus rhythm	85.71% abnormal	14	31.25% abnormal	16	0.0039	**
Heart rate	57 ± 10	14	68 ± 7	16	0.0023	**
PR segment	162 ± 27 ms	14	138 ± 22 ms	16	0.0113	*
QRS segment	78 ± 10 ms	14	65 ± 6 ms	16	0.0002	***
QTc segment	407 ± 16 ms	14	416 ± 20 ms	16	0.2115	ns

ns, not significant.

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Table 3. Characteristics of patients with GHRD and controls at the time of cardiovascular assessment (Ecuador)

	Control (mean ± SD)	*n*	GHRD (mean ± SD)	*n*	*p* value	Significance
Clinical						
Age	51.7 ± 13.1 years	23	46.5 ± 13.0 years	21	0.193	ns
Male gender	26%	23	24%	21	0.862	ns
Height	153.2 ± 7 cm	23	118.5 ± 9.5 cm	21	<0.0001	****
Weight	70.4 ± 18.4 kg	23	43.9 ± 11.7 kg	21	<0.0001	****
Body mass index (BMI)	30.1 ± 8.1	23	31 ± 6.1	21	0.687	ns
BMI ≥30	52%	23	57%	21	0.74	ns
Body surface area (BSA)	1.7 ± 0.2 m^2	23	1.1 ± 0.2 m^2	21	<0.0001	****
Head circumference	54.5 ± 1.8 cm	23	51.8 ± 1.8 cm	21	<0.0001	****
Waist circumference	95.1 ± 15.9 cm	23	83.5 ± 10.8 cm	21	0.009	***
Hip circumference	108 ± 16.5 cm	23	103.4 ± 23.7 cm	21	0.463	ns
Waist-to-hip ratio	0.9 ± 0.1	23	0.8 ± 0.1	21	0.018	*
Pulse rate	73.2 ± 13.6 bpm	23	76.5 ± 7.2 bpm	21	0.334	ns
Clinical systolic blood pressure (SBP)	124.5 ± 16.5 mmHg	23	112.1 ± 18.6 mmHg	21	0.025	*
Clinical diastolic blood pressure (DBP)	72.5 ± 12.3 mmHg	23	66.3 ± 7.8 mmHg	21	0.057	ns
Carotid applanation tonometry						
Test SBP	126.3 ± 15.4 mmHg	23	115.8 ± 17.5 mmHg	21	0.038	*
Test DBP	76.3 ± 8.5 mmHg	23	65.6 ± 6.2 mmHg	21	<0.0001	****
PWV direct carotid-femoral distance × 0.80	468.3 ± 36.4 mm	23	387.3 ± 35.7 mm	21	<0.0001	****
PWV	8.1 ± 2.1 m/s	23	7.2 ± 1.6 m/s	21	0.109	ns
Aortic pulse pressure	37.9 ± 11.4 mmHg	23	40.3 ± 14.6 mmHg	21	0.529	ns
Aortic augmentedpressure	10.6 ± 5.5 mmHg	23	14.1 ± 6.9 mmHg	21	0.07	ns
Augmentation index	26.7% ± 8.8%	23	33.6% ± 7%	21	0.001	**
Augmentation index @75 bpm	25.3% ± 9%	23	33.3% ± 6%	21	0.001	**
Electrocardiogram						
Sinus rhythm	100%	15	100%	14	–	–
Heart rate	68 ± 11	15	77 ± 9	14	0.038	*
PR segment	160 ± 20 ms	15	144 ± 19 ms	14	0.04	*
QRS segment	90 ± 12 ms	15	73 ± 7 ms	14	0.0001	***
QTc segment	419 ± 20 ms	15	414 ± 17 ms	14	0.529	ns
Echocardiography						
Aortic root diameter	28.9 ± 3.1 mm	11	23.4 ± 3.7 mm	14	0.001	***
Interventricular septum diastolic thickness	6.8 ± 1.3 mm	11	5.1 ± 1.3 mm	14	0.003	**
Left ventricular end-diastolic (LVED) diameter	45.9 ± 4.6 mm	11	40.1 ± 2.9 mm	14	0.001	***
Posterior wall diastolic thickness	5.9 ± 1.5 mm	11	4.6 ± 1.1 mm	14	0.021	*
Left ventricular end-systolic (LVES) diameter	24.7 ± 7 mm	11	24.6 ± 2.7 mm	14	0.967	ns
Left ventricle (LV) mass	89.2 ± 29.8 g	11	49.0 ± 9.9 g	14	<0.0001	****
LV mass index	51.6 ± 11.6 g/m^2	11	42.6 ± 12.0 g/m^2	14	0.07	ns
LV hypertrophy	0%	11	0%	14		
E/A ratio	1.2 ± 0.5	11	1.6 ± 0.6	14	0.089	ns
Deceleration time	206.7 ± 72	11	150 ± 47.5	14	0.027	*
Em lateral	12.9 ± 4 cm/s	11	14.8 ± 4.8 cm/s	14	0.295	ns
Em septal	9.5 ± 2.9 cm/s	11	11.5 ± 3.5 cm/s	14	0.129	ns
Em average	11.2 ± 3.2 cm/s	11	13.2 ± 3.9 cm/s	14	0.186	ns
E/Em ratio	9 ± 2	11	8 ± 3	14	0.823	ns
Left atrial (LA) diameter	34.8 ± 7.1 mm	11	31.6 ± 3.2 mm	14	0.138	ns
LA area biplane	17.3 ± 3 cm^2	11	12.2 ± 2.3 cm^2	14	<0.0001	****
LA volume biplane	49.7 ± 15 mL	11	29.5 ± 8.7 mL	14	0.0003	***
LA volume index	28.8 ± 6.7 mL/m^2	11	25.1 ± 7.1 mL/m^2	14	0.1966	ns
LVED volume biplane	72.5 ± 14.8 mL	11	38.9 ± 8.3 mL	14	<0.0001	****
LVES volume biplane	27.1 ± 5.8 mL	11	17.5 ± 8.5 mL	14	0.004	**
LVED volume index	42.5 ± 6.4 mL/m^2	11	32.9 ± 5.0 mL/m^2	14	0.0003	***
LVES volume index	16.1 ± 3.9 mL/m^2	11	14.4 ± 5.3 mL/m^2	14	0.392	ns
LV ejection fraction biplane	64.5% ± 4.7%	11	70.3% ± 5.7%	14	0.014	*

(*Continued on next page*)

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Table 3. *Continued*

	Control (mean ± SD)	*n*	GHRD (mean ± SD)	*n*	*p* value	Significance
Right ventricle (RV) basal diastolic diameter	38.7 ± 5.1 mm	11	31 ± 3.4 mm	14	0.001	***
RV mid diastolic diameter	30.5 ± 6.7 mm	11	24.7 ± 2.6 mm	14	0.006	**
RV length	74.1 ± 7.6 mm	11	60.2 ± 6.2 mm	14	<0.0001	****
RV diastolic wall thickness	6.6 ± 1.1 mm	11	5.2 ± 1.2 mm	14	0.006	**
RV s' (systolic excursion velocity)	15.5 ± 2.8 cm/s	11	13.4 ± 1.8 cm/s	14	0.033	*
Tricuspid valve regurgitation pressure gradient	28.4 ± 10.9 mmHg	11	31.1 ± 12.2 mmHg	14	0.571	ns
Tricuspid annular plane systolic excursion (TAPSE)	23.8 ± 2.6 cm	11	19.9 ± 2.2 cm	14	0.001	***
Inferior vena cava diameter	18.1 ± 3.9	11	13.8 ± 2	14	0.001	***
Carotid ultrasound						
Intima media thickness	0.5 ± 0.2 mm	11	0.3 ± 0.1 mm	14	0.001	***
Carotid plaque (at least one)	36%	11	7%	14	0.1333	ns
Laboratory tests						
Urea	28.8 ± 7.9 mg/dL	15	30.7 ± 5.5 mg/dL	14	0.452	ns
Creatinine	0.8 ± 0.1 mg/dL	15	0.7 ± 0.1 mg/dL	14	0.032	*
Total cholesterol	193.3 ± 35.7 mg/dL	15	214.6 ± 40.4 mg/dL	14	0.142	ns
HDL cholesterol	54 ± 11.3 mg/dL	15	52 ± 12.1 mg/dL	14	0.649	ns
LDL cholesterol	110.5 ± 26.5 mg/dL	15	140.6 ± 33.9 mg/dL	14	0.013	*
Triglycerides	138.3 ± 78.3 mg/dL	15	111.2 ± 42.2 mg/dL	14	0.262	ns
Uric acid	5 ± 1 mg/dL	15	4.7 ± 0.9 mg/dL	14	0.396	ns
Fasting glucose	110.3 ± 24.9 mg/dL	15	90.7 ± 9.8 mg/dL	14	0.002	**
Glucose 120 min	119.6 ± 53.1 mg/dL	15	98 ± 19.6 mg/dL	14	0.164	ns
C-reactive protein	4.9 ± 5.1	15	6.8 ± 3.9	14	0.278	ns
Medications						
Antihypertensive	17.40%	23	23.80%	21	0.598	ns
Lipid lowering	4.40%	23	9.50%	21	0.496	ns
Antiplatelets	0%	23	4.80%	21	0.3	ns

ns, not significant; HDL, high-density lipoprotein.

atherosclerotic plaques compared to controls with at least one plaque present in 7% of GHRD subjects vs. 36% of controls (p = 0.1333) (Table 3), notwithstanding the higher values of LDL cholesterol in GHRD vs. control subjects (140.6 ± 33.9 vs. 110.5 ± 26.5 mg/dL, p = 0.013).

GHRD and control subjects displayed a significant positive correlation of PWV with age and systolic BP. Only the control group showed a significant positive correlation of PWV with glucose (control: Spearman's correlation r = 0.52, p = 0.0485; GHRD: Spearman's correlation r = 0.24, p = 0.41). The control group also showed a correlation of IMT with age (control: Spearman's correlation r = 0.82, p = 0.0032) and glucose level (control: Spearman's correlation r = 0.72, p = 0.0159), while the GHRD group did not (Table S3).

DISCUSSION

Our CVD risk factor assessment of an Ecuadorian cohort of GHRD subjects is the most comprehensive in the literature for both the number of subjects tested and the range of tests.

In general, CV abnormalities observed in conditions of excess GH and IGF-I concentrations, as those seen in patients with acromegaly, have been studied extensively.[18] On the contrary, there are only a few reports on CVD in subjects with GH/IGF-I deficiencies.[19,20] Moreover, there are no studies on the parameters of arterial stiffness in these subjects. Early reports suggested an increased incidence

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of CVD-related mortality in individuals with hypopituitarism, hypothesized to be caused by GH deficiency.[21] However, later studies have shown that individuals with untreated severe isolated GH deficiency, while showing increased levels of cholesterol and obesity, do not show any carotid wall thickness or premature atherosclerosis, raising the possibility that the therapy regimen that includes steroid hormone substitution could be the reason behind increased CVD mortality among individuals suffering from hypopituitarism.[22] Our previous study on the cause of death in the LS/GHRD population in Ecuador indicated that individuals with GHRD have an unusually high rate of alcohol- and accident-related deaths as well as convulsive disorders, whereas cancer- or diabetes-related deaths or incidence was very low. Notably, our earlier studies show a comparable incidence of CVD (cardiac disease + stroke)-related deaths for GHRD subjects as compared to those in their control relatives (30% vs. 33%),[1] although it is difficult to determine whether this may be influenced by the overreporting of cardiac disease as a cause of death when another clear cause of death is not observed.

Our study was performed with advanced echocardiography and state-of-the-art testing for non-invasive investigation of arterial stiffness (i.e., PWV). We found smaller cardiac dimensions in GHRD subjects than those in controls even after normalizing for body size. Similar PWVs were observed in GHRD individuals and the control group, along with a very low prevalence of subjects with carotid plaques as compared to the control group. Age-related changes in PWV and IMT were similar in GHRD subjects and controls. Lower systolic BP, possibly inducing less arterial damage, might explain the trend for fewer arterial plaques in individuals affected by GHRD.

In summary, compared to control subjects, GHRD/LS subjects display enhanced insulin sensitivity, lower BP, no rhythm disturbances, smaller cardiac dimensions, similar PWV, lower CIMT, higher eGFR, lower creatinine, and a trend for a strong reduction in carotid atherosclerotic plaques despite elevated LDL cholesterol levels. Several studies have shown that BMI, and not height, is associated with increased CIMT.[23,24] In our study, BMI is matched in the two groups, and in fact, the GHRD group has a slightly higher BMI as compared to control individuals (31 ± 6.1 vs. 30.1 ± 8.1) and a higher frequency of individuals with a BMI >30 (57% vs. 52%). Hence, CIMT does not appear to require normalization for height.

Here, based on methodologies to diagnose macroscopic organ damage associated with aging and CV risk factors, we find that GHRD subjects have normal or reduced levels of CVD risk factors or markers in comparison to their age- and sex-matched control relatives living under comparable environmental circumstances. In fact, lower BP in addition to the strong tendency for reduced atheroma plaques despite high LDL cholesterol as well as enhanced insulin sensitivity in GHRDs suggests a protective, rather than neutral, effect of GHRD on CV health.

Limitations of the study

One limitation of the study is that GHRD subjects are much smaller in size than controls, although this was largely addressed by normalizations. Another limitation is the lack of genotyping for the control population, although the likely presence of some GHRD heterozygosity is expected to further strengthen the results presented. We have not corrected for multiple hypothesis testing to keep the statistical analysis simple. It is possible that after such correction, several of the marginally significant improvements seen in the GHRD group may no longer be statistically significant.

However, since we are reporting GHRD to have neutral to positive effects on CVD risk, such changes would not affect our general conclusions.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- METHOD DETAILS
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2024.03.022.

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The graphical abstract created in BioRender.com. The study was approved by the Institutional Review Board at the University of Southern California, Los Angeles, USA (HS-14-00127 CR002) and the Ethics Committee of the Institute IEMYR, Quito, Ecuador. All participants provided written informed consent.

AUTHOR CONTRIBUTIONS

V.D.L., J.G.-A., and P.B. designed the study. J.G.-A., V.D.L., and P.B. supervised the study. J.G.-A., C.G., A.V., A.G., G.P., and D.L. contributed to patient recruitment and supervision. J.G.-A., M.C., C.G., A.V., A.G., G.P., and D.L. contributed to data collection. A.M., M.C., and P.B. performed data analysis and prepared tables and figures. A.M. and M.C. performed statistical analyses. A.M. and V.D.L. had unrestricted access to all data. A.M., M.C., J.G.-A., and V.D.L. wrote the paper. J.J.K. contributed to the interpretation and review and editing of the manuscript. All authors read and approved the final article and take responsibility for its content.

DECLARATION OF INTERESTS

V.D.L. has equity interest in L-Nutra, which develops and sells medical food for the prevention and treatment of diseases.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
GraphPad Prism 9.4	GraphPad Software	https://www.graphpad.com/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Valter D. Longo (vlongo@usc.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The patient data reported in this study cannot be deposited in a public repository because of patient privacy concerns. To request access to deidentified patient data, please contact Prof. Valter D. Longo (vlongo@usc.edu). Deidentified data reported in this paper will be shared by the lead contact upon reasonable request and subject to approval by Institutional Review Board at the University of Southern California, Los Angeles, USA. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human studies

Participants information on sex, age, and gender was self-reported. Information on race and socioeconomic status was not collected.

Phase 1: USC Keck Hospital, Los Angeles data collection - For the preliminary study 14 GHRD subjects and 16 control relatives were invited to the USC Keck Hospital in Los Angeles for assessment of complete blood count (CBC), serum parameters, common carotid artery intima-media thickness (CIMT), and flow-mediated dilation (FMD) of the brachial artery measurement.

Phase 2: Ecuador data collection - For a more detailed assessment we collected data from a random group of adult GHRD subjects (n = 21) with GHRD from a historical Ecuadorian sample, and with homozygosity for the ss180 mutation[1] and performed a series of CV examinations. Studies included clinical history and physical examination, carotid applanation tonometry, electrocardiography, echocardiography, carotid ultrasonography, and routine laboratory tests. Depending on logistical variables, subjects were tested either at the Instituto de Endocrinología IEMYR in Quito, or at outpatient medical offices installed in the inland cities of Piñas and Balsas in the Southern province of El Oro in Ecuador. As in previous studies, age- and sex-matched LS relatives (n = 23) (ss180/WT or w/w), living under the same contextual conditions were included as controls. Electrocardiography, echocardiography, carotid ultrasonography, and laboratory tests were only performed on subjects who were able to travel to Quito (25 out of 44). The participants in this cohort

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included 23 subjects (out of 30) previously studied at USC-Keck in Los Angeles and 21 new participants.

Study approval

The Study was approved by the Institutional Review Board at University of Southern California, Los Angeles, USA, and Ethics Committee of the Institute IEMYR, Quito, Ecuador. All participants provided written informed consent.

Rodent studies

None

METHOD DETAILS

High-Resolution B-mode ultrasound carotid artery images for carotid intima-media-thickness were acquired with a Mindray M5 ultrasound imager using a linear array 7.5 MHz transducer. Baseline and reactive hyperemia brachial artery diameters were measured at baseline and at 60 s after cuff inflation. Values are expressed as mean $\pm$ SEM. Student's paired t-tests were used to assess differences between GHRD and Control values.

Carotid artery ultrasound image acquisition: High resolution B mode ultrasound carotid artery images for carotid artery intima-media thickness (CIMT) measurement were acquired with a Mindray M5 ultrasound imager using a linear array 7.5 MHz transducer. The electrocardiogram (ECG) and ultrasound images were simultaneously recorded. Subjects were placed supine and positioned in a 45° molded head block to present the optimal angle for ultrasound examination. Using B mode, the common carotid artery (CCA) was imaged in cross-section and the scanhead moved laterally until the jugular vein and the CCA were stacked with the former above the latter. In this position, the central image line passes along the common diameter of both vessels. The scanhead was then rotated around the central image line 90° maintaining the jugular vein stacked above the CCA while obtaining a longitudinal view of both vessels. In this longitudinal view, the CCA far wall is horizontal. The proximal portion of the carotid bulb was included in all images as an anatomical reference point for standardization of CIMT measurements. Stacking the jugular vein and the CCA determines a repeatable probe angle that allows the same portion of the wall to be imaged at each examination. This leads to further standardization of image acquisition and processing which in turn decreases measurement variability.[25] The minimum gain necessary for clear visualization of structures was used. Images were acquired from the carotid bulb and internal carotid artery, but the emphasis of ultrasound imaging was on the distal centimeter of the CCA because least variability occurs in this area.[26] The far wall was used for statistical purposes since the measurement of near wall thickness is less accurate.[27] Each individual's baseline image was used as a guide to match the vascular and surrounding soft tissue structures of the follow-up examinations. This is a direct visual aid method for reproducing transducer angulation designed for repeat image acquisition for longitudinal studies. These techniques provide a high degree of standardization for image acquisition and processing, resulting in a significant reduction in measurement variability between scans; inter- and intra-sonographer coefficients of variation (CVs) are less than 3%. Even in multicenter studies with multiple acquisition sites, the methodology is highly stable and reproducible with low variability, less than 3% CV.[28–30] To assure the security of data, each ultrasound examination was duplicated and processed images were electronically stored.

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The physical examination consisted of measurements of height (wall-mounted stadiometer), weight (standard scale), waist and hip circumference (metric tape), bicipital, tricipital, subscapular, and supra-iliac skinfolds (millimetric caliper). Blood pressure (BP) was determined according to international guidelines. Briefly, after having the subject rest for at least 5 min in a quiet room under a stable and comfortable temperature, the measurement was taken with an automated, validated device (Microlife BP3MS1-2D, Microlife Corporation, Berneck, Switzerland), and the mean of three consecutive BP measures was recorded. A second set of BP measurements were recorded during the pulse wave analysis to obtain real-time peripheral BP values and performing the test while assessing central BP parameters.[31]

Following a standardized protocol and using a validated device (SphygmoCor CPV system, AtCorMedical, Sydney, Australia), recordings of carotid-femoral pulse wave velocity (PWV) and pulse wave analysis were obtained by carotid applanation tonometry. Carotid-femoral PWV was calculated by dividing traveled distance by transit time (PWV = distance/time), with both parameters measured on the right side according to the previous recommendation that measurement on the right side is more robust compared to left.[32] Traveled distance was estimated by multiplying the direct distance between the carotid and the femoral arterial pulse by 0.80. The distance was measured via an upside-down infantometer, as generally recommended.[33] Pulse wave transit time was calculated as the time difference between the feet of the carotid and femoral arterial waveforms, gated to ECG. Pulse wave analysis was performed by compressing the right radial artery with the tip of the tonometer at the site of maximal pulsation, thereby generating the corresponding central waveform. Aortic systolic and diastolic BP, pulse pressure, augmented pressure and augmentation index were estimated using a validated transfer function.[33]

A resting 12 lead electrocardiogram was recorded before PWV assessment using a standard electrocardiograph apparatus. Heart rate and exclusion of rhythm disturbances or signs of left ventricular hypertrophy were determined. Echocardiography was performed at the Universidad San Francisco de Quito (USFQ) by an experienced cardiologist using a GE Vivid T8 echocardiograph apparatus. Measurements were obtained according to standard recommendations.[17] Carotid ultrasound was performed with the same ultrasound machine, and according to international standards. Maximal intima-media thickness (IMT) was determined, and the presence and degree of atherosclerotic plaques were also evaluated. Blood tests including measurement of circulating lipids, creatinine, urea, and uric acid were also performed.

QUANTIFICATION AND STATISTICAL ANALYSIS

No statistical methods were used to predetermine sample size. Group allocation was not randomized, and investigators were not blinded to allocation during experiments. Investigators were blinded during data analysis and outcome assessments. Statistical analysis was performed using GraphPad Prism 9.4 (GraphPad Software). In all analyses, $p < 0.05$ was considered statistically significant, and the significance of p values was annotated as in GraphPad (≥ 0.05 (NS), 0.01–0.05 (*), 0.001–0.01 (**), 0.0001–0.001 (***), <0.0001 (****)). Continuous variables are presented as the mean $\pm$ SD, and categorical variables as absolute frequencies and percentages. Statistically significant differences between the groups were identified using an unpaired t-test. Categorical variables were compared using Fishers' exact

test. Spearman's correlation analyses were used to determine the association of PWV and IMT with age, systolic BP, blood glucose, and total cholesterol in both study groups. HOMA-IR was calculated using the following formula: HOMA-IR = (insulin(mU/L) × glucose (mg/dL))/405.