Endocrine CONNECTIONS

Brazilian adult individuals with untreated isolated GH deficiency do not have accelerated subclinical atherosclerosis

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Abstract

GH and its principal mediator IGF1 have important effects on metabolic and cardiovascular (CV) status. While acquired GH deficiency (GHD) is often associated with increased CV risk, the consequences of congenital GHD are not known. We have described a large group of patients with isolated GHD (IGHD) due to a homozygous mutation (c.57+1G>A) in the GH releasing hormone receptor gene, and shown that adult GH-naïve individuals have no evidence of clinically evident premature atherosclerosis. To test whether subclinical atherosclerosis is anticipated in untreated IGHD, we performed a cross-sectional study of 25 IGHD and 27 adult controls matched for age and gender. A comprehensive clinical and biochemical panel and coronary artery calcium scores were evaluated by multi-detector tomography. Height, weight, IGF1, homeostasis model assessment of insulin resistance, creatinine and creatininekinase were lower in the IGHD group. Median and interquartile range of calcium scores distribution was similar in the two groups: IGHD 0(0) and control 0(4.9). The vast majority of the calcium scores (20 of 25 IGHD (80%) and 18 of 27 controls (66.6%)) were equal to zero (difference not significant). There was no difference in the calcium scores classification. None of IGHD subjects had minimal calcification, which were present in four controls. Three IGHD and four controls had mild calcification. There were two IGHD individuals with moderate calcification and one control with severe calcification. Our study provides evidence that subjects with congenital isolated lifetime and untreated severe IGHD do not have accelerated subclinical coronary atherosclerosis.

Key Words
- coronary atherosclerosis
- isolated growth hormone deficiency
- calcium score
- multi-detector CT

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Introduction

Growth hormone (GH) and its principal mediator insulin-like growth factor 1 (IGF1) have important effects not only on the acquisition of normal body size, but also on metabolic and cardiovascular (CV) status (1). GH and IGF1 have synergistic anabolic effect on muscle mass, but antagonist effects on insulin action (GH-reducing and IGF1 increasing insulin sensitivity) and lipolysis (GH increasing and IGF1 reducing it) (2). Adult onset GH deficiency (GHD)

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has been described as model of metabolic syndrome, with visceral obesity, insulin resistance, endothelial dysfunction, increased sympathetic activity, and a pro-inflammatory profile (3, 4, 5). Although GH replacement therapy has been shown to reduce the risk profile (6), it is still unclear if this is due to GHD *per se*, or to confounding factors often found in acquired GHD (other pituitary hormone deficiencies, inadequate replacements and pituitary surgery or radiotherapy) (7, 8).

A model of isolated GHD (IGHD) would be preferable to study the relationship between the GH–IGF1 axis and CV risk. In Itabaianinha county, in the northeastern Brazilian state of Sergipe, we have described a large group of subjects with familial IGHD due to a homozygous (c.57+1G>A) mutation in the GHRH receptor gene (*GHRHR*) (9). The untreated IGHD adults have proportionate dwarfism, and otherwise normal pituitary function (10). They exhibit a balance between adverse and beneficial CV risk factors. The adverse factors are increased systolic blood pressure (BP), higher fat mass percentage, increased total and LDL-cholesterol and C-reactive protein (11), visceral obesity and elevated cortisol to cortisone ratio (12). The protective factors include normal serum leptin, increased adiponectin (13) and increased insulin sensitivity (14). The adult IGHD individuals do not present premature clinical carotid (15) or aortic atherosclerosis (16) nor coronary ischemia assessed by stress echocardiography (15, 17), and have similar longevity as non-affected siblings (18). Because coronary disease presents with a long latency period, it is important to know if subclinical atherosclerosis exists in these IGHD subjects. To answer this, it is necessary to assess asymptomatic individuals (19, 20, 21). Measurement of coronary artery calcium by multi-detector tomography has been shown to predict the risk of clinical coronary disease (22, 23, 24, 25). This study tested whether subclinical atherosclerosis is accelerated in these IGHD asymptomatic individuals by comparing calcium scores in IGHD and normal controls.

Subjects and methods

Subjects

In a cross-sectional study, asymptomatic IGHD and age- and sex-matched control subjects (controls) were recruited by advertising in the local Dwarfs Association building and by word of mouth among the inhabitants of Itabaianinha. Inclusion criterion for IGHD was genotype-proven homozygosity for the c.57+1G>A *GHRHR* mutation, whereas controls were normal statured individuals proven to be homozygous for the wt *GHRHR* allele. Exclusion criteria were: previous GH treatment, age under 18 years, CV symptoms or any evidence of active CV diseases. Twenty-five (13 females) IGHD and 27 (15 females) volunteered and were enrolled. None of these IGHD individual have participated in a previous 6-month GH depot trial (17). The Federal University of Sergipe Institutional Review Board approved these studies and all subjects gave written informed consent.

Interview and physical examination

The subjects were submitted to a detailed interview including risk factors for CV disease, hypertension, smoking, dyslipidemia, familiar CV history, co-morbidities and treatments, and to a physical examination, with measurement of body weight and height. BP was obtained by the average of three measurements obtained in the left arm after 10 min of rest in the sitting position by one physicians (U M M C) using a mercury sphygmomanometer with a cuff appropriate for the size of the arm. The Framingham risk score was estimated (26).

Laboratory assessment

Blood was collected after an overnight fast. Total cholesterol, triglycerides, glucose, insulin, urea, creatinine and creatinine kinase were measured by standard techniques. The increase in total cholesterol in this IGHD cohort is due to an increase in LDL cholesterol (11, 13, 15). IGF1 was measured by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (IMMULITE 2000, Siemens Healthcare Diagnostics Products Ltd, Malvern, PA, USA), with intra- and inter-assay variabilities of 3.1 and 6.1% respectively. Insulin was measured by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (IMMULITE 2000 Siemens Healthcare Diagnostics Products Ltd), with intra- and inter-assay variabilities of 4.2 and 5.1% respectively. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMAIR) with the formula: fasting serum insulin (μlU/ml) x fasting plasma glucose (mmol/l)/22.5. All the tests were carried out in the Laboratory of University Hospital of the Federal University of Sergipe, in Aracaju, Sergipe.

Coronary tomography for calcium score

Calcium score was calculated with the use of a 64-slice multi-detector tomography scanner (Siemens Somatom Definition AS) dedicated to ECG-synchronized cardiac

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studies, for a non-contrast ECG-triggered acquisition. Syngovia software (Siemens Healthcare Global) was employed to acquire the Agatston score. All computed tomographies were analyzed by a single reader. The classification of calcium score followed the American College of Cardiology/American Heart Association Task Force criteria. Calcium score >300=severe calcification, 101–300=moderate calcification, 11–100=mild calcification, 1–10=minimal calcification and 0=absence of calcification (25).

Statistical analysis

Data are expressed as mean (s.d.), except for IGF1, insulin, HOMAIR and calcium scores expressed as median (interquartile range). Student's *t*-test was used for variables with normal distribution, and Mann–Whitney *U*-test for variables without Gaussian distribution (IGF1, insulin, HOMAIR and calcium scores). Fisher's exact test was used to analyze classification of CAC sores and percentage of previous smoking, high BP and diabetes. Statistical analysis was performed using the statistical software SPSS 19.0 version. *P* values under 0.05 were considered significant.

Results

Table 1 shows the clinical and biochemical features of the two groups. Height, weight, IGF1, insulin, HOMAIR, creatinine, creatininekinase and were lower in IGHD group.

Table 1 Clinical and biochemical features of IGHD and control subjects.

	IGHD (25)	**Control** (27)	***P***
Age (years)	50.1 (15.9)	51.1 (14.0)	0.799
Sex (F/M)	13/12	15/12	0.764
Smoking	1	2	1
Weight (kg)	39.3 (8.7)	71.8 (14.4)	<0.0001
Diabetes history	4	2	0.411
Dislipidemia history	11	11	1
Arterial hypertension	8	16	0.058
Height (m)	1.2 (0.18)	1.6 (0.1)	<0.0001
BMI (kg/m^2)	25.5 (5.7)	26.7 (4.2)	0.402
Systolic BP (mmHg)	122.1 (18.9)	124.4 (15.0)	0.622
Diastolic BP (mmHg)	78.8 (9.3)	80.4 (6.5)	0.486
IGF1 (ng/ml)	1.9 (0)	132 (55)	<0.0001
Glucose (mg/dl)	105.5 (22.7)	105.9 (70.5)	0.975
Insulin (mUI/ml)	5.35 (3.8)	17.3 (10.9)	<0.0001
Homa IR	1.4 (1.0)	4.3 (3.1)	0.012
Cholesterol (mg/dl)	227.0 (65.1)	210.7 (28.2)	0.257
Tryglicerides (mg/dl)	142.0 (95.5)	135.3 (51.3)	0.762
Urea (mg/dl)	38.6 (9.8)	34.7 (9.0)	0.143
Creatinine (mg/dl)	0.7 (0.1)	0.9 (0.2)	<0.0001
Creatininekinase (IU/l)	82.0 (35.1)	152.8 (141.0)	0.017
Framingham score	5.7 (6.4)	4.9 (4.5)	0.596

Table 2 Classification of calcium scores in IGHD and controls.

Calcium score	**IGHD** (25)	**Controls** (27)
0 (absent)	20	18
1–10 (minimal)	0	4
11–100 (mild)	3	4
101–300 (moderate)	2	0
>300 (severe)	0	1

Table 2 shows the calcium scores. The distribution expressed as median and interquartile range was similar in the two groups: IGHD 0(0) and control 0(4.9). The vast majority of the CAC scores (20 out 25 IGHD (80%) and 18 out 27 controls (66.6%)) were equal to zero, p=0.354. The highest CAC scores were 128.1 in IGHD and 331.5 in controls. Two subjects were further evaluated. A 66-year-old IGHD female (with compliant of chest pain) underwent CT angiography and coronary angiogram, and a clinical three arterial lesion was found. The second, a 61-year-old control male exhibited normal exercise stress echocardiography. Both were recommended lifestyle modifications and statin use.

Discussion

Acquired adult-onset GHD is a model of metabolic syndrome, with abdominal obesity, insulin resistance and dyslipidemia (3, 4, 5). Nevertheless, the relationship between GHD and obesity includes several mechanisms and including some degree of GH resistance in obesity (27). Although GH replacement therapy has been shown to reduce this risk profile (6), it is still unclear if the high risk is due to untreated GHD, or to confounding factors often found in acquired GHD. Some of these factors such as hypogonadism, excessive glucocorticoid replacement, craniopharyngioma, radiotherapy (implicated in cerebrovascular mortality or *de novo* brain tumors) and very recently adrenal crisis (7, 8, 28, 29, 30, 31, 32, 33) have been implicated in the increased mortality risk associated with hypopituitarism (28). Accordingly, very recent national or multicenter studies have suggested that GHD deficiency in the context of hypopituitarism does not cause an increase in CV mortality despite increased general mortality risk (8, 29, 30).

As our previous studies indicated that this IGHD group has no premature clinical atherosclerosis (11, 15, 16, 17), we decided to assess subclinical atherosclerosis, by measuring the calcium content in the coronary arteries. Calcium score is an independent predictor of mortality in a multivariable model controlling for age, gender,

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ethnicity, and cardiac risk factors, and has been shown to provide additional risk prediction beyond the Framingham risk score in individuals with low and high CV risk (22, 23). Most of our IGHD and controls present with calcium score of zero. In subjects with positive values, the majority had mild calcification. There were two IGHD individuals with intermediate calcification (one of them underwent coronary angiography that showed non-critic lesions), and one control with severe calcification (had no symptoms, underwent to resting and exercise echocardiography that showed no ischemia). The lack of subclinical atherosclerosis corroborates our previous finding that congenital IGHD is not a cause of CV mortality (17).

The calcium scores of IGHD individuals expressed as percentage of the negative scores and the median and interquartile range (80% and 0(0)) are very similar to ones of a Portuguese cohort (Lisbon) of asymptomatic normal individuals (82% and 0(0)). Our control values (66.6% and 0(4.9)) were similar to the ones observed in area of southern Brazil (Sao Paulo) (54% and 1(0.68)), and lower than the ones observed in mainland USA (Columbus, Ohio and Nashville, Tennessee) (46% and 4(0.87)), in pooled genders (34). Because the state of Sergipe is one of the regions where the Portuguese colonization of Brazil had begun, 60% of the population is of Portuguese origin (35). It is possible that the Portuguese ancestry influenced the calcium scores in both IGHD and controls. However, as calcium scores parallel the CV mortality rates in the three nations (34), we can conclude that IGHD subjects have an atherosclerosis risk that is similar to non-GHD asymptomatic normal individuals form the same region.

Both IGHD and control groups have low Framingham risk scores, despite the fact that the IGHD group is exposed to higher burden of classical CV risks throughout life (in this particular sample the prevalence of hypertension was not different between the two groups). One explanation for this finding is the increased insulin sensitivity of IGHD subjects despite visceral adiposity (12). Another possible explanation lies on the degree of IGF1 reduction. It is possible that different degrees of IGF1 deficiency may result in different effects on the vascular wall (15). Mild IGF1 reduction in the general population has been linked to increased risk ischemic heart disease (36). Conversely, very severe IGF1 reduction, as found in our IGHD model, may be protective against atherosclerosis. This fits with the increase longevity of animal models of GHD or GH resistance (37). Accordingly, when we treated a subset of these patients with submaximal doses of GH for 6 months, converting a severe in a mild IGF1 deficiency we observed the appearance of atherosclerotic plaques (17).

Calcium scores of patients with IGHD due to large homozygous deletions in the GH-1 gene, or with GH resistance (Laron dwarfism) are not available to compare the frequency of subclinical atherosclerosis in these models with ours. Longevity, influenced not only by clinical atherosclerosis, but also from other conditions such as cancer, infectious diseases and accidents, seems to be reduced in the former model (38), but is not in the latter (39). Differently from subjects with large deletions in the GH-1 gene (complete lack of GH secretion) and Laron dwarfism (lack of GH action), our IGHD subjects have very low, but detectable serum GH levels (10). This residual GH secretion may exert some metabolic actions, resulting in differences between these disease models.

One limitation of our study is that the age of our sample is relatively young. However, near 80% of the IGHD subjects are ≧35 years, age sufficient in other studies to identify individuals who have subclinical atherosclerosis when CV risk factors are present (40, 41). It is also possible that subjects with worse health status would not have volunteered.

Other factors that may influence the development of atherosclerosis include environmental factors and dietary habits. Both GHD and controls reside in the same area, which is becoming progressively more urbanized. While we have recently reported that GHD subjects consume in percentage more proteins, less carbohydrates and equal amount of lipids in comparison with the controls (42), it is difficult to predict the role of such dietary differences on the development of subclinical coronary atherosclerosis.

In conclusion, subjects with congenital isolated lifetime and untreated severe IGHD do not have accelerated subclinical coronary atherosclerosis when compared with the normal controls from the same region.

Declaration of interest
R Salvatori serves in the advisory board of Novo Nordisk, Novartis and Pfizer.

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