Arrest of atherosclerosis progression after interruption of GH replacement in adults with congenital isolated GH deficiency


Department of Medicine, Federal University of Sergipe, Aracaju 49060-100, Brazil, 1Sidney Kimmel Comprehensive Cancer Center and 2Division of Endocrinology, Department of Medicine, The Johns Hopkins University School of Medicine, 1830 East Monument Street, Suite 333, Baltimore, Maryland 21287, USA

(Correspondence should be addressed to R Salvatori; Email: salvator@jhmi.edu)

Abstract

Objective: GH replacement therapy (GHRT) in adult-onset GH deficiency (AOGHD) reduces carotid intima-media thickness (IMT) and increases myocardial mass, with improvement of systolic and diastolic function. These observations have reinforced the use of GHRT on AOGHD. Conversely, we have previously reported that in adults with lifetime congenital and severe isolated GH deficiency (IGHD) due to a mutation in \textit{GHRHR} gene, a 6-month treatment with depot GH increased carotid IMT, caused the development of atherosclerotic plaques, and an increase in left ventricular mass index (LVMI), posterior wall, and septal thickness and ejection fraction. Such effects persisted 12 months after treatment (12-month washout – 12mo).

Methods: We have studied the cardiovascular status (by echocardiography and carotid ultrasonography) of these subjects 60 months after completion of therapy (60-month washout – 60mo).

Results: Carotid IMT reduced significantly from 12 to 60mo, returning to baseline (pre-therapy) value. The number of individuals with plaques was similar at 12 and 60mo, remaining higher than pre-therapy. LVMI, relative posterior wall thickness, and septum thickness did not change between 12 and 60mo, but absolute posterior wall increased from 12 to 60mo. Systolic function, evaluated by ejection fraction and shortening fraction, was reduced at 60mo in comparison with 12mo returning to baseline levels. The \textit{E/A} wave ratio (expression of diastolic function) decreased at 60mo compared with both 12mo and baseline.

Conclusions: In adults with lifetime congenital IGHD, the increase in carotid IMT elicited by GHRT was transitory and returned to baseline 5 years after therapy discontinuation. Despite this, the number of subjects with plaques remained stable at 60mo and higher than at baseline.

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Introduction

GH deficiency (GHD) in adults has been associated with increased cardio- and cerebrovascular mortality (1, 2). This has been attributed to premature atherosclerosis, possibly due to high levels of total and LDL, C-reactive protein (CRP), and abdominal obesity and insulin resistance (3). Accordingly, increased intima-media thickness (IMT) (4) and impaired cardiac performance have been reported in patients with adult-onset GHD (AOGHD) (5). However, most of this literature is based on the studies on AOGHD of different severities and etiologies, often confounded by surgery or radiation, and other associated pituitary deficiencies with multiple hormonal replacement therapies.

We have reported a large extended pedigree from Itabaianinha County (Northeast Brazil) with ~100 affected individuals (over several generations) with congenital isolated GHD (IGHD), due to a homozygous null mutation in the splice donor site of intron 1 (c.57+1G>A) of the \textit{GHRH} receptor gene (\textit{GHRHR}) (6). These individuals have very low serum GH and insulin-like growth factor 1 (IGF1), reduced fat-free mass, increased percentage of fat mass, total and LDL, CRP levels, systolic blood pressure (SBP), and waist:hip ratio. Despite these cardiovascular risk factors, they have no premature atherosclerosis, no insulin resistance, and normal longevity (7, 8, 9, 10, 11).

Differently from AOGHD patients, in whom GH replacement therapy (GHRT) improves carotid IMT and cardiac performance (5), we have found that in these adult IGHD individuals, a 6-month treatment with depot GHRT, despite beneficial effects on metabolic
and body composition parameters, caused worsening of atherosclerosis. We noted an increase in IMT, and in the number of individuals with atherosclerotic plaques (from only one at baseline to five after GH and to fourteen 12 months after completion of therapy), together with an increase in left ventricular mass index, posterior wall, and septum thickness, and a late increase in SBP that persisted at the 12-month washout (12).

The aim of this study was to monitor the status of carotid artery walls, cardiac morphology and function, and blood pressure 60 months after termination of GHRT.

**Subjects and methods**

**Subjects**

Twenty adult GH-naïve IGHD subjects from Itabianinha homozygous for the c.57+1G>A GHRHR mutation (10 men: age: 46 ± 14.5 years; height, 122.1 ± 7.7 cm; weight, 36.7 ± 5.4 kg, body mass index (BMI), 24.8 ± 4.3) were previously studied at baseline, after 6 months of bimonthly depot GH (Nutropin Depot; post-GH), and after 6 and 12 months of washout (6 and 12mo). The initial and final doses were 0.33 and 0.38 mg/kg in women and 0.25 and 0.35 mg/kg in men respectively. This dosing was based on the previous work showing an increase in both GH and IGF1 lasting 14–17 days in adults after a single 0.25 or 0.5 mg/kg dose (13). A similar starting dose (0.3 mg/kg) was used in the only clinical trial that has shown efficacy of this depot GH preparation in adults (14). The average daily dose per body weight was 27.1 and 25 μg/kg per day in women and men respectively. In this study, the cohort was examined at 60-month (60-mo) washout time. One patient died before the 60-mo time point due to respiratory disease (likely pneumonia) without a final autopsy diagnosis. Because of this, the group size was restricted to 19 individuals at the 60-mo time point. Both the Federal University of Sergipe and The Johns Hopkins University institutional review boards approved these studies, and all subjects signed informed consent.

**Study protocol**

**Anthropometric and blood pressure measurements**

Height and body weight were measured and BMI was calculated using the formula: weight (kilograms)/height (meters)$^2$. BP was registered as the mean value of three measurements after 10 min in seated position.

**Laboratory assessment**

Total cholesterol, triglycerides, and glucose were measured by the enzymatic Trinder colorimetric test. IGF1 was measured by an immunoradiometric assay, with double extraction and an assay sensitivity of 0.8 ng/ml (5600; Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The intra- and interassay variabilities were 2.25 and 2.6% respectively.

**Assessment of carotid IMT**

Longitudinal ultrasonography scans of the carotid arteries were performed by the same trained observer as described previously (12). Atherosclerotic plaque was defined as a localized thickening ≥1.3 mm, which did not uniformly involve the whole carotid with or without flow disturbance (12).

**Resting echocardiography**

Echocardiography studies were performed with a commercial machine (HP-Sonos 5500; Hewlett-Packard Co., Palo Alto, CA, USA) according to the standard procedures. M-mode echocardiography of the left ventricle (LV) was performed according to the American Society of Echocardiography recommendations. Only frames with optimal visualization of interfaces and simultaneously visible septum, LV internal diameters, and posterior wall were used for calculations. LV mass was calculated and normalized according to body surface area and height. Relative wall thickness was calculated as ($2 \times$ posterior wall thickness)/LV internal radius (12).

**Statistical analyses**

Anthropometric measurements, blood pressure, carotid IMT, and resting echocardiography measurements are expressed in mean (s.d.), except IGF1 levels expressed in median (interquartile range).

Because in the original study the maximal effects on BP, IMT, and cardiac morphology were found at 12mo, here we were specifically interested in comparing 60 to 12mo. All measurements at all time points were modeled using linear regression models with generalized estimating equations to account for the correlation between measures within individuals. The contrast between 60 and 12mo was estimated from the model with its corresponding 95% confidence interval. Analyses were completed using software SPSS/PC 16.0 (SPSS, Inc., Chicago, IL, USA) and R version 2.13.1 (http://www.r-project.org). P values are shown, not adjusted for multiple comparisons.

**Results**

Weight, BMI, glucose, total cholesterol, triglycerides, and IGF1 did not change from 12- to 60-mo washout (Table 1). In contrast, IMT reduced from 12 to 60mo ($P=0.03$) returning to the baseline values. At 12mo, 14 of 20 individuals had carotid plaques. Subsequently, one more individual presented a plaque, and in another the plaque disappeared. With the death of an individual with a plaque before the 60-mo time point, the number of individuals with plaques was not different at 60mo (13/19) vs at 12mo (14/20).
SBP reduced from 12 to 60mo (P = 0.04), returning to the baseline values. Diastolic blood pressure (DBP) did not change from 12 to 60mo, remaining higher at 60mo in comparison with baseline (P = 0.04). Among the parameters of cardiac mass, no significant difference in LV mass index, relative posterior wall thickness, and septum thickness was observed at 60 vs 12mo, but absolute posterior wall increased from 12 to 60mo, (P < 0.001). Both the parameters of systolic function (ejection fraction and shortening fraction) were reduced at 60mo in comparison with 12mo (P = 0.05 and 0.04 respectively) and were no different from baseline. Of the parameters of diastolic function (E/A (tissue Doppler-derived diastolic velocities) and E'/A' wave ratios), only E/A was decreased at 60mo in comparison with 12mo (P = 0.006), and it was lower than that in baseline value (P = 0.002).

### Discussion

Our previous data show that in adult patients with lifetime untreated IGHD, a 6-month treatment with GH has reversible beneficial effects on body composition and metabolic profile. The beneficial effects on body composition and metabolic profile were also demonstrated in children and adolescents together with a good somatic growth response (15). Despite these positive effects, GHRT in IGHD adults caused a progressive increase in IMT, SBP, and in the number of individuals with atherosclerotic carotid plaques (from one to 14) up to 12 months after GHRT completion (12). Now we show that SBP and IMT reduced from 12 to 60mo, both returning to the baseline values, despite 5 and 1/2 years of aging, and despite cholesterol levels at 60mo being no different from 12mo. Both SBP and IMT reductions (with return to pre-GHRT level) from 12 to 60mo can be attributed to GH withdrawal and disappearance of its effect with time.

Our original findings contrasted with several reports of increased arterial wall thickness in untreated AOGHD (5, 16), and its reduction caused by GHRT (17, 18), and stresses the differences that exist between AOGHD and congenital IGHD. This follow-up study shows that the detrimental effect on carotid IMT is not permanent, similar to the positive effect of GHRT in AOGHD subjects, in whom it increases within 6 months from GH withdrawal (17). We also found that the number of individuals with atherosclerosis plaques did not further increase with longer follow-up, but in one individual the plaque disappeared, showing that despite some conflicting data (19, 20), it is possible not only to reduce vascular wall volume but also to decrease plaques’ volume as shown with some lipid-lowering agents (21, 22). This occurred despite atherogenic conditions, such as abdominal obesity, increased SBP, LDL, and CRP (8, 9, 10).

The cause(s) of the mirror effect of GHRT and GH withdrawal in our IGHD patients when compared with AOGHD can be only speculated. Our patients have extremely low IGF1 levels throughout life, while AOGHD patients have normal levels until they develop GHD, and their IGF1 decreases rarely become extreme. IGF1

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**Table 1** Blood pressure, laboratory, carotid intima-media thickness and resting echocardiographic data in isolated GH-deficient subjects at baseline (n = 20), after 6 months of GH (n = 20), after 12 months (12mo, n = 20), and 60 months (60mo, n = 19) of washout. Values are means (s.d., except for IGF1 levels expressed in median (interquartile range)). Statistics refer to comparison between 12 and 60mo.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Post-GH</th>
<th>12mo</th>
<th>60mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>37.16 (4.85)</td>
<td>36.91 (3.95)</td>
<td>36.57 (4.86)</td>
<td>38.94 (5.74)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 (4.31)</td>
<td>24.75 (3.23)</td>
<td>24.51 (3.68)</td>
<td>26.14 (4.61)</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>95.74 (12.63)</td>
<td>92.11 (10.36)</td>
<td>98.68 (10.11)</td>
<td>102.56 (40.52)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>212.11 (53.91)</td>
<td>185.05 (37.92)</td>
<td>209.42 (50.65)</td>
<td>234.56 (58.59)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>159.05 (105.11)</td>
<td>129.21 (101.52)</td>
<td>140.79 (90.91)</td>
<td>129.61 (57.74)</td>
</tr>
<tr>
<td>IGF1 (ng/ml)</td>
<td>1.0 (0)</td>
<td>27 (21.5)</td>
<td>1.0 (0)</td>
<td>1.0 (0)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>119.84 (18.09)</td>
<td>129.26 (27.06)</td>
<td>134.53 (23.92)</td>
<td>121.25 (15.22)*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>75.32 (11.84)</td>
<td>80.53 (10.68)</td>
<td>78.63 (9.8)</td>
<td>81.56 (9.26)</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.6 (0.10)</td>
<td>0.68 (0.14)</td>
<td>0.8 (0.17)</td>
<td>0.68 (0.15)*</td>
</tr>
<tr>
<td>LVM (g/m²)</td>
<td>62.54 (16.14)</td>
<td>72.57 (15.74)</td>
<td>76.84 (15.57)</td>
<td>79.9 (17.72)</td>
</tr>
<tr>
<td>RWT (%)</td>
<td>26.79 (4.87)</td>
<td>32.55 (4.06)</td>
<td>32.3 (4.29)</td>
<td>32.8 (3.52)</td>
</tr>
<tr>
<td>PWT (cm)</td>
<td>0.56 (0.11)</td>
<td>0.68 (0.08)</td>
<td>0.67 (0.07)</td>
<td>0.74 (0.07)**</td>
</tr>
<tr>
<td>EDD (cm)</td>
<td>4.14 (0.24)</td>
<td>4.19 (0.34)</td>
<td>4.19 (0.43)</td>
<td>4.24 (0.38)</td>
</tr>
<tr>
<td>SWT (cm)</td>
<td>0.6 (0.12)</td>
<td>0.73 (0.09)</td>
<td>0.68 (0.1)</td>
<td>0.72 (0.1)</td>
</tr>
<tr>
<td>E/A</td>
<td>1.36 (0.63)</td>
<td>1.32 (0.52)</td>
<td>1.24 (0.45)</td>
<td>0.88 (0.37)**</td>
</tr>
<tr>
<td>E'/A'</td>
<td>1.07 (0.38)</td>
<td>1.01 (0.42)</td>
<td>1.06 (0.47)</td>
<td>0.88 (0.37)</td>
</tr>
<tr>
<td>EF (%)</td>
<td>0.69 (0.05)</td>
<td>0.69 (0.03)</td>
<td>0.71 (0.04)</td>
<td>0.69 (0.04)*</td>
</tr>
<tr>
<td>SF (%)</td>
<td>38.11 (3.75)</td>
<td>38.25 (2.26)</td>
<td>40.75 (3.48)</td>
<td>38.65 (2.97)*</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; IMT, intima-media thickness; LVM, left ventricle mass index; RWT, relative posterior wall thickness; PWT, posterior wall thickness; EDD, end diastolic diameter; SWT, septal wall thickness; E/A, E/A ratio; E'/A', E'/A' ratio; EF, ejection fraction; SF, shortening fraction. *P < 0.05; **P < 0.01; ***P < 0.001.
has a double influence on the atherosclerotic physiopathology, inducing vasodilatation and promoting atherosclerosis (23). It is possible that different degrees and duration of GHD may result in different effects on the vascular wall, with very severe IGFI deficiency protecting against and milder deficiency accelerating atherosclerosis. Our GHRT depot schedule may have transformed our subjects in a ‘partial’ AOGHD-like condition, and 60 months of washout brought the atherosclerosis to the indolent rhythm typical of this IGHD cohort. The number of treated individuals is small and it is therefore unlikely that in the future we will be able to determine whether these changes translate in a prevalence of cerebrovascular accidents that is different from the never-treated IGHD subjects from the same cohort.

The reported effects of GH replacement on cardiac performance are conflicting (24, 25). A meta-analysis reported an increase in myocardial mass and improvement in systolic and diastolic functions, increase in LV mass, septal thickness, left ventricular posterior wall thickness, and end diastolic volume caused by GHRT in AOGHD (25). On the other hand, no significant changes have been reported in fractional shortening, left ventricular end systolic diameter, and E/A ratio (25). Our study shows that the increase in all the LV mass parameters caused by GHRT persisted at 60mo, suggesting permanent structural changes and showing that even a relatively short-term GHRT may change cardiac anatomy well beyond the end of therapy. We have found a similar long-term effect on thyroid volume, at least at 12mo (26), suggesting that both heart and thyroid are very sensitive to GH replacement. This is different from acromegaly patients, in whom control of GH/IGFI excess is associated with normalization of LV mass and LV dimensions (27). Although the changes in ejection fraction between 60 and 12mo are probably not clinically relevant, systolic function, in agreement with other studies (6, 17), improved with GH therapy and returned to baseline values after 60 months of washout, suggesting a nonanatomically mediated effect of GH on systolic function.

E/A wave ratio did not change after GH treatment but decreased at 60mo, in comparison with baseline. E/A wave ratio is more impaired in clinically controlled than in surgically cured acromegaly patients, suggesting that diastolic dysfunction may occur even with subtle excess of GH secretion (27). This worsening during late GHRT washout may be attributed to aging, as it was accompanied by a simultaneous increase in DBP.

Our study has some limitations, particularly related to the possible confounding effects of aging. However, the fact that IMT returns to pre-treatment levels goes against an age effect. Secondly, our data were generated in middle-aged adults with genetic IGHD at a dose of depot GH only used once previously in adults (13). This may have resulted in a relatively high daily dose per body weight, although this is unlikely, as serum nadir IGFI never actually normalized throughout the study (12). The depot form may have exposed tissues to constant levels of GH, resembling a mild form of acromegaly. Withdrawal from such treatment could therefore be beneficial and account for some of the cardiovascular variables that are improved after 60-month observation. Recently, it has been reported that GH treatment in very low dose (approximately one-tenth of the one we have used) enhances insulin sensitivity with no apparent effects on body composition, lipolysis, and other surrogate cardiovascular risk markers in adults with severe acquired GHD (28). Low-dose short-acting GH regimen could have different vascular effects. Finally, we had found that HDL had increased post-GH but had reduced at 12mo to a lower level than baseline. Regrettably, we do not have fractionated cholesterol data at 60mo to determine whether changes in HDL may have contributed to reversal or arrest of the negative effects of GHRT.

In conclusion, in adults with congenital, lifetime severe IGHD, long-term observation after withdrawal of a 6-month sub-maximal GHRT shows arrest of progression of atherosclerosis and reversal of carotid IMT increase but not of plaques. Similarly, heart function benefits are reversible. Conversely, changes in heart anatomy are not reversible.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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