Growth Hormone Treatment on Atherosclerosis: Results of a 5-Year Open, Prospective, Controlled Study in Male Patients with Severe Growth Hormone Deficiency

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Background: Severe GH deficiency (GHD) is associated with, increased cardiovascular risk and intima-media thickness (IMT) at major arteries.

Objective: The objective of the study was to investigate the 5-yr effects of GH replacement on common carotid IMT and insulin resistance syndrome (IRS) (at least two of the following: triglycerides levels ≥ 1.7 mmol/liter, high-density lipoprotein-cholesterol levels ≤ 1.0 mmol/liter, blood pressure above 130/85 mm Hg, fasting glucose 6.1–7 or 2 hr after glucose 7.7–11.1 mmol/liter).

Design: This was an interventional, open, prospective, controlled study.

Patients: Patients included 35 men with severe GHD and 35 age-matched healthy men as controls.

Intervention: All patients received standard replacement therapy; GH replacement was added in 22 patients (group A) and refused by 13 others (group B).

Measurements: Five-year changes in IMT and IRS prevalence were measured.

Results: At baseline, IMT was higher in the patients with (P < 0.001) and without IRS (P = 0.004) than in controls. Eighteen patients (51.4%) and two controls (5.7%; P < 0.0001) had IRS. At study end, use of lipid-lowering drugs (92.3% vs. 13.6 and 34.3%; P < 0.0001), glucose-lowering drugs (69.2% vs. 31.4 and 22.7%; P = 0.016), and antihypertensive drugs (61.5% vs. 20.0 and 4.5%; P < 0.0001) was higher in group B patients than controls and group A patients. IGF-I levels normalized in all group A patients and remained lower than −1 SD score in 77% of group B patients. IMT significantly decreased only in group A and significantly increased in controls and nonsignificantly in group B patients. IRS prevalence significantly reduced only in group A patients.

Conclusions: Severely hypopituitary GHD men have more frequently increased IMT at common carotid arteries and IRS than controls. After 5 years, only in GH replaced patients, IMT and prevalence of IRS decreased. (J Clin Endocrinol Metab 93: 3416–3424, 2008)
body mass, total and low-density lipoprotein cholesterol levels, and diastolic blood pressure (6). It is accepted that management of dyslipidemia is crucial in primary and secondary prevention of cardiovascular disease and part of the excess vascular risk associated with hypopituitarism is likely to be due to dyslipidemia (7). Besides, GH replacement also induces improvement in cardiovascular markers (8), cardiac performance (9), and surrogate parameters of atherosclerosis such as IMT at major arteries (10–12). Two years of GH replacement was shown to be, however, not adequate to normalize IMT levels at common carotid arteries (13).

We designed this 5-yr observational, prospective, controlled study to verify the likelihood to reverse early atherosclerosis in severe GHD patients. Only men aged 50 yr or younger and with severe GHD were enrolled to avoid gender and aging interference (13). Main outcome measure was IMT at common carotid arteries; secondary measure was prevalence of insulin-resistance syndrome (IRS) according with the American College of Endocrinology (ACE) (14).

Subjects and Methods

Design overview

This is an interventional, open, prospective, controlled study. The inclusion criteria were: 1) male gender; 2) age younger than 50 yr; 3) body mass index less than 30 kg/m²; 4) no familial or personal history of cardiovascular diseases; 5) no concomitant treatment with drugs known to interfere with glucose or lipid metabolism or to influence body mass, total and low-density lipoprotein cholesterol levels, and diastolic blood pressure at the time of study entry to have insights on the known to interfere with glucose or lipid metabolism or to influence body mass, total and low-density lipoprotein cholesterol levels, and diastolic blood pressure; 6) no familial or personal history of cardiovascular disease and part of the excess vascular risk associated with hypopituitarism is likely to be due to dyslipidemia (7). Besides, GH replacement also induces improvement in cardiovascular markers (8), cardiac performance (9), and surrogate parameters of atherosclerosis such as IMT at major arteries (10–12). Two years of GH replacement was shown to be, however, not adequate to normalize IMT levels at common carotid arteries (13).

We designed this 5-yr observational, prospective, controlled study to verify the likelihood to reverse early atherosclerosis in severe GHD patients. Only men aged 50 yr or younger and with severe GHD were enrolled to avoid gender and aging interference (13). Main outcome measure was IMT at common carotid arteries; secondary measure was prevalence of insulin-resistance syndrome (IRS) according with the American College of Endocrinology (ACE) (14).

Setting and participants

All patients and controls were recruited at the Department of Molecular and Clinical Endocrinology and Oncology, University “Federico II” of Naples, Italy.

Patients

Forty-four adult male patients (aged 25–50 yr) with partial or complete hypopituitarism and severe GHD were enrolled in this open prospective study from January 1, 1997, to December 31, 2001 (Fig. 1). Two patients refused to participate, and seven patients discontinued the study. Thus, the study population consists of 35 patients, all having previously undergone surgery for pituitary tumors; nine patients had been irradiated. At study entry, GHD was diagnosed by a GH peak less than 9 μg/liter after arginine plus GHRH test (15). GHD was associated with other hormone deficiencies (Table 1). Hormone replacement therapy with testosterone enanthate (250 mg im monthly), l-thyroxine (75–125 μg orally daily), cortisone acetate (25–37.5 mg/d), and 1-desamino-8-D-arginine vasopressin (5–20 μg/d) was given where appropriate. Adequacy of hormone replacement therapy was periodically assessed by serum-free thyroid hormones, testosterone, serum Na⁺ and K⁺ measurements, and blood pressure. According to previous studies (16–19), the duration of GHD was calculated from the time of diagnosis of the pituitary tumor and was 7.5 ± 3.2 yr (mean ± SD, median 8 yr).

Controls

Thirty-five healthy males, among clerks, and medical and paramedical personnel of the Department of Molecular and Clinical Endocrinology and Oncology of the University “Federico II” of Naples, matched for sex, age (± 1 yr), and body mass index (BMI) (± 1 kg/m²) with the patients agreed to participate in this study and were used as controls. Exclusion criteria for controls were the same of the patients. All patients and controls gave their informed consent to participate in this study that was designed in accordance with the Helsinki II Declaration on human experimentation. Seventeen patients and 18 controls were nonsmokers, two and three were ex-smokers, 16 and 14 were mild smokers (<15 cigarettes/d), and all had a sedentary lifestyle.

Randomization and Interventions

This is a non randomized, open, prospective study that was approved by the Ethical Committee of the University Federico II of Naples. The patients were divided into two groups: A) patients receiving replacement including GH and B) patients receiving replacement excluding GH. Reasons for not giving GH replacement were: 1) very large tumor remnants (n = 4), 2) patient refusal (n = 4), 3) elevated PSA levels at screening (n = 1), and 4) familial colon polyps (n = 1). According to previous studies (12, 13, 20, 21), recombinant GH (Genotropin; Pfizer, Rome, Italy) was given to all patients of group A, at the starting dose of 4–5 μg/kg/d. Subsequently dose titration was performed to maintain IGF-I levels in the range of the 25th to 75th percentile [0.5–1.5 SD score (SDS)] of normality for sex and age throughout the study period. Randomization was not allowed because it was unethical.

Outcomes and follow-up

At study entry, all subjects underwent measurement of serum IGF-I, total- and high-density lipoprotein (HDL)-cholesterol, triglycerides, glucose, and insulin level as fasting and after glucose load (75 g of glucose diluted in 250 ml of saline solution), systolic and diastolic blood pressure measured at the right arm, with the subjects in relaxed sitting position; the average of six measurements was used (13, 19–21) and common carotid arteries ultrasonography [Vingmed Sound CMF 725 equipment (Horten, Norway) using a 7.5 MHz annular phased array transducer]. Details on the technique were reported elsewhere (12, 13, 16).
Presence, location, and size of plaques were also evaluated at the level of common carotid arteries (22). All IMT measurements were made by a single investigator (S.S.) who was blind in respect to the patient or the control examination as well as the patient status to be GH replaced or not. The IMT measurement variability for our instrument was 0.03 mm; our intraobserver variability for repeated measurements of carotid artery diameter is 0.01 ± 0.02 mm.

The conversion factors (milligrams per deciliter to millimoles per liter) for lipids and glucose were as follows: cholesterol 0.02586, triglycerides 0.01129, and glucose 0.05551.

All parameters and carotid ultrasonography were reevaluated after 12, 36, and 60 months in the patients and after 60 months in the control examination as well as the patient status to be GH replaced or not. The IMT measurement variability for our instrument was 0.03 mm; our intraobserver variability for repeated measurements of carotid artery diameter is 0.01 ± 0.02 mm.

The conversion factors (milligrams per deciliter to millimoles per liter) for lipids and glucose were as follows: cholesterol 0.02586, triglycerides 0.01129, and glucose 0.05551.

All parameters and carotid ultrasonography were reevaluated after 12, 36, and 60 months in the patients and after 60 months in the controls.

At study entry and end, in patients and controls we examined the prevalence of IRS (14) based on the presence of at least two criteria of the following: triglycerides levels ≥1.7 mmol/liter or greater, high-density lipoprotein (HDL) cholesterol levels 1.0 mmol/liter or less, blood pressure above 130/85 mm Hg, or fasting glucose 6.1–7 mmol/liter or 2 h after oral glucose tolerance test between 7.7 and 11.1 mmol/liter.

Requirement of treatment with lipid or glucose lowering drugs was not a criterion for withdrawal from the study.

**Assays**

Serum GH levels were measured by immunoradiometric assay (IRMA; HGH-CTK-IRMA; Sorin, Saluggia, Italy) (assay sensitivity 0.05 μg/liter; intra- and interassay variation coefficients (CVs) 4.5 and 7.9%, respectively). Serum IGF-I levels were measured by IRMA after ethanol extraction using Diagnostic System Laboratories Inc. (Webster, TX) (assay sensitivity 0.8 μg/liter; for the low, medium, and high points of the standard curve intraassay CVs of 3.4, 3.0, and 1.5% and interassay CVs of 8.2, 1.5, and 3.7%, respectively). The normal ranges in 20 or less, 21–30, 31–40, and 41–50, old men were 180–625, 118–475, 102–400, and 100–306 μg/liter, respectively, SD of IGF-I levels for age (z-SDS) were calculated using the median and SD results in the Italian population according to the normal ranges reported above.
TABLE 2. Patients’ and controls’ profile at study entry

<table>
<thead>
<tr>
<th></th>
<th>Controls (all)</th>
<th>Patients (all)</th>
<th>Patients with IRS</th>
<th>Patients without IRS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>35</td>
<td>35</td>
<td>18</td>
<td>17</td>
<td>0.63</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>39.5 ± 7.6</td>
<td>39.4 ± 7.6</td>
<td>37.8 ± 7.4</td>
<td>41.1 ± 7.7</td>
<td>0.076</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 1.8</td>
<td>24.6 ± 2.2</td>
<td>24.7 ± 2.3</td>
<td>24.5 ± 2.1</td>
<td>0.78</td>
</tr>
<tr>
<td>GH peak after ARG+GHRH (µg/liter)</td>
<td>57.4 ± 15.5</td>
<td>2.8 ± 2.2</td>
<td>2.6 ± 1.8</td>
<td>3.0 ± 2.5</td>
<td>0.79</td>
</tr>
<tr>
<td>Serum IGF-I levels (µg/liter)</td>
<td>244.6 ± 47.3</td>
<td>60.7 ± 49.3</td>
<td>45.2 ± 31.7</td>
<td>77.1 ± 59.5</td>
<td>0.16</td>
</tr>
<tr>
<td>IGFI SDS score</td>
<td>0.80 ± 0.45</td>
<td>-1.87 ± 0.79</td>
<td>-2.14 ± 0.49</td>
<td>-1.59 ± 0.95</td>
<td>0.081</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>7.5 ± 3.2</td>
<td>9.1 ± 3.0</td>
<td>5.9 ± 2.4</td>
<td>0.002</td>
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</tbody>
</table>

Primary end point

Right carotid artery IMT (mm) 0.62 ± 0.09 0.86 ± 0.24 <0.0001 0.95 ± 0.27 0.76 ± 0.11 0.030
Left carotid artery IMT (mm) 0.63 ± 0.07 0.85 ± 0.18 <0.0001 0.91 ± 0.20 0.78 ± 0.15 0.056
Mean carotid arteries IMT (mm) 0.63 ± 0.07 0.85 ± 0.21 <0.0001 0.93 ± 0.25 0.76 ± 0.11 0.029

Secondary end point

Prevalence of IRS [no. (%)] 2 (5.7%) 18 (51.4%) <0.0001
Systolic blood pressure (mm Hg) 122.0 ± 9.3 130.4 ± 10.7 0.004 134.2 ± 9.3 126.5 ± 11.0 0.020
Diastolic blood pressure (mm Hg) 79.4 ± 4.7 85.1 ± 6.8 0.001 85.7 ± 6.2 83.5 ± 7.2 0.12
Total blood cholesterol levels (mmol/liter) 4.7 ± 0.6 6.0 ± 1.1 <0.0001 6.1 ± 1.1 5.8 ± 0.9 0.48
HDL-cholesterol levels (mmol/liter) 1.4 ± 0.2 1.1 ± 0.2 <0.0001 1.0 ± 0.2 1.2 ± 0.2 0.008
Serum triglyceride levels (mmol/liter) 1.2 ± 0.3 1.6 ± 0.3 <0.0001 1.7 ± 0.2 1.6 ± 0.3 0.024
Fasting blood glucose levels (mmol/liter) 4.7 ± 0.6 5.5 ± 0.6 <0.0001 5.7 ± 0.6 5.2 ± 0.4 0.013
Fasting serum insulin levels (mU/liter) 7.7 ± 2.8 14.1 ± 5.5 <0.0001 15.1 ± 6.6 12.8 ± 4.0 0.49
HOMA index 1.6 ± 0.7 3.7 ± 1.2 <0.0001 3.8 ± 1.2 3.4 ± 0.9 0.077

P values refer to the Wilcoxon matched paired test when patients were compared with controls and Mann-Whitney test when patients with IRS were compared with those without it. ARG, Arginine.

Statistical analysis

Results were expressed as mean ± SD unless otherwise specified. The statistical analysis was performed by MedCalc Software for Windows (MedCalc, Mariakerke, Belgium) package using nonparametric tests. The comparison between controls and patients (at study entry and end) was performed by the Wilcoxon matched paired test; the comparison between the patients of group A vs. B and between those with and without IRS was performed by the Mann-Whitney U test. The Spearman rank correlation test was used to calculate the correlation between prevalence of IRS and baseline characteristics: at this purpose the Spearman’s rho with the 95% confidence interval was calculated. The significance was set at 5% and is reported as two-side P values. Due to the limited number of controls with IRS, this analysis was limited to the patients only. For categorical variables, differences were analyzed by the χ² test and Fisher’s exact test.

Results

Baseline study (Table 2)

In the patients and controls, respectively, well-defined plaques were found in three and none (8.6 vs. 0%; P = 0.24). According to ACE guidelines (14), 18 patients (51.4%) and two controls (5.7%; P < 0.0001) had IRS. The patients with IRS had had a longer estimated duration of GHD and higher IMT (Fig. 2) than those without it; they were also slightly younger and had slightly lower IGF-I SDS. The estimated duration of GHD was correlated with presence of IRS [rho = −0.52; P = 0.0017, 95% confidence interval (CI) (Fisher’s Z transformed) 0.22–0.73]. Mean IMT in the patients without IRS was still significantly higher than that of controls (0.77 ± 0.12 vs. 0.63 ± 0.07 mm, P = 0.0002).

Subgrouping and GH replacement

Patients of group A and group B were comparable for all parameters (Table 3) and had a similar prevalence of IRS (12 of 22 vs. 6 of 13; P = 0.76). In patients of group A, the median GH dose was 6 µg/kg/d (range 4−8 µg/kg/d). Serum IGF-I levels increased progressively during the first year of GH re-
### Table 3. Clinical, biochemical, endocrine, and ultrasonographic features in the patients and controls at study entry and end

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th></th>
<th>Group A patients</th>
<th></th>
<th></th>
<th>Group B patients</th>
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<tbody>
<tr>
<td></td>
<td>Group A patients GH replaced</td>
<td></td>
<td>Group B patients Non-GH replaced</td>
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<td></td>
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<tr>
<td></td>
<td>Study entry</td>
<td>Study end</td>
<td>P</td>
<td>Study entry</td>
<td>Study end</td>
<td>P</td>
<td>Study entry</td>
<td>Study end</td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>35</td>
<td></td>
<td>22</td>
<td>22</td>
<td>0.59</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 1.8</td>
<td>24.5 ± 2.1ª</td>
<td>0.034</td>
<td>23.9 ± 2.2</td>
<td>24.3 ± 1.1ª</td>
<td>0.59</td>
<td>25.7 ± 1.7</td>
<td>26.6 ± 1.9</td>
</tr>
<tr>
<td>Serum IGF-I levels (μg/liter)</td>
<td>244.6 ± 47.3</td>
<td>225.2 ± 52.2ª</td>
<td>0.085</td>
<td>51.8 ± 49.4</td>
<td>232.3 ± 39.3ª</td>
<td>&lt;0.0001</td>
<td>80.3 ± 45.0</td>
<td>87.5 ± 59.3</td>
</tr>
<tr>
<td>IGF-I SDS</td>
<td>0.80 ± 0.45</td>
<td>0.71 ± 0.48ª</td>
<td>0.35</td>
<td>-2.00 ± 0.73</td>
<td>0.88 ± 0.19ª</td>
<td>&lt;0.0001</td>
<td>-1.51 ± 0.82</td>
<td>-1.41 ± 0.92</td>
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<tr>
<td>Primary end point</td>
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<tr>
<td>Right carotid artery IMT (mm)</td>
<td>0.63 ± 0.07</td>
<td>0.66 ± 0.07ª,ª</td>
<td>&lt;0.0001</td>
<td>0.85 ± 0.24</td>
<td>0.76 ± 0.23ª</td>
<td>&lt;0.0001</td>
<td>0.86 ± 0.26</td>
<td>0.88 ± 0.26</td>
</tr>
<tr>
<td>Left carotid artery IMT (mm)</td>
<td>0.63 ± 0.07</td>
<td>0.66 ± 0.07ª,ª</td>
<td>&lt;0.0001</td>
<td>0.83 ± 0.16</td>
<td>0.73 ± 0.16ª</td>
<td>&lt;0.0001</td>
<td>0.87 ± 0.22</td>
<td>0.88 ± 0.21</td>
</tr>
<tr>
<td>Mean carotid artery IMT (mm)</td>
<td>0.63 ± 0.07</td>
<td>0.66 ± 0.07ª,ª</td>
<td>&lt;0.0001</td>
<td>0.84 ± 0.20</td>
<td>0.75 ± 0.20ª</td>
<td>&lt;0.0001</td>
<td>0.87 ± 0.23</td>
<td>0.88 ± 0.23</td>
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<tr>
<td>Secondary end point</td>
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<tr>
<td>Prevalence of IRS (%)</td>
<td>2 (6)</td>
<td>6 (17)</td>
<td>0.26</td>
<td>12 (60)</td>
<td>4 (25)</td>
<td>0.028</td>
<td>8 (54)</td>
<td>3 (23)</td>
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<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>122.0 ± 9.3</td>
<td>128.1 ± 5.7</td>
<td>0.002</td>
<td>129.6 ± 10.9</td>
<td>126.4 ± 6.4</td>
<td>0.28</td>
<td>131.9 ± 10.7</td>
<td>125.8 ± 8.5</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>79.4 ± 4.7</td>
<td>82.3 ± 3.6</td>
<td>0.005</td>
<td>85.0 ± 5.3</td>
<td>81.6 ± 4.5</td>
<td>0.057</td>
<td>85.4 ± 9.0</td>
<td>81.1 ± 4.0</td>
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<tr>
<td>Total blood cholesterol levels (mmol/liter)</td>
<td>4.7 ± 0.6</td>
<td>4.9 ± 0.5</td>
<td>0.079</td>
<td>6.2 ± 1.0</td>
<td>4.4 ± 0.3</td>
<td>&lt;0.0001</td>
<td>5.7 ± 1.1</td>
<td>5.1 ± 0.8</td>
</tr>
<tr>
<td>HDL-cholesterol levels (mmol/liter)</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>0.16</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>&lt;0.0001</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Serum triglyceride levels (mmol/liter)</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>0.036</td>
<td>1.6 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>&lt;0.0001</td>
<td>1.7 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Fasting blood glucose levels (mmol/liter)</td>
<td>4.7 ± 0.6</td>
<td>5.2 ± 0.6</td>
<td>0.003</td>
<td>5.5 ± 0.6</td>
<td>5.0 ± 0.5</td>
<td>0.036</td>
<td>5.4 ± 0.5</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>Fasting serum insulin levels (mU/liter)</td>
<td>7.6 ± 2.8</td>
<td>9.6 ± 3.6</td>
<td>0.086</td>
<td>12.9 ± 5.9</td>
<td>11.2 ± 2.8</td>
<td>0.44</td>
<td>16.0 ± 4.5</td>
<td>14.7 ± 5.5</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.6 ± 0.7</td>
<td>2.3 ± 1.3</td>
<td>0.021</td>
<td>3.8 ± 1.2</td>
<td>2.5 ± 0.6</td>
<td>0.0001</td>
<td>3.9 ± 1.2</td>
<td>3.3 ± 1.3</td>
</tr>
</tbody>
</table>

*P* values refer to the Wilcoxon matched-paired test. Superscript letters indicate significance across groups derived by the Mann-Whitney *U* test.

ª*P* < 0.01 vs. study end group B.

ªª*P* < 0.01 study end group A.
placement, reaching normal levels in all patients. In patients of group B, IGF-I slightly reduced; they persisted less than 2 SDS in five patients and were between 1 and 2 SDS in another five patients. Overall, percent IGF-I levels decreased by 15% in patients of group B.

**Five-year follow-up study (Table 3)**

During the study period, lipid-lowering drugs were given to 12 controls (34.3%), three patients in group A (13.6%), and 12 patients in group B (92.3%; \( P < 0.0001 \)); glucose-lowering drugs were given to 11 controls (31.4%), five patients in group A (22.7%), and nine patients in group B (69.2%; \( P = 0.016 \)); antihypertensive drugs were given to seven controls (20.0%), one patient in group A (4.5%), and eight patients in group B (61.5%; \( P < 0.0001 \)). Significance was derived by the \( \chi^2 \) test.

**Primary outcome measure**

IMT at right and left common carotids significantly decreased only in group A, whereas it was significantly increased both in patients in group B (slightly) and in controls (Table 3). At any rate, 5 yr after GH replacement common carotid IMT was still significantly higher in patients in group A than in controls (Table 3 and Fig. 3). Median percent changes in IMT were 5% (95% CI 3.9–6.7%) in controls, −7.3% (−15.1 to −7.3%) in group A and 2.8% (0.1–3.2%) in group B patients (\( P < 0.0001 \)). Percent increase in mean IMT was similar in controls and patients in group B (\( P = 0.056 \)) after correction for additional treatments. The percent changes of IMT in the three groups are shown in Fig. 4. Of the three patients with atherosclerotic plaques at baseline, two received GH replacement; no significant change was observed in the plaques dimensions and characteristics during the study. In none of the other subjects, atherosclerotic plaques developed during the study period.

**Secondary outcome measure (Table 3)**

The prevalence of IRS significantly reduced only in group A patients. However, it was nonsignificantly reduced also in group B patients, whereas it slightly increased in controls. At study end, the lipid and glucose profile was normalized in all patients, whereas in controls there was a significant increase in blood pressure, triglycerides, and glucose levels. As a consequence, the homeostasis model assessment (HOMA) index increased significantly only in controls, whereas it significantly decreased in patients of group A but not group B.

**Discussion**

The results of this open, observational, prospective, controlled study confirm that adult
hypopituitary men with severe GHD have increased prevalence of cardiovascular risk factors (lipid and glucose profile) and increased surrogate markers of early atherosclerosis (IMT) and demonstrate that 5 yr of GH replacement therapy improves all the parameters above and reduces IRS prevalence, evaluated according to the ACE guidelines (14).

It is worth noting that the presence of IRS at study entry was significantly associated with an increased IMT at common carotid arteries. Patients with IRS were characterized by a slightly younger age, a longer duration of estimated GHD and lower IGF-I levels. None of the previous studies investigating early atherosclerosis in patients with GHD has interpreted the data according to IRS did not include in the analysis an appropriate control group and/or a group of patients with GHD who were not GH treated. This further supports a GH role in controlling some direct and indirect parameters involved in the atherosclerotic process.

The GH/IGF-I axis displays diverse effects on the cardiovascular system (5). Independent epidemiological studies have associated IGF-I levels in the lower normal range with an increased risk of ischemic heart disease or stroke (23–28). Additionally, the prevalence of atherosclerotic plaques was found to be increased in subjects with an IGF-I z-SDS of −2 or less to −1 (29). IMT at any major artery [particularly if ≥1 mm at any age (30)] is acknowledged to be an early marker of generalized atherosclerosis associated with other localizations of atherosclerosis and increased risk of myocardial infarction, as well (31, 32). In the cohort of the European Vascular Aging Study, Bonithon-Kopp et al. (33) reported that the odds ratio for having at least one plaque associated with a 0.10-mm increase in common carotid artery (CCA) IMT was 1.18 (95% CI 1.05–1.32), after adjustment for sex and other risk factors. In this relatively aged population, IMT increase was correlated to locally detected atherosclerosis, and consequently, reduction in IMT has to be considered favorably for the cardiovascular risk profile.

In the current study, we investigated a homogeneous group of healthy adult men, younger than those involved in the European Vascular Aging Study (33): in our controls, IMT increased by 0.03 ± 0.02 mm (95% CI 0.02–0.04), with a median increase by 5% (95% CI 3.9–6.8%) during a period of 5 yr, so less than expected at an older age. In fact, none of our controls developed plaques during the study. Nevertheless, half of our subjects started a treatment with lipid and/or glucose-lowering drugs and/or antihypertensive drugs but were poorly controlled. If these metabolic changes are responsible of increased IMT in our controls cannot be ruled out at present. A longer follow-up is required to draw conclusion about the incidence of atherosclerosis-associated changes in the IGF-I system in this control group. On the other hand, the aim of the current study was investigating the role of GH replacement in GHD patients, and control subjects were required only to better understand the physiological process of metabolic and vascular changes during a 5-yr follow-up because they were not the study population itself.

Interestingly, in our GHD patients who received GH replacement together with other replacement therapies associated with lipid- or glucose-lowering drugs and antihypertensive drugs as appropriate, IMT at CCA was significantly reduced, compared with GHD patients who received all appropriate treatment (as above) except GH. The use of lipid- or glucose-lowering drugs and antihypertensive drugs was significantly greater in patients non-GH replaced than those GH replaced and controls, as expected. Nevertheless, patients non-GH replaced did experience a similar increase in IMT as control subjects: from one side, this result is reassuring that a careful treatment aiming at controlling lipids, glucose, and blood pressure is beneficial on vascular disease in GHD patients and should be performed as in the general population, and from the other side, it further supports the beneficial effect of GH replacement in severely hypopituitary GHD patients. Clearly, the possibility of a negative role of overreplacement of cortisol and thyroxine and underreplacement of testosterone cannot be completely ruled out. Moreover, GHD is associated with an increased cortisol to cortisone metabolite ratio and low-dose GH replacement reverses these abnormalities (34). Thus, alterations in 11β-hydrossisteroid dehydrogenase 1 activity might also contribute in the beneficial effects of GH replacement in GHD.

It is now well accepted that patients with severe GHD have increased IMT at major arteries, increased prevalence of plaques, and increased risk of cardiovascular disease in most (10–13,16,35–37) but not all (38–40) studies. As we demonstrated in the current study, the severity of GHD at diagnosis is crucial to interpret IMT data, which likely correspond to the presence of IRS. In fact, severity and duration of GHD can play a role in the severity of the atherosclerotic profile in hypopituitary patients: patients with IRS (thus with increased lipid, glucose, and blood pressure levels) were those with a longer disease duration and slightly lower IGF-I levels and were those with a higher IMT. In our population, we previously found that increased CCA IMT and presence of atherosclerotic plaques were observed only in patients with IGF-I levels less than 2 SD from the mean or lower (16). On the other hand, GHD patients are known to have abdominal obesity, unfavorable lipid profile, and hyperinsulinemia, all conditions being associated with vascular mortality and morbidity (41–43). Long-term GH replacement was reported to normalize insulin resistance, but results are controversial (44, 45). Similarly, long-term GH replacement does not seem to improve the prevalence of metabolic syndrome, compared with the general population (46). In contrast, after 5 yr of GH replacement in the current cohort, we observed improved insulin sensitivity and reduced IRS prevalence and confirmed that insulin sensitivity deteriorated in controls (so that IRS prevalence was similar in both groups at study end). We also found that some IR improvement (even if significantly less remarkable) was observed in GHD patients non-GH replaced but undergoing a careful treatment with lipid and glucose-lowering and antihypertensive drugs.

Previous data on surrogate markers of atherosclerosis after GH replacement are limited to short period of treatment. Indeed, 12–24 months of GH replacement induced improvement of endothelial function, fell in the serum concentrations of adhesion molecules and inflammatory markers, and also decreased IMT at major arteries (5). In a previous study, we demonstrated that 24 months GH replacement was not sufficient to normalize IMT
In contrast, Pfeifer et al. (10) showed normalized IMT at common carotid artery already after 6 months of GH replacement. However, we (13) previously studied patients with a more severe increase in the IMT (0.85 ± 0.31 mm) than those included in the study by Pfeifer et al. (0.68 ± 0.05 mm) (10). Thus, the entity of vascular thickening is a determinant of GH replacement outcome in GHD patients. Another important limitation of previous studies is the lack of a control group studied with a similar time interval as the patients. In a very recent non-controlled study, decrease of CCA IMT was confirmed after 5 yr of GH replacement in 14 GHD patients (47).

The current study extends our previous observations and also provides new important results in patients with severe GHD non-GH replaced as well as in healthy subjects restudied after 5 yr from the first examination. We confirmed a significant decrease of IMT in all patients GH replaced, even in those with well-defined atherosclerotic plaques, in line with previous reports (12, 13). To note that atherosclerotic plaques did not significantly change after replacement neither developed new plaques in other patients: this finding suggests that GH replacement is started as early as possible in patients with severe GHD to prevent the development of atherosclerotic plaques that do not generally change during treatment. Importantly, in severely hypopituitary GHD patients non-GH replaced increase in IMT after 5 yr was less than that observed in controls. The most likely explanation for such an unexpected finding might be related to two factors: first, that patients were closely followed in our outpatient clinic, titrating the lipid- and glucose-lowering drugs and antihypertensive drugs more carefully than in controls who were examined only at study entry and study end; second, that patients had significantly higher IMT at baseline than controls so that possibly less probably to increase further. Lastly, we noticed that the patients classified as having IRS (14) had the most severe metabolic and atherosclerotic profile, suggesting that the presence of IRS can be considered as a marker of GHD syndrome severity.

Conclusion
Hypopituitary men with severe GHD have more frequent IRS and increased IMT at common carotid arteries than sex- and age-matched controls. A 5-yr GH replacement induced a decrease of IMT and improved insulin sensitivity. No changes in IMT was observed in non-GH-replaced severe GHD patients, whereas increase in IMT is observed in controls.

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