Positive effects of a physiological dose of GH on markers of atherogenesis: a placebo-controlled study in patients with adult-onset GH deficiency

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Abstract

Objective: GH deficiency is associated with an increased cardiovascular mortality. Fifty-five patients with adult-onset GH deficiency (AO-GHD) (24 female, 31 male, mean age 49 years) were enrolled in a placebo-controlled double-blind crossover study to investigate the effects of GH therapy on a variety of cardiovascular risk factors representing different aspects of atherogenesis, including apolipoproteins (Apo A-1, Apo B), markers of subclinical inflammation (high-sensitivity C-reactive protein (CRP) and interleukin-6) and markers of endothelial function (intercellular adhesion molecule-1, von Willebrand factor and sCD40L (a pro-atherogenic factor and marker for plaque destabilization)).

Methods: GH therapy was individually dosed to obtain an IGF-I concentration within the normal range for age and sex. GH and placebo were administered for 9 months each, separated by a 4 month washout period.

Results: The final mean dose of GH was 50% higher for women and IGF-I increased to the same level in both sexes. Compared with placebo, substitution with GH showed a significant effect on Apo B (mean change −0.15 (−0.22 to −0.08) mg/l) and CRP (−1.8 (−3.3 to −0.3) mg/l). The baseline level of and change in IGF-I during treatment with GH contributed significantly to the improvement in both markers. No effects were found on interleukin-6 or Apo A-1, or on markers of endothelial function. No gender differences were observed for any of the markers at baseline or following intervention.

Conclusions: GH substitution to naïve patients with AO-GHD at a low, individually titrated dose aiming at normalizing IGF-I was followed by significant reductions in Apo B and CRP, indicating a positive effect of GH on cardiovascular risk.

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Introduction

Patients with long-standing growth hormone deficiency (GHD) have significantly impaired health compared with the normal population, and long-term follow-up studies of patients with hypopituitarism have shown markedly reduced life expectancy (1–3). GHD may contribute to increased vascular morbidity and mortality (4, 5), as the syndrome is followed by central obesity, insulin resistance, unfavorable lipid alterations, early atherosclerotic changes as shown by increased intima-media thickness of the carotid arteries, reduced cardiac performance and decreased physical capacity (6). Several studies have documented positive effects of GH replacement therapy on all of these parameters (7–14), with the exception of glucose homeostasis, for which most controlled studies have found an increase in glucose and insulin levels (7, 13), at least during short-term treatment.

An unfavorable lipid profile is central in the pathogenesis of atherosclerosis, and the changes in lipids typical of GHD may help to explain the increased cardiovascular risk in untreated GHD, especially in women (15). However, a chronic low-grade inflammation has been considered to play an important role in the atherosclerotic process of the arterial wall (16) and serum markers of inflammation, including C-reactive protein (CRP) and interleukin-6 (IL-6) may predict the risk for
acute cardiovascular and cerebrovascular events (17, 18). Moreover, a correlation between inflammatory markers and markers of endothelial dysfunction has been demonstrated (19). Endothelial dysfunction is thought to be an early and potentially reversible event in the process of atherogenesis (20). Various injurious factors mediate the production of cytokines that stimulate the endothelial cells to express several proteins including the intercellular adhesion molecule-1 (ICAM-1) facilitating the attachment and migration of macrophages and leukocytes to the subendothelial space (21). The activated endothelium is also known to express other markers, such as von Willebrand factor (vWF) (22) and sCD40L, a pro-atherogenic factor and marker for plaque destabilization (23, 24).

The purpose of this post hoc study was to investigate the impact of a physiological and gender-specific GH substitution dose on a variety of cardiovascular risk factors representing different aspects of atherogenesis including CRP, IL-6, ICAM-1, vWF, sCD40L and the non-traditional markers of atherogenic lipids, apolipoproteins Apo A-1 and Apo B.

Subjects and methods

Subjects

Fifty-five subjects (31 men, 24 women; mean age 49 years (range 26–62 years)) who had had severe adult-onset GHD (AO-GHD) for at least 2 years (mean duration 4.0 (range 0.3–12.4 years)) and who had never been treated with GH were randomly assigned to treatment with GH replacement therapy. Severe GHD was defined as a peak GH level to a stimulation test (insulin tolerance test) less than 3 μg/l. All subjects with multiple pituitary insufficiencies had received stable doses of substitution therapy with other hormones for at least 6 months before study entry. The primary diagnosis and treatment of this has previously been described in detail (7). Of the total study population, the vast majority (53/55; 96%) had multiple pituitary deficiencies; only two had isolated GHD. Of the 24 women in the study, 15 received estrogen replacement therapy. Six women were considered postmenopausal and did not undergo estrogen replacement therapy; the remaining women were evaluated as eugonadal and no patients received dehydroepiandrosterone. Of the 31 men, 22 received testosterone substitution; the remaining were considered eugonadal. A total of seven subjects withdrew from the study. However, sufficient data were accumulated and carried forward in the intention-to-treat analyses resulting in a study population of 49 subjects (20 women) for efficacy and safety data.

Study design

The patients underwent treatment with either recombinant human GH therapy (Genotropin; Pfizer Inc., New York, NY, USA) or placebo in a double-blind crossover study. Each treatment period lasted 9 months, with a 4 month washout interval between periods. GH replacement therapy was dosed individually to maintain serum insulin-like growth factor (IGF)-I concentrations between the mean ± 1 S.D. of the sex- and age-related reference range. An independent Ethics Committee approved the study protocol and informed consent was obtained from all subjects (7). GH therapy was initiated at a dose of 0.13 mg/day (0.4 IU/day) for men and at 0.27 mg/day (0.8 IU/day) for women. Dose adjustments were made in increments of 0.13 mg (0.4 IU/day) every 4 weeks (maximum dose, 0.66 mg/day (2.0 IU/day) (men), 0.93 mg/day (2.8 IU/day) (women)) based on IGF-I concentrations and the absence of adverse effects. A physician un-blinded to the study who was not participating in subject care guided all dose adjustments, including dose adjustment for subjects receiving placebo. Patients given placebo received the same adjustments, as a given parallel patient receiving active treatment.

Blood sampling and biochemical measurements

At both the beginning and end of each treatment period, blood samples were drawn after an overnight fast into pyrogen-free vacuum blood collection tubes without additives, allowed to coagulate, centrifuged (1000 g and 4 °C for 15 min) and serum stored at −80 °C in multiple aliquots until analyzed. Apo A-1, Apo B and high-sensitivity CRP assays were performed by accredited laboratories according to standard laboratory methods (Department of Medical Biochemistry, National University Hospital, Oslo). IGF-I analysis was performed using the commercial kit Immulite (Diagnostic Products Corporation, Los Angeles, CA, USA). vWF was analyzed in serum by EIA using antibodies from DakoCytomation (Norway). Briefly, 96-well plates were coated overnight at 4 °C with 100 μl polyclonal rabbit anti-human vWF antibody at 10 mg/l in PBS. Tween 20 (0.1%) in PBS was used as a buffer in all subsequent steps. The standard was pooled serum diluted 1:20–1:1280. After blocking (5 min, assay buffer), 100 μl samples (diluted 1:200) or standard were added and incubated for 2 h. Subsequent steps included peroxidase-conjugated polyclonal rabbit anti-human vWF antibody (1:8000, 1 h) and plates were developed with tetramethylbenzidine, stopped with H2SO4, and read at 450 nm. sCD40L and ICAM-1 in serum were analyzed using antibodies from R&D systems (Minneapolis, MN, USA), according to the manufacturer’s instructions. IL-6 was analyzed by an ultrasensitive EIA from Biosource (Camarillo, CA, USA). All coefficients of variation were less than 10%.

Physical exercise capacity and body composition

Exercise tests and muscle strength were conducted as described (7). Briefly, exercise tests were performed in the subjects from the centers in Oslo (n = 28). Maximal
oxygen uptake and time to exhaustion were determined by treadmill walking or running using a stepwise incremental protocol. The initial workload was chosen individually according to a pre-test, so that the continuous exercising phase lasted from 8 to 12 min. Total body composition was measured by dual-energy X-ray absorptiometry as body fat mass and lean body mass. The two centers in Oslo used a Lunar DPX-L, software version 1.31 (Lunar Corp., Madison, WI, USA). The center in Trondheim used the Hologic QDR-4500 (Waltham, MA, USA) in dual-beam mode. Total body imaging was performed and analyzed by one person at each center.

Statistical analyses

The effect of GH therapy compared with placebo was estimated by the crossover effect, calculated by the difference between GH and placebo (baseline to month 9 changes). Using mean values, a point estimate and a two-sided 95% confidence interval (CI) of the crossover differences were calculated. Only subjects participating in the study for at least 3 months of the second period were included in the analyses. Relationships between variables were tested by Pearson’s correlation analysis. To determine the independent predictors of the changes in circulating Apo B and CRP during GH treatment, a forward stepwise linear regression analysis was performed including baseline levels and changes during GH treatment of the following independent variables: low-density lipoprotein (LDL), fat mass, MaxVO₂ and IGF-I. All tests were performed two-sided and a P-value less than 0.05 was considered significant.

Results

GH dose and IGF-I

The mean IGF-I concentration during the placebo and active treated phase of the study is given in Fig. 1. The final mean dose of GH (±S.D.) was 0.6±0.26 mg/day (1.8±0.8 IU/day) for women and 0.4±0.2 mg/day (1.2±0.6 IU/day) for men (P < 0.03); these doses led to similar levels of IGF-I in both genders (7).

Lipids and body composition

Compared with placebo, GH treatment was associated with improvements in total cholesterol levels (by 8.4%), LDL-cholesterol (LDL-C) (by 11.9%), and in Apo B cholesterol (by 8.2%) (Fig. 2). There were no significant changes in Apo A-1 or Apo A-1/B ratio (Table 1, Fig. 2). No gender difference at baseline or in response to GH for Apo A-1 or Apo B could be demonstrated (Table 2). Regarding body composition, there was a similar decrease in fat mass and an increase in LBM in men and women as previously reported (7).

The results of the stepwise forward regression in relation to Apo B are given in Table 3. The most important independent predictor for the reduction in Apo B was baseline LDL-C, followed by the change in IGF-I, the baseline fat mass, and the baseline physical exercise capacity. The total explanatory power by the model for the change in Apo B was 89% (Table 3).

Inflammatory markers

At baseline, a significant negative correlation between IGF-I and CRP was found (r = −0.44, P = 0.001). Following treatment with GH, CRP decreased by 41% compared with placebo (Table 1 and Fig. 2). As shown in Fig. 3, a negative correlation between the change in IGF-I and the change in CRP was demonstrated (r = −0.43, P = 0.002). The change in IGF-I following treatment could explain 18% of the variation in CRP (Fig. 3). There was no effect of GH compared with placebo on the level of IL-6 (Table 1).
There were no gender differences, either at baseline or following intervention, on any of these parameters. The results of the stepwise forward regression in relation to CRP are given in Table 4. The most important independent predictor for the reduction in CRP was baseline IGF-I followed by the change in IGF-I. In this context, the change in LDL-C was of borderline significance. There were no gender differences, either at baseline or following treatment. Women received a 50% higher dose of GH to reach the same target level of age-adjusted IGF-I (7).

Numerous studies have shown a strong association between the levels of CRP and future vascular events, i.e., coronary, cerebrovascular and peripheral vascular disease (17). In addition to being a powerful risk marker, CRP may directly affect lesion formation through leucocyte activation and endothelial dysfunction (25, 26). Elevated CRP levels have been found for any of the markers studied related to atherogenic lipoproteins, inflammation or endothelial dysfunction either at baseline or following treatment. The most striking effect of GH was the pronounced reduction in CRP, a sensitive marker of subclinical inflammation. Despite a significantly higher level of IGF-I in men at baseline, no gender-specific differences were found for any of the markers studied related to atherogenic lipoproteins, inflammation or endothelial dysfunction either at baseline or following treatment.

Markers of endothelial dysfunction

As given in Table 1, we could not detect any significant effects on the levels of ICAM-1, vWF or sCD40L. There was no gender difference either at baseline or following intervention in any of these parameters (Table 2).

Discussion

A normalization of IGF-I in naïve patients with AO-GHD was in this study followed by improvements in markers representing different aspects of atherogenesis.

Table 2 Cardiovascular risk factors at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-1 (mg/l)</td>
<td>1.28 ± 0.19</td>
<td>1.18 ± 0.19</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B (mg/l)</td>
<td>0.98 ± 0.31</td>
<td>1.01 ± 0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Apo A-1/B ratio</td>
<td>0.78 ± 0.27</td>
<td>0.88 ± 0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Total C (mmol/l)</td>
<td>5.98 ± 1.36</td>
<td>5.44 ± 1.10</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.98 ± 1.23</td>
<td>3.39 ± 1.03</td>
<td>NS</td>
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<tr>
<td>vWF (%)</td>
<td>76 ± 41</td>
<td>81 ± 43</td>
<td>NS</td>
</tr>
<tr>
<td>sCD40L (pg/ml)</td>
<td>136 ± 103</td>
<td>141 ± 113</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>0.40 ± 0.41</td>
<td>0.68 ± 1.62</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>5.2 ± 5.6</td>
<td>3.4 ± 3.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are given as means ± s.d. at baseline.

Table 3 Stepwise forward linear regression showing independent predictors of changes in Apo B during GH substitution to AO-GHD patients for 9 months. Alpha to enter 0.25.

<table>
<thead>
<tr>
<th>Step</th>
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<td>-0.075</td>
<td>-0.066</td>
</tr>
<tr>
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<td>&lt;0.001</td>
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<tr>
<td>Change in IGF-I</td>
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<td>0.009</td>
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<td>0.010</td>
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</tr>
<tr>
<td>P-value</td>
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<td>&lt;0.001</td>
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<tr>
<td>Baseline body fat</td>
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<tr>
<td>P-value</td>
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<td>0.003</td>
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<td></td>
<td></td>
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<tr>
<td>Baseline MaxVO2</td>
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<td>-0.010</td>
<td>-0.012</td>
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<tr>
<td>P-value</td>
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<td>0.022</td>
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<td>Baseline IGF-I</td>
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<td></td>
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<td>P-value</td>
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<td>R-square</td>
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<td>69.68</td>
<td>80.60</td>
<td>85.10</td>
<td>88.59</td>
</tr>
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</table>
more intensive exercise, are reported to reduce CRP levels (27, 28). Usually these interventions are accompanied by increased insulin sensitivity. In our study, the patients did not lose weight, although fat mass was reduced (see below), and glucose homeostasis was if anything worsened, although within the reference range. Of interest is that there was a significant negative correlation between CRP and IGF-I at baseline and also in the changes in CRP in relation to the changes in IGF-I following GH. However, the decrease in body fat did not predict the change in CRP.

Our study showing that GH substitution is followed by improved CRP levels, independently of weight or fat mass, is in line with the anti-inflammatory and immune-modulating actions of GH/IGF-I (29). The finding corroborates recent smaller controlled or open studies using comparable GH replacement doses (30, 31), in controlled studies in men (32), and in long-term open studies (33). However, in the present study of both genders, the effect on CRP seems to be even more pronounced than in the previous controlled study only in men (32). This is important, since women with hypopituitarism and untreated GHD in contrast to healthy women, are at a higher risk of premature mortality due to vascular diseases than men (34). Thus, since women require markedly higher doses, the effects of GH on CRP reduction are most likely mediated by IGF-I. Accordingly, both baseline IGF-I and the change were prominent determinants of the improvement in CRP during active treatment. IL-6 is partly secreted by subcutaneous fat and regulates hepatic CRP production. Although levels of IL-6 have been found increased in GHD and responded in parallel with CRP during replacement therapy in men (14, 35), we could not demonstrate any effect on IL-6 in this study.

As briefly mentioned in our primary report (7), GH replacement therapy was followed by significant improvements in body composition and lipids without alterations in total weight. The changes, however, were less prominent than observed in studies using higher doses (13), underscoring that the effect on adiposity and lipids are dose-dependent (11, 36). The highly atherogenic Apo B has so far only been scanty investigated in GHD. In a cross-sectional observational study Apo B was not found to be increased in AO-GHD, whereas Apo A was found to be lower only in females compared with normal controls (37). In the present study we demonstrate a significant reduction in Apo B following treatment, without gender difference, whereas no effect on Apo A could be shown. Plasma lipid levels are influenced by the clearance of Apo B-containing lipoproteins. Hepatic overproduction and decreased clearance of very-low-density lipoprotein Apo B has been found in hyperlipidemic disorders related to premature atherosclerosis (13). In GHD, it has been suggested that the alteration in lipid profile, and thus the increased cardiovascular risk, may be related to an unfavorable Apo B metabolism (38), in accord with our present data. The change in Apo B in the present study was influenced independently by several factors, as revealed by the forward linear regression analysis. Most interesting, also physical capacity at baseline contributed significantly.

Although most studies have suggested that endothelial dysfunction in general is improved in AO-GHD by GH substitution (8, 10, 30, 39), a recent study found that resistance artery function was unchanged by GH replacement in spite of a decrease in blood pressure and pulse wave velocity (31). Moreover, the decline...
in pulse wave velocity was significantly correlated to the fall in CRP. The authors suggest a differential effect of GH replacement on endothelial function in large and small arteries. This observation might explain the good correlation between biophysical and biochemical parameters of endothelial dysfunction at baseline in AO-GHD (40), in spite of improvement of the biophysical parameters without significant changes in the biochemical markers of endothelial dysfunction (30).

In conclusion, GH substitution to naïve patients with AO-GHD at a low, individually titrated dose aiming at normalizing IGF-I, was followed by a significant reduction in Apo B and CRP. The baseline level of and change in IGF-I during treatment with GH contributed significantly to the improvement in both markers. These findings suggest a positive effect of GH replacement therapy on cardiovascular risk in patients with AO-GHD.

Acknowledgements

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