Short-term effects of GH treatment on coagulation, fibrinolysis, inflammation biomarkers, and insulin resistance status in prepubertal children with GH deficiency

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Abstract

Objective: The aims of this study was to determine whether prepubertal GH deficiency (GHD) children showed any impairment in coagulation- and fibrinolysis-related parameters and in inflammatory and insulin resistance markers and to evaluate the effect of short-term GH therapy on these parameters.

Design: This was a 6-month, prospective, observational, case–control study (36 prepubertal children with GHD and 38 healthy prepubertal children with no differences in BMI). Comparison of study parameter values in GHD and control groups at baseline and after 6 months of GH treatment in the GHD group. The following were analyzed: glucose, insulin, fibrinogen, absolute plasminogen activator inhibitor type 1 (aP AI-1), von Willebrand factor (vWF), homeostasis model assessment for insulin resistance (HOMA-IR) index, C-reactive protein (CRP), and interleukin 6 (IL6) levels.

Results: Children with GHD showed higher baseline levels of aP AI-1 and fibrinogen and lower levels of glucose, insulin, and HOMA-IR index than healthy controls. No intergroup differences were found for vWF. After 6 months of treatment, aP AI-1 levels were lower but no changes were observed in fibrinogen or vWF levels, which were similar to those of controls. Glucose levels increased, though not significantly, while insulin levels and HOMA-IR index rose to normal levels. A positive correlation was found between changes in insulin status/HOMA-IR index and levels of aP AI-1, fibrinogen, vWF, CRP, and IL6.

Conclusions: At early ages, GH therapy appears to exert beneficial effects on the amount of aP AI-1. At the same time, it increases the state of insulin resistance (HOMA-IR index) without modifying the levels of fibrinogen, vWF, CRP, and IL6.

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Introduction

Adult patients with GH deficiency (GHD) are at greater risk of cardiovascular disease; this is reflected especially in increased levels of absolute plasminogen activator inhibitor type 1 (aP AI-1) (1), fibrinogen (2), and von Willebrand factor (vWF) (3). An increase in the level of inflammatory markers such as interleukin 6 (IL6) and C-reactive protein (CRP) has also been reported in these patients (4). Changes in various parameters linked to coagulation, fibrinolysis, and inflammation in adults with untreated GHD contribute to an increase in cardiovascular and cerebrovascular mortality. GH treatment has been associated with an improvement in patient status (5, 6, 7). Research has shown that adolescents with GHD have elevated baseline fibrinogen levels compared with healthy controls (8, 9). Few studies have focused on changes in aP AI-1 and vWF levels following GH treatment in preadolescent children with GHD.

In adults with GHD, GH therapy reportedly leads to the normalization of inflammatory factor levels (10). Adolescent GHD patients show low-grade inflammation, evidencing increased circulating CRP levels, which may lead to premature arteriosclerosis (11, 12); replacement therapy is associated with some improvement with respect to untreated controls (11). Few studies have addressed this issue in prepubertal children.

Both long- and short-term GH therapy have a beneficial effect on several of these parameters in GHD adults, prompting a reduction in fibrinolytic activity, increased peripheral insulin resistance (5), and even some improvement in inflammatory marker levels.
In GH-deficient children and in children with other pathologies, including those born small-for-gestational age, GH therapy is associated with impairment – albeit moderate – of the insulin metabolism (13). Metabolic disorders in GHD children and adolescents are currently the subject of more detailed research (14). This study sought to determine whether prepubertal GHD children showed any impairment in coagulation- and fibrinolysis-related parameters and in inflammatory and insulin resistance markers and to evaluate the effect of short-term GH therapy on these parameters.

Materials and methods

This was a 6-month, prospective, observational, case-control study on prepubertal small-for-gestational age children with GHD treated with GH.

A total of 43 small-for-gestational age children with idiopathic GHD (22 boys and 21 girls) were initially included in the study and started a 6-month course of GH therapy. All subjects were at Tanner stage I (prepubertal). Five of the subjects initially recruited were later excluded due to the onset of puberty during the follow-up, while two further subjects abandoned the study. The final group comprised 36 patients (22 boys and 14 girls).

The control group comprised 38 healthy children (21 boys and 17 girls). The study included only nonobese prepubertal children (Tanner stage 1). Local schools were informed that the study was to be carried out, and parents were asked permission for their children to participate. Children who agreed to participate in the study were classified by BMI and assigned to one of the two groups (GHD and control).

The following criteria for diagnosing GHD were used:

- Short stature – 2 S.D. from the reference population (15); height – up to 1.5 S.D. from the target height.
- Growth rate, at least during the last year, 1 S.D. below the mean of the chronological age; and bone age, calculated by an X-ray of the left wrist and hand, at least <1 year of the chronological age. Two GH provocation tests were applied, with 1 week in between, with clonidine and 8 ng/ml measured by means of a monoclonal assay. All girls underwent karyotyping and showed 46,XX. In addition, IGF1 and IGFBP3 were determined. Other causes, age between 6 and 12 years, and nonobese, prepubertal, and nonobese subjects, of short stature were excluded (15, 16) (BMI <85). These subjects received GH therapy at 0.030 mg/kg per day for 6 months. Any subject showing signs of puberty during the 6-month treatment period was withdrawn from the study. Exclusion criteria were as follows: failure to comply with any of the above criteria, concomitant metabolic disorder or general disease, abnormal length or weight at birth, obesity, or BMI over p85.

Study parameters

Subjects in the GHD group made two visits, one before the initiation of the GH therapy and the other 6 months later. Subjects in the control group made only one baseline visit. The following data were recorded for each subject: age, sex, weight (W) in kilograms, height (H) in centimeters, and BMI obtained by dividing weight in kilograms by the square of height expressed in meters. The control group was used to compare values with those recorded for the GHD group at baseline and after 6 months of GH therapy. Glucose and insulin levels were measured, and the homeostasis model assessment (HOMA) index was calculated. To assess fibrinolysis and coagulation, levels of aPAI-1, fibrinogen, and vWF were measured. Ultrasensitive CRP and IL6 were measured as inflammation biomarkers.

Blood samples were collected after 12 h of fasting from a vein in the antecubital fossa, without venous occlusion. All collections were made between 0800 and 0900 h. The samples were separated into aliquots and frozen immediately at −45 °C until analysis. The following parameters were measured in all children: glucose, insulin, HOMA-insulin resistance (IR) index, fibrinogen, aPAI-1, vWF, CRP, and IL6.

Glucose concentrations were measured using a random access analyzer (Axon, Bayer Diagnostics) with reagents from Bayer Diagnostics. Insulin was quantified using an Access2-Immunoassay System (Beckman Coulter, Brea, CA, USA). The HOMA-IR was used to detect the degree of insulin resistance. Resistance was assessed from fasting glucose and insulin concentrations using the formula: resistance (HOMA-IR) = (insulin (mU/l) × glucose (mmol/l)) / 22.5.

Antigenic immunoassay methods were used to measure the aPAI-1 (Asserachrom PAI-1, Diagnostica Stago, Asnieres-Sur-Seine, France). Fibrinogen was measured by quantitative assay using thrombin in an automatic analyzer (Electra 1600; Ortho Clinical Diagnostics, Madrid, Spain), and vWF was measured by enzyme immunoassay (R&D, Wiesbaden-Nordenstadt, Germany) using a Biokit automatic microplate processor from Biochem Immunosystems. Inflammation biomarkers were measured as follows: i) ultrasensitive CRP by nephelometry using the Dade Behring Nephelometer Analyzer II; and ii) IL6 by enzyme immunoassay (R&D) using a Biokit automatic microplate processor from Biochem Immunosystems.

Statistical analysis was performed using Microstat (Ecosoft, Indianapolis, IN, USA) or GraphPAD InStat (GraphPAD Software, San Diego, CA, USA) software packages. Abnormal values (outliers) were excluded. Results were expressed as mean ± S.E.M. with a 95% confidence interval (95% CI). The distribution of each variable was tested for departure from Gaussian distribution, and equality of variance was tested using Snedecor’s F-test. Mean values for groups were compared using Student’s unpaired t-test. Statistical
Discussion

It is essential to establish all the cardiovascular risk factors associated with GHD in prepubertal children and to carefully evaluate the potentially beneficial effects of GH replacement therapy for the prevention of cardiovascular disease.

This study focused exclusively on prepubertal children, in order to rule out the possible influence of changes in insulin action and other parameters as a result of pubertal development. For that reason, all subjects showing any sign of puberty during the follow-up period were withdrawn from the study.

Children with BMI greater than p85 were excluded from the study, in order to rule out the influence of obesity on all cardiovascular risk factors; for example, obesity is known to be associated with elevated aPAI-1 and inflammation biomarker levels. The use, for both groups, of selection criteria such as Tanner stage 1 and nonobesity meant that there could be significant differences in age but not in BMI.

GHD is associated with a range of auxological, clinical, biochemical, and molecular anomalies caused by abnormal GH secretion. Studies on hypopituitary patients with untreated GHD report reduced longevity and an elevated incidence of cardiovascular disease and premature death in these patients. Increased mortality has been linked to an increase in the risk factors for atherosclerosis: dyslipidemia, reduced fibrinolytic activity (elevation of aPAI-1 levels), increased fibrinogen levels, increased abdominal obesity, high blood pressure, increased glucose intolerance, and peripheral insulin resistance. Additionally, elevated CRP and IL6 levels are reported in adults with GHD. Although fewer data are available for prepubertal children, GHD in this age group is

Table 1 Clinical and anthropometric data for GHD and control groups. Results are expressed as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Variable</th>
<th>GHD group (n=36)</th>
<th>Control group (n=38)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.02 ± 0.31</td>
<td>8.22 ± 0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>28.64 ± 1.31</td>
<td>27.48 ± 0.63</td>
<td>0.717</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>127.33 ± 1.82</td>
<td>127.21 ± 1.02</td>
<td>0.954</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.3 ± 0.44</td>
<td>16.98 ± 0.21</td>
<td>0.507</td>
</tr>
</tbody>
</table>

significant was set at P < 0.05. Correlation between variables was evaluated using Pearson’s correlation coefficient and regression analysis.

All parents gave their written consent, and the study was authorized by the Hospital Ethics Research Committee. Follow-up of nonobese children (longitudinal study) was not authorized.

Results

Age, weight, height, and BMI were recorded for both the groups (GHD and control) at the initiation of the study (Table 1). No significant change in BMI was observed in the GHD group after 6 months of GH therapy.

Baseline glucose levels were significantly lower in the GHD group than in the control group (Table 2). After 6 months of GH treatment, no significant intergroup difference was observed (Table 3).

Both insulin levels and the HOMA-IR index were significantly lower at baseline in the GHD group than in the control group (Table 2). After 6 months of GH therapy, both had risen significantly, approaching the control group levels (Table 3).

Children in the GHD group had significantly higher baseline aPAI-1 levels than controls. By the end of 6 months of GH therapy, levels had undergone a statistically significant decline and approached those recorded for the control group (Tables 2 and 3). Baseline fibrinogen levels were also significantly higher in GHD children than in controls but were not significantly modified by GH treatment (Tables 2 and 3).

No significant differences were found in baseline vWF levels between the GHD and control groups, and levels did not change significantly as a result of GH therapy (Tables 2 and 3). No significant intergroup differences in CRP and IL6 levels were observed either before or after treatment (Tables 2 and 3).

Changes (Δ) in all these variables were expressed as the difference between baseline and posttreatment values. A significant positive correlation was observed between changes (Δ) in insulin levels/HOMA-IR index and changes in aPAI-1, fibrinogen and vWF levels (Fig. 1). Variations in insulin levels also correlated with changes in CRP (r = 0.5481; P < 0.001) and IL6 levels (r = 0.4042; P = 0.020). Correlations were also observed between changes in the HOMA index and changes in both CRP (r = 0.5572; P < 0.001) and IL6 (r = 0.3572; P = 0.041). There was no correlation between changes in BMI and changes in other variables.

Table 2 Baseline analytical data for GHD and control groups. Results are expressed as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Variable</th>
<th>GHD group (n=36)</th>
<th>Control group (n=38)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.45 ± 0.08</td>
<td>4.82 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>4.15 ± 0.39</td>
<td>6.31 ± 0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA index</td>
<td>0.822 ± 0.075</td>
<td>1.352 ± 0.088</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>aPAI-1 (ng/ml)</td>
<td>27.65 ± 2.91</td>
<td>19.16 ± 2.81</td>
<td>0.039</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.9 ± 0.11</td>
<td>2.3 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vWF (%)</td>
<td>71.21 ± 4.87</td>
<td>77.3 ± 4.93</td>
<td>0.387</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.55 ± 0.17</td>
<td>0.67 ± 0.24</td>
<td>0.687</td>
</tr>
<tr>
<td>IL6 (pg/ml)</td>
<td>1.45 ± 0.32</td>
<td>1.62 ± 0.24</td>
<td>0.669</td>
</tr>
</tbody>
</table>

aPAI-1, absolute plasminogen activator inhibitor type 1; vWF, von Willebrand factor; CRP, C-reactive protein; IL6, interleukin 6.
associated with greater frequency of central obesity and hypercholesterolemia (22). Other metabolic disorders linked to GHD in adults have not been studied in children.

Adult GHD patients tend to show elevated baseline insulin levels and increased insulin resistance (6, 10), as well as lower glucose levels (23). In this study, GHD children had significantly lower glucose levels than controls; however, insulin levels and the HOMA-IR index were also lower in controls. This may be because children in the GHD group did not show the changes in body composition reported in GHD adults (24). Obesity is in itself associated with hyperinsulinemia and reduced insulin sensitivity, this being among the first alterations observed in obese adults and children (25).

Administration of GH in adults with GH deficiency prompts a short-term increase (between 6 weeks and 3 months) in insulin and glucose levels, a reduction in insulin sensitivity, and an increase in insulin resistance (26). Studies to determine the effect of GH therapy over 1 and 2 years on insulin resistance status and other parameters in children have reported a statistically significant, duration-dependent increase in insulin levels and in the HOMA-IR index (27). Here, a rising trend in glucose levels was observed following 6 months of GH therapy in GHD children, but the increase was not statistically significant. By contrast, significant increases were noted in insulin levels and in the HOMA index. These results coincide with the findings reported elsewhere in adults and, more recently, in children (27). This way and using more accurate methods than the HOMA-IR index to measure insulin sensitivity, Hoffman (1995) describes a decrease in insulin sensitivity after 6 months of GH therapy in a small group of children with GHD without affecting other cardiovascular risk factors (28). However, due to the difficulty of using more invasive methods in our specific age group, we were forced to use the HOMA-IR index for the ease of its execution and general application.

Elevated aP AI (2), fibrinogen, and vWF levels (29) are reported in adults with GHD. Hyperfibrinogenemia in GHD adolescents has proved to respond to GH replacement therapy (30), which has also been found to reduce aP AI-1 activity (8).

In this study, baseline fibrinogen levels were significantly higher in GHD children than in healthy controls, a finding also reported in adolescents (8) and adults (5) but not hitherto in prepubertal children. Similarly, GHD children had significantly higher aP AI-1 levels, although values for vWF were similar to those of controls. This suggests impaired coagulation and fibrinolysis in GHD patients, even before puberty. It should be stressed that hyperfibrinogenemia and elevated aP AI-1 may favor the development of cardiovascular disease in adult life.

After 6 months of GH therapy, aP AI-1 levels in the GHD group had declined and approached those of healthy controls. By contrast, no significant changes were observed for fibrinogen and vWF. GH therapy thus appears beneficial, in that it normalizes aP AI-1 in GHD children.

Table 3 Posttreatment data in the GHD group. Results are expressed as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>GHD group (baseline)</th>
<th>GHD group (posttreatment)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=36)</td>
<td>(n=36)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>127.33 ± 1.82</td>
<td>131.84 ± 1.95</td>
<td>0.086</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.45 ± 0.083</td>
<td>4.58 ± 0.074</td>
<td>0.248</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>4.15 ± 0.39</td>
<td>7.01 ± 0.67</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HOMA index</td>
<td>0.822 ± 0.075</td>
<td>1.426 ± 0.145</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>27.65 ± 2.91</td>
<td>19.67 ± 2.73</td>
<td>0.049</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.9 ± 0.11</td>
<td>2.97 ± 0.07</td>
<td>0.605</td>
</tr>
<tr>
<td>vWF (%)</td>
<td>71.21 ± 4.87</td>
<td>70.9 ± 4.73</td>
<td>0.871</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.55 ± 0.17</td>
<td>0.49 ± 0.21</td>
<td>0.826</td>
</tr>
<tr>
<td>IL6 (pg/ml)</td>
<td>1.45 ± 0.32</td>
<td>1.12 ± 0.22</td>
<td>0.378</td>
</tr>
</tbody>
</table>

aP AI-1, absolute plasminogen activator inhibitor type 1; vWF, von Willebrand factor; CRP, C-reactive protein; IL6, interleukin 6.
thus reducing the attendant risk of atherothrombosis. A long-term follow-up may be required to establish whether GH therapy prompts in children any significant changes of the kind reported in adults (2).

Elevated aPAl-1 levels have been reported in patients showing insulin resistance, including obese children and adults with type II diabetes (17). The mechanisms prompting increased aPAl-1 in patients with metabolic syndrome remain unclear, though they may involve various metabolic disorders: dyslipidemia, hyperinsulinemia, glucose intolerance, and high blood pressure, all of which could favor aPAl-1 synthesis and release (18). A correlation has been reported between fibrinogen and insulin levels in non-diabetic subjects and also between vWF levels, insulin levels, and insulin resistance (30). In this study on prepubertal children with GHD, a positive correlation was observed between coagulation- and fibrinolysis-related factors (aPAl-1, fibrinogen, and vWF) and changes in insulin levels and the HOMA-IR index.

Adolescents with untreated GHD have higher baseline CRP levels than healthy controls, and GH therapy appears to prompt significant changes (11). There is insufficient published data on CRP levels – and IL6 levels – in prepubertal GHD children. The results obtained here revealed no significant changes in CRP or IL6 levels following GH therapy, perhaps because subjects were prepubertal and follow-up was relatively short. However, changes in inflammation markers (IL6 and CRP) were significantly correlated with changes in basal insulin levels and HOMA-IR index values, although not with changes in BMI. Insulin resistance may be involved in the onset of metabolic disorders accompanying GHD in adulthood, independently of obesity.

In summary, from an early age, children with GHD show changes in the fibrinolysis system similar to those reported in adult GHD, with elevated fibrinogen and aPAl-1 levels. GH therapy appears to exert beneficial effects on the amount of aPAl-1. At the same time, it increases the state of insulin resistance (HOMA-IR index) without modifying the levels of fibrinogen, vWF, CRP, and IL6. The positive correlation between changes in insulin/HOMA-IR index levels and changes in parameters related to coagulation, fibrinolysis, and inflammation (fibrinogen, aPAl-1, vWF, IL6, and CRP) could suggest a potential contribution of insulin in the pathophysiology of these parameters. However, long-term observation is necessary to observe potential changes in the above-mentioned variables. More research is needed to clarify this aspect. Further research should focus on the duration of GH therapy in GH-deficient patients with metabolic disturbances at risk for premature atherosclerosis.

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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References


