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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1514 – 1517
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Serum resistin concentrations in growth hormone–deficient children during growth hormone replacement therapy

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Received 3 January 2007; accepted 5 June 2007

Abstract

We performed this study to examine whether the serum resistin concentrations in growth hormone (GH)–deficient (GHD) children are influenced by administration of GH and to assess the relationship between serum resistin and free fatty acid levels during GH replacement therapy. The study included 20 prepubertal GHD children (16 boys and 4 girls) who were treated with recombinant human GH (hGH). The serum levels of resistin, insulin-like growth factor I, free fatty acid (FFA), triglyceride, cholesterol and glucose levels, leukocyte counts, and hemoglobin A_{1c} were measured at baseline and after 1 month of hGH treatment. The serum resistin levels after hGH therapy were significantly higher than the basal resistin levels (median [range], 6.2 [4.9-11.8] vs 5.6 [4.4-8.3] ng/mL; $P < .05$), whereas the serum FFA levels were unchanged before and after treatment (0.51 [0.34-0.76] vs 0.37 [0.24-0.60] mEq/L). No significant relationship was found between serum resistin and FFA levels after hGH therapy. Body mass index, serum triglyceride, cholesterol and glucose levels, leukocyte counts, and hemoglobin A_{1c} showed no significant differences before and after hGH treatment. Our results suggest that elevated serum resistin levels after 1-month hGH therapy in GHD children are not associated with the GH-induced lipolysis as found in GHD adults during short-time hGH therapy.

1. Introduction

Resistin is a 12.5-kd cysteine-rich polypeptide that is secreted from adipose tissues. Secretion of resistin in mice is increased in obesity and diabetes mellitus, and is decreased by administration of peroxisome proliferator-activated receptor-γ agonists [1]. Resistin treatment aggravates glucose intolerance in mice and absorption of sugar in 3T3-L1 cells [1]. Because increased levels of serum resistin have been reported in human patients with obesity and type 2 diabetes mellitus, serum resistin levels are thought to be correlated with insulin resistance [2]. On the contrary, some studies in humans have found no relationship between resistin gene expression and body weight or insulin sensitivity [3]. In addition, in humans, resistin is expressed strongly in monocytes rather than in adipocytes [4]. Thus, a definite role of resistin in human metabolism has yet to be established [5].

Increased insulin resistance has been shown 6 weeks after human growth hormone (hGH) replacement therapy in growth hormone (GH)–deficient adults [6], and the mechanism through which hGH increases insulin resistance is presumed to involve an increase in free fatty acid (FFA) level caused by hGH-induced lipolysis [7]. In rats, resistin levels are increased by administration of hGH [8] and also by administration of FFA [9]. Thus, a mechanism involving resistin may be associated with the transient increase in insulin resistance after administration of hGH.

Given this background, we carried out the current study to examine whether resistin levels in GHD children are influenced by administration of hGH. To our knowledge, there have been no previous reports regarding the effects of hGH on resistin in GHD children, and therefore, this is the first study to examine resistin levels after hGH therapy.

2. Materials and methods

2.1. Subjects

The study included 20 short children (16 boys and 4 girls) with GH deficiency who were treated with hGH. The mean age (±SD) of the subjects was 6.8 ± 3.2 years. The diagnosis of GHD was based on the following criteria: height of less

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doi:10.1016/j.metabol.2007.06.018

Table 1
Changes in biochemical variables and leukocyte counts after hGH therapy

	Baseline	GH-treated	*P*
Resistin (ng/mL)	5.6 (4.4-8.3)	6.2 (4.9-11.8)	.03*
IGF-I (ng/mL)	182 ± 120	267 ± 157	.02*
FFA (mEq/L)	0.51 (0.34-0.76)	0.37 (0.24-0.60)	.16
Triglycerides (mg/dL)	75 ± 58	96 ± 58	.07
Cholesterol (mg/dL)	164 ± 26	161 ± 20	.79
Glucose (mg/dL)	93 ± 14	98 ± 16	.56
Leukocyte (/μL)	6700 (6350-9300)	7900 (6000-9450)	.71
HbA_{1c} (%)	5.0 ± 0.6	5.1 ± 0.5	.49

Data are shown as mean ± SD or median (interquartile range).
* $P < .05$.

than −2 SD score; peak GH of less than 10 μg/L in at least 2 standard GH-provocative tests. The average height was 104.8 ± 17.4 cm, and the height SD score was −2.66 ± 0.67. The average weight was 18.8 ± 9.3 kg, and the body mass index (BMI) was 16.3 ± 2.1. The pubertal stage was Tanner 1 in all subjects. The supplemented dose of hGH ranged from 0.151 to 0.203 mg/kg per week, with an average of 0.174 mg/kg per week. Biosynthetic GH was given subcutaneously once daily at bedtime. The serum levels of resistin, insulin-like growth factor I (IGF-I), FFA, triglyceride, cholesterol and glucose levels, leukocyte counts, and hemoglobin A_{1c} (HbA_{1c}) were measured at baseline and after 1 month of hGH treatment. Blood samples for measuring resistin, IGF-I, and FFA were collected in the fasting state, and serum was separated immediately by centrifugation and kept at −20°C until analysis. The other parameters were measured immediately after blood sampling. Informed consent was obtained from the parents of all children, and the study was approved by the local ethics committee.

2.2. Laboratory analysis

The serum resistin concentration was assayed with an enzyme-linked immunosorbent assay kit (EZHR-95K, Linco Research, St Charles, MO). The sensitivity of the assay was 0.16 ng/mL and the limit of linearity was 10 ng/mL, with inter- and intra-assay coefficients of variation of 7.1% to 7.7% and 3.2% to 7.0%, respectively. The serum FFA concentration was determined using the ACS·ACOD method (Wako Pure Chemical Industries, Osaka, Japan) with intra-assay coefficients of variation of less than 3.0%. The serum IGF-I concentration was assayed with an enzyme-linked immunosorbent assay kit (Biocode-Hycel, Liège, Belgium). The sensitivity of this assay was 4.9 ng/mL, and inter- and intra-assay coefficients of variation were 11.3% to 13.7% and 6.6% to 9.7%, respectively. The serum triglyceride, cholesterol and glucose levels, leukocyte counts, and HbA_{1c} were measured by conventional methods in our hospital laboratory.

2.3. Statistical analysis

Results are expressed as median and interquartile range or means ± SD. The Wilcoxon matched-pair rank sign test was used to determine statistical significance for non-normally distributed variables (resistin, FFA, leukocyte), and paired *t* test for normally distributed variables. Relationships between parameters were evaluated by Spearman rank correlation analysis or by simple linear regression analysis. All analyses were performed using Stat View 5.0 (SAS Institute, Cary, NC). $P < .05$ was accepted as statistically significant.

3. Results

The serum resistin levels after hGH therapy were significantly higher than basal resistin levels (median [range], 6.2 [4.9-11.8] vs 5.6 [4.4-8.3] ng/mL; $P < .05$), whereas the serum FFA levels were unchanged before and after treatment (0.51 [0.34-0.76] vs 0.37 [0.24-0.60] mEq/L, $P = .16$) (Table 1). Of 20 subjects, 14 showed increased levels of serum resistin after hGH therapy. Changes in log-transformed serum resistin levels during 1 month of hGH replacement therapy are shown in Fig. 1. Serum IGF-I levels were significantly elevated after hGH therapy compared with basal IGF-I levels (267 ± 157 vs 182 ± 120 ng/mL; $P < .05$). Body mass index, serum triglyceride, cholesterol and glucose levels, leukocyte counts, and HbA_{1c} showed no significant differences before and after hGH therapy (Table 1). No significant relationship was found between serum resistin and FFA levels after hGH therapy. Neither the serum resistin nor the FFA levels showed a significant correlation with hGH dose, age, BMI, or IGF-I after hGH therapy. The serum resistin levels did not correlate with

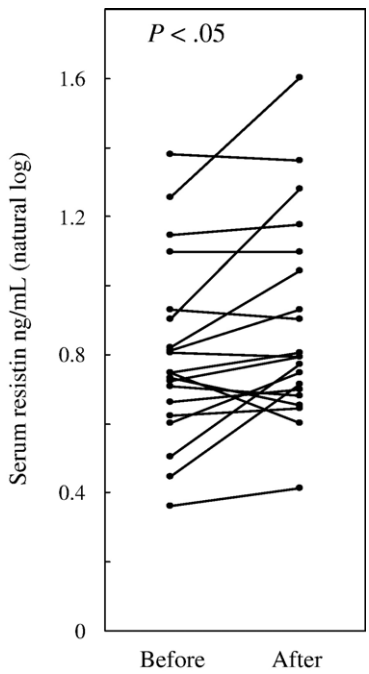

Fig. 1. The changes in log serum resistin levels after 1 month of hGH replacement therapy.

leukocyte counts before and after hGH therapy. A tendency toward a positive correlation between the ratio of the posttreatment to the basal resistin levels (median [range], 1.1 [1.0-1.5]) and the ratio of the posttreatment to the basal leukocyte counts (1.0 [0.9-1.3]) was found, but it did not reach statistical significance ($\rho = 0.44$, $P = .055$).

4. Discussion

There have been only a few studies regarding the effects of GH replacement on resistin in GHD adults, and no data are available on GHD children. Hana et al [10] have reported that there is no effect of GH replacement (0.31 mg/d) over a 12-month period on the plasma levels of resistin in adult GHD patients. Svensson et al [11] have reported that 1-week GH replacement therapy (0.067 mg/kg per week) in GHD adults induces glucose intolerance but does not significantly change the serum levels of resistin. In contrast, Delhanty et al [8] have demonstrated that GH treatment for GH-deficient rats causes a marked rapid and sustained induction of resistin gene expression in white adipose tissue, suggesting that GH has a direct effect on resistin. This difference in the effects of GH replacement on resistin between humans and rats may be explained by resistin being expressed primarily in white adipose tissues in rats [1] but mainly in macrophages in humans [4]. In our GHD children, the serum resistin levels were significantly higher after 1-month GH therapy than at baseline. This disparate finding on resistin between the previous studies in adults and our study may be related to differences in age, heterogeneity of GHD subjects, dose of hGH, or duration of GH replacement.

The effects of GH replacement in GHD adult patients on insulin sensitivity appear to be biphasic [11]. The short-term (<6 months) effects of hGH, with increased lipid oxidation and increased circulating FFA levels, decrease insulin sensitivity, whereas the long-term (>1 year) effects, with a reduction in body fat, are beneficial for insulin sensitivity. Nielsen et al [7] also reported that the direct effects of GH on insulin sensitivity and increased circulating FFA levels through GH-induced lipolysis were possible mechanisms for the transient, rapidly reversed decrease in insulin sensitivity shortly after starting GH replacement therapy. Only a few studies on insulin sensitivity in GHD children during GH replacement therapy have been reported. Heptulla et al [12] have assessed the effects of GH treatment on insulin secretion and action using a hyperglycemic clamp procedure in 5 GHD and 3 non-GHD children, demonstrating the decreased insulin sensitivity and compensatory hyperinsulinemia 6 months after the start of hGH therapy. Radetti et al [13] have reported that the quantitative insulin sensitivity check index during the first year of GH treatment was significantly decreased in GHD children, with no further reduction over the following years. In our study, both serum FFA levels and BMI were unchanged after 1 month of GH therapy, suggesting that the GHD children did not present the increased lipolysis as found in GHD adults after short-time GH treatment.

Resistin has been shown to enhance insulin resistance in mice [1], predominantly in the liver in rats [14]. Some studies in human adults have demonstrated positive correlations between resistin and body fat mass or insulin resistance [2,15,16], whereas others have failed to detect an association between resistin concentrations and body fat or markers for insulin sensitivity [3,17,18]. Gerber et al [19] could not find any significant correlation of resistin levels in obese children with markers of insulin resistance and glucose homeostasis. Alternatively, resistin is well known as a cytokine that plays an important role in inflammatory reactions because it is primarily expressed in monocytes and macrophages in humans [20]. Yang et al [21] have reported resistin expression in leukocytes of acute myelomonocytic and lymphoblastic leukemia origin. Kunnari et al [22] have demonstrated a positive correlation of serum resistin levels with leukocyte counts and highly sensitive C-reactive protein in middle-aged subjects. Furthermore, Kaser et al [23] demonstrated that in human mononuclear cells, resistin messenger RNA expression is regulated by proinflammatory cytokines such as interleukin (IL)–1, IL-6, and tumor necrosis factor α. On the other hand, an increase in neutrophils via the action of granulocyte colony-stimulating factor has been shown 2 months after the start of hGH administration in GHD adults [24]. Pagani et al [25] have reported that serum levels of proinflammatory cytokines IL-6 and tumor necrosis factor α produced particularly by monocytes/macrophages are significantly increased in GHD children 6 hours after hGH administration. We paid attention to the changes in serum resistin levels and leukocyte counts during hGH therapy and assessed the ratio of posttreatment to basal values in each parameter. Because our results showed a tendency toward a positive correlation between the ratio of the posttreatment to the basal resistin levels and the ratio of leukocyte counts before and after hGH treatment, the increase in resistin after GH therapy might be partly explained by a production of leukocyte-derived cytokines. To verify this hypothesis, however, further investigations are needed in a larger sample size.

In conclusion, our results showed that serum resistin concentrations significantly increased following 1-month administration of hGH in GHD children, and this increase in resistin was not associated with the GH-induced lipolysis as found in GHD adults during hGH therapy.

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