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Leptin responses to insulin administration in children with short stature

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Abstract

The aim of the study was to investigate the effect of standard insulin tolerance test on plasma leptin levels in children with idiopathic short stature (ISS) and in children with growth hormone deficiency (GHD). Furthermore, plasma leptin levels were analyzed with regard to age, body mass index (BMI), and plasma levels of human growth hormone and of insulin-like growth factor-1 (IGF-1).

Sixty-three patients with a height below the third percentile, an age of 10.24 ± 0.40 years and a BMI standard deviation score (SDS) of −0.78 ± 0.13 (weight SDS −0.07 ± 0.12; height SDS −2.39 ± 0.10) were investigated (mean ± SD). Based on responses to insulin tolerance test, the patients were classified as ISS (n = 49) or GHD (n = 14).

Plasma leptin levels were significantly lower in all patients 60 minutes ($P < .001$) and 120 minutes ($P < .001$) after insulin administration. This effect was independent of GHD, and no difference in leptin decrease was found when comparing patients with ISS to those with GHD. A correlation was found when comparing plasma leptin levels of all patients to BMI SDS (r = 0.43; $P < .001$) and plasma IGF-1 levels (r = 0.31; $P < .01$). Furthermore, positive correlation was found when BMI SDS was compared to IGF-1 (r = 0.25; $P < .05$).

In summary, we found that insulin administration in children with short stature decreases plasma leptin levels, equally in those with and without GHD.

1. Introduction

Leptin is a protein secreted from the adipose tissue [1]. Since the discovery of leptin, the understanding of its physiological role has evolved from a satiety signal to that of an integrative hormone that responds to and regulates different endocrine pathways with direct metabolic effects on peripheral tissues [2-5].

Leptin plays an integral role in energy homeostasis and may even be important during stress [6]. Although it is evident that caloric deprivation decreases plasma leptin levels [7,8], responses of the hormone during repeated episodes of physiological stress such as hypoglycemia remain undetermined. A confounding variable when studying the impact of hypoglycemia on plasma leptin levels is that experimental hypoglycemia may cause both an increase and a decrease in plasma leptin levels in adults [9-11].

To evaluate the possible role of acute alterations of plasma glucose and insulin for the regulation of circulating plasma leptin levels in children, we investigated the effect of insulin administration on plasma leptin levels. For this issue, we performed a standard insulin tolerance test (ITT) in children with short stature. Furthermore, plasma leptin levels were analyzed with regard to age, body mass index (BMI), and plasma levels of human growth hormone (GH) and of insulin-like growth factor-1 (IGF-1).

2. Patients and methods

2.1. Subjects

The study population consisted of 63 children who were admitted to our department because of short stature (below the third percentile).

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Table 1
Standard insulin tolerance test: characteristics of response in patients with GHD and patients with ISS

	GHD	ISS
Number	14	49
Age (y)	8.98 ± 0.89	10.60 ± 0.44
Gender		
Female (n)	6	23
Male (n)	8	26
Weight SDS	−2.06 ± 0.30	−2.07 ± 0.12
Height SDS	−2.72 ± 0.23	−2.29 ± 0.12
BMI SDS	−0.35 ± 0.30	−0.90 ± 0.15
GH basal (ng/mL)	2.06 ± 0.69	4.10 ± 0.64*
GH peak (ng/mL)	6.76 ± 0.49	21.01 ± 1.65**
IGF-1 (ng/mL)	184.27 ± 42.41	248.80 ± 27.64
Leptin 0 (ng/mL)	6.21 ± 1.52	4.31 ± 0.58
Leptin 60 (ng/mL)	5.18 ± 1.32	3.52 ± 0.51
Leptin 120 (ng/mL)	4.97 ± 1.39	3.35 ± 0.50
Leptin decrease (%) after 60 min	21.56 ± 1.02	22.51 ± 0.98
Leptin decrease (%) after 120 min	27.79 ± 1.18	27.05 ± 1.03

Data are shown as mean ± SD. Weight, height, and BMI (kg/m^2) are related to age and sex and given as SDS.
GH basal indicates plasma GH level before insulin administration; GH peak, highest plasma GH level during ITT; leptin 0, basal plasma leptin level before insulin administration; leptin 60, plasma leptin level 60 min after insulin administration; leptin 120, plasma leptin level 120 min after insulin administration; IGF-1, plasma insulin-like growth factor-1 level before insulin administration.
* $P < .05$.
** $P < .001$ (patients with GHD vs patients with ISS).

To evaluate GH deficiency (GHD), a standard insulin stimulation test was performed. The present study was designed as a pilot study, and blood samples were obtained according to our routines for the ITT. All subjects were free of other disorders as assessed by their medical history, physical examination, and relevant laboratory data.

Growth hormone deficiency was defined by an insufficient GH response (<10 ng/mL) during ITT. The diagnosis of GHD was approved by another provocative test (arginine stimulation test). Because of these results, patients were divided into 2 groups (Table 1):

1. Patients with GHD
2. Patients with idiopathic short stature (ISS)

Fully informed consent was obtained from all patients and/or their parents, respectively.

2.2. Methods

We measured height and weight for evaluation of BMI (kg/m^2). Body mass index, height, and weight were related to age and sex, and indicated as standard deviation score (SDS).

Insulin tolerance test was performed as follows: after an overnight fast, 0.1 IU/kg insulin (Actrapid, Novo Nordisk, Rued, Norway) was injected intravenously at 8:00 AM. Venous blood samples were drawn before and every 15 minutes from 0 to 120 minutes, and at the time when patients had symptoms of hypoglycemia. Nadir plasma glucose was less than 2.2 mmol/L in all patients.

From these samples, leptin levels (ng/mL) were assessed before (leptin 0), 60 minutes (leptin 60), and 120 minutes (leptin 120) after insulin administration. Decrease in plasma leptin was calculated as follows:

$$\text{Leptin decrease (\%)} = [(\text{leptin 0} - \text{leptin 60 \{120\}}) / \text{leptin 0}] \times 100$$

Furthermore, GH (ng/mL) and IGF-1 (ng/mL) were assessed before insulin administration as well as highest plasma levels of GH (GH peak) during ITT.

Leptin, IGF-1, and GH were measured by means of radioimmunoassay using commercially available kits (leptin, IGF-1: Diagnostic Systems Laboratories, Inc, USA; GH: Nichols Institute Diagnostics, San Clemente, Calif). Intra- and interassay coefficients of variation for all parameters were less than 3% in our laboratory.

Fasting plasma glucose was determined by an automated enzymatic method (Hitachi 917, Roche Diagnostics, Austria).

2.3. Statistics

Wilcoxon test was used for statistical analysis of plasma leptin levels before and after insulin administration. Mann-Whitney U test was used to compare parameters of interest between groups. Correlations between variables of interest were calculated using Pearson's correlation coefficient. The independence and significance of variables were tested by stepwise, multiple regression analysis based on results of the bivariate correlations. P values less than 5% were considered significant. Calculations were performed using WinStat 3.1 (Kalmia Co Inc, Cambridge, UK). Results are given as mean (± SD).

3. Results

Sixty-three patients with an age of 10.24 ± 0.40 years and a BMI SDS of −0.78 ± 0.13 (weight SDS, −0.07 ± 0.12; height SDS, −2.39 ± 0.10) were included in the study (mean ± SD) (Table 1). After insulin administration, glucose levels fell beneath the defined hypoglycemia threshold of 2.2 mmol/L in all patients, with a nadir of 1.4 ± 0.1 mmol/L.

Basal plasma leptin levels were not significantly different between the groups. Plasma leptin levels decreased in all patients and were significantly lower 60 minutes ($P < .001$) and 120 minutes ($P < .001$) after insulin administration. No statistically significant difference was found when comparing patients with GHD and children with ISS with regard to plasma leptin levels before insulin administration (leptin 0), 60 minutes (leptin 60), and 120 minutes (leptin 120) thereafter, as well as to leptin decrease (Table 1). When normalized for BMI and expressed as leptin/BMI ratio, similar results were obtained.

A correlation was found when comparing the plasma leptin levels of all patients (leptin 0, leptin 60, leptin 120)

to age (r = 0.42, r = 0.42, r = 0.43; all $P < .001$), weight (r = 0.54, r = 0.54, r = 0.56; all $P < .001$), height (r = 0.38, r = 0.38, r = 0.41; all $P < .001$), BMI SDS (r = 0.43, r = 0.43, r = 0.42, all $P < .001$), and IGF-1 plasma levels (r = 0.31, r = 0.31, r = 0.31; all $P < .01$). Furthermore, positive correlation was found when BMI SDS was compared to IGF-1 (r = 0.25; $P < .05$). In stepwise multiple regression analysis using leptin as a dependent variable, weight explained 30% and BMI SDS 20% of the variation in leptin ($P < .001$).

4. Discussion

We studied the relationship between insulin administration and plasma leptin levels in children with short stature. Our main finding was a decrease in plasma leptin levels after insulin administration, independent of GHD.

Studies describing leptin responses to hyperinsulinemia and hypoglycemia in healthy men have been conflicting, with reports of both an increase and a decrease of plasma leptin levels [10]. Prolonged elevations of insulin under euglycemic conditions have been shown to increase plasma leptin levels [9-11]. It has been demonstrated that a 6-hour hyperinsulinemic-euglycemic clamp caused a 147% ± 7% increase over baseline in plasma leptin levels in healthy lean males, but 6 hours of hyperinsulinemia with graded hypoglycemia blunted the increase in plasma leptin levels [10]. In response to hypoglycemia, plasma leptin levels decreased in healthy adults, but not in patients with insulin-dependent diabetes [8]. Wellhoener et al [11] exposed subjects to 4 different conditions: high insulin with either euglycemia or graded hypoglycemia, and low insulin with either euglycemia or graded hypoglycemia. With both high and low insulin levels, the insulin-induced increase in plasma leptin levels was attenuated by 50% during hypoglycemia [11]. Thus, hypoglycemia counteracts the stimulatory effect of hyperinsulinemia on plasma leptin levels. The evidence that hypoglycemia induced by hyperinsulinemia inhibits rises in plasma leptin supports the hypothesis that low glucose levels during a prolonged fast, directly or indirectly, signal the adipocyte to reduce leptin secretion [8]. We have shown these effects in children with short stature for the first time. It is well known that changes in leptin have marked effects on metabolism. Increases in leptin can elevate hepatic glucose production in rats in the postabsorptive state [12]. Reports also demonstrate leptin-induced fat mobilization and fatty acid oxidation in mice [13]. Thus, a major function of leptin may be to switch fuel use from carbohydrates to fat [14,15]. This metabolic impact could be important during hypoglycemia, where leptin decrease could contribute to a shift toward carbohydrate use allowing quicker generation of ATP [8].

Leptin levels have been shown to be inversely related to pituitary function [16]. However, in our study there were no obvious differences between patients with GHD and children with ISS. This has also been shown in adults [16]. This indicates that GH has no direct effect on short-term leptin regulation, as found by others [17,18]. Although it has been reported that in adults with GHD there was no difference in leptin levels when adjusted for BMI and sex compared to healthy subjects [19], in adolescents with GHD, GH treatment caused a substantial increase in the nocturnal leptin peak during the second night after commencing substitution [20]. Whether this effect was due to an increase in insulin or directly caused by GH remains unclear. In children with isolated GHD, leptin levels were normal before therapy and decreased after 4 weeks of GH therapy, which could not be explained by changes in body composition [21]. In vitro, GH led to a moderate reduction in leptin release from human adipocytes. This inhibitory effect was only observed in the presence of insulin, suggesting that GH interacts with the stimulatory effect of insulin [22].

The relationship between plasma leptin levels and IGF-1 is discussed controversially in the literature. Insulin-like growth factor-1 has been used as an indicator of nutritional status in children and adults [23,24]. Leptin was thought primarily to regulate IGF-1 secretion, the inverse relationship suggesting a feedback mechanism [25]. However, Dagogo-Jack et al [26] showed that basal plasma leptin levels and IGF-1 levels were not correlated. Otherwise, long-term administration of recombinant human IGF-1 was associated with an early and sustained decrease in plasma leptin levels [26,27]. Insulin-like growth factor-1 at supraphysiological concentrations mimicked the insulin effect, indicating that this effect was probably mediated via the insulin receptors of the adipocytes [28]. Iglesias et al [29] confirmed the presence of high circulating plasma leptin in dialysis patients and showed that plasma leptin levels were further increased by exogenous administration of GH. This suggested that the increase in plasma leptin levels after GH therapy might be related to GH-induced changes in insulin. Others have demonstrated that in patients with GH receptor defect (Laron syndrome), IGF-1 treatment did not cause significant changes in plasma leptin levels [30]. Our observation of a positive correlation between plasma leptin levels and IGF-1 in children with short stature complements previous studies in prepubertal [31] and peripubertal [32] children and in children with severe malnutrition [33]. As it has been demonstrated that body fat mass is a main determinant for plasma leptin levels [4,5,19,28,34] and IGF-1 is positively correlated with nutritional status [23,35], the close relationship between plasma leptin levels and IGF-1 can be explained. This finding is supported by our data demonstrating higher IGF-1 levels in patients with higher BMI.

In conclusion, we found that insulin administration decreases plasma leptin levels in children with short stature, independent of GHD.

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