Leptin levels are strongly correlated with those of GH-binding protein in prepubertal children

Ragnar Bjarnason1,2, Margaret Boguszewski1, Jovanna Dahlgren1, Lars Gelanders1, Berit Kristrom1,1, Sten Rosberg1, Björn Carlsson2, Kerstin Albertsson-Wikland1 and Lena M S Carlsson2

1International Pediatric Growth Research Centre, Department of Pediatrics, 2Research Centre for Endocrinology and Metabolism, Department of Internal Medicine, Sahlgrenska University Hospital, University of Göteborg, S-413 45 Göteborg, Sweden and 3Department of Pediatrics, University of Umeå, S-901 87 Umeå, Sweden

(Correspondence should be addressed to R Bjarnason, International Pediatric Growth Research Centre, Department of Pediatrics, Sahlgrenska University Hospital/Ostra, S-416 85 Göteborg, Sweden)

Abstract

Objective: Nutritional status is an important determinant of growth, and previous studies have indicated that this is due, at least in part, to an increased target-tissue sensitivity to GH. An attractive candidate for mediating this effect is leptin, a hormone secreted by the adipose tissue. The aim of this study was to investigate if there was a connection between GH-binding protein (GHBP) and leptin.

Design and Methods: We investigated the relationship between serum levels of leptin and those of GHBP in 229 prepubertal children. These included 107 healthy children with normal GH secretion, 55 GH-deficient (GHD) children and 55 children born small for gestational age (SGA) sampled on one occasion for GHBP and leptin, and 12 healthy children followed longitudinally at monthly intervals for 1 year.

Results: In the healthy children and in those born SGA, the serum concentration of GHBP was positively correlated with that of leptin (r = 0.65, P < 0.001; r = 0.74, P < 0.001 respectively). There was no correlation between GHBP and leptin in the group of children with GHD (r = 0.27, not significant). This means that leptin alone explained 42% of the variation of GHBP in the healthy group and 55% in the SGA group. The correlation remained after adjustment for body mass index and age in the healthy children (r = 0.57, P < 0.0001, r² = 0.33) and for children born SGA (r = 0.74, P < 0.0001, r² = 0.55). There was a positive correlation between the intra-individual monthly changes in GHBP and changes in leptin respectively; in the 12 healthy children followed longitudinally, the mean of the correlation coefficients was 0.38 (median = 0.29; range 0.03 to 0.86; P < 0.05).

Conclusions: There was a highly significant correlation between serum levels of leptin and those of GHBP, except in children with GHD. The possibility that leptin could mediate the effects of body fat mass on GH sensitivity, therefore, merits further investigation.

European Journal of Endocrinology 137 68–73

Introduction

Obesity in children is associated with an increased linear growth rate (1). This cannot be explained by altered growth hormone (GH) secretion, as heavy children tend to have reduced GH concentrations in the blood (2). Furthermore, in GH-deficient (GHD) children, the response to GH therapy is correlated to body mass index (BMI) (3), and it has therefore been suggested that an increased BMI is associated with an increased responsiveness to GH. Thus, several findings suggest that the amount of body fat influences growth in children, possibly by increasing GH responsiveness, although the mechanisms behind this are unknown.

Characterization of the GH-binding protein (GHBP) (4, 5) and the cloning of the GH receptor (6) have allowed studies of the initial steps in GH action. It is difficult to study GH receptor abundance and function directly in man. However, several observations indicate a relationship between GHBP levels and GH receptor function/GH responsiveness (7, 8). For example, GHBP concentrations in the blood are positively correlated with the response to GH treatment in children with GHD (3), and there is an inverse relationship between GH secretion and GHBP levels in children of normal height (9). In GH-‘sufficient’ children with idiopathic short stature, GHBP levels are low (10), and we recently reported that in adults undergoing abdominal surgery both mRNA levels for the GH receptor in skeletal muscle and plasma levels of GHBP decrease in parallel (8). Studies of the regulation of GHBP may therefore indirectly provide information about the regulation of the GH receptor.

Many investigators have reported that body composition plays a key role in the regulation of GHBP levels. There is a positive correlation between GHBP and BMI both in children (9) and in adults (11, 12). Anorexia
nervosa (11, 13) and critical illness (8, 14) result in decreased GHBP levels, and refeeding restores GHBP levels to normal in patients with anorexia nervosa (13).

An attractive candidate for the link between growth and body fatness is leptin, the product of the recently cloned obese (ob) gene (15). Leptin, produced by adipose tissue (15), is a hormone belonging to the cytokine superfamily (16). Leptin concentrations in the blood correlate directly with measures of body fatness in both adults (17, 18) and children (19). Leptin receptor expression has been demonstrated in several tissues (20–22), including those that express GH receptors and are targets for GH action. Leptin is believed to be a regulator of appetite and energy expenditure (15, 23–25) but there is also increasing evidence for a more general role in the regulation of growth (26) and sexual maturation (27, 28).

The aim of this study was to analyse the relationship between GHBP and leptin in children in order to test the hypothesis that leptin is the link between adipose tissue and serum concentration of GHBP.

**Patients and methods**

**Subjects**

The children included in this study were investigated at the International Pediatric Growth Research Centre, Göteborg, Sweden, except for a minor number of the GHD children and the small for gestational age (SGA) children who were investigated at other referral centres in Sweden. All children were classified as prepubertal, according to Tanner & Whitehouse (29) regarding breast and pubic hair development, and according to Zachmann et al. (30) regarding testicular volume as measured by an orchidometer. Height, weight and weight for height (W/H) at the time of the study were expressed as standard deviation scores (SD score) compared with Swedish reference values (31–33). All the children were healthy and did not deviate on weight charts nor had they anamnesis or signs of undernutrition. Blood and serum values were normal, including thyroid, liver and kidney functions.

The study was approved by the Ethical Committee of the Medical Faculty, University of Göteborg. Informed consent was obtained from each child and his/her parents.

**Longitudinal study** Blood samples were taken once a month from 12 healthy children (five girls and seven boys) with a mean and median age of 9.3 and 10.0 years (range 8.5 to 11.1 years). The number of mean, medium and range of samples available (10.8, 11, range 8 to 13) allowed the calculation of 125 monthly changes for both leptin and GHBP. In this group, height SD score was 1.2, 0.8 (—3 to 2.9), weight SD score was 1.1, 1.1 (0.2 to 2.9), W/H SD score was 0.3, 0.4 (0.1 to 2.5) and BMI was 16.9, 17.0 (14.9 to 20.1).

**Cross-sectional studies** The study groups were: healthy GH-'sufficient' children, children with GHD and SGA children. The detailed characteristics of the children included in the cross-sectional studies are shown in Table 1. The group of healthy children (n=107) consisted of normal controls and children investigated for short stature. All had normal GH secretion on 24-h sampling. GHD was defined as a failure of GH concentrations to exceed 20 mU/l (measured by an immunoradiometric assay; Pharmacia & Upjohn, Uppsala, Sweden, using the WHO First International Reference Preparation hGH 66/217 as standard) in response to an arginine-insulin tolerance test.

**Measurements of GHBp and leptin**

All blood samples were taken between 1000 and 1600 h. After withdrawal, blood samples were kept at room temperature and centrifuged within 24 h. Serum was stored at −20°C until assayed. In each subject, samples for GHBP and leptin were obtained on the same occasion.

**GHBP assay** Total GHBP was measured by a ligand-mediated immunofunctional assay (LIFA) as previously described (34). The detection range of the LIFA was 15.6-1000 pmol/l. The intra- and interassay

<table>
<thead>
<tr>
<th>No. (% female)</th>
<th>Healthy children</th>
<th>SGA children</th>
<th>GHD children</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>107 (27%)</td>
<td>6.2 (2.0 to 12.8)</td>
<td>8.3 (2.8 to 13.2)</td>
</tr>
<tr>
<td>Height SD score</td>
<td>—1.3 to —2.1 (—3.8 to 4.9)</td>
<td>—3.3 to —3.0 (—5.4 to —1.9)</td>
<td>—2.8 to —2.7 (—6.0 to —1.1)</td>
</tr>
<tr>
<td>Weight SD score</td>
<td>—1.0 to —1.4 (—3.0 to 3.4)</td>
<td>—2.9 to —2.9 (—5.3 to —1.3)</td>
<td>—1.5 to —1.5 (—3.9 to 0.0)</td>
</tr>
<tr>
<td>W/H SD score</td>
<td>0.0 to 0.0 (—2.5 to 4.8)</td>
<td>—0.6 to —0.7 (—4.0 to 2.33)</td>
<td>0.9 to 0.8 (—0.9 to 3.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.0; 15.8 (12.7 to 23.0)</td>
<td>14.2; 14.2 (11.7 to 17.4)</td>
<td>15.6; 15.3 (12.7 to 20.5)</td>
</tr>
<tr>
<td>GHBP (pmol/l)</td>
<td>124; 108 (23 to 419)</td>
<td>122; 112 (43 to 328)</td>
<td>160; 128 (43 to 387)</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>3.8; 3.2 (1.5 to 13.3)</td>
<td>3.1; 2.6 (1.4 to 7.1)</td>
<td>3.6; 2.8 (1.7 to 16.2)</td>
</tr>
</tbody>
</table>
coefficients of variation were 7.3% and 11.3% respectively. Samples from each study group were analysed in the same assay.

**Leptin assay** Leptin was measured by RIA (Linco Research Inc, St Charles, MO, USA). The assay has a detection range of 0.22 to 100 μg/l and, in our hands, intra-assay coefficients of variation of 7.0% at 2.4 μg/l and 4.9% at 14.0 μg/l. The corresponding interassay coefficients of variation were 9.6% and 6.7% respectively. Samples from each study group were analysed using the same assay batch.

**Statistical methods**

If not stated otherwise, values are given as mean, median, with range in parentheses. Pitman’s non-parametric permutation test (35) was used for correlation analysis. Pearson’s correlation coefficient was calculated for descriptive purpose. A non-parametric partial correlation analysis, using Mantel’s technique of pooling (36) applied to Pitman’s permutation test (35) was used to study the correlation between two variables adjusted for other variables. To test the intra-individual correlation of the changes in GHBP and changes in leptin over individuals in the longitudinal study, an r value was calculated for each individual. Wilcoxon’s signed rank test was then used on these r values to test the correlation. Tests were considered significant with a P value of 0.05 or less.

**Results**

**Longitudinal study**

**Variation in GHBP and leptin in healthy prepubertal children over 1 year** Serum concentrations of GHBP and leptin were measured in monthly samples taken over 1 year from 12 healthy prepubertal children. Mean, median serum GHBP concentrations and mean, median serum leptin concentrations in this group were 325.331 pmol/l (88 to 461 pmol/l) and 6.1. 5.5 μg/l (2.3 to 12.2 μg/l) respectively. Both leptin and GHBP concentrations varied during the sampling period in all children, with median intra-individual coefficients of variation of 15.2% (7 to 25%) for GHBP and 28.1% (15 to 45%) for leptin. There was a significant positive intra-individual correlation between the monthly changes in GHBP concentrations and those of leptin concentrations. The mean of r was 0.38 (median = 0.29; range, 0.03 to 0.86; P < 0.05). Monthly changes in GHBP versus monthly changes in leptin are given in Fig. 1, for descriptive purpose.

**Cross-sectional studies**

**Healthy GH-‘sufficient’ children** In this group of children there was a positive correlation between serum GHBP and leptin concentrations (r = 0.65, P < 0.001; Fig. 2a). There were also significant correlations between GHBP and BMI (r = 0.37, P < 0.001), leptin and BMI (r = 0.59, P < 0.001) and leptin and W/H SD score (r = 0.35, P < 0.01). The results are summarized in Table 2.

There were still significant non-parametric partial correlations (P < 0.001) between leptin and GHBP after adjustment for age, BMI, gender, W/H SD score, height SD score and weight SD score. After simultaneous adjustment for all the above variables the partial correlation coefficient between leptin and GHBP was 0.48 (P < 0.0001). This implies that leptin explained 23% of the variation in GHBP after adjustment for all other variables. After adjustment for only BMI and age the partial correlation coefficient was 0.57, with 33% of the variation explained (P < 0.0001).

**Short children born SGA** Serum GHBP concentrations correlated positively with serum leptin levels (n = 55, r = 0.74, P < 0.001; Fig. 2b) and negatively to height SD score (r = −0.32, P < 0.05). The results are summarized in Table 2. There was no significant correlation between leptin and BMI (r = −0.04, not significant) or leptin and W/H SD score (r = 0.15, not significant) in these children.

There were still significant non-parametric partial correlations (P < 0.001) between leptin and GHBP after adjustment for age, BMI, gender, W/H SD score, height SD score and weight SD score. After simultaneous adjustment for all the above variables the partial correlation coefficient between leptin and GHBP was 0.69 (P < 0.0001). This implies that leptin explained 48% of the variation in GHBP after adjustment for all other variables. After adjustment for only BMI and age.
the partial correlation coefficient was 0.74, with 55% of the variation explained ($P < 0.0001$).

**Children with GHD** In the 55 children with GHD there was no correlation between GHBP and leptin ($n = 55$, $r = 0.27$, not significant; Fig. 2c). None of the other available parameters correlated to GHBP, summarized in Table 2, nor was there correlation between leptin and BMI ($r = 0.00$, not significant) or leptin and W/H SD score ($r = 0.20$, not significant) in the children with GHD.

**Discussion**

In this study, serum concentrations of leptin were found to be highly correlated with serum concentrations of GHBP in healthy prepubertal children and in children born SGA, but not in children diagnosed as GHD.

Several studies have demonstrated that the concentration of GHBP (6) in the blood correlates with different measures of body fatness (9, 11, 12), but the mechanisms behind this correlation are unknown. Both GHBP and the GH receptor are products of the GH receptor gene (6), and it is well established that this gene is expressed in a large number of tissues, including adipose tissue, reviewed by Kelly et al. (37). It is likely that GHBP in the blood is derived from many different tissues, and the simplest explanation for the increase in GHBP concentrations with increasing fat mass is that the adipose tissue itself is a major producer of GHBP. However, it has been suggested that the liver is the major source of GHBP, and this is supported by the results of a recent study showing that GH receptor gene expression is much higher in the liver than in adipose tissue and skeletal muscle (8). An alternative explanation for the connection between adiposity and GHBP levels is that the adipose tissue may provide a signal that regulates the expression of the GH-receptor gene, and thereby also the amount of GHBP in the circulation.

In the cross-sectional study of healthy children and children born SGA, there were strong correlations between serum GHBP and serum leptin levels. This relationship between GHBP and leptin was not seen in children with GHD. The reason for this discrepancy is not known. Further studies are needed to clarify what role GH might have in this context. It could be argued that this relationship only reflects the fact that both GHBP and leptin are correlated with body composition (i.e. BMI). However, this relationship was maintained after adjustment for age and BMI, when tested with non-parametric partial correlation analysis. Interestingly, the relationship between leptin and GHBP persisted in children born SGA, in whom correlations between GHBP and BMI, and leptin and BMI were absent. In addition, there was also a correlation between the monthly changes in serum leptin and changes in serum GHBP levels in healthy children followed over 1 year. This observation not only confirms the observation from the cross-sectional studies that there is a correlation between leptin and GHBP, but also demonstrates that there is short-term correlation between leptin and GHBP. Although the present results clearly show that there is a close relationship between GHBP and leptin this does not infer causality between
the two. However, the relationship between leptin and GHBP, taken together with the clinically well-recognized connection between nutrition and growth, opens the possibility that leptin may be involved in growth regulation.

Acknowledgements
This work was supported by grants from the Swedish Medical Research Council (7509, 11285, 11502, 11331, 11576), Swedish Medical Society, Göteborgs Läkaresällskap, University of Göteborg, Medical Faculty, Firma AB, Childrens Hospital and to all the children and parents who made this study possible. The authors are grateful to the staff at Ward 34, Children’s Hospital and to Ingela Larsson, Birgit Lidvall, Carina Ankarberg and Birgitta Svensson for technical support and Nils-Gunnar Pehrsson for statistical advice and constructive discussions.

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Table 2 Correlation analysis and non-parametric permutation tests between different variables and GHBP in the cross-sectional study groups.

<table>
<thead>
<tr>
<th></th>
<th>Normal group (n = 107)</th>
<th>SGA group (n = 55)</th>
<th>GHD group (n = 55)</th>
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<tbody>
<tr>
<td></td>
<td>r¹</td>
<td>P value²</td>
<td>r¹</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.65</td>
<td>0.001</td>
<td>0.74</td>
</tr>
<tr>
<td>BMI</td>
<td>0.37</td>
<td>0.001</td>
<td>–0.03</td>
</tr>
<tr>
<td>Age</td>
<td>–0.04</td>
<td>NS</td>
<td>–0.04</td>
</tr>
<tr>
<td>Height SD score</td>
<td>0.42</td>
<td>0.001</td>
<td>–0.32</td>
</tr>
<tr>
<td>Weight SD score</td>
<td>0.50</td>
<td>0.001</td>
<td>–0.06</td>
</tr>
<tr>
<td>W/H SD score</td>
<td>0.19</td>
<td>NS</td>
<td>0.09</td>
</tr>
</tbody>
</table>

¹ Pearson’s correlation coefficient; ² P value according to Pitman’s non-parametric permutations test; NS, not significant.
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Received 3 February 1997
Accepted 20 March 1997