Clinical Study

Associations between body mass, leptin, IGF-I and circulating adrenal androgens in children with obesity and premature adrenarche

Dagmar l’Alemand, Stefan Schmidt, Valentin Rousson1, Georg Brabant2, Theo Gasser1 and Annette Grüters

Children’s Hospital, Charité Campus Virchow, Humboldt University, Berlin, Germany, 1 Department of Biostatistics, University of Zurich, Switzerland and 2 Department of Medicine, University of Hannover, Germany

(Correspondence should be addressed to A Grüters-Kieslich, Charité Kinderklinik, Campus Virchow, Humboldt Universität, Augustenburger Platz 1, D-13353 Berlin, Germany; Email: annette.grueters@charite.de)

Abstract

Objective: To explain why adrenal androgens rise with increasing adiposity during childhood, the role of body mass index (BMI), leptin and IGF-I was studied. We also tested whether these parameters contribute to inducing premature adrenarche (PA).

Design: In a cross-sectional study, 26 prepubertal obese children were compared with a group of 26 prepubertal children of normal weight, and 30 children under observation for PA were compared with 30 healthy children, matched for gender, bone age and BMI.

Methods: Relative contributions of BMI standard deviation scores (SDS) and height SDS, as well as unbound leptin and IGF-I, to the levels of androgens, dehydroepiandrosterone sulfate (DHEAS) and Δ4-4-androstenedione (AD) were investigated by means of stepwise regression models. Logarithms of all hormones were standardised for age using residuals of a simple regression analysis, labelled by the suffix ‘res’.

Results: In the obese children, height SDS, IGF-I res, DHEASres (all \( P < 0.05 \)), leptinres (\( P < 0.01 \)), and ADres (\( P = 0.07 \)) were higher than in the controls, and covariates were correlated with each other (leptinres versus BMI SDS \( r = 0.71 \), IGF-Ires versus height SDS \( r = 0.61 \)). In the stepwise regression analysis of control and obese children, BMI SDS explained 26% and leptin res explained 12% of the variability of DHEAS res, but this percentage remained at 26% when both variables were simultaneously introduced into the model. In contrast, IGF-I res and BMI SDS alone each accounted for 15% of the variability of AD, and their joint influence accumulated to explain 28% of the variability of AD res. In PA, neither BMI SDS nor leptin res were correlated with the increased androgens.

Conclusion: Before the onset of gonadal activity in obese and control children, DHEAS levels, to some extent, are explained by BMI and leptin, while IGF-I in addition to BMI in part accounts for AD levels. Enhanced adrenal androgen secretion in children with PA, however, may be explained by parameters other than leptin or BMI.

European Journal of Endocrinology 146 537–543

Introduction

Adrenal androgens are increased in children with obesity (1, 2), and they have been made responsible for their accelerated growth before puberty (3), but the underlying regulatory mechanism of their stimulation remains unknown. Since fat cells produce leptin, it is reasonable to study whether leptin interferes with endocrine alterations observed in obesity. In humans, leptin deficiency accounts for hyperphagia, decreased sympathetic activity and hypogonadism (4, 5). Leptin seems to trigger pubertal development in primates, as before puberty it increases nightly to induce enhanced gonadotrophin-releasing hormone pulsatility and luteinizing hormone secretion (6). Thus leptin may represent the metabolic gate to initiate puberty (7). In humans, data to support a comparable function of leptin are scarce. It was deduced from cross-sectional studies that leptin levels may rise before the onset of puberty (7–10), but this has been clearly documented in only one longitudinal study of boys (11).

In addition, the adrenal gland contributes to pubertal development and sex steroid secretion. Longitudinal studies have shown that, independent of age and genetic determination (12), the individual increase of body mass as such is a factor that markedly affects the rise of adrenal androgen secretion at the time of adrenarche. Leptin may represent one factor mediating...
Table 1 Clinical data given as medians and ranges (in parentheses).

<table>
<thead>
<tr>
<th>Group</th>
<th>Prepubertal obese</th>
<th>Prepubertal controls</th>
<th>PA 1</th>
<th>Matched controls for PA 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. (girls)</td>
<td>26 (16)</td>
<td>26 (17)</td>
<td>30 (21)</td>
<td>30 (21)</td>
</tr>
<tr>
<td>Chronological age</td>
<td>8.5 (2.9–13.1)</td>
<td>8.0 (2.7–11.4)</td>
<td>9.1 (5.0–17.1)</td>
<td>9.3 (5.1–15.0)</td>
</tr>
<tr>
<td>Bone age</td>
<td>10.0 (4.0–13.8)</td>
<td>8.5 (4.3–11.5)</td>
<td>10.5 (5.0–18.0)</td>
<td>10.0 (5.0–15.0)</td>
</tr>
<tr>
<td>P stage</td>
<td>1</td>
<td>1</td>
<td>3** (2–6)</td>
<td>1 (1–5)</td>
</tr>
<tr>
<td>B or G stage</td>
<td></td>
<td></td>
<td>1 (1–5)</td>
<td>1 (1–5)</td>
</tr>
<tr>
<td>Height SDS</td>
<td>2.5* (2.3–4.1)</td>
<td>0.4 (1.9–3.9)</td>
<td>0.7 (2.1–5.2)</td>
<td>1.1 (1.9–4.4)</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>5.6*** (3.1–9.5)</td>
<td>0.5 (0.9–2.8)</td>
<td>1.5 (2.9–8.1)</td>
<td>1.8 (1.0–7.2)</td>
</tr>
</tbody>
</table>

1 13/30 children with breast or genital stage ≥ 2.
PA controls = controls matched with PA for gender, BMI and bone age.

P, B, G = Tanner pubertal stages of pubic hair, breast and genitalia respectively.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significant differences versus matched control group.
Bone age was determined according to Greulich & Pyle (30). There were no statistical differences between the obese children or the children with PA and their respective control groups regarding age, bone age, pubertal stage, as analysed by Mann–Whitney test, and sex distribution, as analysed by a chi-square test (Table 1). The only significant difference was found for the advanced pubic hair stage in the PA group, which is inherent to the diagnosis.

The following hormones were measured by direct specific radioimmunoassays with the inter-assay coefficients of variation (CV) as indicated, and slightly lower intra-assay CV values (data not shown): DHEAS (IBL/RSL, Hamburg, Germany, CV = 9.2% at 1605 ng/ml, n = 25), AD (DSL, Sinsheim, Germany, CV = 5.5% at 1.21 ng/ml, n = 30) and IGF-I (Mediagnost, Tuebingen, Germany, CV = 7.2% at 114 ng/ml, n = 14) and unbound leptin as published by Horn et al. (31), (CV = 8.3% at 110 pmol/l, n = 6).

Age-dependent reference ranges for the methods used have been established in our laboratory (Table 2), with the exception of AD and leptin. For these methods, normative data of children younger than 3 and 8 years respectively were not available; however, all hormones were dependent on age. The hormones entered into the regression analysis were therefore standardised for age as described below. There was no significant sex difference in hormone levels, as assessed by the Mann–Whitney test. As a consequence, all hormones were calculated on boys and girls combined.

### Table 2 Hormone levels in the study groups and reference ranges.

<table>
<thead>
<tr>
<th></th>
<th>Leptin (nmol/l)</th>
<th>IGF-I (nmol/l)</th>
<th>DHEAS (µmol/l)</th>
<th>AD (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prepubertal obese</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>26</td>
<td>16</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Median</td>
<td>192***</td>
<td>26.4*</td>
<td>2.47*</td>
<td>1.88(*)</td>
</tr>
<tr>
<td>Range</td>
<td>41–498</td>
<td>11.1–55.4</td>
<td>0.61–8.1</td>
<td>0.17–12.6</td>
</tr>
<tr>
<td><strong>Prepubertal controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>26</td>
<td>16</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Median</td>
<td>36.5</td>
<td>17.7</td>
<td>0.48</td>
<td>0.84</td>
</tr>
<tr>
<td>Range</td>
<td>12–392</td>
<td>7.9–38.3</td>
<td>0.21–4.54</td>
<td>0.17–3.98</td>
</tr>
<tr>
<td><strong>Normal reference values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>8–10</td>
<td>2–10</td>
<td>2–10</td>
<td>3–10</td>
</tr>
<tr>
<td>Median</td>
<td>19.5</td>
<td>17.4</td>
<td>0.61</td>
<td>0.98</td>
</tr>
<tr>
<td>Range</td>
<td>10–92</td>
<td>5.1–43.2</td>
<td>0.21–3.4</td>
<td>0.17–2.6</td>
</tr>
<tr>
<td><strong>PA patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>30</td>
<td>n.d.</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>Median</td>
<td>48</td>
<td>6.18**</td>
<td>4.05**</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12–733</td>
<td>0.59–14.6</td>
<td>1.05–14.7</td>
<td></td>
</tr>
<tr>
<td><strong>PA controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>30</td>
<td>n.d.</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>Median</td>
<td>54</td>
<td>3.08</td>
<td>2.06</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12–431</td>
<td>0.29–9.5</td>
<td>0.17–6.35</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001, significant differences versus controls (Student’s t-test); (*) trend, P = 0.07 versus controls; †P < 0.05 difference from controls only significant by non-parametric Mann–Whitney test.

n.d. = not done.

### Statistics

Because most of the variables showed a skew distribution, data were summarised by medians and ranges. Logarithms of hormone variables, i.e. leptin, IGF-I, DHEAS and AD, showed an approximately normal distribution. Because all these variables were clearly age dependent, we established age-standardised values. To do so, we calculated a least squares fit with age as explanatory variable and the logarithm of the hormone as the response. This regression was made using all the children except those of the PA group because, in the latter, the relationship between age and hormonal parameters is atypical. Age-standardised values were then defined as the residuals with respect to this fit (this was done also for the PA group). We refer to them as leptinres, IGF-Ires, DHEASres and ADres. Note that this standardisation technique is equivalent to considering age as a covariate.

In order to compare the obese and the control group, as well as the PA and the PA control group with respect to leptinres, IGF-Ires, DHEASres and ADres, height SDS and BMI SDS, we used two sample t-tests.

In order to investigate the relative contribution of anthropometric variables (BMI SDS and height SDS) and hormonal variables (leptinres and IGF-Ires) to the levels of DHEASres and ADres, we considered stepwise regression models with two explanatory variables. The relationship between an ‘exploratory’ variable (leptinres, IGF-Ires, BMI SDS or height SDS) and a response (DHEASres or ADres) was characterised by the Pearson correlation coefficient \( r_{(\text{expl})} \) or equivalently by its square \( R^2 \times 100 \), which represents the percentage of variability of the response indicated by the explanatory variable. We also investigated the relationship between two explanatory variables in this way. In the regression models with two explanatory variables, we used F tests to assess the joint influence, and partial F tests to assess the contribution of each explanatory variable. All regression models with one or two explanatory variables were calculated using both the obese and the control groups. We also calculated such models in the PA group. However, hormonal variables scattered unevenly because 13 children had already entered puberty with breast (B) or genital (G) development; hence, regression analysis was additionally tested in the separate ‘prepubertal’ PA group (B1, G1, pubic hair stage \( \geq 2 \)).

Throughout the analyses, \( P \) values below 0.05 were considered significant.

### Results

**Anthropometric and hormonal data of prepubertal obese and control children**

In children with obesity, BMI SDS was increased by definition and height SDS was also significantly elevated.
The anthropometric data were reflected by the pattern of leptin and IGF-I (Table 2); leptin_{res} and IGF-I_{res} were significantly higher in the obese group compared with controls. DHEAS_{res} (Table 2) was significantly higher in the obese children than in the controls. There was a trend for higher AD_{res} in the obese group ($P = 0.07$).

**Regression analyses to explain DHEAS and AD in the combined group of obese and control children**

Before entering explanatory variables into the regression model to recognise their relative contribution to explain the variability of adrenal androgens, we tested the influence of age. As mentioned above, all hormones were clearly age dependent: in the combined prepubertal group (obese and controls), age accounted for about half of the variability of the logarithms of IGF-I, DHEAS and AD ($\%R^2$ (square of Pearson regression coefficient $\times 100$) = 48, 55 and 55 respectively, with $P < 0.0001$), and for 16% of leptin ($P < 0.0001$). We also tested the potential relationships between the covariates themselves. As shown by others, we found strong correlations between some covariates: leptin_{res} was correlated with BMI SDS ($r = 0.71$, $P < 0.001$), and IGF-I_{res} with height SDS ($r = 0.61$, $P < 0.001$). In the regression model, leptin_{res} was significantly correlated with DHEAS_{res}, explaining about 12% of the androgen's variability (Table 3); the introduction of BMI SDS into the model significantly improved the explanation of the DHEAS_{res} variability, yielding 28% together with IGF-I_{res}. Neither leptin_{res} nor height SDS, in any combination, were found to make significant contributions to improving the association between IGF-I_{res} and DHEAS_{res} or AD_{res}.

**PA group**

To examine the relation between leptin or body mass and increased adrenal androgen secretion in children with PA (Table 4), we adopted two approaches. First, hormonal data of the patients with PA were compared with controls matched for gender, bone age and BMI, to minimise the influence of these covariates. Second, the interaction between adrenal androgens, leptin and several auxological parameters was tested by multiple regression analysis in the PA group only.

First, BMI in the PA group (Table 1) was increased, on average, as can be deduced from the fact that the median BMI SDS was 1.5 times higher than the average of the ZLS references. Then, as the PA group was compared with the matched control group, BMI SDS did not differ significantly and leptin was also similar in both groups, while DHEAS and AD were still elevated in the entire PA group (Table 2), or in 'prepubertal' PA children (data not shown; $P < 0.01$ and $P < 0.05$ respectively). Therefore, leptin had no specific effect, beyond mediating obesity, to account for increased adrenal androgen levels in PA subjects.

This observation was further tested by multiple regression analysis, as explained in the section on Patients and methods. Despite the fact that in the PA group the covariates leptin and BMI SDS were significantly correlated ($r = 0.71$, $P < 0.001$), neither

<table>
<thead>
<tr>
<th>Response</th>
<th>1st explanatory variable ($x_1$)</th>
<th>Model with $x_1$</th>
<th>2nd explanatory variable ($x_2$)</th>
<th>Model with $x_2$</th>
<th>Model with $x_1$ and $x_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_{\text{Pea}}$</td>
<td>$%R^2$</td>
<td>$P$</td>
<td>$r_{\text{Pea}}$</td>
<td>$%R^2$</td>
</tr>
<tr>
<td>DHEAS_{res}</td>
<td>Leptin_{res}</td>
<td>0.35</td>
<td>12</td>
<td>*</td>
<td>BMI SDS</td>
</tr>
<tr>
<td>DHEAS_{res}</td>
<td>IGF-I_{res}</td>
<td>0.20</td>
<td>4</td>
<td>n.s.</td>
<td>BMI SDS</td>
</tr>
<tr>
<td>DHEAS_{res}</td>
<td>BMI SDS</td>
<td>0.51</td>
<td>26</td>
<td>***</td>
<td>Height SDS</td>
</tr>
<tr>
<td>AD_{res}</td>
<td>Leptin_{res}</td>
<td>0.25</td>
<td>6</td>
<td>n.s.</td>
<td>BMI SDS</td>
</tr>
<tr>
<td>AD_{res}</td>
<td>IGF-I_{res}</td>
<td>0.39</td>
<td>15</td>
<td>*</td>
<td>BMI SDS</td>
</tr>
<tr>
<td>AD_{res}</td>
<td>BMI SDS</td>
<td>0.28</td>
<td>8</td>
<td>n.s.</td>
<td>Height SDS</td>
</tr>
</tbody>
</table>

Response = dependent variable considered; $x_1$, $x_2$ = 1st and 2nd explanatory or independent variables brought into the regression model; $r_{\text{Pea}}$ = Pearsons correlation coefficient; $\%R^2$ = square of $r_{\text{Pea}} \times 100$, indicating the percent of variation of the response being explained by the 1st or the 2nd explanatory variable, both together; $P$ = associated significance; *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. significant relationship between explanatory variable and response in bold type. n.s. = not significant.

$P_{x_1}$ = significance of introducing the 1st explanatory variable into the model, when the 2nd one is already in. $P_{x_2}$ = significance of introducing the 2nd explanatory variable into model, when the 1st one is already in.

No. = sample size available for the analysis of correlation.

www.eje.org
leptin$_{res}$ nor BMI SDS alone or in combination were correlated with DHEAS$_{res}$ or AD$_{res}$, no matter whether testing was done for the entire PA group (data not shown) or the ‘prepubertal’ PA children (Table 4). Height SDS was significantly correlated with DHEAS$_{res}$ or AD$_{res}$, no matter whether BMI or IGF-I was significantly correlated with DHEAS$_{res}$ or AD$_{res}$, no matter whether BMI or IGF-I was significantly correlated with fat tissue or height, at least in lean children. Since IGF-I was significantly correlated with DHEAS$_{res}$ and BMI, whereas the levels of AD are more closely related to IGF-I and BMI, BMI, however, does not precisely represent fat mass, but also mirrors height, at least in lean children. Since IGF-I was significantly correlated with height, our results may suggest that the effects of fat mass are mediated by leptin, and those of height or lean mass by IGF-I. In fact, more studies will be necessary to establish the contribution of each compartment of body composition to affect adrenal androgen secretion. The sequence of these associations cannot be extrapolated from the present cross-sectional study but from other publications as follows. From in vitro experiments, it can be deduced that leptin (13, 14) and IGF-I (16, 34) each directly account for changes in adrenal enzyme activity or expression. Longitudinal studies have revealed in vivo that BMI SDS changes precede adrenarche (12), and that leptin (11) and IGF-I (6) increase before steroid hormones are secreted. Leptin and IGF-I both increase before puberty and might provide the brain with information on body composition and size to start pituitary, adrenal and gonadal maturation (6, 12). The involvement of IGF-I in the regulation of adrenal androgens is confirmed by a clinical study on girls with Turner’s syndrome, which showed an increased stimulation of adrenal androgens in parallel with a rise of IGF-I levels after the initiation of GH therapy (35).

In children with PA, neither BMI nor leptin alone explain the precocious adrenal maturation. Nevertheless, the sample size of the present study may be too limited to reveal weak associations. As a matter of fact, there is no reason why obesity or BMI should not have an additional stimulating effect on adrenal androgen formation in children with PA, as demonstrated in healthy children. Altogether, we suggest that, in PA, additional factors are involved in the regulation of androgen secretion, e.g. mild enzyme defects, which might have been missed by routine ACTH testing in this study, or hyperinsulinism, as discussed below.

The positive correlation of adiposity, to the extent indicated by BMI, to androgens, observed in prepubertal children, contrasts with the regulation observed in adults. As discussed above, DHEAS levels, in relation to enhanced insulin secretion, may even be reduced as a result of increased metabolic turnover of this hormone (22–26). In fact, there are yet other factors in addition to IGF-I or leptin, which increase with body size or obesity and stimulate adrenal steroids, but their investigation could not be carried out in this study. Two candidate hormones have been shown to be associated with increased androgen levels in adolescent girls: corticotrophin-releasing hormone, which is...
activated in obesity and has recently been suggested to increase adrenal androgen secretion directly (36), and hyperinsulinism due to insulin resistance (20, 37). In prepubertal children, however, no correlation between insulin and DHEAS could be found (38). Puberty may lead to different, positive relationships of leptin to androgens, as shown recently (39). However, published data are contradictory: according to another study in adult women with polycystic ovary syndrome, leptin does not affect circulating sex hormones or insulin levels independently of BMI (40).

Nonetheless, the secretion of gonadal steroids in response to the pubertal increase of gonadotrophins is assumed to have a much stronger effect on circulating androgen levels, and may mask secretion and regulation of adrenal androgens (41). For that reason, a pubertal group was not included in the present study.

Moreover, as in PA, the leptin—BMI SDS—androgen interaction is complicated by testosterone itself, suppressing leptin secretion from adipocytes (42). Also, very high levels of leptin (100–1000 ng/ml) directly decrease adrenal steroid formation (13) by inhibiting the expression of the 17α-mRNA in vitro. Such high leptin levels, as encountered in extremely obese adults, would outweigh the stimulation of androgen formation at low leptin levels. Therefore, the switch from a stimulated prepubertal to a different, ‘adult’ type of androgen regulation by leptin may be caused by higher doses of this peptide.

Further research on this issue is required, all the more since several other studies call attention to the consequences of an adrenal androgen increase in prepubertal and pubertal girls, affecting ovarian function in later life and representing a risk factor for ovarian hyperandrogenism (43, 44).

The causal relationship between prepubertal growth-related signals and steroid secretion cannot be assessed by a cross-sectional study. However, when combining the present results with recent longitudinal studies on the onset of adrenal and gonadal maturation, it turns out that leptin and IGF-I signalling body mass are significant regulators of steroid secretion before the onset of puberty. This has also to be born in mind when GH is administered in non-classical indications, since one of the potential effects of IGF-I could be to induce adrenal androgen secretion.

Acknowledgements

Our grateful acknowledgements go to Bettina Tippel, Bernd Berger and Gabi Sahm of the paediatric endocrine laboratory for their assistance in collecting the data, and to the nursing staff of the endocrine outpatient department for their care of the patients. Parts of this work were supported by a grant from the Deutsche Forschungsgemeinschaft DFG AL332/2-2.

References

16 L’Allemand D, Penhoat A, Lébrethon MC, Ardevol R, Baehr V, Oelkers W et al. Insulin-like growth factors enhance steroidogenic enzyme and corticotropin receptor messenger ribonucleic acid

Received 27 March 2001
Accepted 11 January 2002