CLINICAL STUDY

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

Dagmar l'Allemand, 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 Δ 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}, DHEAS_{res} (all $P < 0.05$), leptin_{res} ($P < 0.01$), and AD_{res} ($P = 0.07$) were higher than in the controls, and covariates were correlated with each other (leptin_{res} versus BMI SDS $r = 0.71$, IGF-I_{res} 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.

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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

in this event, since the ob receptor is expressed in adrenal tissue (13). Recent data indicate that leptin has a specific, dose-dependent role to promote the formation of adrenal androgens, stimulating 17,20-lyase activity of cytochrome P45017 (14). In addition leptin acts in concert with other growth-derived signals, such as growth hormone (GH) and insulin-like growth factor-I (IGF-I) (6, 15), to regulate the onset of puberty in humans and primates. IGFs, *in vitro*, activate the expression of adrenal enzymes and increase the activity of the 17,20-lyase selectively, yielding a higher rate of $\Delta 4$ -androstenedione (AD) formation (16).

Furthermore, dehydroepiandrosterone sulfate (DHEAS) and AD themselves are correlated with adiposity, as indicated in the following. First, fat cells can store and metabolise steroid hormones (17, 18). Second, insulin resistance, as is often observed in obesity (19), results in hyperinsulinism and enhanced activity of IGF-I, and has been shown to be associated with increased androgen levels in adolescent girl (20). However, these interrelationships may differ according to age or gender. In obese women, DHEAS levels are slightly increased and independent of insulin secretion, but positively correlated with IGF-I (21). In contrast, in adult men with obesity (22) and hyperinsulinaemia (23, 24) DHEA(S) is decreased. Levels of dehydroepiandrosterone or DHEAS are similarly reduced in women with severe obesity or type 2 diabetes (25, 26).

So far, it has not yet been shown in prepubertal children how leptin, IGF and adrenal androgens, as well as parameters of obesity are interrelated. The aim of the present study was to measure adrenal androgen levels in children of normal weight and with obesity, and to correlate them with leptin and anthropometric variables. A second aim was to analyse the influence of IGF-I on adrenal androgens in lean and obese children. Furthermore, additional investigations were carried out on a group of children with premature adrenarche (PA), to answer the question as to whether the elevation of adrenal androgens in this disorder is the consequence of obesity, or directly to a rise of leptin, or both.

Patients and methods

In this cross-sectional study, 52 healthy children were investigated in our outpatient department in the following groups. Obese group, 26 prepubertal children with obesity, defined by body mass index (BMI) standard deviation score (SDS) above 3 and control group, 26 children considered as normal weight controls, comprising 19 children with congenital hypothyroidism, detected by newborn thyrotrophin screening, and treated with thyroxine since the first 2 weeks of life, in whom normal growth and pubertal development have recently been described (27), and seven children with untreated familial tall stature, defined by a height above the 97th percentile for age (28). Tall children may be included as controls, since growth is accelerated in obese children (3). In addition, 30 children with idiopathic PA or hirsutism with a history of PA were included for comparison, because of their precocious, increased secretion of adrenal androgens. This group included 13 children who had already entered puberty with breast or genital stage ≤ 2 . An adrenal enzyme defect had previously been excluded by an adrenocorticotrophin (ACTH) stimulation test (29). This group was compared with 30 healthy children as described above, and matched for gender, bone age and BMI to each PA subject.

The study was approved by the Ethics Committee of the Humboldt University Hospital, Campus Virchow, and informed consent was given by the parents. No more than 1 ml serum was available from each child and therefore not all hormones could be determined in each patient. IGF-I was not determined in the PA group.

Height, weight and pubertal stages were assessed according to standard methods (28) (Table 1). To adjust for gender and age, height and BMI (weight in kg divided by the square of the height in m) were given as SDS using the First Zurich Longitudinal Study (ZLS) as reference (28). Obesity was defined by a BMI SDS above 3, because this is the only limit that allows for the selection of markedly obese children.

Table 1 Clinical data given as medians and ranges (in parentheses).

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

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 public 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.

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

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, 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 leptin_{res}, IGF-I_{res}, DHEAS_{res} and AD_{res}. 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 leptin_{res}, IGF-I_{res}, DHEAS_{res}, AD_{res}, 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 (leptin_{res} and IGF-I_{res}) to the levels of DHEAS_{res} and AD_{res}, we considered stepwise regression models with two explanatory variables. The relationship between an 'explanatory' variable (leptin_{res}, IGF-I_{res}, BMI SDS or height SDS) and a response (DHEAS_{res} or AD_{res}) was characterised by the Pearson correlation coefficient (r_{Pea}) 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 ≥ 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

(Table 1). 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} response to 26 %, but neither the introduction of height SDS nor of IGF-I_{res} (not shown) in addition to leptin changed the explanation of DHEAS variance. BMI SDS alone explained about 26% of the variability of DHEAS_{res}, and this relationship was not changed if leptin_{res} was introduced into the model. Hence, we concluded that leptin, at least in part, could be a mediating signal of BMI with respect to its contribution in

explaining DHEAS levels. The model with BMI SDS was also not improved by the addition of IGF-I_{res} or height SDS. While IGF-I_{res} was not correlated with DHEAS_{res}, it was an important variable to explain AD_{res}, accounting for about 15% of its variance (Table 3). BMI SDS significantly added about 13% to the explanatory model, 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 3 Regression models in prepubertal children: combined group of control and obese children.

Response	1st explanatory variable (x_1)	Model with x_1			2nd explanatory variable (x_2)	Model with x_2			Model with x_1 and x_2				No.
		r_{Pea}	$\%R^2$	P		r_{Pea}	$\%R^2$	P	$\%R^2$	P	P_{x1}	P_{x2}	
DHEAS _{res}	Leptin _{res}	0.35	12	*	BMI SDS	0.51	26	***	26	**	n.s.	*	37
DHEAS _{res}	IGF-I _{res}	0.20	4	n.s.	BMI SDS	0.47	22	*	23	*	n.s.	*	27
DHEAS _{res}	BMI SDS	0.51	26	***	Height SDS	0.17	3	n.s.	27	**	**	n.s.	37
AD _{res}	Leptin _{res}	0.25	6	n.s.	BMI SDS	0.28	8	n.s.	9	n.s.	n.s.	n.s.	35
AD _{res}	IGF-I _{res}	0.39	15	*	BMI SDS	0.39	15	*	28	*	*	n.s.	27
AD _{res}	BMI SDS	0.28	8	n.s.	Height SDS	0.20	4	n.s.	11	n.s.	n.s.	n.s.	35

Response = dependent variable considered; x_1 , x_2 = 1st and 2nd explanatory or independent variables brought into the regression model; r_{Pea} = Pearson's correlation coefficient; $\%R^2$ = square of $r_{Pea} \times 100$, 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_{x1} = significance of introducing the 1st explanatory variable into the model, when the 2nd one is already in. P_{x2} = 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.

Table 4 Regression models in prepubertal children of the PA group.

Response	1st explanatory variable (x_1)	Model with x_1			2nd explanatory variable (x_2)	Model with x_2			Model with x_1 and x_2				No.
		r_{Pea}	%R ²	P		r_{Pea}	%R ²	P	%R ²	P	P_{x1}	P_{x2}	
DHEAS _{res}	Leptin _{res}	0.17	6	n.s.	BMI SDS	0.14	2	n.s.	6	n.s.	n.s.	n.s.	17
DHEAS _{res}	BMI SDS	0.14	2	n.s.	Height SDS	0.33	11	n.s.	11	n.s.	n.s.	n.s.	17

See Table 3 for explanation of terms.

The sample size in this group was too small to perform the regression analyses with AD.

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} in the entire PA group ($r_{Pea} = 0.42$, $P < 0.05$), apparently as a consequence of the effects of DHEA/DHEAS on growth promotion.

Discussion

The present data for the first time support the hypothesis that leptin and IGF-I may act as mediators to signal changes in body composition or size and to increase adrenal androgen secretion. Our study provides data for prepubertal girls and boys over a broad range of body weight, to demonstrate that adrenal androgens increase with adiposity, as assessed by BMI SDS. Previous studies merely included selected cohorts of girls with a specific range of age and weight (1, 32), or of boys (2). The present study provides a methodological advantage in so far as it is the first to measure free leptin in children, which is more accurately correlated with fat tissue than total leptin (33). Our main results may be summarised as follows. (a) In prepubertal children with obesity, DHEAS and AD as well as leptin and IGF-I are elevated. Before puberty, BMI SDS and leptin together explain 26% of the DHEAS response in obese and control children after correction for age. Leptin, in its own right, has a weak influence on DHEAS (12%), but leptin is not an independent factor in addition to BMI to explain DHEAS, while BMI SDS alone explains 26%. The variability of the response of AD is essentially (28%) explained by IGF-I in concert with BMI, but also by IGF-I (15%) and BMI SDS (15%) alone. (b) Children with PA have an increased BMI on average, but BMI is not significantly correlated with adrenal androgens in this group. After control for age and adiposity, leptin has no significance in explaining the increased adrenal androgen levels in PA subjects.

Before the pubertal onset of gonadal activity, the levels of DHEAS seem to be mainly related to leptin and BMI, whereas the levels of AD are more closely linked 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 17 α -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.

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