Steroid Hormone Profiles in Prepubertal Obese Children Before and After Weight Loss

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Context: Little information is available on the steroid hormone profiles in obese children and their changes after weight loss.

Objective: We compared liquid chromatography-tandem mass spectrometry of serum steroid hormone profiles between obese and normal-weight children and studied the differential effects of weight loss on these hormones.

Design: This study was a cross-sectional comparison between obese and normal-weight children and a longitudinal 1-year follow-up study during lifestyle intervention in obese children.

Setting: The setting of the study was primary care.

Patients: Forty obese prepubertal (mean age 8.5 $\pm$ 2.1 years, 48% female, mean body mass index 24.8 $\pm$ 3.5 kg/m^2) and 40 normal-weight children matched for gender, age, and pubertal stage.

Intervention: The study consisted of an outpatient 1-year intervention program based on exercise, behavior, and nutrition therapy.

Main Outcomes Measures: Progesterone, 17-hydroxyprogesterone, 11-deoxycorticosterone, corticosterone, aldosterone, 11-deoxycortisol, cortisol, cortisone, dehydroepiandrosterone sulfate (DHEAS), androstenedione, T, dihydrotestosterone, insulin resistance index of the homeostasis model assessment, and blood pressure were measured.

Results: Prepubertal obese children showed significantly increased androgens (DHEAS, androstenedione, T), mineralocorticoid precursor corticosterone, and glucocorticoids (11-deoxycortisol, cortisol, cortisone) compared with normal-weight children. In contrast to 20 obese children without weight loss, the 20 obese children with substantial weight loss demonstrated a significant decrease of cortisol, cortisone, and corticosterone. Androstenedione and T decreased but DHEAS remained elevated. Changes of the homeostasis model assessment correlated significantly positively with changes of cortisol ($r = 0.38$) and cortisone ($r = 0.43$) in partial regression analyses adjusted to changes of weight status.

Conclusions: In obese prepubertal children, the increased androgens, mineralocorticoid precursors, and glucocorticoids were responsive to weight loss in contrast to DHEAS, suggesting that DHEAS does not seem to be regulated by changes in body mass index. (***J Clin Endocrinol Metab*** **98: E1022–E1030, 2013)**

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Abbreviations: BMI, body mass index; BMI-SDS, BMI SD score; BP, blood pressure; DHEAS, dehydroepiandrosterone sulfate; HOMA, homeostasis model assessment; HPA, hypothalamo-pituitary-adrenal; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; IQR, interquartile range; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

Obesity is a complex condition associated with changes in steroid hormone synthesis: it has been documented that cortisol levels are moderately increased in obese children (1) and adults (2–4). Furthermore, T concentrations are increased in obese children (5) and obese women (6). In contrast, obese men have demonstrated decreased androgen levels (5, 7).

Interestingly, obese children with increased cortisol levels and obese adolescent females and women with increased androgens as well as obese men with low T levels are more prone to metabolic disturbances such as insulin resistance, type 2 diabetes mellitus, lipid abnormalities, and hypertension and may therefore be at particular risk of developing atherosclerotic complications (1, 5, 8, 9). On the other hand, there is increasing evidence that T treatment improves insulin sensitivity and lipid profiles in obese men (10). Furthermore, a high androgenic activity is discussed to be associated with precocious puberty, premature adrenarche, and accelerated bone age with relatively tall stature in children (11). Additionally, increased androgens have even been postulated to cause obesity in childhood (11). To understand these complex relationships between steroid hormones, obesity, and its comorbidities, it would be ideal to study the differential regulation of steroid hormone concentrations in obese children, which are naïve to drugs and other comorbidities.

The differential regulation of steroid hormone concentrations encompassing glucocorticoids, mineralocorticoids, androgens, and their specific precursors in obesity is largely unknown. In particular, little and controversial information is available on steroid hormones in obese children. Some investigators stated that hyperandrogenemia occurs only after menarche (12). One study reported lower T levels in male and female obese prepubertal children as compared with normal-weight children (13). Other studies revealed similar T concentrations in obese and normal-weight prepubertal boys and girls but increased dehydroepiandrosterone sulfate (DHEAS) levels in obese prepubertal children (14, 15).

Because only sparse and contradicting data exist on the different classes of steroid hormones in prepubertal obese children, we analyzed a comprehensive serum profile of 12 steroid hormones, androgens, mineralocorticoids, glucocorticoids, and their precursors using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) steroid profiling method (16). We hypothesized that androgen and glucocorticoid concentrations are increased in obese prepubertal children as compared with age-matched normal-weight prepubertal children and decreased in substantial weight loss, suggesting that changes of steroids in childhood obesity are a consequence and not a cause of obesity.

Materials and Methods

Subjects

Written informed consent was obtained from all parents of the participants prior to inclusion of the study. The study was approved by the local Ethics Committee of the University of Witten/Herdecke in Germany.

We examined 40 obese prepubertal Caucasian children [median age 8.8 (interquartile range [IQR]) 6.9–9.8 years, 48% female, median body mass index (BMI) 24.9 (IQR 22.3–26.8) kg/m^2] and 40 normal-weight prepubertal children matched for age and gender. All boys were stages G1 and P1 and all girls were stages B1 and P1 according to the pubertal stages of Tanner and Whitehouse.

The 40 prepubertal obese children were recruited randomly from our obesity cohort (17) including 20 obese children with substantial weight loss and 20 obese children without weight loss. Substantial reduction of overweight was defined by a decrease in SDS score of BMI (BMI-SDS) of 0.5 or greater ,and no reduction of overweight was defined by a decrease in BMI-SDS less than 0.1. This classification was used because in a reduction of less than 0.5 BMI-SDS, no improvement of insulin resistance and cardiovascular risk factors could be measured and hormones like leptin or cortisol did not tend to normalize below this cutoff point in obese German children (1, 18, 19). Children with changes of BMI-SDS between 0.1 and less than 0.5 were not included in this study.

All 40 obese children participated in the lifestyle intervention, Obeldicks, which has been described in detail elsewhere (20). Briefly, this outpatient intervention program for obese children is based on physical exercise, nutrition education, and behavior therapy including the individual psychological care of the child and his or her family. The nutritional course is based on a fat- and sugar-reduced diet as compared with the everyday nutrition of German children.

None of the children in the current study suffered from endocrine disorders, premature adrenarche, or syndromal obesity or received any drug. None of the obese children entered into puberty during the study period.

Measurements

We analyzed BMI, blood pressure (BP), and fasting serum steroid hormones (progesterone, 17-hydroxyprogesterone, 11-deoxycorticosterone, corticosterone, 11-deoxycortisol, cortisol, cortisone, androstendione, T, dihydrotestosterone, and DHEAS) in 40 obese and 40 normal-weight children. Aldosterone was determined only in the 40 obese patients with and without weight loss but not in normal-weight children.

These hormones, body fat based on skinfold thickness, waist circumference, and the insulin resistance index of homeostasis model assessment (HOMA) were determined in the 40 obese children at baseline and after the 1-year lifestyle intervention Obeldicks.

Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured unclothed to the nearest 0.1 kg using a calibrated balance scale. BMI was calculated as weight in kilograms divided by the square of height in meters. The degree of overweight was quantified using Cole's least mean square method, which normalized the BMI skewed distribution and expressed BMI as a SD score (BMI-SDS) (21). Reference data for German children were used (22). All of the children in the study

were obese according to the definition of the International Obesity Task Force (23). Waist circumference was measured halfway between lower rib and iliac crest.

BP was measured using a validated protocol (24). Systolic and diastolic BP were measured at the right arm twice after a 10-minute rest in the supine position by using a calibrated sphygmomanometer and averaged. The cuff size was based on the length and circumference of the upper arm and was as large as possible without having the elbow skin crease obstructing the stethoscope (24). The intra- and interoperator variability was less than 5% for systolic and diastolic BP.

Triceps and subscapularis skinfold thickness was measured twice using a caliper and averaged to calculate the percentage of body fat using a skinfold thickness equation with the following formulas (25): for boys, body fat percentage = 0.783 × (subscapularis skinfold thickness + triceps skinfold thickness in millimeters) + 1.6; for girls, body fat percentage = 0.546 × (subscapularis skinfold thickness + triceps skinfold thickness in millimeters) + 9.7.

Blood sampling was performed in the fasting state at 8:00 AM. After clotting, blood samples were centrifuged for 10 minutes at 8000 rpm. Serum was stored at −81°C for later determination of steroid hormones and insulin. All samples were thawed only once. Insulin concentrations were measured by microparticle enhanced immunometric assay (MEIA; Abbott, Wiesbaden, Germany). Glucose levels were determined by colorimetric test using a Vitros analyzer (Ortho Clinical Diagnostics, Neckargmuend, Germany). Except for DHEAS, all steroid hormones were determined in a parallel assay by LC-MS/MS as previously described (16). In brief, 0.1 mL serum was extracted using an Oasis SPE system (Waters, Milford, Massachusetts). The steroid measurements were carried out using an UPLC Quattro Premier/Xe system (Waters). DHEAS was determined in a separate LC-MS/MS assay using the same UPLC Quattro Premier/Xe system. Intra- and interassay coefficients of variation were less than 5% for all measurements.

To interpret enzyme activities in the steroid metabolism, we calculated ratios between precursor steroids and their resulting downstream products in the steroid pathway.

HOMA was used to detect the degree of insulin resistance using the formula: resistance (HOMA) = [insulin (milliunits per liter) × glucose (millimoles per liter)]/22.5 (26).

Statistics

Statistical analyses were performed using the Winstat software package (R. Fitch Software, Bad Krozingen, Germany). Normal distribution was tested by the Kolmogorov-Smirnov test. Changes of steroid hormones in the 1-year follow-up were correlated to changes of insulin and changes of BP by partial regression analyses adjusted to changes of BMI-SDS. To compare variables at baseline or in the course of 1 year, a Fisher exact test and a Student's *t* test for paired and unpaired observations, Wilcoxon and Mann-Whitney *U* test were used as appropriate. A $P < .01$ was considered as significant to account for multiple testing. Data were presented as median and IQR.

Results

The prepubertal obese children demonstrated significantly increased concentrations of the glucocorticoids 11-deoxycortisol, cortisol, and cortisone compared with normal-weight children (Table 1). Although the mineralocorticoid precursor corticosterone was significantly increased in obese children, its direct precursor 11-deoxycorticosterone tended to show lower levels compared with

Table 1. Weight Status and Steroid Hormones in Obese vs Age- and Gender Matched Normal-Weight Prepubertal Children [Aged 8.8 (IQR 6.9–9.8) Years, 48% Female]

	Obese	Normal Weight	*P* Value
n	40	40	—
BMI, kg/m²	**24.9 (IQR 22.3–26.8)**	**16.1 (IQR 14.8–17.4)**	**<.001[b]**
BMI-SDS	**2.33 (IQR 2.05–2.58)**	**−0.01 (IQR −0.49 to +0.40)**	**<.001[b]**
11-Deoxycortisol, ng/mL	**0.56 (IQR 0.37–0.86)**	**0.20 (IQR 0.12–0.63)**	**.001[a]**
Cortisol, ng/mL	**104 (IQR 67–132)**	**63 (IQR 36–85)**	**<.001[b]**
Cortisone, ng/mL	**25 (IQR 21–36)**	**16 (IQR 9–21)**	**<.001[b]**
11-Deoxycorticosterone, ng/mL	0.077 (IQR 0.033–0.124)	0.105 (IQR 0.090–0.156)	.010[a]
Corticosterone, ng/mL	**2.22 (IQR 1.58–3.56)**	**0.97 (IQR 0.42–1.66)**	**<.001[a]**
Androstenedione, ng/dL	**34 (IQR 20–50)**	**20 (IQR 8–34)**	**<.001[b]**
T, ng/dL	**15 (IQR 11–19)**	**8 (IQR 5–11)**	**<.001[a]**
DHEAS, μg/dL	**39.7 (IQR 17.2–82.1)**	**22.1 (IQR 13.5–31.6)**	**.009[a]**
Dihydrotestosterone, ng/dL	2.01 (IQR 0.29–4.33)	2.9 (IQR 2.9–3.1)	.025[a]
Progesterone, ng/mL	0.10 (IQR 0.07–0.14)	0.07 (IQR 0.06–0.10)	.130[a]
17-Hydroxyprogesterone, ng/mL	0.29 (IQR 0.23–0.42)	0.23 (IQR 0.10–0.40)	.052[a]

Data are reported as median and IQR. Bold indicates significant changes.

[a] *P* values are derived from Mann-Whitney *U* test (not normally distributed variables).

[b] *P* values are derived from unpaired *t* test (normally distributed variables); conversation factors to SI units: cortisol, milligrams per deciliter × 27.6 = nanomoles per liter; cortisone, micrograms per deciliter × 0.0277 = micromoles per liter; 11-deoxycorticosterone, nanograms per milliliter × 0.03 = nanomoles per liter; corticosterone, nanograms per deciliter × 0.0289 = nanomoles per liter; aldosterone, nanograms per milliliter × 0.0277 = nanomoles per liter; androstenedione, nanograms per deciliter × 0.0349 = nanomoles per liter; T, nanograms per deciliter × 0.0347 = nanomoles per liter; DHEAS, micrograms per deciliter × 0.0256 = micromoles per liter; dihydrotestosterone, nanograms per milliliter × 0.0344 = nanomoles per liter; progesterone, nanograms per deciliter × 0.0318 = nanomoles per liter; 17-hydroxyprogesterone, nanograms per milliliter × 3 = nanomoles per liter.

Table 2. Age, Gender, Weight Status, and Steroid Hormones in 40 Obese Prepubertal Children at Baseline and 1 Year Later Separated to Weight Loss

	Substantial Weight Loss		
n	20		
Age, years	8.9 (IQR 7.2–9.8)		
Gender	45% female		
	Baseline	**1 Year Later**	***P* Value**[c]
BMI, kg/m²	**25.8 (IQR 23.4–26.7)**	**24.3 (IQR 21.0–25.7)**	**<.001**[d]
BMI-SDS	2.54 (IQR 2.28–2.68)	1.94 (IQR 1.61–2.16)	**<.001**[d]
Tricep thickness, mm	28 (IQR 21–32)	24 (IQR 19–28)	.013[d]
Subscapularis thickness, mm	28 (IQR 19–32)	24 (IQR 20–30)	.028[d]
Body fat, %	**41 (IQR 34–47)**	**37 (IQR 32–45)**	**.005**[d]
Waist circumference, cm	83 (74–88)	82 (72–86)	.265[d]
11-Deoxycortisol, ng/mL	0.56 (IQR 0.38–0.86)	0.34 (IQR 0.25–0.46)	.093[e]
Cortisol, ng/mL	**109 (IQR 80–139)**	**75 (61–96)**	**.007**[d]
Cortisone, ng/mL	**28 (IQR 24–35)**	**23 (19–28)**	**.004**[d]
11-Deoxycorticosterone, ng/mL	0.077 (IQR 0.036–0.119)	0.066 (IQR 0.025–0.110)	.627[e]
Corticosterone, ng/mL	**2.20 (IQR 1.87–3.56)**	**1.70 (IQR 1.22–2.22)**	**.009**[e]
Aldosterone, ng/mL	0.059 (IQR 0.025–0.141)	0.048 (IQR 0.004–0.173)	.687[e]
Androstenedione, ng/dL	**39 (IQR2 9–50)**	**26 (IQR-21–30)**	**.005**[d]
T, ng/dL	**15 (IQR 12–20)**	**12 (IQR 10–14)**	**<.001**[d]
DHEAS, μg/dL	41.6 (IQR 17.2–79.1)	51.6 (IQR 32.7–82.9)	.970[e]
Dihydrotestosterone, ng/dL	0.39 (IQR 0.29–3.07)	1.51 (IQR 0.03–2.79)	.936[e]
Progesterone, ng/mL	0.11 (IQR 0.07–0.14)	0.09 (IQR 0.05–0.12)	.135[e]
17-Hydroxyprogesterone, ng/mL	0.27 (IQR 0.23–0.47)	0.19 (IQR 0.14–0.33)	.019[e]
Insulin, mU/L	**11 (IQR 8–16)**	**8 (IQR 6–11)**	**.001**[d]
HOMA	**2.5 (IQR 2.0–3.0)**	**1.6 (IQR 1.1–2.3)**	**.005**[d]
Systolic BP, mm Hg	**110 (IQR 105–114)**	**103 (IQR 96–110)**	**.008**[d]
Diastolic BP, mm Hg	63 (IQR 60–71)	60 (IQR 56–70)	.018[d]

Data are reported as median and IQR. Bold indicates significant changes. Conversation factors to SI units: cortisol, milligrams per deciliter × 27.6 = nanomoles per liter; cortisone, micrograms per deciliter × 0.0277 = micromoles per liter; 11-deoxycorticosterone, nanograms per milliliter × 0.03 = nanomoles per liter; corticosterone, nanograms per deciliter × 0.0289 = nanomoles per liter; aldosterone, nanograms per milliliter × 0.0277 = nanomoles per liter; androstenedione, nanograms per deciliter × 0.0349 = nanomoles per liter; T, nanograms per deciliter × 0.0347 = nanomoles per liter; DHEAS, micrograms per deciliter × 0.0256 = micromoles per liter; dihydrotestosterone, nanograms per milliliter × 0.0344 = nanomoles per liter; progesterone, nanograms per deciliter × 0.0318 = nanomoles per liter; 17-hydroxyprogesterone, nanograms per milliliter × 3 = nanomoles per liter.

[a] *P* value baseline compared between children with and without substantial weight loss.

[b] *P* value 1 year compared between children with and without substantial weight loss.

[c] *P* value baseline compared with 1 year later.

[d] *P* values derived from paired *t* test (normally distributed variables).

[e] *P* values derived from Wilcoxon test (not normally distributed variables).

the normal-weight group (Table 1). Among the androgens, androstenedione, T, and DHEAS were all significantly increased in the obese children, whereas dihydrotestosterone did not differ significantly. Progesterone and 17-hydroxyprogesterone also did not differ significantly between the 2 groups (Table 1).

Substantial weight loss in 20 obese children [in median −0.52 (IQR −0.84 to −0.50) BMI-SDS] was associated with significant decreases of body fat, BP, insulin, and HOMA (Table 2). The 3 different classes of steroid hormones showed distinct patterns of changes after weight loss. Among the glucocorticoids, cortisol and cortisone levels decreased significantly. Also, 11-deoxycortisol decreased but this change was not statistically significant (Table 2). The mineralocorticoids, 11-deoxycorticosterone and aldosterone, did not change significantly. However, the corticosterone levels fell significantly (Table 2). In the androgen group of steroid hormones, androstenedione and T levels dropped significantly after the weight loss (Table 2). In contrast, the elevated DHEAS remained at its high level during the 1-year follow-up period of our study. Dihydrotestosterone, progesterone, and 17-hydroxyprogesterone did not change significantly after the weight loss (Table 2).

In the obese children without substantial weight loss [BMI-SDS in median +0.03 (IQR −0.09 to +0.10)], neither significant changes of body fat, insulin, HOMA, and BP nor steroid hormone concentrations were observed. Waist circumference tended to increase in obese children without substantial weight loss in contrast to obese children with substantial weight loss (Table 2). The obese children with and without substantial weight loss did not differ at baseline con-

Table 2. Continued

No Substantial Weight Loss		*P* Value[a]	*P* Value[b]
20			
8.6 (6–9.8)		.895	.895
50% female		.755	.755
Baseline	**1 Year Later**	***P* Value[c]**	***P* Value[a]**	***P* Value[b]**
24.1 (IQR 20.9–27.1)	**25.4 (IQR 22.6–27.8)**	**<.001**[d]	.353[d]	.010[d]
2.12 (IQR 2.17–2.42)	2.14 (IQR 2.12- 2.50)	.202[d]	.001[d]	.001[d]
29 (IQR 26–34)	30 (IQR 24–34)	.202[d]	.222[d]	.013[d]
29 (IQR 21–35)	30 (IQR 23–34)	.532[d]	.128[d]	.076[d]
45 (IQR 39–50)	46 (IQR 36–52)	.431[d]	.072[d]	.003[d]
83 (IQR 79–95)	90 (77–95)	.025[e]	.509[e]	.009[e]
0.54 (0.027–0.99)	0.47 (IQR 0.29–1.05)	.734[e]	.473[e]	.185[e]
96 (IQR 58–123)	95 (IQR 71–137)	.524[d]	.742[d]	.024[d]
22 (IQR 21–29)	23 (IQR 21–29)	.911[d]	.160[d]	.756[d]
0.080 (IQR 0.029–0.139)	0.076 (IQR 0.028–0.116)	.940[e]	.981[e]	.666[e]
2.15 (IQR 1.38–3.69)	2.26 (IQR 1.34–4.17)	.832[e]	.607[e]	.018[e]
0.123 (IQR 0.051–0.244)	0.054 (IQR 0.004–0.150)	.030[e]	.247[e]	.967[e]
23 (IQR 17–48)	27 (IQR 19–40)	.970[e]	.185[e]	.871[e]
13 (IQR 10–19)	14 (IQR 9–23)	.737[e]	.330[e]	.279[e]
32.5 (IQR 17.2–82.1)	47.5 (IQR 23.2–98.2)	.126[e]	.805[e]	.892[e]
3.11 (IQR 0.03–4.96)	2.74 (IQR 0.66–5.59)	.744[e]	.605[e]	.135[e]
0.09 (IQR 0.06–0.13)	0.11 (IQR 0.08–0.23)	.073[e]	.912[e]	.051[e]
0.30 (IQR 0.24–0.41)	0.29 (IQR 0.18–0.51)	.654[e]	.566[e]	.110[e]
10 (IQR 5–15)	12 (IQR 8–18)	.122[d]	.644[d]	.009[d]
2.2 (IQR 1.0–3.6)	2.2 (IQR 1.8–3.9)	.116[d]	.618[d]	.025[d]
110 (IQR 101–114)	110 (105- 115)	.317[d]	.274[d]	.054[d]
61 (IQR 55–77)	63 (IQR 56–70)	.916[d]	.087[d]	.045[d]

cerning their weight status (BMI), body fat, waist circumference, HOMA, or steroid hormones (Table 2).

The ratios between cortisol and cortisone did not differ significantly ($P = .087$) between normal-weight [4.3 (IQR 3.3–5.5)] and obese children [3.3 (IQR 2.8–4.9)]. The ratios between precursor steroids and their resulting downstream products in the steroid pathway did not change significantly in the 20 obese children with and without substantial weight loss and did not differ between the children with and without substantial weight loss at baseline or in follow-up (Table 3).

In the 1-year follow-up, changes of waist circumference were significantly positively correlated with changes of T ($r = 0.44$, $P = .002$) in partial regression analyses adjusted to changes of BMI-SDS but not with changes of any other steroid hormone. Changes of insulin were correlated significantly positively with changes of cortisol ($r = 0.35$, $P = .014$), cortisone ($r = 0.43$, $P = .003$), and dihydrotestosterone ($r = 0.34$, $P = .018$) in partial regression analyses adjusted to changes of BMI-SDS but not with changes of any other steroid hormone. Changes of HOMA were correlated significantly positively with changes of cortisol ($r = 0.38$, $P = .010$), cortisone ($r = 0.43$, $P = .004$), and dihydrotestosterone ($r = 0.42$, $P = .005$) in partial regression analyses adjusted to changes of BMI-SDS but not with changes of any other steroid hormone. The changes of systolic BP did not significantly correlated with any steroid hormone in partial regression analyses adjusted to changes of BMI-SDS. The changes of diastolic BP were correlated significantly positively with changes of corticosterone ($r = 0.50$, $P < .001$) and 11-desoxycortisol ($r = 0.44$, $P = .003$) in partial regression analyses adjusted to changes of BMI-SDS but not with changes of any other steroid hormone.

Discussion

To the best of our knowledge, this is the first study analyzing the longitudinal relationships of a comprehensive steroid profile encompassing glucocorticoids, mineralocorticoids and androgens, several precursors of the active steroids, insulin, the insulin resistance index HOMA, and BP in obese prepubertal children participating in a lifestyle intervention.

Compared with normal-weight children of the same age and gender, obese prepubertal children demonstrated significantly increased androgens and glucocorticoids and significantly increased levels of the mineralocorticoid precursor corticosterone. Normalization of concentrations in response to weight loss as seen for the glucocorticoids and corticosterone as well as for androstenedione and T indicate that their elevation in obese prepubertal children is

Table 3. Ratios Between Precursor Steroids and Their Resulting Downstream Products in the Steroid Pathway in 20 Obese Prepubertal Children With and 20 Obese Prepubertal Children Without Substantial Weight Loss at Baseline and 1 Year Later

	Substantial Weight Loss		
	Baseline	**1 Year Later**	***P* Value[a]**
Cortisol/cortisone	3.1 (IQR 2.8–3.4)	3.5 (IQR 2.8–4.2)	.179
11-Deoxycortisol/cortisol	0.005 (IQR 0.004–0.007)	0.006 (IQR 0.004–0.006)	.501
Corticosterone/aldosterone	30 (IQR 11–93)	133 (IQR 18–494)	.044
Androstenedione/T	2.3 (IQR 1.7–3.4)	2.4 (IQR 1.4–31)	.970
T/dihydrotestosterone	8 (IQR 3–20)	30 (IQR 5–51)	.379
Progesterone/11-deoxycorticosterone	1.3 (IQR 0.5–4.8)	1.2 (IQR 0.9–2.7)	.709
Progesterone/ 17-hydroxyprogesterone	0.4 (IQR 0.2–0.8)	0.3 (IQR 0.2- 0.5)	.167

Data are reported as median and IQR.

[a] *P* value baseline compared with 1 year later.

[b] *P* value baseline compared between children with and without substantial weight loss.

[c] *P* value 1 year compared between children with and without substantial weight loss; *P* values derived from Wilcoxon test ([a]) and Mann-Whitney *U* test ([b,c]).

rather the consequence than the cause of obesity. However, the persistently elevated DHEAS after weight loss is in marked contrast to these findings. It indicates an irreversible maturation of the zona reticularis and the formation of DHEAS in the prepubertal obese children.

The reversibility of the elevation of glucocorticoids after weight loss may point toward a reversible activation of the hypothalamo-pituitary-adrenal (HPA) axis. In women, the increased production of ACTH has been described in obesity (27). Moreover, a slightly increased sensitivity of the HPA axis has been reported in obese adults (28). In obese rats, in vivo and in vitro enhanced adrenocortical response to ACTH stimulation, an in vitro adrenal fasciculata-reticularis cell hypersensitivity to ACTH stimulus, hyperplasia of their adrenal zona fasciculata cells, and adrenal fasciculata-reticularis cell refractoriness to the inhibitory effect of leptin on ACTH-stimulated glucocorticoid production due, at least in part, to decreased adrenal leptin receptor expression have been reported (29). However, because HOMA was related to glucocorticoids in our study, insulin resistance may also contribute to higher glucocorticoid levels, for example influencing the levels of peripheral enzymes (30).

Evidence has been presented that activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts hormonally inactive cortisone to active cortisol (30, 31). This process may be of particular relevance in central obesity due to 11β-HSD1 activity in the omental fat tissue (32). However, Wiegand et al (33) reported that 11β-HSD1 activity is reduced in obese boys. In our study, we did not find a significant relationship between waist circumference and cortisol or cortisone. Additionally, we neither found significant differences of cortisol to cortisone ratios between normal-weight children and obese children nor significant changes of this ratio after weight loss according to another study of obese prepubertal children (34). On the other hand, the higher glucocorticoid levels in obesity may also be cause of insulin resistance in obese prepubertal children. Our study protocol does not allow differentiating whether insulin resistance follows increased glucocorticoids in obesity or whether increased glucocorticoids are a consequence of insulin resistance.

The predominant feature of the mineralocorticoid pattern was the significant elevation of corticosterone in the obese prepubertal children vs the normal-weight children and its significant drop to about the range seen in normal-weight children after weight loss. In contrast, 11-deoxycorticosterone and aldosterone showed only marginal changes after weight loss. This hormone pattern argues against a relevant activation of the renin-angiotensin pathway in obesity. In contrast, our data support an ACTH-mediated activation of the CYP11B1 enzymatic step in the zona fasciculata giving rise to increased corticosterone formation. Our additional observation that changes of the diastolic BP were correlated with corticosterone levels supports that higher concentrations of these mineralocorticoids in prepubertal obese children may contribute to their elevated BP.

There are different potential mechanisms that may act alone or in concert causing the elevated concentrations of the androgens DHEAS, androstenedione, and T in the prepubertal obese children. In addition to an increased central activation of the HPA axis, CRH has been suggested to increase adrenal androgen secretion directly in children (35). In addition, leptin may also act at the level of adrenal steroid biosynthesis because it has a documented dose-dependent role to promote the formation of adrenal androgens by stimulating 17,20-lyase activity of CYP17 (36). Therefore, leptin secreted by the adipose tissue may be an important functional link between obesity and in-

Table 3. Continued

No Substantial Weight Loss				
Baseline	**1 Year Later**	***P* Value[a]**	***P* Value[b]**	***P* Value[c]**
4.3 (IQR 3.2–5.6)	3.6 (IQR 3.0–5.1)	.455	.016	.433
0.005 (IQR 0.004–0.010)	0.006 (IQR0.004–0.009)	.823	.482	.626
25 (IQR 8–40)	102 (IQR 19–102)	.079	.417	.725
2.1 (IQR 1.5–3.1)	2.0 (IQR 1.3–3.7)	.823	.387	.646
3 (IQR 2–12)	3 (IQR 2–5)	.249	.279	.543
1.8 (IQR 1.0–4.8)	1.4 (IQR 0.6–4.1)	.526	.279	.978
0.4 (IQR 0.3 - 0.6)	0.3 (IQR 0.2- 0.5)	.232	.850	.665

creased androgens in prepubertal obese children. Another important enzymatic step for androgen formation is the Δ5 to Δ4 conversion of dehydroepiandrosterone to androstenedione. It has been shown that 3β-hydroxysteroid dehydrogenase expression can be enhanced via an insulin receptor substrate-1- and -2-dependent pathway in different tissue types (37). Therefore, the increased insulin concentrations in obesity as also shown in our present study could activate this enzymatic step in the adrenals and in the periphery, respectively, thus contributing to the observed increased prepubertal androstenedione formation. After puberty, insulin has additional documented roles in gonadal androgen formation (38).

One of the most intriguing findings of our long-term study is the fact that the elevated DHEAS in obesity did not normalize after weight loss as compared with all other steroids. In contrast, short-term studies in children and adolescents revealed decreasing DHEAS concentrations in weight loss (14, 39). Our data support a premature, enhanced, and irreversible differentiation of the zona reticularis in the prepubertal obese children. Adrenal androgen production normally starts to increase before gonadal production at the age of 6–8 years (40). Once the developmental path of adrenarche has been initiated, it continues irreversibly as reflected by the increasing DHEAS concentrations during the study period, even after successful weight loss. We cannot decipher, however, whether obesity has been the first event that has only secondarily initiated preterm adrenarche or whether preterm adrenarche has actually been the first hit promoting development of obesity as the secondary event, which has also been suggested by others (11, 14).

Strengths and limitations of the study

The strengths of this study are its longitudinal design; the analyses of a comprehensive profile of glucocorticoids, mineralocorticoids, and androgens by a state-of-the-art LC-MS/MS multisteroid profiling method (16); and the study of a homogenous cohort of prepubertal obese children naive to drugs. However, our study presents some potential limitations. First, BMI percentiles were used to classify overweight. Although BMI is a good measure for overweight, one needs to be aware of its limitations as an indirect measurement of fat mass. Second, androgens are bound to SHBG, the concentrations of which are influenced by many factors. SHBG is decreased in obese children, regardless of pubertal stage or gender (5, 13). Therefore, a measurement of increased androgens in obese children points to an increased androgen activity. Third, androgen concentrations correlate with fat distribution (39). This probably explains the different findings concerning androgens in childhood obesity. However, only T concentrations were associated with waist circumference in our study. Fourth, our study sample was too small to study gender differences on the changes of steroids. Finally, weight loss may be too small to measure effects on DHEAS levels. On the other hand, a reduction of the BMI-SDS of 0.5 or greater, as achieved in our children with substantial weight loss, is reported to normalize many hormonal and metabolic changes in childhood obesity like insulin sensitivity, the cardiovascular risk factor profile, hormones, and adipocytokines (1, 18, 19).

In summary, moderately increased steroid hormones were found in obese prepubertal children. Weight loss induced a decrease of most of these steroid hormones pointing to a reversible increase of these hormones in obesity. In contrast, DHEAS remained constantly elevated after weight loss pointing to an irreversible increased production in the adrenals in prepubertal obese children. Based on the profiles of steroids, the adrenals are likely to be the major source for the elevated hormone concentration. However, relevant biosynthesis may also occur in peripheral tissues, which seems particularly relevant for androstendione and T. There is still a great need for further research to understand the pathogenesis of age-dependent alterations of steroid hormones in obesity and the contributions of different tissues therein.

Acknowledgments

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T.R. and P.-M.H. developed the study design; T.R., N.L., and B.W. performed the anthropometrical measurements; and A.K. performed the laboratory measurements. All of the authors discussed the findings and participated in the writing of the paper. T.R. wrote the first draft of the manuscript. He and P.-M.H. were the leading writers of the paper. The hypothesis development, analysis, interpretation, and conclusions contained in this study are those of the authors alone.

This study is registered at http://clinicaltrials.gov (NCT00435734).

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References

1. **Reinehr T, Andler W.** Cortisol and its relation to insulin resistance before and after weight loss in obese children. *Horm Res*. 2004; 62(3):107–112.
2. **Bjorntorp P, Rosmond R.** Neuroendocrine abnormalities in visceral obesity. *Int J Obes Relat Metab Disord*. 2000;24(suppl 2):S80–S85.
3. **Chiodini I.** Clinical review: diagnosis and treatment of subclinical hypercortisolism. *J Clin Endocrinol Metab*. 2011;96(5):1223–1236.
4. **Stewart PM.** Cortisol, hypertension and obesity: the role of 11β-hydroxysteroid dehydrogenase. *J R Coll Physicians Lond*. 1998; 32(2):154–159.
5. **Reinehr T, de Sousa G, Roth CL, Andler W.** Androgens before and after weight loss in obese children. *J Clin Endocrinol Metab*. 2005; 90(10):5588–5595.
6. **Samojlik E, Kirschner MA, Silber D, Schneider G, Ertel NH.** Elevated production and metabolic clearance rates of androgens in morbidly obese women. *J Clin Endocrinol Metab*. 1984;59(5):949–954.
7. **Gapstur SM, Gann PH, Kopp P, Colangelo L, Longcope C, Liu K.** Serum androgen concentrations in young men: a longitudinal analysis of associations with age, obesity, and race. The CARDIA male hormone study. *Cancer Epidemiol Biomarkers Prev*. 2002;11(10 Pt 1):1041–1047.
8. **de Sousa G, Brodoswki C, Kleber M, Wunsch R, Reinehr T.** Association between androgens, intima-media thickness and the metabolic syndrome in obese adolescent girls. *Clin Endocrinol (Oxf)*. 2010;72(6):770–774.
9. **Ehrmann DA.** Polycystic ovary syndrome. *N Engl J Med*. 2005; 352(12):1223–1236.
10. **Saad F.** Androgen therapy in men with testosterone deficiency: can testosterone reduce the risk of cardiovascular disease? *Diabetes Metab Res Rev*. 2012;28(suppl 2):52–59.
11. **Saenger P, DiMartino-Nardi J.** Premature adrenarche. *J Endocrinol Invest*. 2001;24(9):724–733.
12. **Richards GE, Cavallo A, Meyer WJ III, et al.** Obesity, acanthosis nigricans, insulin resistance, and hyperandrogenemia: pediatric perspective and natural history. *J Pediatr*. 1985;107(6):893–897.
13. **Gascon F, Valle M, Martos R, et al.** Sex hormone-binding globulin as a marker for hyperinsulinemia and/or insulin resistance in obese children. *Eur J Endocrinol*. 2000;143(1):85–89.
14. **Pintor C, Loche S, Faedda A, Fanni V, Nurchi AM, Corda R.** Adrenal androgens in obese boys before and after weight loss. *Horm Metab Res*. 1984;16(10):544–548.
15. **Genazzani AR, Pintor C, Corda R.** Plasma levels of gonadotropins, prolactin, thyroxine, and adrenal and gonadal steroids in obese prepubertal girls. *J Clin Endocrinol Metab*. 1978;47(5):974–979.
16. **Kulle AE, Riepe FG, Melchior D, Hiort O, Holterhus PM.** A novel ultrapressure liquid chromatography tandem mass spectrometry method for the simultaneous determination of androstenedione, testosterone, and dihydrotestosterone in pediatric blood samples: age- and sex-specific reference data. *J Clin Endocrinol Metab*. 2010; 95(5):2399–2409.
17. **Reinehr T, Hinney A, de Sousa G, Austrup F, Hebebrand J, Andler W.** Definable somatic disorders in overweight children and adolescents. *J Pediatr*. 2007;150(6):618–622.
18. **Reinehr T, Kratzsch J, Kiess W, Andler W.** Circulating soluble leptin receptor, leptin, and insulin resistance before and after weight loss in obese children. *Int J Obes (Lond)*. 2005;29(10):1230–1235.
19. **Reinehr T, Andler W.** Changes in the atherogenic risk factor profile according to degree of weight loss. *Arch Dis Child*. 2004;89(5): 419–422.
20. **Reinehr T, de Sousa G, Toschke AM, Andler W.** Long-term follow-up of cardiovascular disease risk factors in children after an obesity intervention. *Am J Clin Nutr*. 2006;84(3):490–496.
21. **Cole TJ.** The LMS method for constructing normalized growth standards. *Eur J Clin Nutr*. 1990;44:45–60.
22. **Kromeyer-Hauschild K, Wabitsch M, Geller F, et al.** Percentiles of body mass index in children and adolescents evaluated from different regional German studies. *Monatsschr Kinderheilkd*. 2001;149: 807–818.
23. **Cole TJ, Bellizzi MC, Flegal KM, Dietz WH.** Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000;320(7244):1240–1243.
24. **National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents.** The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics*. 2004;114(suppl 2, 4th report):555–576.
25. **Slaughter M, Lohmann T, Boileau R, Horswill C, Stillmann R, Bemben D.** Skinfold equations for estimation of body fatness in children and youth. *Hum Biol*. 1998;60:709–723.
26. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412–419.
27. **Pasquali R, Cantobelli S, Casimirri F, et al.** The hypothalamic-pituitary-adrenal axis in obese women with different patterns of body fat distribution. *J Clin Endocrinol Metab*. 1993;77(2):341–346.
28. **Pasquali R, Ambrosi B, Armanini D, et al.** Cortisol and ACTH response to oral dexamethasone in obesity and effects of sex, body fat distribution, and dexamethasone concentrations: a dose-response study. *J Clin Endocrinol Metab*. 2002;87(1):166–175.
29. **Perello M, Moreno G, Camihort G, et al.** Nature of changes in adrenocortical function in chronic hyperleptinemic female rats. *Endocrine*. 2004;24(2):167–175.
30. **Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP.** Clinical review: the pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *J Clin Endocrinol Metab*. 2009; 94(8):2692–2701.
31. **Bujalska IJ, Kumar S, Stewart PM.** Does central obesity reflect "Cushing's disease of the omentum"? *Lancet*. 1997;349(9060): 1210–1213.
32. **Bujalska IJ, Walker EA, Hewison M, Stewart PM.** A switch in dehydrogenase to reductase activity of 11β-hydroxysteroid dehydrogenase type 1 upon differentiation of human omental adipose stromal cells. *J Clin Endocrinol Metab*. 2002;87(3):1205–1210.

33. **Wiegand S, Richardt A, Remer T, et al.** Reduced 11β-hydroxysteroid dehydrogenase type 1 activity in obese boys. *Eur J Endocrinol*. 2007;157(3):319–324.
34. **Chalew SA, Nagel H, Burt D, Edwards CR.** The integrated concentration of cortisone is reduced in obese children. *J Pediatr Endocrinol Metab*. 1997;10(3):287–290.
35. **Ibanez L, Potau N, Marcos MV, de Zegher F.** Corticotropin-releasing hormone as adrenal androgen secretagogue. *Pediatr Res*. 1999;46(3):351–353.
36. **Biason-Lauber A, Zachmann M, Schoenle EJ.** Effect of leptin on CYP17 enzymatic activities in human adrenal cells: new insight in the onset of adrenarche. *Endocrinology*. 2000;141(4):1446–1454.
37. **Gingras S, Cote S, Simard J.** Multiple signal transduction pathways mediate interleukin-4-induced 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase in normal and tumoral target tissues. *J Steroid Biochem Mol Biol*. 2001;76(1–5):213–225.
38. **Pasquali R, Casimirri F, de Iasio R, et al.** Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. *J Clin Endocrinol Metab*. 1995;80(2):654–658.
39. **Wabitsch M, Hauner H, Heinze E, et al.** Body fat distribution and steroid hormone concentrations in obese adolescent girls before and after weight reduction. *J Clin Endocrinol Metab*. 1995;80(12):3469–3475.
40. **Remer T, Manz F.** Role of nutritional status in the regulation of adrenarche. *J Clin Endocrinol Metab*. 1999;84(11):3936–3944.