Truncal Distribution of Fat Mass, Metabolic Profile and Hypothalamic-Pituitary Adrenal Axis Activity in Prepubertal Obese Children

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Objective To investigate whether truncal distribution of fat mass (TDFM) is associated with variations of the hypothalamic-pituitary-adrenocortical (HPA) axis activity in prepubertal obese children.

Study design TDFM, assessed with dual energy X-ray absorptiometry and a comprehensive set of measures of HPA axis activity and reactivity have been studied in 45 prepubertal obese children aged 6 to 11 years (girls) and 6 to 13 years (boys).

Results After adjustment for whole body fat mass (%) (WBFM), TDFM correlated positively with insulin (r = 0.50, 95% CI [0.23; 0.70]) and homeostasis model assessment of insulin resistance (r = 0.52, 95% CI [0.25; 0.71]). When adjusted for WBFM, TDFM correlated positively with morning plasma cortisol (r = 0.38, 95% CI [0.15; 0.64]) in the total population. TDFM correlated negatively with the rise of salivary cortisol after a standard meal (r = −0.43, 95% CI [−0.71; −0.02]), obviously in girls. When adjusted for WBFM and TDFM, morning plasma cortisol correlated positively with total cholesterol (r = 0.41, 95% CI [0.11; 0.65]) and triglyceride (r = 0.44, 95% CI [0.14; 0.67]). The rise of salivary cortisol after a standard meal was negatively (r = −0.56, 95% CI [−0.85; −0.01]) and positively (r = 0.74, 95% CI [0.16; 0.94]) correlated with homeostasis model assessment of insulin resistance in boys and girls, respectively.

Conclusions Association exists in prepubertal obese children between TDFM and markers of HPA axis activity. These data suggest that HPA axis could be involved early in life in obesity associated with pejorative metabolic profile. *(J Pediatr 2007;150:535-9)*

Excess of trunk fat in obesity is most closely associated with the metabolic syndrome.[1] This led many authors to look for factors involved in the accumulation of visceral adipose tissue. Because of the prevalence of abdominal obesity in Cushing's syndrome, the hypothalamic-pituitary-adrenal (HPA) axis has been extensively studied in adult obesity. On the one hand, abdominal obesity has been linked with stress-related cortisol secretion[2] and hyperactivity of the HPA axis.[3-6] On the other hand, changes in peripheral glucocorticoid metabolism have been shown in human obesity, perhaps stimulating excess production.[7-10] Nevertheless, whether cortisol changes are primary causes or secondary effects in abdominal obesity is still debated.

Studying abdominal obesity early in life could contribute to our understanding. Even in prepubertal children, visceral fat accumulation is related to metabolic risk factors, independently of obesity, which are in turn implicated in cardiovascular morbidity and death later in life.[11,12] However, little is known about the factors involved in fat mass distribution in prepubertal children, and, particularly, the HPA axis has been inadequately studied in this population. The objective of this study was to investigate whether truncal distribution of fat mass (TDFM), assessed with dual-energy X-ray absorptiometry (DEXA), is associated with metabolic profile and HPA axis activity and reactivity in prepubertal obese children, independently of percent fat mass.

ACTH	Adrenocorticotropin hormone	HPA	Hypothalamic-pituitary-adrenocortical
BMI	Body mass index	QUICKI	Quantitative insulin sensitivity check index
CBG	Corticosteroid binding globulin	TDFM	Truncal distribution of fat mass
DEXA	Dual energy-X ray absorptiometry	THE	Tetrahydrocortisone
DXM	Dexamethasone	THF	Tetrahydrocortisol
HOMA	Homeostasis model assessment of insulin resistance	WBFM	Whole body fat mass

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METHODS

Inclusion Criteria

Children were recruited in the Departments of Paediatrics of Bordeaux and Toulouse from obesity clinics before their participation in a program of care coordination. Inclusion criteria were ages 6 to 11 years for girls and 6 to 13 years for boys, prepubertal stage with breast, genital, and pubic hair stage 1 according to Marshall and Tanner and obesity defined by body mass index (BMI) as proposed by the International Obesity Task Force[13]; subjects had BMI above international cutoff points defined to pass through BMI of 30 kg/m^2 at age 18. Exclusion criteria were oral or inhaled steroids in the last 6 months, bulimia, dieting for at least 3 months before the study (>30% variation of caloric intake), hypothyroidism, Cushing syndrome and, for girls, hyperandrogenia. The study had the approval of the local ethics committee. Informed consent was obtained from the children and from the parents of each study participant.

Clinical Protocol

Forty-five obese children (n = 29 in Bordeaux; n = 16 in Toulouse) were included in this study. The average BMI was 27.8 ± 5.9 kg/m^2 for fathers and 27.5 ± 5.4 kg/m^2 for mothers. All children had normal physical examination results apart from obesity. In the Bordeaux and Toulouse hospitals, children were admitted in the evening of day 0. On day 1 at 0800 hours after an overnight fast, blood pressure was measured with the patient in the supine position, and 11 mL of blood was drawn to determine plasma cortisol, adrenocorticotropin hormone (ACTH), glucose, insulin, lipid profile, corticosteroid binding globulin (CBG), and leptin levels. Then, body weight and height and waist and hip circumferences were measured to the nearest 0.1 kg or cm, respectively. On the same morning, total fat mass and truncal distribution of fat mass were determined with DEXA.

In addition, children in Bordeaux had further exploration of HPA axis activity and reactivity. Twenty-four hour urine collection was started at 2000 hours on day 0. On day 1, saliva samples were collected at 0800 hours before blood sample and at 1130 and 1200 hours. A standardized meal (950 Kcal; 55% carbohydrate, 15% protein, 30% fat) was served at noon, and saliva samples were repeated every 30 minutes for 2 hours. The highest value of saliva cortisol after the meal was used to determine the increment of saliva cortisol after a meal as follows: Δ meal $\text{Cortisol}_{\text{saliva}}$ (%) = 100 × (highest $\text{Cortisol}_{\text{saliva}}$ − 1200 h $\text{Cortisol}_{\text{saliva}}$)/1200 h $\text{Cortisol}_{\text{saliva}}$. On day 1 at 2000 hours, urine collections stopped and urine aliquots were stored without preservative at −20°C until assayed for free urinary cortisol and total glucocorticoid metabolites. On day 1 at 2300 hours, oral dexamethasone (DXM) was given (0.25 mg). On day 2 at 0800 hours, blood samples were collected for cortisol measurements. We measured the decrease of plasma cortisol after $\text{DXM}_{(0.25\ \text{mg})}$ as followed: ratio $\text{DMX}_{(0.25\ \text{mg})}$ $\text{Cortisol}_{\text{plasma}}$ = day 2 $\text{Cortisol}_{\text{plasma}}$/day 1 $\text{Cortisol}_{\text{plasma}}$.

DEXA

On day 1, a standard whole-body DEXA examination (Bordeaux: Hologic QDR 4500A, Waltham, Mass; Toulouse: Lunar DPX-L, Madison, Wis) included total body and regional measurements to analyze body composition according to a 3-compartment model: fat mass, lean tissue, and bone mineral content. The coefficient of interassay variation for the percentage of fat mass between the 2 instruments was less than 1% in a high fat configuration using a phantom. The percentage of whole body fat mass (WBFM) was calculated as follows: WBFM/whole body (fat mass + lean tissue + bone mineral content) × 100. To evaluate TDFM, 4 regions of interest were manually determined by the same operator. These regions included the subscapular, waist, hip, and thigh regions. The regions were defined by anatomic bony landmarks. The height of each region was equivalent and was defined as one third the distance from the top of iliac crest to the knee. The waist region was placed on the iliac crest, with the subscapular region placed on top of that. The hip region was placed at the middle of the pelvis, with the thigh region just below that. The width of each region was adjusted to include all soft tissue in that region. TDFM was calculated as followed: (Subscapular + Waist)/(Hip + Thigh) fat mass,[14] this ratio being increased when trunk fat distribution predominates. TDFM was available for all 45 children. The percentage of body fat mass was available for 43 children.

Laboratory Methods

Cortisol, ACTH, insulin, CBG, and leptin measurements were measured by the centralized laboratories in Bordeaux. Blood glucose was measured at the bedside with a glucose dehydrogenase oxidation technique (Olympus Diagnostica GmbH, Lismeehan, O'Callaghan'Mills Co. Clare, Ireland). Serum insulin was analyzed with a commercial IRMA (Dia Sorin, Saluggia, Italy). Homeostasis model assessment of insulin resistance (HOMA-IR = [fasting insulin × fasting glucose]/22.5) and quantitative insulin sensitivity check index (QUICKI = 1/[log(fasting insulin) + log(fasting glucose)]) were calculated as previously described. Commercial colorimetric method was used for blood cholesterol (Olympus Diagnostica GmbH) and triglycerides (Olympus Diagnostica GmbH). Serum leptin was assayed with commercial RIA (Mediagnost, Tuebingen, Germany). Blood was collected in serum separator tubes for cortisol determination and serum was stored at −20°C until assayed with a commercial RIA (Dia Sorin, Stillwater, MN). Samples for ACTH determination were taken in prechilled EDTA test tubes and spun in a centrifuge immediately, and plasma was stored at −70°C until assayed with a commercial IRMA (Nichols Institute Diagnostics, San Clemente, Calif). The binding capacity of CBG (Bmax) and its affinity (Kd) for cortisol were measured at 4°C by a solid phase assay with Concanavalin A-Sepharose.[15] Salivary cortisol was then extracted into dichloromethane (cortisol recovery >95%) and assayed after evaporation and resuspension of the dried extract in human desteroidized serum with a commercial RIA Coat-A-Count (DPC, Los

Table II. Correlations between fat mass, truncal distribution of fat mass, and metabolic data in total population

	Body fat mass (%)			Truncal distribution of fat mass				
				Unadjusted for fat mass		Adjusted for body fat mass (%)		
	n	r	95% CI	n	r	n	r	95% CI
Leptin	36	0.67*	[0.43; 0.82]	38	0.38*	36	0.24	[−0.10; 0.53]
Glucose	43	0.18	[−0.13; 0.46]	45	0.26	43	0.23	[−0.07; 0.50]
Insulin	41	0.21	[−0.10; 0.49]	43	0.54*	41	0.50*	[0.23; 0.70]
HOMA	41	0.25	[−0.06; 0.52]	43	0.56*	41	0.52*	[0.25; 0.71]
QUICKI	41	−0.10	[−0.40; 0.22]	43	−0.23	41	−0.21	[−0.48; 0.11]
Cholesterol	43	0.02	[−0.29; 0.32]	45	−0.13	43	−0.15	[−0.43; 0.16]
LDL-C	43	0.14	[−0.17; 0.42]	45	−0.07	43	−0.12	[−0.41; 0.18]
Triglyceride	43	0.13	[−0.18; 0.42]	45	0.20	43	0.16	[−0.15; 0.44]

r, Pearson correlation coefficient.
*The r is significantly different from 0 ($P < .05$).

Angeles, Calif). Free urinary cortisol was assayed in Bordeaux using a commercial kit: CORT-CT2 (Cis Bio International, Gif sur Yvette, France). Glucocorticoid metabolites were measured by gas chromatography and electron impact mass spectrometry after Sep-Pak C18 extraction, hydrolysis with β-glucuronidase, and formation of methoxime trimethylsilyl derivative, as previously described.[16] Epi-cortisol and epi-tetrahydrocortisol were used as internal standards, which were added to samples before extraction. Total cortisol metabolite excretion was calculated as tetrahydrocortisols (α and β THFs) + tetrahydrocortisone (THE) + cortols + cortolones. Relative metabolism by 5α and 5β-reductases were inferred from the 5α-THF/5β-THF ratio. A-ring reduction of cortisol was inferred from the ratios of THFs/cortisol and 5α-reductase activity from the ratio of THE/cortisone. Whole-body equilibrium between cortisol and cortisone, determined by the balance of tissue-specific activities of 11β-reductase and 11β-dehydrogenase activities, was inferred from the ratio of THFs/THE. Renal 11β-dehydrogenase activity was inferred from the urinary cortisol/cortisone ratio.[17]

Statistical Analysis

Statistical analysis was performed by using SAS system software (version 8.2, SAS Institute, Inc., Cary, NC). Patients' characteristics were described by mean and standard deviation (SD) and compared between boys and girls by a Student t test except for HPA axis activity, which was compared by an analysis of variance, adjusted for fat mass percentage. Associations between fat mass, TDFM, anthropometric and metabolic data and HPA axis were evaluated with Pearson correlation coefficients. Ninety-five percent confidence intervals (95% CI) were estimated with Gaussian assumption after Fisher transformation. A correlation coefficient was considered as significantly different from 0 if its 95% CI did not include 0. Correlations were adjusted for the fat mass percentage, with partial Pearson correlations.

RESULTS

Forty-five obese children (26 boys, 19 girls) were recruited. Table I (available at www.jpeds.com) shows the clinical and metabolic characteristics, as well as HPA axis activity and reactivity variables in boys and girls. None of them had abnormal blood pressure, plasma glucose, total and LDL-cholesterol, or triglycerides values.

WBFM (%) was not different between boys and girls. When adjusted for WBFM (%), ACTH and morning plasma cortisol were higher in boys than in girls. No other statistically significant difference was seen between boys and girls for other HPA axis data.

In boys, TDFM was correlated significantly with BMI (r = 0.47; 95% CI [0.09; 0.73]), waist circumference (r = 0.48; 95% CI [0.07; 0.75]), and waist-to-hip ratio (r = 0.71; 95% CI [0.41; 0.87]) but not with WBFM (%) (r = 0.34; 95% CI [−0.08; 0.66]). In girls, TDFM was correlated significantly with waist circumference (r = 0.64; 95% CI [0.25; 0.85]), and waist-to-hip ratio (r = 0.64; 95% CI [0.24; 0.85]) but not with BMI (r = 0.34; 95% CI [−0.15; 0.70]) nor WBFM (%) (r = 0.25; 95% CI [−0.24; 0.64]). Because there is no sex difference for correlations between fat mass, truncal distribution of fat mass, and metabolic data, all data are presented for the total population (Table II). Serum leptin was significantly correlated with WBFM (%) but not with fat distribution when adjusted for WBFM (%). No significant correlation was found between WBFM (%) and glucose, insulin, insulin sensitivity indexes or lipid measures. By contrast, TDFM adjusted for WBFM (%) was significantly correlated with insulin and HOMA.

No significant correlation was found between WBFM (%) and any of the HPA axis activity and reactivity variables in the total population (Table III). On the other hand, when adjusted for WBFM (%), TDFM was positively correlated with morning plasma cortisol in the total population but not in boys and in girls studied separately. TDFM was negatively correlated with salivary cortisol response to lunch in the total population with significant correlation in girls but not in boys. In

Table III. Correlations between fat mass, truncal distribution of fat mass and HPA axis

	Body fat mass (%)			Truncal distribution of fat mass adjusted for body fat mass (%)						
	Total population			Boys		Girls		Total population		
	n	r	95% CI	n	r	n	r	n	r	95% CI
0800 h $Cortisol_{plasma}$	39	0.17	[−0.15; 0.46]	22	0.33	17	0.40	39	0.38*	[0.07; 0.62]
0800 h $Cortisol_{saliva}$	19	0.38	[−0.09; 0.71]	7	−0.33	12	0.53	19	0.35	[−0.12; 0.69]
0800 h ACTH	39	0.06	[−0.26; 0.37]	20	0.15	19	0.41	39	0.25	[−0.07; 0.52]
B max_{CBG} (nM)	32	0.21	[−0.15; 0.52]	21	0.05	11	0.14	32	0.02	[−0.33; 0.37]
Kd_{CBG}	32	−0.11	[−0.44; 0.26]	21	0.30	11	−0.52	32	0.03	[−0.33; 0.38]
Δ meal $Cortisol_{saliva}$ (%)	23	0.27	[−0.16; 0.61]	14	−0.28	9	−0.78*	23	−0.43*	[−0.71; −0.02]
DMX (0.25 mg) $Cortisol_{plasma}$	24	0.15	[−0.27; 0.52]	17	0.17	7	−0.77*	24	−0.04	[−0.44; 0.37]
Free urinary cortisol	28	−0.28	[−0.59; 0.10]	14	0.09	14	0.25	28	0.14	[−0.24; 0.49]
Total glucocorticoid metabolites	22	0.08	[−0.36; 0.48]	14	0.06	8	0.92*	22	0.24	[−0.20; 0.60]
Cortisol/Cortisone	22	0.25	[−0.19; 0.61]	14	−0.24	8	0.47	22	−0.11	[−0.51; 0.32]
(5α-THF + 5β-THF)/THE	22	−0.41	[−0.71; 0.01]	14	0.11	8	−0.41	22	−0.08	[−0.49; 0.35]
5α-THF/5β-THF	22	−0.33	[−0.66; 0.12]	14	−0.03	8	−0.29	22	0.17	[−0.29; 0.56]

r, Pearson correlation coefficient.
*The r is significantly different from 0 ($P < .05$).

girls, TDFM was negatively correlated with the ratio of plasma cortisol after $DXM_{0.25\ mg)}$ (day 2)/cortisol day 1 (r = −0.77; 95% CI [−0.96; −0.03]) and positively correlated with total glucocorticoid metabolites (r = 0.92; 95% CI [0.62; 0.99]).

Because of the number of potential variables to analyze, we limited our study to morning plasma cortisol and salivary cortisol response to lunch because of their association with trunk fat distribution in the total population. When adjusted for WBFM (%) and TDFM, morning plasma cortisol was positively correlated with total cholesterol (r = 0.49, 95% CI [0.01; 0.78]) in girls and total cholesterol (r = 0.41, 95% CI [0.11; 0.65]) and triglyceride values (r = 0.44, 95% CI [0.14; 0.67]) in the total population. When adjusted for WBFM (%) and TDFM, no significant correlation was found between salivary cortisol response to lunch and metabolic data in the total population (data not shown). However, we found inverse correlations when comparing boys (n = 17) and girls (n = 9). Salivary cortisol response to lunch (%) was negatively correlated with HOMA (r = −0.56, 95% CI [−0.85; −0.01]) and positively correlated with QUICKI (r = 0.58, 95% CI [0.04; 0.86]) in boys and positively correlated with HOMA (r = 0.74, 95% CI [0.16; 0.94]) and negatively correlated with QUICKI (r = −0.71, 95% CI [−0.93; −0.08]) in girls.

DISCUSSION

Our results demonstrate that TDFM is positively associated with insulin resistance and morning plasma cortisol and negatively associated with the increase of saliva cortisol after lunch in obese prepubertal children, and that this effect is independent of the percentage of WBFM. DEXA, taking into account subcutaneous and visceral truncal fat together, has already been used in children and adolescents aged 9 to 17 years, demonstrating that TDFM is a more important independent correlate of cardiovascular risk factors than percent body fat.[14] Here, we used the same method to evaluate TDFM in strictly prepubertal obese children and confirmed the independent association between TDFM and plasma insulin levels and insulin resistance estimated with HOMA in this population.

We made the choice not to include non-obese control subjects because we were more interested in changes of HPA axis activity and reactivity with body fat distribution in obese children than changes of HPA axis with obesity. The associations we found between morning plasma cortisol and TDFM but also between morning plasma cortisol and some lipid profile measures, independently of TDFM, suggest that HPA axis is involved early in life in truncal obesity, even in prepubertal children. However, because our results were independent of the percentage of fat mass, it would be of interest to perform the same study in non-obese children.

Saliva cortisol closely reflects the free/active plasma cortisol. In spite of positive correlation with morning plasma cortisol, we found no significant correlation between morning salivary cortisol and trunk fat distribution. However, salivary cortisol was not obtained for all children because children have more difficulty than adults spitting saliva early in the morning and having fasted. To complete the assessment of extracellular cortisol availability, we studied CBG level and its affinity for cortisol. Serum CBG levels measured by RIA are known to correlate negatively with BMI, waist-to-hip ratio, and HOMA in adults.[18] In this study, we found no correlation between fat mass, TDFM, and CBG levels.

The relationship we found between morning plasma cortisol and TDFM was the same in boys and girls. However, other significant results suggest a sexual dimorphism in HPA axis activity and reactivity, even in prepubertal children. In adults, morning plasma cortisol is reduced in women compared with men.[19] Here, cortisol and ACTH were reduced in girls, independently of the percentage of fat mass. Moreover, we found strong relationships between TDFM and urinary total

glucocorticoid metabolites, sensitivity to DXM or cortisol responsiveness after lunch in girls but not in boys. We also found opposite relationships in boys and girls between salivary cortisol response to lunch and indexes of insulin resistance.

The urinary cortisol metabolites provide insights into activities of cortisol metabolizing enzymes. 11β-HSD1 activity is increased in adipose tissue of obese adults[7] and is preferentially increased in omental than subcutaneous adipose stroma cells which may explain the specific action of glucocorticoids on different adipose tissue depots.[20] However, 11β-HSD1 activity in the liver is decreased in obese human beings and rodents, perhaps as a compensation to reduce the local intrahepatic load of glucocorticoids.[7,21] The ratio of (5α-THF + 5β-THF)/THE indicates the balance of 11β-HSD1 and 11β-HSD2 in all tissues but is also influenced by the activities of A-ring reductases. Hence, correlations between the values and degree of obesity have been inconsistent.[7,22,23] In this study we found a trend for a negative correlation between (5α-THF + 5β-THF)/THE ratio and the percentage of WBFM. This tendency disappeared when correlating with TDFM.

HPA axis reactivity after a meal is known to be higher at lunch time.[4,24] In premenopausal obese women, cortisol responsiveness after lunch has been shown to be the same[4] or enhanced[25] in women with abdominal fat distribution compared with women with peripheral obesity. This response of salivary cortisol after lunch is the same between non-obese and obese children.[26] Interestingly, salivary cortisol responsiveness to a standardized lunch was negatively correlated with TDFM in the total population but more clearly in girls. This result is the opposite of what we have reported in premenopausal women.[25] If this difference as compared with adults is confirmed in a larger population, this suggests that HPA axis reactivity after a meal evolves in accordance with age, at least in females.

Our study confirms that, in prepubertal obese children, an association exists between some markers of HPA axis activity and TDFM, independently of WBFM. Morning plasma cortisol is associated with the lipid profile, independently of fat mass and fat mass distribution. Moreover, salivary cortisol response to lunch is associated with insulin resistance. Both truncal distribution of fat mass and lipid profile are criteria defining the metabolic syndrome in adults.[27] Therefore our data suggest that HPA axis could be involved early in life in an obesity associated with pejorative metabolic profile. To gain better insight into this relationship between HPA axis and abdominal obesity, both the sexual dimorphism of the HPA axis activity in prepubertal obese children and the evolution of the HPA axis reactivity after a meal from childhood to adulthood need to be investigated.

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Table I. Subject characteristics, DEXA, biochemical and hormonal data in boys and girls

	Boys			Girls			
	n	Mean	SD	n	Mean	SD	*P* value
Age (y)	26	10.1	1.7	19	7.9	1.1	
Weight (kg)	26	58.0	15.3	19	43.4	7.8	
Height (cm)	26	143.2	9.2	19	130.4	8.0	
Body mass index (kg/m^2) [range]	26	27.9 [20.8-40.9]	4.8	19	25.3 [21.0-30.1]	2.3	
Waist circumference (cm)	23	90.0	11.6	19	78.3	7.4	
Waist/hip ratio	23	0.96	0.06	19	0.91	0.06	
Systolic blood pressure (mm Hg)	23	110	13	17	102	14	
Diastolic blood pressure (mm Hg)	23	61	8	17	59	11	
Body fat mass (DEXA, %)	24	40.5	4.8	19	41.6	4.1	.42
Truncal distribution of fat mass (DEXA)	26	0.90	0.18	19	0.87	0.18	.55
Glucose (mmol/L)	26	4.8	0.3	19	4.3	0.7	.07
Insulin (mU/L)	24	13.1	7.0	19	11.3	5.4	.37
HOMA	24	2.8	1.6	19	2.3	1.2	.21
QUICKI	24	0.69	0.09	19	0.68	0.33	.23
Cholesterol (mmol/L)	26	4.7	0.7	19	4.4	1.1	.39
LDL cholesterol (mmol/L)	26	2.9	0.6	19	2.9	0.7	.87
Triglyceride (mmol/L)	26	1.0	0.7	19	0.8	0.2	.16
Leptin (ng/mL)	22	23.0	17.9	16	18.9	8.3	.36
0800 h $Cortisol_{plasma}$ (nmol/L)	24	431	164	17	311	135	$<$.01
0800 h $Cortisol_{saliva}$ (nmol/L)	9	11.8	5.4	12	10.6	4.8	.34
0800 h ACTH (pg/mL)	22	53.6	37.2	19	22.2	12.6	$<$.001
Cortisol binding globulin Bmax (nmol/L)	22	191.6	84.4	11	236.3	68.2	.09
Kd	22	0.78	0.28	11	1.00	0.40	.06
Δ meal $Cortisol_{saliva}$ (%)	14	58	61	9	63	59	.86
DMX (0.25 mg) $Cortisol_{plasma}$	17	0.59	0.31	7	0.46	0.23	.26
Urine cortisol metabolites							
Free urinary cortisol (μg/d)	15	30.9	15.3	14	20.6	7.5	.10
Total glucocorticoid metabolites (mg/d)	14	10.6	4.2	8	7.8	3.6	.12
Cortisol/Cortisone	14	0.66	0.11	8	0.62	0.07	.23
(5α-THF + 5β-THF)/THE	14	1.23	0.37	8	1.02	0.34	.26
5α-THF/5β-THF	14	1.67	0.93	8	2.07	0.88	.22

NS, Nonsignificant difference between boys and girls.