The sexual dimorphism in cortisol secretion starts after age 10 in healthy children: urinary cortisol metabolite excretion rates during growth

Stefan A. Wudy, Michaela F. Hartmann, and Thomas Remer

From the Steroid Research Unit, Center of Child and Adolescent Medicine, Justus-Liebig-University, Giessen, Germany (S.A.W., M.F.H.) and the Department of Nutrition and Health, Research Institute of Child and Nutrition, Dortmund, Germany (T.R.).

Address of author responsible for correspondence about the manuscript:
Prof. Dr. Stefan A. Wudy
Steroid Research Unit
Center of Child and Adolescent Medicine
Justus-Liebig-University
Feulgenstr. 12
D-35392 Gießen
Germany

Phone: +49 641 99 43476
Fax: +49 641 99 43659
E-mail: stefan.wudy@paediat.med.uni-giessen.de

Supported by a research grant from the Deutsche Forschungsgemeinschaft (RE 753/5) to T.R. and S.A.W., and by the Ministry of Science and Research of the State of North Rhine-Westphalia, Germany

Key words: steroid, glucocorticoid, gas chromatography, mass spectrometry

Short running head: cortisol secretion during childhood
**Abbreviations**

BMI, body mass index

BSA, body surface area

DONALD study, Dortmund Nutritional and Anthropometric Longitudinally Designed study;

GC-MS, gas chromatography-mass spectrometry;

HSD, hydroxysteroid dehydrogenase;

$5\alpha$-Red, $5\alpha$-reductase;

SD, standard deviation;

**Steroid nomenclature:**

F, cortisol (pregn-4-ene-11$\beta$,17$\alpha$,21-triol-3,20-dione);

$F_{\text{comb}}$, combined urinary free and conjugated cortisol as measured by GC-MS

11-hydroxy cortisol metabolites:

THF, tetrahydrocortisol (5$\beta$-pregnane-3$\alpha$,11$\beta$,17$\alpha$,21-tetrol-20-one);

a-THF, allo-tetrahydrocortisol (5$\alpha$-pregnane-3$\alpha$,11$\beta$,17$\alpha$,21-tetrol-20-one);

$\alpha$-Cortol (5$\beta$-pregnane-3$\alpha$,11$\beta$,17$\alpha$,20$\alpha$,21-pentol);

$\beta$-Cortol (5$\beta$-pregnane-3$\alpha$,11$\beta$,17$\alpha$,20$\beta$,21-pentol);

11-oxo cortisol metabolites:

THE, tetrahydrocortisone (5$\beta$-pregnane-3$\alpha$,17$\alpha$,21-triol-11,20-dione);

$\alpha$-Cortolone (5$\beta$-pregnane-3$\alpha$,17$\alpha$,20$\alpha$,21-tetrol-11-one);

$\beta$-Cortolone (5$\beta$-pregnane-3$\alpha$,17$\alpha$,20$\beta$,21-tetrol-11-one);

C21: sum of major 11-hydroxy and 11-oxo metabolites;
Abstract:

Detailed data on the physiological pattern of adrenocortical activity during normal growth are lacking. An established method to determine adrenocortical glucocorticoid secretion is the measurement of 24-h excretion rates of major urinary cortisol metabolites (C21). To test the hypothesis that the frequently reported higher cortisol secretion in men than in women develops during puberty, we examined C21 together with excretions of combined urinary free and conjugated cortisol (Fcomb) in 400 healthy boys and girls aged 3-18 yr using gas chromatography-mass spectrometry (GC-MS).

Daily excretion rates of C21, Fcomb, and body surface area (BSA)-corrected Fcomb significantly increased with age in both sexes. In contrast, C21/BSA (µg m⁻² d⁻¹) declined from the age of 3-4 yr to 7-8 yr in boys and girls (P<0.01; e.g., in boys: from 3991±1167 to 3193±804), then increased in both sexes and finally became discordant after the age of 11-12 yr with a further rise in males only (17-18 yr olds: males, 5275±1414; girls 3939±1586, P<0.01). This pattern was associated with the occurrence of a lower index for 5α-reductase activity (allo-tetrahydrocortisol/tetrahydrocortisol) in females compared to males.

Our results demonstrate dynamical changes in adrenocortical activity in healthy children resulting in an emerging sex dimorphism in cortisol secretion after age 11. The latter can be explained, at least partly, by diverging 5α-reductase activities in boys and girls. Fcomb, a frequently analyzed GC-MS parameter, proved not to reflect dynamical changes in cortisol secretion. In conclusion the varying metabolic need for cortisol during normal growth may have implications for future improvements in glucocorticoid replacement therapy.
Introduction

It has so far been assumed that cortisol secretion corrected for body surface area is constant throughout childhood and adolescence. This assumption has been based on studies investigating urinary excretion rates of selected glucocorticoid metabolites in mostly small samples of children (2, 15, 17). However, detailed data on the physiological pattern of daily cortisol secretion, i.e. of adrenocortical activity during normal growth, are lacking.

Assessment of cortisol secretion in a huge sample of healthy children and adolescents requires not only a non stressful and non invasive protocol, but also a practical approach to permit successful realization. The application of invasive techniques based on isotope dilution requires venipuncture and infusions of either stable or radioactively labeled cortisol in specialized hospitals or research units with subsequent sampling of blood and/or urine to recover labeled metabolites (19, 22, 50). Additionally, administration of isotope labeled tracers might influence endogenous cortisol production by negative feed back (18). However, it has been shown that the determination of urinary excretion rates of the major endogenous glucocorticoid metabolites presents a suitable alternative to isotopic methods for the determination of cortisol secretion (15, 19, 50) and gas chromatography-mass spectrometry (GC-MS) urinary steroid profiling has been successfully used in determining cortisol secretion in preterm infants (14).

Meanwhile, measurement of urinary 24-h major cortisol metabolites is an established method, used by numerous endocrine research groups (9, 16, 23, 29, 39, 41, 43, 49) to examine adrenocortical activity and/or glucocorticoid metabolism in healthy and ill children and adults. This relies on the fact that the sum of the 7 major urinary cortisol metabolites (for details see Subjects and methods) encompasses almost 80 % of the cortisol secreted per day
by the adrenal gland (27, 40). Although recent observations in children with congenital adrenal hyperplasia have suggested an increased cortisol clearance at puberty and have shown problems with glucocorticoid replacement therapy (6, 7), possible changes in cortisol secretion and cortisol metabolism in normal growing children have not been specifically investigated, so far. In addition, it is unclear at what time the known sex dimorphism with higher cortisol secretion rates in men than in women develops. This sex dimorphism has been demonstrated in adults with stable isotope tracer infusions (45) as well as with 24-h urinary cortisol metabolite excretion measurements (1, 30, 38).

To examine the dynamics in cortisol secretion during growth and to test the hypothesis that the higher cortisol secretion in men than in women develops during puberty, we determined urinary glucocorticoid metabolite excretion rates non invasively in a large sample of healthy children of the DONALD study (4, 34).
Subjects and Methods

Subjects and urine collections

The study group comprised 400 healthy children and adolescents (200 boys, 200 girls aged 3-18 yr) participating in the DOrtmond Nutritional and Anthropometric Longitudinally Designed (DONALD) Study (4, 34). Data from these 400 children have already been published on urinary markers of adrenarche (32). Fifty 24-h urine samples (25 from boys and 25 from girls) were randomly selected for each of the 8 equally wide age groups. The study was approved by the institutional review board of the Research Institute for Child Nutrition Dortmund, and parental consent and child's assent were obtained before entry into the study. The participating children and their parents received instruction and written guidance to ensure compliance in the 24-h urine collection which was performed at home by each subject (31, 34).

Measurements, calculations, and background for adjustments

Anthropometric and dietary data were obtained as described in detail elsewhere (33, 34). Urinary steroid profiles were determined using quantitative data produced by GC-MS analysis according to the method described previously (32, 37, 48). In brief, after extraction of free and conjugated urinary steroids and enzymatic conjugate hydrolysis, GC-MS analysis was performed using the selected ion monitoring (SIM) mode.

Daily urinary excretion rates were determined for cortisol, i.e., combined free and conjugated cortisol (F_comb) and its major metabolites (Fig. 1), such as its tetrahydro-reduced metabolites, 5α-tetrahydrocortisol (α-THF) [m/z 652], tetrahydrocortisol (THF) [m/z 652], tetrahydrocortisone (THE) [m/z 578], and its hexahydro-reduced metabolites α-cortol [m/z 523], β-cortol [m/z 523], α-cortolone [m/z 449], and β-cortolone [m/z 449].
cortisol secretion these analyzed major urinary glucocorticoid metabolites were summed (40, 41, 43, 50). Global activity of the enzymatic system of 11β-hydroxysteroid dehydrogenase (11β-HSD) was conventionally assessed by the ratio a-THF + THF / THE. The net balance between 5α- and 5β-reductase was assessed by the ratio a-THF / THF (28, 37, 47). Intraassay precision (n=6) varied between 2.2 % (for THF) and 3.6 % (for THE) and interassay precision (n=6) between 1.1 % (for α-cortol) and 2.3 % (for a-THF).

For comparison, mean 24-h excretion data of the variables determined were provided (in tables and figure legends) also for healthy normal-weight adults aged around 30 years. These data were taken from Tomlinson et al (41) and Tsilchorozidou et al (43) for men and women, respectively. The index for 5α-reductase of men was obtained by division of the mean value for 5α-THF (2025 µg/d) by the mean for THF (2469 µg/d) (41). Body surface area (BSA)-corrected excretions for adults were determined on the basis of average BSA values. For woman an average BSA of 1.73 m² was assumed, for men an average BSA of 1.98 m² was calculated from height and weight means reported (41).

Since there is a close functional-anatomic correlation between adrenal volume and body surface area (21, 35) glucocorticoid excretion rates were normalized to BSA. Accordingly, correction with BSA of 24-h urinary free cortisol, a marker that grossly integrates the plasma free (bioactive) cortisol concentration during the entire day (26), has yielded relatively constant, age-independent values between ages 2 years and 17 years in healthy children (13). Correction with urinary creatinine, however, yields the typical age-dependent decline in the cortisol/creatinine ratio (13), which is present for many urinary analytes, due to the fact that muscle mass and thus 24-h creatinine excretion show physiologically markedly steeper increases during growth than BSA and/or energy intake (24, 33, 34). This age-related
physiological variability is the reason why the ratio of glucocorticoid to creatinine (without further adjustments) is no useful index for glucocorticoid status assessment, especially in children (3, 13). Correcting urinary glucocorticoids by body mass index (BMI) would not yield useful ratios too, since this index of fatness shows a nadir around 5-6 years (adiposity rebound) which would create cortisol/BMI variations with age which are not associated with growth processes, energy needs, or cortisol secretion.

**Statistical analysis**

Data are presented as mean ± SD and/or median and percentiles. Overall gender and age group effects were tested by two-way ANOVA. Subsequent analyses of the influence of age on hormonal and enzymatic variables during specific periods of ≈ 4 yr to 6 yr duration were done (with age as a continuous predictor) using regression analysis. Gender differences in particular age groups were tested by unpaired t-test. P < 0.05 was considered statistically significant. All tests were two-tailed. Calculations and analyses were performed using SAS for Windows (version 8.2; Statistical Analysis System, Cary, NC).
Results

Mean values ± SD for BMI, body surface area, daily energy intake and urine volume of the subjects according to age and gender are shown in Table 1. These data reflect normal developmental changes in the corresponding basic characteristics of healthy children and adolescents.

In Table 2 24-hr urinary excretion rates of the 3 most important single glucocorticoid metabolites THE, THF, and a-THF are given according to age and sex. Table 3 shows the sums of total daily renal output (C21) of all seven major glucocorticoid metabolites (THF, a-THF, α-cortol, β-cortol, THE, α-cortolone, β-cortolone) reflecting overall cortisol secretion. Significant overall effects of age and gender were observed by two-way ANOVA for all individual glucocorticoid metabolites and the sums of metabolites presented in Tables 2-3. Age and sex remained strong predictors for cortisol secretion (C21) even after urinary 24-h excretion rates of C21 were corrected for BSA (Table 4). No significant sex difference is discernible for Fcomb excretion, whether corrected for BSA or not (Table 4). While the ratio Fcomb/BSA showed significant variation over the whole age range (3 – 18 yr), it was no longer significant for the age range from 3 yr to 15 yr (n = 330; P = 0.1; Table 4).

Figure 2 shows a scatter plot of age dependent 24-h excretion rates of the sum of the major urinary cortisol metabolites (C21) when normalized for BSA. In both sexes, cortisol secretion rate – reflected by the 24-h urinary excretion of C21 – decreased significantly (P < 0.01) until the age of 8 yr. Thereafter, cortisol secretion rate showed a clear increase for both sexes (P < 0.0001 in boys and P < 0.05 in girls). After the age of 11-12 yr, dissociation in cortisol secretion rates between both sexes could be noted: while cortisol secretion increased further in boys, it stagnated in girls. Sex differences for C21 adjusted for body surface area were significant in age groups 15-16 yr (P < 0.05) and 17-18 yr (P < 0.01).
Furthermore, we investigated indices for activities of the two primary enzyme systems involved in cortisol metabolism. Figure 3a depicts the age-dependent global activities of 11β-HSD. The corresponding (α-THF + THF)/THE ratios were not constant during childhood and showed an u-shaped course in both sexes. To assess age-dependent changes in the activity of the glucocorticoid-catabolic 5α-reductase, we conventionally determined the α-THF/THF ratio which in the first instance reflects the balance between 5α- and 5β-reductase. Increases in this ratio could therefore imply a decrease in 5β-reductase or an increase in 5α-reductase. However, since the 5β-metabolites THF and THE do not decline in our children (relative to total cortisol secretion, compare Tables 2 and 3), changes in the ratio α-THF/THF represent altered 5α-reductase activity. Fig. 3b shows that a dissociation emerges for 5α-reductase between boys and girls after age 11-12 yr (sex difference: P < 0.05 in 15-16 yr olds and P < 0.01 in 17-18 yr olds).
Discussion

In this paper we have demonstrated that daily cortisol secretion – when corrected for body surface area (C21/BSA) – is by no means constant throughout childhood and adolescence, but shows a dynamic pattern with a nadir around the age of 7-8 yr. Thereafter, C21/BSA started to rise again in boys and in girls, and after the age of 11-12 yr it became divergent between both sexes with significantly higher levels in males.

No such dynamics is discernible for urinary cortisol (Fcomb) as conventionally measured by GC-MS. Fcomb/BSA was relatively constant over a wide age range in both sexes, especially during the period with the most dynamic changes in cortisol secretion, i.e., between age 3 yr and 15 yr. Since Fcomb is a combined parameter consisting only partly of urinary free cortisol, it is comprehensible that Fcomb/BSA is not necessarily totally age-independent as might be expected if this measure would closely reflect integrated plasma free (bioactive) cortisol over 24-h. A considerable part of Fcomb consists of glucuronidated and sulfated cortisol, implying that changes in hepatic conjugation activity during growth may confound Fcomb/BSA as an accurate index for the non invasive assessment of circulating bioactive free cortisol.

Our results corroborate the hypothesis that the reported higher cortisol secretion of men compared to women develops during puberty, a period during which the rough indicator of bioactive free cortisol in circulation Fcomb/BSA remains fairly constant. This strongly suggests that metabolic differences between males and females, which emerge during puberty may be responsible. In accordance with this, significant differences between the sexes were discernible for the index of 5α-reductase activity that we assessed, too (see below).

Measurement and summation of the major GC metabolites of 24-h urine samples (C21)
allows a time-integrated, stress-free, in-vivo examination of the overall amount of cortisol and cortisone originally secreted by the adrenal gland in large samples of healthy and/or ill subjects (9, 16, 23, 29, 39, 41, 43). This is the reason that we could confirm in healthy children and adolescents, stable isotope infusion results (hitherto obtained mostly in small groups of normal and obese adults) which suggest that daily cortisol production rates vary considerably between individuals (10, 44, 45). Vierhapper et al, for example, found daily cortisol production rates ranging between 1680 and 7440 μg m-2 d-1 in 7 healthy women (45). This range corresponds closely with the variation range that we observed (Fig. 2). In line with a commonly large inter-individual variability are other results by stable isotope dilution methodology demonstrating that patients with proven Cushing’s syndrome can have normal and patients without clinical and biochemical symptoms can have elevated cortisol productions (36). The large inter-individual variation in cortisol status parameters can be explained by genetic (8, 11, 20) and metabolic factors. Shifts in glucocorticoid metabolism are frequently responsible for marked changes in cortisol secretion in various illnesses. For example, elevated a-THF and normal THF excretion rates in patients with hyperthyroidism indicate an increased 5a-reductase activity and thus a reduction in cortisol half-life (16). This stimulated cortisol degradation explains why these patients frequently show an elevated cortisol secretion, necessary to maintain an appropriate bioactive free cortisol level in circulation. A similar mechanism operates in patients with polycystic ovary syndrome (43).

Earliest estimates of cortisol production rates in normal children and adolescents using isotope dilution with i.v. radiolabelled tracers yielded values around 12.1 ± 2.9 mg m−2 d−1 (mean ± SD) which seemed to be constant over time. The number of subjects studied (n = 20) was too low to permit reliable information about dynamic variations or sex differences regarding cortisol production (17). Later, in a study employing high performance liquid chromatography-mass spectrometry and i.v. stable isotope dilution much lower cortisol
production rates (mean ± SD; 6.8 ± 1.9 mg m\(^{-2}\) d\(^{-1}\)) were determined. In this (22) and further studies (5, 10) cortisol production was not found to vary with sex or pubertal stage, probably because of the small numbers of children studied.

Significant sex differences in the production of cortisol could be demonstrated in a study investigating 24 h urinary steroid profiles by high resolution GC (30). The authors found higher total cortisol metabolites in men than in women (mg m\(^{-2}\) d\(^{-1}\); mean ± SD; men: 4.2 ± 1.4; women: 2.9 ± 1.0). These values were similar to those found in our adolescents 17-18 yr of age. Similar gender differences in urinary excretion rates of cortisol metabolites measured by GC were documented in another study in adults (38) and in a further investigation (45) using stable isotope dilution with deuterium labeled cortisol and analysis by GC-MS (11.5 ± 2.2 versus 5.3 ± 1.9 mg m\(^{-2}\) d\(^{-1}\) in men and women, respectively). However, concerning the dynamic course of cortisol secretion throughout childhood and adolescence our study is the first report on the point of time, i.e. the age of 11-12 years, when this divergence in cortisol secretion begins. Earlier investigations on 24-h urinary steroid excretions in children (2, 15) did not report corresponding dynamic variations probably due to inhomogeneous study population (hospitalized and non hospitalized children; partly small numbers in certain age groups) and the fact that only selected glucocorticoid metabolites were evaluated.

Several studies in adults have tried to relate the observed sex dimorphism to a change in 11\(\beta\)-HSD-dependent cortisol metabolism (30, 42, 46). However, the strictly parallel behavior of 11\(\beta\)-HSD that we observed in both sexes does not allow any plausible explanation for the sexual dimorphism in cortisol secretion in adolescence. Therefore, our results do not support concepts explaining the sex dimorphism in human cortisol metabolism on the basis of sex specific regulation of 11\(\beta\)-HSD. Our findings are consistent with previous observations of cortisol metabolite excretion in young adults (12) showing that differences in cortisol
metabolite excretion between men and women could not be attributed to alterations in 11β-HSD.

Concerning further cortisol metabolizing enzymatic systems, studies in adults have shown a predominance of 5α-reduced cortisol metabolites over 5β-reduced metabolites in males (12, 30, 42). Our data showed (Fig. 2B) that net activity of 5α-/5β-reductases did not differ between boys and girls below the age of 11-12 yr. Thereafter it became different between sexes with a higher ratio of 5α-/5β-metabolites in males, thus confirming the above mentioned observations in adults. Consecutively, our findings corroborate the hypothesis that sexual dimorphism in cortisol metabolite excretion is attributable to less 5α-reductase activity in women, which means that cortisol is cleared less rapidly from plasma in females than in males (12). The fact that we observed the start of divergence in net activity of 5α-reductases around the beginning of puberty suggests an influence of gonadal steroids. In an earlier study, a higher increase in 5α-metabolites of testosterone compared to 5β-metabolites has been observed after administration of human chorionic gonadotrophin in humans (25) indicating that androgens might increase 5α-reductase activity. However, data on the regulation of 5α-/5β-reductases by sex steroids are scarce, frequently inconsistent, and mostly originating from animal experiments (12).

In summary, we have measured urinary markers of cortisol secretion and metabolism in children and adolescents using GC-MS. We found that overall cortisol secretion varies considerably with age and sex despite adjustment for BSA. Decreases of BSA-corrected C21 excretion rates in prepubertal children and diverging increases in adolescent males and females suggest that development- and sex-dependent changes in cortisol secretion exist during normal childhood and adolescence. Our results corroborate the hypothesis that the sex dimorphism of cortisol secretion reported for adults develops during puberty. Changes in
steroid metabolizing enzymes appear to be at least partly responsible. The findings point to a varying metabolic need for cortisol during growth and may bear importance for glucocorticoid replacement therapy.

Acknowledgement

This work was supported by a research grant from the Deutsche Forschungsgemeinschaft (RE 753/5 to T.R. and S.A. W.) and by the Ministry of Science and Research North Rhine-Westphalia, Germany
References


19. Kraan GP, Dullaart RP, Pratt JJ, Wolthers BG, Drayer NM, and De Bruin R. The


Table 1. Characteristics of the 400 children and adolescents

Legend:
Data are presented as the means ± SD. Each age group includes 25 boys and 25 girls. BMI, body mass index.
**TABLE 2.** Absolute 24-h excretion rates [μg/d] of the 3 most important single glucocorticoid metabolites (THE, THF and α-THF) in boys and girls

Legend:
Each age group includes 25 boys and 25 girls.

- $^a P < 0.0001$ for age and $P < 0.05$ for sex (two-way ANOVA).
- $^b P < 0.0001$ for age and $P < 0.001$ for sex (two-way ANOVA).
- $^c P < 0.0001$ for age and $P < 0.0001$ for sex (two-way ANOVA).
- $^d$ Reported data for healthy adults aged around 30 years; men: mean values ±SE (41); women: median and [range] (43).
TABLE 3. Urinary 24-h excretion rates [μg/d] of C21, i.e., the sum of all seven major glucocorticoid metabolites (THF, α-THF, α-cortol, β-cortol, THE, α-cortolone, β-cortolone) according to age and sex

Legend:
Data are presented as percentiles, median (P50), and means ± SD. Each age group includes 25 boys and 25 girls.

\(^a\) \(P < 0.0001\) for age, \(P < 0.0001\) for sex, and \(P < 0.0001\) for sex by age interaction (two-way ANOVA).

\(^b\) Reported data for healthy adults aged around 30 years; men: mean values ±SE (41); women: median and [range] (43).
TABLE 4. Urinary 24-h excretion rates of C21 (body surface-corrected) and cortisol (absolute and body surface-corrected) according to age and sex

Legend:
Data are presented as the means ± SD. Each age group includes 25 boys and 25 girls. C21 is the sum of all seven major glucocorticoid metabolites (THF, α-THF, α-cortol, β-cortol, THE, α-cortolone, β-cortolone); F denotes cortisol.

\(^a\) Body surface-corrected urinary 24-h excretion rate \([\mu g \cdot m^{-2} \cdot d^{-1}]\)

\(^b\) \(P < 0.0001\) for age, \(P = 0.002\) for sex, and \(P = 0.01\) for sex by age interaction (two-way ANOVA).

\(^c\) \(P < 0.0001\) for age, \(P = 0.11\) for sex, and \(P = 0.4\) for sex by age interaction (two-way ANOVA).

\(^d\) \(P < 0.0001\) for age, \(P = 0.45\) for sex, and \(P = 0.08\) for sex by age interaction (two-way ANOVA).

\(^e\) \(P = 0.1\) for age, \(P = 0.11\) for sex (regression analysis [with sex as dummy] for the age range 3yr - 15 yr, \(n = 330\)).

\(^f\) Reported data for healthy adults aged around 30 years; men: values are either mean ± SE or calculated from the mean (division by BSA, see methods) (41); women: calculated from the median by division by BSA, no \(F_{comb}\) data available (43).
FIG. 1: Metabolism of cortisol. Major catabolic products are the tetrahydro-reduced compounds tetrahydrocortisol (THF), allo(5α)-tetrahydrocortisol (a-THF) and tetrahydrocortisone (THE) as well as the hexahydro-reduced compounds α-cortol, β-cortol, α-cortolone and β-cortolone. The black dots indicate intermediate metabolic products.

FIG. 2: Age dependent BSA-adjusted 24-h excretion rates ($\mu$g d$^{-1}$ m$^{-2}$) of the sum of the major urinary cortisol metabolites (THE, a-THF, THF, α-cortol, β-cortol, α-cortolone, β-cortolone) in 400 healthy children (boys ●; girls ○).

FIG 3: (A) Global activity of 11α-hydroxysteroiddehydrogenase (urinary ratio of (a-THF + THF) / THE); $P < 0.0001$ for age, $P < 0.01$ for sex, and $P = 0.93$ for sex by age interaction (two-way ANOVA). Reported mean values for healthy adults aged around 30 yr; men: 0.98 (41); women: 0.84 (43). (B) Activity of 5α-reductase (urinary ratio of a-THF / THF) according to age and sex (two-way ANOVA: $P < 0.0001$ for age, $P < 0.01$ for sex, and $P < 0.05$ for sex by age interaction ). Reported mean values for healthy adults aged around 30 yr; men: 0.82 (41); women: 0.47 (43). The total number of 400 children applies for both panels A and B (boys ●; girls ○). Reported data for healthy adults aged around 30 years; men: mean values ±SE ; women: median and [range].
Table 1. Characteristics of the 400 children and adolescents

<table>
<thead>
<tr>
<th>Age</th>
<th>Boys (n = 200)</th>
<th>Girls (n = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>BMI (kg/m²)</td>
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<tr>
<td>3-4y</td>
<td>15.5 ±1.12</td>
<td>15.9 ±1.59</td>
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<td>0.70 ±0.06</td>
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<td>5-6y</td>
<td>15.2 ±1.06</td>
<td>15.5 ±1.44</td>
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<tr>
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<td>0.83 ±0.07</td>
<td>0.80 ±0.07</td>
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<td>7-8y</td>
<td>16.3 ±1.77</td>
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<td>0.97 ±0.12</td>
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<tr>
<td>9-10y</td>
<td>17.3 ±2.00</td>
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<td></td>
<td>1.18 ±0.13</td>
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<td>11-12y</td>
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<td>18.7 ±3.29</td>
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<td>1.39 ±0.17</td>
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<td>15-16y</td>
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<td>17-18y</td>
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<td>1.92 ±0.14</td>
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Data are presented as the means ± SD. Each age group includes 25 boys and 25 girls. BMI, body mass index.
TABLE 2. Absolute 24-h excretion rates [µg/d] of the 3 most important single glucocorticoid metabolites (THE, THF and a-THF) in boys and girls

<table>
<thead>
<tr>
<th>Age</th>
<th>THEa (P50)</th>
<th>THFb (P50)</th>
<th>a-THFc (P50)</th>
<th>THEa (Mean, SD)</th>
<th>THFb (Mean, SD)</th>
<th>a-THFc (Mean, SD)</th>
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<th>THFb (P50)</th>
<th>a-THFc (P50)</th>
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<td>544</td>
<td>674</td>
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<td>446</td>
<td>545</td>
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<td>11-12y</td>
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<td>597</td>
<td>744</td>
<td>2040</td>
<td>518</td>
<td>620</td>
<td>2343</td>
<td>640</td>
<td>792</td>
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<tr>
<td>13-14y</td>
<td>3037</td>
<td>834</td>
<td>989</td>
<td>2909</td>
<td>790</td>
<td>861</td>
<td>2905</td>
<td>897</td>
<td>1096</td>
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<tr>
<td>15-16y</td>
<td>3364</td>
<td>1199</td>
<td>1440</td>
<td>2391</td>
<td>1019</td>
<td>885</td>
<td>3367</td>
<td>1186</td>
<td>1561</td>
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<td>17-18y</td>
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<td>1561</td>
<td>1665</td>
<td>2003</td>
<td>1087</td>
<td>766</td>
<td>3511</td>
<td>1616</td>
<td>1654</td>
</tr>
<tr>
<td>Adults</td>
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<td>2469</td>
<td>2025</td>
<td>1810</td>
<td>960</td>
<td>510</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each age group includes 25 boys and 25 girls.

\( ^a \) \( P < 0.0001 \) for age and \( P < 0.05 \) for sex (two-way ANOVA).

\( ^b \) \( P < 0.0001 \) for age and \( P < 0.001 \) for sex (two-way ANOVA).

\( ^c \) \( P < 0.0001 \) for age and \( P < 0.0001 \) for sex (two-way ANOVA).

\( ^d \) Reported data for healthy adults aged around 30 years; men: mean values ±SE (41); women: median and [range] (43).
**TABLE 3.** Urinary 24-h excretion rates [µg/d] of C21, i.e., the sum of all seven major glucocorticoid metabolites (THF, α-THF, α-cortol, β-cortol, THE, α-cortolone, β-cortolone) according to age and sex

<table>
<thead>
<tr>
<th>AGE</th>
<th>P5</th>
<th>P25</th>
<th>P50</th>
<th>P75</th>
<th>P95</th>
<th>Mean</th>
<th>SD</th>
<th>P5</th>
<th>P25</th>
<th>P50</th>
<th>P75</th>
<th>P95</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-4y</td>
<td>1534</td>
<td>2091</td>
<td>2703</td>
<td>3279</td>
<td>3924</td>
<td>2781</td>
<td>±859</td>
<td>1298</td>
<td>1991</td>
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<td>3184</td>
<td>3901</td>
<td>2471</td>
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<tr>
<td>5-6y</td>
<td>1145</td>
<td>2315</td>
<td>2604</td>
<td>3131</td>
<td>3988</td>
<td>2709</td>
<td>±804</td>
<td>1300</td>
<td>1833</td>
<td>2925</td>
<td>3493</td>
<td>4294</td>
<td>2784</td>
<td>±958</td>
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<tr>
<td>7-8y</td>
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<td>2968</td>
<td>3604</td>
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<td>1817</td>
<td>2234</td>
<td>2825</td>
<td>3493</td>
<td>4294</td>
<td>2784</td>
<td>±640</td>
</tr>
<tr>
<td>9-10y</td>
<td>2633</td>
<td>3542</td>
<td>4029</td>
<td>4876</td>
<td>5401</td>
<td>4068</td>
<td>±889</td>
<td>2534</td>
<td>3051</td>
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<td>11-12y</td>
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<td>6586</td>
<td>8096</td>
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<td>±1595</td>
<td>3078</td>
<td>3808</td>
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<td>6174</td>
<td>9114</td>
<td>5139</td>
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<td>13-14y</td>
<td>2926</td>
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<td>15-16y</td>
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<td>±3101</td>
<td>3785</td>
<td>5490</td>
<td>6763</td>
<td>9610</td>
<td>10356</td>
<td>7348</td>
<td>±2425</td>
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<tr>
<td>17-18y</td>
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<td>3288</td>
<td>4365</td>
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<td>9749</td>
<td>11104</td>
<td>6870</td>
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<tr>
<td>Adults</td>
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<td></td>
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<td>±2686</td>
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<td></td>
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<td>5960</td>
<td>[2870 11000]</td>
</tr>
</tbody>
</table>

Data are presented as percentiles, median (P50), and means ± SD. Each age group includes 25 boys and 25 girls.

*a* P < 0.0001 for age, *P* < 0.0001 for sex, and *P* < 0.0001 for sex by age interaction (two-way ANOVA).

*b* Reported data for healthy adults aged around 30 years; men: mean values ±SE (41); women: median and [range] (43).
TABLE 4. Urinary 24-h excretion rates of C21 (body surface-corrected) and cortisol (absolute and body surface-corrected) according to age and sex

<table>
<thead>
<tr>
<th>AGE</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C21/BSA&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>F&lt;sub&gt;comb&lt;/sub&gt; (µg/d)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-4y</td>
<td>3991 ±1167</td>
<td>20.5 ±6.63</td>
</tr>
<tr>
<td>5-6y</td>
<td>3255 ±949</td>
<td>30.5 ±10.2</td>
</tr>
<tr>
<td>7-8y</td>
<td>3193 ±804</td>
<td>36.9 ±12.9</td>
</tr>
<tr>
<td>9-10y</td>
<td>3455 ±659</td>
<td>35.5 ±9.26</td>
</tr>
<tr>
<td>11-12y</td>
<td>3871 ±949</td>
<td>54.1 ±13.8</td>
</tr>
<tr>
<td>13-14y</td>
<td>4655 ±1679</td>
<td>53.2 ±21.4</td>
</tr>
<tr>
<td>15-16y</td>
<td>5474 ±1648</td>
<td>68.5 ±24.0</td>
</tr>
<tr>
<td>17-18y</td>
<td>5275 ±1414</td>
<td>75.0 ±25.3</td>
</tr>
<tr>
<td>Adults</td>
<td>6331</td>
<td>76 ±16</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD. Each age group includes 25 boys and 25 girls. C21 is the sum of all seven major glucocorticoid metabolites (THF, α-THF, α-cortol, β-cortol, THE, α-cortolone, β-cortolone); F denotes cortisol.

<sup>a</sup> Body surface-corrected urinary 24-h excretion rate [µg · m⁻² · d⁻¹]

<sup>b</sup> P < 0.0001 for age, P = 0.002 for sex, and P = 0.01 for sex by age interaction (two-way ANOVA).

<sup>c</sup> P < 0.0001 for age, P = 0.11 for sex, and P = 0.4 for sex by age interaction (two-way ANOVA).

<sup>d</sup> P < 0.0001 for age, P = 0.45 for sex, and P = 0.08 for sex by age interaction (two-way ANOVA).

<sup>e</sup> P = 0.1 for age, P = 0.11 for sex (regression analysis [with sex as dummy] for the age range 3yr - 15 yr, n = 330).

<sup>f</sup> Reported data for healthy adults aged around 30 years; men: values are either mean ±SE or calculated from the mean (division by BSA, see methods) (41); women: calculated from the median by division by BSA, no F<sub>comb</sub> data available (43).