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Sexual dimorphism in cortisol metabolism throughout pubertal development: a longitudinal study

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Abstract

Objective: Sex differences in disease susceptibility might be explained by sexual dimorphism in hypothalamic-pituitary-adrenal axis activity, which has been postulated to emerge during puberty. However, studies conducted thus far lacked an assessment of Tanner pubertal stage. This study aimed to assess the contribution of pubertal development to sexual dimorphism in cortisol production and metabolism.

Methods: Participants (n = 218) were enrolled from a population-based Netherlands Twin Register. At the ages of 9, 12 and 17 years, Tanner pubertal stage was assessed and early morning urine samples were collected. Cortisol metabolites were measured with GC-MS/MS and ratios were calculated, representing cortisol metabolism enzyme activities, such as A-ring reductases, 11β-HSDs and CYP3A4. Cortisol production and metabolism parameters were compared between sexes for pre-pubertal (Tanner stage 1), early pubertal (Tanner stage 2–3) and late-pubertal (Tanner stage 4–5) stages.

Results: Cortisol metabolite excretion rate decreased with pubertal maturation in both sexes, but did not significantly differ between sexes at any pubertal stage, although in girls a considerable decrease was observed between early and late-pubertal stage (P < 0.001). A-ring reductase activity was similar between sexes at pre- and early pubertal stages and was lower in girls than in boys at late-pubertal stage. Activities of 11β-HSDs were similar between sexes at pre-pubertal stage and favored cortisone in girls at early and late-pubertal stages. Cytochrome P450 3A4 activity did not differ between sexes.

Conclusions: Prepubertally, sexes were similar in cortisol parameters. During puberty, as compared to boys, in girls the activities of A-ring reductases declined and the balance between 11β-HSDs progressively favored cortisone. In addition, girls showed a considerable decrease in cortisol metabolite excretion rate between early and late-pubertal stages. Our findings suggest that the sexual dimorphism in cortisol may either be explained by rising concentrations of sex steroids or by puberty-induced changes in body composition.

Key Words
- glucocorticoid
- metabolites
- steroid
- Tanner
- sex differences

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Introduction

Males and females differ in their susceptibility to develop specific diseases. While females are more likely to develop auto-immune diseases and neuropsychiatric disorders like anxiety and depression, males are more susceptible to infectious diseases and are more likely to engage in violent competition (1, 2, 3). Moreover, males and females differ in cardiovascular disease susceptibility (4). Sex differences in HPA-axis settings have been hypothesized to play a role in these differences (5, 6, 7, 8, 9, 10).

Sexual dimorphism in HPA-axis activity has been suggested to be already present in early childhood. A recent meta-analysis suggested that boys and girls differed in basal HPA-axis activity, as assessed by salivary cortisol levels. Compared to girls, boys, up to age 8, had higher salivary cortisol levels and lower levels beyond this age (11). The timing of this change suggests that sex steroids influence the HPA axis. Surprisingly, to the best of our knowledge, there are no studies that have reported on HPA-axis activity across pubertal development.

HPA-axis activity is determined by the net effect of cortisol production and metabolism. Cortisol is metabolized by various enzymes (Fig. 1). The A-ring reductases (5α- and 5β-reductase), together with cytochrome P (CYP) 3A4, eliminate cortisol from the circulation primarily in the liver (12). The 11beta-hydroxysteroid dehydrogenase (11β-HSD) isozymes regulate the interconversion between cortisol and its inactive metabolite cortisone. 11β-HSD type 1 is mainly expressed in the liver and adipose tissue, where it regenerates cortisol, and 11β-HSD type 2 catalyzes the reverse reaction in renal epithelial cells.

In adulthood, females were found to have a lower urinary excretion rate of cortisol metabolites than males (13, 14, 15). This is likely to be attributed to less A-ring reduction in females, resulting in a prolonged half-life of cortisol and, hence, enhanced central feedback suppression (14). In contrast, CYP3A4 activity is higher in women than in men. However, CYP3A4 is known to eliminate only a small proportion of circulating cortisol (16, 17). There is controversy as to whether men and women differ in the activities of 11β-HSDs (14, 18, 19, 20).

Interaction between gonadal steroids and the metabolism of cortisol has been suggested by several studies (21, 22, 23, 24). However, there is only one cross-sectional study that has investigated glucocorticoid metabolism in children of various ages (18). This study demonstrated that the sex differences in the elimination rate of cortisol, as observed in adulthood, began around the age of 11–12 years and were attributable to a progressive difference in 5α-reductase activity (being lower in older girls). Therefore, sexual dimorphism in cortisol metabolism was postulated to emerge during pubertal maturation, suggesting an interplay of adrenal and gonadal axes (18). However, information on pubertal stage was not available in that study (18). To the best of our knowledge, a longitudinal follow-up study of cortisol metabolism from pre- to post-puberty has never been conducted. The aim of this study was to assess the contribution of pubertal development to sexual dimorphism in cortisol production and metabolism.

Materials and methods

Participants

We conducted a prospective follow-up study and recruited healthy mono- and dizygotic twin pairs from the Netherlands Twin Register (NTR), a population-based registry (25, 26). Twins born between 1995 and 1996 were invited to participate in the BrainScale study of cognition, hormones, and brain development (27, 28). BrainScale is a...
Sexual dimorphism in cortisol Cytochrome P450 3A4 activity

Sum of cortisol metabolites

Balance of 11α-HSD type 2 activity

Statistical analysis

In line with previous analyses in this sample, extreme outliers (>3 s.d. above the phenotypic mean or twin pairs with highly discordant outcomes; on average six and one per index, respectively) were excluded from the statistical

Table 1 Summary of outcomes.

| Parameter | Index |
|-----------|-------|
| (THF + allo-THF + THE + α-cortol + β-cortol + α-cortolone + β-cortolone)/creatinine | Sum of cortisol metabolites (cortisol metabolite excretion rate) |
| allo-THF/F | 5α-reductase activity |
| THF/F | 5β-reductase activity (a) |
| THE/E | 5β-reductase activity (b) |
| F/E | 11β-HSD type 2 activity |
| (THF + allo-THF)/THE | Balance of 11β-HSD activities |
| 6β-OH cortisol/F | Cytochrome P450 3A4 activity |

E, cortisol; F, cortisol; HSD, hydroxysteroid dehydrogenase; THE, tetrahydrocortisone; THF, tetrahydrocortisol.
Sexual dimorphism in cortisol

Next, the data were corrected for batch effects by fitting a random effects model, in which batch was treated as a random effect (42).

**Statistical modeling**

Our main aim was to determine the effect of pubertal stage on the outcomes. To this end, we implemented a discrete-time Markov model, in which we estimated the mean and s.d. of each metabolite conditional on the pubertal stage. In this model, each participant was assigned to stage A, B, or C. We took into account that a given participant may transition from stage A to B or C and from stage B to stage C between ages 9 and 12 and between 12 and 17 years, respectively. The parameters of the Markov model are the probabilities of being in stage A, B, or C at the age of 9 years, transition probabilities from ages 9 to 12 years, and transition probabilities from ages 12 to 17 years. Because no 9-year olds were assigned to stage C and no 17-year olds were assigned to stage A, the Markov model includes stages A and B at the age of 9 years, stages A, B, and C at the age of 12 years, and stages B and C at the age of 17 years. Figure 2 depicts the model. In Fig. 2, \( p_1 \) is the probability of being in stage A at the age of 9 years, \( q_{11} \) and \( q_{12} \) are conditional probabilities governing the transition between ages 9 and 12 years, and \( r_{11} \) and \( r_{21} \) are conditional probabilities governing the transition between ages 12 and 17 years. For instance, \( q_{12} \) is the probability of transitioning from stage A at the age of 9 years to stage B at the age of 12 years.

As we expected sex differences in probability of the pubertal stages at the age of 9 years and in the transition probabilities from ages 9 to 12 years and from 12 to 17 years, we allowed these parameter to vary with sex (i.e. the probabilities \( p_1, q_{11}, q_{12}, q_{22}, r_{11}, \) and \( r_{21} \) varied with sex). In addition, we allowed for sex differences in the means of the metabolites within a given pubertal stage (i.e. the means \( m_A, m_B, \) and \( m_C \) varied with sex). s.d. were constrained to be equal over sexes.

The Markov transition model was implemented in Mplus 6.0 (43). The parameters were estimated by means of maximum likelihood (ML) estimation. For this study, twins were treated as individuals. Therefore, the s.e., CIs, and test statistics were corrected for family clustering. For all outcomes, means were calculated for girls and boys along with their 95% CIs. Differences between sexes were tested and reported by a two-tailed \( P \)-value, whereby a \( P \)-value of < 0.05 was considered as statically significant. Given the sample size, correction for multiple testing was not conducted. In addition, changes in cortisol parameters were calculated during pubertal development for both sexes.

**Results**

A total of 218 participants (50% females) were included in this study, including 94 monozygotic and 124 dizygotic twins. The monozygotic twin pairs included 23 male and 24 female pairs. The dizygotic twin pairs included 22 male-, 21 female-, and 19 opposite-sex pairs. Participants were tested at 9.1 (±0.1), 12.2 (±0.3), and 17 years.
17.2 (±0.2) years of age. In total 542 samples were analyzed, of which 213 (50% females), 167 (50% females), and 162 (63%) females) were obtained at the ages of 9, 12, and 17 years, respectively. Mean S.D. score (Z score (±S.D.) BMI (weight(kg)/height(m)) was 0.14 (± 0.93), 0.45 (± 1.00), and 0.27 (± 1.08) at the ages of 9, 12 and 17 years, respectively (44). Table 2 displays the characteristics for boys and girls separately at the ages of 9, 12, and 17 years.

**Pubertal development**

The Markov model parameter estimates are shown in Table 3. At the age of 9 years, as expected, boys were more likely to be pre-pubertal than girls (0.944 vs 0.811). At the age of 12 years, the entire spectrum of pubertal stages were observed in both sexes, although in girls puberty was generally more advanced. At the age of 17 years, all girls were classified as late-pubertal, while, contrary to expectation, still 20% of the boys were early pubertal.

Consequently, sex differences in the transition probabilities from 9 to 12 years and from 12 to 17 years were also observed. For instance, the probability of remaining in stage A between the ages of 9 and 12 years was markedly higher in boys (0.259) than in girls (0.127). The probability of moving from stage A to C between 9 and 12 years was higher in girls than in boys (0.282 vs 0.025).

**Sexual dimorphism in cortisol production and metabolism during pubertal development**

Table 4 displays the sex-specific means for cortisol metabolite excretion rate and cortisol metabolite ratios by pubertal stage. Cortisol metabolite excretion rate did not statistically differ between sexes at any stage. The cortisol metabolite ratios were similar between sexes at stage A, but diverged during pubertal development. In boys at stages B and C, as compared to girls of the same stage, the balance of 11β-HSD activities ((THF+allo-THF)/THE ratio) favored cortisol. At stage B, this difference could be partially attributable to a lower 11β-HSD type 2 activity (cortisol/cortisone ratio) in boys. Girls at stage C, as compared to boys of the same stage, had lower activities of 5α- (allo-THF/F ratio) and 5β-reductases (THE/E ratio). There were no differences in CYP3A4 activity (6-OH cortisol/cortisol ratio) between sexes at stages B or C.

**Table 2** Characteristics of participants.

| Length (cm) | SDS | Weight (kg) | SDS | Body mass index (kg/m²) | SDS | Tanner stage (%) |
|-------------|-----|-------------|-----|------------------------|-----|------------------|
| Boys | 9 years (n = 106) | 12 years (n = 83) | 17 years (n = 77) | Girls | 9 years (n = 106) | 12 years (n = 86) | 17 years (n = 95) |
| cm | | | | | | | |
| 139.2 ± 5.5 | 151.8 ± 7.2 | 179.6 ± 6.2 | 138.2 ± 4.8 | 152.5 ± 7.2 | 168.6 ± 6.1 |
| 0.03 ± 0.89 | −0.57 ± 0.93 | −0.23 ± 0.83 | 0.06 ± 0.76 | −0.60 ± 1.05 | −0.10 ± 0.93 |
| 31.6 ± 4.3 | 42.5 ± 8.4 | 67.3 ± 9.4 | 31.3 ± 4.6 | 43.8 ± 8.4 | 61.3 ± 8.88 |
| 0.34 ± 0.95 | 0.38 ± 0.91 | 0.12 ± 1.03 | 0.29 ± 0.91 | 0.05 ± 2.91 | 0.37 ± 1.09 |
| 16.2 ± 1.4 | 18.6 ± 2.0 | 20.9 ± 2.5 | 16.4 ± 2.0 | 18.8 ± 2.9 | 21.6 ± 3.3 |
| 0.21 ± 0.83 | 0.58 ± 0.86 | 0.21 ± 1.03 | 0.09 ± 1.00 | 0.32 ± 1.11 | 0.30 ± 1.13 |
| A | 94 | 24 | 0 | 81 | 10 | 0 |
| B | 6 | 70 | 20 | 19 | 62 | 0 |
| C | 0 | 5 | 80 | 0 | 28 | 100 |

Values represent mean ± s.d. or %.

*Percentage of patients in Tanner stages are based on the transition probabilities according our Markov model. A = pre-pubertal (Tanner stage 1); B = early pubertal (Tanner stage 2–3); C = late-pubertal (Tanner stage 4–5).
Sexual dimorphism in cortisol

In this longitudinal study, we have demonstrated that the excretion of cortisol metabolites diverges between sexes with advancing pubertal maturation. Therefore, our study suggests that the sexual dimorphism in cortisol metabolism that generally starts around the age of 11 is a hormonally driven process, either directly, by influencing gene expression, or indirectly, by impacting on body composition.

Previous studies showed that adult men and women differ in the excretion rate of cortisol metabolites, which was higher in males (13, 45). Wudy et al. found that these differences emerged from the age of 11 to 12 years (18), suggestive of an important role of gonadal hormones. However, their study lacked an assessment of pubertal status. In our study, which included repeated assessment of Tanner pubertal stage, we found that the excretion rate of cortisol metabolites decreased significantly in girls between early and late-pubertal stage, suggestive of an effect of pubertal development on the excretion rate of cortisol metabolites in girls. In contrast to previous research (18), we were not able to detect statistically significant differences in cortisol metabolite excretion rate between sexes which might be due to the use of morning instead of 24-h urine or the relatively small sample size.

Our data provided evidence for a sexual dimorphism in cortisol metabolism, as assessed by ratios reflecting the activities of the enzymes involved. We found that with advancing pubertal maturation in females, as compared to males, the balance of 11β-HSDs progressively favored cortisone and that the proportion of A-ring reduced metabolites was lower, in line with data in adults (13, 14, 15, 20, 45). During pubertal maturation, levels of sex steroids increase gradually along with the development of A-ring reductases (ratios of allo-THF/F, THF/F, and THE/E) increased (with the exception of 5α-reductase activity) between stages A and B, while in girls it decreased considerably between stages B and C. In both sexes, the balance of 11β-HSD type 2 activity ((THF + allo-THF)/THE) changed in the direction of cortisol, which could partially be explained by decreased 11β-HSD type 2 activity (cortisol/cortisone ratio). In girls, the activities of A-ring reductases (ratios of allo-THF/F, THF/F, and THE/E) increased (with the exception of 5α-reductase activity) between stages A and B and decreased between stages B and C. In boys, these parameters did not change during pubertal development. In both sexes, CYP3A4 activity (6-OH cortisol/cortisol ratio) was stable across pubertal development.

**Discussion**

In this longitudinal study, we have demonstrated that the excretion of cortisol metabolites diverges between sexes with advancing pubertal maturation. Therefore, our study suggests that the sexual dimorphism in cortisol metabolism that generally starts around the age of 11 is a hormonally driven process, either directly, by influencing gene expression, or indirectly, by impacting on body composition.

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Table 5  Changes in cortisol metabolite excretion rate and cortisol metabolite ratios during pubertal development.

| Parameter                      | Pubertal stage* | Girls Mean difference (95% CI)          | P value | Boys Mean difference (95% CI)          | P value |
|--------------------------------|-----------------|----------------------------------------|---------|----------------------------------------|---------|
| Cortisol metabolite excretion rate | A -> B          | −0.004 (−0.069–0.060)                 | 0.913   | −0.059 (−0.160–0.012)                 | 0.040   |
| Sxr-reductase activity         | B -> C          | −0.128 (−0.188–0.068)                 | <0.001  | −0.034 (−0.097–0.048)                 | 0.580   |
| Sjr-reductase activity (a)     | A -> B          | 0.928 (−0.234–2.090)                  | 0.189   | −1.150 (−2.367–1.150)                 | 0.120   |
| Sjr-reductase activity (b)     | A -> B          | −4.406 (−5.703–3.109)                 | <0.001  | 0.303 (−0.945–3.030)                  | 0.690   |
| 5β-reductase activity (a)      | B -> C          | 1.953 (1.097–2.808)                   | <0.001  | 1.172 (−2.209–2.553)                  | 0.163   |
| 5β-reductase activity (b)      | B -> C          | −1.325 (−2.366–0.284)                 | 0.036   | −0.638 (−2.113–0.837)                 | 0.477   |
| 11β-HSD type 2 activity       | A -> B          | 4.416 (1.501–7.331)                   | 0.013   | 2.056 (−0.359–4.471)                  | 0.161   |
| 11β-HSD activities             | B -> C          | −5.422 (−8.335–2.508)                 | 0.002   | 1.003 (−2.180–4.186)                  | 0.604   |
| Balance of 11β-HSD activities  | A -> B          | −0.124 (−0.213–0.036)                 | <0.001  | 0.043 (−0.220–0.433)                  | <0.001  |
| Cytochrome P450 3A4 activity   | B -> C          | 0.492 (0.363–0.620)                   | 0.079   | 0.326 (0.200–0.443)                   | <0.001  |
|                                 | A -> B          | 0.013 (−0.205–0.232)                  | 0.919   | 0.016 (−0.031–0.063)                  | 0.566   |
|                                 | B -> C          | −0.134 (−0.400–0.133)                 | 0.410   | 0.153 (0.089–0.218)                   | 0.304   |

*A = pre-pubertal (Tanner stage 1); B = early pubertal (Tanner stage 2–3); C = late-pubertal (Tanner stage 4–5).

of secondary sexual characteristics (46). Therefore, our findings suggest that sex steroids influence cortisol metabolism either directly, for example, by influencing gene expression, or indirectly, by impacting on body composition (47, 48, 49).

With advancing pubertal development, differences in body composition emerge between sexes; girls gain more mass and boys acquire more fat-free mass and skeletal mass (50). These differences are regulated by endocrine factors, including gonadal steroids and growth hormone (51), in addition to genetic and environmental factors. Body composition is strongly associated with HPA-axis activity, both in adulthood and childhood (52, 53), and the observed differences between sexes in cortisol parameters that emerge during pubertal development could (partially) be explained by progressive differences in body composition. Although males and females did not differ in BMI at any age, unfortunately our study lacked a more detailed assessment of body composition.

It is unclear whether the sex differences in cortisol metabolism that we observed are androgen- or estrogen mediated. A study in adult men showed that testosterone reduced the CRH-stimulated rise in serum cortisol, in spite of increased ACTH, suggestive of adrenal hyporesponsiveness (54). Evidence for an effect of androgens on glucocorticoid metabolism was provided by other studies (55, 56, 57). It has been demonstrated by multiple studies that women with polycystic ovary syndrome – a condition characterized by increased androgen production – had a higher Sxr-reductase activity than BMI-matched controls (55). However, this may be part of the PCOS trait rather than an effect of hyperandrogenism, as their daughters already had a greater Sxr-reductase activity, in spite of a similar androgen metabolite excretion rate, from early childhood than age-matched controls (56). In contrast, experiments in gonadectomized male rats suggested that androgens suppress the expression and/or the activity of hepatic A-ring reductases (57). In addition, the same experiments suggested that androgens increase 11β-HSD type 1 in liver and adipose tissue (57).

Studies in rats and humans suggest that estrogens could also influence glucocorticoid metabolism, though findings were contradictory. Several studies in rodents have shown that the expression and/or the activity of A-ring reductases and 11β-HSD type 1 was upregulated and downregulated by estrogen, respectively (19, 22, 58, 59, 60, 61). In contrast, in humans the activity of these enzymes was independent of the phase of the menstrual cycle or, after menopause, use of estrogen replacement therapy (14, 15, 20).

Our study has several strengths and limitations. The major strength of our study was the long follow-up period of 8 years, enabling us to assess the contribution of pubertal maturation to sexual dimorphism in HPA-axis functioning. Moreover, participants were recruited from a population-based twin register and the numbers remaining into follow-up were relatively high for an age group that is notoriously difficult to engage in longitudinal studies. Another strength is that all measurements were performed in the same laboratory at the same time. Samples were frozen as soon as possible and were thawed only once prior to analysis, which enhances stability (62).

Our study also has its limitations. First, the Brain Scale study was not powered specifically for the present study and, hence, a sample size calculation was not performed prior to analysis, which might explain our inability to detect differences in cortisol metabolite excretion rate between sexes. Second, some limitations related to our
Sexual dimorphism in cortisol

With advancing pubertal maturation, sexual dimorphism in cortisol metabolism became increasingly manifest, while no differences were seen prepubertally. These differences could emerge from direct or indirect effects of sex steroids on cortisol metabolism.

Conclusion

With advancing pubertal maturation, sexual dimorphism in cortisol metabolism became increasingly manifest, while no differences were seen prepubertally. These differences could emerge from direct or indirect effects of sex steroids on cortisol metabolism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

1 Fairweather D, Petri MA, Coronado MJ & Cooper LT. Autoimmune heart disease: role of sex hormones and autoantibodies in disease pathogenesis. Expert Review of Clinical Immunology 2012 8 269–284. (https://doi.org/10.1586/eci.12.10)
2 World Health Organization. Gender and Women’s Mental Health. Gender Disparities and Mental Health: The Facts. Geneva, Switzerland: WHO. (available at: https://www.who.int/mental_health/prevention/genderwomen/en/)
3 Schacht R, Rauch KL & Bergerhoff Mulder M. Too many men: the violence problem? Trends in Ecology and Evolution 2014 29 214–222. (https://doi.org/10.1016/j.tree.2014.02.001)
4 Appelman Y, van Rijn BB, Ten Haaf ME, Boersma E & Peters SA. Sex differences in cardiovascular risk factors and disease prevention. Atherosclerosis 2015 241 211–218. (https://doi.org/10.1016/j.atherosclerosis.2015.01.027)
5 Silverman MN & Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Annals of the New York Academy of Sciences 2012 1261 55–63. (https://doi.org/10.1111/j.1749-6632.2012.06633.x)
6 Malhi GS & Mann JJ. Depression. Lancet 2018 392 2299–2312. (https://doi.org/10.1016/S0140-6736(18)31948-2)
7 Liyanarachchi K, Ross R & Debono M. Human studies on hypothalamo-pituitary-adrenal (HPA) axis. Best Practice and Research: Clinical Endocrinology and Metabolism 2017 31 459–473. (https://doi.org/10.1016/j.beem.2017.10.011)
8 Dekker MJ, Koper JW, van Aken MO, Pols HA, Hofman A, de Jong FH, Kirschbaum C, Wittman JC, Lamberts SW & Themeeier H. Salivary cortisol is related to atherosclerosis of carotid arteries. Journal of Clinical Endocrinology and Metabolism 2008 93 3741–3747. (https://doi.org/10.1210/j.c.2008-0496)
9 Zorn JV, Schur RR, Boks MP, Kahn RS, Joels M & Vinkers CH. Cortisol stress reactivity across psychiatric disorders: a systematic review and meta-analysis. Psychoneuroendocrinology 2017 77 25–36. (https://doi.org/10.1016/j.psyneuen.2016.11.036)
10 World Health Organization. Biological Mechanisms Related to Cardiovascular and Metabolic Effects by Environmental Noise. Geneva, Switzerland: WHO, 2018. (available at: http://www.euro.who.int/en/health-topics/environment-and-health/noise/publications/2018/biological-mechanisms-related-to-cardiovascular-and-metabolic-effects-by-environmental-noise)
11 van der Voorn B, Hollanderds JJ, Ket JC, Rotteveel J & Finken MJ. Gender-specific differences in hypothalamus-pituitary-adrenal axis activity during childhood: a systematic review and meta-analysis. Biology of Sex Differences 2017 8 3. (https://doi.org/10.1186/s13293-016-0123-5)
12 Methile P, Husebye EE, Hustad S, Lien EA & Lovas K. Grapefruit juice and licorice increase cortisol availability in patients with Addison’s disease. European Journal of Endocrinology 2011 165 761–769. (https://doi.org/10.1530/EJE-11-0158)
13 Shamim W, Yousufuddin M, Bakhai A, Coats AJ & Honour JW. Gender differences in the urinary excretion rates of cortisol and androgen metabolites. Annals of Clinical Biochemistry 2000 37 770–774. (https://doi.org/10.1258/0004563001900084)
14 Finken MJ, Andrews RC, Andrew R & Walker BR. Cortisol metabolism in healthy young adults: sexual dimorphism in activities of A-ring reductases, but not 11beta-hydroxysteroid dehydrogenases. Journal of Clinical Endocrinology and Metabolism 1999 84 3316–3321. (https://doi.org/10.1210/jc.84.9.6009)
15 Andrew R, Phillips DL & Walker BR. Obesity and gender influence cortisol secretion and metabolism in man. Journal of Clinical Endocrinology and Metabolism 1996 81 1806–1809. (https://doi.org/10.1210/jcem.81.5.8667)
16 Lutz U, Bittner N, Ufer M & Lutz WK. Quantification of cortisol and 6 beta-hydroxycortisol in human urine by LC-MS/MS, and gender-specific evaluation of the metabolic ratio as biomarker of CYP3A activity. Journal of Chromatography: B, Analytical Techniques in the Biomedical and Life Sciences 2010 878 97–101. (https://doi.org/10.1016/j.jchromb.2009.11.023)
17 Inagaki K, Inagaki M, Kataoka T, Sekido I, Gill MA & Nishida M. A wide interindividual variability of urinary 6beta-hydroxycortisol to free cortisol in 487 healthy Japanese subjects in near basal condition. Therapeutic Drug Monitoring 2002 24 722–727. (https://doi.org/10.1097/00007691-200212000-00007)
18 Wudy SA, Hartmann MF & Remer T. Sexual dimorphism in cortisol secretion starts after age 10 in healthy children: urinary cortisol metabolite excretion rates during growth. American Journal of Physiology: Endocrinology and Metabolism 2007 293 E970–E976. (https://doi.org/10.1152/ajpendo.00495.2006)

19 Low SC, Chapman KE, Edwards CR, Weils T, Robinson IC & Seckl JR. Sexual dimorphism of hepatic 11 beta-hydroxysteroid dehydrogenase in the rat: the role of growth hormone patterns. Journal of Endocrinology 1994 143 541–548. (https://doi.org/10.1677/joe.01430541)

20 Toogood AA, Taylor NF, Shalet SM & Monson JP. Sexual dimorphism of cortisol metabolism is maintained in elderly subjects and is not oestrogen dependent. Clinical Endocrinology 2000 52 61–66. (https://doi.org/10.1046/j.1365-2265.2000.00087.x)

21 Yokoi H, Tsuruo Y, Miyamoto T & Ishimura K. Steroid S alpha-reductase type 1 immunolocalized in the adrenal gland of normal, gonadotropinized, and sex hormone-supplemented rats. Histochemistry and Cell Biology 1998 109 127–134. (https://doi.org/10.1007/s004180050210)

22 Jamieson PM, Nyirenda MJ, Walker BR, Chapman KE & Seckl JR. Interactions between oestradiol and glucocorticoid regulatory effects on liver-specific glucocorticoid-inducible genes: possible evidence for a role of hepatic 11beta-hydroxysteroid dehydrogenase type 1. Journal of Endocrinology 1999 160 103–109. (https://doi.org/10.1677/joe.016000103)

23 Nowak KW, Neri G, Nussdorfer GG & Malendowicz LK. Effects of sex hormones on the steroidogenic activity of dispersed adrenocortical cells of the rat adrenal cortex. Life Sciences 1995 57 833–837. (https://doi.org/10.1016/0024-3205(95)02015-b)

24 Oyola MG & Handa RJ. Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. Stress 2017 20 476–494. (https://doi.org/10.1080/10253890.2017.169523)

25 Boomsla DM, de Geus EJ, Vink JM, Stubbie JH, Distel MA, Hottenga JJ, Posthuma D, van Beijsterveldt TC, Huizkja J J, Bartels M, et al. Netherlands Twin Register: from twins to twin families. Twin Research and Human Genetics 2006 9 849–857. (https://doi.org/10.1375/183242706779462426)

26 Boomsla DM, Vink JM, van Beijsterveldt TC, de Geus EJ, Beem AL, Mulder EJ, Derks EM, Riese H, Willemsen GA, Bartels M, et al. Netherlands Twin Register: a focus on longitudinal research. Twin Research 2002 5 401–406. (https://doi.org/10.1375/136998102299996174)

27 van Soelen IL, Brouwer RM, Peper JS, van Leeuwen M, Koenis MM, van Beijsterveldt TC, Swagerman SC, Kahn RS, Hulshof Pol HE & Boomsla DM. Brain SCALE: brain structure and cognition: an individual differences. Twin Research and Human Genetics 2012 15 453–467. (https://doi.org/10.1017/thg.2012.4)

28 Teeuw J, Brouwer RM, Koenis MMG, Swagerman SC, Boomsla DM & Hulshof Pol HE. Genetic influences on the development of cerebral cortical thickness during childhood and adolescence in a Dutch longitudinal twin sample: the Brainscan study. Cerebral Cortex 2019 29 978–993. (https://doi.org/10.1093/cercor/bhy0015)

29 Marshall WA & Tanner JM. Variations in the pattern of pubertal changes in boys. Archives of Disease in Childhood 1970 45 13–23. (https://doi.org/10.1136/adc.45.239.13)

30 Marshall WA & Tanner JM. Variations in pattern of pubertal changes in girls. Archives of Disease in Childhood 1969 44 291–303. (https://doi.org/10.1136/adc.44.235.291)

31 Ernst A, Lauridsen LLB, Brix N, Kjersgaard C, Olsen J, Parner ET, Clausen N, Olsen LH & Ramlaun-Hansen CH. Self-assessment of pubertal development in a puberty cohort. Journal of Pediatric Endocrinology and Metabolism 2018 31 763–772. (https://doi.org/10.1515/jpem-2018-0178)

32 Wu Y, Schreiber GR, Klementowicz V, Biro F & Wright D. Racial differences in accuracy of self-assessment of sexual maturation among young black and white girls. Journal of Adolescent Health 2001 28 197–203. (https://doi.org/10.1016/s1054-139x(00)00163-4)

33 Desmangles JC, Lappe JM, Lipacizewski G & Haynatzki G. Accuracy of pubertal Tanner staging self-reporting. Journal of Pediatric Endocrinology and Metabolism 2006 19 213–221. (https://doi.org/10.1515/jpem.2006.19.3.213)

34 Hegeneroder AC, Hill RB, Wong WW, Sangi-Haghpeyak H & Taylor W. Validity of self-assessment of pubertal maturation in African American and European American adolescents. Journal of Adolescent Health 1999 24 201–205. (https://doi.org/10.1016/s1054-139x(98)00011-0)

35 Schlossberger NM, Turner RA & Irwin JF CE. Validity of self-report of pubertal maturation in early adolescents. Journal of Adolescent Health 1992 13 109–113. (https://doi.org/10.1016/s1054-139x(92)80075-m)

36 Bonat S, Pathamvanich A, Keil MF, Field AE & Yanovsky JA. Self-assessment of pubertal stage in overweight children. Pediatrics 2002 110 743–747. (https://doi.org/10.1542/peds.110.4.743)

37 Sun Y, Tao FR, Su PY & China Puberty Research Collaboration. Self-assessment of pubertal Tanner stage by realistic colour images in representative Chinese obese and non-obese children and adolescents. Acta Paediatrica 2012 101 e163–e166. (https://doi.org/10.1111/j.1651-2227.2011.02568.x)

38 Slora EJ, Bocian AB, Herman-Giddens ME, Harris DL, Pedlow SE, Dowshen SA & Wasserman RC. Assessing inter-rater reliability (IRR) of Tanner staging and orchidometer use with boys: a study from PROS. Journal of Pediatric Endocrinology and Metabolism 2009 22 291–299. (https://doi.org/10.1515/jpem.2009.22.4.291)

39 Homer N, Kothiya S, Rutter A, Walker BR & Andrew R. Gas chromatography tandem mass spectrometry offers advantages for urinary steroids analysis. Analytical Biochemistry 2017 538 34–37. (https://doi.org/10.1016/j.ab.2017.09.002)

40 Apple F, Bandt C, Prosch A, Erlandsen G, Holmström V, Scholen J & Googins M. Creatinine clearance: enzymatic vs Jaffe determinations of creatinine in plasma and urine. Clinical Chemistry 1986 32 388–390. (https://doi.org/10.1093/clinchem/32.2.388)

41 van Keulen BJ, Dolan CV, Andrew R, Walker BR, Hulshof Pol HE, Boomsla DM, Rotteveel J & Finken MJ. Heritability of testosterone production and metabolism throughout adolescence. Journal of Clinical Endocrinology and Metabolism 2020 105. (https://doi.org/10.1210/clinem/dga0316)

42 Dzintsova VV, Willemsen G, Dolan CV, Hottenga JJ, Martin NG, Slagboom PE, Ordonana JR & Boomsla DM. Establishing a twin register: an inclusive resource for behavior (genetic, epidemiological, biomarker, and ‘omics’ studies. Twin Research and Human Genetics 2018 21 239–252. (https://doi.org/10.1017/thg.2018.23)

43 Muthén LK & Muthén BO. Mplus User’s Guide, 6th ed. Los Angeles, CA, USA: Muthén & Muthén, 2007. (available at: https://www.statmodel.com/download/usersguide/mplus%20Users%20Guide%20v6.pdf)

44 TNO. Vijfde Landelijke Groeistudie. The Hague, Netherlands: TNO, 2010. (available at: https://www.tno.nl/nl/aandachtgebieden/gezond-leven/roadmaps/landelijke-groeistudie-toont-toename-overgewicht-kinderen/)

45 Raven PW & Taylor NF. Sex differences in the human metabolism of cortisol. Endocrine Research 1996 22 751–755. (https://doi.org/10.1089/107435809609043772)

46 Varlinskaya EJ, Vetter-O’Hagen CS & Spear IP. Puberty and gonadal hormones: role in adolescent-typical behavioral alterations. Hormones and Behavior 2013 64 343–349. (https://doi.org/10.1016/j.yhbeh.2012.11.012)

47 Johnstone AM, Faber P, Andrew R, Gibney ER, Elia M, Lobley G, Stubbs RJ & Walker BR. Influence of short-term dietary weight loss on hormones and inflammatory markers. Journal of Parenteral and Enteral Nutrition 2012 36 123–128. (https://doi.org/10.1177/0148607111430270)
loss on cortisol secretion and metabolism in obese men. European Journal of Endocrinology 2004 150 185–194. (https://doi.org/10.1530/eje.0.1500185)

48 Walker BR & Andrew R. Tissue production of cortisol by 11beta-hydroxysteroid dehydrogenase type 1 and metabolic disease. Annals of the New York Academy of Sciences 2006 1083 165–184. (https://doi.org/10.1196/annals.1367.012)

49 Bredella MA. Sex differences in body composition. Advances in Experimental Medicine and Biology 2017 1043 9–27. (https://doi.org/10.1007/978-3-319-70178-3_2)

50 Veldhuis JD, Roemmich JN, Richmond EJ, Rogol AD, Lovejoy JC, Veldhuis JD, Roemmich JN, Richmond EJ, Rogol AD, Lovejoy JC. Sex differences in body composition. Current Opinion in Endocrinology and Metabolism 2009 16 10–15. (https://doi.org/10.1097/MED.0b013e328320d54c)

51 Loomba-Albrecht LA & Styne DM. Effect of puberty on body composition. Current Opinion in Endocrinology, Diabetes, and Obesity 2009 16 10–15. (https://doi.org/10.1097/MED.0b013e328320d54c)

52 Reinehr T, Kulke A, Wolters B, Lass N, Wezel M, Riepe F & Holterhus PM. Steroid hormone profiles in prepubertal obese children before and after weight loss. Journal of Clinical Endocrinology and Metabolism 2013 98 E1022–E1030. (https://doi.org/10.1210/jc.2013-1173)

53 Kumari M, Chandraa T, Brunner E & Kivimaki M. A nonlinear relationship of generalized and central obesity with diurnal cortisol secretion in the Whitehall II study. Journal of Clinical Endocrinology and Metabolism 2010 95 4415–4423. (https://doi.org/10.1210/jc.2009-2105)

54 Rubinow DR, Roca CA, Schmidt PJ, Danaceau MA, Putnam K, Cizza G, Chrousos G & Nieman L. Testosterone suppression of CRH-stimulated cortisol in men. Neuropsychopharmacology 2005 30 1906–1912. (https://doi.org/10.1038/sj.npp.1300742)

55 Wu C, Wei K & Jiang Z. Salpha-reductase activity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Reproductive Biology and Endocrinology 2017 15 21. (https://doi.org/10.1186/s12958-017-0242-9)

56 Torchon LC, Idkowiak J, Fogel NR, O’Neil DM, Shackleton CH, Arlt W & Dunaii A. Evidence for increased Salpha-reductase activity during early childhood in daughters of women with polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism 2016 101 2069–2075. (https://doi.org/10.1210/jc.2015-3926)

57 Barat P, Livingstone DE, Ellerink CM, McDonell CR, Walker BR & Andrew R. Effects of gonadectomy on glucocorticoid metabolism in obese Zucker rats. Endocrinology 2007 148 4836–4843. (https://doi.org/10.1210/en.2007-0597)

58 Lax EK, Rumstadt F, Plassczyk H, Peetz A & Schriefers H. Antagonistic action of estrogens, flutamide, and human growth hormone on androgen-induced changes in the activities of some enzymes of hepatic steroid metabolism in the rat. Endocrinology 1983 113 1043–1055. (https://doi.org/10.1210/end-113-3-1043)

59 Mode A & Norstedt G. Effects of gonadal steroid hormones on the hypothalamo-pituitary-liver axis in the control of sex differences in hepatic steroid metabolism in the rat. Journal of Endocrinology 1982 95 181–187. (https://doi.org/10.1677/joe.0.0950181)

60 Tsuji M, Terada N, Yamamoto H, Takeyama M & Matsumoto K. Hormonal regulation of activities of 4-ene-5 beta and 5 alpha-reductases and 17 beta-ol-dehydrogenase in immature golden hamster ovary. Journal of Steroid Biochemistry 1983 18 777–781. (https://doi.org/10.1016/0022-4731(83)90259-5)

61 Albiston AL, Smith RE & Krozowski ZS. Sex- and tissue-specific regulation of 11 beta-hydroxysteroid dehydrogenase mRNA. Molecular and Cellular Endocrinology 1995 109 183–188. (https://doi.org/10.1016/0303-7207(95)03501-w)

62 Miki K & Sudo A. Effect of urine pH, storage time, and temperature on stability of catecholamines, cortisol, and creatinine. Clinical Chemistry 1998 44 1759–1762. (https://doi.org/10.1093/clinchem/44.8.1759)

63 van Keulen BJ, Dolan CV, Andrew R, Walker BR, Hulshoff Pol HE, Boomsma DI, Rotteveel J & Finken MJJ. Long-term stability of cortisol production and metabolism throughout adolescence: longitudinal twin study. Twin Research and Human Genetics 2020 23 33–38. (https://doi.org/10.1017/thg.2020.6)

64 Ross KM, Murphy MLM, Adam EK, Chen E & Miller GE. How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. Psychoneuroendocrinology 2014 39 184–193. (https://doi.org/10.1016/j.psyneuen.2013.09.016)

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