RESEARCH

Role of glucocorticoid metabolism in childhood obesity-associated hypertension

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Abstract

Objective: Childhood obesity is associated with alterations in hypothalamus–pituitary–adrenal axis activity. We tested the hypothesis that multiple alterations in the metabolism of glucocorticoids are required for the development of hypertension in children who become overweight.

Methods: Spot urine for targeted gas chromatography-mass spectrometry steroid metabolome analysis was collected from (1) overweight/hypertensive children (n = 38), (2) overweight/non-hypertensive children (n = 83), and (3) non-overweight/non-hypertensive children (n = 56).

Results: The mean (± S.D.) age of participants was 10.4 ± 3.4 years, and 53% of them were male. Group 1 and group 2 had higher excretion rates of cortisol and corticosterone metabolites than group 3 (869 (interquartile range: 631–1352) vs 839 (609–1123) vs 608 (439–834) μg/mmol creatinine × m^2 body surface area, $P < 0.01$, for the sum of cortisol metabolites), and group 1 had a higher excretion rate of naive cortisol than group 3. Furthermore, groups differed in cortisol metabolism, in particular in the activities of 11β-hydroxysteroid dehydrogenases, as assessed from the ratio of cortisol:cortisone metabolites (group 2 < group 3), 5α-reductase (group 1 > group 2 or 3), and CYP3A4 activity (group 1 < group 2 or 3).

Discussion: The sequence of events leading to obesity-associated hypertension in children may involve an increase in the production of glucocorticoids, downregulation of 11β-hydroxysteroid dehydrogenase type 1 activity, and upregulation of 5α-reductase activity, along with a decrease in CYP3A4 activity and an increase in bioavailable cortisol.

Key Words
- glucocorticoids
- obesity
- hypertension
- children

Endocrine Connections (2022) **11**, **e220130**

Introduction

Rates of childhood obesity have steeply increased over the past decades (1). Nowadays, an estimated number of 107.7 million children suffer from obesity worldwide (1), and approximately 20% of them progress to hypertension (2, 3, 4). Studies show that obesity and hypertension tend to track from childhood to adulthood (5, 6), which is worrisome.

Glucocorticoids may be involved in the pathophysiology of obesity and obesity-associated hypertension, given their effects on body fat disposition and vascular reactivity. Indeed, childhood obesity has been associated with alterations in hypothalamus–pituitary–adrenal (HPA) axis activity, including increased cortisol

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production and flattening of early-morning peak cortisol (7, 8, 9). Furthermore, in childhood obesity, indices of cortisol production were positively associated with components of the metabolic syndrome, that is, a cluster of metabolic abnormalities characterized by abdominal fat distribution, raised blood pressure, insulin resistance, and dyslipidemia (7, 10).

There is controversy as to whether increased HPA axis activity is a cause or a consequence of childhood obesity. It has been demonstrated that the rise in serum corticosteroids associated with obesity was partly reversible when children managed to lose weight, suggesting that increased HPA axis activity is a consequence rather than a cause of obesity (11). A similar conclusion was drawn from a study of twins who were followed up at ages 9, 12, and 17 years (12). In this study, BMI positively predicted later cortisol metabolite excretion, and not vice versa (12). Conversely, data in adults demonstrated that hair glucocorticoid levels were positively associated with future gains in BMI and waist circumference (13).

The need for glucocorticoids in the tissues is regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes. Cortisol is generated from inert cortisone by 11β-HSD type 1 in the liver and adipose tissue, while the reverse reaction is catalyzed by the type 2 isozyme in the kidney (14). Only few studies have investigated whether childhood obesity-associated hypertension is accompanied by alterations in 11β-HSDs. A small study (of $n = 41$) found that children with obesity and hypertension had a higher ratio of (tetrahydrocortisol + allo-tetrahydrocortisol)/tetrahydrocortisone in urine than children with obesity but without hypertension and controls with a normal weight (15), suggesting that the cortisol–cortisone shuttle favors cortisol in childhood obesity-associated hypertension. However, other studies on this topic did not include a normal-weight control group or lacked information on metabolic syndrome components (10, 16, 17).

In addition to alterations in the metabolism of glucocorticoids, it could be possible that rate-limiting steps in their synthesis contribute to the development of hypertension in children with obesity. Patients with 11β-hydroxylase deficiency congenital adrenal hyperplasia become hypertensive due to the accumulation of 11-deoxycorticosterone, which has a strong affinity for the mineralocorticoid receptor (18, 19). Likewise, mineralocorticoid excess is also apparent in 17α-hydroxylase deficiency congenital adrenal hyperplasia. To our knowledge, there are no reports that have described the alterations in the activities of 11β-hydroxylase or 17α-hydroxylase in children with obesity.

With the present study, we aimed to test the hypothesis that the development of obesity-associated hypertension requires various concerted steps that could result in an increased availability of bioactive glucocorticoids. Therefore, in the present study using targeted gas chromatography-mass spectrometry (GC-MS) steroid metabolome analysis, we compared urinary corticosteroid profiles (enabling assessment of the activities of various enzymes involved in the metabolism and synthesis of glucocorticoids, including 11β-HSDs, A-ring reductases, CYP3A4, 20α-HSD, 11β-hydroxylase, and 17α-hydroxylase) from children with overweight, with or without hypertension, to those of controls without overweight or hypertension.

Materials and methods

Population

A convenience sample of 5–17-year olds was recruited for the study of glucocorticoid parameters, as assessed from urine and/or saliva, in childhood obesity-associated hypertension. Children with overweight or obesity were recruited at a pediatric outpatient obesity clinic, and normal-weight controls of the same age were recruited at two schools and at a general pediatric outpatient clinic, as described previously (8). Children with conditions known to affect blood pressure were not eligible for inclusion.

The study was approved by the medical ethics committee of VU University Medical Center (protocol number 2015.121). Written informed consent was obtained from at least one parent and from all children aged 12 years or older.

Study protocol

Height was measured to 0.1 cm accuracy by trained personnel using a fixed stadiometer, according to standardized procedures. Weight was measured to the nearest 0.1 kg on a balance scale, while wearing light clothing. After 5 min of rest in sitting position, blood pressure was measured three times consecutively using an oscillometric device. The cuff size was adjusted to the upper arm circumference, according to the guidelines of the National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents (20). Based on the lowest of three consecutive blood pressure measurements, hypertension was defined as

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a value greater than or equal to the 95th percentile for age, sex, and height. Spot urine was collected at the study site at a time between the end of the morning and the beginning of the afternoon, which was not the first urine output.

BMI was converted to SD score (SDS) using an LMS method, based on World Health Organization reference data (21). BMI status was determined based on the International Obesity Task Force criteria (22). Blood pressure SDSs were calculated using the equations provided by the NHBPEP Working Group (20).

Laboratory analysis

Urine samples were stored at −80°C and thawed only once just before analysis. Steroids in urine samples were analyzed using quantitative data generated by targeted GC-MS analysis, as described previously (10, 23, 24). In brief, free and conjugated urinary steroids were extracted by solid-phase extraction, and conjugates were enzymatically hydrolyzed. After recovery of hydrolyzed steroids by solid-phase extraction, known amounts of internal standards (5α-androstane-3α,17α-diol, stigmasterol) were added to each extract before the formation of methyloxime-trimethylsilyl ethers. GC was performed using an Optima-1 fused silica column (Macherey-Nagel, Dueren, Germany) housed in an Agilent Technologies 6890 series GC that was directly interfaced to an Agilent Technologies 5975 inert XL mass selective detector. Quantification was performed in the selected ion monitoring mode.

Statistical analysis

We calculated excretion rates and enzyme activities based on the measured steroid levels. The excretion rates were subsequently divided by the product of urine creatinine concentration (mmol/L) and body surface area (according to the Mosteller formula (25)), to account for inter-individual differences in urine production and body size, respectively.

Non-normally distributed variables were 10log-transformed prior to analysis. Based on evidence demonstrating that the sex difference in A-ring reductase activity, as observed in young adulthood (26), emerged from an age of approximately 11 years (27), groups were compared using linear regression analysis adjusted for sex and age (< or ≥11 years). Next, a sex × age (< or ≥11 years) interaction term was added to the model. Statistical significance was defined as a P value ≤0.05. For interaction models, a P value ≤0.10 was deemed significant.

Results

Characteristics

Of a total of 270 children (8), 177 (of whom 38 were overweight and hypertensive, 83 were overweight and non-hypertensive, and 56 were non-overweight and non-hypertensive) provided urine samples for targeted GC-MS steroid metabolome analysis. The mean (±S.D.) age of participants in this specific study was 10.4 ± 3.4 years, and 53% of them were male, similar to the entire sample (8). Table 1 presents their characteristics by group, showing differences in sex distribution, and, by definition, in BMI and/or blood pressure.

Associations

Table 2 presents the outcomes by group. Boys and girls did not differ in outcomes (data not shown). Moreover, findings from tests of sex × age interaction were inconsistent (i.e. of 36 comparisons, only 4 had a P value ≤0.10), so that there is no need to stratify analyses.

Overweight/hypertensive vs overweight/non-hypertensive

The overweight/hypertensive group excreted more 5α-reduced metabolites (in absolute terms as well as relative to the excretion of 5β-reduced metabolites) than the overweight/non-hypertensive group. CYP3A4 activity was lower in the overweight/hypertensive group than in the overweight/non-hypertensive group.

Overweight/hypertensive vs non-overweight/non-hypertensive

The overweight/hypertensive group had higher excretion rates of naive cortisol, cortisol metabolites, corticosterone metabolites, and 5α-reduced metabolites than the non-overweight/non-hypertensive group. In the overweight/hypertensive group, as compared to the non-overweight/non-hypertensive group, 11β-hydroxylase activity was higher and CYP3A4 activity was lower.

Overweight/non-hypertensive vs non-overweight/non-hypertensive

The overweight/non-hypertensive group had higher excretion rates of cortisol metabolites and corticosterone metabolites than the non-overweight group. The

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Table 1 Characteristics of participants by weight and blood pressure status.

Characteristic	Groups			*P* value[a]		
	Overweight and hypertensive (1)	Overweight and non-hypertensive (2)	Non-overweight and non-hypertensive (3)	1 vs 2	1 vs 3	2 vs 3
N/males (%)	38/15 (40%)	83/41 (49%)	56/38 (68%)	0.31	0.006	0.03
Age (years)	9.9 ± 3.8	10.7 ± 3.4	10.3 ± 3.2	0.25	0.59	0.48
BMI (kg/m^2)	27.7 ± 7.6	27.3 ± 5.2	17.1 ± 2.3	0.72	<0.001	<0.001
BMI (SD score)	2.8 ± 0.7	2.6 ± 0.9	−0.2 ± 1.0	0.40	<0.001	<0.001
Severity of obesity				0.43	–	–
Overweight (%)	9 (24%)	23 (28%)	–			
Obese (%)	12 (32%)	33 (40%)	–			
Morbidly obese (%)	17 (45%)	27 (33%)	–			
Mean systolic blood pressure (SD score)	2.0 ± 0.5	0.4 ± 0.9	0.2 ± 0.8	<0.001	<0.001	0.15
Mean diastolic blood pressure (SD score)	1.2 ± 0.8	0.3 ± 0.6	−0.1 ± 0.7	<0.001	<0.001	0.001

Values represent *N* (%) or mean ± SD.
[a]Groups were compared by the independent samples *t*-test or the chi-square test, as appropriate.

overweight/non-hypertensive group, as compared to the non-overweight group, had a lower ratio of cortisol to cortisone metabolites, higher 20α-HSD activity, and lower 17α-hydroxylase activity.

Discussion

Our study provided evidence for a model in the pathophysiology of childhood obesity-associated hypertension that includes increased production and impaired clearance of glucocorticoids.

Childhood obesity, analogous to adult obesity, may be accompanied by metabolic perturbations that include insulin resistance and dyslipidemia in addition to hypertension. In childhood obesity, increased insulin resistance over time predicted higher blood pressure (28), implying that the overweight/hypertensive group might be more insulin-resistant than the overweight/non-hypertensive group. Previous studies addressing the impact of childhood obesity on indices of HPA axis activity either did not include a normal-weight control group or lacked information on metabolic syndrome components (10, 16, 17). Therefore, these studies were unable to make inferences about the role of HPA axis dysregulation in childhood obesity-associated metabolic perturbation.

We showed that childhood obesity was associated with an increase in the excretion rate of glucocorticoids. Those obese children who were hypertensive had, analogous to obese children who fulfilled the International Diabetes Federation definition of the metabolic syndrome, a higher excretion rate of naive cortisol (7), which could most likely be attributed to increased cortisone-to-cortisol conversion by 11β-HSDs and lower CYP3A4 activity.

In our study, the overweight/non-hypertensive group, as compared to the non-overweight/non-hypertensive group, had evidence of a cortisol-cortisone shuttle that favors cortisone. A previous study in the same sample demonstrated that the cortisol/cortisone ratio in spot urine, which provides an estimate of renal 11β-HSD type 2 activity, did not differ between these groups (8). Therefore, our findings, along with data demonstrating that weight loss increased the expression and activity of 11β-HSD type 1 in omental cells of adults with obesity (29), lend support to previous observations showing that childhood obesity, in particular in the presence of insulin resistance (as assessed from fasting glucose and insulin levels), reduces 11β-HSD type 1 activity (16, 30). More detailed studies in middle-aged adults with obesity linked deterioration of glucose tolerance during 4 years of follow-up to lower 11β-HSD type 1 expression in subcutaneous-tissue biopsies (31). This might represent a compensatory mechanism to decrease local glucocorticoid exposure in the face of an emerging adverse metabolic phenotype.

We found that the overweight/hypertensive group had higher 5α-reductase activity than the overweight/non-hypertensive group. A study among adults with obesity showed that weight loss decreased interstitial fluid glycerol concentrations during a hyperinsulinemic–euglycemic clamp, suggestive of insulin sensitization, along with decreases in 5α-reductase activity and cortisol production (32). These as well as other data (30, 33) suggest that insulin resistance rather than obesity *per se* upregulates 5α-reductase activity, leading to decreased central feedback suppression, and, consequently, increased cortisol production.

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Table 2 Outcomes by weight and blood pressure status.

Outcome	Calculation	Groups			*P* value[a]		
		Overweight and hypertensive (1)	Overweight and non-hypertensive (2)	Non-overweight and non-hypertensive (3)	1 vs 2	1 vs 3	2 vs 3
Excretion rates (in μg/mmol × m^2)							
Cortisol	F/ creatinine × BSA	7.6 (5.3–12.2)	6.9 (4.9–9.6)	6.3 (4.5–8.2)	0.11	0.03	0.54
Sum of cortisol metabolites	(F + THF + a-THF + THE + α-C + β-C + α-Cl + β-Cl + 6β-OH-F + 20α-DHF)/creatinine × BSA	869 (631–1352)	839 (609–1123)	608 (439–834)	0.27	0.001	0.007
Sum of corticosterone metabolites	(THA + a-THB + THB)/creatinine × BSA	72 (48–137)	69 (51–124)	51 (36–77)	0.99	0.008	0.002
Sum of 5α-reduced metabolites	(An + 11-OH-An + a-THF + a-THB + Po-5α,3α)/ creatinine × BSA	329 (218–407)	252 (178–326)	205 (142–286)	0.04	0.009	0.27
Sum of 5β-reduced metabolites	(Et + 11-OH-Et + THF + THB + Po-5β,3α)/ creatinine × BSA	176 (133–278)	196 (142–251)	169 (118–222)	0.89	0.17	0.14
Enzyme activities							
Global 11β-HSD activity	(F + THF + a-THF + α-C + β-C + 6β-OH-F + 20α-DHF)/(THE + α-Cl + β-Cl)	0.56 (0.44–0.67)	0.47 (0.42–0.59)	0.60 (0.49–0.75)	0.13	0.16	<0.001
Relative 5α-/5β-reductase activity	(An + 11-OH-An + a-THF + a-THB + Po-5α,3α)/ (Et + 11-OH-Et + THF + THB + Po-5β,3α)	1.56 (1.18–2.17)	1.23 (1.02–1.56)	1.47 (1.01–1.84)	0.004	0.06	0.69
CYP3A4 activity	6β-OH-F/ F	2.13 (1.65–2.72)	2.46 (1.95–3.13)	2.43 (1.96–3.04)	0.05	0.02[b]	0.49
20α-HSD activity	20α-DHF/ F	0.57 (0.47–0.73)	0.59 (0.49–0.72)	0.53 (0.47–0.61)	0.69	0.08	0.001[b]
11β-hydroxylase activity	(THF + a-THF)/ THS	27.5 (21.5–37.1)	25.1 (17.5–33.2)	23.4 (15.3–31.6)	0.16[b]	0.005	0.13
Global 17-hydroxylase/17,20-lyase activity	(THA + THB + a-THB)/ (An + Et)	0.72 (0.44–1.62)	0.86 (0.34–1.86)	0.61 (0.29–2.05)	0.62	0.52[b]	0.28
Global 17α-hydroxylase activity	(THA + THB + a-THB)/ (a-THF + THF + THE)	0.13 (0.09–0.20)	0.14 (0.11–0.18)	0.12 (0.10–0.17)	0.14	0.83	0.05

Values represent median (interquartile range).

[a]After 10log-tranfsormation, groups were compared by linear regression analysis adjusted for sex and age. [b]*P* value for sex × age interaction ≤0.10.

An, androsterone; a-THB, allo-tetrahydrocorticosterone; a-THF, allo-tetrahydrocortisol; BSA, body surface area; Et, etiocholanolone; F, cortisol; THA, 5b-Pregnane-3a,21-diol-11,20-dione; THB, tetrahydrocorticosterone; THE, tetrahydrocortisone; THF, tetrahydrocortisol; Po-5α,3α, 5a-Pregnane-3a,17a-diol-20-one; Po-5β,3α, 5b-Pregnane-3a,17a-diol-20-one; 6β-OH-F, 6β-hydroxycortisol; 11-OH-An, 11-hydroxyandrosterone; 11-OH-Et, 11-hydroxyetiocholanolone; 20α-DHF, 4-Pregnene-11b,17a,20a,21-tetrol-3-one; α-C, α-cortol; α-Cl, α-cortolone; β-C, β-cortol; β-Cl, β-cortolone.

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We made a couple of other observations. The overweight/non-hypertensive group had slightly lower 17α-hydroxylase activity than the non-overweight/non-hypertensive group, which is expected to have a limited clinical impact but contrasts with our observation that glucocorticoid production is increased in overweight children. Furthermore, the overweight/hypertensive group had higher 11β-hydroxylase activity than the non-overweight/non-hypertensive group, which could likely be attributed to greater glucocorticoid production.

Our study has several strengths and limitations. The major strength of our study was the inclusion of three study groups, enabling us to unravel the relative contributions of obesity and hypertension on selected outcomes. Another strength of our study was the use of GC-MS steroid metabolome analysis. Our study also has its limitations. First, a diagnosis of hypertension was based on blood pressure measurements obtained on only one occasion, instead of the gold standard 24-h ambulatory blood pressure monitoring, which might have caused misclassification. Nonetheless, to avoid stress and anxiety falsely elevating blood pressure readings, only the lowest of three measurements was used after 5 min of rest (34). Secondly, our study was based on a convenience sample of children, resulting in lacking information on important factors, such as socio-economic status, ethnicity, health status, pubertal stage, and metabolism, for example, an estimate of body composition or insulin sensitivity, and heterogeneity in age and sex distribution (although statistically controlled for). Thirdly, spot urine was collected in our study, in contrast to previous studies on children collecting 24-h urine samples (7, 10, 16). Although 24-h urine sampling seems superior to spot urine, it has been estimated that approximately half of 24-h urine sample collections are inaccurate (35). In our study, the timing of urine sampling was rather fixed, and the excretion rates were corrected for creatinine output.

In summary, based on our and previous data we speculate that the sequence of events leading to obesity-associated hypertension in children follows a certain route, involving (i) an increase in the production of glucocorticoids, (ii) downregulation of 11β-HSD type 1 activity, probably in an early attempt to preserve insulin sensitivity (although more detailed studies are necessary to link tissue-specific 11β-HSD type 1 expression to metabolic disease progression in childhood obesity), and (iii) upregulation of 5α-reductase activity, probably due to worsening of insulin resistance, along with a decrease in CYP3A4 activity and an increase in bioavailable cortisol.

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.

Funding

This work was supported by the Dutch Kidney Foundation (VR12.03).

References

1 NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet* 2017 **390** 2627–2642. (https://doi.org/10.1016/S0140-6736(17)32129-3)

2 Sorof JM, Lai D, Turner J, Poffenbarger T & Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics* 2004 **113** 475–482. (https://doi.org/10.1542/peds.113.3.475)

3 Maldonado J, Pereira T, Fernandes R & Carvalho M. Blood pressure distribution of a sample of healthy Portuguese children and adolescents: the AVELEIRA registry. *Revista Portuguesa de Cardiologia* 2009 **28** 1233–1244.

4 Polat M, Yikilkan H, Aypak C & Gorpelioglu S. The relationship between BMI and blood pressure in children aged 7–12 years in Ankara, Turkey. *Public Health Nutrition* 2014 **17** 2419–2424. (https://doi.org/10.1017/S1368980014000846)

5 Singh AS, Mulder C, Twisk JW, van Mechelen W & Chinapaw MJ. Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obesity Reviews* 2008 **9** 474–488. (https://doi.org/10.1111/j.1467-789X.2008.00475.x)

6 Chen X & Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. *Circulation* 2008 **117** 3171–3180. (https://doi.org/10.1161/CIRCULATIONAHA.107.730366)

7 Reinehr T, Kulle A, Wolters B, Knop C, Lass N, Welzel M & Holterhus PM. Relationships between 24-hour urinary free cortisol concentrations and metabolic syndrome in obese children. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 2391–2399. (https://doi.org/10.1210/jc.2013-4398)

8 Wirix AJ, Finken MJ, von Rosenstiel-Jadoul IA, Heijboer AC, Nauta J, Groothoff JW, Chinapaw MJ & Kist-van Holthe JE. Is there an association between cortisol and hypertension in overweight or obese children? *Journal of Clinical Research in Pediatric Endocrinology* 2017 **9** 344–349. (https://doi.org/10.4274/jcrpe.4802)

9 Noppe G, van den Akker EL, de Rijke YB, Koper JW, Jaddoe VW & van Rossum EF. Long-term glucocorticoid concentrations as a risk factor for childhood obesity and adverse body-fat distribution. *International Journal of Obesity* 2016 **40** 1503–1509. (https://doi.org/10.1038/ijo.2016.113)

10 Gawlik A, Shmoish M, Hartmann MF, Malecka-Tendera E, Wudy SA & Hochberg Z. Steroid metabolomic disease signature of nonsyndromic childhood obesity. *Journal of Clinical Endocrinology and Metabolism* 2016 **101** 4329–4337. (https://doi.org/10.1210/jc.2016-1754)

11 Reinehr T, Kulle A, Wolters B, Lass N, Welzel 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)

12 Van Keulen BJ, Dolan CV, Andrew R, Walker BR, Hulshoff Pol HE, Boomsma DI, Rotteveel J & Finken MJJ. Exploring the temporal relation between body mass index and corticosteroid metabolite excretion in childhood. *Nutrients* 2020 **12** 1525. (https://doi.org/10.3390/nu12051525)

13 van der Valk ES, van der Voorn B, Iyer AM, Mohseni M, Leenen PJM, Dik WA, van den Berg SAA, de Rijke YB, van den Akker ELT, Penninx BWJH, *et al.* Hair cortisol, obesity and the immune system: results from a 3 year longitudinal study. *Psychoneuroendocrinology* 2021 **134** 105422. (https://doi.org/10.1016/j.psyneuen.2021.105422)

14 Seckl JR & Walker BR. Minireview: 11beta-hydroxysteroid dehydrogenase type 1 – a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 2001 **142** 1371–1376. (https://doi.org/10.1210/endo.142.4.8114)

15 Csabi GY, Juricskay S & Molnar D. Urinary cortisol to cortisone metabolites in hypertensive obese children. *Journal of Endocrinological Investigation* 2000 **23** 435–439. (https://doi.org/10.1007/BF03343752)

16 Wiegand S, Richardt A, Remer T, Wudy SA, Tomlinson JW, Hughes B, Gruters A, Stewart PM, Strasburger CJ & Quinkler M. Reduced 11beta-hydroxysteroid dehydrogenase type 1 activity in obese boys. *European Journal of Endocrinology* 2007 **157** 319–324. (https://doi.org/10.1530/EJE-07-0193)

17 Paize F, Isherwood D, Honour J & Jones CA. Abnormal urinary steroid profiles in four hypertensive obese children. *Journal of Pediatric Endocrinology and Metabolism* 2010 **23** 237–244. (https://doi.org/10.1515/jpem.2010.23.3.237)

18 Bongiovanni AM & Eberlein WR. Plasma and urinary corticosteroids in the hypertensive form of congenital adrenal hyperplasia. *Journal of Biological Chemistry* 1956 **223** 85–94. (https://doi.org/10.1016/S0021-9258(18)65119-1)

19 New MI & Levine LS. Congenital adrenal hyperplasia. *Advances in Human Genetics* 1973 **4** 251–326. (https://doi.org/10.1007/978-1-4615-8261-8_4)

20 National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004 **114** (2 Supplement 4th Report) 555–576.

21 de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C & Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bulletin of the World Health Organization* 2007 **85** 660–667. (https://doi.org/10.2471/blt.07.043497)

22 Cole TJ, Bellizzi MC, Flegal KM & Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000 **320** 1240–1243. (https://doi.org/10.1136/bmj.320.7244.1240)

23 Remer T, Boye KR, Hartmann MF & Wudy SA. Urinary markers of adrenarche: reference values in healthy subjects, aged 3-18 years. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 2015–2021. (https://doi.org/10.1210/jc.2004-1571)

24 Wudy SA, Schuler G, Sanchez-Guijo A & Hartmann MF. The art of measuring steroids: principles and practice of current hormonal steroid analysis. *Journal of Steroid Biochemistry and Molecular Biology* 2018 **179** 88–103. (https://doi.org/10.1016/j.jsbmb.2017.09.003)

25 Mosteller RD. Simplified calculation of body-surface area. *New England Journal of Medicine* 1987 **317** 1098. (https://doi.org/10.1056/NEJM198710223171717)

26 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/jcem.84.9.6009)

27 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)

28 de Giorgis T, Marcovecchio ML, Giannini C, Chiavaroli V, Chiarelli F & Mohn A. Blood pressure from childhood to adolescence in obese youths in relation to insulin resistance and asymmetric dimethylarginine. *Journal of Endocrinological Investigation* 2016 **39** 169–176. (https://doi.org/10.1007/s40618-015-0351-2)

29 Tomlinson JW, Moore JS, Clark PM, Holder G, Shakespeare L & Stewart PM. Weight loss increases 11beta-hydroxysteroid dehydrogenase type 1 expression in human adipose tissue. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2711–2716. (https://doi.org/10.1210/jc.2003-031376)

30 Gawlik AM, Shmoish M, Hartmann MF, Wudy SA & Hochberg Z. Steroid metabolomic signature of insulin resistance in childhood obesity. *Diabetes Care* 2020 **43** 405–410. (https://doi.org/10.2337/dc19-1189)

31 Crowley RK, Woods CP, Hughes BA, Gray J, McCarthy T, Taylor AE, Gathercole LL, Shackleton CHL, Crabtree N, Arlt W, *et al.* Increased central adiposity and decreased subcutaneous adipose tissue 11beta-hydroxysteroid dehydrogenase type 1 are associated with deterioration in glucose tolerance – a longitudinal cohort study. *Clinical Endocrinology* 2019 **91** 72–81. (https://doi.org/10.1111/cen.13939)

32 Tomlinson JW, Finney J, Hughes BA, Hughes SV & Stewart PM. Reduced glucocorticoid production rate, decreased 5alpha-reductase activity, and adipose tissue insulin sensitization after weight loss. *Diabetes* 2008 **57** 1536–1543. (https://doi.org/10.2337/db08-0094)

33 Wu C, Wei K & Jiang Z. 5Alpha-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)

34 Parker ED, Kharbanda EO & Sinaiko AR. Are we measuring blood pressure correctly in children, particularly in obesity? *Archives of Disease in Childhood* 2016 **101** 990–991. (https://doi.org/10.1136/archdischild-2016-310682)

35 Boyd C, Wood K, Whitaker D, Ashorobi O, Harvey L, Oster R, Holmes RP & Assimos DG. Accuracy in 24-hour urine Collection at a Tertiary Center. *Reviews in Urology* 2018 **20** 119–124. (https://doi.org/10.3909/riu0807)

Received in final form 24 May 2022
Accepted 13 June 2022
Accepted Manuscript published online 14 June 2022