The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. 106, No. 9, e3400–e3413
doi:10.1210/clinem/dgab379
Clinical Research Article

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Associations of Hair Cortisol Concentrations With Cardiometabolic Risk Factors in Childhood

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Abbreviations: BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; OR, odds ratio; SDS, SD score.

Received: 29 January 2021; Editorial Decision: 24 May 2021; First Published Online: 29 May 2021; Corrected and Typeset: 28 June 2021.

Abstract

Context: Biological stress is related to cardiovascular disease in adults. The associations of stress with cardiovascular and metabolic diseases may originate in childhood.
Objective: This work aims to examine the associations of hair cortisol concentrations at age 6 years with cardiometabolic risk factors at ages 6 and 10 years.
Methods: Cortisol concentrations were measured in hair of 6-year-old children (n = 2598) participating in the Generation R Study, a population-based prospective cohort study in Rotterdam, the Netherlands. Main outcome measures included blood pressure, heart rate, concentrations of insulin, glucose, lipids, and C-reactive protein in blood at ages 6 and 10 years.
Results: Higher hair cortisol concentrations at age 6 years were associated with higher systolic blood pressure at age 10 years (difference 0.17 SD score; 95% CI, 0.03-0.31). The association attenuated into nonsignificance after adjustment for childhood body mass index (BMI) at age 6 years. Higher hair cortisol concentrations at age 6 years were associated with an increase in total and low-density lipoprotein cholesterol between ages 6 and 10 years but not with those measurements at age 6 or 10 years. Hair cortisol concentrations were not associated with other cardiometabolic risk factors at age 6 or 10 years.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Conclusion: Hair cortisol concentrations were not independent of BMI associated with cardiometabolic risk factors at 6 or 10 years. The associations of biological stress with cardiometabolic risk factors may develop at later ages.

Key Words: hair cortisol, child, cardiometabolic risk, blood pressure, heart rate, lipids, cholesterol, insulin, glucose, C-reactive protein

Stress is associated with cardiometabolic disease in adults (1, 2). Results from a study among 136 637 individuals showed that stress-related disorders were robustly associated with multiple types of cardiovascular disease, such as hypertensive diseases and heart failure (3). Similarly, a prospective cohort study, among 10 308 men and women, reported that those with chronic work stress were twice as likely to develop metabolic syndrome (4). It has also been suggested that long-term exposure to elevated cortisol concentrations may lead to long-term physiological alterations compromising the anatomy and function of the cardiovascular and metabolic systems (5, 6). Cortisol concentrations measured in saliva, serum, and urine are subject to situational and intraindividual fluctuations (7). Hair cortisol concentrations reflect long-term cumulative cortisol concentrations and are therefore a useful biomarker of long-term systemic cortisol exposure, which is mainly determined by hypothalamic-pituitary-adrenal-axis activity (7, 8). A recent review among 11 cross-sectional studies shows positive associations of hair cortisol with adverse cardiometabolic outcomes including higher systolic blood pressure, diabetes, metabolic syndrome, and adiposity (1). In addition, hair cortisol concentrations have been shown to be associated with an increased risk of having cardiovascular diseases, such as coronary heart disease, stroke, and peripheral arterial disease, in elderly (9). The associations of chronic stress with adverse cardiometabolic outcomes may originate in early life. It is well known that adverse exposures in early life are associated with cardiovascular risk factors development from childhood onward (10-12). Also, cardiometabolic risk factors tend to track from childhood into adulthood (13-16). We previously reported associations of hair cortisol concentrations with childhood body mass index (BMI) and fat mass distribution at ages 6 and 10 years (17, 18). Thus far, studies in children have not reported associations between hair cortisol concentrations and cardiometabolic risk factors (19-21). These previous studies had small sample sizes and, mostly, a cross-sectional design.

We hypothesized that chronic exposure to higher cortisol concentrations is associated with an adverse cardiometabolic risk profile already in school-aged children, and thereby predispose individuals to later-life cardiovascular disease. We examined, in a population-based prospective cohort study among 2598 children, the associations of hair cortisol concentrations at age 6 years with blood pressure, heart rate, lipid profile, glucose metabolism, and C-reactive protein concentrations at ages 6 and 10 years, and explored the potential mediating role of childhood BMI.

Materials and Methods

Study Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward in Rotterdam, the Netherlands (22). Written informed consent was provided for all children. The medical ethics committee of Erasmus MC approved the study (MEC 198.782/2001/31). This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. In total, 2984 children had information on hair cortisol concentrations at age 6 years. Twins (N = 58) and children without any measurement of cardiometabolic risk factors at ages 6 and 10 years (N = 23) were excluded. Since the highest values of hair cortisol concentrations may be incorrect because of external factors such as glucocorticoid use, we excluded the extreme values of cortisol (N = 305) using the Tukey definition of outliers (Q1-1.5 * interquartile range [IQR] and Q3 + 1.5 * IQR) (23). There were no substantial differences in the cardiometabolic risk factors between the population of analysis and the excluded outliers (data not shown). The population for analysis consisted of 2598 children. The same selection procedure was followed for the cortisone analyses (N = 2605). The flowchart of participants is given in Supplementary Fig. 1 (24).

Hair Cortisol Concentration Measurements

As described previously, in children aged 6 years, a hair strand of approximately 100 hairs was cut from the posterior vertex using small surgical scissors, as close to the scalp as possible (25). Details on collection, sample preparation, extraction, and analysis using the liquid chromatography–tandem mass

spectrometry method are provided in the Supplementary Methods (24). To reduce variability and account for right skewedness of the distribution, cortisol and cortisone concentrations outliers defined by the Tukey definition of outliers (Q1-1.5 * IQR and Q3 + 1.5 * IQR) were excluded, after which values were either divided in quintiles, or natural log-transformed and further standardized by the IQR to ease the interpretation of effect sizes (23).

Cardiometabolic Risk Factors

Outcome assessments were performed at ages 6 and 10 years (22). Blood pressure and heart rate were measured at the right brachial artery 4 times with 1-minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus (26). We calculated the mean value for systolic and diastolic blood pressure and heart rate using the last 3 measurements for each participant. Thirty-minute fasting venous blood samples were collected to measure serum concentrations of insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, C-reactive protein at ages 6 and 10 years, and glucose only at age 10 years (22). Because the blood samples were collected at different time points during the day, it was not possible to have fasting samples. Participants were asked to stop eating and drinking 30 minutes before the blood draw. Unfortunately, we do not have information on the exact time between the last meal and samples, nor on the nutrient composition of the meals. Glucose, total cholesterol, HDL cholesterol, triglycerides, and C-reactive protein concentrations were measured using the c702 module on the Cobas 8000 analyzer. Insulin was measured by electrochemiluminescence immunoassay on the e601 module (Roche) (27). Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula (28, 29). We defined children with a clustering of cardiometabolic risk factors as being at risk for the metabolic syndrome phenotype, in line with other studies (30, 31). Clustering of cardiometabolic risk factors was defined as having 3 or more out of the following 4 adverse risk factors: android fat mass percentage above the 75th percentile; systolic or diastolic blood pressure above the 75th percentile; HDL cholesterol below the 25th percentile or triglycerides above the 75th percentile; and insulin above the 75th percentile of our study population. We measured total body fat mass and fat mass in the abdomen (android fat mass) using a dual-energy x-ray absorptiometry scanner (iDXA, GE140 Lunar, 2008, enCORE software v.12.6) according to standard procedures (32). We calculated android fat mass percentage as android fat mass divided by total body fat mass. For the clustering of cardiometabolic risk factors at age 10 years, we also had visceral fat mass obtained by magnetic resonance imaging scans available, as described previously (22). Because the distribution of insulin and triglycerides concentrations was skewed, we used their natural logged values. Since C-reactive protein was not normally distributed and transformation did not yield an acceptable distribution, we categorized C-reactive protein concentrations into less than 3 mg/L (normal levels) or greater than or equal to 3 mg/L (high levels) in line with previous studies (33, 34). To enable comparison of effect sizes of different measures, we constructed SDS ([observed value – mean] / SD) for all variables.

Covariates

Information on child sex was obtained from midwife/obstetric records. Maternal height was assessed at the first visit. Information about maternal weight just before pregnancy was obtained by questionnaire. Maternal prepregnancy BMI was calculated as weight in kilograms divided by height in meters squared. Information on maternal education, family income, child ethnicity, and television watching time was obtained by questionnaires. Hair color was partially coded through parent report and was completed by 2 raters using photographs made at the research center. We calculated BMI at age 6 years from height and weight, both measured without shoes and heavy clothing. Parents completed a questionnaire about their child on factors that can potentially influence hair cortisol concentrations, such as hair washing frequency, time since last wash, hair product use, and use of and administration route of glucocorticoid medications at age 6 years. We tested whether birth weight was a confounder in the associations of hair cortisol concentrations and cardiometabolic risk factors but birth weight did not change the effect estimates more than 10% and thus was not included in the final confounder model.

Statistical Analysis

First, we examined differences in participant characteristics between hair cortisol concentration quintiles with analysis of variance tests for continuous variables and chi-squared tests for categorical variables. For nonresponse analyses, we compared participants and nonparticipants using chi-square tests, *t* tests, and Mann-Whitney tests. Second, we used linear regression models to assess the associations of hair cortisol concentrations at age 6 years in quintiles with cardiometabolic risk factors at ages 6 and 10 years, and the change in cardiometabolic risk factor SD scores between these ages (systolic blood pressure, diastolic blood pressure, heart rate, total cholesterol, HDL and LDL cholesterol, triglycerides, insulin, glucose). Third, we used logistic regression models to assess the

associations of hair cortisol concentrations at age 6 years in quintiles with the odds of increased C-reactive protein concentrations (≥ 3 mg/L) and the odds of having clustered cardiometabolic risk factors at ages 6 and 10 years. Only cases with complete data on cardiometabolic outcomes were used for the analyses with clustered cardiometabolic risk factors. Tests for trend across quintiles were performed by analyzing cortisol quintiles as a continuous variable. Fourth, we performed linear regression models to assess the associations of continuous hair cortisol concentrations (the natural log-transformed hair cortisol measures further standardized with IQR) with all cardiometabolic outcomes. The basic models were adjusted for child sex, age at cortisol measurement, and age at assessment of cardiometabolic outcomes. The confounder models were additionally adjusted for maternal prepregnancy BMI, maternal education, family income, child ethnicity, hair color, and average duration of television watching per day. We performed an additional model to assess whether any significant association in the confounder model was explained by childhood BMI. We visualized potential covariates by drawing a directed acyclic graph and included the covariates in the models that were associated with exposure and outcome at age 6 years and changed the effect estimates more than 10% (Supplementary Fig. 2) (24). We tested if there was an interaction of cortisol with sex by adding an interaction term to the basic model. After taking multiple testing into account, the interaction was only significant for insulin and triglyceride concentrations at 6 years ($P < .01$). For these associations, we performed sex-stratified analyses. As a sensitivity analysis, we only included children without any glucocorticoid use in the 3 months prior to the hair sample collection (N = 2296). Also, we repeated all analyses for cortisone, the less active form of cortisol (N = 2605). Considering 3 groups of outcomes (blood pressure and heart rate, lipids, and glucose metabolism), multiple testing adjustment would lead to a P value cutoff of less than .017. We depicted both significance levels (.05 and .017) in the tables and figures. Missing data of covariates were multiple-imputed using a Markov chain Monte Carlo approach (35). Five imputed data sets were created and analyzed together. All statistical analyses were performed using the Statistical package of Social Sciences version 24.0 for Windows (IBM Corp, released 2016; IBM SPSS Statistics for Windows, version 24.0).

Results

Participant Characteristics

As compared to children in the lower cortisol quintiles, children in the upper cortisol quintiles more often had a mother who had a higher prepregnancy BMI, was lower educated, and had a lower family income. Also, these children more often had non-European ethnicity, a higher BMI and systolic blood pressure at ages 6 and 10 years, brown or black hair color, and a higher average duration of television watching at age 6 years (Tables 1 and 2) (24). Nonresponse analyses showed that, compared to mothers of participants, mothers of nonparticipants more often had a higher BMI, a lower family income, and lower education. Nonparticipants more often were boys, had non-European ethnicity, a higher BMI, brown or dark hair, watched more television, and used more often glucocorticoids in the 3 months prior to hair sampling (Supplementary Table 2) (24).

Cardiovascular Risk Factors

Results from the basic models showed that, as compared to the lowest quintile of hair cortisol concentrations at age 6 years, children in the highest quintile had a higher systolic and diastolic blood pressure and heart rate at age 6 years (see Supplementary Table 2) (24). When we adjusted these models for potential confounders, these associations attenuated into nonsignificance (Table 3) (24). As compared to the lowest quintile of hair cortisol concentrations at age 6 years, children in the highest quintiles had a higher systolic blood pressure at age 10 years in the basic models (see Supplementary Table 2) (24). This association remained significant after adjustment for confounders (difference 0.15 SDS; 95% CI 0.00-0.29; and 0.17 SDS; 95% CI, 0.03-0.31) for the fourth and fifth quintile, respectively) but attenuated into nonsignificance after additional adjustment for childhood BMI at age 6 years (see Table 3 and Supplementary Table 3) (24). Associations for continuous cortisol measures showed similar results (Supplementary Table 4) (24). The tests for trend across the quintiles were not significant.

Metabolic Risk Factors

Hair cortisol concentrations were not associated with lipid and glucose metabolism biomarkers in the basic and main models (Supplementary Tables 4 and 5, respectively) (24). In the sex-stratified analyses, higher hair cortisol concentrations were associated with lower triglycerides and insulin concentrations among boys only at age 6 years (differences –0.11 SDS; 95% CI, –0.21 to –0.01; and –0.09 SDS; 95% CI, –0.18 to 0.00, respectively) (Supplementary Table 6) (24). As compared to the lowest quintile of hair cortisol concentrations at age 6 years, children in the highest quintile had a higher increase in total cholesterol and LDL cholesterol concentrations

Table 1. Family and birth characteristics (N = 2598)

		Hair cortisol concentrations					
	Total group[a]	Quintile 1[a]	Quintile 2[a]	Quintile 3[a]	Quintile 4[a]	Quintile 5[a]	*P*[b]
	0.131-6.764	0.131-0.744 pg/mg	0.745-1.173 pg/mg	1.174-1.831 pg/mg	1.832-2.925 pg/mg	2.926-6.764 pg/mg	
	(N = 2598)	(N = 519)	(N = 520)	(N = 520)	(N = 520)	(N = 519)	
Family characteristics							
Prepregnancy BMI, median (95% range)	22.6 (18.2-35.1)	22.1 (18.8-33.9)	22.7 (18.1-34.6)	22.6 (18.1-35.1)	22.3 (18.1-35.9)	23.0 (17.5-36.2)	.039
Maternal education, %							< .001
Primary school	98 (4.5)	8 (1.7)	15 (3.4)	22 (5.0)	34 (7.9)	19 (4.6)	
Secondary school	815 (37.2)	151 (32.7)	150 (34.0)	167 (38.2)	169 (39.1)	178 (42.8)	
High education	1275 (58.3)	303 (65.6)	276 (62.6)	248 (56.8)	229 (53.0)	219 (52.6)	
Family income, %							< .001
Low (< €1600/mo)	327 (15.8)	39 (8.8)	51 (12.3)	58 (14.1)	90 (22.1)	89 (22.6)	
Medium (€1600-4000/mo)	981 (47.4)	202 (45.5)	186 (44.7)	212 (51.7)	197 (48.3)	184 (46.8)	
High (> €4000/mo)	763 (36.8)	203 (45.7)	179 (43.0)	140 (34.1)	121 (29.7)	120 (30.5)	
Birth characteristics							
Sex, No., %							.054
Boys	1237 (47.6)	223 (43.0)	248 (47.7)	247 (47.5)	247 (47.5)	272 (52.4)	
Girls	1361 (52.4)	296 (57.0)	272 (52.3)	273 (52.5)	273 (52.5)	247 (47.6)	
Ethnicity, %							< .001
European	1644 (65.0)	419 (82.2)	355 (70.9)	302 (59.2)	283 (55.6)	285 (57.1)	
Non-European	885 (35.0)	91 (17.8)	146 (29.1)	208 (40.8)	226 (44.4)	214 (42.9)	

Abbreviation: BMI: body mass index.

[a]Values are means (SD), medians (95% range), or numbers of participants (valid %).

[b]*P* values for differences in participant characteristics between cortisol quintiles were tested using one-way analysis of variance tests for continuous variables and chi-square tests for categorical variables.

Table 2. Child characteristics (N = 2598)

		Hair cortisol concentrations					
	Total group[a]	Quintile 1[a]	Quintile 2[a]	Quintile 3[a]	Quintile 4[a]	Quintile 5[a]	*P*[b]
	0.131-6.764	0.131-0.744 pg/mg	0.745-1.173 pg/mg	1.174-1.831 pg/mg	1.832-2.925 pg/mg	2.926-6.764 pg/mg	
	(N = 2598)	(N = 519)	(N = 520)	(N = 520)	(N = 520)	(N = 519)	
Child characteristics at age 6 y							
Age at measurements, median (95% range), y	5.9 (5.7-8.1)	5.9 (5.6-8.1)	5.9 (5.7-8.1)	5.9 (5.7-8.2)	5.9 (5.7-8.2)	5.9 (5.7-8.1)	.013
Body mass index, median (95% range)	15.8 (13.6-21.2)	15.7 (13.7-19.0)	15.7 (13.6-21.0)	16.0 (13.7-20.9)	15.9 (13.7-21.5)	16.1 (13.6-22.4)	< .001
Hair cortisol concentrations, median (95% range), pg/mg	1.46 (0.33-5.62)	0.56 (0.23-0.74)	0.96 (0.75-1.17)	1.46 (1.18, 1.81)	2.28 (1.86-2.88)	3.98 (2.98-6.60)	< .001
Hair cortisone concentrations, median (95% range), pg/mg	7.50 (2.63-29.00)	4.65 (2.00-8.78)	6.19 (2.95, 10.66)	8.25 (3.31, 14.56)	11.52 (3.60, 23.18)	16.00 (3.65-44.58)	< .001
Systolic blood pressure, mean (SD), mm Hg	102.6 (8.4)	101.7 (8.1)	102.1 (8.2)	103.1 (8.2)	103.0 (8.2)	103.2 (9.0)	.007
Diastolic blood pressure, mean (SD), mm Hg	60.5 (6.7)	60.1 (6.4)	60.1 (6.6)	60.4 (7.1)	60.4 (6.6)	61.2 (7.0)	.072
Heart rate, mean (SD), beats/min	82.7 (9.8)	82.0 (9.0)	83.6 (10.1)	83.1 (10.4)	82.4 (9.6)	82.7 (9.9)	.091
Insulin, median (95% range), pmol/L	116.90 (18.58-405.28)	110.70 (24.60-401.89)	130.30 (15.27-411.94)	93.49 (13.30-388.15)	128.60 (18.26-422.35)	115.30 (19.53-455.04)	.014
Total cholesterol, mean (SD), mmol/L	4.25 (0.65)	4.27 (0.63)	4.28 (0.62)	4.28 (0.66)	4.23 (0.63)	4.22 (0.68)	.571
HDL cholesterol, mean (SD), mmol/L	1.38 (0.32)	1.38 (0.33)	1.37 (0.32)	1.39 (0.33)	1.35 (0.30)	1.39 (0.30)	.640
LDL cholesterol, mean (SD), mmol/L	2.37 (0.56)	2.37 (0.55)	2.41 (0.54)	2.39 (0.59)	2.37 (0.54)	2.35 (0.58)	.690
Triglycerides, median (95% range), mmol/L	0.99 (0.41-2.35)	0.97 (0.41-2.64)	0.97 (0.40-2.35)	1.02 (0.42-2.14)	0.99 (0.41-2.37)	1.00 (0.38-2.30)	.973
C-reactive protein, No., %							.025
< 3 mg/L	1591 (89.2)	334 (92.0)	313 (90.2)	301 (85.8)	319 (91.1)	324 (86.9)	
≥ 3 mg/L	193 (10.8)	29 (8.0)	34 (9.8)	50 (14.2)	31 (8.9)	49 (13.1)	
Prevalence cardiometabolic clustering, No., %[c]	218 (10.8)	34 (8.4)	44 (11.2)	47 (11.6)	45 (11.1)	48 (11.7)	.529
Glucocorticoid use in 3 mo prior to hair sample collection, No., %							.622
No	2296 (93.0)	452 (92.2)	469 (94.6)	460 (92.4)	455 (93.0)	460 (92.7)	
Yes	173 (7.0)	38 (7.8)	27 (5.4)	38 (7.6)	34 (7.0)	36 (7.3)	
Hair color, No., %							< .001
Red	78 (3.0)	20 (3.9)	16 (3.1)	20 (3.9)	11 (2.1)	11 (2.1)	
Blond	1381 (53.2)	376 (72.4)	281 (54.0)	241 (46.4)	246 (47.4)	237 (45.7)	
Brown	857 (33.0)	111 (21.4)	175 (33.7)	207 (39.9)	180 (34.7)	184 (35.5)	
Black	280 (10.8)	12 (2.3)	48 (9.2)	51 (9.8)	82 (15.8)	87 (16.8)	

Table 2. Continued

	Total group[a]	Hair cortisol concentrations					P^b
		Quintile 1[a]	Quintile 2[a]	Quintile 3[a]	Quintile 4[a]	Quintile 5[a]	
	0.131-6.764	0.131-0.744 pg/mg	0.745-1.173 pg/mg	1.174-1.831 pg/mg	1.832-2.925 pg/mg	2.926-6.764 pg/mg	
	(N = 2598)	(N = 519)	(N = 520)	(N = 520)	(N = 520)	(N = 519)	
Television watching time, No., %							< .001
< 2 h/d	1631 (81.7)	381 (88.6)	338 (83.5)	318 (79.5)	303 (78.1)	291 (78.0)	
≥ 2 h/d	365 (18.3)	49 (11.4)	67 (16.5)	82 (20.5)	85 (21.9)	82 (22.0)	
Child characteristics at age 10 y							
Age at measurements, median (95% range), years	9.7 (9.3-10.6)	9.7 (9.3-10.7)	9.7 (9.3-10.4)	9.7 (9.4-10.6)	9.8 (9.2-10.7)	9.7 (9.3-11.0)	.155
Body mass index, median (95% range)	16.9 (14.0-24.8)	16.5 (13.9-22.4)	16.7 (14.2-23.9)	17.0 (14.0-23.5)	17.0 (14.1-25.4)	17.2 (13.8-26.6)	< .001
Systolic blood pressure, mean (SD), mm Hg	103.2 (8.1)	102.4 (7.8)	102.7 (7.4)	103.0 (8.3)	103.9 (8.4)	104.2 (8.4)	.005
Diastolic blood pressure, mean (SD), mm Hg	58.6 (6.6)	58.4 (6.8)	58.8 (6.3)	58.4 (6.7)	58.5 (6.8)	59.0 (6.6)	.698
Heart rate, mean (SD), beats/min	74.3 (10.1)	74.3 (10.0)	74.3 (9.8)	74.2 (10.4)	74.8 (10.6)	74.2 (10.0)	.928
Insulin, median (95% range), pmol/L	193.2 (33.37-709.27)	177.10 (37.21-613.68)	189.50 (30.72-618.40)	201.40 (37.03-698.83)	202.00 (28.19-718.82)	199.55 (30.81-777.55)	.341
Glucose, mean (SD), mmol/L	5.38 (0.92)	5.42 (0.91)	5.30 (0.84)	5.47 (0.99)	5.35 (0.92)	5.38 (0.94)	.227
Total cholesterol, mean (SD), mmol/L	4.30 (0.65)	4.28 (0.64)	4.32 (0.64)	4.29 (0.68)	4.28 (0.63)	4.34 (0.66)	.790
HDL cholesterol, mean (SD), mmol/L	1.47 (0.33)	1.48 (0.33)	1.49 (0.35)	1.46 (0.31)	1.46 (0.32)	1.47 (0.33)	.831
LDL cholesterol, mean (SD), mmol/L	2.33 (0.57)	2.31 (0.57)	2.32 (0.56)	2.34 (0.59)	2.33 (0.53)	2.36 (0.58)	.835
Triglycerides, median (95% range), mmol/L	0.93 (0.42-2.56)	0.95 (0.39-2.63)	0.93 (0.43-2.84)	0.93 (0.37-2.43)	0.95 (0.45-2.38)	0.93 (0.44-2.65)	.910
C-reactive protein, No., %							.140
< 3 mg/L	1303 (93.9)	272 (96.1)	266 (94.7)	254 (94.8)	267 (92.1)	244 (91.7)	
≥ 3 mg/L	85 (3.3)	11 (3.9)	15 (5.3)	14 (5.2)	23 (7.9)	22 (8.3)	
Prevalence cardiometabolic clustering, No., %[c]	172 (13.3)	26 (10.0)	35 (13.4)	40 (15.7)	31 (11.7)	40 (15.7)	.219

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; pg/mg, picogram per milligram.

[a]Values are means (SD), medians (95% range), or numbers of participants (valid %).

[b]*P* values for differences in participant characteristics between cortisol quintiles were tested using one-way analysis of variance tests for continuous variables and chi-square tests for categorical variables.

[c]Clustering of cardiometabolic risk factors was defined as having 3 or more out of the following 4 adverse risk factors: android fat mass percentage above the 75th percentile; systolic or diastolic blood pressure above the 75th percentile; HDL cholesterol below the 25th percentile or triglycerides above the 75th percentile; and insulin above the 75th percentile of our study population. We used android fat mass as a percentage of total body fat mass as a proxy for waist circumference because this was not available. For the clustering of cardiometabolic risk factors at 10 years, we also had visceral fat mass obtained by magnetic resonance imaging scans available, as described previously (22). (N = 2022 at 6 years and N = 1.298 at 10 years).

Table 3. Association of hair cortisol quintiles at age 6 years with blood pressure and heart rate at ages 6 years and 10 years and with the change between ages 6 and 10 years, confounder models

	Cardiovascular risk factors at age 6 y		
	Difference (95% CI) in SDS		
Hair cortisol Q at age 6 y	Systolic blood pressure[a]	Diastolic blood pressure[a]	Heart rate[a]
	(N = 2466)	(N = 2466)	(N = 2465)
Q1	Reference	Reference	Reference
Q2	0.02 (–0.11 to 0.14)	–0.02 (–0.14 to 0.11)	**0.17 (0.05 to 0.29)**[e]
Q3	0.11 (–0.02 to 0.23)	–0.01 (–0.13 to 0.11)	0.09 (–0.03 to 0.21)
Q4	0.09 (–0.04 to 0.22)	–0.02 (–0.14 to 0.10)	0.01 (–0.11 to 0.12)
Q5	0.09 (–0.04 to 0.21)	0.09 (–0.03 to 0.22)	0.05 (–0.07 to 0.17)
	Cardiovascular risk factors at age 10 y		
	Difference (95% CI) in SDS		
Hair cortisol Q at age 6 y	Systolic blood pressure[b]	Diastolic blood pressure[b]	Heart rate[b]
	(N = 1938)	(N = 1938)	(N = 1891)
Q1	Reference	Reference	Reference
Q2	0.03 (–0.11 to 0.17)	0.04 (–0.10 to 0.19)	–0.01 (–0.15 to 0.14)
Q3	0.04 (–0.11 to 0.18)	–0.04 (–0.19 to 0.11)	–0.04 (–0.19 to 0.11)
Q4	**0.15 (0.00 to 0.29)**[d]	–0.04 (–0.19 to 0.11)	0.01 (–0.13 to 0.16)
Q5	**0.17 (0.03 to 0.31)**[d]	0.05 (–0.11 to 0.19)	–0.01 (–0.16 to 0.13)
	Cardiovascular risk factors between ages 6 and 10 y		
	Difference (95% CI) in SDS		
Hair cortisol Q at age 6 y	Change in systolic blood pressure[c]	Change in diastolic blood pressure[c]	Change in heart rate[c]
	(N = 1829)	(N = 1828)	(N = 1789)
Q1	Reference	Reference	Reference
Q2	0.02 (–0.12 to 0.16)	0.14 (–0.02 to 0.30)	–0.08 (–0.22 to 0.07)
Q3	–0.06 (–0.20 to 0.08)	0.01 (–0.15 to 0.18)	–0.13 (–0.27v0.02)
Q4	0.06 (–0.09 to 0.20)	–0.00 (–0.17 to 0.16)	0.02 (–0.13 to 0.16)
Q5	0.05 (–0.10 to 0.19)	–0.05 (–0.22 to 0.11)	–0.07 (–0.21 to 0.07)

Confounder models are adjusted for child sex, child age at cortisol assessment, child age at assessment of cardiometabolic outcomes, maternal prepregnancy BMI, maternal education, family income, child ethnicity, television watching time, and hair color.

Abbreviations: Q, quintile; SDS, SD score.

[a]Values are linear regression coefficients (95% CI) and reflect the change in blood pressure and heart rate at age 6 years in SDS for the cortisol quintiles compared to the first quintile.

[b]Values are linear regression coefficients (95% CI) and reflect the change in blood pressure and heart rate at age 10 years in SDS for the cortisol quintiles compared to the first quintile.

[c]Values are linear regression coefficients (95% CI) and reflect the change in blood pressure and heart rate between ages 6 and 10 years in SDS for the cortisol quintiles compared to the first quintile.

[d]*P* less than .05.

[e]*P* less than .017.

from ages 6 to 10 years (differences 0.19 SDS; 95% CI, 0.05-0.34; and 0.15 SDS; 95% CI, 0.00-0.29, respectively), but no difference in change of other metabolic risk factors (Table 4) (24). The association for change in total cholesterol was independent of childhood BMI at age 6 years (see Supplementary Table 3) (24). The associations for continuous cortisol measures showed similar results (Supplementary Table 4) (24).

Increased C-Reactive Protein Concentrations and Clustering of Cardiovascular Risk Factors

Results from the basic models showed that, as compared to the lowest quintile of hair cortisol concentrations at age 6 years, children in the highest quintile had a higher risk of increased C-reactive protein at age 6 years (odds ratio [OR]: 1.76; 95% CI, 1.08-2.86) and a higher risk of increased C-reactive protein (OR: 2.23; 95% CI, 1.05-4.70) and cardiometabolic clustering (OR 1.73; 95% CI, 1.01-2.97) at age 10 years (Supplementary Table 7) (24). When we further adjusted the models for potential confounders, these associations attenuated into nonsignificance (Table 5) (24). The associations for continuous cortisol measures showed similar results (Supplementary Table 8) (24).

Sensitivity Analyses

In the confounder models, excluding children with all types of glucocorticoid use in the 3 months prior to hair sample collection (N = 173), we observed similar but slightly stronger results for systolic blood pressure at age 10 years (differences 0.20 SDS; 95% CI, 0.05-0.34) (Supplementary Table 9) (24). When we further adjusted the model for childhood BMI, the association attenuated into nonsignificance (Supplementary Table 10) (24). In these analyses, results from the confounder model showed that children in the highest quintile of hair cortisol concentrations at age 6 years, compared to those in the lowest quintile, had a higher risk of increased C-reactive protein at age 6 years (OR: 1.83; 95% CI: 1.06-3.13) and age 10 years (OR: 2.53; 95% CI, 1.11-5.77) (Supplementary Table 11), independent of childhood BMI at age 6 years (see Supplementary Table 10) (24). Hair cortisone concentrations at age 6 years were not associated with any of the cardiometabolic outcomes at age 6 or 10 years (results not shown).

Discussion

In this population-based, prospective cohort study among 2598 children, we observed that hair cortisol concentrations at age 6 years were not consistently associated with cardiometabolic risk factors at ages 6 and 10 years. The association of higher hair cortisol concentrations at age 6 years with higher systolic blood pressure at age 10 years was explained by childhood BMI.

Interpretation of Main Findings

A meta-analysis in 2832 adults from 11 studies showed that higher hair cortisol concentrations were associated with higher systolic blood pressure, but not with diastolic blood pressure (36). Also, a review including 20 studies investigating the relationships between various cortisol measures and cardiometabolic parameters in adults reported that 3 out of 6 studies found positive associations between cortisol measures and systolic blood pressure and reported inconclusive results for the other outcomes (37). Previous studies in children did not find associations between hair cortisol concentrations and blood pressure, heart rate, lipids, C-reactive protein, or glucose metabolism (19-21). These studies in children had smaller sample sizes and most of them had a cross-sectional design. Results of studies into the associations of salivary, serum, or urinary cortisol with cardiometabolic risk factors in childhood were not consistent (38-42).

In the present study, we observed that higher hair cortisol concentrations at age 6 years were associated with a higher systolic blood pressure at age 10 years. This finding is in line with the findings of 3 cross-sectional studies in children that showed a positive association between serum cortisol concentrations and systolic blood pressure but not, or less clearly, with diastolic blood pressure (40-42). However, we did not find an association between hair cortisol concentrations at age 6 years and systolic blood pressure at age 6 years. Thus, it may be that higher cortisol concentrations lead to increased systolic blood pressure later in childhood, which is known to track into adulthood (13). Additional adjustment of the association between hair cortisol concentrations at age 6 years and systolic blood pressure at age 10 years for childhood BMI at age 6 years resulted in attenuation of this association. This is in contrast with the findings of the 3 cross-sectional studies mentioned earlier, which showed that the association remained after adjustment for BMI or total body fat mass (40-42). Childhood BMI can be either an intermediate or a confounder in the association of hair cortisol concentration at age 6 years and systolic blood pressure at age 10 years. We know from previous studies in our cohort that higher hair cortisol concentrations are associated with higher childhood BMI at ages 6 and 10 years (17, 18). A bidirectional association between cortisol and adiposity may

Table 4. Association of hair cortisol quintiles at 6 years with lipids, insulin and glucose at ages 6 years and 10 years and with the change between 6 and 10 years, confounder models

	Metabolic risk factors at age 6 y				
	Difference (95% CI) in SDS				
Hair cortisol Q at age 6 y	Total cholesterol[a]	HDL[a]	LDL[a]	Triglycerides[a]	Insulin[a]
	(N = 1781)	(N = 1781)	(N = 1782)	(N = 1773)	(N = 1766)
Q1	Reference	Reference	Reference	Reference	Reference
Q2	0.01 (–0.14, 0.16)	–0.04 (–0.19, 0.11)	0.08 (–0.06, 0.23)	–0.01 (–0.16, 0.14)	0.10 (–0.05, 0.24)
Q3	–0.02 (–0.17, 0.13)	0.00 (–0.15, 0.15)	0.01 (–0.13, 0.16)	0.02 (–0.13, 0.16)	–0.10 (–0.25, 0.04)
Q4	–0.08 (–0.23, 0.07)	–0.11 (–0.26, 0.04)	0.01 (–0.14, 0.16)	0.02 (–0.13, 0.17)	0.13 (–0.02, 0.27)
Q5	–0.10 (–0.25, 0.05)	–0.01 (–0.16, 0.14)	–0.04 (–0.19, 0.10)	–0.03 (–0.18, 0.12)	–0.04 (–0.19, 0.11)

	Metabolic risk factors at age 6 y					
	Difference (95% CI) in SDS					
Hair cortisol Q at age 6 y	Total cholesterol[b]	HDL[b]	LDL[b]	Triglycerides[b]	Insulin[b]	Glucose[b]
	(N = 1388)	(N = 1387)	(N = 1377)	(N = 1380)	(N = 1382)	(N = 1387)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	0.05 (–0.11 to 0.22)	0.05 (–0.11 to 0.20)	0.02 (–0.14 to 0.18)	0.01 (–0.15 to 0.18)	–0.03 (–0.20 to 0.14)	–0.12 (–0.28 to 0.05)
Q3	0.01 (–0.16 to 0.17)	–0.03 (–0.19 to 0.13)	0.05 (–0.11 to 0.21)	–0.07 (–0.24 to 0.09)	0.08 (–0.09 to 0.26)	0.06 (–0.11 to 0.22)
Q4	–0.01 (–0.18 to 0.15)	–0.01 (–0.16 to 0.15)	0.02 (–0.14 to 0.18)	–0.06 (–0.23 to 0.10)	0.05 (–0.12 to 0.22)	–0.05 (–0.21 to 0.12)
Q5	0.10 (–0.07 to 0.27)	0.04 (–0.12 to 0.20)	0.11 (–0.06 to 0.27)	–0.05 (–0.22 to 0.12)	0.03 (–0.15 to 0.20)	–0.02 (–0.18 to 0.15)

	Metabolic risk factors change between ages 6 and 10 y				
	Difference (95% CI) in SDS				
Hair cortisol Q at age 6 y	Change in total cholesterol[c]	Change in HDL[c]	Change in LDL[c]	Change in triglycerides[c]	Change in insulin[c]
	(N = 1051)	(N = 1050)	(N = 1043)	(N = 1041)	(N = 1036)
Q1	Reference	Reference	Reference	Reference	Reference
Q2	0.09 (–0.05 to 0.23)	0.03 (–0.11 to 0.18)	–0.01 (–0.15 to 0.13)	0.08 (–0.14 to 0.30)	–0.14 (–0.39 to 0.10)
Q3	0.10 (–0.05 to 0.25)	0.07 (–0.08 to 0.22)	0.06 (–0.08 to 0.20)	–0.01 (–0.24 to 0.21)	0.15 (–0.10 to 0.40)
Q4	0.12 (–0.03 to 0.26)	0.13 (–0.02 to 0.28)	0.06 (–0.09 to 0.20)	–0.10 (–0.32 to 0.13)	–0.08 (–0.33 to 0.17)
Q5	**0.19 (0.05 to 0.34)**[e]	0.02 (–0.13 to 0.16)	**0.15 (0.00 to 0.29)**[d]	0.04 (–0.18 to 0.27)	0.08 (–0.16 to 0.33)

Confounder models are adjusted for child sex, child age at cortisol assessment, child age at assessment of cardiometabolic outcomes, maternal prepregnancy body mass index, maternal education, family income, child ethnicity, television watching time, and hair color.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; Q, quintile; SDS, SD score.

[a]Values are linear regression coefficients (95% CI) and reflect the change in lipids and insulin concentrations at age 6 years in SDS for the cortisol quintiles compared to the first quintile.

[b]Values are linear regression coefficients (95% CI) and reflect the change in lipids, insulin, and glucose concentrations at age 10 years in SDS for the cortisol quintiles compared to the first quintile.

[c]Values are linear regression coefficients (95% CI) and reflect the change in the delta of lipids and insulin concentrations between ages 6 and 10 years in SDS for the cortisol quintiles compared to the first quintile.

[d]*P* less than .05.

[e]*P* less than .017.

Table 5. Association of hair cortisol quintiles at age 6 years with risk of increased C-reactive protein and risk of cardiometabolic clustering at ages 6 and 10 years, confounder models

Hair cortisol Q at age 6 y	Odds ratio (95% CI) for outcomes at age 6 y	
	Risk of C-reactive protein ≥ 3 mg/L[a]	Risk of cardiometabolic clustering[b]
	(N = 1784)	(N = 2022)
Q1	Reference	Reference
Q2	1.19 (0.70 to 2.01)	1.39 (0.86 to 2.27)
Q3	**1.69 (1.04 to 2.77)**[c]	1.32 (0.81 to 2.14)
Q4	0.98 (0.57 to 1.68)	1.21 (0.74 to 1.98)
Q5	1.54 (0.94 to 2.54)	1.29 (0.79 to 2.11)
Hair cortisol Q at age 6 y	**Odds ratio (95% CI) for outcomes at age 10 y**	
	Risk of C-reactive protein ≥ 3 mg/L[a]	**Risk of cardiometabolic clustering[b]**
	(N = 1389)	**(N = 1299)**
Q1	Reference	Reference
Q2	1.34 (0.59 to 3.02)	1.34 (0.75 to 2.39)
Q3	1.14 (0.50 to 2.61)	1.50 (0.86 to 2.64)
Q4	1.76 (0.82 to 3.79)	1.00 (0.55 to 1.82)
Q5	1.91 (0.88 to 4.13)	1.32 (0.74 to 2.35)

Confounder models are adjusted for child sex, child age at cortisol assessment, child age at assessment of cardiometabolic outcomes, maternal prepregnancy body mass index, maternal education, family income, child ethnicity, television watching time, and hair color.

Abbreviation: Q, quintile.

[a]Values are odds ratios (95% CI) and represent the risk of childhood high C-reactive protein concentrations (≥ 3 mg/L) at ages 6 and 10 years for the cortisol quintiles compared to the first quintile.

[b]Values are odds ratios (95% CI) and reflect the odds of cardi-metabolic clustering at ages 6 and 10 years defined as having 3 or more out of the following 4 adverse risk factors: android fat mass percentage above the 75th percentile; systolic or diastolic blood pressure above the 75th percentile; high-density lipoprotein cholesterol below the 25th percentile or triglycerides above the 75th percentile; and insulin above the 75th percentile of our study population for the cortisol quintiles compared to the first quintile.

[c]*P* less than .05.

[d]*P* less than .017.

be present that should be further explored in future studies (43). Future studies are also needed to obtain further insight into the role of BMI in the association of hair cortisol concentrations with blood pressure.

We observed an association between higher hair cortisol concentrations and the increase in total cholesterol and LDL concentrations between ages 6 and 10 years, but not with any of the lipid concentrations at age 6 or 10 years. Studies that used different types of samples to measure cortisol did not find an association with lipid concentrations in children (20, 21, 38, 39, 42). Studies in adults are not consistent about the association between cortisol and lipids, but most provide evidence for a positive association between cortisol and total cholesterol and LDL (44-48). It may be that the association between higher hair cortisol and higher total cholesterol and LDL concentrations becomes more apparent at later ages.

In sex-stratified analyses, we observed that higher hair cortisol concentrations were associated with lower triglyceride and insulin concentrations among boys at age 6 years, independent of childhood BMI, and higher concentrations of triglycerides and insulin among girls at age 6 years. These findings were significant only among boys and not among girls, which may be explained by a higher variability in hair cortisol concentrations among boys. In our study and similarly to previous studies, hair cortisol concentrations were significantly higher among boys than girls (36, 49). It has been hypothesized that sex differences in reactivity to psychological stress might contribute to the sex differences in morbidity and mortality rates of cardiovascular diseases. However, studies in adults have not reported differences in the associations between hair cortisol concentrations and cardiometabolic risk factors after stratification on sex (36, 37, 50, 51). The sex-specific associations of cortisol concentrations with cardiovascular risk factors and disease need further study.

Metabolic syndrome shares many characteristics of Cushing syndrome, caused by the endogenous overproduction of cortisol, such as impaired glucose tolerance, dyslipidemia, abdominal fat distribution, and hypertension (52). Therefore, it has been suggested that altered activity of the hypothalamus-pituitary-adrenal axis leading to the

hypersecretion of glucocorticoids may play an important role in the development of metabolic syndrome (52-55). However, we did not find clear evidence for an association of higher cortisol concentrations and characteristics of metabolic syndrome in childhood.

Strengths and Limitations

One of the strengths of this study was the prospective data collection from early pregnancy onward. We had a large sample size and detailed measurements of hair cortisol concentrations and childhood cardiometabolic risk factors. A limitation of our study is the lack of hair cortisol measurements at age 10 years. Therefore, we do not know how cortisol concentrations develop over time. To prevent contamination of data caused by hair cortisol outliers, we excluded cortisol values using the Tukey definition of outliers (23). Excluding these values would have affected the effect estimates if cardiometabolic risk factors were different for the excluded children and the population of analysis. However, there were no substantial differences in the characteristics of these groups. Also, the hair cortisol concentration values in the population of analysis were all within the liquid chromatography–tandem mass spectrometry–based reference interval for children aged 6, provided by a recent study that aimed to establish age-adjusted reference intervals for hair cortisol in children (56). We used nonfasting venous blood samples to measure the serum concentrations of the cardiometabolic risk factors. The blood samples were collected at different time points during the day, depending on the time of the study visit. Since glucose and insulin concentrations change easily during the day and in response to carbohydrate intake, this may have caused nondifferential misclassification. We think the effect of this potential misclassification will be minor. A previous study reported that insulin resistance or sensitivity in semifasted blood samples are moderately correlated with fasting values (57). Also, studies have reported that nonfasting lipid concentrations can predict increased risks of cardiovascular events later in life (58, 59). Overall, results should be interpreted with caution and this study should be replicated using fasting samples. Even though the analyses were adjusted for a large number of potential confounding factors, residual cofounding may still be a concern, as in any observational study. Owing to the observational design of the study, we cannot establish causality of the observed associations.

Conclusion

Our results suggest that hair cortisol concentrations at age 6 years are not consistently associated with cardiometabolic risk factors at ages 6 and 10 years. The association between hair cortisol concentrations and systolic blood pressure was explained by childhood BMI. The associations of stress with cardiometabolic risk factors may develop at later ages.

Acknowledgments

The Generation R Study is conducted by the Erasmus MC, University Medical Center Rotterdam, in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam; the Municipal Health Service Rotterdam area, Rotterdam; the Rotterdam Homecare Foundation, Rotterdam; and the Stichting Trombosedienst and Artsenlaboratorium Rijnmond (STAR), Rotterdam. We gratefully acknowledge the contribution of participating parents, children, general practitioners, hospitals, midwives, and pharmacies in Rotterdam.

Financial Support: This work was supported by the European Union's Horizon 2020 Research and Innovation Programme (Nos. 848158, EarlyCause; 733206, LifeCycle; and 874739 LongITools). The general design of the Generation R Study is supported by the Erasmus Medical Center, University Medical Center, Rotterdam, the Netherlands; Organization for Health Research and Development (ZonMw); the Netherlands Organization for Scientific Research (NWO); the Ministry of Health, Welfare and Sport; and the Ministry of Youth and Families. Dr Jaddoe has received funding from the European Research Council (grant No. ERC-2014-CoG-648916). Dr van den Akker and Dr van Rossum are supported by the Elisabeth Foundation. Dr van Rossum received a Vidi Grant (No. 91716453) from the Netherlands Organization of Scientific Research NWO.

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Disclosures: The authors have no conflicts of interest relevant to this article to disclose.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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