The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. 106, No. 2, e551–e561
doi:10.1210/clinem/dgaa785
Clinical Research Article

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Associations of Hair Cortisol Concentrations with General and Organ Fat Measures in Childhood

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Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; BMI, body mass index; CI, confidence interval; DXA, dual-energy X-ray absorptiometry; HCC, hair cortisol concentration; IQR, interquartile range; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; SDS, standard deviation score.

Received: 23 June 2020; Editorial Decision: 20 October 2020; First Published Online: 29 October 2020; Corrected and Typeset: 2 December 2020.

Abstract

Context: Stress may lead to an adverse body fat distribution from childhood onwards.
Objective: To examine the associations of hair cortisol concentration (HCC) at 6 years with general and organ fat measures, risk of overweight, and nonalcoholic fatty liver disease (NAFLD) at 10 years and to assess whether these were independent of adiposity measures at 6 years.
Design, Setting and participants: HCCs were measured in hair of 6-year-old children (n = 2042) participating in the Generation R Study, a population-based prospective cohort study.
Main Outcome Measures: Body mass index (BMI), fat mass index measured by dual-energy X-ray absorptiometry scan, and visceral fat index, pericardial fat index, liver fat fraction measured by magnetic resonance imaging and risk of overweight and NAFLD were obtained at 10 years.
Results: The associations of higher HCC at 6 years, with higher BMI, fat mass index, and increased risk of overweight at age 10 years are explained by the relationships observed at 6 years. HCCs at 6 years were associated with a higher liver fat fraction (difference 0.11 liver fat fraction standard deviation score; 95% confidence interval [CI] 0.03, 0.18) and a

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

higher risk of NAFLD at 10 years (odds ratio 1.95; 95% CI 1.06, 3.56), independent of fat mass index at 6 years. HCCs were not associated with pericardial or visceral fat indices.
Conclusions: Higher HCCs at 6 years were associated with higher BMI, fat mass index, liver fat fraction, and higher risks of overweight and NAFLD at 10 years. Only the associations for liver fat fraction and NAFLD were independent of fat mass index at 6 years.

Freeform/Key Words: hair cortisol, hair cortisone, child, adiposity, organ fat, nonalcoholic fatty liver disease

Obesity is a major public health problem and is associated with short- and long-term morbidity and mortality (1). Previous studies suggested that stress is associated with adiposity among adults (2). Cortisol and cortisone, both glucocorticoids, are objective biomarkers of stress (3). Long-term dysregulated cortisol secretion can contribute to the development of obesity through insulin resistance of peripheral target tissues and accumulation of visceral fat (4, 5). Unlike the traditional cortisol measures in saliva, serum, and urine, hair cortisol concentrations (HCCs) reflect long-term cumulative cortisol concentrations (3, 6, 7). Cortisol can be converted into inactive cortisone (8). The assessment of both glucocorticoids, which are highly correlated, may give more insight into the amount of active and inactive corticosteroids (9, 10). Previous studies reported associations of HCCs with body mass index (BMI), and other adiposity measures in adults (2, 6, 11). Thus far, studies in children have been of a modest sample size, have used a cross-sectional design, did not show consistent results, and did not look into the association of cortisol with organ fat measures (12). We have previously reported cross-sectional associations of higher HCCs with higher BMI and fat mass index at 6 years (13).

Based on these previous results, we hypothesized that chronic exposure to higher cortisol concentrations leads prospectively to an adverse body fat distribution. We examined, in a population-based prospective cohort study among 2042 children, the associations of HCCs at 6 years with BMI, fat mass index measured by dual-energy X-ray absorptiometry (DXA), and pericardial fat index, visceral fat index, and liver fat fraction measured by magnetic resonance imaging (MRI) and the risks of overweight and nonalcoholic fatty liver disease (NAFLD) at 10 years. We additionally examined whether any association was independent of the previously reported cross sectional associations at 6 years.

Materials and Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, The Netherlands (14). 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 reporting guideline. In total 2984 children had information on HCCs at 6 years. Twins (N = 58) and children without any measurement of adiposity at 10 years (N = 648) were excluded. Also, children with extreme values of cortisol (N = 236) were excluded using Tukey's definition of outliers (Q1 – 1.5 × IQR and Q3 + 1.5 × IQR) (15). The population for analysis consisted of 2042 children. The same selection procedure was followed for the cortisone analyses (N = 2051). The flowchart of participants is given elsewhere (all supplementary material and figures are located in a digital research materials repository (16).

Hair cortisol and cortisone concentration measurements

As described previously, hair cortisol and cortisone concentrations were measured in proximal scalp hair (17). Details on collection, sample preparation, extraction, and analysis are provided elsewhere (16). To reduce variability and account for right skewedness of the distribution cortisol and cortisone concentrations outliers defined by Tukey's 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 interquartile range (IQR) to ease the interpretation of effect sizes (15). The Spearman correlation coefficient between the original variables of hair cortisol and cortisone concentration was 0.63.

General, visceral, and organ fat

Outcome assessments were performed at ages 6 and 10 years (14). We calculated BMI (kg/m^2) at this age from height and weight, both measured without shoes and heavy clothing. We calculated sex- and age- adjusted standard deviation scores (SDSs) of childhood BMI based

on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation) (18). BMI categories (underweight, normal weight, overweight, and obesity) were calculated using the International Obesity Task Force cut-offs (19, 20). We measured total body fat mass using a DXA scanner (iDXA, GE140 Lunar, 2008, Madison, WI, enCORE software v.12.6), according to standard procedures (21).

Visceral and organ adiposity were obtained from MRI scans performed at 10 years, as described previously (14). Briefly, all children underwent imaging using a 3.0-T MRI scanner (Discovery MR750w; GE Healthcare). Pericardial fat imaging in short axis orientation was performed using an electrocardiogram-triggered black-blood–prepared thin-slice single-shot fast-spin echo acquisition with multibreath-hold approach. An axial 3-point Dixon acquisition for fat and water separation (IDEAL IQ) was used for liver fat imaging (22). An axial abdominal scan from lower liver to pelvis and a coronal scan centered at the head of the femurs were performed with a 2-point Dixon acquisition (LavaFlex). The scans were analyzed by the Precision Image Analysis company (PIA, Kirkland, WA,), using the sliceOmatic software package (TomoVision, Magog, Canada). Details on methods and measurements are provided elsewhere (16).

To create measures independent of height, we estimated the optimal adjustment by log-log regression analyses and subsequently we divided total fat mass at 10 years by height (4) (fat mass index) and visceral and pericardial fat mass by height (3) (visceral and pericardial fat indices) (23-25). We log-transformed the non-normally distributed childhood DXA and MRI adiposity measures. We constructed SDS [(observed value—mean)/SD] of the sample distribution for DXA and MRI outcomes to enable comparisons of effect sizes. We used Spearman's rank correlation coefficients to estimate correlations of BMI and fat mass index at 6 years with BMI, fat mass index, pericardial fat mass index, visceral fat mass index and liver fat fraction at 10 years (16).

Covariates

Information on child sex was obtained from midwife/obstetric records. We collected information on maternal prepregnancy BMI and psychological distress during pregnancy by questionnaires. Information on maternal education and marital status, child ethnicity and television watching time was obtained by questionnaires at the age of 6 years completed by the mother. Hair color was partially coded through parent report and was completed by two raters using front desk photographs at the research center. Parents completed a questionnaire for their child on use and administration route of glucocorticoid medications at the age of 6 years.

Statistical analysis

First, we examined differences in subject characteristics between hair cortisol concentration quintiles with analysis of variance tests for continuous variables and Chi-square tests for categorical variables. For nonresponse analyses, we compared participants and nonparticipants using chi-squared tests, Student t tests and Mann–Whitney tests. Second, we used linear regression models to assess the associations of HCCs at 6 years with adiposity measures at 10 years (BMI, fat mass index, visceral and pericardial fat indices, and liver fat fraction). Third, we used logistic regression models to assess the associations of HCCs at 6 years with the risk of childhood overweight or obesity at 10 years, to which we further refer as overweight. Tests for trends across quintiles were performed by analyzing cortisol quintiles as a continuous variable. Fourth, we performed linear regression models to assess the associations of continuous HCCs (the natural log transformed hair cortisol measures further standardized with the IQR) with all adiposity measures. For NAFLD we only assessed the association with the continuous cortisol measurement since the number of children with NAFLD was too small for some of the cortisol quintiles. Fifth, we examined whether HCCs were associated with change in BMI and fat mass index SD scores between 6 and 10 years. Next, we used conditional regression analyses to assess whether the associations of HCCs at 6 years with adiposity outcomes at 10 years were independent of adiposity measures at 6 years. For these models, we first estimated the standardized residuals from the regression models with the 6 years adiposity measurements as exposures and the 10 years adiposity measurements as outcomes. Subsequently, these residuals were used as outcomes for the associations with HCCs (26). These residuals should be interpreted as excess in fat measures at 10 years, as would be expected based on the cross-sectional analyses at 6 years. Since the organ fat measurements were only available at 10 years, these were conditioned on fat mass index at 6 years based on the strongest correlation (16). For all continuous and dichotomous adiposity outcomes, we performed sensitivity analyses by adjusting the models focused on the associations of HCCs at 6 years with adiposity outcomes at 10 years for adiposity measures at 6 years. The basic models included child sex and age at cortisol measurement as confounders. The confounder model was additionally adjusted for maternal prepregnancy BMI, maternal psychological distress during pregnancy, maternal education and marital status, child ethnicity, hair color and average duration of television watching. We identified potential covariates based on the graphical criteria for confounding

by visualizing a directed acyclic graph and included the covariates in the models that were associated with exposure and outcome and changed the effect estimates >10% (16, 27, 28). We assessed which covariates had the strongest effects in the associations of continuous HCCs at 6 years with childhood general and organ fat measures at 10 years. We did not observe statistically significant interactions of hair cortisol levels with child ethnicity and sex. As sensitivity analysis, we excluded children with any glucocorticoid use in the 3 months prior to the hair sample collection (N = 1805). Also, we repeated all analyses for cortisone (N = 2051). Because of the correlations between the outcomes (16), we did not perform Bonferroni adjustment (29). However, considering 3 groups of outcomes (BMI, fat mass index, organ fat measures), multiple testing adjustment would lead to $P < .017$. We depicted both significance levels (.05 and .017) in the tables and figures. In order to maintain statistical power and reduce bias related to missing data on covariates (16), we performed multiple imputation according to Markov Chain Monte Carlo method (16, 30). Five imputed datasets were created and pooled results are presented. All statistical analyses were performed using the Statistical Package of Social Sciences (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp).

Results

Subject characteristics

Table 1 shows that compared with children in the lower cortisol quintiles, children in the upper cortisol quintiles more often had a mother who was younger, lower educated, without a partner, and who reported more psychological distress during pregnancy. Also, these children more often had a lower birth weight, a non-European ethnicity, a brown or black hair color and a higher average duration of television watching at age 6 years. Nonresponse analyses showed that, compared with participants, nonparticipants had mothers who were slightly younger, with a higher BMI, who reported more psychological distress during pregnancy and were more often lower educated. Nonparticipants more often had a higher BMI, a non-European ethnicity, brown or dark hair, and an increased average duration of television watching (16).

Hair cortisol concentrations and general adiposity measures

Compared with the lowest quintile, children in the highest quintile of HCCs at 6 years, had a higher BMI and fat mass index (differences 0.22 SDS; 95% confidence interval [CI] 0.09, 0.36, and 0.21 SDS; 95% CI 0.09, 0.33, respectively) (Fig. 1A and 1B). Tests for trends were significant for BMI and fat mass index (P for trend ≤.001). Associations of continuous cortisol concentrations with general adiposity outcomes showed similar results (an IQR increase in the natural log–transformed HCCs was associated with a 0.10 (95% CI 0.04 0.26) SDS higher BMI and a 0.09 (95% CI 0.04, 0.15) SDS higher fat mass index (16). Maternal prepregnancy BMI, maternal education, and child's sex and child's age were the strongest covariates (16). Results from basic models were in the same direction and slightly stronger (16). HCCs were not associated with the change in BMI and fat mass index SD scores between 6 and 10 years (16). Results from the conditional regression analyses showed that the associations of HCCs with BMI and fat mass index residuals were not consistently significant anymore after conditioning the outcomes on adiposity measures at 6 years (16).

Hair cortisol concentrations and visceral and organ fat measures

Also, compared with the lowest quintile, children in the highest quintile of HCCs at 6 years had higher liver fat fraction at 10 years (difference 0.26 liver fat fraction SDS; 95% CI 0.10, 0.43). HCCs were not associated with pericardial or visceral fat indices (Fig. 2A-C). Test for trends was significant for liver fat fraction (P for trend <.001). Associations for continuous cortisol measures showed similar results: An IQR increase in the natural log–transformed HCC was associated with a 0.15 (95% CI 0.07, 0.22) SDS higher liver fat fraction and a significantly higher risk of NAFLD (odds ratio [OR] 2.35; 95% CI 1.31, 4.22) (16). Results from basic models were in the same direction and slightly stronger (16). The associations of HCCs with liver fat fraction residuals remained significant after conditioning liver fat fraction on fat mass index at 6 years, suggesting these associations were independent of fat mass index at 6 years (16).

Hair cortisol concentrations and risk of childhood overweight

The prevalence of overweight at 10 year increased from 11.4% in the first quintile to 25.8% in the fifth quintile of HCCs (Fig. 3). Compared with the lowest quintile, children in the highest quintile of HCCs at 6 years, had a higher risk of overweight (OR 1.87; 95% CI 1.23, 2.86) at 10 years (Fig. 3). Test for trend was significant for the risk of overweight (P for trend <.001). Associations for continuous cortisol measures showed similar results. Results from basic models were in the same direction and slightly stronger (16).

Table 1. Subject characteristics (N = 2042)

	Total group[a] (N = 2042)	Hair cortisol concentrations					
		Quintile 1[a] 0.137-0.727 pg/mg (N = 408)	Quintile 2[a] 0.728-1.150 pg/mg (N = 409)	Quintile 3[a] 1.151-1.820 pg/mg (N = 408)	Quintile 4[a] 1.827-2.927 pg/mg (N = 409)	Quintile 5[a] 2.928-6.799 pg/mg (N = 408)	*P* value[b]
Family characteristics							
Maternal age, mean (SD), years	31.2 (4.8)	31.8 (4.5)	31.5 (4.7)	30.7 (4.8)	31.0 (4.9)	30.8 (5.1)	.003
Prepregnancy BMI, median (95% range), kg/m^2	22.5 (18.2, 35.0)	22.1 (18.8, 34.2)	22.6 (18.4, 34.5)	22.3 (17.8, 35.5)	22.3 (18.3, 33.9)	23.0 (17.7, 36.2)	.04
Psychological distress during pregnancy, N (%)							.003
Yes	560 (28.6)	95 (24.1)	95 (24.2)	116 (29.8)	118 (29.8)	136 (35.2)	
No	1397 (71.4)	299 (75.9)	297 (75.8)	273 (70.2)	278 (70.2)	250 (64.8)	
Maternal education (%)							<.001
Primary school	68 (3.8)	4 (1.1)	10 (2.8)	15 (4.2)	25 (6.9)	14 (4.0)	
Secondary school	619 (34.3)	115 (31.0)	113 (31.2)	122 (33.8)	131 (36.4)	138 (39.1)	
High education	1120 (62.0)	2592 (67.9)	239 (66.0)	224 (62.0)	204 (56.7)	201 (56.9)	
Marital status, N (%)							<.001
Partner	1576 (87.4)	342 (92.7)	323 (89.0)	323 (89.2)	293 (82.5)	295 (83.1)	
No partner	228 (12.6)	27 (7.3)	40 (11.0)	39 (10.8)	62 (17.5)	60 (16.9)	
Birth characteristics							
Sex, N (%)							.09
Boys	970 (47.5)	176 (43.1)	188 (46.0)	194 (47.5)	197 (48.2)	215 (52.7)	
Girls	1072 (52.5)	232 (56.9)	221 (54.0)	214 (52.5)	212 (51.8)	193 (47.3)	
Birth weight, mean (SD), weeks	3439 (542)	3521 (497)	3441 (571)	3417 (525)	3409 (556)	3405 (555)	.01
Ethnicity (%)							<.001
European	1391 (69.2)	347 (86.3)	302 (74.9)	253 (62.8)	240 (59.4)	249 (62.6)	
Non-European	619 (30.8)	55 (13.7)	101 (25.1)	150 (37.3)	164 (40.6)	149 (37.4)	
Child characteristics at 6 years							
Age at measurements, median (95% range), years	5.9 (5.7, 8.0)	5.9 (5.6, 7.8)	5.9 (5.7, 8.3)	5.9 (5.7, 8.1)	5.9 (5.7, 7.9)	5.9 (5.6, 8.0)	.15
Body mass index, median (95% range), kg/m^2	15.8 (13.6, 20.7)	15.7 (13.7, 18.8)	15.7 (13.6, 20.0)	15.8 (13.6, 20.4)	15.9 (13.7, 21.4)	16.0 (13.4, 22.4)	<.001
Hair cortisol concentrations, median (95% range), pg/mg[c]	1.43 (0.32, 5.63)	0.54 (0.23, 0.71)	0.94 (0.73, 1.14)	1.43 (1.16, 1.80)	2.27 (1.84, 2.89)	3.98 (2.99, 6.62)	<.001
Hair cortisone concentrations, median (95% range), pg/mg[c]	7.30 (2.64, 29.03)	4.57 (2.02, 8.76)	5.91(2.87, 10.32)	7.93 (3.16, 14.73)	11.77 (3.61, 23.66)	16.27 (3.92, 45.13)	<.001
Cortisol/cortisone ratio, median (95% range)	0.17 (0.07, 0.73)	0.11 (0.05, 0.24)	0.16 (0.10, 0.33)	0.18 (0.10, 0.43)	0.19 (0.10, 0.65)	0.24 (0.10, 1.20)	<.001
Glucocorticoid use in the 3 months prior to hair sample collection, N (%)							.66
No	1805 (92.6)	354 (91.9)	369 (93.7)	363 (91.9)	358 (93.7)	361 (91.6)	
Yes	145 (7.4)	31 (8.1)	25 (6.3)	32 (8.1)	24 (6.3)	33 (8.4)	

Table 1. Continued

	Total group[a] (N = 2042)	Hair cortisol concentrations					
		Quintile 1[a] 0.137-0.727 pg/mg (N = 408)	Quintile 2[a] 0.728-1.150 pg/mg (N = 409)	Quintile 3[a] 1.151-1.820 pg/mg (N = 408)	Quintile 4[a] 1.827-2.927 pg/mg (N = 409)	Quintile 5[a] 2.928-6.799 pg/mg (N = 408)	*P* value[b]
Hair color, N (%)							<.001
Red	63 (3.1)	16 (3.9)	11 (2.7)	17 (4.2)	10 (2.5)	9 (2.2)	
Blond	1166 (57.1)	310 (76.0)	240 (58.7)	207 (50.7)	204 (50.0)	205 (50.2)	
Brown	620 (30.4)	76 (18.6)	127 (31.1)	149 (36.5)	136 (33.3)	132 (32.4)	
Black	192 (9.4)	6 (1.5)	31 (7.6)	35 (8.6)	58 (14.2)	62 (15.2)	
Television watching time, N (%)							<.001
<2 hours per day	1381 (83.3)	310 (90.4)	289 (86.5)	270 (80.6)	257 (79.8)	255 (78.9)	
≥2 hours per day	276 (16.7)	33 (9.6)	45 (13.5)	65 (19.4)	65 (20.4)	68 (21.1)	
Child characteristics at 10 years							
Age at measurements, median (95% range), years	9.7 (9.3, 10.6)	9.7 (9.3, 10.4)	9.7 (9.3, 10.4)	9.7 (9.4, 10.5)	9.7 (9.2, 10.7)	9.7 (9.3, 11.0)	.10
Height, mean (SD), cm	141.4 (6.4)	141.8 (5.8)	141.1 (6.4)	140.9 (6.2)	141.4 (6.9)	141.7 (6.7)	.21
Weight, median (95% range), kg	33.6 (25.4, 54.0)	32.8 (25.4, 49.6)	33.4 (25.1, 51.2)	33.6 (25.2, 50.2)	34.0 (24.9, 56.6)	36.7 (25.4, 57.6)	<.001
Body mass index, median (95% range), kg/m^2	16.9 (14.0, 24.8)	16.5 (13.9, 22.2)	16.7 (14.2, 24.0)	16.9 (14.0, 23.5)	17.0 (14.1, 25.4)	17.2 (13.8, 26.6)	<.001
Total body fat mass, median (95% range), kg	8.48 (4.46, 21.88)	8.18 (4.59, 18.98)	8.39 (4.44, 20.07)	8.61 (4.45, 20.49)	8.67 (4.31, 24.03)	9.05 (4.62, 24.47)	<.001
Pericardial fat mass, median (95% range), g	10.13 (4.48, 22.15)	10.03 (4.60, 20.66)	10.42 (4.62, 20.67)	10.09 (4.45, 22.57)	9.72 (3.95, 21.70)	10.49 (4.29, 24.22)	.49
Visceral fat mass, median (95% range), kg	0.36 (0.16, 1.01)	0.36 (0.16, 0.91)	0.35 (0.17, 1.06)	0.35 (0.15, 0.98)	0.35 (0.15, 0.99)	0.37 (0.15, 1.19)	.14
Liver fat fraction, median (95% range), %	1.97 (1.20, 5.04)	1.92 (1.24, 4.45)	1.88 (1.19, 3.63)	1.90 (1.15, 6.16)	2.03 (1.22, 4.23)	2.07 (1.26, 7.91)	<.001
Nonalcoholic fatty liver disease, N (%)							<.001
No	1327 (97.5)	274 (99.6)	265 (98.9)	263 (94.9)	267 (99.6)	258 (94.5)	
Yes	34 (2.5)	1 (0.4)	3 (1.1)	14 (5.1)	1 (0.4)	15 (5.5)	

[a]Values are means (standard deviation), medians (95% range) or numbers of subjects (valid %).
[b]*P* values for differences in subject characteristics between cortisol quintiles were tested using 1-way analysis of variance tests for continuous variables and Chi-square tests for categorical variables.
[c]pg/mg = picogram per milligram.

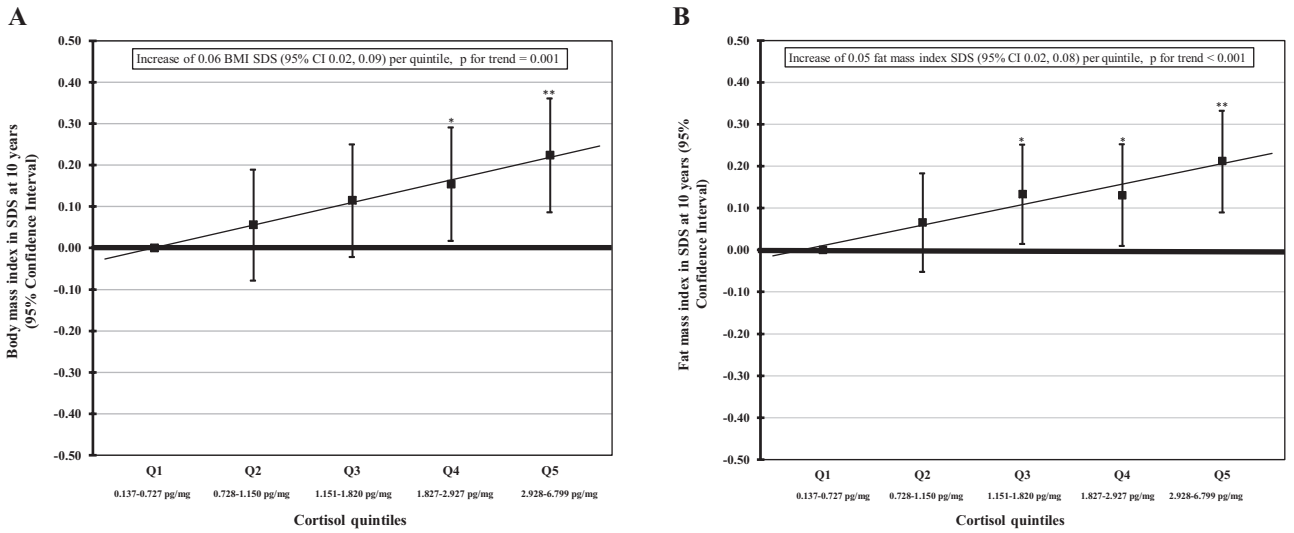

Figure 1. Associations of hair cortisol concentrations with general fat measures at 10 years (N = 2042). Values are linear regression coefficients (95% confidence interval) and reflect the change in SDS childhood BMI (A, N = 2037) and fat mass index (B, N = 2013) at 10 years for the cortisol quintiles. Models are adjusted for child's sex and age (except for sex- and age adjusted body mass index SDS), maternal prepregnancy BMI, psychological distress during pregnancy, maternal educational level and marital status at 6 years, child's ethnicity, hair color, and television watching time. Tests for trend were based on multiple linear regression models with hair cortisol concentration quintiles as a continuous variable. **P* < .05, ***P* < .017.

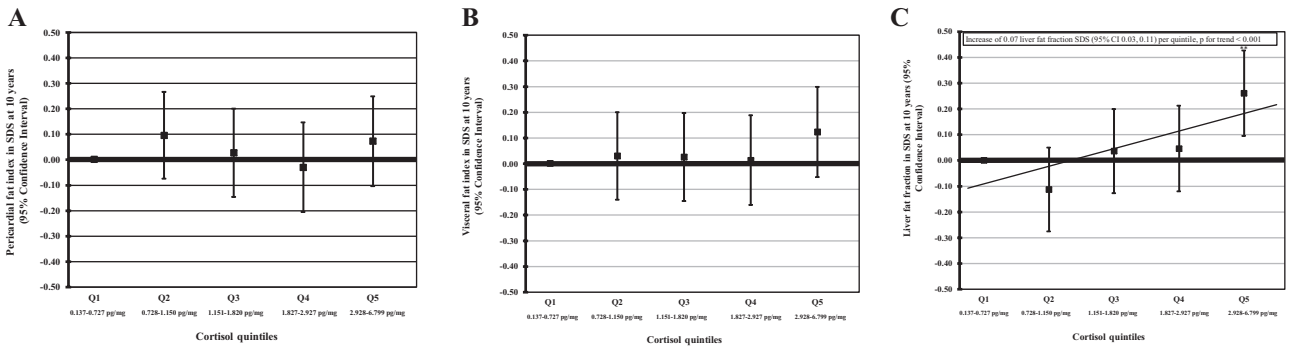

Figure 2. Association of hair cortisol quintiles with visceral and organ fat measures at 10 years (N = 1523). Values are linear regression coefficients (95% confidence interval) and reflect the change in SDS childhood pericardial fat (A, N = 1278), visceral fat (B, N = 1237) indices and liver fat fraction (C, N = 1361) for the cortisol quintiles. Models are adjusted for child's sex and age, maternal pre-pregnancy BMI, psychological distress during pregnancy, maternal educational level and marital status at 6 years, child's ethnicity, hair color, and television watching time. Test for trend was based on a multiple linear regression model with hair cortisol concentration quintiles as a continuous variable. **P* < .05, ***P* < .017.

Sensitivity analyses

Results from the models excluding children with all types of glucocorticoid use in the 3 months prior to hair sample collection were in the same direction and slightly stronger (16). Higher hair cortisone concentrations were associated with higher BMI and fat mass index, but with a lower pericardial fat index. Higher hair cortisone concentrations were not associated with liver fat fraction, and visceral fat index, or the risk of overweight, although effect estimates were in the same direction as the results for cortisol (16). The sensitivity analyses, in which we adjusted the main models for adiposity measures at 6 years, showed similar results as the conditional analyses (16). Also, these sensitivity analyses suggested that the associations with the risk of NAFLD remained significant, whereas the association with risk of overweight attenuated into nonsignificance (16).

Discussion

In this population-based prospective cohort study among 2042 children, we observed that higher HCCs at 6 years were associated with higher BMI, fat mass index, liver fat fraction, and increased risk of overweight and NAFLD at age 10. HCCs were not associated with visceral fat or pericardial fat indices.

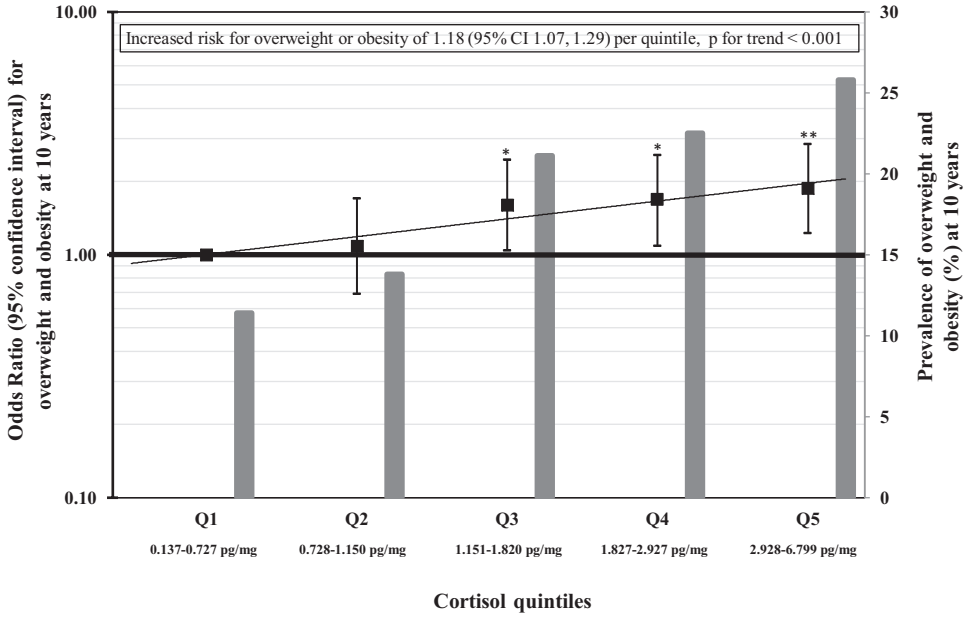

Figure 3. Associations of hair cortisol concentrations with risk of overweight and obesity at 10 years (N = 1898). Values on the left y-axis are odds ratios (95% confidence interval) on a logarithmic scale and represent the risk of childhood overweight at 10 years for the cortisol quintiles. Models are adjusted for child's sex and age, maternal pre-pregnancy BMI, psychological distress during pregnancy, maternal educational level and marital status at 6 years, child's ethnicity, hair color, and television watching time. Values on the right y-axis are percentages and represent the prevalence (%) of overweight at 10 years. Test for trend was based on a logistic regression model with cortisol quintiles as a continuous variable. *$P < .05$, **$P < .017$.

Interpretation of main findings

Previous studies reported associations of long-term cortisol concentrations in hair with BMI, and other adiposity measures in adults (2, 6, 11). A previous study in our cohort used a cross-sectional design and reported that higher hair cortisol and cortisone concentrations were associated with a higher BMI, fat mass index, and increased risk of overweight at age 6 years (31). We extended this study by examining the prospective associations of hair cortisol and cortisone concentrations at age 6 years with general and organ fat measures measured by MRI at 10 years.

We observed that HCCs at age 6 years were positively associated with childhood BMI, fat mass index, and the risk of overweight at 10 years. These findings are in line with the previous study, although we observed somewhat smaller effect sizes (31). This may partly be explained by a different design and smaller numbers of subjects. Additional conditional analyses and adjustment for BMI at 6 years showed that the associations of HCCs with BMI and risk of overweight were explained by the associations already present at 6 years. Also, we did not observe associations of HCCs with change in BMI or fat mass index SD score between 6 and 10 years, suggesting that the associations already observed at 6 years persist during childhood. A recent review including 12 cohort studies in children reported that a majority of studies showed a positive relationship between HCCs and BMI (12). Altogether, results from previous studies and our study suggest that higher cortisol concentrations are associated with higher BMI, fat mass index, and risk of overweight throughout childhood.

We observed that higher HCCs at 6 years were positively associated with liver fat fraction and a higher risk of NAFLD at 10 years. Additional conditional regression analyses and adjustment for fat mass index at 6 years suggest that these association were independent of fat mass at 6 years. To our knowledge, this study is the first to report associations of HCCs with visceral and organ fat in children. NAFLD is the most common liver disease in western populations, among both children and adults, and closely linked to the development of the metabolic syndrome (32, 33). Our findings are in line with an adult study showing that increased serum cortisol concentration was associated with an increased prevalence of NAFLD (34). It has been suggested that overactivity of the hypothalamic–pituitary–adrenocortical axis and increased glucocorticoids have an important role in the development of NAFLD (34-36). We did not observe an association of HCCs with visceral adiposity, a well-known consequence of hypercortisolism (34, 37). Pericardial fat is, next to visceral and liver fat, related to adverse cardiometabolic outcomes in adults which we know are associated with increased hair cortisol levels in adults (38, 39). We did not observe an association between hair cortisol and pericardial fat in childhood. It may be that the associations

between hair cortisol and visceral and pericardial fat become more apparent at older ages.

We performed 2 sensitivity analyses. The slightly stronger effect estimates after excluding children who used glucocorticoid medication might be explained by the exclusion of a more heterogeneous group of children with sometimes elevated cortisol concentrations due to exogenous causes. Also, hair cortisone concentrations were associated with childhood adiposity measures, but the effect estimates were weaker than for cortisol. This might be explained by the differences in biological activity.

There are various mechanisms through which cortisol concentrations may affect childhood adiposity. Increased cortisol levels increase appetite, specifically for very sweet and fatty foods, stimulate adipogenesis, induce insulin resistance, and negatively affect brown adipose tissue (2, 40). However, a bidirectional association, where changes in the cortisol metabolism, are consequences of metabolic changes accompanying adiposity, might also be present (41). Future research should explore the potential of reversed causation and examine underlying mechanisms of these associations. The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is expressed in the brain, adipose tissue and the liver and converts cortisone into active cortisol (42, 43). Regeneration of cortisol from inactive cortisone has been found to be increased in adipose tissue in obese individuals (41, 44). The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is expressed in the kidneys but also the placenta and fetus highly express this enzyme which converts cortisol into inactive cortisone, protecting the body from mineralocorticoid excess (44, 45). By inactivating the majority of maternal glucocorticoids passing to the fetus, 11β-HSD2 may prevent premature maturation of fetal tissues, decreased birth weight and consequent developmental "programming of later life diseases" (44, 46). Adverse circumstances during pregnancy such as maternal psychological distress may induce persistent changes and affect fetal programming of the hypothalamic–pituitary–adrenocortical axis and subsequent body composition and metabolic function (47-52). Future studies should identify fetal and early-childhood factors that influence cortisol concentrations and thereby lead to developmental adaptations with persistent consequences.

We observed that higher HCCs are associated with an adverse body fat profile, increased liver fat fraction and increased risk of overweight and NAFLD during childhood. These findings seem important, since it is well known that body fat distribution tracks from childhood into adulthood and is associated with cardiovascular disease in later life. (53, 54). Future research is needed to obtain further insight into the causality and underlying mechanisms of these associations and to assess whether childhood cortisol concentrations have effect on body fat development throughout adult life.

Strengths and limitations

Strengths of this study were the prospective data collection from early pregnancy onwards, the large sample size, detailed measurements of HCCs and childhood adiposity measures including organ fat measures assessed by MRI. This study also has limitations. Of all children who had information on hair cortisol at 6 years (N = 2926) only 2278 had information on at least 1 measurement of adiposity at 10 years. Selective nonresponse could lead to selection bias if the associations of HCCs at 6 years with childhood adiposity at 10 years differ between participants and nonparticipants. This seems unlikely, but cannot excluded. Another limitation of our study is the lack of hair cortisol measurements at the age of 10 years. Therefore we do not know how cortisol concentrations develop over time and if they partly explain the effects seen at the age of 10 years. Higher cortisol concentrations at both ages could be caused by continued stress but also genetic variation in genes such as *HSD11B1*, *HSD11B2*, *SPERINA6*, or *SPERINA1* may be a cause (44, 55). *HSD11B1* and *HSD11B2* encode enzymes 11β-HSD1,and 11β-HSD2, which are involved in the cortisol and cortisone metabolism (44). Between approximately ages 4 and 7 children undergo an adiposity rebound, resulting in accelerated increase in BMI. An early adiposity rebound is associated with an increased risk of obesity in later life (56, 57). The age at adiposity rebound may be important in the relation between cortisol and adiposity development and this should be addressed in future studies (58). Detailed information about a large number of potential confounding factors was available in this study. However, residual confounding, for example, by maternal and child stress prior to or around the time of hair cortisol examination, might still be present. Also, because of the observational design, no conclusions can be drawn yet on the causality and directionality of the observed associations.

Conclusion

Our results suggest that the associations of higher HCCs at age 6 years, with higher BMI, fat mass index and increased risk of overweight at age 10 years, are explained by the associations already observed at 6 years. The associations of higher HCCs at 6 years with liver fat fraction and NAFLD at 10 years were independent of fat mass index at 6 years. Future studies are needed to assess the causal pathways underlying these associations, the determinants of early-life cortisol concentrations and the long-term body fat and cardiometabolic consequences.

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: The general design of the Generation R Study is made possible by financial support from 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 received funding from grant ERC-2014-CoG-648916 from the European Research Council. This project received funding from the European Union's Horizon 2020 research and innovation program (848158, EarlyCause; 733206, LifeCycle).

Additional Information

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Disclosure Summary: 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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