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# Increased Cortisol and Cortisone Levels in Overweight Children

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Statistical Analysis C  
Data Interpretation D  
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## Background:

It has been unclear whether relatively high cortisol and cortisone levels are related to overweight in childhood, parental body mass index (BMI), and family dietary habits. The aim of this study was to compare cortisol and cortisone levels in urine and saliva from overweight and normal children, as well as correlations between children's BMI, parental BMI and family dietary behavior questionnaire score (QS).

## Material/Methods:

We analyzed the data from 52 overweight children and 53 age- and sex-matched normal-weight children aged 4–5 years. The concentrations of salivary cortisol (SF), salivary cortisone (SE), urinary cortisol (UF) and urinary cortisone (UE) were measured using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The family dietary behavior QS was answered by the parent mainly responsible for the family diet.

## Results:

Average cortisol and cortisone levels were significantly higher in overweight children. There was no significant difference in the ratio of cortisol to cortisone ( $R_{cc}$ ) and the marker of  $11\beta$ -hydroxysteroid dehydrogenase type 2 ( $11\beta$ -HSD2) activities. The results displayed correlations among cortisol, cortisone, and  $R_{cc}$ . Positive correlations were weak-to-moderate between BMI and SF, SE, UF, and UE. There were correlations between BMI and maternal BMI (mBMI), and BMI was significantly associated with QS.

## Conclusions:

Our results suggest that cortisol and cortisone levels are associated with overweight in children, but the  $11\beta$ -HSD2 activities showed no significant differences. Unhealthy family diet was associated with higher BMI, UF, and UE, and families with maternal overweight or obesity had a higher prevalence of children's overweight or obesity.

## MeSH Keywords:

**Child • Cortisol • Cortisone • Overweight**

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## Background

The prevalence of overweight and obesity in children is high and continues to increase. The prevalence of childhood overweight (36%) in Shanghai, China is higher than that in the USA [1]. Overweight and obesity often become latent or established at an early age, and few children actually develop overweight and obesity during adolescence [2]. Overweight children usually remain overweight or obese as adults [3]. Consequently, they are at risk of developing obesity-related illnesses such as cardiovascular disease and type 2 diabetes [3,4]. Additionally, childhood obesity is often accompanied by comorbidities including hypertension, impaired glucose tolerance, and dyslipidemia. The clustering of these cardiometabolic abnormalities is known as metabolic syndrome [5].

It is reported that individuals with excess systemic glucocorticoid are at risk for developing visceral obesity. Some studies have shown a positive association between serum cortisol levels and obesity in children or adolescents [6]. However, other studies presented an inverse association between serum cortisol level and adiposity [7]. A recent study demonstrated overweight and obese children have lower salivary cortisol levels than normal-weight children [8]. On the other hand, a recent review concluded that there is no strong relationship between urinary cortisol and obesity or metabolic syndrome [9]. These inconsistent conclusions have caused great interest in such researchers. Cushing's syndrome has the obvious characteristic of higher cortisol than normal [5], so simultaneous sampling and measurement of salivary and urinary cortisol in overweight children were not reported. Additionally, we should note that there is an inter-conversion between cortisol and cortisone catalyzed by 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD).

11 $\beta$ -HSD catalyzes non-adrenal, tissue-specific metabolism of glucocorticoids, containing 2 isozymes of 11 $\beta$ -hydroxysteroid dehydrogenases type 1 (11 $\beta$ -HSD1) and type 2 (11 $\beta$ -HSD2). 11 $\beta$ -HSD1 acts in metabolically important tissues, including liver, adipose, vasculature, brain, and macrophages, and catalyzes the conversion of cortisone to cortisol for specific cell types to utilize circulating cortisone for intracellular, non-adrenal cortisol production, resulting in high local cortisol concentration [10]. 11 $\beta$ -HSD2 is present in other epithelial tissues, mainly in the kidney, sweat glands, and intestinal epithelium tissues, and inactivates cortisol to cortisone [11]. A series of reports have shown that 11 $\beta$ -HSD has close association with obesity, and increased activity of 11 $\beta$ -HSD2 is closely correlated with obesity and its detrimental consequences [12]. The activities of 11 $\beta$ -HSD2 *in vivo* can be assessed by the ratio of cortisol to cortisone ( $R_{cc}$ ) [13], and studies have demonstrated that  $R_{cc}$  is related to obesity. Additionally, most studies utilized  $R_{cc}$  from urine or saliva matrices to assess the activities

of 11 $\beta$ -HSD2 because collecting urine and saliva is non-invasive and easy [14].

Recent research reported family eating behavior was related to parent's BMI, but not to children's adiposity [15]. However, more recent research results suggest that the mother's BMI is an important correlate of children's intake of energy [16]. Parental BMI showed a weak correlation with BMI and their children's adiposity, but children's risk of becoming overweight increased with parental overweight and obesity [17]. A large population-based study showed similar parental-offspring BMI associations when the offspring were 3 years old [18]. Research shows that girls growing up in families with vs. without parental overweight had divergent developmental trajectories for BMI and disinherited overeating [19]. Associations have been reported between parental feeding practices, dietary intake, and problematic eating behaviors in overweight children [20]. Therefore, it is important to research links among cortisol, cortisone, BMI, parental BMI, and family dietary behavior.

Herein, we studied cortisol and cortisone concentrations and  $R_{cc}$  in urine and saliva from children aged 4–5 years. The present study aimed to utilize a simultaneous assay of cortisol and cortisone, which is suitable for both saliva and urine matrices, and to determine whether overweight children have higher salivary cortisol (SF), salivary cortisone (SE), urinary cortisol (UF), urinary cortisone (UE), and  $R_{cc}$  than normal-weight children. We also examined associations among SF, SE, UF, UE, the ratio of cortisol to cortisone from saliva matrix ( $R_{scc}$ ), the ratio of cortisol to cortisone from urine matrix ( $R_{ucc}$ ), maternal BMI (mBMI), paternal BMI (pBMI), family dietary behavior questionnaire scores (QS), and children's BMI.

## Material and Methods

### Subjects and sampling

Three kindergartens in Suzhou of Jiangsu, China, were chosen to participate in the study. Taken together, these 3 kindergartens had essentially the same demographic make-up as the Chinese population at large regarding socioeconomic level. Informed consent in the study was obtained from the legal guardians of children. According to Cole et al. [21], 55 overweight and 55 normal-weight children aged 4–5 years were chosen, and the sex ratio was close to 1 (males/females, 27/28) in each group. Sex was not considered a factor in the statistical analysis of the data. Every subject's pBMI and mBMI was obtained by measuring their parents' height and weight. Additionally, a Family Eating Behavior Questionnaire (FEBQ) was answered by the adult mainly responsible for the whole family diet. The FEBQ for the Chinese family designed by the China Hi-Tech Health Industry Committee (CHHC) was used for assessment

**Table 1.** Cortisol and cortisone levels and  $R_{cc}$  for normal and overweight children (mean  $\pm$ SD).

	Overweight (n=52)	Normal weight (n=53)	P value of difference
SF (ng/mL)	1.97 $\pm$ 0.86	1.24 $\pm$ 0.63	<0.001
SE (ng/mL)	32.25 $\pm$ 10.13	21.75 $\pm$ 9.84	<0.001
$R_{scc}$	0.068 $\pm$ 0.035	0.062 $\pm$ 0.039	NS
UF/cr ( $10^{-5}$ )	5.46 $\pm$ 1.92	3.74 $\pm$ 1.43	<0.001
UE/cr ( $10^{-5}$ )	8.37 $\pm$ 3.31	6.01 $\pm$ 2.06	0.002
$R_{ucc}$	0.67 $\pm$ 0.19	0.68 $\pm$ 0.17	NS

SF – salivary cortisol; SE – salivary cortisone;  $R_{scc}$  – ratio of cortisol to cortisone in saliva matrix; UF/cr – urinary cortisol/creatinine; UE/cr – urinary cortisone/creatinine;  $R_{ucc}$  – ratio of cortisol to cortisone in urine matrix; NS – not significant.

of the health habits of family eating behavior. The eating behavior score calculated using the FEBQ scale is inversely proportional to the health level of eating behavior, so higher QS means less healthy family eating behavior.

To achieve a high rate of participation and homogeneous sampling conditions, sampling was conducted during school hours (8: 30 a.m.). Before sampling, the children were instructed to remain as calm as possible for half an hour, during which eating was prohibited. Self-study activities such as drawing, reading, and writing were encouraged to keep children calm. Teachers supervised and experimenters sampled at kindergartens. A commercial Salivette tube containing a cotton wool swab was used to collect saliva. Subjects gargled twice with purified water and then a swab was rotated in the mouth for 2–4 min. When the swab was saturated with saliva, it was inserted back into the tube. Then, subjects were instructed to collect a urine sample with a urine cup having an interior for collecting urine, a lid, and a chamber for holding liquid. All samples were stored upon collection in a -50°C freezer until analysis.

### Concentration measurements

The quantitation of cortisol and cortisone was analyzed by HPLC-MS/MS method [22] with some modifications, mainly in sample pre-treatment, so that samples were thawed at room temperature and centrifuged twice at 35 000 rpm for 5 min, then supernatants were removed for assay. Urine creatinine (cr) concentrations were determined using a colorimetric reaction (WS/T 97-1996, China). All samples were assayed in duplicate. The average within-assay coefficient of variation for creatinine was 3.9%, and the inter-assay variability was 7.9%.

### Statistical analysis

Five subjects were excluded: 3 due to lack of salivary sample and 2 due to lack of urinary sample. Statistical analysis was performed using SPSS version 22 (IBM SPSS Statistics). Data

that were not normally distributed were logarithmically transformed. Differences in SF, SE,  $R_{scc}$ , UF, UE, and  $R_{ucc}$  between the 2 groups were analyzed with one-way ANOVA. Correlations were analyzed using Pearson's coefficients rho. A p value lower than 0.05 was regarded as statistically significant.

## Results

As Table 1 shows, the levels of SE, SF, UE, and UF differed significantly between the 2 groups. The overweight children displayed significantly higher levels compared with their normal-weight counterparts. No significant differences in  $R_{scc}$  or  $R_{ucc}$  were found between the 2 groups.

Table 2 shows the association among measurements and values. SF and SE were moderately correlated ( $P=0.657$ ,  $r<0.001$ ), as were UF/cr and UE/cr ( $P=0.753$ ,  $r<0.001$ ). Positive correlations were weak-to-moderate between  $R_{scc}$  and SF ( $P=0.488$ ,  $r<0.001$ ), as were  $R_{ucc}$  and UF/cr ( $P=0.466$ ,  $r=0.004$ ). Positive correlations were weak-to-moderate between children BMI and SF ( $P=0.426$ ,  $r=0.001$ ), and SE ( $P=0.435$ ,  $r<0.001$ ), and UF/cr ( $P=0.446$ ,  $r<0.001$ ), and UE/cr ( $P=0.423$ ,  $r=0.001$ ). There were correlations between BMI and mBMI ( $P=0.376$ ,  $r=0.003$ ). pBMI was not significantly correlated to any value in Table 2. Positive correlations were found between QS and UF/cr ( $P=0.276$ ,  $r=0.044$ ) but a weak correlation was found between QS and UE/cr ( $P=0.361$ ,  $r=0.007$ ). Children's BMI was significantly related to QS ( $P=0.350$ ,  $r=0.009$ ).

## Discussion

This study shows that overweight children display higher cortisol levels in salivary and urinary samples. The results support the observation by Veldhorst et al. [23], who found higher hair cortisol concentration in obese children than in normal-weight children. However, our findings contradict the results

**Table 2.** Pearson's correlation coefficients between concentrations and measures.

	SF	SE	$R_{scc}$	UF/cr	UE/cr	$R_{ucc}$	BMI	mBMI	pBMI	QS
SF	1									
SE	0.657* <0.001**	1								
$R_{scc}$	0.488 <0.001	-0.226 0.028	1							
UF/cr	-0.161 NS	-0.227 NS	0.015 NS	1						
UE/cr	-0.155 NS	-0.184 NS	0.000 NS	0.753 <0.001	1					
$R_{ucc}$	0.009 NS	-0.043 NS	0.026 NS	0.466 0.004	-0.280 0.028	1				
BMI	0.426 0.001	0.435 <0.001	0.105 NS	0.446 <0.001	0.423 0.001	-0.057 NS	1			
mBMI	0.187 NS	0.182 NS	0.045 NS	-0.086 NS	-0.113 NS	0.056 NS	0.376 0.003	1		
pBMI	0.128 NS	-0.030 NS	0.295 0.023	-0.026 NS	-0.040 NS	0.016 NS	0.212 NS	0.061 NS	1	
QS	0.089 NS	0.042 NS	0.058 NS	0.276 0.044	0.361 0.007	0.094 NS	0.350 0.009	0.006 NS	0.268 0.048	1

\* The first number is the *p* value and \*\* the second is the *r* value. SF – salivary cortisol; SE – salivary cortisone;  $R_{ucc}$  – ratio of cortisol to cortisone in urine; UF/cr – urinary cortisol/creatinine; UE/cr – urinary cortisone/creatinine;  $R_{scc}$  – ratio of cortisol to cortisone in saliva; BMI – body mass index; mBMI – maternal BMI; pBMI – paternal BMI; QS – questionnaire score; NS – not significant.

of Kjölhede et al. [8], who reported lower salivary cortisol levels in overweight and obese children than in normal-weight children, and also contradict the results of Chalew et al. [24], who found lower plasma cortisol levels in obese children than in normal-weight children. Knutsson et al. [25] found no association between cortisol levels and body composition, but recent studies tend to show a positive association between cortisol levels and obesity [6,26], body fat distribution [27], insulin resistance [6,28], and the metabolic syndrome [29] in children and adolescents. Table 1 shows that the salivary cortisol concentration levels were significantly different between overweight and normal-weight children ( $P=0.001$ ), as well as a significant difference in urinary cortisol concentration ( $P=0.025$ ). This raises the questions of whether elevated cortisol concentrations play a role in the development of obesity, and whether higher cortisol levels in overweight children are relevant to subsequent obesity. It appears that cortisol concentration maintains a higher level for a long time before a child becomes obese.

Our study also displays a significant difference in cortisone concentration between overweight and normal-weight children ( $P<0.05$ ) and a significant difference in urinary cortisone concentration ( $P<0.05$ ). To the best of our knowledge, our study is the first to show increased cortisol and cortisone concentrations of simultaneous saliva and urine samples in

overweight children compared with normal-weight children aged 4–5 years. The ratio of cortisol to cortisone ( $R_{cc}$ ) is an important indicator [13]. Table 1 shows the  $R_{scc}$  and  $R_{ucc}$  values. The mean  $R_{scc}$  value was 0.068 or 0.062 and it is lower than reported values using similar detection methods [30] with adult subjects, which may mean more cortisol is converted to cortisone, and the 11 $\beta$ -HSD2 is more active in children. However, the study found no difference in  $R_{scc}$  and  $R_{ucc}$  between the 2 groups, which may mean that the activity of 11 $\beta$ -HSD2 is not abnormal for overweight children.

Due to the conversion of cortisol to cortisone catalyzing by 11 $\beta$ -HSD2, cortisol is positively related to cortisone and, which is clearly shown in Table 2. Additionally, BMI is significantly related to SF, SE, UF/cr, and UE/cr, which supports the results of Margriet et al. [23], who found higher cortisol levels with increasing BMI. This study showed a correlation between BMI and mBMI, which is also consistent with the reported studies [31]. This finding may simply mean that families with maternal overweight are more likely to have overweight children. Thus, the association between children's weight and maternal BMI confirms the need for prevention and intervention efforts for childhood obesity in families, especially with overweight mothers. Furthermore, we found pBMI has no correlation with children's values, consistent with the observation [32] that the strength of the association

between maternal obesity and the overweight or obesity children is higher than that of paternal obesity.

In China, fathers usually are not mainly responsible for the whole family diet, and mothers are usually the primary caregivers for young children and take the main responsibility for children's food intake [33]. Therefore, mothers are prone to choose their favorite food for themselves and children, which increases the likelihood of eating too much [31]. Mothers might influence children's diets more than fathers do [34]. Thus, the correlation between mothers' weight and QS and children's weight was stronger. The correlation shown in Table 2 between QS and BMI, UF/cr, and UE/cr may reflect less healthy family eating behavior, with higher BMI and higher urine cortisol and cortisone levels. Further study should be continued to explore whether children's weight reducing will lead to cortisol level dropping or not when family dietary habit and strategy are changed.

This study concerns children 4–5 years old, but one weakness was that our findings were based on a relatively small number of children, which may have limited the statistical power. Second, the cross-sectional design limits any inferences about the direction of causality for the association (e.g., between salivary cortisol and urinary cortisol). Third, our study focused on children in China, and our findings may not be generalizable to other populations. Furthermore, unmeasured or residual confounding is also possible. To sum up, the factors mentioned above pose difficulties when comparing results from different studies. More research (e.g., longitudinal study design and covariates consideration) is needed and research is warranted to investigate whether increased cortisol levels in overweight children lead to obesity, whether the  $R_{scc}$  and/or  $R_{ucc}$  is consistent as the overweight children become obese, and whether a bidirectional association between elevated cortisol or  $R_{cc}$  and overweight, as well as the influences of family dietary habit and parent's BMI on children's weight. This study showed the correlation among overweight, unhealthy diet, and higher cortisol, which is analogous to an observation reported that diet-induced weight loss was associated with lower cortisol [35]. Therefore, further research is necessary to explore the interactions among BMI, cortisol, cortisone, and diet.

### Measurement method consideration

One problem in cortisol measurements is the presence of a higher concentration of potentially interfering steroids. Higher specificity implies lower and more comparable results, assuming the use of HPLC-MS/MS technology. In this paper, simultaneous measurement of cortisol and cortisone using an HPLC-MS/MS approach was conducted, and the analyte concentration was lower than that reported by Kjölhede et al. [8] using commercial enzyme immunoassay (EIA), which agrees with a report comparing radioimmunoassay (RIA) and LC-MS/MS methods for salivary cortisol and cortisone measurement [30].

Therefore, an additional factor to be considered when comparing results from different studies is the use of a multitude of analytical methods, for example RIA [9,25], DELFIA [36], EIA [8], ELISA [37–39], chemical immune luminescence assay [40], and LC-MS/MS [9,22,41]. We used HPLC-MS/MS method for simultaneous measurement of these biomarkers, which would be a valuable tool for children due to difficulty in taking blood samples. Furthermore, simultaneous measurements of more than 1 biomarker in several samples could provide greater study strength. Fortunately, our results are comparable to the ones recently reported in the literature [20–23]. Salivary cortisol values obtained using the HPLC-MS/MS were significantly lower than the ones obtained with the direct RIA technique. That result was expected in light of the greater analytical specificity of the HPLC-MS/MS methodology. The HPLC-MS/MS method compares favorably with the immunity assay for cortisol measurement, with the additional possibility of concomitant cortisone measurement and the evaluation of 11 $\beta$ HSD2 activity, which is consistent with the latest research results [30].

### Conclusions

The results suggest that compared with normal-weight children, overweight children have higher cortisol and cortisone levels in simultaneously collected saliva and urine sample, but  $R_{scc}$  and  $R_{ucc}$  were not different. We also found a weak correlation between urinary cortisol and salivary cortisol but no correlation between  $R_{ucc}$  and  $R_{scc}$ . These results suggest that it might be relevant to consider cortisol and cortisone promotion in overweight children, but the 11 $\beta$ -HSD activities are not abnormal compared with normal-weight children. We also found correlations among cortisol, cortisone, and  $R_{cc}$ . Positive correlations were weak-to-moderate between BMI and salivary cortisol, salivary cortisone, urinary cortisol, and urinary cortisone. There were correlations between BMI and maternal BMI, and BMI was significantly related to family dietary behavior questionnaire scores. Our association study shows that less healthy family eating behavior is associated with higher BMI, as well as higher cortisol and cortisone levels. Our findings emphasize the need for further research on the relationship between overweight/obesity in children and cortisol, cortisone, and  $R_{cc}$ , as well as the influences of parent's BMI and family dietary habits on children's weight.

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### Conflict of interest

The authors declare no conflict of interest.



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