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Salivary Cortisol does not Correlate with Metabolic Syndrome Markers or Subjective Stress in Overweight Children

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Abstract

Objective: Being overweight is a risk factor for metabolic syndrome in children, but not all overweight children develop metabolic syndrome. Cortisol excess from chronic psychological stress has been proposed as an independent risk factor for metabolic syndrome in this already at-risk population.

The present study assesses the relationship of biochemical and body composition radiographic markers of metabolic syndrome to salivary cortisol and self-reports of chronic psychological stress in a cohort of overweight children.

Methods: This prospective, cross-sectional study took place in a multi-disciplinary pediatric obesity clinic at a tertiary care hospital, and involved fifteen children with BMI at or above the 85th percentile for age and sex, 10 of whom provided salivary cortisol samples. The main outcomes measured were salivary bedtime cortisol, first-waking cortisol, and cortisol awakening response (CAR-the rise in cortisol in the first half hour after waking); fasting serum triglycerides, HDL cholesterol, glucose and insulin for HOMA-IR; the ratio of abdominal fat to total body fat by DXA scan; and scores of validated stress and bullying questionnaires (PANAS-C, PSS, and SEC-Q).

Results: In this pilot study, no correlation was found between salivary cortisol measures and questionnaire scores of subjective stress or bullying, and no correlation was found between any of these measures and markers of metabolic syndrome (dyslipidemia, insulin resistance, increased abdominal fat).

Conclusions: These results suggest that measures of psychological stress, whether biochemical or subjective, do not appear to predict risk of metabolic syndrome in overweight children. While ease of collection and demonstrated utility both in detection of pediatric Cushing disease and in adult psychological research make salivary

cortisol assessment an attractive clinical tool, further investigation into the value of salivary measures in pediatric stress research is needed.

Keywords: Salivary cortisol; Psychological stress; Chronic hypercortisolemia; Metabolic syndrome; CAR (Cortisol awakening response)

Introduction

Pediatric obesity is leading to a significant increase in early-onset type 2 diabetes, non-alcoholic fatty liver disease, and other metabolic derangements, and these in turn strongly correlate with risk of cardiovascular disease in adulthood [1]. It is poorly understood, however, why some children experience greater morbidity than others from similar degrees of adiposity.

Obesity as defined by body mass index (BMI) does not distinguish between differences in body composition and fat distribution, and thus is an inaccurate predictor of insulin resistance, fatty liver disease, and other components of the metabolic syndrome (MetS). In both obese and non-obese children [2,3] adipose tissue located in ectopic sites (e.g. liver, viscera, muscle) predicts insulin resistance and risk for type 2 diabetes better than BMI. Differential ectopic vs. subcutaneous deposition of fat is influenced by ethnicity [4] genetic polymorphisms such as PNPLA [3,5] and psychological states such as depression in both adolescents and adults [6-8].

Psychological stress leading to chronic amplification of the hypothalamic-pituitary-adrenal (HPA) axis has been proposed as another potential factor promoting deposition of fat into viscera and other ectopic sites, thereby increasing the risk for insulin resistance, hepatic steatosis and dyslipidemia [9,10]. While precise mechanisms remain poorly understood, chronic cortisol elevation increases central (especially visceral) adipose tissue deposition while peripheral subcutaneous depots recede [11]. Visceral fat has more inflammatory cells (macrophages) than

subcutaneous fat [12] and more actively produces adipokines [13] that contribute to a chronic inflammatory state and insulin resistance.

An association between psychological stress, chronic amplification of “stress physiology” (i.e. HPA axis activity, sympatho-adrenal medullary activity, and cardiac autonomic activity), and metabolic syndrome is supported primarily by animal and adult human studies, some of which also demonstrate reversibility [14-17]. A small number of pediatric studies show variable relationships between chronic psychological stress and obesity [18-24]. To our knowledge, no study has explored in a single group the relationship between self-perceived stress, objective bullying, chronic activation of the HPA axis, adipose tissue distribution, and biochemical components of MetS. This study examines the degree to which insulin resistance, dyslipidemia and ectopic fat deposition correlate with chronic psychological stress and salivary cortisol measures of HPA axis activation in overweight children and adolescents.

Methods

Subjects

This is a prospective, cross-sectional study of fifteen male and female patients' aged 10-18 years (average 13.7) with BMI \geq 85th percentile, recruited from the University of Wisconsin Pediatric Fitness Clinic. Boys (8) and girls (7) were nearly equally represented, and the racial make-up included African Americans (1 male, 2 females), Latino Americans (2 males, 1 female), and European Americans (5 boys, 4 girls) in proportion to their relative presence in the local community.

All subjects showed pubertal levels of LH. Exclusion criteria included recent oral or inhaled glucocorticoid therapy, medications for dyslipidemia or blood sugar management, and a diagnosis of diabetes. Ten subjects completed the salivary cortisol portion of the study. The study was reviewed and approved by the Human Subjects Committee Institutional Review Board (IRB) of the University of Wisconsin-Madison School of Medicine and Public Health.

Measurements

Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm. Weight was measured on a calibrated beam balance platform scale to the nearest 0.1 kg. BMI z-score (BMI z) and BMI-for-age percentiles were computed using the CDC reference values.

Laboratory

A fasting morning blood sample was drawn for glucose, insulin, HDL, triglycerides, and LH. All serum labs were performed in the UW Health Sciences Core Laboratory. Insulin was measured by chemiluminescent immunoassay.

Salivary cortisol samples were collected by subjects at home using SARSTEDT Salivette-Blue Cap tubes upon first waking (fasting), 30 minutes after waking (fasting), and again between 9

and 11 PM (at least 3 hours since last food ingestion). Salivary cortisol analysis was performed at Mayo Medical Laboratories.

Imaging

The ratio of abdominal fat to total body fat was determined via DXA using the Norland XR-800 whole body bone densitometer (Norland Corporation, Ft. Atkinson, Wisconsin USA) and tissue masses were analyzed using software Illuminatus version 4.5.0. Scans were performed by the same investigator, and each scan session was preceded by a calibration routine using multiple quality control phantoms that simulate soft tissue and bone.

Body composition values of total bone, muscle and fat mass, as well as %FAT were measured, with abdominal fat being calculated using the midriff region extending from the bottom of the ribcage to the top of the pelvis (iliac crest).

Psychological Stress and Bullying Assessment

Psychological stress was assessed by three measures: The Perceived Stress Scale (PSS) [25], a 10-item self-report measure of stress perception/appraisal [26]; The Positive and Negative Affect Schedule for Children (PANAS-C) [27], a 30-item self-report completed by both the child and the parent [28]; and the Social Experience Questionnaire (SEQ), a 15-item self-report assessment of bullying, an objective source of stress [29-31]. Questionnaires were administered by clinic staff within a week of the clinic appointment.

Statistical Analysis

All study outcomes were summarized in terms of means \pm standard deviations. Nonparametric Spearman's rank correlation analyses were conducted to evaluate the associations between markers. Fisher z-transformation was utilized to construct the 95% confidence intervals for the correlation coefficients. All reported P-values are two-sided and $P < 0.05$ was used to define statistical significance. Statistical analyses were conducted using SAS software version 9.4 (SAS Institute Inc., Cary NC).

Results

There were no significant correlations of MetS risk markers with either salivary cortisol measures of HPA axis elevation or self-reported chronic stress and bullying (**Table 1**). In addition, salivary cortisol measures of HPA axis activity did not correlate with self-reported chronic stress or bullying.

Lack of correlations persisted when boys and girls were studied separately (data not shown). Separate analyses were made with each of the separate subsections of the SEQ-S (bullying) questionnaire, and lack of significance persisted (results not shown).

Finally, an additional analyses (data not shown) for correlation between the questionnaires showed strong and significant correlation (+0.82, $p < 0.001$) between the two stress

questionnaires (PSS and PANAS-S), but no correlation between these and the SEQ-S questionnaire.

Table 1: Correlations between self-reported stress, HPA activity, and markers of metabolic syndrome.

Comparison	Correlation Coefficient (95% CI)	P-value
Ratio abdominal/total fat vs. waking cortisol	-0.23 (-0.70-0.41)	0.477
Ratio abdominal/total fat vs. CAR	+0.20 (-0.37-0.66)	0.482
Ratio abdominal/total fat vs. PSS	-0.11 (-0.64-0.50)	0.737
Ratio abdominal/total fat vs. PANAS-S	-0.40 (-0.77-0.21)	0.180
Ratio abdominal/total fat vs. SEQ-S	-0.18 (-0.67-0.41)	0.542
HOMA-IR vs. waking cortisol	-0.54 (-0.84-0.08)	0.073
HOMA-IR vs. CAR	+0.46 (-0.11-0.79)	0.094
HOMA-IR vs. PSS	+0.38 (-0.27-0.78)	0.224
HOMA-IR vs. PANAS-S	+0.02 (-0.56-0.59)	0.949
HOMA-IR vs. SEQ-S	+0.22 (-0.40-0.71)	0.478
HDL vs. waking cortisol	-0.08 (-0.62-0.52)	0.803
HDL vs. CAR	-0.13 (-0.62-0.43)	0.646
HDL vs. PSS	-0.08(-0.62-0.52)	0.810
HDL vs. PANAS-S	+0.31 (-0.33-0.74)	0.328
HDL vs. SEQ-S	-0.02 (-0.58-0.56)	0.962
Triglycerides vs. waking cortisol	-0.43 (-0.80-0.21)	0.166
Triglycerides vs. CAR	+0.49 (-0.08-0.80)	0.078
Triglycerides vs. PSS	+0.16 (-0.46-0.67)	0.616
Triglycerides vs. PANAS-S	-0.05 (-0.61-0.54)	0.871
Triglycerides vs. SEQ-S	+0.03 (-0.55-0.59)	0.924

Discussion

This study found no correlation between markers of metabolic syndrome and either salivary markers of HPA axis activity or self-reports of chronic stress and bullying. The results suggest that any potential correlation between these parameters in overweight pubertal children is not robust enough to manifest in a small sample size. It is also possible that salivary cortisol levels do not correlate with subjective measures of stress in children as well as in adults, perhaps because the HPA axis in children is more resilient to chronic stress, or because the duration of stress has been insufficient to result in chronic alteration of HPA activity. Furthermore, while salivary cortisol is validated for detection of cortisol excess in conditions such as Cushing syndrome, it has not yet been proven to reliably detect lower levels of hypercortisolemia [32] in children and adolescents.

Despite the negative results of this study, further exploration of these relationships is supported by a trend toward significant correlation between salivary cortisol measures and HOMA-IR in this study, as well as findings of other studies. For example, in

rats and primates, stress-induced cortisol hyper-secretion increases central fat deposition [16,17] In adult humans, cortisol secretion and self-report of psychological stress have been positively associated with central adiposity [33] and both cardiovascular and inflammatory stress responses have shown correlation with increasing waist circumference [34].

Studies in children show conflicting results [23] and are limited in number, sample size, and scope of parameters studied (e.g. to a single serum cortisol sample [18,22] or to questionnaire data alone [19,35]). For example, school-related stressful events were positively associated with visceral and subcutaneous adipose tissue in peri-pubertal Latino girls with high CAR, and not in girls with low CAR [20] but laboratory assessment for MetS were not performed. In overweight (BMI>85th percentile) Latino youth, markers of MetS (increasing BMI, body fat mass by DXA, abdominal adipose tissue by MRI, fasting lipids, decreasing insulin sensitivity) were associated with higher morning serum cortisol [18] but psychological stress was not assessed. In obese peri-pubertal children, fasting morning serum cortisol and especially 24-hour urine free cortisol were associated with increasing waist circumference and laboratory markers of MetS [21] but body composition and psychological stress were not assessed.

Associations between activation of the HPA axis and psychological stress in children also show variable results depending on testing methods. For instance, in adolescent girls, increasing BMI and central adiposity by DXA scan correlated positively with overnight urine free cortisol, but correlated negatively with mid-day pre- and post-venipuncture serum cortisol (controlled stress event) [24]. An explanation offered for these divergent findings is that chronic stress-induced elevation of baseline diurnal activity of the HPA axis eventually leads to blunting of acute reactivity [36]. On the other hand, in a longitudinal study of adolescents, increasing BMI was associated with decreases in salivary cortisol at all times of the day [37]. Further, in a large cohort of obese children and adolescents, while a weak correlation was found between fasting morning serum cortisol and laboratory markers of MetS, no correlation was found with waist circumference [22].

The measurement of salivary cortisol is now considered a feasible, reliable, and accurate indicator of serum free cortisol, and late-night salivary sampling has emerged as a valuable screening test for hyperactivity of the HPA axis in suspected Cushing disease and other states of cortisol excess in adults and children [38]. Salivary cortisol measurement for stress research especially in adults has also expanded significantly in recent years [36,39-41] but while its use has also become more prevalent in pediatric research, the interpretation of both the awakening salivary cortisol and CAR in children has not been reliably established [32]. Self-reported stress in adults is associated with both increased salivary cortisol upon first waking and CAR [36] but the association in adults of waist circumference with both waking salivary cortisol and CAR has been conflicting, with some finding a positive correlation [42] and some finding no correlation [43].

This study was limited by small sample size; an expanded study would provide more power to discern subtler associations

between chronic stress, HPA axis activation, and markers of MetS. Furthermore, while the study population included children with ethnic and economic characteristics that have been associated with stress, it is reasonable to speculate that levels of stress in this study population-organized and motivated enough to attend obesity clinic-were insufficient to illuminate associations. Finally, while the questionnaires used have all been validated for this type of research, simultaneous use of three questionnaires with different Likert scales (e.g. from 0-4 vs. from 1-5), time periods assessed (e.g. over the past 1 week vs. 1 month vs. in general), and formats may have been confusing to the subjects. The finding of high correlation between the PSS and PANAS-S, however, suggests that at least these stress questionnaires were in fact accurate gauges of stress in these children.

Conclusion

In conclusion, while theoretically attractive, precise relationships between psychological stress, activation of the HPA axis, deposition of ectopic fat and risk for metabolic syndrome remain unresolved. Optimal and feasible approaches to measuring perceived stress and HPA axis activation in children also merit further study. If psychological stress in children can be linked to chronic HPA activation and components of MetS including ectopic fat distribution, insulin resistance and dyslipidemia, a potential role for stress-reducing interventions (e.g. mindfulness training) to reduce the morbidity of obesity could emerge.

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Competing and Conflicting Interests

The authors report no conflicts of interest.

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