RESEARCH PAPER

Adiposity and Cortisol Response to Stress in Indian Adolescents

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Objective: We examined associations of different adiposity measures with cortisol responses during the Trier Social Stress Test for children (TSST-C).

Design: Descriptive study.

Setting: Holdsworth Memorial Hospital, Mysore, India.

Participants: Adolescents aged 13.5y from a birth cohort were recruited (*N*=269, 133 boys).

Methods: The stressor (TSST-C) was 5-minutes each of public speaking and mental arithmetic tasks in front of two unfamiliar 'judges'. Salivary cortisol concentrations were measured at baseline and at regular intervals after TSST-C. Weight, height, sub scapular and triceps skinfold thickness, and waist and hip circumference were measured, and percentage body fat was estimated (fat%; bioimpedance). Body mass index (BMI) and Waist-to-hip ratio (WHR) were calculated. All variables were converted into within-cohort SD scores before analysis. Stress-induced change in cortisol concentrations from baseline (cortisol response) was examined in relation to adiposity.

Results: Stress increased cortisol concentrations significantly from baseline (mean (SD): 5.5 (6.4) ng/mL; *P*<0.001). Higher WHR was associated with lower cortisol response at 20 and 30-minutes after stress (~0.13 SD decrease in cortisol response per SD higher WHR, *P*<0.05). Higher fat% was also associated with lower cortisol response only in girls 20-minutes post-stress (0.23 SD lower response per SD higher fat%, *P*=0.004). Sum of skinfold thickness and BMI were not associated with cortisol responses.

Conclusions: Abdominal adiposity is associated with reduced hypothalamic-pituitary-adrenal axis reactivity to stress in this adolescent population.

Keywords: *Obesity, Trier Social Stress Test-Children, Stress response, Waist-to-hip ratio.*

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Psychological stress is a well-recognized risk factor for adult non-communicable diseases (NCD). Chronic stress results in dysregulated hypothalamic-pituitary-adrenal (HPA) axis activity and abnormal cortisol release, which trigger the phenotypic aberrations of stress-related disorders [1]. Increased central/ abdominal adiposity is one of the proposed consequences of chronic stress. Central adiposity in turn may alter HPA axis responses [2]. This may then amplify NCD risk in obese individuals.

Indians have higher truncal and abdominal adiposity relative to lean body mass and this is thought to contribute to their increased susceptibility to NCDs [3]. Indians may be particularly sensitive to the effects of cortisol, especially in the presence of higher adiposity, which may add to their disease risk [4]. Both adiposity and stress levels are increasing steadily in Indian children and adolescents. We aimed to test the hypothesis that higher adiposity is associated with altered cortisol response to stress in Indian children. We examined associations of different adiposity measures on cortisol responses measured during the Trier Social Stress Test for Children (TSST-C) in adolescents from the Mysore Parthenon Cohort.

METHODS

The Parthenon cohort was established at Holdsworth Memorial Hospital (HMH), Mysore during 1997-1998 to examine early-life factors associated with adult NCD risk [5]. The original cohort comprised 663 normal singleton babies born to mothers whose anthropometry and gestational diabetes (GDM) status were assessed at ~30 weeks of gestation (***Fig.* 1**). The babies were followed-up regularly from birth. At 13.5 years, 545 children were available for anthropometry, and cardio-metabolic and cognitive assessments. During 2011-2012, in a subsample (*N*=273), we adapted and administered the TSST-C, a well-accepted method of standardising the stressor component in a research setting [6]. The TSST has been

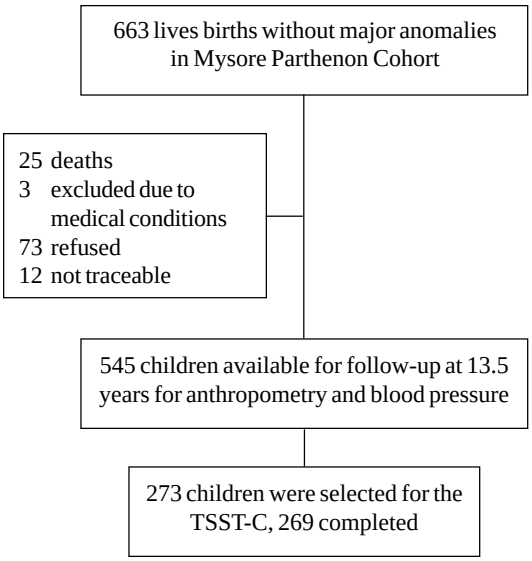

FIG. 1 *Flow chart of the study participants.*

shown in European populations to produce reliable cortisol response in adolescents [7]. All cohort children living in Mysore city (*N*=354) were eligible for the study. Equal number of eligible boys and girls representing different birth weight quartiles were recruited consecutively in the chronological order of their birth until the target number was reached.

A baseline salivary sample was collected 10 minutes before the TSST-C, after the children had watched a calming video for 5 minutes. For the TSST-C, each child completed 5-minute each of public speaking (imaginative story telling) and mental arithmetic tasks (serial subtraction) in front of two unfamiliar adult 'evaluators' as described before [8]. Post-test salivary samples were collected at 10, 20, 30, 40 and 70 minutes after stress induction.

Weight (Salter, UK), height (Microtoise, CMS instruments, UK), subscapular and triceps skinfold thickness (Harpenden callipers, CMS instruments), and waist and hip circumference (anthropometric tape) were measured. Body mass index (BMI) and waist-to-hip circumference ratio (WHR) were estimated. Percentage body fat (fat%) was measured using the Bioimpedance method (Bodystat, Quadscan 4000, UK). Resting systolic and diastolic blood pressures (BP) were measured using an automated BP monitor (Dinamap 8100, Criticon, USA). Pubertal development was assessed as the stage of breast development (girls) or genital development (boys) using Tanner's method [9]. Socio-economic status (SES) was determined using the Standard of Living Index designed by the National Family Health Survey-2 [10]. Fasting blood samples were collected the following day.

Laboratory assays were carried out at the Diabetes Unit, KEM Hospital Research centre, Pune. Salivary cortisol concentrations were measured using an ELISA method (Alpco Diagnostics, USA). The assay sensitivity was 1 ng/mL; inter- and intra-assay coefficients of variation were 10.0% and 6.6%, respectively. Plasma glucose, insulin and lipid concentrations were measured, as described elsewhere [11]. Insulin resistance was estimated using the Homeostasis Model Assessment (HOMA-IR) equation [12].

The ethics committee of Holdsworth Memorial Hospital approved the study; informed written consent from parents and assent from children were obtained.

Statistical methods: Cortisol and insulin concentrations and HOMA-IR were log-transformed to satisfy the assumption of normality. Partial correlations were used to examine associations between adiposity measures and cardio-metabolic outcomes. Associations of BMI, fat%, and sum of subscapular and triceps skin fold thickness (subcutaneous adiposity) and WHR (central/abdominal adiposity) with repeated cortisol measures were examined using linear mixed-model analyses to account for within group correlations. Cortisol concentrations at all time points were included in the models to examine the change in cortisol from baseline over time (stress response). Exposure and outcome variables were converted into within-cohort SD scores (SDS) before analysis. The data represent SD change in cortisol response per SD change in adiposity. All analyses were adjusted for age, sex, pubertal stage, SES, birth weight, gestational age at birth and maternal BMI and GDM status. These were chosen as *a priori* covariates likely to be associated with children's adiposity or outcome measurements. Analyses were done using SPSS v 21.0 and STATA v 12.

RESULTS

The TSST-C was completed by 269 children. Girls had greater BMI, fat% and skinfold thickness and higher HOMA-IR; boys had higher WHR, fasting glucose and resting systolic and diastolic BP (***Table* I**). There were no differences in baseline or post-stress cortisol concentrations between boys and girls.

Generally, higher adiposity was associated with higher fasting insulin, triglyceride and total cholesterol concentrations, HOMA-IR and systolic BP, and lower HDL-cholesterol concentrations ($P<0.05$). Higher fat% was associated with lower baseline cortisol concentrations (-0.22 SD per SD increase in fat%, 95% CI: -0.39, -0.06 SD; $P=0.008$). There were no associations between other adiposity measures and baseline cortisol.

TABLE I GENERAL CHARACTERISTICS OF THE STUDY POPULATION (*N*=269)

	Boys (N=133)	*Girls (N=136)*
Age (yr)	13. 6 (0.2)	13.6 (0.1)
Birth weight (g)	2890 (490)	2883 (456)
Height (cm)	154.7 (8.2)	153. 7 (5.7)
*Body mass index (kg/m^2)	17.0 (2.3)	18.6 (3.1)
*Body fat (%), *n*=268	17.4 (6.7)	26.6 (5.7)
*Sum of skinfolds (mm)	23.1 (11.7)	32.3 (10.7)
*Waist-to-hip ratio	0.90 (0.05)	0.87 (0.05)
Socioeconomic status (score)	38.4 (6.7)	37.8 (6.6)
*Fasting glucose (mmol/L), *n*=265	5.2 (0.5)	5.0 (0.4)
*Fasting Insulin (pmol/L)#, *n*=265	36.7 (26.2,48.9)	49.4 (39.4,64.7)
Insulin resistance (HOMA-IR), *n*=265	1.4 (1.0,1.8)	1.8 (1.5,2.4)
*Systolic blood pressure (mmHg)	111.3 (8.7)	107.7 (7.2)
*Diastolic blood pressure (mmHg)	63.1 (6.7)	59.3 (6.5)
Total cholesterol (mmol/l), *n*=268	3.6 (0.7)	3.7 (0.6)
Triglycerides (mmol/l), *n*=268	0.83 (0.43)	0.89 (0.36)
HDL cholesterol (mmol/l), *n*=268	1.10 (0.24)	1.07 (0.23)
Baseline cortisol (ng/mL)#, *n*=266	6.7 (4.6,8.9)	6.6 (5.2,9.1)
Mean post-stress cortisol (ng/mL)*	11.5 (7.9,18.2)	10.7 (7.6,16.3)

*HOMA-IR: Homeostasis Model Assessment for Insulin Resistance; All values in mean (SD) or # median (IQR);*P<0.001; $P=0.009; n=269, unless stated otherwise.*

Overall, cortisol concentrations increased from baseline after inducing stress (mean (SD) increase: 5.5 (6.4) ng/mL, *P*<0.001) (***Web Fig.* 1**). Adolescents with higher WHR had lower cortisol responses at all time points after stress induction, strongest at 20 and 30 minutes post-stress (***Table* II, *Fig.* 2**). Associations appeared somewhat stronger in girls (***Web Table* I**) but sex-specific differences in these associations were not supported by formal interaction testing. Higher fat% was associated with lower cortisol response to stress only in girls, especially 20 minutes after inducing stress (*P* for interaction by sex=0.02) (***Web Table* I**). BMI and sum of skinfold thickness were not associated with cortisol responses.

DISCUSSION

In this group of healthy adolescents, greater abdominal adiposity and total fat% were associated with diminished cortisol responses to acute stress. There was no association of either subcutaneous adiposity or BMI with cortisol responses.

Higher abdominal/visceral adiposity is a major risk factor for adult NCDs [13]. Release of excess free fatty acids into the circulation is one of the suggested mechanisms. Greater adiposity is also thought to increase cortisol response to stress [2], thus adding to disease risk. Indeed, studies in adults have shown an association between higher abdominal adiposity and greater cortisol reactivity [14]. In contrast, our study observed a reduced cortisol response to stress. Previous studies have

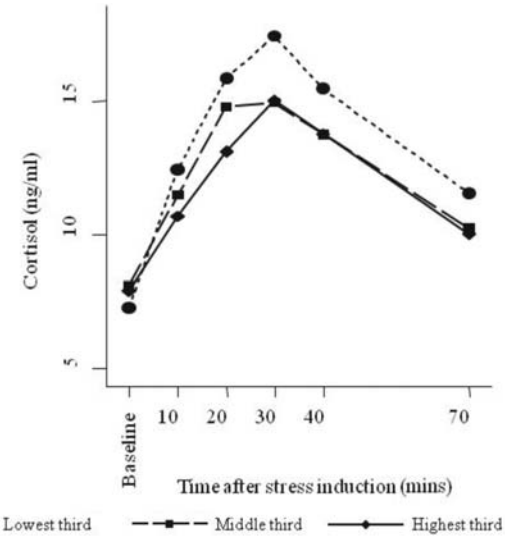

FIG.2 *Change in salivary cortisol concentration from baseline after stress-induction according to children's waist-to-hip ratio (using sex-specific thirds).*

Table II CORTISOL RESPONSES TO STRESS ACCORDING TO DIFFERENT ADIPOSITY MEASURES

	*Salivary cortisol concentrations (SDS)**				
	10 min	*20 min*	*30 min*	*40 min*	*70 min*
Waist-hip ratio (SDS)					
Model 1: β (95% CI)	-0.08 (-0.18,0.03)	-0.12 (-0.22,-0.01)	-0.11(-0.21,-0.01)	-0.09 (-0.20,0.01)	-0.09 (-0.19,0.02)
P value	0.2	0.03	0.04	0.08	0.1
Model 2: β (95% CI)	-0.09 (-0.19,0.02)	-0.13 (-0.24,-0.02)	-0.13 (-0.25,-0.02)	-0.10 (-0.21,0.00)	-0.09 (-0.21,0.02)
P value	0.1	0.02	0.02	0.07	0.09
Body fat% (SDS)					
Model 1: β (95% CI)	-0.04 (-0.13,0.06)	-0.03 (-0.12,0.07)	-0.00 (-0.10,0.10)	-0.00 (-0.10,0.09)	-0.03 (-0.13,0.06)
P value	0.5	0.6	1.0	1.0	0.5
Model 2: β (95% CI)	-0.05 (-0.15,0.06)	-0.04 (-0.14,0.06)	-0.01 (-0.10,0.10)	-0.01 (-0.11,0.09)	-0.03 (-0.13,0.07)
P value	0.4	0.5	0.9	0.9	0.6
Sum of skinfolds (SDS)					
Model 1: β (95% CI)	-0.00 (-0.10,0.10)	-0.05 (-0.15,0.05)	-0.05 (-0.15,0.05)	-0.03 (-0.13,0.07)	-0.04 (-0.14,0.06)
P value	1.0	0.3	0.3	0.6	0.4
Model 2: β (95% CI)	-0.02 (-0.13,0.08)	-0.06 (-0.16,0.05)	-0.05 (-0.16,0.05)	-0.04 (-0.15,0.06)	-0.06 (-0.16,0.05)
P value	0.7	0.3	0.3	0.4	0.3
Body Mass Index (SDS)					
Model 1: β (95% CI)	-0.02 (-0.12,0.09)	-0.05 (-0.16,0.06)	-0.05 (-0.16,0.05)	-0.03 (-0.13,0.08)	-0.04 (-0.15,0.06)
P value	0.8	0.4	0.3	0.6	0.4
Model 2: β (95% CI)	-0.04 (-0.15,0.07)	-0.05 (-0.16,0.06)	-0.05 (-0.16,0.07)	-0.03 (-0.14,0.08)	-0.05 (-0.16,0.06)
P value	0.5	0.4	0.4	0.6	0.4

*SDS: Standard Deviation Score; β: represents SDS change in cortisol response per SDS change in fat%; *Logged variable; Model 1: adjusted for children's age and sex; Model 2 adjusted for children's age, sex, pubertal stage, birth weight, gestational age, socioeconomic status, and maternal BMI and gestational diabetes status*

consistently shown inverse associations between body weight and adiposity, and circulating cortisol concentrations in the non-stressed state, possibly resulting from increased peripheral metabolism of cortisol [15]. A few studies have also observed similar inverse associations during stress. In the Dutch Famine Birth cohort adults, there was a 20% decrease in cortisol response to stress in relation to skinfold thickness [16]. In UK, higher visceral adiposity was associated with a blunted cortisol response to stress tasks [17]. Even in children, salivary cortisol response to behavioral stress tasks was inversely associated with higher BMI (0.17 SD per SD decrease in cortisol) in one study [18].

Mechanisms underlying a diminished cortisol response during stress in relation to adiposity are speculative. Researchers suggest that repeated stress exposure, which is a risk factor for higher adiposity, eventually 'burns out' the HPA axis, leading to a blunted cortisol response [2]. However, such an extreme manifestation of chronic stress is unlikely in these young participants. On the other hand, reduced stress responses may be related to their behavior and perception. Motivation to perform well and a greater effort to engage in the stress-inducing tasks are important triggers for cortisol release during TSST-C [7]. Adolescents with lower motivation may have a blunted stress response. Lower awareness may result in lower perceived stress, and thus reduced cortisol response. Higher adiposity has been shown to be associated with lower cognitive ability in children [19], though it was associated with better cognitive performance in our participants during childhood [20].

A chronically elevated HPA axis response and higher circulating cortisol are associated with cardiometabolic and psychological abnormalities that increase NCD risk [1,21]. In this context, lower cortisol response in our adipose adolescents appears to be protective. Some researchers argue that physiologically decreased cortisol may be an adaptive mechanism to minimise its harmful effects in potentially pathological conditions [22]. In particular, higher cortisol release may amplify the cardiometabolic risks associated with higher adiposity.

WHAT IS ALREADY KNOWN?

- Indian children and adults have higher central adiposity relative to lean mass, which increases their chronic disease risk.

WHAT THIS STUDY ADDS?

- Higher central adiposity is associated with altered hypothalamic-pituitary-adrenal axis (cortisol) response to stress in Indian adolescents.

However, a few studies have shown associations between blunted cortisol response and a variety of adverse psychological health outcomes such as depression and substance abuse behaviours [23]. An optimum HPA axis activity prepares body's physiological systems to cope with stressful situations. Researchers suggest that a hypo-reactive HPA axis represents a 'less-adaptive' neuro-endocrine system, which fails to perform optimally during a challenge [23]. Hence, a reduced reactivity may indicate a reduced ability to deal with daily stresses in adipose adolescents.

We used salivary method for cortisol assessment as it is non-invasive and enabled multiple sampling required for this study, and is a reliable marker of the level of circulating free cortisol concentrations [24]. Stress responses were measured only in urban children which reduces the generalizability of our findings. Adolescents' background stresses that may have influenced their stress response were not measured. Measurement of abdominal adiposity was based on anthropometry; however, our findings correspond to those observed using magnetic resonance imaging [17]. Several biological and environmental factors including age, sex and timing of the test may induce variability in salivary cortisol. However, a comprehensive range of measurements during pregnancy, at birth and current follow-up and standardised stress test conditions enabled relevant adjustments.

In conclusion, our findings, in the light of existing evidence, indicate that increased abdominal adiposity reduces stress reactivity which may compromise their ability to maintain homeostasis during challenging situations. This combined with cardio metabolic risks associated with visceral adiposity may increase future NCD consequences in these adolescents. Our study was not designed to examine the causal associations between adiposity and stress responses, hence we cannot rule out the effect of residual confounding on these findings. Our continued follow-up of this cohort may provide clues to the role of optimised stress responses in reducing NCD risks in vulnerable children.

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Contributors: GVK, AJ, CHDF: conceived and designed the study; GVK, SRV, RS, SCK acquired the data; GVK, AJ, CHDF: analyzed and interpreted data; GVK, CHDF drafted the article. All authors revised the manuscript critically for important intellectual content, and approved the final version.

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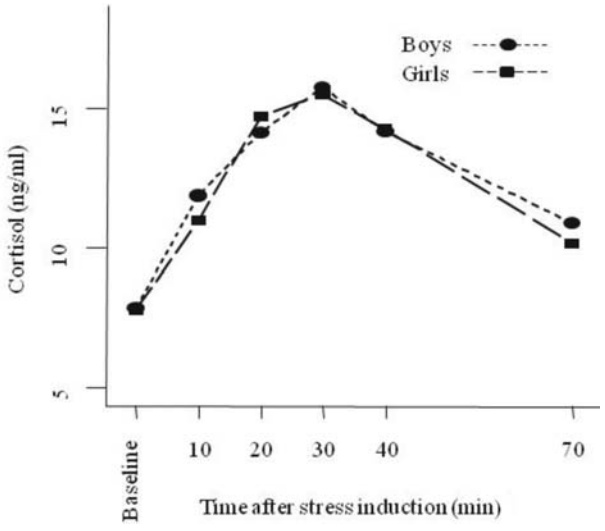
WEB TABLE I ASSOCIATION BETWEEN WAIST-TO-HIP RATIO (WHR), FAT% AND CORTISOL RESPONSE TO STRESS BY GENDER

	*Salivary cortisol concentrations (SDS)**				
	10 min	20 min	30 min	40 min	70 min
WHR (SDS)					
Girls					
β (95% CI)	-0.17 (-0.32,-0.01)	-0.16 (-0.32,-0.01)	-0.19 (-0.35,-0.04)	-0.11 (-0.27,0.04)	-0.21 (-0.36,-0.05)
P value	0.04	0.04	0.01	0.1	0.008
Boys					
β (95% CI)	-0.06 (-0.23,0.12)	-0.11 (-0.29,0.06)	-0.09 (-0.27,0.08)	-0.11 (-0.29,0.06)	-0.04 (-0.22,0.13)
P value	0.5	0.2	0.3	0.2	0.6
Fat% (SDS)					
Girls					
β (95% CI)	-0.16 (-0.33,0.02)	-0.23 (-0.40,-0.05)	-0.13 (-0.30,0.05)	-0.06 (-0.23,0.12)	-0.12 (-0.30,0.05)
P value	0.08	0.01	0.1	0.5	0.2
Boys					
β (95% CI)	0.10 (-0.08,0.28)	0.08 (-0.10,0.26)	0.12 (-0.06,0.30)	0.03 (-0.15,0.21)	0.15 (-0.03,0.33)
P value	0.3	0.4	0.2	0.7	0.1

*SDS: Standard Deviation Score;β represents SDS change in cortisol response per SDS change in adiposity;*logged variable; Models adjusted for children's age, pubertal stage, birth weight, gestational age, socioeconomic status, and maternal BMI and gestational diabetes status.*



WEB FIG. 1 *Cortisol responses during the Trier Social Stress Test in boys and girls.*