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Stress and abdominal Fat: Preliminary Evidence of Moderation by the Cortisol awakening Response in Hispanic Peripubertal Girls

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Abstract

Stress and the cortisol awakening response (CAR) have been independently linked to increases in abdominal fat depots. This cross-sectional study examined the CAR as a moderator of the association between stress, visceral adipose tissue (VAT), and subcutaneous abdominal adipose tissue (SAT) in a sample ($N = 23$) of female peripubertal Hispanic girls aged from 8 to 11. The study included: (i) monitored salivary cortisol collection, (ii) VAT and SAT obtained by multislice magnetic resonance imaging, and (iii) a stressful life events checklist with four domain-specific subscales: peer, family, personal, and school. Regression analysis indicated an interaction of school-related life events and CAR on VAT and SAT, with greater numbers of school-related events being related to greater VAT and SAT for girls with high CAR, but no association with VAT or SAT for girls with low CAR. Similar to job stress in adults, school-related stress in children may contribute to central adiposity, especially for girls with high CAR.

INTRODUCTION

Central adiposity has been indicated as a strong predictor of negative cardiovascular risk profiles beyond age- and sex-specific BMI among children (1). Central adiposity is associated with diabetes and is one of the five components of the metabolic syndrome in children (2).

Visceral adipose tissue (VAT) is central fat interspersed in the abdominal cavity around organs, and is particularly damaging because it alters metabolic regulatory mechanisms (3), and may reduce neuroendocrine responses to psychological stress (4,5). Subcutaneous abdominal adipose tissue (SAT), or fat located beneath the skin, may also be related to disease processes. Some research indicates SAT may be a protective factor for cardiovascular disease risk (6), yet other research indicates SAT is related to increased metabolic dysfunction (7,8). These discrepancies may be attributable to abdominal adipose

DISCLOSURE

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tissue being deleterious to metabolic health, while subcutaneous adipose tissue in the lower extremities (gluteofemoral fat) may be protective (9).

General measures of central adiposity, such as waist circumference and waist-to-hip ratio, are used as proxy measures for abdominal fat; however, they lack the ability to differentiate various tissues in the abdomen such as VAT, SAT, bone, muscle, and organs (10). During pubertal development, there are significant changes in regional fat distribution, irrespective of changes in waist circumference (11). Thus, adequate differentiation and precise measurement of VAT, SAT, and whole body fat are key to understanding the relationship between central adiposity and metabolic health in children.

It is hypothesized that central adiposity is modified by biological and behavioral responses to stress, such as altered hypothalamic-pituitary-adrenal axis (HPAA) activity, sympathetic neural activity, and consumption of foods high in fat and/or sugar (4,5). Hyperactivity of the HPAA results in increased exposure to circulating levels of cortisol, a hormone associated with stress, increased appetite, and the mobilization of fat from the periphery to the central region. Cushing's disease is a case of extreme hypercortisolism and is known to cause abdominal obesity, peripheral wasting, and insulin resistance.

One known marker of basal HPAA activity is the cortisol awakening response (CAR), an increase in cortisol secretion in the 30–60 min after awakening (12). The CAR is associated with chronic stress (13) and central adiposity (14,15), and has shown a moderate degree of heritability (40–60%) (16,17). The CAR has state-like qualities, notably, variation with anticipated stress of the upcoming day (18,19); however, it has shown little to no relationship with HPAA measures of induced acute stress (e.g., the Trier Social Stressor Task). Although the purpose of the CAR is unknown, it appears to be related to the mobilization of energy for the transition from sleep to wake, in order to meet the demands of the upcoming day (12). Thus, for those with high CAR and chronic stress, increased central adiposity may be expected through increased exposure to cortisol in the morning.

The present study examines the independent and interactive effects of stress and HPAA activity in predicting VAT and SAT among a sample of Hispanic peripubertal girls. Hispanic girls were studied because they have the greatest risk of having the metabolic syndrome in adolescence, compared to black and white girls (20), and it is estimated that over half of Hispanic girls will develop diabetes in their lifetime (21). Females also consistently report higher levels of interpersonal stress than males, a pattern that begins in adolescence and continues through adulthood (22). We hypothesized girls with high stress would have greater amounts of VAT and SAT, and high CAR would interact with stress to exacerbate this relationship.

METHODS AND PROCEDURES

Participants

Participants were recruited through participating physicians from clinics, churches, schools, and community centers in Los Angeles County. Study participants were taking part in a longitudinal study of psychosocial and physiological determinants of physical activity and insulin resistance in minority youth. Inclusion criteria were: female gender, Tanner breast stage 1 or 2 (19), nonmenstruating, between the ages of 8 to 11, and African-American or Hispanic ethnicity. To meet criteria for African-American or Hispanic ethnicity, participants' parents had to self-report all four grandparents as African-American or Hispanic, respectively. Additionally, participants were required to meet one of the following criteria: (i) BMI $\geq$ 85th percentile (overweight or obese); or (ii) BMI $<$ 85th percentile (normal weight) with at least one parent meeting adult criteria for overweight (BMI $\geq$ 25), or

diagnosed with type 2 diabetes (23). Exclusion criteria were diagnosis of diabetes at screening, assessed by fasting plasma glucose ≥ 126 mg/dl (24), or diagnosis of a major illness or disease that could effect body composition, fat distribution, or insulin action or secretion. Inclusion criteria for the analyses presented in this paper were: (i) Hispanic ethnicity, and (ii) data from the first annual overnight stay at the University of Southern California General Clinical Research Center (GCRC) that included extensive biological measures. We chose not to include African-American participants ($n = 11$) due to marked racial/ethnic differences in fat distribution between Hispanic and African-American children (11).

Before any testing procedure, parents and children were provided with detailed written and verbal information about the study in the primary language spoken by the parent. Informed written consent from the parent and assent from the child was obtained, and participants were informed that they could withdraw from the study at any time. The institutional review board of the University of Southern California approved the study.

Procedure

Participants arrived for an overnight GCRC visit at ~1300 h. Parents and participants were each asked to individually complete demographic and psychosocial questionnaires. After completing the questionnaires, a licensed pediatric health-care provider performed a complete medical examination and health history. Anthropometric and body composition measures were taken (detailed below) and participants were given a standardized dinner and a snack before 2000 h. Participants were informed of morning saliva collection times and were instructed to refrain from drinking water or brushing teeth prior to the saliva collection. Staff awakened participants at 0600 h and collected the first saliva sample, followed by the second sample at 0630 h. Final study procedures followed the morning salivary samples, and participants were offered lunch and discharged from the GCRC.

Measures

Weight and height were measured three times to the nearest 0.1 kg and 0.1 cm, respectively, using a beam medical scale and wall-mounted stadiometer. The mean of the three measurements was used to calculate BMI and BMI percentiles for age and gender using Epi Info 2000, version 1.1 (CDC, Atlanta, GA).

Total body composition was measured by air displacement plethysmography (BodPod; Life Measurement Instruments, Concord, CA). This system uses computerized pressure sensors to determine body volume and body density through an air displacement. BodPod provides an accurate measure of body fat and lean body mass in children without dunking, pinching, or exposure to electric currents or radiation, and has been validated in children against hydrostatic weighing, dual-energy X-ray absorptiometry, and multicompartment models (25).

Abdominal fat volume was obtained by multislice magnetic resonance imaging, using a Siemens Magnetom 1.5T Symphony Maestro Class Syngo 2004A (Siemens, Erlangen, Germany) with a Numaris/4 software at the USC-HCCII imaging center. Patients were positioned supine, and 19 axial images of the abdomen with a thickness of 10 mm were taken. Multislice imaging is an accurate, reliable measure of abdominal adipose tissue, and is recommended for small studies where each subject cannot serve as its own control (26). After image acquisition, VAT and SAT were segmented using image analysis software (SliceOmatic Tomovision, Montreal, Canada) at Image Reading Center (New York City, NY). A localizer was used to identify the L4–L5 space. Axial images were acquired from L4–L5 through the pelvis and from L4–L5 through the liver. Trained image analysts with

medical or scientific backgrounds analyzed all acquired images. Images were analyzed with commercially available medical imaging software using a semiautomated, threshold method. The technical errors for three repeated readings of the same scan by the same observer for VAT and SAT volumes were 1.97% and 0.96%, respectively. The intraclass correlation coefficients among the analysts (who each read the same scans twice, separated by a 3-month interval) were 0.95 and 0.99 for VAT and SAT, respectively.

Salivary cortisol samples were collected with salivettes (Sarstedt, Newton, NC). A dry dental roll was placed in the participant's inner cheek for 2 min, and was then placed into a plastic vial. After collecting the morning samples, the salivettes were centrifuged at 2,500 rpm for 10 min, and saliva supernatant was then frozen at -70°C until assayed. Samples were assayed for cortisol using an automated enzyme immunoassay (Tosoh AIA 600 II analyzer, Tosoh Bioscience, South San Francisco, CA) in the GCRC laboratory. The assay sensitivity was 0.02 $\mu\text{g/dl}$, interassay coefficient of variation was 11.2%, and intra-assay coefficient of variation was 8.2%. Each participant's cortisol samples were completed in the same assay run.

The CAR is a rise in cortisol secretion from awakening to 30 min after awakening. The CAR was calculated by subtracting cortisol at awakening from cortisol at 30 min after awakening (27).

Negative life events were measured using a life events checklist developed for and validated in multiethnic urban adolescent children (28). This checklist measures four domains of negative life events using four subscales (example items in parentheses): school (argued with a teacher, had to study for a big test, got into a fight at school), family (family member lost a job, was ignored by my family, parents fought with each other), personal (got sick, lost something or had something stolen, someone insulted me or my family), and peer (argued with friends, someone threatened to beat me up, friend moved). For each item of the scale, the participant was instructed to check if the event had occurred in the past 6 months. School, family, personal, and peer-related scales contained 7, 19, 13, and 16 items, respectively. Items of the scale were summed for each domain and for the total score.

Analytic technique

The primary dependent variables in these analyses were VAT and SAT. The primary interest was to examine the relationships between negative life events, CAR, VAT, and SAT. A natural log transformation was used to normalize the distribution of VAT and SAT, which are referred to as log VAT and log SAT in all analyses, unless otherwise specified. Bivariate associations were examined, followed by hierarchical regression to determine whether negative life events and CAR were independent or interactive in their prediction of current log VAT and log SAT. Tanner stage, and percent body fat were entered as control variables. We controlled for percent body fat in order to examine the effect of negative life events on log VAT and log SAT, irrespective of total body fat. To characterize the relationship between negative life events, the CAR, and adiposity, we used percent body fat as a dependent variable, controlling for Tanner stage and total abdominal adiposity, as measured by SAT and VAT combined (29). This allowed us to examine the effect of negative life events on peripheral body fat (i.e., percent body fat, after controlling for total abdominal fat). All continuous independent variables were normally distributed. Variables used to create interaction terms were standardized, and the cross-product terms were created using standardized values (30).

Two observations were multivariate outliers and were removed from further analyses based on (i) the examination of jackknife residuals, leverage, Cook's D , DFBETAS (standardized difference of the β), and DFFITS and (ii) being consistently observable in bivariate

scatterplots (30). The omitted cases were both Tanner stage 2, with extreme CAR values ($-0.72, 2.03$), moderate VAT scores ($1.2, 0.42$), and school LE scores ($3, 1$), respectively. This yielded a final analytic sample size of 23. Examination of variance inflation factors indicated no issues of multicollinearity between any of the independent variables or the interaction term.

RESULTS

Sample characteristics are presented in Table 1 and bivariate associations are presented in Table 2. All three measures of adiposity were significantly correlated. Percent body fat was correlated with log VAT ($r = 0.57, P = 0.004$) and log SAT ($r = 0.67, P = 0.001$), and log SAT and log VAT were correlated ($r = 0.91, P < 0.001$). The personal, peer, and family-related negative life events subscales were significantly correlated ($r = 0.45$ to $0.82, P < 0.05$); however, the school-related life events subscale was not significantly correlated with the other subscales ($r = -0.10$ to 0.32). Multivariate models using the family, peer, personal, and total negative life events subscales to predict VAT and SAT were not significant (data not shown); however, school-related life events was consistently related to VAT, SAT, and percent body fat in multivariate models.

Tables 3–5 display results from multivariate hierarchical regression analyses using school-related life events to predict the three measures of adiposity: VAT, SAT, and percent body fat. The school-related life events scale was independently associated with log VAT ($b = 0.39, P = 0.009$), and the addition of the interaction did not substantially reduce the association ($b = 0.37, P = 0.01$). The school-related life events scale was not independently associated with SAT or percent body fat. The CAR was not independently associated with log VAT, log SAT, or percent body fat. The interaction of school-related life events and the CAR was significant for log VAT, log SAT, and percent body fat. The interaction was positively associated with log VAT ($b = 0.43, P = 0.05$) and accounted for over 9% of the variance after accounting for percent body fat, Tanner stage, and the independent effects of school-related life events and the CAR ($\Delta R^2 = 0.09, F(1, 17) = 4.64, P = 0.05, f^2 = 0.26$). Table 4 displays these results using log SAT as the dependent variable. The interaction was positively associated with log SAT ($b = 0.42, P = 0.01$) accounted for 14% of the variance ($\Delta R^2 = 0.14, F(1, 17) = 7.86, P = 0.01, f^2 = 0.49$). Using percent body fat as the dependent variable, the interaction of school-related life events and CAR was significant ($b = -5.59, P = 0.03$) and accounted for over 13% of the variance in percent body fat, after accounting for abdominal fat, Tanner stage, and the independent effects of school-related life events and CAR ($\Delta R^2 = 0.13, F(1, 17) = 5.62, P = 0.03, f^2 = 0.32$).

Simple regression equations were used to plot exponentiated values for the regression of log VAT and log SAT on school-related life events at low (one standard deviation below the mean) and high (one standard deviation above the mean) CAR (30). For girls with low CAR, school-related life events did not predict VAT or SAT. For girls with high CAR, school-related life events was associated with increased VAT (Figure 1) and increased SAT (Figure 2), controlling for percent body fat, and decreased percent body fat (Figure 3), controlling for abdominal fat. For girls with high CAR, we estimated the difference between being one standard deviation below the mean and one standard deviation above the mean (roughly equivalent to reporting 1.3 school-related life events compared to reporting 3.5 school-related life events), would be 0.5 l of VAT and 3.7 l of SAT.

DISCUSSION

Central adiposity confers increased risk of mortality from cardiovascular disease, cancer, and all causes among women (31). This cross-sectional study provides preliminary evidence

that school-related stress and CAR interact to play a role in the determination of body fat distribution among Hispanic female youth. The combination of high CAR and school stress was related to unhealthy fat distribution profiles characterized by higher volumes of VAT and SAT, and reduced peripheral fat stores. This is consistent with known effects of cortisol to redistribute fat from the periphery to the abdominal region (5).

Our hypothesis was that overall stress would interact with CAR in predicting current abdominal fat, and the use of domain-specific stressful life events was exploratory. Stress has strongly and consistently been related to abdominal fat accumulation in well-controlled animal studies, including nonhuman primates (32) and rodents (33) consuming a palatable high-fat diet; however, evidence from human studies is not as clear. For example, a large, prospective study of adults found chronic work stress to be associated with increases in central fat stores over a 19-year period (34). However, a 5-year prospective study of adolescents (aged 11–15) found perceived stress to be associated with waist circumference in cross-sectional analyses, but not in longitudinal analyses (35). These discrepancies in human studies may be due to differences in measuring stress. Animal studies typically study objective stress (e.g., tail pinching, restraint), whereas human studies use subjective measures of stress (e.g., perceived stress). Stress is generally defined as the inability to meet or cope with environmental demands (36). Life events represent more objective measures of stress because they measure events that are generally considered to be stressful, whereas perceived stress measures the subjective evaluation of an event as being stressful (36).

Interestingly, of the four domains of stressful life events examined, school-related life events subscale was the only significant predictor of abdominal fat in multivariate models, and this was moderated by CAR. It is notable that the relationship between school-related stress, CAR, and abdominal fat parallels research in the adult literature, where work-related stress has been associated with central adiposity (34) and CAR (14,37). A considerable amount of time is spent at work and school, for adults and children respectively; thus, strain in these domains is likely to have a considerable degree of chronicity. Increased CAR has been noted on weekdays compared to weekends, and appears to be greater in those experiencing stress at work (38).

Anticipating stress at school or work may result in higher CAR over time or a greater number of days of high CAR, especially for those with polymorphisms in genes associated with both the CAR and stress (e.g., 5-HTT gene variants) (39). Thus, gene–environment interactions are a plausible explanation for the interaction of school-related life events, the CAR, and the accumulation of visceral and subcutaneous abdominal fat. In the current study, the interaction of school stress and the CAR revealed that for children with high CAR, but not for children with low CAR, school stress was related to increased VAT and SAT, and decreased percent body fat, after controlling for abdominal fat. A potential explanation is that the overnight stay was stressful for the participants and that participants predisposed to stress-induced increases in the CAR would have elevated CAR during the study. Therefore, girls that experience increased CAR associated with stress may have chronically high CAR when they are consistently experiencing school stress, leading to increases in abdominal fat. Girls that experience increased CAR associated with stress, but do not have chronic stress at school, may not have consistently high CAR and therefore would not have increases in abdominal fat. It would be useful for future research to examine the frequency of each school-related event in order to assess the chronicity of each stressor, and the participant's appraisal of each stressor. It would also be useful to measure the CAR at home over several school days to determine whether a dose–response relationship exists between school-related stress, the CAR, and central adiposity.

Children reporting greater numbers of stressful school events may be experiencing chronic stress due to academic or social difficulty in school. One of the most frequently reported items on the school-related life events measure was, “Had to study for a big test.” In previous research, exam stress has been associated with total cortisol and weight gain among female nursing students (40), increases in weight and waist circumference among female medical students (41), and increases in CAR for women, but not men. Thus, similar to previous studies examining adult women, academic stress may play a role in increased central adiposity among female children with high CAR.

This study was strengthened by the use of precise, well-validated, measures of central adiposity, total body fat, and the CAR, as well as the use of a previously validated subjective measure of stress for urban minority youth. Despite these strengths, there are notable limitations concerning causality, sample size, and study population. This was a cross-sectional analysis, and therefore ambiguity of causal direction is a concern; however, longitudinal evidence in highly controlled animal studies indicates that cortisol and stress lead to subsequent increases in VAT (32). The most important limitation of this study was the small sample size. Maxwell cautions, “... the tendency to conduct underpowered studies will tend to produce an inconsistent body of literature (42).” Because the nature of the data collection (i.e., minority children required to stay overnight and provide blood samples) make it difficult to obtain large samples, we used two strategies to increase power and the reliability of our estimates: (i) We used precise measurement techniques (multislice magnetic resonance imaging and BodPod) and well controlled study variables (monitored cortisol collection), and (ii) reported 95% confidence intervals around regression coefficients. Although we recognize that Maxwell recommends the use of confidence intervals around standardized regression coefficients (42), the standardized coefficients are improper for an interaction term due to centering. Therefore, the interaction was created using the cross-product of the *z*-scores, which gives the proper standardized regression coefficients and corresponding confidence intervals in the non-standardized/raw solution (30).

The study population was urban Hispanic peripubertal females, either overweight/obese or at potential risk for developing overweight. Racial/ethnic differences in stressful life events were not reported in previous research using this negative life events inventory among urban youth (28); however, stress may vary by race/ethnicity and location. For example, in schools where educators are not experienced in teaching children speaking another language in the home, or where racial/ethnic diversity is not common, school may be more stressful for children. It would be helpful to examine stressors specific to Hispanic youth in academic settings to understand whether differences in stress contribute to disparities in metabolic health.

We encourage similar studies with longitudinal methods and larger, more diverse samples, in order to delineate the independent and interactive effects of stressors and HPA activity in the development and proliferation of abdominal fat and nonabdominal subcutaneous fat. These preliminary data are not strong enough to recommend large scale interventions; however, studies utilizing stress-reduction interventions (e.g., stress-reduction guided imagery, reduction in school stress) may be helpful in understanding the malleability of CAR and school stress, and how these mechanisms may lead to changes in adiposity and fat distribution over time.

According to the US Census Bureau (43), it is projected that the majority of youth in the United States will be Hispanic by 2050 and it is estimated that over half of Hispanic female youth will develop diabetes in their lifetime (21). Thus, it is important to understand the psychological and physiological mechanisms that lead to diabetes in this important

population. This study provides evidence that for Hispanic female youth, school stress may play a significant role in the accumulation visceral fat, a well-known precursor to diabetes.

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Figure 1.

The interaction of the cortisol awakening response (CAR) and school-related life events on visceral adipose tissue, at 1 s.d. above (high) and below (low) the mean of CAR and school-related life events.

Figure 2.

The interaction of the cortisol awakening response (CAR) and school-related life events on subcutaneous abdominal adipose tissue, at 1 s.d. above (high) and below (low) the mean of CAR and school-related life events.

Figure 3. The interaction of the cortisol awakening response (CAR) and school-related life events on percent body fat, at 1 s.d. above (high) and below (low) the mean of CAR and school-related life events.

Table 1Descriptive characteristics ($N = 23$)

Study variables	Mean (s.d.)	Range
Age (years)	9.2 (0.90)	8–11
VAT (l) ^a	0.75 (0.61)	0.12–2.1
SAT (l) ^a	4.75 (3.18)	0.96–11.7
CAR ($\mu\text{g/dl}$)	0.85 (0.40)	–0.09–1.64
% Body fat	27.7 (9.3)	11.0–40.6
BMI (kg/m^2)	21.3 (4.4)	13.5–29.9
BMI (kg/m^2) z-score	1.17 (1.08)	–2.2–2.4
Negative life events (total)	16.5 (7.3)	6–33
School	2.4 (1.1)	1–5
Family	6.1 (3.1)	1–12
Personal	4.4 (2.2)	1–9
Peer	3.7 (2.2)	0–10
Number (%)		
Tanner breast stage		
Tanner I	13 (61.9)	
Tanner II	10 (38.1)	
Number (%) endorsing		
School life events items		
Argued with a teacher	1 (4.3)	
Bad/worse grades	9 (38.5)	
Fight at school	3 (13.0)	
In trouble at school	8 (34.8)	
Study for big test	18 (82.6)	
New school/changed class	7 (30.4)	
Bad teacher	5 (26.1)	

CAR, cortisol awakening response; SAT, subcutaneous abdominal adipose tissue; VAT, visceral adipose tissue.

^aVAT and SAT are not transformed here, but are natural log transformed in other analyses.

Table 2

Bivariate correlations of major study variables

	VAT	SAT	Body fat(%)	CAR	Life events		
					School	Family	Peer
SAT	0.91						
Body fat (%)	0.57	0.67					
CAR	0.17	0.23	−0.03				
School	0.49	0.32	0.05	0.13			
Family	−0.13	−0.20	0.04	−0.03	0.25		
Peer	−0.03	0.06	0.05	0.00	−0.10	0.45	
Personal	−0.07	−0.13	−0.10	−0.04	0.32	0.82	0.55
Cumulative	−0.01	−0.05	0.01	0.00	0.33	0.89	0.73
							0.92

Boldface items indicate statistical significance ($P < 0.05$).

CAR, cortisol awakening response; SAT, subcutaneous abdominal adipose tissue; VAT, visceral adipose tissue.

Table 3Regression of school-related life events, the CAR, and their interaction on log VAT ($N = 23$)

Variable	Model 1	Model 2	Model 3
	<i>b</i> (95% CI)	<i>b</i> (95% CI)	<i>b</i> (95% CI)
Constant	-2.23 (-3.39 to -1.08)	-2.18 (-3.18 to -1.18)	-2.75 (-3.82 to -1.68)
% Body fat	0.05* (0.01 to 0.09)	0.05** (0.01 to 0.09)	0.06** (0.02 to 0.09)
Tanner breast stage ^b	0.18 (-0.59 to 0.96)	0.07 (-0.61 to 0.74)	0.27 (-0.38 to 0.92)
School-related life events ^d		0.39* (0.10 to 0.68)	0.37** (0.10 to 0.63)
CAR ^d		0.16 (-0.27 to 0.58)	0.11 (-0.28 to 0.50)
School-related life events ^d × CAR ^d			0.43* (0.01 to 0.86)
R^2_{total}	0.34	0.56	0.65
ΔR^2	0.34**	0.22**	0.09**

Model 1 includes percent body fat, and Tanner breast stage; model 2 includes percent body fat, Tanner breast stage, school-related life events, and CAR; model 3 includes percent body fat, Tanner breast stage, school-related life events, CAR, and the interaction of school-related life events and CAR.

CAR, cortisol awakening response; log VAT, visceral adipose tissue, natural log.

^a Standardized

^b Tanner breast stage 1 is the comparison group.

* $P < 0.05$;

** $P < 0.01$.

Table 4Regression of school-related life events, the CAR, and their interaction on log SAT ($N = 23$)

Variable	Model 1	Model 2	Model 3
	<i>b</i> (95% CI)	<i>b</i> (95% CI)	<i>b</i> (95% CI)
Constant	−0.11 (−0.95–0.74)	−0.07 (−0.85–0.72)	−0.62 (−1.41–0.17)
% Body fat	0.05** (0.02–0.08)	0.05** (0.02–0.08)	0.06** (0.03–0.08)
Tanner breast stage ^a	0.08 (−0.49–0.65)	0.01 (−0.53–0.54)	0.20 (−0.28–0.68)
School-related life events ^b		0.18 (−0.05–0.41)	0.16 (−0.03–0.36)
CAR ^b		0.22 (−0.12–0.56)	0.17 (−0.12–0.46)
School-related life events ^b × CAR ^b			0.42** (0.10–0.72)
R^2_{total}	0.45	0.57	0.71
ΔR^2	0.45**	0.12	0.14*

Model 1 includes percent body fat, and Tanner breast stage; model 2 includes percent body fat, Tanner breast stage, school-related life events, and CAR; model 3 includes percent body fat, Tanner breast stage, school-related life events, CAR, and the interaction of school-related life events and CAR.

CAR, cortisol awakening response; SAT, subcutaneous adipose tissue.

^aTanner breast stage 1 is the comparison group.

^bStandardized.

* $P < 0.05$;

** $P < 0.01$.

Table 5Regression of school-related life events, the CAR, and their interaction on percent body fat ($N = 23$)

Variable	Model 1	Model 2	Model 3
	<i>b</i> (95% CI)	<i>b</i> (95% CI)	<i>b</i> (95% CI)
Constant	11.92 (1.72 to 22.11)	10.61 (21.09 to 0.13)	15.57 (25.95-5.20)
Tanner breast stage ^a	7.52* (-0.70 to 4.36)	7.21* (14.32 to 0.09)	2.65 (10.20 to -4.90)
Abdominal adipose tissue (l)	0.90 (0.10 to 14.38)	1.26* (2.37-0.15)	1.63** (2.68 to 0.58)
School-related life events ^b		-2.45 (2.79 to -7.69)	-2.22 (2.47 to -6.92)
CAR ^b		-1.74 (1.98 to -5.46)	-2.00 (1.34 to -5.34)
School-related life events ^b × CAR ^b			-5.59* (-0.62 to -10.57)
R^2_{total}	0.40	0.46	0.59
ΔR^2	0.40**	0.06	0.13*

Model 1 includes total abdominal adipose tissue and Tanner breast stage; model 2 includes total abdominal adipose tissue, Tanner breast stage, school-related life events, and CAR; model 3 includes total abdominal adipose tissue, Tanner breast stage, school-related life events, CAR, and the interaction of school-related life events and CAR.

CAR, cortisol awakening response.

^aTanner breast stage 1 is the comparison group.

^bStandardized.

* $P < 0.05$;

** $P < 0.01$.