Higher Levels of Physical Activity Are Associated With Lower Hypothalamic-Pituitary-Adrenocortical Axis Reactivity to Psychosocial Stress in Children

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Context: Children who undertake more physical activity (PA) not only have more optimal physical health but also enjoy better mental health. However, the pathways by which PA affects well-being remain unclear.

Objective: To address this question, we examined whether objectively measured daytime PA was associated with diurnal hypothalamic-pituitary-adrenocortical axis (HPAA) activity and HPAA responses to psychosocial stress.

Design and Setting: We conducted a cross-sectional study in a birth cohort in Helsinki, Finland.

Participants: We studied 258 8-year-old children.

Main Outcome Measures: PA was assessed with wrist-worn accelerometers. Overall PA and percentage of time spent in vigorous PA (VPA) were categorized by sex into thirds. Salivary cortisol was measured diurnally and in response to the Trier Social Stress Test for Children.

Results: The children in different PA groups did not show differences in diurnal salivary cortisol ($P > .10$ for overall PA and VPA). Children with the highest levels of overall PA or VPA showed no, or only small, increases over time in salivary cortisol after stress ($P = .10$ and $P = .03$ for time in analyses of PA and VPA, respectively), whereas children belonging to the lowest and intermediate thirds showed significant increases over time in salivary cortisol after stress ($P \leq .002$ for time in the analyses of overall PA and VPA).

Conclusions: These results suggest that children with lower levels of daytime PA have higher HPAA activity in response to stress. These findings may offer insight into the pathways of PA on physical and mental well-being. **(*J Clin Endocrinol Metab* 98: E619–E627, 2013)**

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Abbreviations: AUC, area under the curve; BMI, body mass index; cpm, counts per minute; HPAA, hypothalamic-pituitary-adrenocortical axis; PA, physical activity; TSST, Trier Social Stress Test; TSST-C, TSST for Children; VPA, vigorous physical activity.

Children who undertake more physical activity (PA) not only have more optimal physical health but also enjoy better mental health (1–7). However, the pathways by which PA affects well-being are still unclear. One explanatory mechanism might be the hypothalamic-pituitary-adrenocortical axis (HPAA) activity, which plays a key role in physical and mental well-being (8).

PA has been suggested to serve as both a stressor and a modifier of stress; the adaptation of the HPAA, caused by physical activity and exercise might generalize to other stressors as well, including psychosocial ones (9). For example, studies in adults have found that physically trained men exhibit lower cortisol responses to acute physical exercise when compared with moderately trained or sedentary men (10). Trained men also showed significantly lower cortisol responses to a psychosocial stress test [Trier Social Stress Test (TSST)] when compared with their untrained counterparts (11). In another study, older physically fit women showed lower cortisol responses to a different psychosocial stress test (Matt Stress Reactivity Protocol) when compared with unfit older women (12). However, the same study also found that younger fit and unfit women did not show differences in cortisol responses to stress when compared with each other (12).

Because HPAA function (13) and the quantity and quality of PA (14) change by age and pubertal maturation, the generalization of the previous results to prepubertal children is precluded and studies examining these associations in children are needed. To our knowledge, the associations between daytime PA and diurnal HPAA activity in children have not been investigated previously. Similarly, no studies appear to have examined the associations between objectively measured daytime PA levels on HPAA reactivity to psychosocial stress in children.

Accordingly, we examined objectively measured daytime PA and HPAA activity in a community sample of 8-year-old children. Our study had 3 aims. First, we examined whether overall daytime PA, referring to the child's habitual activity level, was associated with diurnal salivary cortisol pattern. Second, we examined whether overall daytime PA was associated with salivary cortisol responses to a standardized psychosocial stress test, the TSST for Children (TSST-C). Finally, we examined whether children who occupy a higher percentage of time in vigorous physical activity (VPA) showed different diurnal patterns of salivary cortisol and different salivary cortisol responses to stress.

Materials and Methods

Participants

The children came from an urban community-based cohort comprising 1049 infants born between March and November 1998 (15). Because a primary objective of the initial study was to examine the effects of maternal licorice consumption during pregnancy on their offspring's developmental outcomes, subsequent participants were recruited to overrepresent children whose mothers consumed higher amounts of licorice. The recruitment has been described in detail before: of the 413 invited children, 321 (77.7%) participated in the 2006 follow-up (16).

For the current analyses, we excluded children with parent-reported, physician-diagnosed developmental delay (n = 3) or Asperger syndrome (n = 1) and those who did not provide at least 4 days of valid PA data including at least 1 weekend day (n = 54). Of the remaining 263 children, 11 in the diurnal sampling and 15 in the sampling during the TSST-C were excluded for having more than 1 missing cortisol value. Thus, complete data on PA and diurnal cortisol was available for 252 (48.8% boys) and on PA and TSST-C for 248 (48.4% boys) children. The included children did not differ from those excluded in birth characteristics or current body anthropometry, and there were no differences in maternal consumption of tobacco, alcohol, or licorice during pregnancy. The Ethics Committees of the City of Helsinki Health Department and Helsinki University Hospital of Children and Adolescents approved the study protocol. Each child and her/his parent gave written informed consent.

Physical activity

PA was objectively measured by an omnidirectional accelerometer (Actiwatch AW4; Cambridge Neurotechnology Ltd, Cambridge, United Kingdom). The accelerometer was worn on the nondominant wrist and activity counts were recorded using 1-minute epochs. All periods with no detected movement during 10 consecutive epochs (ie, 10 minutes) were recorded as missing values. PA was calculated daily over 12 hours from 9:00 AM onward. Only days with data available for at least 10 hours were included in the analyses. Further details on the PA assessment have been described before (17).

The outcome variables of PA were overall PA as counts per minute (cpm) and percentage of time spent in VPA. Overall PA described as counts per minute is an indicator of the total volume of PA (ie, average intensity of PA). This variable was calculated by dividing total counts by monitoring time (min) per day and averaged over the measurement period (mean number of d = 5.93; SD 1.05; range 4–9). We used a cutoff of 1624 cpm representing the 6 metabolic equivalents threshold for VPA, based on a calibration study for a wrist-worn Actiwatch by Ekblom et al (18). To calculate the percentage of time spent in VPA, we divided the number of epochs accumulated in VPA by the total number of epochs measured for each day included in our analysis. The mean percentage of VPA was then averaged over the measurement period for each participant.

Diurnal cortisol sampling

Parents were shown how to collect salivary samples for determination of cortisol using cotton swabs (Salivette, Sarstedt, Nümbrecht, Germany). Salivary samples were obtained during a 1-day period, at awakening (mean 7:53 AM; SD 50 minutes), 15 and 30 minutes thereafter, and at 10:30 AM, 12:00 PM, 5:30 PM, and bedtime (mean 9:15 PM; SD 75 minutes): 81% of the children underwent the cortisol sampling during the PA assessment. The range of time between sampling and measurement of PA varied from 0 to 151 days.

Cortisol sampling during the TSST-C stressor

The TSST-C elicits reliable HPAA and autonomic responses and has been described in detail elsewhere (19, 20). Details on cortisol sampling from the participants of this study have been described earlier (21, 22). In brief, the children were scheduled to arrive in the clinic at 10:00 AM, 12:00 PM, or at 2:00 PM and were asked to abstain from eating for 2 hours before arrival. After the child and parent/guardian had signed an informed consent, a saliva sample, termed arrival hereafter, was obtained, and weight and height of the child were measured. After this, the baseline saliva sample was obtained (mean 36.5; SD 6.2 minutes after the arrival sample). The actual stress test consisted of story-telling and arithmetic tasks. Salivary samples (Salivette) were obtained at arrival and at baseline, as described above, and 0, 10, 20, 30, and 45 minutes after stress. The stress protocol was performed with the child as standing and the recovery period after stress with the child in a sitting position.

Biochemical analyses

Samples were collected between January 2006 and December 2006. The samples were stored at −20°C and analyzed in August 2007. Salivary cortisol concentrations were determined by use of a competitive solid-phase, time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA; Wallac, Turku, Finland) (23). The assay has 0.6% cross-reactivity for cortisone. The intraassay coefficient of variation was between 4.0% and 6.7%, and the interassay coefficients of variation were between 7.1% and 9.0%. Cortisol concentrations were measured in duplicate, and the mean coefficient of variation between duplicate analyses was 5.0%.

Cortisol parameters

Cortisol concentrations were log transformed to attain normality. Diurnal variables were cortisol peak value after awakening (peak of values 15 and 30 minutes after awakening), cortisol awakening response (peak value after awakening minus value at awakening), awakening time weighted area under the curve (AUC) (AUC of 0, 15, and 30 minutes after awakening, calculated as the AUC above zero under trapezoidal rule), awakening AUC increment (AUC minus awakening value), and nadir (minimum of diurnal values). TSST-C stressor variables were baseline, peak value after stress, increment (peak value after stress minus baseline value), time-weighted AUC (calculated as the AUC above zero under trapezoidal rule), and AUC increment (AUC minus baseline value).

Statistical analyses

To facilitate the interpretation of the results, both overall PA and VPA were categorized into thirds by sex. In addition to testing linear associations, we computed quadratic contrasts to assess possible nonlinear associations.

The diurnal hormonal pattern and hormonal response to the TSST-C were first analyzed by a mixed-model analysis (SAS Proc Mixed; SAS Institute Inc, Cary, North Carolina) (24), which is designed for analyzing repeated-measures data with variation arising from both intra- and interindividual differences. One of the main advantages of using mixed models is in the capability to directly measure and specify covariance structure. To test whether the diurnal and the TSST-C hormonal patterns varied according to PA, we included an interaction term, PA as linear × sampling time, into the regression equation, followed by the main effects. To assess quadratic effects, an interaction term, PA as quadratic × sampling time, was tested in the presence of linear effects. In case of significant interactions, subanalyses were used to test if sampling time associated differently with salivary cortisol diurnal pattern and responses to stress in the 3 groups of overall PA and VPA.

Second, multiple linear regression analyses were used to determine whether the more traditional indices of the diurnal and the TSST-C hormonal patterns (described above) varied according to overall PA and VPA. If significant associations were found, we compared children in the lowest third with the children in the intermediate and highest thirds to address linear associations, and to address nonlinear associations, we compared the children in the intermediate third with the children in the highest and lowest thirds.

Because obesity might be associated with the PA and HPAA function, we also performed the analyses after excluding children with obesity (n = 10) (25). However, because this did not affect any of the results, we will present our findings with these children included. Finally, because associations with salivary cortisol may vary according to sex (13), we tested whether sex moderated any of the associations.

Potential confounders

All analyses were adjusted for time of day (at awakening for diurnal analyses; at baseline for analyses of TSST-C) (26), sex and age at testing (13), body mass index (BMI) (mean 16.5, SD 2.2 kg/m^2) (27), and maternal occupational status, which was categorized according to a modified classification system of Statistics Finland (low, n = 18, 7.0%; intermediate, n = 79, 30.6%; upper, n = 161, 62.4%). In addition, we adjusted for mothers' weekly licorice consumption during pregnancy, which is known to be associated with HPAA function in this study population (28). Sleep duration [in minutes, measured with the same device as PA (17)] was included as a covariate because it has been associated with overall daytime PA (17) and HPAA activity (22).

Results

Table 1 shows the sample characteristics according to sex. Boys in comparison with girls had a higher overall PA ($P = .01$) and mean percentage of VPA ($P < .001$), and they displayed lower levels at awakening ($P = .02$), peak after awakening ($P = .03$), and lower AUC ($P = .02$) of diurnal salivary cortisol as well as lower AUC ($P = .004$) and peak after stress ($P = .007$). Categorized overall PA and mean percentage of VPA were highly correlated (Pearson $r = 0.87$, $P < .001$), and the categories were largely overlapping: of the children belonging to the lowest, intermediate, and highest thirds in overall PA, 90%, 73%, and 84%, respectively, belonged to the corresponding third in VPA.

PA and diurnal salivary cortisol

Interactions between overall PA level and sampling time were not significant [PA (linear term) × sampling time, $P = .55$; PA (quadratic term) × sampling time, $P =$

Table 1. Descriptive Statistics[a]

	Boys (n = 126) Mean (SD)	Girls (n = 132) Mean (SD)	P[b]
Child characteristics			
Age, y	8.2 (0.3)	8.1 (0.3)	.04
Height, m	132.0 (5.8)	130.6 (5.3)	.05
BMI, kg/m^2	16.5 (2.2)	16.5 (2.3)	.85
Overall physical activity			
Mean daytime physical activity, cpm	665 (250)	583 (262)	.01
Overall PA thirds			
Low, cpm	390 (185)	238 (175)	
Intermediate, cpm	693 (40)	625 (46)	
High, cpm	913 (115)	841 (116)	
VPA			
Measurement time, %	9.7 (6.3)	6.6 (5.4)	< .001
VPA thirds			
Low,%	3.0 (2.5)	1.2 (1.2)	
Intermediate,%	9.2 (1.5)	5.9 (1.3)	
High, %	16.8 (4.0)	12.6 (4.3)	
Daily measurement time, min	705 (13)	703 (14)	.22
Measurement period, d	5.9 (1.0)	5.9 (1.1)	.93
Weekdays	4.0 (0.9)	4.0 (0.9)	.78
Weekend days	1.9 (0.4)	1.9 (0.5)	.72
Diurnal salivary cortisol (nmol/L)[c]			
Upon awakening	6.8 (1.7)	8.0 (1.7)	.02
Peak after awakening	9.2 (1.7)	10.8 (1.7)	.03
Awakening response	1.4 (1.6)	1.3 (1.7)	.86
Awakening AUC	7.8 (1.7)	9.1 (1.6)	.02
Awakening AUC increment	1.2 (1.4)	1.1 (1.5)	.88
Nadir	0.8 (2.4)	0.8 (2.4)	.72
Salivary cortisol during the TSST-C stressor (nmol/L)[d]			
Arrival	3.0 (1.8)	2.7 (1.8)	.15
Baseline	2.2 (2.0)	2.5 (1.9)	.18
Peak after stress	3.4 (2.0)	4.5 (2.4)	.007
Increment	1.5 (1.8)	1.8 (2.3)	.07
AUC	2.3 (1.8)	3.0 (2.0)	.004
AUC increment	1.1 (1.6)	1.2 (1.9)	.09

[a] Salivary cortisol values are presented as geometric means.

[b] *P* value for the difference between girls and boys.

[c] Diurnal variables: peak after awakening, peak of 15 and 30 minutes after awakening; awakening response, peak value after awakening minus value upon awakening; awakening AUC, awakening time-weighted AUC of 0, 15, and 30 minutes after awakening, calculated as the AUC above zero under trapezoidal rule; awakening AUC increment, AUC minus awakening value; nadir, minimum of diurnal values.

[d] Stress response variables: peak after stress, peak of 0, 10, 20, 30, and 45 minutes after stress; increment, peak after stress minus baseline value; AUC, time-weighted AUC of baseline, 0, 10, 20, 30, and 45 minutes after stress calculated as the AUC above zero under trapezoidal rule; AUC increment, AUC minus baseline value.

.07]. Figure 1A shows that the diurnal salivary cortisol pattern was similar in the 3 PA groups of children by decreasing significantly as the day progressed ($P < .001$ for time in each group). Table 2 shows that there were no differences between groups in the traditional indices of diurnal salivary cortisol ($P > .12$).

Interactions between VPA and sampling time were not significant either [VPA (linear term) × sampling time; $P = .61$; VPA (quadratic term) × sampling time, $P = .15$]. Figure 1B shows that the diurnal salivary cortisol pattern was similar in the 3 VPA groups of children by decreasing significantly as the day progressed ($P < .001$ for time in each group); Table 3 shows that there were no differences between groups in the traditional indices of diurnal cortisol ($P > .64$).

Sex did not moderate the associations of overall PA and VPA with diurnal salivary cortisol levels ($P > .06$ for sex × overall PA/VPA × time interactions).

PA and salivary cortisol responses to TSST-C stressor

Overall PA interacted significantly with sampling time [PA (linear term) × sampling time, $P = .01$], and nonlinear interaction with sampling time was not found [PA (quadratic term) × sampling time, $P = .22$]. Figure 2A shows that children, belonging to the lowest and intermediate thirds in overall PA, showed a significant increase in salivary cortisol in response to stress ($P < .001$ for time). Children belonging to the highest third in overall PA did not show a significant increase in sal-

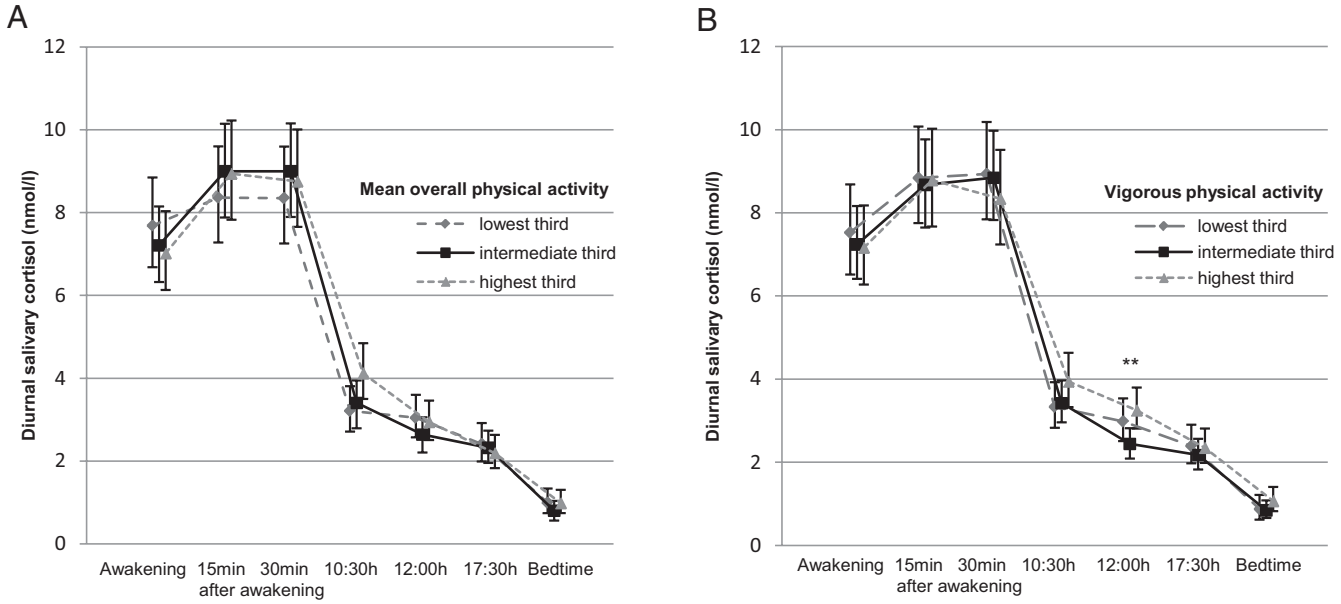

Figure 1. Diurnal salivary cortisol values in children by thirds of mean overall physical activity (A) and thirds of time spent in vigorous physical activity (B). Values are geometric means, and error bars are 95% confidence intervals adjusted for the time at awakening, sex, age, BMI, sleep duration, mother's occupational status, and licorice use during pregnancy. $^{**}P < .01$ for quadratic trend.

ivary cortisol in response to stress ($P = .10$ for time). Table 2 shows that salivary cortisol increment and AUC increment were lower in children with higher PA (P for linear trend $< .02$).

VPA also interacted significantly with sampling time [VPA (linear term) × sampling time, $P = .003$], and nonlinear interaction with sampling time was not found [VPA (quadratic term) × sampling time, $P = .45$]. Figure 2B shows that salivary cortisol in response to stress increased significantly in children belonging to the lowest ($P = .002$) and intermediate ($P < .001$) thirds in VPA. Although salivary cortisol also increased in children belonging to the highest third in VPA, the increase was smaller ($P = .03$). Table 3 shows that salivary cortisol increment and AUC

Table 2. Geometric Means and 95% CIs of Diurnal Salivary Cortisol and Salivary Cortisol During the TSST-C According to the Amount of Overall Physical Activity

Variable	Physical Activity Thirds, Mean (95% CI)			*P* Linear[a]
	Lowest	Intermediate	Highest	
Diurnal salivary cortisol (nmol/L)				
Upon awakening	7.7 (6.7, 8.9)	7.3 (6.4, 8.2)	7.0 (6.1, 8.0)	.41
Peak after awakening	9.6 (8.4, 11.0)	10.2 (9.0, 11.4)	9.9 (8.7, 11.2)	.82
Awakening response	1.2 (1.1, 1.4)	1.4 (1.2, 1.6)	1.4 (1.2, 1.6)	.26
Awakening AUC	8.1 (7.2, 9.2)	8.5 (7.6, 9.5)	8.4 (7.4, 9.4)	.80
Awakening AUC increment	1.1 (1.0, 1.2)	1.2 (1.1, 1.3)	1.2 (1.1, 1.3)	.13
Nadir	0.9 (0.7, 1.1)	0.7 (0.6, 0.9)	0.8 (0.7, 1.0)	.67
Salivary cortisol during the TSST-C stressor (nmol/L)				
Baseline	2.3 (2.0, 2.7)	2.3 (2.0, 2.6)	2.5 (2.1, 2.9)	.57
Peak after stress	4.8 (4.0, 5.9)	3.5 (3.0, 4.2)	3.7 (3.0, 4.4)	.09
Increment	2.1 (1.7, 2.5)	1.5 (1.3, 1.8)	1.5 (1.2, 1.7)	.02[b]
AUC	3.1 (2.6, 3.6)	2.4 (2.1, 2.8)	2.5 (2.1, 2.9)	.12
AUC increment	1.3 (1.2, 1.5)	1.1 (0.9, 1.2)	1.0 (0.9, 1.1)	.01[c]

Abbreviation: CI, confidence interval. Diurnal variables: peak after awakening, peak of 15 and 30 minutes after awakening; awakening response, peak value after awakening minus value upon awakening; awakening AUC, awakening time-weighted AUC of 0, 15, and 30 minutes after awakening, calculated as the AUC above zero under trapezoidal rule; awakening AUC increment, AUC minus awakening value; and nadir, minimum of diurnal values. Stress response variables: peak after stress, peak of 0, 10, 20, 30, and 45 minutes after stress; increment, peak after stress minus baseline value; AUC, time-weighted AUC of baseline 0, 10, 20, 30, and 45 minutes after stress calculated as the AUC above zero under trapezoidal rule; AUC increment, AUC minus baseline value. Associations are adjusted for time of day (at awakening for diurnal analyses; at baseline for analyses of TSST-C), sex, age, BMI, sleep duration, mother's occupational status and licorice use during pregnancy

[a] *P* values are for linear trend, and all *P* values for quadratic trend were nonsignificant ($P > .09$).

[b] $P = .01$ for lowest vs intermediate third; $P = 0.01$ for lowest vs highest third; $P = .69$ for intermediate vs highest third.

[c] $P = .02$ for lowest vs intermediate third; $P = .01$ for lowest vs highest third; $P = .52$ for intermediate vs highest third.

Table 3. Geometric Means and 95% CIs of Diurnal Salivary Cortisol and Salivary Cortisol During the TSST-C According to the Amount of Vigorous Physical Activity

Variable	Vigorous Physical Activity Thirds, Mean (95% CI)			*P* Linear[a]
	Lowest	Intermediate	Highest	
Diurnal salivary cortisol (nmol/L)				
Upon awakening	7.5 (6.6, 8.6)	7.2 (6.4, 8.2)	7.2 (6.3, 8.2)	.65
Peak after awakening	10.1 (8.8, 11.5)	9.8 (8.7, 11.0)	9.7 (8.5, 11.0)	.72
Awakening response	1.3 (1.2, 1.5)	1.4 (1.2, 1.5)	1.4 (1.2, 1.5)	.91
Awakening AUC	8.5 (7.5, 9.6)	8.3 (7.5, 9.3)	8.2 (7.3, 9.3)	.75
Awakening AUC increment	1.1 (1.0, 1.2)	1.2 (1.1, 1.2)	1.2 (1.1, 1.3)	.77
Nadir	0.8 (0.7, 1.0)	0.7 (0.6, 0.9)	0.9 (0.7, 1.1)	.68
Salivary cortisol during the TSST-C stressor (nmol/L)				
Baseline	2.2 (1.9, 2.6)	2.1 (1.9, 2.5)	2.7 (2.3, 3.2)	.09
Peak after stress	4.3 (3.5, 5.2)	3.8 (3.2, 4.5)	3.9 (3.2, 4.7)	.56
Increment	1.9 (1.6, 2.3)	1.8 (1.5, 2.0)	1.4 (1.2, 1.7)	.02[b]
AUC	2.8 (2.4, 3.3)	2.6 (2.2, 3.0)	2.6 (2.2, 3.0)	.59
AUC increment	1.3 (1.1, 1.4)	1.2 (1.1, 1.4)	0.9 (0.8, 1.1)	.007[c]

Abbreviation: CI, confidence interval. Diurnal variables: peak after awakening, peak of 15 and 30 minutes after awakening; awakening response, peak value after awakening minus value upon awakening; awakening AUC, awakening time-weighted AUC of 0, 15, and 30 minutes after awakening, calculated as the AUC above zero under trapezoidal rule; awakening AUC increment, AUC minus awakening value; and nadir, minimum of diurnal values. Stress response variables: peak after stress, peak of 0, 10, 20, 30, and 45 minutes after stress; increment, peak after stress minus baseline value; AUC, time-weighted AUC of baseline 0, 10, 20, 30, and 45 minutes after stress calculated as the AUC above zero under trapezoidal rule; AUC increment, AUC minus baseline value. Associations are adjusted for time of day (at awakening for diurnal analyses; at baseline for analyses of TSST-C), sex, age, BMI, sleep duration, mother's occupational status and licorice use during pregnancy.

[a] *P* values are for linear trend, and all *P* values for quadratic trend were nonsignificant ($P > .10$).

[b] $P = 0.48$ for lowest vs intermediate third; $P = 0.03$ for lowest vs highest third; $P = 0.06$ for intermediate vs highest third.

[c] $P = 0.69$ for lowest vs intermediate third; $P = 0.008$ for lowest vs highest third; $P = 0.006$ for intermediate vs highest third.

increment were lower in children with higher VPA (*P* for linear trend $< .03$).

Sex did not moderate the associations of overall PA and VPA with salivary cortisol responses to stress ($P > .10$ for sex × overall PA/VPA × time interactions).

Discussion

To our knowledge, this is the first study to show that PA levels in healthy 8-year-old children are associated with altered HPAA reactivity to stress. After adjusting for major covariates, children with the highest levels of objec-

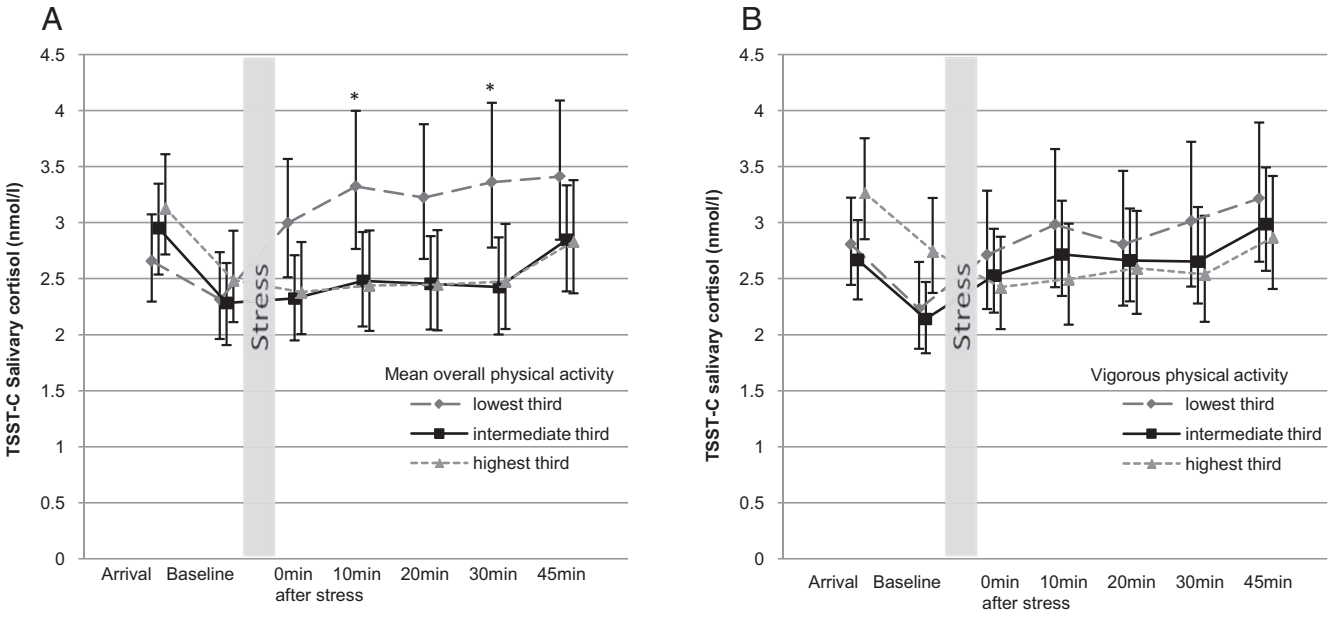

Figure 2. Salivary cortisol responses to TSST-C by thirds of mean overall physical activity (A) and thirds of vigorous physical activity (B). Values are geometric means, and error bars are 95% confidence intervals adjusted for the time at baseline, sex, age, BMI, sleep duration, mother's occupational status, and licorice use during pregnancy. $*P < .05$ for linear trend.

tively measured overall daytime PA or VPA showed no or only small increases in salivary cortisol levels in response to stress. In contrast, children with less PA showed a significant increase in salivary cortisol levels after stress. This finding was also reflected in the higher levels of the salivary cortisol increment and AUC increment in response to stress, which suggests that stress reactivity was higher for individuals with lower PA, although the baseline and peak after stress did not vary between the groups. The diurnal salivary cortisol pattern did not differ according to the level of the overall daytime PA and VPA.

The association between PA and HPAA was similar in girls and boys, although the level of their activity differed, which indicates that sex does not moderate the association between PA and HPAA responsivity at this age. However, the possibility of sex-specific results in later development should be investigated in the follow-up studies.

Our findings contribute to the existing literature on the role of PA as both a stressor and a modifier of stress (9). The lower reactivity of HPAA to the psychosocial stress protocol in children with a higher level of PA indicates that PA modifies responses to psychosocial stress. This finding is in line with previously reported findings for adults, in which physically trained men exhibited significantly lower cortisol responses to the TSST when compared with their untrained counterparts (11) and older physically fit women showed lower cortisol responses to another psychosocial stress test when compared with unfit older women (12). Consequently, PA might serve as a protective factor in stressful day-to-day experiences, which may be 1 explanatory mechanism behind the association of higher PA and better psychological well-being in children and youth (1–7). However, as HPAA function (13) and the quantity and quality of PA (14) change by age and pubertal maturation, the role of the HPAA in the association of PA and well-being among prepubertal children still requires confirmation by further research.

Future research should focus on cortisol responses to acute PA in prepubertal children and whether a lower cortisol response to physical exercise can be found in a highly physically active prepubertal group. At present, the results are scarce and contradictory. One study has previously found that acute physical exercise was not associated with postexercise cortisol levels in 53 prepubertal (9 years old) girls and boys (29). However, another study including 38 prepubertal (10 years old) boys found this association for highly fit participants but not for those with average fitness (30).

We found that in terms of diurnal cortisol pattern across the measurement day, the cortisol levels did not vary between children with different levels of PA. However, because diurnal cortisol assessment was conducted outside the clinic, we are not able to know whether all the children have been in similar PA during the salivary extractions, and therefore, we cannot verify to what extent acute exercise might have affected the children's cortisol levels. Similarly, the direct effects of acute exercise on HPAA cannot be assessed with our protocol, and thus, our study is not comparable with the above-mentioned research on children. In addition, the physical condition of the participants was not assessed. Not being able to adjust the data for physical fitness is a limitation because it might affect the cortisol response of the participants.

As we have reported earlier on the same participants (22), the children's salivary cortisol response to the TSST-C stressor was significantly lower than that seen in a similarly aged and sized study of healthy children in the United Kingdom (20). It has been recognized recently that many typically developing low-risk children have relatively low HPAA responses to a variety of stressors, including the TSST-C (31). In some children, salivary cortisol levels may even decrease in response to stress (31). In our cohort, the children with higher levels of PA might also represent a more optimally developing group of children, who demonstrate a hyporesponsivity to stress typically found in the prepubertal period of development in both animals and humans (32).

To answer our question regarding the relevance of PA intensity, we found that both the overall habitual level of PA and the amount of VPA associated with HPAA reactivity of our participants. However, in our study the overall PA and percentage of VPA were highly correlated, and data on the actual exercise activities were not collected. Thus, our method might not precisely identify the children initiating the highest intensity activities. Consequently, further research is needed to focus on the significance of different physical activity patterns and intensities in producing favorable health effects.

The strengths of our study include the relatively large, population-based sample with a narrow prepubertal age range. We collected data on salivary cortisol levels across various time points, both diurnally and after a robust stress test, and used objective measures to assess PA. Motion detector measurement is almost as good in predicting PA as direct observation (33), which has previously been reported as the most accurate way of assessing children's PA (34).

Accelerometers are poor in assessing static exercise and certain types of dynamic activities, eg, cycling and water sports (the devices are not water resistant), and they produce possible measurement error caused by vehicle transportation. When assessing the mean level of PA over multiple days, however, the measurement error caused by these factors is relatively low. In addition, the positioning

of the device on the wrist may not give similarly accurate information on different activities (35). All positions of the devices, however, have been shown to predict energy expenditure accurately (35, 36).

It should be acknowledged that no gold standard for threshold values of counts per minute for VPA has been established thus far. We used 6 metabolic equivalents as a cutoff for VPA, based on a recent calibration study with the same device worn on the nondominant wrist (18). Using a 1-minute epoch in recording activity counts might underestimate the high intensity activities as compared with a shorter epoch length (37). A reasonable agreement was found, however, when the ability of different epoch lengths to measure VPA were compared with each other (37). Furthermore, owing to its cross-sectional nature, the causality of the effect cannot be identified in this study. Prospective controlled studies are required to focus on the causal relationships, for example, by investigating whether increasing exercise would moderate the cortisol balance.

Because we have used multiple measures of diurnal cortisol pattern and cortisol responses to the TSST-C at different time points, multiple testing was carried out. We decided not to use the correction for multiple testing (eg, Bonferroni correction), however: first, because the number of type I errors cannot decrease without increasing type II errors (38) and second, theoretical assumption behind the correction for multiple testing is that all null hypotheses are true simultaneously, which was not of interest for our study. It is suggested that simply describing the analyses carefully is usually the most rational way of dealing with multiple testing (39).

In conclusion, our study shows that increased levels of PA are associated with decreased HPAA reactivity to stress in a community sample of 8-year-old children. Although children with different levels of PA show similar diurnal patterns of salivary cortisol, the children with the lowest activity levels are more responsive to psychosocial stress. Consequently, PA may contribute to the psychological well-being of children by regulating their neuroendocrine reactivity to stress.

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