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Research Article

A Longitudinal Study on Teenagers´ Salivary Cortisol and Psychosocial Health in a Swedish County

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Abstract

Previous studies have found that somatic and mental health in schoolchildren is not good, mostly in girls. The teenage period is most vulnerable. Salivary cortisol is a well-known biomarker for stress and psychosocial distress. The aims of this study were to analyse salivary cortisol, mental and somatic health, and progress in healthy students (15 - 17 years age), and look for differences between gender, age, type of school, seasonal variations and correlations between psychometric questionnaires and salivary cortisol concentrations.

Sixty-four children (32 girls) participated from one urban school with mostly low socioeconomic immigrants and one rural school with moderate socioeconomic status families and few immigrants. Salivary cortisol samples were collected between 8 and 9 am in the classroom, after which the students answered three questionnaires (Stress In Children (SIC), Beck Youth Inventory psychosocial questionnaire (BYI) and the Teenage Stress Questionnaire, and finally completed a physical body investigation. Each child was included at six occasions.

Salivary cortisol concentration increased with age, and was higher in females. It was neither seasonal variation nor correlation between salivary cortisol and psychometric tests. Psychometric index scores on the BYI and Global mean scores (GMS) for SIC were generally high. Headache and gastrointestinal disturbance were frequent (35 - 60%), but decreased during follow up. Headaches were significant more frequent in girls. The intensity was mild to moderate, and it was at a frequency of mostly less than twice a week. In the urban school, height, weight, BMI, the waist/hip ratio, rate-pressure-products and salivary cortisol were higher, but only in girls.

We conclude that the present study confirm different grades of stress in girls and boys. Both subjective (more psychosomatic symptoms, BYI, SIC) and objective findings (heart rate, blood pressure, cortisol) points in this direction and that girls are more prone to react with markers for stress. The reason behind this phenomena needs to be better explored and the possible reasons for this in the school environment has to be studied.

Keywords: *Hypothalamic Pituitary Adrenal Axis (HPA axis); Psychometric Test; School-Age Children; Body Mass Index, Blood Pressure, Rate Pressure Product (RPP)*

Abbreviations

BMI: Body Mass Index Defined as Body Weight/Height^2; BYI: Beck Youth Inventory (Psychometric Test); CAR: Cortisol Arousal Reaction: Cortisol Awakening Response; HPA: Hypothalamic-Pituitary-Adrenal Axis; RPP: Rate Pressure Product (Heart Rate *Systolic Blood Pressure); Salivary Cortisol: Saliva Sample Analyzing Cortisol (RIA method); SIC: Stress in Children (Psychometric Test); GMS: Global Mean Score in SIC

Introduction

The experienced life satisfaction of schoolchildren is at a reasonably high level, although stress-related complaints are common problems and have been reported to increase particularly among girls [1-5]. In a Swedish study, the degree of stress-related disturbances also tended to increase with age, especially from the 8th to 9th (15 years of age) grade in primary school. In girls, the frequency of self-reported and stress-related symptoms increased from eight to twenty-five percent in the pupils [6]. This development is in accordance with the predicted future scenario of the global burden of diseases. The World Health Organisation has ranked psychiatric disease to be one of the leading causes for disability in the world in the next decade [7]. For this reason, it is crucial to find appropriate methods and instruments to measure and follow the development of stress and related disorders, especially in children. In this study, we defined stress as the degree of all the wear and tear caused by life. The disrupted biological homeostasis caused by this exposure is defined as allostasis, i.e. the constantly adjusting levels of physiological activity and arousal in order to adapt to environmental factors. In the presence of stress, this process creates a physiological overload, which is eventually expressed as chronic disease states [8,9].

A number of previous studies have shown salivary cortisol to be an excellent mirror of HPA-axis function [9,10]. In children, cortisol has been shown to react to psychosocial stress related to the mother´s socioeconomic status and a depressive state [11]. Also, an alteration of cortisol release with psychosomatic origins has been found in children with recurrent abdominal pain [12] and those exposed to a relatively low degree of psychosocial stress load [13]. Since cortisol is one of the best studied biomarkers for psychosocial stress load in children, and since one of the authors has developed reference values for morning salivary cortisol concentrations in healthy school-aged children, we decided to study cortisol as a marker for stress in this study [14].

Significance of the study. There are sparse studies following repetitively a cohort of teenager´s during a long period with a multimodal design analysing both quantative and qualitative aspects. Therefore, we have performed this study to look for some.

Differences between gender, age specific and individual trends, dependence on school atmosphere, and socioeconomic situations. We also wanted to highlight the possible usefulness of cortisol as a possible marker for stress and stress related problems in the young. Finally, could salivary cortisol be the only outcome factor and reduce the need of more time consuming questionnaires?

The primary aim with this study was to longitudinally analyse salivary cortisol concentrations in two socially different schools and to collect and analyse physiological as well as psychometric data from validated questionnaires during the same time. By doing this, we wanted to study changes and co-variations in the teenagers regarding psychosocial health, psychosomatic condition and cortisol concentrations. The second aim was to compare the cortisol concentrations with age and sex matched healthy schoolchildren in the long term perspective in 1990 and 2000 and the time point for this study (2010).

We had the following hypotheses for our study:

- Salivary cortisol increases with age and grade in school
- Salivary cortisol depends on type of school situations
- Salivary cortisol differs with gender
- Salivary cortisol correlates with psychometric instruments measuring stress and poor psycho-socio-mental health [e.g., Stress in Children (SIC) and Beck Youth Inventory (BYI) scores].
- Salivary cortisol concentrations have increased during the last two decades

Study Population and Methods

The present study was conducted as a longitudinal study with the aim to test a number of hypotheses in two different schools.

Demographic and clinical data are shown in table 1. Five school classes with pupils in the 8th grade in primary school were approached. One school was situated in a suburban area of a city with approximately 50,000 inhabitants in the south-western part of Sweden. This area is considered to be mainly populated by working class people and immigrants (B-School). In contrast, the other school was located in a rural area (A-school) considered to be mainly populated by middle-class people and few immigrants. A significant part of the population in this area is occupied within the agricultural sector.

One hundred pupils were approached with the question to participate in the study. Pupils from one class did not want to participate. In total 87 pupils were eligible to be included in the study. Of these, 64 (together with their parents) gave their oral and written informed consent to participate in the study. At the final examination, performed two and a half years later, 22 pupils were lost to follow up due to unwillingness for further participation. In one case, a pupil moved to another part of the country, and it was, therefore, impossible to follow up due to administrative reasons. The first examination of the pupils took place in December 2007, and they were all examined each spring and autumn thereafter until June 2010. The pupils were examined on six occasions.

Primary outcome was salivary cortisol and secondary outcome were psychosomatic and psychosocial variables.

Ethics

The Regional Ethics Committee of Gothenburg University approved the study (No 544-07). All participants and their parents gave informed consent before inclusion. In the classroom all participants were informed orally about the investigation and they were free to participate or not. Salivary cortisol samplings and answering questionnaires were performed for all participants in the classroom as a group. In contrast, somatic investigations were performed individually. The investigation conforms to the principles outlined in the Declaration of Helsinki [15].

Clinical data

All participants were followed with a case record form containing all information collected within the study. The participants were also asked to respond to fifteen questions regarding their subjective experience of their physical and emotional health, frequency of health care contacts, pharmacological treatment, and presence of gastrointestinal pain and/or headache (frequency, intensity, localisation and treatment).

These questions were based on response categories with two to four alternatives (Likert scale). The children were asked to indicate the category that best applied to them. These categories were: yes (1) or no (0) and frequency; daily (1), weekly (2), monthly (3) or less often (4), diurnal time point; morning (1), during day (2), evening (3), night (4) or a mixture (more than one mark), duration; < 5 minutes (1), < 15 minutes (2), < 1 hour (3) and > 1 hour (4). The intensity was graded as little (1), moderate (2) or intensive (3). The questions were expected to be completed in five to ten minutes.

All clinical data were collected in the school, i.e. in the classroom or at a nearby expedition for the nurse and doctor attached to the nurse and doctors office. The examinations were performed in the following order:

- Sampling of salivary cortisol
- Psychometric examination
- Physical examination

Salivary cortisol

Measurements of salivary cortisol have been done since the 1980´s [16,17]. The incitement to measure cortisol in saliva is that it is free and independent of different binding globulins such as transcortin and the influence of hormone shifts for oestrogen during puberty. The free fraction is the biologically active and its concentration oscillates in saliva in the same manner as in blood ($r > 0.7$) [18,19]. Another advantage with measuring cortisol in saliva, especially in children, is that it is painless and convenient [14].

We collected saliva between 8 - 9 am in the classrooms in order to eliminate the cortisol awakening response (CAR), i.e. the maximal spontaneous increase of cortisol concentration during the day, normally taking place within one hour after awakening [20-22]. Another rationale for this procedure was to avoid pitfalls connected with the measurement at home such as not remembering the measurement time, procedure and other violations against the strict protocol necessary to achieve valid data. By performing all measurements in this manner, we also ensured standardisation in the collection procedure and minimised missing data due to lack of saliva in the test tube or other technical problems. This procedure has been applied by us in our previous studies for more than two decades, which makes it possible to do comparisons with our earlier findings [12,14].

Collection of salivary samples was done using a previously described technique [12,14]. In summary, saliva was collected in neutral cotton based Salivette tubes™, and the participants were asked to wet the cotton swab for two minutes while in a standing position. This was done simultaneously for all participating children. Thereafter, the saliva was collected in a freezer (-80^0C) until the analyses of all six samples/children. We used cotton-based swabs without any stimulator of saliva secretion. A commercial RIA-based technique for salivary cortisol was used for analyses (Spectria™ Cortisol I^{125} coated tubes, Landskrona, Sweden).

Psychometric measurements

A questionnaire with the aim to capture self-perceived stress in children has been developed by one of the authors (PW) and has been found to be valid and reliable. It has been considered possible to apply this instrument for screening of stress in schoolchildren. The SIC questionnaire is a self-rating instrument comprising 21 items covering physical, emotional and symptomatic aspects of stress, as well as experienced degree of social support. It is based on response categories on a Likert scale with four alternatives. The children were asked to indicate the category that best applied to them. These categories were: never (1), sometimes (2), often (3) and very often (4). The questionnaire can be completed in a few minutes. When analysing data from SIC, each participant´s total score was calculated and divided by the number of questions answered to provide a mean score, i.e. SIC-Global Mean Score (GMS). A factor analysis revealed three factors in the instrument: (I) well-being, (II) distress and (III) social support. Internal consistency ad modum Cronbach has been reported to reveal an alpha-coefficient of 0.86 for the complete instrument [22].

The BYI [23] was introduced as five self-completion scales that can be used to assess and screen for anger, anxiety, depression, disruptive behaviour and self-perception in children aged 9 - 18. Each subscale of 20 items requires less than 10 minutes to complete. The items are written at a 2nd-grade reading level. The subject rates each statement on a 4-point Likert scale ranging from never (0), sometimes (1), often (2) to always (3). The children were asked to indicate the category that best applied to them. The resulting scores are calculated for each inventory by summing their 20 responses, and these scores can range from 0 to 60.

The psychometric properties of the BYI were studied in a nationally drawn, standardised sample of 800 children in the USA. The internal consistencies of the five inventories were all high (coefficients > 0.84), and the magnitudes for the median seven-day, test-retest reliability correlations for the five inventories were large (r > 0.73). Evidence supporting the construct validity of the BYI was reported in a study of paediatric psychiatric outpatients [24]. The inventories have been translated into Swedish, and this version was studied intensively (n = 2400) among standardised samples aged 9 - 18 years drawn from different Swedish regions and from different clinical and school settings [25]. Four of the scales were applied in this study: anger, anxiety, depression and disruptive behaviour.

Physical examination

Physical examination was performed individually directly after sampling of saliva and after filling in the questionnaires at each occasion in a nearby quiet classroom. Blood pressure (BP) and heart rate (HR) were measured after five minutes of rest in a supine position with two measurements. The second measurement was recorded. An automatic oscillation method for registration/estimation of blood pressure was applied (DINAMAP compact T, Criticon, Gwent, UK).

All measurements were performed at the same time during the day for each child.

Rate pressure product (RPP) was used as a measure of the stress put on the cardiac muscle based on the number of times it needs to beat per minute and the arterial blood pressure that it is pumping against. It was considered an indication of the energy demand of the heart, and thus a good measure of the energy consumption of the heart. RPP was calculated as Resting Heart Rate (RHR) * Systolic Blood Pressure (SBP) [26,27].

Anthropometric measurements were performed in the following manner. Weight was recorded to the nearest 0.5 kg using a calibrated standard balance scale when the subjects had bare feet and were wearing light indoor clothing. Height was measured to the nearest 0.5 cm using a ruler attached to the scales. Body mass index (BMI) was calculated as body weight in kg divided by height^2 (kg/m^2). Waist circumference was measured at the umbilical level and hip circumference at the widest point over the buttocks by using a plastic measuring tape.

Statistical Methods

Descriptive statistics were generated and STATISTICA version 8.0 and 10.0 (STAT soft Inc. Uppsala, Sweden) and SPSS version 18.0 (SPSS Inc. Chicago, IL, USA) were used for the statistical evaluations. Multiple regression analysis and non-parametric tests, including the Mann-Whitney U test (Tables 1, 3, 4), median test (Table 5), Friedman`s ANOVA (Figure 1), Kruskal-Wallis one-way ANOVA (Tables 3 and 5), and Wilcoxon´s matched-pairs signed ranks test (Table 2, Figure 1), were used. Chi^2 test was used for comparing gender (Table 2). A Spearman rank order correlation test comparing salivary cortisol, SIC and BYI was also used. Bonferroni method for adjustment of mass-significance was used.

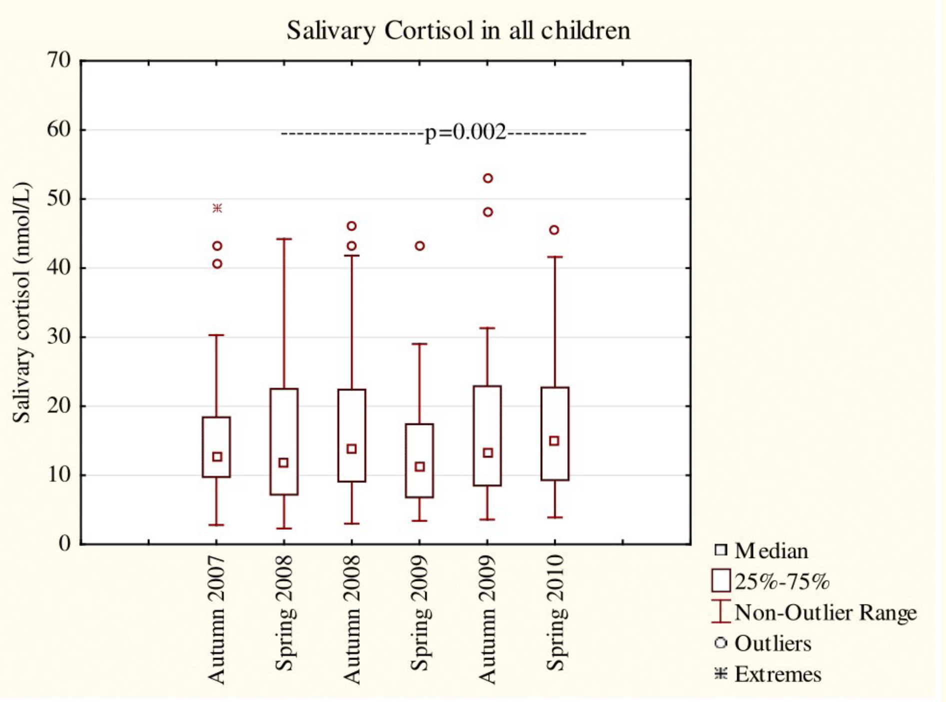

Figure 1: *Changes in salivary cortisol (nmol/L) during the study period: all participants. Statistics; Friedmann´s ANOVA Chi square (All, (N = 24; df = 5, Z = 14,034; p = 0.015), females (N = 13, df = 5, Z = 19,052; p = 0.002), males (N = 11, df = 5, Z = 3,259; p = 0.66).*

Results

Physical characteristics at baseline are shown in table 1.

For all children, there were significant differences between gender in height, weight, BMI, hip and waist/hip ratio. In addition, the heart rate, systolic, diastolic and median blood pressure and the rate pressure product were significantly different. Age at menarche was 12.0 years (11.0 - 13.0; median, 10/90 percentiles).

Sex	Boys (A) [16]	Boys (B) [14]	Girls (A) [13]	Girls (B) [19]
Age (y)	14.5 (13.9 - 14.9)	14.9 (14.3 - 15.2)	14.6 (14.2 - 14.9)	14.9 (14.2 - 15.5)
Height (cm)	167.8 (151.0 - 178.8)	175.0[1] (163.2 - 188.2)	161.4 (150.4 - 174.5)	164.2[1] (153.0 - 178.4)
Weight (kg)	53.3 (45.0 - 72.0)	63.0[2] (50.5 - 103.0)	51.8 (44.5 - 59.1)	53.0[2] (43.0 - 70.5)
BMI	19.8 (16.1 - 23.5)	20.5[3] (17.2 - 30.4)	19.9 (17.8 - 24.0)	19.2[3] (17.5 - 24.2)
Waist (cm)	71.7 (66.2 - 88.0)	79.4 (68.8 - 105.0)	72.0 (67.5 - 75.2)	74.8 (64.0 - 88.4)
Hip (cm)	84.5[4] (81.0 - 92.3)	96.7 (82.0 - 115.4)	90.8[4] (84.5 - 98.0)	91.5 (81.8 - 99.8)
Waist/Hip ratio	0.83[5] (0.8 - 0.93)	0.85 (0.76 - 0.97)	0.79[5] (0.75 - 0.85)	0.82 (0.76 - 0.91)
SBP (mmHg)	114 (92 - 132)	122 (102 - 144)	115 (97 - 124)	126 (99 - 153)
DBP (mmHg)	59 (43 - 74)	64 (54 - 77)	63 (48 - 83)	65 (54 - 97)
Heart rate (bpm)	71 (50 - 93)	88 (64 - 106)	74 (60 - 96)	81 (31 - 109)
Rate Pressure Product	7 684[6] (5 100 - 11 067)	10 302[6] (7 560 - 12 920)	8 450 (5 820 - 10 788)	9 775 (3 224 - 16 677)

Table 1: *Physical characteristics at baseline given as [n], mean and range in school A and B.*

SBP: Systolic blood pressure; DBP; Diastolic blood pressure.

The statistical method used was Mann - Whitney U test,

[1,2]comparison between boys and girls in school B; p < 0.001

[3]comparison between boys and girls in school B; p < 0.05

[4,5]comparison between boys and girls in school A; p < 0.05

[6]comparison between boys in school A and school B; p < 0.01

A detailed description of clinical symptoms and treatments at baseline are shown in table 2. A significant difference in frequency of headaches were found between the gender with a higher prevalence among the girls in both schools (Chi-squared = 8.16, DF = 1, p = 0.0043) but not for abdominal pain (Chi-squared = 1.54, DF = 1, p-value = 0.215).

		Boys		Girls	
School		**A [16]**	**B [14]**	**A [13]**	**B [20]**
Are you healthy?	Yes	85	91	92	100
Do you have any medical therapy?	Yes	0	0	8[1]	0
Do you have any pharmacotherapy?	Yes	12	7	8	0
[2]Do you have gastrointestinal pain sometimes?	Yes	18	35	54	45
How often?	Daily	0	14	8	0
	Twice weekly	6	7	8	15
	Twice monthly	13	7	24	30
	Sometimes	38	57	48	50
	Never	43	14	16	5
Duration of pain?	< 5 min	25	21	0	20
	< 15 min	19	0	38	25
	< 60 min	13	36	23	20
	> 60 min	0	29	23	25
What time point in the day/night?	In the morning	25	14	8	15
	At midday	19	35	46	40
	Evening	13	28	15	5
	Night	0	0	8	5
	Several time points	44	7	8	20
[2]Do you have headache?	Yes	6	28	54	55
How often?	Daily	0	7	8	15
	Twice weekly	0	28	16	35
	Twice monthly	20	0	31	10
	Sometimes	43	28	24	35
	Never	36	35	23	5
Intensity of pain?	Little	43	22	31	35
	Medium	13	50	31	50
	Severe	0	0	16	10
	No pain	43	28	23	5

Table 2: *Clinical symptoms and treatment at baseline are presented in percentages, [n].*

A significant difference in frequencies of headaches were found between gender, with a higher prevalence in girls, (Chi-squared = 8.16, DF = 1, p = 0.0043).

[1]Physiotherapy. [2]Duration from hours to 24 months

Salivary cortisol-association with age, gender, season and time trends

Salivary cortisol concentrations in all children during the whole study period split by school and gender are shown in table 3. Totally, 24 of the present children had collected 6 salivary samples and another group of 19 children had collected five salivary samples during the study period.

Salivary cortisol concentrations at baseline (mean, SD) differed significantly between boys (n = 25, 12.0 (5.2) nmol/L) and girls (n = 31, 17.1(11.1) nmol/L; MW-U (2;56), Z = -2,42; *p* = 0.015). During the following two and half years, the cortisol concentrations increased significantly in the whole group mean 14.1 to 19.3 nmol/l; ANOVA Chi sqr (N = 24, DF = 5, Z = 14,03; *p* = 0.015) and in girls mean 17.7 to 23.9 nmol/L;(N = 13, DF = 5, Z = 19,05; *p* = 0.002), but not in boys, mean 9,9 to 13.9 nmol/L (N = 11, DF = 5, Z = 3,26; *p* = 0.66)) (Figures 2A+2B). There was a significant difference between boys and girls in the B-school but not in the A-school in salivary cortisol concentrations in autumn 2009, in boys and girls 10.1 versus 21.1 nmol/L (KW-H (1;22) Z = 5.94; p = 0.015) and and in spring 2010, 10.5 versus 20.2 nmol/L (KW-H (1;21) Z = 6,55;p = 0.01), respectively.

There was no seasonal variation in salivary cortisol concentration when comparing all children for each period (autumn 2007 against spring 2008, 2009 and 2010, and autumn 2008 against spring 2008, 2009 and 2010, and autumn 2009 against spring 2008, 2009 and 2010 (p = 0.057-0.55)).

School	(A)		(A)	(B)	(B)	(B)	(A+B)
Sex	Boys	Girls	All	Boys	Girls	All	All
Time							
Autumn 2007	[15] 12.7 (5.5)	[11] 18.5 (10.5)	[26] 15.1 (8.3)	[10] 10.8 (4.9)	[18] 17.6 (11.5)	[28] 15.2 (10.1)	[55] 15.1 (9.2)
Spring 2008	[14] 10.8 (7.3)	[11] 15.8 (8.0)	[25] 13.0 (7.8)	[14] 14.0 (10.3)	[18] 16.8 (9.9)	[32] 15.6 (10.0)	[58] 14.7 (9.2)
Autumn 2008	[12] 11.5 (4.6)	[12] 16.2 (12.0)	[24] 13.9 (9.2)	[13] 11.9 (10.0)	[19] 18.4 (10.9)	[32] 18.2 (10.4)	[58] 16.7 (10.5)
Spring 2009	[12] 12.8 (7.4)	[13] 16.6 (8.6)	[25] 14.7 (8.1)	[12] 12.5 (8.3)	[18] 12.6 (8.6)	[30] 12.6 (8.4)	[57] 13.5 (8.1)
Autumn 2009	[12] 11.3 (5.4)	[11] 19.0 (12.6)	[23] 15.0 (10.1)	[9] 11.5 (7.8)[1]	[11] 21.7 (12.4)[1]	[20] 17.1 (11.6)	[45] 16.2 (10.7)
Spring 2010	[11] 11.7 (8.0)	[9] 20.5 (11.8)	[20] 15.7 (10.6)	[10] 15.3 (11.5)[2]	[10] 20.7 (7.9)[2]	[20] 18.0 (10.0)	[42] 17.7 (10.8)

Table 3: *Morning (8 - 9 am) salivary cortisol concentrations (nmol/L) in all children split by school and gender, given as [n], mean (SD). The statistical method used was MW-U test. We found significant different results between boys and girls only at school B.*

[1]p = 0.015, [2]p = 0.010

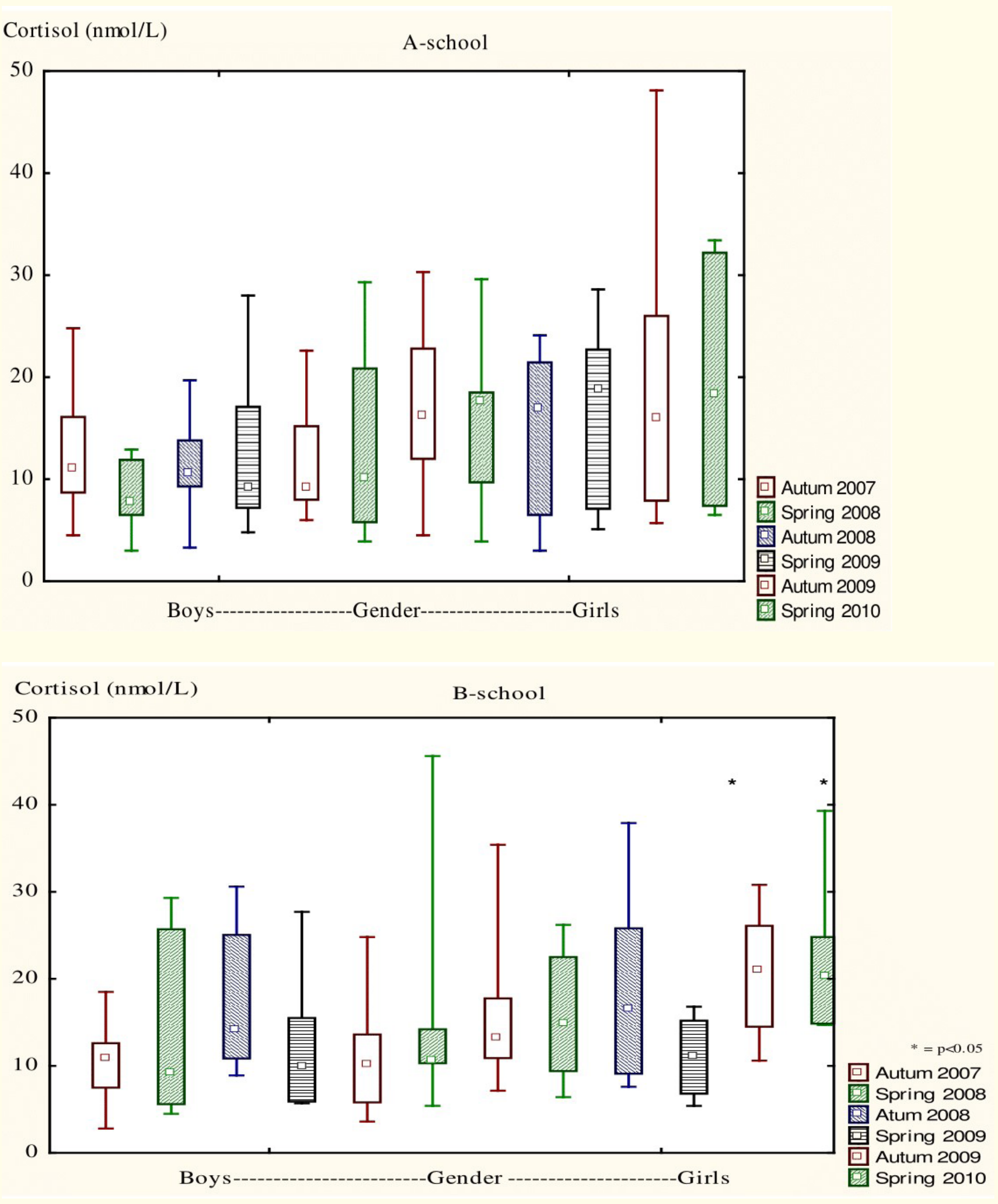

Figure 2: *Salivary cortisol concentration (nmol/L) at 8-9 am from baseline and during the study period, split by gender and school.*

Statistics: (Wilcoxon matched pairs test) Spring 2008 versus spring 2010, N = 37, df = 1, Z = 3.06; p = 0.002

Salivary cortisol concentrations trends - we found that the morning cortisol were higher in girls but not in boys in 2010 compared to the 1990 and the 2000 periods.

Psychometric assessments at baseline and during the study period

Stress in Children questionnaire

At baseline, the GMS for the complete SIC questionnaire was, for all children (mean, (SD) (n = 63, 2.45 (0.26), for boys (n = 30, 2.38 (0.28) and for girls (n = 33, 2.50 (0.24): there was no gender difference (p = 0.07). There was a significant increase in GMS over time for all children (p = 0.004) with no difference between gender (Table 4). GMS values over 2.5 is higher than normal and suspected/ indication for abnormal stress. (22, 28).

The three factors in the SIC questionnaire were assessed at all six time points within the study. In Factor 1 (lack of well-being), a worsened well-being over time was found for both boys and girls with no difference between gender. In Factor 2 (distress), no increase was found during the study period. Neither in Factor 3 (lack of social support) was differences found over time in any gender (Table 4).

Time point	Factor 1	Factor 2	Factor 3
Baseline 2007 [63]	2.95 (0.84)	2.02 (0.69)	2.31 (0.73)
Spring 2008 [58]	2.95 (0.84)	2.04 (0.59)	2.31 (0.75)
Autumn 2008 [61]	2.92 (0.80)	2.05 (0.63)	2.33 (0.71)
Spring 2009 [58]	2.88 (0.83)	2.05 (0.68)	2.20 (0.74)
Autumn 2009 [43]	3.06 (0.79)	2.09 (0.64)	2.39 (0.72)
Spring 2010 [41]	3.05 (0.76)	1.97 (0.62)	2.44 (0.73)

All children

Baseline 2007 [33]	2.97 (0.84)	2.14 (0.78)	2.36 (0.67)
Spring 2008 [29]	3.06 (0.82)	2.16 (0.59)	2.44 (0.71)
Autumn 2008 [31]	3.01 (0.79)	2.16 (0.65)	2.38 (0.68)
Spring 2009 [32]	2.96 (0.83)	2.13 (0.63)	2.36 (0.66)
Autumn 2009 [21]	3.04 (0.75)	2.19 (0.63)	2.44 (0.70)
Spring 2010 [20]	3.06 (0.78)	2.17 (0.57)	2.52 (0.66)

Girls

Baseline 2007 [30]	2.94 (0.82)	1.90 (0.55)	2.26 (0.72)
Spring 2008 [28]	2.87 (0.83)	1.90 (0.52)	2.24 (0.72)
Autumn 2008 [28]	2.81 (0.79)	1.93 (0.57)	2.26 (0.68)
Spring 2009 [25]	2.77 (0.82)	1.94 (0.72)	2.30 (0.78)
Autumn 2009 [22]	3.09 (0.82)	1.99 (0.63)	2.34 (0.73)
Spring 2010 [21]	3.09 (0.76)	1.81 (0.58)	2.38 (0.76)

Boys

Table 4: *A summary of the three SIC factors from baseline and during the study period.*

Values are given as [n], mean (SD).

Reference values, GMS < 1.5 = low level, 1.5 to 2.5 = normal level, > 2.5 = increased level of stress (Währborg, 2007).

When we analysed separate questions, there were differences between gender and time points. In Factor 1, the answer "When I´m happy, I show it" was more frequently occurring in girls than in boys (autumn 2007 p = 0.025, spring 2008 p = 0.001, autumn 2008 p = 0.013, spring 2009 p < 0.001, autumn 2009 p = 0.037 and spring 2010 p = non-significant).

In Factor 2, there were two answers more frequently occurring with girls than in boys: "I get sad" (spring 2008 p = 0.009, autumn 2008 p = 0.002, spring 2009 p = 0.002 and spring 2010 p < 0.001), and "I´m feeling alone" (spring 2010 p = 0.008).

In Factor 3, there were two answers more frequent in girls than in boys: "When there are problems, it helps to be together with my friends" (autumn 2007 p = 0.001 and autumn 2008 p = 0.002) and "When I´m sorry I show it" (autumn 2007 p = 0.019, spring 2008 p < 0.001 and autumn 2009 p = 0.019).

BECK Youth Inventory Swedish version

In the BYI questionnaire, four factors were studied; anxiety, depression, anger and disruptive behaviour.

In table 5, we present the level of BYI factors at baseline split for school and gender. Levels are given as mean +/- SD. Significant differences are printed in italics.

There were lower levels in anxiety, depression and anger in boys in the A-school compared to the B-school. It was clear that there were significant differences between gender in the A-school, but not in the B-school. There were no differences in BYI levels from baseline and during the study period in the B-school (not shown).

School	Anxiety	Depression	Anger	Disruptive behaviour
(A)	[29] 10.6 (8.4)	[29] 9.6 (8.7)	[29] 8.6 (8.4)	[28] 4.6 (4.9)
Boys	[16] 6.9 (3.2)	[16] 5.8 (3.5)	[16] 5.1 (4.4)	[15] 4.5 (5.3)
Girls	[13] 15.2 (10.5)	[13] 14.4 (10.9)	[13] 12.8 (10.3)	[13] 4.6 (4.7)
(B)	[34] 12.4 (8.9)	[33] 10.2 (7.9)	[33] 9.5 (7.2)	[33] 4.1 (4.3)
Boys	[13] 11.0 (7.4)	[12] 9.3 (7.4)	[12] 10.3 (7.3)	[12] 6.3 (5.1)
Girls	[21] 13.3 (9.7)	[21] 10.7 (8.3)	[21] 9.1 (7.3)	[21] 2.9 (3.2)
(A+B)	[63] 11.6 (8.6)	[62] 9.9 (8.3)	[62] 9.1 (7.7)	[61] 4.3 (4.6)
Boys	[29] 8.7 (5.8)	[28] 7.3 (5.7)	[28] 7.3 (6.2)	[27] 5.3 (5.2)
Girls	[34] 14.0 (9.9)	[34] 12.1 (9.4)	[34] 10.5 (8.6)	[34] 3.5 (3.9)
p-values[1]	*0.016*	*0.031*	0.146	0.184
p-values[2]	*0.006*	*0.036*	0.070	0.375

Table 5: *Beck-Youth Inventory levels at baseline. Values are given as [n], mean (SD). p-values (Kruskal Wallis test[1] , Median test[2]) for differences between gender in all (A+B) boys and girls. Significant differences in italics.*

Abdominal pain and headache

The prevalence of abdominal pain and headache from baseline are shown in table 2. One-third to half of the students had abdominal and/or headache pain. The pain occurred mostly some days/week and lasted mostly less than one hour/attack. Central abdominal pain dominated, and headaches were localised to the frontal and/or temporal part of the brain. Pain was most pronounced during midday and afternoon. Few had pain during the night and early in the morning. Prevalence of abdominal pain but not headache seemed to decrease during the study period in boys but not in girls.

Correlations between salivary cortisol, psychometric scores and clinical symptoms

No significant correlations were found between salivary cortisol and SIC-GMS or BYI levels at any time point.

Differences between the two schools

Physical characteristics

As shown in table 1, the children in the B-school were significantly heavier and taller. There was also a significant difference in waist- and hip-measures, and waist/hip ratio. Even the heart rates, blood pressures and the RPP were higher in the B-school compared to the A-school (Table 1). For girls, there was only one difference between schools: the waist/hip ratio, $p = 0.040$. There were significant differences between boys in the two schools regarding height, weight, hip measurement, systolic, median blood pressure, heart rate and RPP.

Salivary cortisol concentrations

There was a significant difference in cortisol concentrations between the schools at baseline ($p = 0.013$) as shown in table 3. A significant difference was also found between boys (mean, SD) (10.5, (5.1) nmol/L) and girls (22.3, (22.2) nmol/L in the B-school with higher concentrations for girls ($p = 0.033$). Changes in salivary cortisol during the study period for the B-school are presented in figure 2.

Psychometric measures

Stress in Children questionnaire

In the SIC questionnaire, no differences between the schools at baseline or during the study period were found. During the study period, no significant changes in GMS were found in the SIC.

Beck Youth Inventory

Table 5 shows BYI levels for all children split for gender and school at baseline. There were lower levels in anxiety, depression and anger in boys in the A-school compared to the B-school. In the BYI, a significant difference (N16/12; adjusted Z = -2,00; p = 0.042) was found regarding anger. The children in the B-school scored higher values on items measuring anger. A significant difference was found between gender in the A-school but not in the B-school. There were no significant changes from baseline during the study period in any school.

Blood pressure, heart rate and RPP

In addition, the heart rate, systolic, diastolic and median blood pressure and the rate pressure product were significantly different between the schools with higher values in the B-school compared to the A-school.

Headache and abdominal pain

There were no significant differences in the prevalence of headache and/or abdominal pain between schools. Headaches were dominant in girls in both schools (Table 2).

Discussion

The present study supports our first hypothesis that cortisol concentrations in children increase with age and grade in school. The most pronounced increase was found in the higher-grade girls and in the B-school, i.e. the school that was populated with working class people and immigrants to a greater extent than the rural A-school, which mainly was populated by middle-class people and fewer immigrants.

Our findings also support the second hypothesis that salivary cortisol concentration differs with gender. Already at baseline, a significant difference was found with higher values in girls. A more pronounced increase of cortisol concentrations over time was found in girls compared with boys, which was seen in the B-school (less socio-economic privileged school). Interestingly, no seasonal variation in salivary cortisol concentration was found in our study. Median salivary cortisol was significantly higher in this population of girls compared to similar groups in the early 1990s and 2000s. This seems to be an indirect sign of more stress in girls nowadays compared to 10 - 20 years ago.

We were not able to confirm our third hypothesis, since we could not find any correlation between salivary cortisol concentration and psychometric instruments with the aim to measure stress (SIC) and poor psychosocial health (BYI). However, the psychometric questionnaires applied in this study indicated lower psychosocial health and well-being in girls as compared to boys.

Clinical signs of ill health, i.e. headache and abdominal pain, were more common among girls, but only headaches were significantly more frequent among girls independent of school. The reported episodes of such pain had a low frequency, short duration and were mostly present during midday and evenings. Furthermore, very few of the studied population had any kind of medical treatment. Physical health differences were found between the two schools in the study. Anthropometric as well as hemodynamic measures performed in the study indicated values bearing higher risk for cardiovascular diseases in the B-school (lowest socio-economic status), especially among the boys.

The occurrence of headache and gastrointestinal pain in this study is in agreement with Hjern, who analysed more than 2,500 school-aged children in grades 3 - 6, 7 - 9 and upper secondary school in 2002 - 2003 in Sweden [29]. They found a total prevalence of 13 - 17% for weekly and about 40% for monthly complaints. Pain was more frequent in girls and increased with age/grade in agreement with this study [29]. They found that school stressors, such as harassment by peers, schoolwork pressure and being treated poorly by teachers were associated with psychosomatic pain as well as psychological complaints, such as sadness, irritability and feeling unsafe and nervous, with a two- to eight-fold increased risk of having psychosomatic pain. However, in contrast, the family situation and the socioeconomic status (SES) did not influence the occurrence or grade of psychosomatic pain.

A number of earlier studies have shown a relationship between social disadvantage and an increase in cortisol concentrations. Evans., *et al*. found higher overnight cortisol in young children in poor families m [30]. In addition, the duration of poverty has been found to predict increased secretion of cortisol [31]. Most studies but not all [32] have shown higher cortisol levels in unprivileged children [33].

In 1995, Kiess showed that cortisol levels were age-dependent, but also correlated significantly to pubertal stages [34]. However, they found no sex differences. Törnhage and Alfvén [12], among others, partly confirmed this finding and concluded that salivary cortisol concentration in healthy children was dependent on time, age and menarche. Östberg., *et al*. have shown that the perceived stress, diurnal cortisol secretion and students´ own account of stress differed between 14 - 15 year-olds boys and girls with disadvantage of girls in Sweden [35]. Therefore, our present results are in line with earlier findings.

We expected a correlation between cortisol concentration and the questionnaires applied in this study. Such a correlation has been found in earlier study [12], but with a number of limitations. Results using self-completed instruments should, however, be interpreted with caution since there is a risk for over- or under-estimation of perceived adversities. Since this study had a limited number of participants (a majority of whom experienced a rather "normal" degree of stress), the power to show such a correlation might be too low.

Given the fact that stress and stress related problems are common in school and, as confirmed in this study, the following practical implications seems to be of importance.

- Stress has to be continuously followed and assessed throughout the time in school.
- Individualized arrangements should be provided for those pupils who need counselling or any other kind of support during their time in school.
- Further research has to be implemented in this population in order to identify stressors of importance for the development of stress related ill health.
- The differences in health related to socioeconomic differences has to be paid certain attention to avoid unwanted differences in school achievements due to inequalities between pupils

Strengths and Limitations

In the present study, all participants were examined by one person (CJT), and all samples were taken at the same time and under supervised circumstances. The samples were further analysed at the same time after been frozen. The longitudinal design and the number of investigations during different seasons increased the validity and reliability of the study.

The major limitation of the study is its size, with a small number of dropouts due to practical reasons.

Conclusions

- Cortisol concentrations in children increase with age and grade in school.
- Salivary cortisol concentration differs with gender.
- Salivary cortisol depends on type of school situations.
- Salivary cortisol concentrations have increased in girls during the last two decades.
- No seasonal variation in salivary cortisol concentration was found.
- There was no correlations between salivary cortisol concentration and psychometric instruments with the aim to measure stress (SIC) and poor psychosocial health (BYI).
- Psychosomatic pain was more frequent in girls and increased with age/grade and it was of low frequency, short duration and was mostly present during midday and evenings.

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Authors' Contributions

CJT was responsible for the study conception, data collection and design. PW was responsible for the design of the SIC protocol and decided on the type of other psychometric programs. CJT collected, programmed and performed the data analysis. CJT and PW were responsible for drafting the manuscript. CJT supervised the study.

Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. The financial sponsor had no influence on study population, randomization, analysis of cortisol and psychometric samples, and writing of the manuscript.

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