Intercorrelations between serum, salivary, and hair cortisol and child-reported estimates of stress in elementary school girls

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Abstract

To evaluate the impact of stress on children's well-being, it is important to have valid and reliable stress assessment methods. Nevertheless, selection of an appropriate method for a particular research question may not be straightforward, as there is currently no consensus on a reference method to measure stress in children. This article examined to what extent childhood stress can be estimated accurately by stressor questionnaires (i.e., Coddington Life Events Scale) and biological markers (serum, salivary, and hair cortisol) using the Triads (a triangulation) method in 272 elementary school girls. Salivary cortisol was shown to most accurately indicate true childhood stress for short periods in the past (i.e., last 3 months), whereas hair cortisol may be preferred above salivary measurements for periods more distant and thus for chronic stress assessment. However, applicability should be confirmed in larger and more heterogeneous populations.

Descriptors: Children/infants, Stress, Social factors, Biochemical, Cortisol

Childhood stress and its effects on children's physical and psychological well-being have been studied extensively over the past years. In particular, adverse events with a chronic or cumulative character may strongly affect children's health, with effects potentially persisting into adolescence and adulthood (Schilling, Aseltine, & Gore, 2007; Schneiderman, Ironson, & Siegel, 2005; Teicher et al., 2003). The combined increase in the prevalence of childhood stress with the prevalence of psychosomatic complaints (Alfven, Ostberg, & Hjern, 2008; Hesketh et al., 2010), obesity (Gundersen, Mahatmya, Garasky, & Lohman, 2011), and behavioral or mental health problems in children is therefore of special concern (Grant, Compas, Thurm, McMahon, & Gipson, 2004; Schilling et al., 2007; Timmermans, van Lier, & Koot, 2010; Vanaelst, De Vriendt, Ahrens, et al., 2012).

To evaluate the impact of stress on children's well-being, it is important to have valid and reliable stress assessment methods that can be easily implemented in large-scale epidemiological studies. In general, stressor questionnaires and laboratory measurements of cortisol have been widely performed in childhood epidemiological research, though both approaches measure distinct aspects of the stress response (Cohen, Kessler, & Gordon, 1997; Vanaelst, De Vriendt, Huybrechts, Rinaldi, et al., 2012). Stressor questionnaires assess the occurrence of stressful events during a predefined time period, whereas laboratory cortisol measurements in biological samples (e.g., blood, saliva, and hair) represent the activation of the physiological stress system provoked by stressor exposure: Activation of the hypothalamus–pituitary–adrenal axis, a main pathway of the body's stress system, results in the release of cortisol by the adrenal glands (Figure 1). As shown in Figure 1, the different biological samples for cortisol measurement reflect cortisol levels of a different time frame (e.g., serum cortisol for acute stress and salivary cortisol and hair cor-
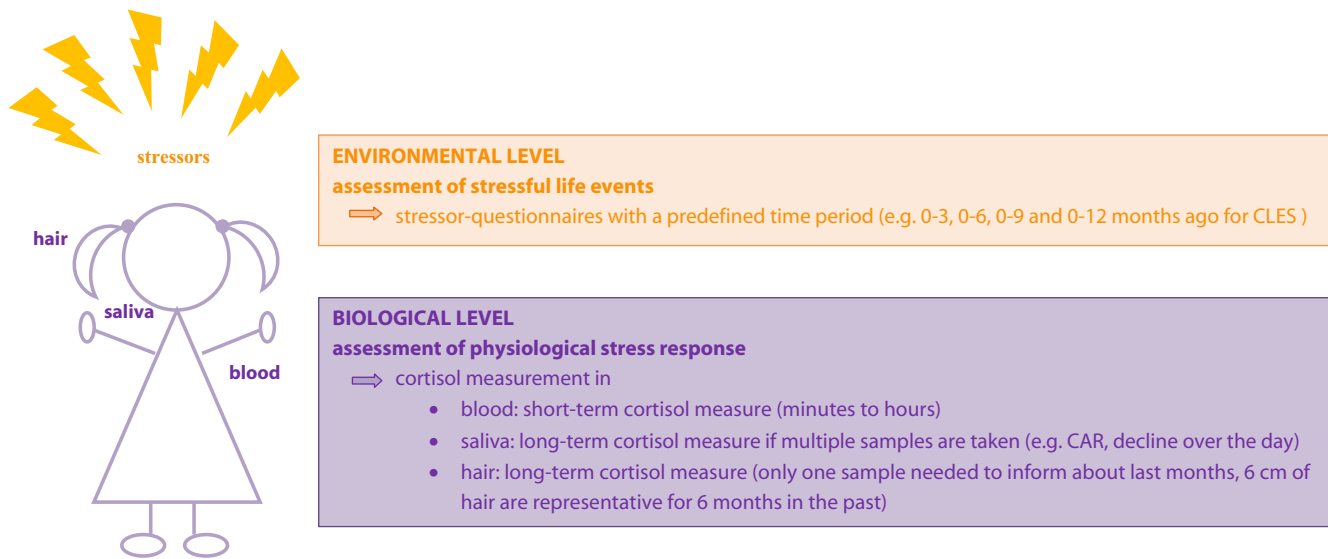
Figure 1. Overview of common childhood stress assessment methods at an environmental versus a biological level. CLES: Coddington Life Events Scale; CAR: cortisol awakening response.

tisol for longer term to chronic stress). A more detailed overview of epidemiological approaches to measure (chronic) childhood stress is described elsewhere (Vanaelst, De Vriendt, Huybrechts, Rinaldi, et al., 2012).

As questionnaires and cortisol measurements reflect partially different information, correlations between (a) questionnaires and cortisol measurements and (b) cortisol intercorrelations in different biological samples have often been contradictory (Hellhammer, Wust, & Kudielka, 2009; Oldehinkel et al., 2011; Schlotz et al., 2008; Vanaelst, De Vriendt, Huybrechts, Rinaldi, et al., 2012). Nevertheless, the correlation between salivary and free serum cortisol, both biomarkers of short-term measurement and both representing diurnal cortisol fluctuations, is well supported in literature (Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007; Poll et al., 2007; Tunn, Mollmann, Barth, Derendorf, & Krieg, 1992) and in studies of children (Chou et al., 2011; Delcorral, Mahon, Duncan, Howe, & Craig, 1994). Hair cortisol, a measure of long-term cortisol production, has not been shown to correlate well with serum cortisol, but it was observed to be associated with the salivary cortisol awakening response (CAR) or average salivary cortisol (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011; van Holland et al., 2011; Xie et al., 2012).

Although salivary cortisol has repeatedly been measured in children in relation to childhood stress or behavioral problems (Gunnar, Sebanc, Tout, Donzella, van Dulmen, 2003; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Gustafsson, Anckarsater, Lichtenstein, Nelson, & Gustafsson, 2010; Gustafsson, Gustafsson, & Nelson, 2006; Hatzinger et al., 2007; Jessop & Turner-Cobb, 2008; Lupien, King, Meaney, & Mcewen, 2001; Maldonado et al., 2008; Ruttle et al., 2011; Smeekens, Riksen-Walraven, & van Bakel, 2007; Wolf, Nicholls, & Chen, 2008), hair cortisol analysis as a marker of chronic stress is relatively new (Dettenborn, Tietze, Bruckner, & Kirshbaum, 2010; Karlen, Ludvidsson, Frostell, Theodorsson, & Faresjo, 2011; Manenschijn, van Kruysbergen, de Jong, Koper, & van Rossum, 2011; Russell, Koren, Rieder, & Van Uum, 2012; Steudte, Kolassa, et al., 2011; Steudte, Stalder, et al., 2011). Recently, a positive correlation was observed between hair cortisol concentrations and

major childhood life events in children (Vanaelst, De Vriendt, Huybrechts, Michels, et al., 2012).

Despite their specific characteristics as regards cost effectiveness, logistics, invasiveness, bias, and so on (Vanaelst, De Vriendt, Huybrechts, Rinaldi, et al., 2012), questionnaires and cortisol measurements have both been shown to be valid indicators of childhood stress. Nevertheless, selection of an appropriate stress assessment method for a particular research question may not be straightforward and should be well considered, as there is currently no consensus on a reference method to measure stress in children. Therefore, this article first investigates cortisol intercorrelations in different biological samples (i.e., serum, saliva, and hair) and, second, examines to what extent childhood stress can be estimated accurately by stressor questionnaires and biological markers in elementary school girls using the Triads method.

The Triads method has particularly been applied in dietary validation studies and was developed to get a valid estimation of a true, unknown exposure if a gold standard method is lacking (Kaaks, 1997; Yokota, Miyazaki, & Ito, 2010). As the Triads method may also be of value for other research fields, this study expanded its application to stress research. More specifically, through the calculation of "validity coefficients," this study examines which stress assessment method may most accurately indicate true childhood stress by comparing questionnaires and biological markers triangularly.

Methods

Participants

The Children's Body composition and Stress (ChiBS) project was designed at Ghent University and investigates the relationship between chronic psychosocial stress and changes in body composition in young children (5–11 years old) living in Aalter (a city in Flanders, Belgium), over a 2-year follow-up period (2010–2012; Michels, Vanaelst, et al., 2012). The ChiBS project offered the opportunity to study the feasibility and interrelationships of dif-
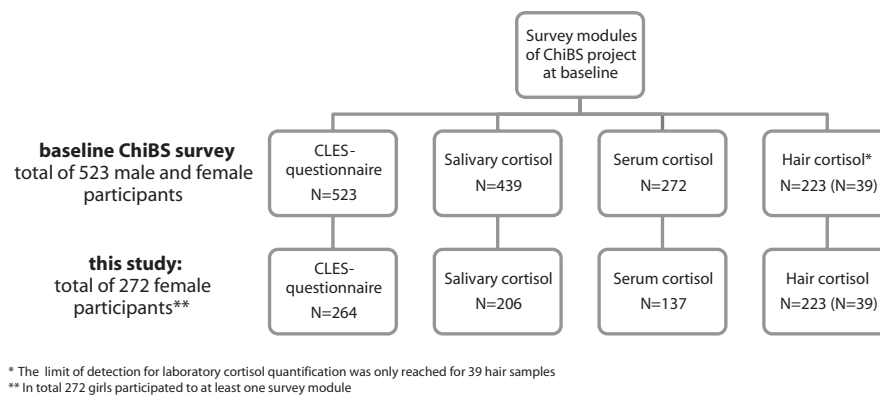
Survey modules of ChiBS project at baseline

baseline ChiBS survey
total of 523 male and female participants

| CLES-questionnaire N=523 | Salivary cortisol N=439 | Serum cortisol N=272 | Hair cortisol* N=223 (N=39) |

this study:
total of 272 female participants**

| CLES-questionnaire N=264 | Salivary cortisol N=206 | Serum cortisol N=137 | Hair cortisol N=223 (N=39) |

* The limit of detection for laboratory cortisol quantification was only reached for 39 hair samples
** In total 272 girls participated to at least one survey module

Figure 2. Participation numbers for the different measurement modules in the baseline ChiBS survey and the present study. CLES: Coddington Life Events Scale.

ferent stress assessment methods in children. Parents were asked to sign a consent form in which the option was offered to participate in the full ChiBS program or in a selected set of measurement modules, resulting in distinct participation numbers for the different measurement modules, as presented in Figure 2.

In total, 523 healthy children participated to the 2010 baseline survey of the ChiBS project. Analyses in this study were, however, limited to the female participants of the ChiBS project, as one of the survey modules, more specifically hair sampling, was only performed in girls ($n = 272/523$ or 52%; M age = 8.37 years, $SD = 1.19$; Figure 2). No differences were found between boys and girls for age, body mass index (BMI), parental education, family structure, or migrant status. Detailed sociodemographic information on the ChiBS study population is described elsewhere (Michels, Vanaelst, et al., 2012). The ChiBS project was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the Ethics Committee of the Ghent University Hospital.

Instruments

Coddington Life Events Scale for Children (CLES-C). Child-reported estimates of stress were collected with the CLES-C, completion of which was assisted by a trained researcher. The CLES questionnaires is a validated questionnaire on the occurrence of stressors or life events (Coddington, 1972). It measures the frequency and timing of 36 positive and negative life events relevant for this age group during the last year (four trimesters) and results in a "life change units" score per trimester and for the time periods of 0–3, 0–6, 0–9, and 0–12 months ago. Apart from the total event score, a score for only negative life events was also calculated.

Serum, salivary, and hair cortisol. To cover short- and long-term stress exposure, three different biological samples were collected for cortisol analyses, namely, serum, saliva, and hair samples. Detailed information on the strategies for sample collection and cortisol analyses were previously described (Michels, Sioen, De Vriendt, et al., 2012; Michels, Vanaelst, et al., 2012; Vanaelst, De Vriendt, Huybrechts, Michels, et al., 2012).

In short, salivary samples were collected with Salivette swabs during two consecutive weekdays at four time points, that is, immediately at awakening (T0), 30 min after waking up (T30), 60 min after waking up (T60), and in the evening between 7 and 8 p.m.

(Tev). Salivary area under the curve (AUC) cortisol was calculated on the basis of the morning samples, as the total area under the curve between T0 and T60 and cortisol declines over the day as (Tev – T0) divided by the number of hours, taking into account the salivary samples of the 2 days (mean value). Blood samples were obtained after an overnight fasting period through venipuncture. Hair samples with a diameter of approximately 5 mm were cut from the vertex posterior region of the scalp. Only the most proximal 6 cm were used for cortisol analyses. Hair samples were only taken from girls to maximize the probability that the hair reached the required length of 6 cm. Cortisol was analyzed by electro-chemoluminescence immunoassays for serum and salivary samples (in nanomoles per liter) and by liquid chromatography–tandem mass spectrometry for hair samples (in picograms per milligram).

Statistical Procedures

Statistical analyses were performed with the PASW Statistics Program, version 19.0.0 and SAS version 9.3 for bootstrapping analyses. P values < .05 were considered statistically significant for all tests. Because of the specific formulation of the informed consent, different participation numbers for each measurement module were obtained (Figure 2). To maximize the sample size and power of calculations, analyses were performed on the largest sample size possible and not restricted to the subsample of children for whom data on all measurement modules were available (as n would only be 19 for this latter approach). The cortisol concentrations in serum, saliva, and hair and the CLES scores are presented by their median and interquartile range, as these are not normally distributed (Kolmogorov–Smirnov, Shapiro–Wilk). For all variables, Z scores were calculated to improve the linearity of the distribution. These Z scores were used for all tests.

Cortisol intercorrelations in different biological samples. The correlations and agreement between serum, salivary, and hair cortisol concentrations in corresponding samples were investigated using Spearman rank correlations and Bland–Altman analyses. Bland–Altman plots were created to graphically present the agreement or comparability between the quantitative measurements of cortisol in (a) hair versus saliva (AUC), (b) hair versus serum, and (c) serum versus saliva (AUC). In these charts, the difference of the paired two measurements is plotted on the vertical axis against the mean of the two measurements on the horizontal axis. Three reference lines are superimposed on the plot (i.e., the upper limit of
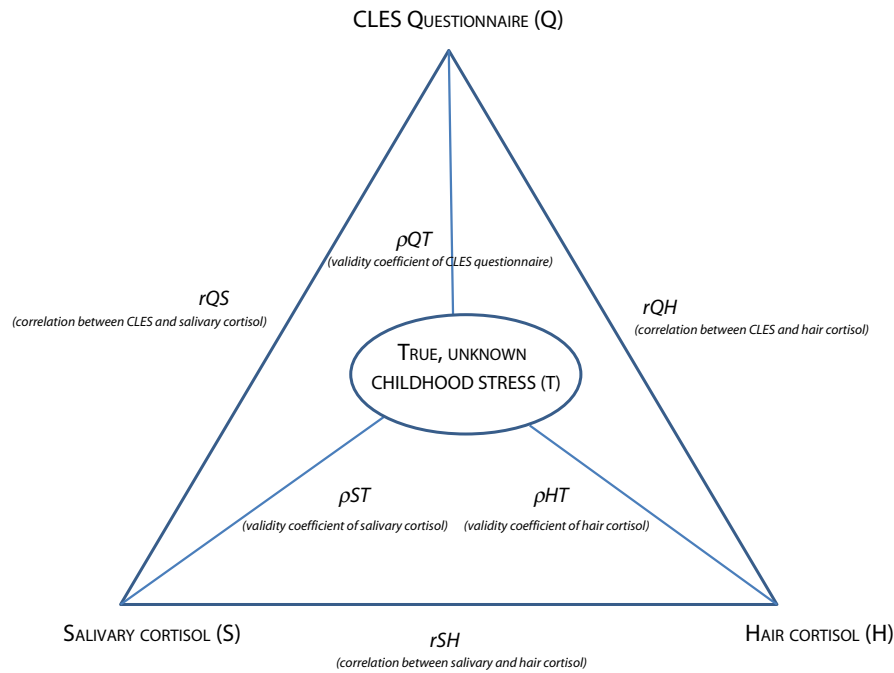
Figure 3. Schematic representation of the Triads method comparing the CLES questionnaire and salivary and hair cortisol triangularly with the true, but unknown childhood stress. The figure is adapted from Yokota et al. (2010).

agreement [mean + 2 *SD*], the average difference between the measurements [mean], and the lower limit of agreement [mean − 2 *SD*]). If the two methods are comparable, the mean of the differences will be close to 0. Bland–Altman plots were not created for salivary decline, as no correlation for this variable with hair or serum cortisol was observed (Altman & Bland, 1983; Bland & Altman, 2010).

Correlations between cortisol measurements and child-reported estimates of stress: Triad analyses. This study applied the Triads technique (a triangulation method) to examine which stress assessment method may most accurately indicate true childhood stress. The idea is that, although it is impossible to measure true childhood stress directly, it can be estimated by stressor questionnaires (CLES scores) and biological markers such as salivary and hair cortisol (Michels, Sioen, Huybrechts et al., 2012; Vanaelst, De Vriendt, Huybrechts, Michels, et al., 2012). These are, therefore, the three variables being compared in this triangulation technique, as presented in Figure 3.

The aim of the Triads method is to obtain validity coefficients (ρ) that estimate the correlation between each measurement method and the subject's true but unknown stress, with higher values indicating a better approximation of true exposure (range 0–1; Kaaks, 1997; Yokota et al., 2010). Serum cortisol was not included in the Triad analysis because of the absence of a relationship with the CLES scores (data not shown).

The Triad approach assumes that (a) correlations between the three measurements are explained entirely by the fact that all are linearly related to true stress and (b) that their random measurement errors are mutually independent (Kaaks, 1997; Ocke & Kaaks, 1997). In a first step, pair-wise Spearman's correlation coefficients (*r*) are calculated between each of the measurement methods. These correlation coefficients are then used to calculate validity coefficients (ρ) using the formulas

$$\rho QT = \sqrt{\frac{rQS * rQH}{rSH}}$$

$$\rho HT = \sqrt{\frac{rQH * rSH}{rQS}}$$

$$\rho ST = \sqrt{\frac{rSH * rQS}{rQH}}$$

in which *Q* stands for questionnaire, *S* for salivary cortisol, *H* for hair cortisol, and *T* for true childhood stress and where ρ*QT*, ρ*HT*, and ρ*ST* are the validity coefficients of, respectively, the stressor questionnaire, hair cortisol, and salivary cortisol in relation to true stress and *rQS*, *rQH*, and *rSH* are the correlation coefficients between, respectively, the questionnaire and salivary cortisol, the questionnaire and hair cortisol, and salivary and hair cortisol (Figure 3). Validity coefficients are always equal to or greater than the sample correlations between that type of measurement and the other two: If all three sample correlations are high, the measurements are expected to have validity coefficients close to 1; likewise, low correlations are expected to result in lower validity coefficients. Validity coefficients higher than 1 are known as "Heywood cases" and can emerge when the product of two of the three samples correlations is larger than the third and can be explained by two factors: (a) random sampling fluctuations in the observed correlations between measurements, in which case validity coefficients higher than 1 are acceptable, and (b) violation of one or more of the model assumptions, in which case the estimated validity coefficients are biased (Ocke & Kaaks, 1997).

The Triads method was applied for four models. In the first model salivary AUC cortisol, hair cortisol, and CLES negative

Table 1. *Information on Sociodemographics and Stress Measurements in the Studied Girls (N = 272)*

Sociodemographic information	*N*	*%*
Parental education (missing *n* = 12)		
ISCED 1	3	1.2
ISCED 2	4	1.5
ISCED 3	74	28.5
ISCED 4	49	18.8
ISCED 5	130	50
Age of child		
5	5	1.9
6	29	10.7
7	67	24.6
8	77	28.3
9	68	25
10	24	8.8
11	2	0.7
BMI category of child (COLE) (missing *n* = 1)		
Underweight	36	13.3
Normal	208	76.8
Overweight	19	7
Obese	8	2.9

Stress measurements	valid *N*[a]	Min	Max	P25	Median	P75
Cortisol analyses						
Serum total cortisol (nmol/L)	137	105.38	801.65	194.62	257.1	337.51
Serum free cortisol (nmol/L)	136	3.01	40.55	5.36	7.61	10.3
Salivary cortisol: AUC (nmol/L)	206	6.67	102.62	19.97	22.59	28.93
Salivary cortisol: decline (nmol/L)	189	−27.70	−0.19	−1.04	−0.81	−0.63
Hair cortisol (pg/mg)	39[b]	5.34	1330.48	7.30	8.80	36.58
CLES questionnaire						
Total event score last 3 months	264	0	281	0	28	61.75
Total event score last 6 months	264	0	451	0	39	74.75
Total event score last 9 months	264	0	479	17.25	45	88.75
Total event score last 12 months	264	0	499	30.25	66	105.75
Negative event score last 3 months	264	0	210	0	0	47
Negative event score last 6 months	264	0	348	0	21.5	52
Negative event score last 9 months	264	0	376	0	28	59
Negative event score last 12 months	264	0	396	10	43	75

Note. AUC = area under the curve; CLES = Coddington Life Events Scale; ISCED = International Standard Classification of Education (1 = primary education, 2 = lower secondary education, 3 = upper secondary education, 4 = postsecondary nontertiary education, 5 = first stage of tertiary education). [a]The number of samples/questionnaires valid for analyses is presented. Children could participate to a selected set of measurement modules, resulting in distinct participation numbers. [b]The limit of quantification for laboratory cortisol (LOQ = 5 pg/mg) was not reached in the majority of hair samples (*n* = 39/223; Vanaelst, De Vriendt, Huybrechts, Rinaldi, et al., 2012).

event scores were studied as measurement methods. The second model was similar to the first except for salivary cortisol diurnal decline as a new salivary measure. These two models were then repeated after replacing the CLES negative event scores by the CLES total event scores, representing the third and fourth models, respectively. These four models were performed for four different time periods in the past (0–3, 0–6, 0–9, and 0–12 months ago, respectively), thus resulting in a total of 16 models that were studied.

For all models, 95% confidence intervals (95% CIs) were calculated as the 2.5th–97.5th percentile for the replicates of estimated validity coefficients from 1,000 bootstrap samples of equal size (*N* = 272; nonparametric bootstrap method). For a number of bootstrap samples, validity coefficients could not be estimated because of negative sample correlation coefficients, leading to 95% CIs based on less than 1,000 samples. When the estimated validity coefficients were higher than 1 (Heywood cases), their value was set to 1 in order to keep the 95% CI within the theoretical range of [0–1] (Andersen et al., 2005; Bhakta et al., 2005; Kabagambe et al., 2001; Ocke & Kaaks, 1997).

Results

Population Characteristics

Table 1 presents sociodemographic data about the participating girls and the number of girls included for each measurement module of this study as well as the serum, salivary, and hair cortisol concentrations and the CLES scores.

Cortisol Intercorrelations in Different Biological Samples

As presented in Table 2, serum (free and total) cortisol was positively correlated with salivary cortisol measures. Hair cortisol was correlated with salivary cortisol but showed no correlation with serum cortisol (*n* = 19; data not shown). Neither serum nor hair cortisol was correlated with the salivary cortisol decline (data not shown). The Bland-Altman plots in Figure 4 present the agreement between serum cortisol, hair cortisol and salivary AUC measurements.

The Bland-Altman plot for hair and salivary measurements (Figure 4a) demonstrated that cortisol concentrations in hair are

Table 2. *Significant Cortisol Intercorrelations in Different Biological Samples*

	N	Spearman's ρ	p value
Serum free cortisol			
Salivary T0 cortisol	122	.196	.031
Salivary T30 cortisol	121	.270	.003
Salivary T60 cortisol	118	.274	.003
Salivary AUC cortisol	114	.303	.001
Serum total cortisol			
Salivary T30 cortisol	122	.272	.002
Salivary T60 cortisol	118	.289	.002
Salivary AUC cortisol	114	.299	.001
Hair cortisol			
Salivary T30 cortisol	33	.398	.022
Salivary AUC cortisol	32	.398	.024

Note. AUC = area under the curve.

lower compared to salivary analyses (reference line of the average difference is lower than 0, i.e., −0.38). More specifically, the divergent pattern indicates that the higher the salivary cortisol concentrations, the higher the difference between cortisol in hair versus salivary samples (larger horizontal scattering). Similar findings were observed in Figure 4b representing hair versus serum cortisol concentrations. Figure 4c illustrates that cortisol concentrations from serum and salivary samples do not agree well on the individual level (large upper and lower limits of agreement, i.e., 2.61 and −2.77), although on the population level these sample types give similar information on the mean cortisol concentration (reference line of the average difference close to 0 and the majority of data points nicely located within upper and lower limit of agreement). The disagreement between saliva and serum increases with an increasing mean cortisol.

Correlations between Cortisol Measurements and Child-Reported Estimates of Stress: Triad Analyses

Table 3 presents the correlation and validity coefficients (ρQT, ρHT, ρST) for the different models investigated by the Triads technique, as well as the 95% CI for the estimated validity coefficients. Both models indicated that for elementary school girls, salivary cortisol measurements presented the highest validity coefficient in relation to true (but unknown) stress for a short period in the past (i.e., last 3 months; $\rho = .74$ and $\rho = .63$, for salivary AUC and cortisol decline, respectively), whereas for periods more distant in the past, hair cortisol showed the highest validity coefficients ($\rho = .79$, $\rho = 1$, $\rho = .94$ and $\rho = .49$, $\rho = .55$, $\rho = .60$ for the last 6, 9 and 12 months in the past for both models, respectively). Analyses were repeated with the CLES total event scores and resulted in similar observations (data not shown).

Discussion

Although it is quite common to measure childhood stress, this study is, to our knowledge, the first to compare cortisol measurements in serum, saliva, and hair in elementary school girls and to examine their relationship with child-reported stressors in order to identify the most accurate indicator of childhood stress.

Cortisol Intercorrelations in Different Biological Samples

In line with previous research, we have shown a correlation between serum free cortisol and salivary morning cortisol (both
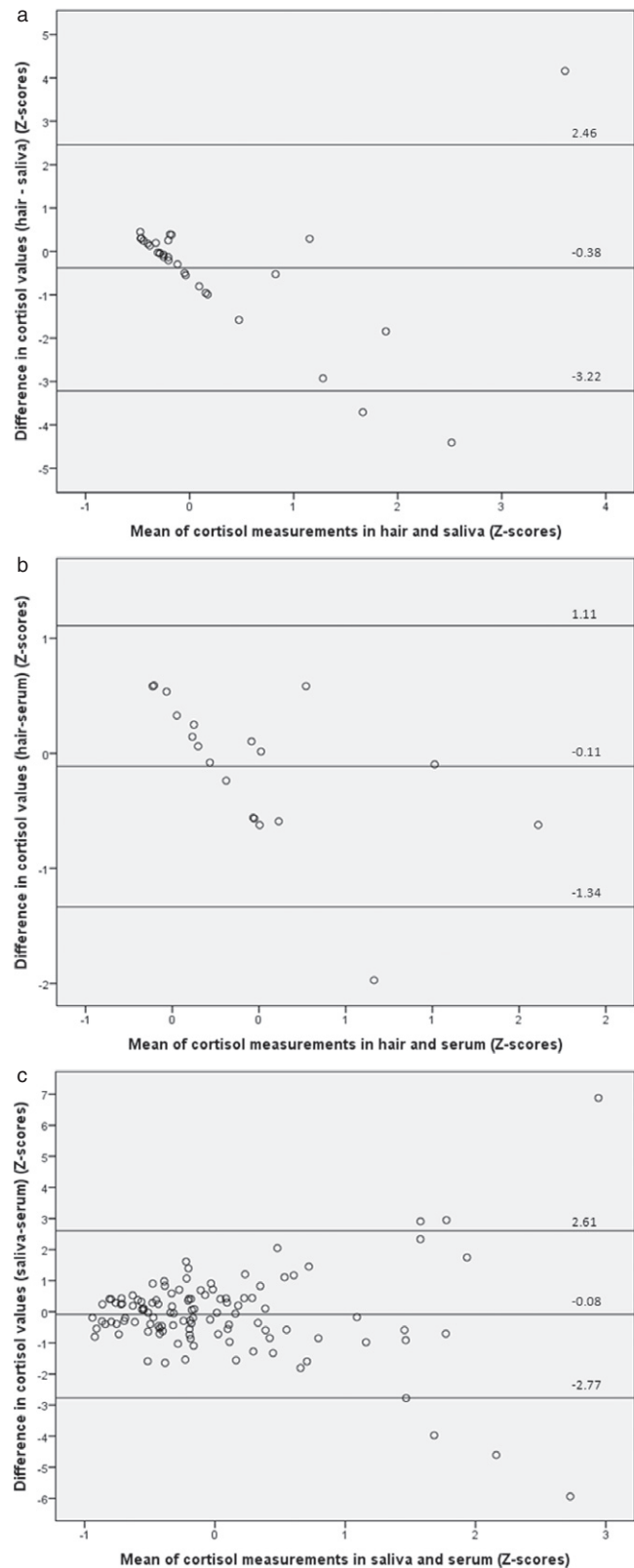
Figure 4. Bland–Altman plots for (free) serum cortisol, salivary (AUC) cortisol, and hair cortisol concentrations. The horizontal reference lines represent the upper limit of agreement (mean + 2 *SD*), the average difference between the measurements (mean), and the lower limit of agreement (mean − 2 *SD*), respectively.

Table 3. *Triad Analyses: Spearman's Rank Correlation Coefficients and Validity Coefficients for CLES Scores, Salivary Cortisol (AUC and Decline), and Hair Cortisol*

	CLES vs. saliva		CLES vs. hair		Hair vs. saliva		Triad analyses		
	n	Spearman's ρ	*n*	Spearman's ρ	*n*	Spearman's ρ	ρ*ST* [95% CI]	ρ*HT* [95% CI]	ρ*QT* [95% CI]
Model salivary AUC—hair cortisol—CLES questionnaire									
CLES score negative events last 3 months	202	.140*	39	.103	32	.398*	**0.74** [0.14–1]	0.54 [0.12–1]	0.19 [0.03–0.59]
CLES score negative events last 6 months	202	.132	39	.208	32	.398*	0.50 [0.11–1]	**0.79** [0.19–1]	0.26 [0.05–0.62]
CLES score negative events last 9 months	202	.099	39	.259	32	.398*	0.39 [0.08–1]	**1**[a] [0.21–1]	0.25 [0.05–0.61]
CLES score negative events last 12 months	202	.117	39	.26	32	.398*	0.42 [0.09–1]	**0.94** [0.22–1]	0.28 [0.06–0.71]
Model salivary decline—hair cortisol—CLES questionnaire									
CLES score negative events last 3 months	186	.188*	39	.103	32	.218	**0.63** [0.12–1]	0.35 [0.07–1]	0.30 [0.07–1]
CLES score negative events last 6 months	186	.191*	39	.208	32	.218	0.45 [0.11–1]	**0.49** [0.10–1]	0.43 [0.09–1]
CLES score negative events last 9 months	186	.186*	39	.259	32	.218	0.40 [0.10–1]	**0.55** [0.11–1]	0.47 [0.12–1]
CLES score negative events last 12 months	186	.157*	39	.26	32	.218	0.36 [0.08–1]	**0.60** [0.12–1]	0.43 [0.11–1]

Note. Values highlighted in bold represent the highest validity coefficients for the studied model (one bold value per model, i.e., per row). AUC = area under the curve; CLES = Coddington Life Events Scale; ρ*ST* = validity coefficient of salivary cortisol; ρ*HT* = validity coefficient of hair cortisol; ρ*QT* = validity coefficient of CLES questionnaire.
[a]Heywood case: original value of 1.02.
*Significant at the *p* < .05 level.

short-term measurements, representing actual cortisol changes) (Levine et al., 2007; Poll et al., 2007; Tunn et al., 1992) as well as between serum free cortisol and salivary AUC cortisol (Hellhammer et al., 2009). Although salivary cortisol reflects the unbound, free cortisol fraction (Levine et al., 2007), correlations with serum total cortisol were also observed. This relationship has previously been shown to be of a nonlinear nature, depending on the relative saturation of the corticosteroid binding globulin protein in blood (Hellhammer et al., 2009). Our observations also confirmed the previously reported lack of correlation between hair cortisol (long-term measure) with single-point serum or salivary cortisol measurements (Sauve, Koren, Walsh, Tokmakejian, & Van Uum, 2007; Steudte, Stalder, et al., 2011). However, as shown before (D'Anna-Hernandez et al., 2011; van Holland et al., 2011), hair cortisol strongly correlated with salivary AUC cortisol, which is a longer term representation of cortisol production and stress (Chida & Steptoe, 2009; Xie et al., 2012). However, the Bland–Altman plot did not indicate a good agreement between these two measures, probably due to the small sample size for this analysis (*n* = 32). In summary, this study has demonstrated that the previously observed cortisol intercorrelations are also applicable in this population of young healthy girls.

Correlations between Cortisol Measurements and Child-Reported Estimates of Stress: Triad Analyses

The four models investigated with the Triads method demonstrated that salivary cortisol measurements (both AUC and decline) may more accurately indicate true childhood stress than hair cortisol measurements for short periods in the past (i.e., last 3 months); hair cortisol may, however, be preferred above salivary measurements for periods more distant and thus for chronic stress assessment (i.e., more than 3 months ago). Although single salivary samples

represent single-point or short-term cortisol measurements (hours), salivary AUC measurements are assumed to represent cortisol exposure on a longer term (days or weeks; Chida & Steptoe, 2009), as confirmed by our results (i.e., indicator for stress for up to 3 months ago). For hair cortisol, different research groups have described its potential as a proper chronic stress measure for several months retrospectively (Dettenborn et al., 2010; Karlen et al., 2011; Manenschijn et al., 2011; Russell et al., 2012; Steudte, Kolassa, et al., 2011; Steudte, Stalder, et al., 2011), which is in line with our observations.

We limited hair cortisol analyses to the 6 most proximal centimeters as this is assumed to be the maximum length of hair being a reliable estimate of systemic cortisol concentrations in the past (Russell et al., 2012). Based on an average growth rate of 1 cm per month (Harkey, 1993), a 6-cm hair sample thus represents a 6-month period prior to sampling. Theoretically, our study could thus not report on periods over 6 months ago, even though hair cortisol also presented the highest validity coefficient for periods of 9 and 12 months ago. A possible delay period between stressor exposure and cortisol incorporation in hair may partly explain our observations, although misconceptions and generalizations on hair growth rate may be a more plausible factor involved in this observed associations between "nonmatching periods": LeBeau, Montgomery, and Brewer (2011) have described the influence of genetic and external variables on the hair growth rate and the effect of fluctuations in sample collection. They demonstrated that a 1-cm hair segment may correspond to hair formed 1.3 to 2.2 months earlier, which varies considerably from the generally accepted 1 cm/1 month hypothesis. This way, hair hormonal concentrations may be associated with stressor exposure in more distant periods than theoretically possible. Given the small sample size for the hair cortisol analyses (*n* = 39/223), the above mentioned ("nonmatching") results should, however, be interpreted with caution.

Biological quantification of stress using salivary or hair cortisol has been increasingly used to overcome limitations inherent to the more subjective nature of questionnaires and potential difficulties in implementing checklists in younger age groups. However, salivary and hair cortisol measurements are no clear-cut diagnostic media for childhood stress and measure different aspects of the stress response compared to questionnaires (Figure 1). Although environmental, psychological, and biological stress responses are theoretically strongly interconnected through the human stress system, some differentiation between these assessment levels should be made because (a) of the different dynamics of the psychological and biological stress system (i.e., the endocrine stress response lagging behind the psychological response; Oldehinkel et al., 2011; Schlotz et al., 2008), (b) not all stressors conclusively produce a (measurable) psychological or biological stress response (e.g., if it is not perceived stressful; Gunnar, Talge, & Herrera, 2009), and additionally (c) inter- and intra-individual differences in response to stressors may exist, depending on characteristics of both the stressor and the person facing it (Cohen & Hamrick, 2003; Kudielka, Hellhammer, & Wust, 2009; Michaud, Matheson, Kelly, & Anisman, 2008; Miller, Chen, & Zhou, 2007). Therefore, we want to emphasize the added value of including both stressor questionnaires and biological markers in stress research, as simultaneous application may provide a more aggregated and complementary view on stress in children (Vanaelst, De Vriendt, Huybrechts, Rinaldi, et al., 2012). After all, measures of stressor occurrence may only provide partial knowledge about the physiological stress responsiveness and vice versa (Oldehinkel et al., 2011; Schlotz et al., 2008).

Strengths and Limitations

This study was the first to examine both the environmental (i.e., major life events) and biological (i.e., serum, salivary, and hair cortisol) stress dimensions in young girls using a standardized methodology. As there is currently no gold standard method to measure childhood stress and each approach has its strengths and limitations, we examined which studied stress measures could most accurately indicate childhood stress. For this purpose, we used the Triads method, which was previously only used in the area of dietary research, but now we expanded its application to stress research and gained a clearer insight in the applicability of salivary and hair cortisol measurements for childhood stress research.

Nevertheless, there were some specific methodological limitations. First, results cannot be generalized to boys or children in general, as hair samples were exclusively taken from girls. This implicates that our observations should be confirmed in a more heterogeneous population including boys.

Hair cortisol analyses were performed using LC-MS/MS methodology, which is considered the gold standard technique for hair analyses (Kushnir et al., 2011; Society of Hair Testing, 2011). Yet,

a large percentage of the hair samples did not reach the limit of quantification (LOQ = 5 pg/mg), with only 39 of the 223 hair samples with quantifiable cortisol concentrations. As a result, the sample size for some of the analyses was small, which could have affected the power of the statistical analyses. As the accuracy of the applied LC-MS/MS method was demonstrated in validation experiments (Vanaelst, Rivet, Ludes, De Henauw, & Raul, 2012), the low physiological levels of hair cortisol in girls represent true observations rather than a methodological issue. This raises questions, however, regarding the general utility of hair cortisol analyses for childhood populations. Keeping this limitation in mind, we recommend confirmation of our observations in a larger population sample with less drop-out for (laboratory) biological measurements (e.g., hair cortisol).

Concerning the precision of the Triad analyses (i.e., the 95% CI of the estimated validity coefficients), all intervals were very large and included the value 1 (except for the 95% CI for ρQT of Model 1), indicating low sample correlations (Yokota et al., 2010). According to Ocke and Kaaks (1997), a sample size of 100 to 200 individuals may, in many situations, be insufficient to estimate the validity coefficients with reasonable precision, particularly in the view of low sample correlations that often arise using biological markers. So, further research should confirm our observations using a larger and more heterogeneous population to gain a more complete and accurate insight in the applicability of salivary and hair cortisol measurements for childhood stress research.

Conclusions

This article investigated the relationship between cortisol measurements in different biological samples, showing a lack of association and disagreement between measures of single-point, short-term cortisol versus long(er) term cortisol. In addition, this article examined to what extent childhood stress can be accurately estimated by stressor questionnaires and biological markers in girls. Salivary cortisol was shown to most accurately indicate true childhood stress for short periods in the past (i.e., last 3 months) whereas hair cortisol may be preferred above salivary measurements for periods more distant and thus for chronic stress assessment. As a result, we suggest differentiating the type of biological matrix (i.e., saliva, hair) according to the time period under investigation. Nevertheless, our observations should be confirmed in future research investigating more heterogeneous populations (e.g., including both boys and girls and children from different sociodemographic backgrounds) and in large-scale settings with small drop-out for biological measurements, implicating further improvement of the hair cortisol laboratory analyses. Moreover, analyses should be extended to other age groups (e.g., adolescent populations), and other stressor questionnaires or forms of self-report should be included into the Triad analyses.

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