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Children's stress-related reports and stress biomarkers interact in their association with metabolic syndrome risk

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Abstract

The purpose was to examine the cross-sectional associations of stress-related reports and stress biomarkers with metabolic syndrome (MetS) risk in children while also testing the interaction between stress biomarkers and stress reports. In 353 children (5–10 years old, 7.9% overweight/obese), MetS risk was measured by blood pressure, waist circumference, glucose homeostasis, triglycerides, and high-density cholesterol. Stress was measured by stress-related reports (events, emotions, and internalizing/externalizing problems) and two biomarkers: salivary cortisol (total-day and morning output) and heart rate variability (percentage of consecutive normal RR intervals differing more than 50 ms and low-to-high-frequency ratio). Cross-sectional regression analyses with *z* scored total MetS risk as outcome were adjusted for age, sex, and socio-economic status. Only internalizing problems were directly related to a higher MetS risk score (β = 0.236). Cortisol and heart rate variability were significant moderators: High cortisol morning output resulted in a positive (unfavourable) report–MetS relationship (β = 0.259–0.552), whereas low percentage of consecutive normal RR intervals differing more than 50 ms resulted in a negative (favourable) report–MetS relationship (β = −0.298) and low low-to-high-frequency ratio in a positive (unfavourable) report–MetS relationship (β = 0.478). In conclusion, stress can sometimes be a disadvantageous factor in metabolic health of otherwise healthy children. The cortisol biomarker seems relevant because metabolic risk was highest when stress-related reports were accompanied by high morning cortisol output.

KEYWORDS

autonomic nervous system, cardiovascular disease prevention, cortisol, metabolic health, moderation, psychophysiology, psychosocial stress

1 | INTRODUCTION

The presence of cardiovascular risk factors or metabolic syndrome (MetS) factors, such as central obesity, hyperglycaemia, elevated blood pressure, and abnormal plasma lipid levels in childhood, strongly influence the probability of acquiring cardiovascular disease in adulthood (Hong, 2010). Therefore, it is pivotal to know the underlying predictors of these MetS factors in childhood. Apart from genetic and lifestyle predictors (Hong, 2010), there is increasing evidence since 1992 (Chrousos & Gold, 1992) that psychosocial stress might also lead to these metabolic dysregulations and this both in adults (Hemingway & Marmot, 1999; Rosmond, 2005) and in children (Pervanidou & Chrousos, 2011). Especially in children, there is evidence that alterations of biological stress systems by chronic stress may have permanent effects because organs, such as the brain, are still developing (Teicher, Andersen, Polcari, Anderson, & Navalta, 2002). The body's goal in stress situations is not only to maintain stability through changes in the immune system, nervous system, and endocrine system

for an appropriate amount of time but also to turn off these reactions immediately afterwards. In contrast, chronic stress leads to prolonged activation or inefficient management of these systems, with detrimental physiological consequences, such as MetS (Ghike, 2016; McEwen, 1998, 2007). Negative stress–MetS relations have also been found in conditions such as hypertension in youth (Berendes, Meyer, Hulpke-Wette, & Herrmann-Lingen, 2013) using both internalizing/emotional problems and conduct problems as stress measure.

Biological stress measures are necessary in this type of research because the nature and the chronicity of the stressor, as well as the individual's vulnerability and stress perception, are important variables in determining the chronic adverse effects of stress (Miller, Chen, & Zhou, 2007). There are two main physiological stress systems (Charmandari, Tsigos, & Chrousos, 2005). The first stress system is the hypothalamic–pituitary–adrenal axis with cortisol as the end product. The second stress system is the autonomic nervous system with the catecholamines adrenaline and noradrenaline as end products, but often, heart rate variability (HRV) is used as a non-invasive biomarker to indirectly measure cardiac parasympathetic and sympathetic activities (Task Force of European Society of Cardiology [ESC]/North American Society of Pacing and Electrophysiology [NASPE], 1996). Both HRV and salivary cortisol values are thus well-accepted stress biomarkers in children and adults (Aguilar Cordero et al., 2014; Hellhammer, Wust, & Kudielka, 2009; Marques, Silverman, & Sternberg, 2010; Porges, 1995).

In the MetS-related literature, the theory on cortisol has received the most attention by general reviews (Anagnostis, Athyros, Tziomalos, Karagiannis, & Mikhailidis, 2009; Bjorntorp & Rosmond, 1999; Chrousos, 2000). Theoretically, cortisol can be associated with all MetS components due to stimulation of the lipoprotein lipase enzyme, adipogenesis induction, decreased vasodilator production, decreased pancreatic insulin production, and glycogen synthase activation (Anagnostis et al., 2009). Cortisol was not related to MetS in some youth populations (Boyne et al., 2009; DuBose & McKune, 2013) but was related to overall MetS in other (mainly obese) youth populations (Guzzetti, Pilia, Ibba, & Loche, 2014; Villarreal, Guevara, Lopez, & Saenz, 2012).

Most theoretical evidence for the role of the autonomic nervous system is found in blood pressure and hyperglycaemia because sympathetic activity mediates vasoconstriction, resulting in higher peripheral resistance, and it antagonizes insulin-mediated glucose uptake via effects on skeletal muscle blood flow (Lambert & Lambert, 2011; Thayer, Yamamoto, & Brosschot, 2010). Indeed, some reviews emphasize the role of the autonomic nervous system in MetS (Lambert & Lambert, 2011; Thayer et al., 2010). For example, HRV was lower in adult MetS cases (Brunner, 2002) and was positively related with a MetS risk in youth (Zhou, Xie, Wang, & Yang, 2012). Concerning MetS components, HRV was related to all MetS aspects except high-density lipoprotein (HDL) in adults (Licht et al., 2010), whereas in youth, the focus has been on blood pressure (Ryder et al., 2016; Zhou et al., 2012).

Importantly, this stress physiology might act as moderator. In this concept of moderation, there might only be an effect of stress on metabolic health when a stressor is combined with a high biological stress response, that is, when the stress gets under the skin. After all, the physiological response of stress is determined by the individual's vulnerability and stress perception (Miller et al., 2007). Cortisol has already been mentioned as a moderator in the effects of depressive symptoms on adults' MetS (Vogelzangs et al., 2007; Weber-Hamann et al., 2002). For HRV, no moderation effects on MetS have been published, but heart rate (not HRV) was used as a moderator between stress reports and mental health problems in a sample of (pre)adolescents (Oldehinkel, Verhulst, & Ormel, 2008).

Taken together, in-depth stress–MetS research using both questionnaires and biomarkers is necessary. For example, it may be useful to determine whether the cortisol system and the autonomic nervous system are equally important for MetS risks to identify at-risk cases. Furthermore, information on biomarker–report interaction is lacking, but it is important for prevention/intervention (e.g., psychological interventions are not able to decrease stressors but might reduce biomarker increases), and missing this complete picture might bias observed stress–MetS relations. Especially in children, less research has been done, and the research has primarily been conducted in very small populations (DuBose & McKune, 2014; Holmes, Eisenmann, Ekkekakis, & Gentile, 2008; Villarreal et al., 2012) using only stress reports (i.e., questionnaires; Holmes et al., 2008; Suchday, Bellehsen, Friedberg, Almeida, & Kaplan, 2014) or by rather low-quality biomarker collection, such as a single sample of serum cortisol (Guzzetti et al., 2014; Weigensberg, Toledo-Corral, & Goran, 2008). Even in the stress reports, several aspects such as stressors, perceived stress, and internalizing (emotions such as anxiety and depression and social isolation) and externalizing problems (undercontrolled behaviour such as aggression) have been tested although they have been differently related to the stress biomarkers (Michels, Sioen, Huybrechts, et al., 2012; Michels et al., 2013). Interestingly, one study suggests that associations might be more visible in adolescence than in childhood (Guzzetti et al., 2014).

Therefore, the main hypothesis of the current study was the positive association of different stress-related reports (events, emotions, and internalizing and externalizing problems) and stress biomarkers (high cortisol and low parasympathetic activity) with a MetS score in children. An additional hypothesis was that stress biomarkers interact with stress reports towards the development of MetS.

2 | MATERIAL AND METHODS

2.1 | Participants and general procedures

Participating children were part of the European Identification and Prevention of Dietary- and Lifestyle-induced Health Effects in Children and Infants (IDEFICS) study and were recruited for the Belgian Children's Body Composition and Stress (ChiBS) study that examines the association between stress and body composition. Detailed aims, methods, and population characteristics were described elsewhere (Michels, Vanaelst, et al., 2012). Data for the current cross-sectional analyses were collected between February and June 2010 (the ChiBS baseline survey) in the selected city of Aalter in Belgium when the children were between 5 and 10 years old. All measurements (MetS, HRV, anthropometrics, stress-related reports, and other questionnaires) were collected during one individual appointment;

only salivary cortisol was collected some days earlier or later. Parental education was used as the proxy variable for the socio-economic status using the classification by the International Standard Classification of Education (United Nations Educational, Scientific and Cultural Organization, 2010). Data were further categorized into two groups: low (= up to secondary education) and high (= tertiary education) status. Weight and height were measured in bare feet and light underwear with an electronic scale (TANITA BC 420 SMA, TANITA Europe GmbH, Sindelfingen, Germany) and a stadiometer (Seca 225, SECA GmbH & Co. KG., Hamburg, Germany) to the nearest 0.1 kg and 0.1 cm, respectively. Age- and sex-specific body mass index *z* scores were calculated according to the method from Cole, Freeman, and Preece (1998). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The project protocol was approved by the Ethics Committee of the Ghent University Hospital. Written informed consent was obtained from the parents of all participants. The authors declare that they have no conflict of interest.

2.2 | Sample size

In total, 523 children participated in the ChiBS survey (no exclusion criteria existed). The biological measurement modules (i.e., salivary cortisol, HRV, and blood withdrawal) were optional. Exclusion criteria for the current paper were lack of data on MetS risk due to blood withdrawal refusal (n = 169; 51% boys), diagnosed Cushing/Addison disease (n = 0), diagnosed heart disease (n = 0), diagnosed attention deficit hyperactivity disorder (n = 1), cardiovascular-related medication use (n = 0), or corticosteroid use (n = 5). Because of high-quality control for HRV and cortisol data (see respective sections) and the fact that the modules were optional, 264 of the children (48% boys) had complete HRV data, and 284 (54% boys) had complete cortisol data. Cases with missing information on HRV or cortisol were only excluded for the cortisol and HRV regression analyses. No difference in sex, parental education, MetS score, body mass index *z* score, and overweight prevalence was observed between children included and not included in these two sets of analyses, but those included were somewhat older.

2.3 | Metabolic syndrome

We have used the recently validated IDEFICS definition of paediatric MetS (Ahrens et al., 2014), which applies the criteria of several international definitions. This MetS definition considers four components: central obesity (by waist circumference), blood pressure (based on systolic blood pressure [SBP] and diastolic blood pressure [DBP]), lipid homeostasis (based on triglycerides and HDL), and insulin resistance (based on insulin and glucose using the homeostasis model assessment [HOMA]). Following this definition, each continuous component was transformed in age- and sex-specific *z* scores using the European IDEFICS childhood cohort, and then the sum was obtained using the following formula: $\text{MetS} = z_{\text{waist}} + (z_{\text{SBP}} + z_{\text{DBP}}) / 2 + (z_{\text{Triglycerides}} - z_{\text{HDL}}) / 2 + z_{\text{HOMA}}$. As a result, each component had the same weight in the total score, and higher scores indicate a less favourable metabolic profile. This MetS demonstrates a clear association with anthropometry (Bornhorst et al., 2016).

2.3.1 | Waist circumference

The measurement of waist circumference was obtained twice in an upright position with relaxed abdomen and feet together, with the measurement taken from midway between the lowest rib margin and the iliac crest to the nearest 0.1 cm with an inelastic tape (Seca 200).

2.3.2 | Blood pressure

Blood pressure was measured with an electronic sphygmomanometer (Welch Allyn 4200B-E2, Welch Allyn Inc., New York, USA). The cuff length for blood pressure measurement was chosen according to the arm circumference value. Children were asked to sit for at least 5 min before the measurement. Two measurements were taken at 2-min intervals, and a third measurement was taken if measurements differed by >5%. The average of the two (or three) measurements was used for statistical analysis.

2.3.3 | Blood collection for lipid and glucose homeostasis

Fasting blood was used to assess blood glucose, HDL, and triglycerides on site within 5 min of blood withdrawal by placing one drop of blood in the "point-of-care" analyser (Cholestech, Hayward, USA). Serum insulin concentrations were measured by luminescence immunoassay in a central laboratory using an AUTO-GA Immulite 2000 (Siemens, Eschborn, Germany). As an indicator of insulin resistance, the HOMA was calculated following this formula: HOMA = fasting insulin (μIU/ml) × fasting glucose (mg/dl)/405.

2.4 | HRV as a biomarker of the autonomic nervous system stress axis

HRV is defined as the variability of the distance between consecutive R peaks of the electrical heart beat signal as a result of the activity in parasympathetic and sympathetic cardiac innervations (Task Force of ESC/NASPE, 1996). Interbeat RR intervals were recorded at a sampling rate of 1,000 Hz with the Polar Wearlink 31 elastic electrode belt, which has been validated against an electrocardiogram device in children (Gamelin, Baquet, Berthoin, & Bosquet, 2008). Each child was individually examined in a quiet room in the supine position during 10 min while breathing normally and not speaking or moving. Children were asked to refrain from strenuous physical activity on the measurement day and had some time to relax before the measure. Further data processing was performed with the professional HRV Analysis Software of the University of Kuopio, Finland. Low-frequency (LF) and high-frequency (HF) bands were analysed between 0.04 to 0.15 Hz and 0.15 to 0.4 Hz, as default (Task Force of ESC/NASPE, 1996). The RR series were detrended using the smoothness priors method with $\alpha = 300$, and a cubic interpolation at the default rate of 4 Hz was performed. The middle 5 min were manually checked for quality, and if necessary, another appropriate 5-min interval was selected. Quality was defined as no large RR interval outliers, an equidistance between consecutive RR interval points and Gaussian

RR intervals, and heart rate distribution graphics. In this way, disturbing phenomena such as the Valsalva manoeuvre were excluded. Data of 13 children were discarded by this quality control, and one child with a functional heart disease was also excluded.

Two HRV parameters were used in the statistical analyses. Using time domain methods, the percentage of consecutive normal RR intervals differing more than 50 ms (pNN50) was determined as representing the vagal innervation (parasympathetic activity). Consequently, high pNN50 values are desirable. Using spectral domain methods, parametric autoregression (forward–backward least squares with a personally optimized model order) was performed obtaining the LF/HF ratio, representing the approximate sympathovagal balance (Task Force of ESC/NASPE, 1996). Consequently, low LF/HF ratios are desirable. These HRV measures (pNN50, LF/HF, and others such as root mean square of the successive differences) have already been tested as stress marker in this sample (Michels et al., 2013).

2.5 | Salivary cortisol as biomarker of hypothalamic–pituitary–adrenal stress axis

Saliva was collected at home into Salivette synthetic swabs for cortisol analysis (Sarstedt, Germany) during two consecutive weekdays at four time points: immediately after wake up, 30 min after wake up, 60 min after wake up, and in the evening between 7 and 8 p.m. For the four consecutive time points, the within coefficients of variation were 36.2%, 33.6%, 43.0%, and 41.5%, respectively. Standardized sampling instructions have been published (Michels, Sioen, De Vriendt, et al., 2012). Quality control was executed by excluding morning samples collected more than 5 min from the requested time point and evening samples not collected between 7 and 9 p.m. (271 out of 3,290 samples; Michels, Sioen, De Vriendt, et al., 2012).

Centrifuged saliva filtrates were assayed in the routine laboratory of the Ghent University Hospital on a Modular E 170 immunoanalyser system (Roche Diagnostics, Mannheim, Germany) using the Roche Cobas Cortisol assay. The area under the curve with respect to the ground (AUCg) was calculated as the total area under the curve between the first (awakening) and third (60 min after awakening) samples as a measure of the cortisol awakening response (Fekedulegn et al., 2007). The area under the curve between the first and last samples of the day was also investigated as a measurement of the "total-day cortisol output."

2.6 | Stress-related reports

A broad definition of stress was developed by measuring the different aspects of the stress process: negative events and also outcome aspects (i.e., negative emotions and more generally internalizing problems). The stress appraisal phase ("are you stressed?") was not measured because this subjective representation seemed to be too difficult for this age group. Children were individually interviewed by a trained researcher to obtain information about their negative events and emotions. Parents were asked to report on their child's internalizing problems over the past 6 months.

2.6.1 | Negative events (child report)

The "Coddington Life Events Scale" for children (Coddington, 1999) was used to identify potential physical and mental health problems arising from life events: events concerning illness, death, family, school, and social domains. This validated (3-month test–retest intraclass correlation (ICC) = 0.69, construct validity = 0.45) 36-item questionnaire measured the self-reported frequency and timing of neutral and negative events in the last year relevant for this age group. The resulting "life change units" score was a sum of all events but with very recent events and serious events receiving higher scores (following the manual instructions). For the current paper, only the negative events were used.

2.6.2 | Negative emotions (child report)

Children were asked to indicate how they mostly feel (not only today) on a Likert scale. For each of the three negative emotions (anger, anxiety, and sadness), they could rate themselves from 0, *not at all*, to 10, *very strong* (Zimmer-Gembeck, Lees, Bradley, & Skinner, 2009). The total negative emotions score was obtained via a sum and ranged between 0 and 30. To help the children understand these distinct feelings, pictures of a social skills training game for very young children were displayed next to the question (one picture for each emotion). These basic emotions are already understandable during infancy and can be uncomplicatedly used in this age group. The sum of the three negative emotions was validated against the well-known Positive and Negative Affect Scale for Children questionnaire that can be used for children of at least 9 years old. A Spearman correlation of $r = .48$ $p < .001$ with the negative affect score of the Positive and Negative Affect Scale for Children questionnaire was found in a sample of 153 children who were between 9 and 13 years old at follow-up.

2.6.3 | Internalizing and externalizing problems (parental report)

Parents were asked to complete the "Strengths and Difficulties Questionnaire" (Goodman, 1997) on their child's behavioural problems over the past 6 months. Parents could answer the statements with *not true* (0), *somewhat true* (1), and *certainly true* (2). An "internalizing problem" score was calculated by summing up the subscales for emotional symptoms and peer problems, in accordance with the questionnaire manual. The externalizing problem score is reflected by conduct problems. This questionnaire has shown good test–retest stability ($r = .88$) and good validity when compared with the Child Behavior Checklist (r between .87 and .7).

2.7 | Statistical analyses

All statistical calculations were performed in SPSS software, version 22.0 (IBM, NY, USA), with a significance set at two-sided $p < .05$. Residues from regression were normally distributed without transformation.

As the main hypothesis, linear regressions were performed with the MetS risk score as outcome and each stress parameter (negative events, negative emotions, internalizing problems, externalizing problems, AUCg, total-day cortisol output, LF/HF, and pNN50) as a predictor in a separate model while adjusting for age, sex, and parental

education. Only on this main hypothesis, Bonferroni correction ($p < .006$) has been tested once. The regressions for the separate MetS components (blood pressure, HOMA, HDL, triglycerides, and waist) are given in Appendix A.

For all stress-related reports (events, emotions, internalizing problems, and externalizing problems), moderation by stress biomarkers (AUCg, total cortisol, pNN50, and LF/HF) in the total MetS outcome was tested. A moderator is a third variable affecting the direction and/or strength of the relationship between a predictor and an outcome variable (as tested by interaction). Consequently, a significant predictor–outcome association might be present only at certain values of the moderator. For the significant interactions, graphs have been created by plotting predicted outcome values for three representative groups of the moderator and predictor: those at the mean, at 1 *SD* below the mean, and 1 *SD* above the mean.

3 | RESULTS

3.1 | Descriptive data

Table 1 shows the descriptive data of the sample on stress and MetS parameters. Sex differences were found in several parameters. Only 2.6% of the children were "at risk" following the MetS calculation (i.e., three or four components above the 90th percentile), but 14.4% were at risk for central adiposity, 10.5% for high blood pressure, 15.4% for disordered lipid homeostasis, and 9% for disordered glucose homeostasis; thus, 46.2% had at least one risk factor. Following the International Obesity Task Force classification, 7.4% were considered overweight, and 0.5% were obese. Using cut-offs of the questionnaires, 28% experienced a considerable number of stressors, 18% had an increased risk for externalizing problems, and 21% for internalizing problems.

3.2 | The association between stress (reports and biomarkers) and MetS

Table 2 shows the zero-order Spearman correlations between the main parameters. The MetS score was only associated with internalizing problems. Additionally, significant associations between stress measures were found. The two HRV parameters were associated with each other, the two cortisol parameters were associated with each other, and the some questionnaire parameters were associated with each other. Associations between these three different stress measures (cortisol, HRV, and questionnaires) were mutually limited, and only the HRV parameters were significantly associated with negative emotions.

Table 3 shows the linear regression results on the relation between stress measures (reports, cortisol, and HRV) and MetS. Similar to the results presented in Table 2, the MetS score was only associated with internalizing problems (B = 0.214; β = 0.236). This even stayed significant after Bonferroni correction ($p < .006$ as there were eight regression analyses; no Bonferroni correction was implemented

TABLE 1 Descriptive characteristics

	Full group		Boys (51.3%)		Girls		Sex difference
	Mean	*SD*	Mean	*SD*	Mean	*SD*	*p* value
Age (years)	8.4	1.1	8.4	1.2	8.4	1.1	.750
Parental education (tertiary)	72.7%		75.4%		69.8%		.049
Stress-related reports (*N* = 353; higher scores reflect higher stress reports)							
Negative events (0–144)[a]	20.7	5.8	16.1	6.4	26.9	5.1	.170
Negative emotions (0–30)	7.6	5.9	6.7	5.5	8.6	6.2	.005
Internalizing problems (0–20)	3.6	2.8	3.6	2.7	3.7	2.8	.494
Externalizing problems (0–6)	1.6	1.1	1.4	1.2	1.9	1.0	<.001
Stress biomarkers cortisol (*N* = 284)							
Cortisol AUCg (nmol/L)[a]	22.6	1.4	22.0	1.4	23.2	1.5	.352
Total cortisol output (nmol/L)[a]	357,928	156,580	333,307	127,395	381,250	187,461	.020
Stress biomarkers heart rate variability (*N* = 264)							
pNN50 (%)	39.7	17.4	40.1	17.6	39.3	17.3	.013
LF/HF (%)[a]	1.0	0.8	1.1	1.8	0.9	1.9	.498
Metabolic syndrome (*N* = 353)							
HOMA	1.0	0.7	0.9	0.5	1.1	0.8	.001
Systolic blood pressure (mmHg)	102.9	7.7	103.5	7.8	102.4	7.6	.052
Diastolic blood pressure (mmHg)	64.5	5.7	64.5	5.9	64.6	5.7	.587
High-density cholesterol (mg/dl)	61.9	13.7	63.2	13.1	60.6	14.4	.190
Triglycerides (mg/dl)	64.5	20.6	61.6	16.7	66.5	22.9	.001
Waist circumference (cm)	57.2	5.7	56.5	4.3	57.9	6.9	.048
Total metabolic risk score (*z* score)	0.72	2.55	0.3	2.0	1.0	2.9	.028

Note. AUCg = area under the curve of the three morning samples with respect to the ground; HOMA = homeostasis model assessment; LF/HF = ratio of low-frequency to high-frequency heart rate variability measures; pNN50 = percentage of consecutive normal RR intervals differing more than 50 ms.

[a]These variables were log-transformed because of nonnormal distribution, but the mean and *SD* were back-transformed to the original units.

TABLE 2 Zero-order Spearman correlations between stress variables and MetS

		Negative events	Negative emotions	Externalizing problems	Internalizing problems	AUCg	Total-day cortisol	pNN50	LF/HF
Negative emotions	Rho	.152*							
	p	<.001							
Externalizing problems	Rho	.061	.087						
	p	.176	.055						
Internalizing problems	Rho	.136*	.095*	.601					
	p	.003	.036	<.001*					
AUCg	Rho	.095	−.050	−.025	−.016				
	p	.060	.324	.619	.467				
Total-day cortisol	Rho	.101	.003	−.024	−.016	.668*			
	p	.054	.950	.656	.761	<0.001			
pNN50	Rho	.012	−.112*	.008	−.024	.004	.048		
	p	.824	.034	.871	.613	.949	.438		
LF/HF	Rho	−.085	.142*	.027	.014	−.044	−.078	−0.394*	
	p	.107	.007	.582	.767	.460	.206	<0.001	
MetS	Rho	.125	−.011	.096	.171*	.178	−.064	.069	−.047
	p	.184	.905	.239	.036	.084	.552	.481	.629

Note. AUCg = area under the curve of the three morning samples with respect to the ground; LF/HF = ratio of low-frequency to high-frequency heart rate variability measures; MetS = total metabolic syndrome risk score; pNN50 = percentage of consecutive normal RR intervals differing more than 50 ms.

*$p < .05$.

TABLE 3 Linear regressions for stress as predictor of the metabolic syndrome

	Metabolic syndrome risk score	
	β	*p*
Stress-related reports (*N* = 353)		
Negative events	0.137	.165
Negative emotions	−0.033	.736
Internalizing problems	0.236	.003*
Externalizing problems	0.016	.154
Salivary cortisol as stress biomarker (*N* = 284)		
AUCg	0.089	.411
Total-day cortisol	−0.006	.957
Heart rate variability as stress biomarker (*N* = 264)		
pNN50	0.175	.082
LF/HF	−0.067	.511

Note. The eight regressions (each predictor was tested separately) were adjusted for sex, age, and parental education. β = standardized regression coefficient; AUCg = area under the curve of the three morning samples with respect to the ground; LF/HF = ratio of low-frequency to high-frequency heart rate variability measures; pNN50 = percentage of consecutive normal RR intervals differing more than 50 ms.

*$p < .05$.

in the table). In the supplement, the linear regression results on the separate MetS components are given: events positive with HOMA, internalizing problems positive with HOMA and triglycerides, AUCg positive with HOMA, and LF/HF positive with blood pressure but negative with triglycerides. When splitting internalizing problems into peer and emotional problems, similar results were seen as for internalizing problems (Table 3).

3.3 | Moderation in the stress–MetS associations

Significant report–biomarker interactions in predicting MetS were found for the following combinations: AUCg emotions ($p = .048$), AUCg problems ($p = .015$), LF/HF events ($p = .010$), and pNN50 emotions ($p = .037$). These interactions are depicted in Figure 1 to clarify the direction of this interactive effect. Only in the cases of high or moderate values for cortisol (AUCg) were the stress-related reports significantly positively associated with MetS. In contrast to the hypothesis, low pNN50 resulted in a negative report–MetS relationship and low LF/HF in a positive report–MetS relationship.

4 | DISCUSSION

This study examined the role of high-quality stress biomarkers and stress-related reports in overall MetS risk within a relatively large sample of 5- to 10-year-old "healthy" children. Only the parent-reported "internalizing problems" were directly related to a higher MetS risk score, but some MetS component-specific results were also found. Nevertheless, a significant interaction between stress biomarkers and stress reports was detected. Especially in the case of rather high salivary cortisol output in the morning (AUCg), a positive (unfavourable) MetS association with stress-related reports existed. This moderation effect means that MetS was thus more influenced when stress reached a critical level, as reflected by a combination of high stress-related reports and high morning cortisol output, rather than by high stress-related reports alone.

4.1 | Stress-related reports in relation to MetS

From the set of questionnaires, only the internalizing problem score was positively related to total MetS. After moderation by biomarkers, also events and child-reported emotions seemed relevant. Other studies in children/adolescents found no association between MetS and reported stress (Suchday et al., 2014), depressive symptoms (Holmes et al., 2008), anxiety (Holmes et al., 2008), or perceived stress (Holmes et al., 2008), but positive associations with appearance-related teasing

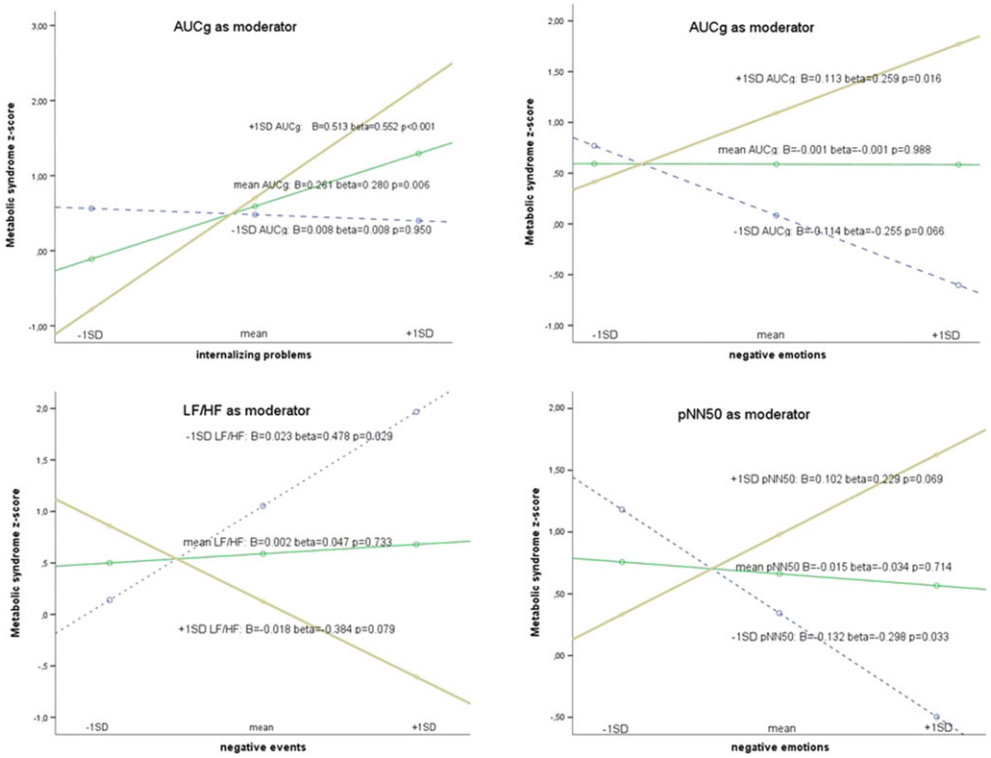

FIGURE 1 Moderation by stress biomarkers in the association between stress-related reports and metabolic syndrome. Moderation was tested by introducing the "stress report biomarker" interaction in the linear regressions for total metabolic syndrome score as outcome. In this way, two biomarkers from the salivary cortisol sampling (AUCg and total-day cortisol output; *N* = 284) and two biomarkers from the heart rate variability measures (pNN50 and LF/HF; *N* = 264) were tested as moderators. All stress-related reports (questionnaires on internalizing/externalizing problems, negative emotions, and negative events) have been tested in this interaction. For the significant reporting of biomarker interactions, graphs have been created by plotting predicted outcome values for three representative groups of the moderator and predictor: those at the mean, at 1 SD below the mean (=low), and 1 SD above the mean (=high). For each of these three moderator values, significance level and unstandardized and standardized regression coefficients of the association between stress-related report and metabolic syndrome is mentioned next to the respective line. AUCg = area under the curve of the three morning samples with respect to the ground; LF/HF = ratio of low-frequency to high-frequency heart rate variability measures; pNN50 = percentage of consecutive normal RR intervals differing more than 50 ms

(Holmes et al., 2008) and school-related self-esteem (Holmes et al., 2008) were found. In fact, different aspects can have different physiological effects as specificity in the report–cortisol association has been found in this population depending on the type of stress report (Michels, Sioen, Huybrechts, et al., 2012) and as the effect of negative events depends on the perception of the individual. Apart from this, an informant-based bias (child vs. parent vs. teacher) is well known in paediatric research (van Dulmen & Egeland, 2011).

4.2 | Cortisol in relation to MetS: Direct and interaction effects

Although cortisol was not directly related to total MetS, cortisol was related to higher HOMA. Cortisol as a glucocorticoid modulates the expression of approximately 10% of the human genes (Buckingham, 2006) and can be associated with all MetS components due to stimulation of the lipoprotein lipase enzyme, adipogenesis induction, decreased vasodilator production, decreased pancreatic insulin production, and glycogen synthase activation (Anagnostis et al., 2009). Comparisons with the literature are often difficult due to smaller samples sizes and different quality in biomarker measurement. In other youth populations, cortisol has been reported as a predictor of blood pressure (Boyne et al., 2009), lipids (DuBose & McKune, 2013; Villarreal et al., 2012), waist circumference/obesity (Barat et al., 2007; Veldhorst et al., 2014), and insulin (Huybrechts et al., 2014). Cortisol was not related to overall MetS in some youth populations (Boyne et al., 2009; DuBose & McKune, 2013) but was related to overall MetS in other (mainly obese) youth populations (Guzzetti et al., 2014; Villarreal et al., 2012).

The most prominent added value of this study is the revealed moderator role of cortisol: The stress biomarker cortisol enhanced the association of stress-related reports with MetS. Indeed, biological measures are necessary because the physiological response and chronic adverse effects of stress in humans are determined by the stressor nature, stressor chronicity, and the individual's vulnerability and stress perception (Miller et al., 2007). A high stress report/experience without related high stress biomarkers can reflect a good buffering system to avoid stress-related long-term allostatic load as shown in women (e.g., due to a good coping capacity or good social support; Sladek, Doane, Jewell, & Luecken, 2016). Genetic aspects can also play a role herein as several genetic polymorphisms are related to cortisol reactivity (Derijk, 2009). Further, high cortisol values can be due to factors other than stress, also in children (Jessop & Turner-Cobb, 2008). Taken together, the combination of high stress-related reports and high stress biomarker levels reflects the highest vulnerability to stress-induced health effects in the current paediatric sample. This

emphasizes the need to consider both stress reports and biomarkers. Similar conclusions are almost nonexistent in literature, but cortisol has previously been mentioned as a moderator in the effects of depressive symptoms on adults' MetS (Vogelzangs et al., 2007; Weber-Hamann et al., 2002).

4.3 | HRV in relation to MetS: Direct and interaction effects

Overall, HRV results in our study were less promising. HRV parameters were not directly related to total MetS, but LF/HF was related to higher blood pressure (thus in the theoretically sound direction), and a rather counterintuitive relationship was found between LF/HF and lower triglycerides. Indeed, the autonomic nervous system (here, measured via HRV) is responsible for providing blood to the heart, lungs, and muscles in preparing the fight/flight reaction (Charmandari et al., 2005). In an adult sample, HRV has been related to overall MetS risk and to all MetS components except HDL (Licht et al., 2010). Published studies in youth consistently reported a higher blood pressure with LF/HF, although also other MetS domains such as waist and lipids have been reported (Rodriguez-Colon et al., 2015; Ryder et al., 2016; Zhou et al., 2012).

The findings for HRV as a moderator (low pNN50 resulted in a negative report–MetS relationship and low LF/HF in a positive report–MetS relationship) were in contrast to the hypothesis. Primarily, low parasympathetic tone (e.g., low pNN50) and high sympathetic tone (approximately reflected by high LF/HF) have been found to be related to metabolic risk in youth (Ryder et al., 2016) and adults (Brunner et al., 2002; Lambert & Lambert, 2011; Licht et al., 2010; Thayer et al., 2010; Yoo et al., 2016). In literature, one study in adults reported counter-intuitive results (i.e., sympathetic activity as negatively associated with triglycerides; Min, Min, Paek, & Cho, 2008). Further, in the current population sample, LF/HF was related to lower triglycerides; thus, a healthier status was achieved (nonpublished data). Consequently, HRV seems not a reliable marker in measuring stress-related metabolic health within a children's population with low overweight prevalence.

4.4 | Strengths and limitations

Apart from its focus on a population-based sample of children, the central asset of this study is the combination of stress-related reports and strictly measured biomarkers of the two main stress pathways. This allowed us to test which of the two pathways is the most relevant in explaining MetS and whether this stress physiology interacts with stress-related reports.

Caution should be used when generalizing the results of this population from one city to the overall population because cultural variations have been described in stressors and coping, and our population showed a low overweight prevalence. Stronger associations between stress and MetS might be encountered in populations with more extreme health differences. Nevertheless, the observed overweight/obesity prevalence did not strongly deviate from the estimated 10% for this age group in this region (Drieskens et al., 1997), and 34% of the normal weight children had at least one elevated MetS component. Indeed, the MetS score variation in our population (*SD* of 2.5) was not deviating much from the MetS score variation in a larger European population of this age group (*SD* of 2.9; Ahrens et al., 2014). A considerable decrease in sample size was experienced in the biological sampling, although this seemed not to introduce a large bias (overweight prevalence not significantly different for excluded and included participants). The cross-sectional design limits statements concerning causality, but there was, for example, no interaction between MetS and stress-related reports in predicting stress biomarkers (data not shown). Another limitation is the use of a rather broad definition of stress, including events, emotions, and internalizing/externalizing problems, and each aspect might have other physiological outcomes. Finally, the LF/HF variable as a parameter of sympatho-vagal balance should be interpreted with caution because of non-linear interactions between sympathetic and parasympathetic nerve activity.

5 | CONCLUSION AND TRANSLATIONAL POTENTIAL

In a low overweight sample of children, the evidence for a direct stress–MetS relationship was small, that is, only when using parent-reported internalizing problems as a stress parameter or when looking at specific MetS components. Nevertheless, a prominent finding is the importance of the stress biomarker cortisol. The autonomic system as measured by HRV seemed less relevant. Indeed, cortisol enhanced the adverse effects of stress-related reports on MetS by moderation. MetS was thus more influenced when stress reached a critical level, as reflected by a combination of high stress-related reports and high morning cortisol output, rather than by high stress-related reports alone. These findings should be replicated in other population samples.

For future studies on metabolic health, this underlines the importance of including both cortisol and stress-related reports, although often only one of them is collected. From a public health perspective, stressful events are difficult to avoid, but the prevention of a chronic physiological stress reaction might be beneficial in maintaining optimal metabolic health. One such opportunity is by incorporating education on stress management and emotion regulation in prevention programmes and in school curricula (Aparicio, Canals, Arija, De Henauw, & Michels, 2016). After all, good stress and emotion coping might prevent chronic cortisol increases and stress-related behaviours such as emotional eating. This type of curricular programme should focus on learning and encouraging awareness and understanding of feelings, identifying internal strengths, and learning effective emotion regulation skills such as acceptance, cognitive reframing, self-compassion, and problem solving.

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CONFLICTS OF INTEREST

The authors have declared that they have no conflict of interest.

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APPENDIX A

Linear regressions for stress as predictor of the metabolic syndrome components

	Blood pressure (*z* score)		HOMA (*z* score)		HDL (*z* score)		Triglycerides (*z* score)		Waist circumference (*z* score)	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Stress reports (*N* = 353)										
Negative events	−0.027	.614	**0.126**	**.021**	0.017	.752	0.136	.176	0.058	.284
Negative emotions	0.072	.190	−0.014	.791	−0.025	.642	−0.063	.533	−0.056	.302
Internalizing problems	**0.099**	**.016**	0.068	.142	−0.089	.055	**0.207**	**.010**	0.013	.749
Peer	0.071	.066	0.059	.207	**−0.106**	**.023**	**0.187**	**.018**	*0.041*	*.320*
Emotional	**0.089**	**.030**	0.053	.251	−0.048	.297	**0.155**	**.043**	*−0.012*	*.776*
Externalizing problems	0.015	.716	0.044	.350	−0.033	.483	−0.124	.120	0.057	.173
Salivary cortisol as stress biomarker (*N* = 284)										
AUCg	0.077	.207	**0.173**	**.003**	0.063	.305	0.023	.832	−0.084	.169
Total-day cortisol	−0.051	.421	0.113	.072	0.054	.395	−0.028	.803	−0.031	.626
Heart rate variability as stress biomarker (*N* = 264)										
pNN50	−0.048	.366	0.041	.470	−0.052	.354	0.151	.128	0.073	.162
LF/HF	**0.093**	**.048**	0.010	.854	0.047	.406	**−0.229**	**.021**	−0.094	.069

Note. The regressions were adjusted for sex, age, and parental education. β = standardized regression coefficient; AUCg = area under the curve of the three morning samples with respect to the ground; HDL = high density lipoprotein cholesterol; HOMA = homeostasis model assessment; LF/HF = ratio of low frequency to high frequency of heart rate variability measure; pNN50 = percentage of consecutive normal RR intervals differing more than 50 ms. Bold = $p < .05$.