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Hair cortisol as a biomarker of chronic stress in preadolescents: influence of school context and bullying

Izaro Babarro ^{a,b}, Jesus Ibarluzea ^{a,b,c,d}, Elvar Theodorsson^e, Eduardo Fano^{a,b}, Andrea Lebeña^{a,e}, Monica Guxens ^{c,f,g,h}, Jordi Sunyer^{c,f,g,i} and Ainara Andiarena ^{a,b}

^aFaculty of Psychology, University of the Basque Country, UPV/EHU, Donostia/San Sebastian, Spain; ^bBiodonostia Health Research Institute, Group of Environmental Epidemiology and Child Development, Donostia/San Sebastian, Spain; ^cSpanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain; ^dHealth Department of Basque Government, Public Health of Gipuzkoa, Donostia/San Sebastian, Spain; ^eDepartment of Biomedical and Clinical Science, Clinical Chemistry, Linköping, Sweden; ^fDepartment of Experimental and Health Sciences, Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain; ^gDepartment of Experimental and Health Sciences, University of Pompeu Fabra (UPF), Barcelona, Spain; ^hDepartment of Child and Adolescent Psychiatry/Psychology, Erasmus MC University Medical Centre, Rotterdam, The Netherlands; ⁱMunicipal Institute of Medical Research (IMIM-Hospital del Mar), Barcelona, Spain

ABSTRACT

Bullying has been identified as the most common form of aggression and a major source of stress among children and adolescents. The main objective of this study was to analyze the association that school context in general and bullying in particular might have with hair cortisol concentration (HCC), examining the effect of executive function and sex on this association. The study included 659 11-year-old preadolescents from the cohorts of Gipuzkoa and Sabadell of the INMA (INfancia y Medio Ambiente-Children and Environment) project. We gathered information about school-related factors (bullying, school environment, problems with peers and academic performance) and executive function (risky decision-making). Hair samples were collected to measure cortisol concentrations and Structural Equation Modeling was used to examine associations between school-related factors, executive function and HCC. Results showed that being involved as a bully/victim was related to higher HCC and, higher HCC was associated with poorer executive function. This study may contribute to a better understanding of the consequences that chronic exposure to a stressful factors may have on preadolescents' health and developmental outcomes. Besides, our results are relevant for designing programs for prevention and intervention, which could modify individual physiological responses to stress and reduce the effects of stress on the health.



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Hair cortisol; school context; bullying; executive function; preadolescents

During childhood and adolescence school has been the most studied developmental context. Apart from being a place where children and adolescents develop relationships with peers, it can also be the place where students may experience several stressful demands. Therefore, it is not surprising that school-related stress negatively affects

CONTACT Izaro Babarro  izaro.babarro@ehu.eus  Faculty of Psychology, University of the Basque Country, UPV/EHU, Plaza. de Elhuyar, 2, Donostia-San Sebastian, Gipuzkoa 20018, Spain

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students' health (García-Moya et al., 2013). Although acute stress can be beneficial and adaptive, chronic stress can be detrimental to several health outcomes, including the biological systems involved in the stress response. The Hypothalamic–Pituitary–Adrenal (HPA) axis and Sympathetic Adrenal Medullary (SAM) system work together to facilitate adaptation to stress. Specifically, when our bodies identify a stressor, involuntary processes mobilize a rapid SAM response and activation of the HPA axis, leading to cortisol synthesis. This glucocorticoid has been identified as a valuable biomarker of HPA function and it has been studied in relation to multiple indicators of social adversity (Bryson et al., 2021; Lugarinho et al., 2017).

Bullying, defined as a type of aggressive behavior that occurs in the school environment, has been identified as one of the main stress sources among children and adolescents (Vanaelst et al., 2012). The association between bullying and cortisol levels has been explored in some studies, finding mixed results (Kliewer et al., 2019). The inconsistencies in the associations may derive, among others, from the way cortisol levels were measured. Various samples can be used to measure cortisol (blood, urine, saliva, and hair), being saliva and hair samples the most commonly used. With regard to saliva samples, results can also differ based on the amount and timing of samples collected. In order to understand this, it is essential to know that cortisol follows a daily cycle in order to maintain healthy physiological functioning. Specifically, cortisol levels sharply increase in the morning, approximately 30 min after waking up. This is known as the cortisol awakening response (CAR). After this, cortisol levels decline throughout the day, reaching their lowest points after sleep begins. As long as the HPA axis is functioning properly, stress response and cortisol secretion follow predictable patterns; however, this can change under chronic stress conditions.

Regarding the association between cortisol levels and bullying, most research has used saliva samples to determine cortisol levels. Some authors concluded that victims or cybervictims present lower reactivity of cortisol (Calhoun et al., 2014; Knack et al., 2011; Ouellet-Morin et al., 2011, 2013), lower morning cortisol levels (Knack et al., 2011) and flattened cortisol patterns (González-Cabrera et al., 2017; Knack et al., 2011; Peters et al., 2011). However, others have suggested that victimized children have higher cortisol reactivity (Chen et al., 2018; Kliewer, 2006). One recent study which analyzed cortisol in hair samples found that highly victimized boys had higher levels of hair cortisol concentration (HCC) (Ouellet-Morin et al., 2021). With respect to the role of bullies, some researchers suggested that aggressive behaviors in children and adolescents were associated with low HPA axis activity (McBurnett et al., 2000; Platje et al., 2013). In the same vein, González-Cabrera et al. (2017) found that cyberbullies showed flattened cortisol curves. As far as bully/victim roles are concerned, González-Cabrera et al. (2017) found that the HPA axis was over activated in cyberbully/victims. Finally, there are also studies that found no association between victimization and cortisol levels (Vaillancourt & Schmidt, 2011; Williams et al., 2017).

The relationship between bullying and cortisol was found to be sex-dependent by some researchers. According to Vaillancourt et al. (2008) while victimized girls presented lower cortisol levels, victimized boys had higher cortisol levels. Östberg et al. (2018) showed that victims displayed lower cortisol levels and lower CAR. On the contrary, Arbel et al. (2019) found that victimization was related to higher total

cortisol levels. In both studies, however, the associations were only significant among boys.

In addition to bullying, some other school-related factors have been studied in relation to cortisol levels. Flattered cortisol slopes have been described to be related to non-supportive classrooms and conflictive relationships with teachers and peers (Ahnert et al., 2012; Bai et al., 2017). McHale et al. (2012) in their study found that spending more time than usual doing school homework was associated with higher cortisol secretion during the day.

When examining the relationship between social stressors and HPA axis activity, a number of individual differences may be of particular importance due to their association with both, cortisol levels, and social stressors. Among these differences genetic factors, personality traits, or cognitive abilities could be found. Particularly, the effect of stress in executive function has been amply documented. Executive functioning in general, and risky decision-making in particular, has shown correlation not only with hormone levels (e.g., sex hormones or cortisol) but also with various psychopathological conditions, such as aggressive behavior. A meta-analysis showed that despite its impairment on working memory and cognitive flexibility, a main effect of stress on inhibition was not shown. According to this study, stress affects cognitive inhibition, but response inhibition is enhanced (Shields et al., 2016). In the same vein, it has been found that moderate increases in stress levels were related to better executive functioning, however, high increases of cortisol levels were found to cause impaired performance on executive functioning (Pyle Hennessey et al., 2020). Moreover, executive function has also been studied in relation to bullying, concluding that a low executive function was a significant predictor of being involved in bullying behavior, as a victim, as a bully or as a bully/victim (Kloosterman et al., 2014; Verlinden et al., 2014). However, in a previous study an association between executive function and bullying behavior was not found (Babarro et al., 2020). In this line, previous studies have showed that executive functions, peer problems, and bullying experiences also impair childrens academic achievement (Gomes et al., 2020; Pascual et al., 2019). Additionally, sex differences may also impact the association that school-related variables and bullying have with hair cortisol, as well as with individual variables affecting this relationship. Regarding cortisol levels, most of the previous studies concluded that boys had higher HCC than girls (Anand et al., 2020; Gerber et al., 2017; Rippe et al., 2016; Simmons et al., 2016). Although there is also a study who find no difference in HCC by sex (Noppe et al., 2014). Sex differences have also been observed in bullying, generally boys being more involved than girls (Álvarez-García et al., 2015). Moreover, sex differences have been also noted regarding school environment' perception, executive function, and academic achievement. Girls perceived generally the school environment as better than boys (Yates, 2003) and are more likely to succeed at school (Parajuli & Thapa, 2017). Finally, a systematic review concluded that although sex differences exist in brain structures and neural networks underlying executive control functioning, there were mixed results in sex differences in executive function (Gaillard et al., 2021).

As aforementioned, school is the primary context in which children and adolescents are exposed to numerous stressors. In fact, bullying has been identified as the first source of stress during these developmental stages. Previous evidence has shown that various school factors (Ahnert et al., 2012; Bai et al., 2017; McHale et al., 2012) and bullying

(Kliewer et al., 2019) are related to cortisol levels. It has also been noted that when looking at the impact of school factors and bullying on cortisol levels, it is important to keep in mind that they are interrelated (Babarro et al., 2020) and that some individual variables could be associated with both stressors and cortisol levels. Among the individual variables, executive function has been linked to cortisol levels (Shields et al., 2016) and bullying (Kloosterman et al., 2014; Verlinden et al., 2014). Sex is another individual variable that has been found to affect cortisol levels (Anand et al., 2020; Grebe et al., 2019), executive function (Gaillard et al., 2021), school-related factors (Yates, 2003) and bullying (Álvarez-García et al., 2015). Therefore, the main objective of this study was to determine whether bullying, along with other school-related factors (problems with peers, school environment, academic performance) could predict chronic stress in 11-year-old preadolescents, examining the effect of executive function and sex on this association. It was hypothesized that school-related factors (problems with peers, poorer school environment, and poorer academic performance) and bullying involvement would predict HPA axis dysregulation. In addition, we expected that higher HCC would predict poorer executive function; and that poorer executive function would be associated with school-related factors (more problems with peers, poorer school environment, and poorer academic performance) and higher bullying involvement.

Methods

Participants

The study participants were preadolescents of the INMA project, from the cohorts of Gipuzkoa (Basque Country, Spain) and Sabadell (Catalonia, Spain). Participants' mothers were recruited in their first trimester of pregnancy in health centers or hospitals of the public health system. Since recruitment, data have been collected in several follow-up phases, and in this study, we used data from the 11-year follow-up phase, where 871 preadolescents and their families were visited and 212 respondents were excluded due to missing HCC ($n = 164$), or other data ($n = 48$), yielding a final sample of 659. We compared the differences between participants included and excluded from the study (Appendix A) and found that those excluded from the study had more problems with peers, poorer perceptions of the school environment, lower academic scores, and more people involved as victims than those included.

Measures

Hair cortisol

To date, in most studies, serum, saliva, or urine samples have been used to analyze HPA axis activity. Blood and saliva samples provide a momentary measure of cortisol concentrations, and lack the ability to retrospectively quantify HPA axis activity during a certain period of time. In order to overcome this problem, hair cortisol analysis was proposed as an alternative. Because hair grows at an average rate of 1 cm per month, hair samples provide a reliable measure of stress system activity retrospectively (Anand et al., 2020).

For this study, trained staff cut hair strands of 3 cm in length from the posterior vertex area of the participants. The hair was enclosed in sealed plastic packages marked with

identification numbers and stored at room temperature until analysis. All analyses were performed in the Clinical Chemistry Laboratory of the University of Linköping (Sweden). Hair samples were first cut into small pieces and were put into a 2 mL QiaGenRB sample tube with a 0.5 mm QuiGen stainless steel bead. The sample tubes were placed into aluminum cylinders and frozen in liquid nitrogen for 2 min and the hair samples were thereafter homogenized for 2 min, producing fine hair powder. 1 mL of the methanol was added to each tube and the samples were extracted overnight on a moving board. Afterward, 0.8 mL of methanol supernatant was pipetted off and lyophilized using a Savant Speed Vac Plus SC210A. The samples were dissolved in radioimmunoassay buffer and analyzed. The primary antibody used was Rabbit Cortisol 3 Polyclonal Antibody (MyBiosource, San Diego, USA). The secondary antibody which was anti-rabbit IgG was Sac Cell AA-Sac 1 (ImmunoDiagnostic System Ltd, Bordon, England). Hair samples between 3 and 10 mg were required to maintain a total inter-assay coefficient of variation below 8% for hair extraction and measurement of cortisol by the radioimmunoassay.

Bullying: Olweus Bully Victim Questionnaire (OBVQ)

This version consist of a standard definition of bullying and 16 questions (e.g., “I was called mean names, was made fun of, or teased in a hurtful way”) about bullying experiences in the past 2 months. The first eight items refer to different victimization behaviors (physical, verbal, social, sexual, and cyberbullying) and, the following eight to physical, verbal, social, sexual, or cyber harassment to another student. Items are rated on a 5-point Likert scale (0 “it has not happened to me in the past couple of months,” 1 “it happens once or a few times,” 2 “it happens 2 or 3 times a month (every month),” 3 “it happens “every week” and 4 “it happens several times a week”). A dichotomized variable was created following criteria of Solberg and Olweus (2003): (0) not involved or (1) frequently involved in bullying. So, for this study we used three variables of two categories, one for each role participant could take in bullying situations: victim (not involved (0) or frequently involved as a victim (1)), bully (not involved (0), or frequently involved as a bully (1)). The OBVQ showed adequate internal consistency in the present sample: $\alpha = 0.82$ for victim scale and $\alpha = 0.67$ for bully scale.

School environment: Kidscreen-27 questionnaire (Kidscreen-27, n.d.)

This self-reported questionnaire consisted of 27 items based on the previous week that are rated on a 5-point Likert scale (1 “never,” 2 “seldom,” “quite often,” 4 “very often” and 5 “always”-reflecting the frequency of behaviors or feelings and 1 “not at all,” 2 “slightly,” 3 “moderately,” 4 “very,” and 5 “extremely”-reflecting the intensity of a belief or attitude). The items were divided into five dimensions: Physical well-being, Psychological well-being, Peers and social support, Parents and autonomy, and School environment. In all cases, a higher score was indicative of higher quality of the measured construct. For this study, we used only the subscale of school environment (e.g., “Have you enjoyed going to school?”). The questionnaire showed acceptable internal consistency in the present sample for each of the subscales, being $\alpha = 0.70$ for school environment.

Problems with peers: Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997)

Parents were asked to complete the questionnaire to assess the general behavioral development of their children. The SDQ comprises 25 items in total, divided into 5 separate subscales: emotional symptoms, conduct problems, hyperactivity-inattention, peer relationship problems, and prosocial behavior. The items are rated on a 3-point Likert scale (0 “not true,” 1 “somewhat true,” and 2 “certainly true”). In this study, the subscale about peer relationship problems was used (e.g., “Generally liked by other children”). The internal consistency for the questionnaire was acceptable in the present sample ($\alpha = 0.78$).

Risky decision making. Cups Task Roulette Version Test (Levin et al., 2007)

2007 This computer task, consisted of 54 trials that assessed risky decision-making. More specifically, this task assesses hot executive function (cognition influenced by emotion), analyzing whether the participant adjusts his risky behavior according to the probabilities and importance of the outcome. In this task, the participants were presented with two wheels divided into segments of equal size and each associated with an amount of money. On each trial, the participants were asked to choose which wheel to spin, to gain, or avoid losing money. After the response, the wheel selected spun for 2 s and then ended on the amount of money to be won or lost. For this study, we took into account the total number of risky decisions each child made.

Academic achievement: ad hoc questionnaire

Only for children from Gipuzkoa cohort, we assessed their academic achievement skills using an *ad hoc* questionnaire. Using a 6-point Likert scale (1 “much less than his/her peers,” 2 “less than his/her peers,” 3 “slightly less than the average of his/her peers,” 4 “slightly higher than the average of his/her peers,” 5 “higher than his/her peers,” and 6 “much higher than his/her peers”) the students’ tutor should evaluate each preadolescent regarding their ability in reading, attention, mathematics, Spanish and Basque. For this study, we created a punctuation for academic achievement based on the average each preadolescent achieved in all the areas. The higher the score, the better was the students academic achievement.

Data analysis

SPSS 27 and AMOS 23 (IBM, Armonk, NY, USA) were used to conduct data analysis. First, we carried out the descriptive analysis and explore the differences by cohort and sex. Besides, we carried out bivariate analysis after making descriptive analysis. Then, we conduct Structural Equation Modeling (SEM). SEM makes the assumptions of linearity in the relationships between continuous variables and Gaussian error terms, in the first step we studied the symmetry of each relevant variable, transforming data, when appropriate. We also analyzed the linearity using GAM plots and we found that associations between the variables were linear.

After constructing the metamodel (Figure 1) based on *a priori* theoretical knowledge and after exploratory data analysis to ensure that SEM assumptions were met, we obtained global estimates. Data to model consistency was evaluated using a chi-square

test comparing the tested model with a saturated model. Apart from the aforementioned chi-square test, the root mean square error of approximation (RMSEA) was used. The final models were accepted only when the following conditions were met: chi-square test p -value > .05, the CFI > 0.95, and RMSEA p -value < .05.

Multigroup analysis was used to check if the proposed relationship among the variables would vary across different conditions in some variables: sex and cohort. The effect of the cohort was assessed by the study design. Sex was tested for the influence they could have on both the variables identified as stressors, cortisol levels, and executive function.

In addition, a sensitivity analysis was conducted incorporating to the models the data about academic achievement that was only available for the Gipuzkoa sample.

Metamodel

The purpose of the study was to explore if preadolescents' HCC was determined not only by bullying implication but also by other school-related variables. We also aimed to study the role of risk seeking and sex for its relation not only with bullying behavior but also with cortisol levels. With this objective and based on previous literature, we designed a metamodel (Figure 1).

Previous evidence showed that school-related factors (Ahnert et al., 2012; Bai et al., 2017; McHale et al., 2012) and bullying can affect cortisol levels. School-related factors such as peer relationships or school environments may also contribute to bullying (Babarro et al., 2020). Therefore, we incorporated these associations into our model. In addition, some individual variables may also be of interest when studying the

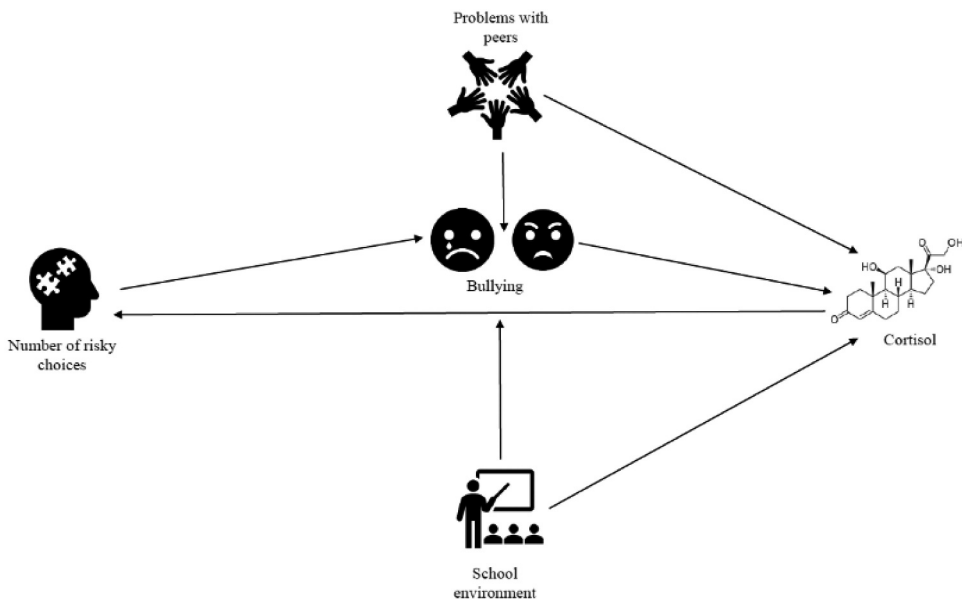


Figure 1. Metamodel summarizing the hypothesized relationships among <bullying involvement> (victim role, bully role, bully/victim role), <school variable> (school environment measured with *KidScreen 27*, peer problems with *SDQ*) and <individual variables> (number of risky decisions assessed with *Cups Task*), whose hypothesized direct and indirect effect on hair cortisol levels.

relationships between stress factors and cortisol levels. These include executive function and sex. Executive function has been examined in relation to cortisol levels (Shields et al., 2016) and bullying (Kloosterman et al., 2014; Verlinden et al., 2014). Furthermore, sex has been found to be related to cortisol levels (Anand et al., 2020; Grebe et al., 2019), executive function (Gaillard et al., 2021), bullying (Álvarez-García et al., 2015) and other school-related factors (Yates, 2003). All of these previous evidences are incorporated into the present complex model.

Multigroup analysis was used to test whether the proposed model differed by sex and cohort. After testing multigroup analysis we concluded that there were no effect of cohort or sex, so we decided to test the models in the total sample.

Results

Sample description

The study sample consisted of 659 children (58.7% girls and 41.3% boys) of 11 years ($M = 10.95$; $SD = 0.46$) from the Gipuzkoa ($n = 335$) and Sabadell ($n = 324$) cohorts of the INMA project. The mean HCC was 10.69 pg/mg ($SD = 11.71$). Regarding prevalence of bullying, 8% were considered victims of bullying, 1.7% were bullies and another 1.7% were classified as bully/victims.

An initial analysis was conducted to assess differences between the cohorts. The only statistically significant differences were children's age and risky decisions score. Specifically, participants from Sabadell were older, attended more to public school and made less risky choices, what means that they had better executive function.

Sex differences

Our results did not show statistically significant differences by sex in hair cortisol. Regarding predictor variables, differences were observed in executive function, school perception, and academic achievement. On average, boys made more risky decisions what means, poorer executive function. They also have more problems with peers, worse perceptions of the school environment and were more involved in bullying as a bully/victim (Table 1).

Table 1. Differences in the variables by sex.

Variable		Female	Male	Statistic
Cortisol (pg/mg)		2.20 (0.47)	2.23 (0.44)	$t(657) = -1.014$; $p = .311$
Number of risky decisions (Cups Task)		29.57 (9.72)	31.75 (8.01)	$t(640.41) = -3.141$; $p = .002$
Problems with peers (SDQ)		0.67 (0.74)	0.78 (0.77)	$t(657) = -1.89$; $p = .059$
School environment (Kidscreen-27)		1.68 (0.40)	1.58 (0.43)	$t(657) = 3.091$; $p = .002$
Academic skills		4.38 (0.86)	4.17 (0.89)	$t(333) = 2.221$; $p = .028$
Victim total (OBVQ)	Not involved	356 (58.7)	250 (41.3)	$Chi(1) = 0.001$; $p = .971$
	Frequent	31 (58.5)	22 (41.5)	
Bully total (OBVQ)	Not involved	378 (58.4)	269 (41.6)	$Chi(2) = 0.913$; $p = .339$
	Frequent	8 (72.7)	3 (27.3)	
Bully_victim total (OBVQ)	Not involved	383 (59.2)	264 (40.8)	$Chi(2) = 4.546$; $p = .033$
	Frequent	3 (27.3)	8 (72.7)	

M = mean; SD = Standard Deviation; F = frequency.

Table 2. Bivariate associations between predictor variables and hair cortisol concentrations.

Variable		M (SD)	Statistic
Cohort	Gipuzkoa	2.24 (0.48)	$t(657) = 1.42; p = .156$
	Sabadell	2.19 (0.43)	
Age		10.95 (0.46)	$r = -0.032; p = .41$
Sex	Girl	2.20 (0.47)	$t(657) = -1.01; p = .311$
	Boy	2.23 (0.44)	
Problems with peers (SDQ)			$r = 0.003; p = .941$
School environment (Kidscreen-27)			$r = -0.036; p = .362$
Number of risky decisions (Cups Task)			$r = 0.112; p = .004$
Academic skills		4.29 (0.88)	$r = -0.047; p = .393$
Victim (OBVQ)	Not involved	2.21 (0.45)	$t(657) = 0.145; p = 0.885$
	Victim	2.20 (0.52)	
	Bully	2.15 (0.58)	
Bully (OBVQ)	Not involved	2.21 (0.45)	$t(656) = 0.420; p = .675$
	Bully	2.15 (0.58)	
Bully/victim (OBVQ)	Not involved	2.21 (0.46)	$t(656) = -1.970; p = .049$
	Bully/Victim	2.48 (0.42)	

M = mean; SD = Standard Deviation; F = frequency.

Bivariate findings

The examination of bivariate relationships showed that two of the variables included in the model was positively associated with HCC: the number of risky decisions (measured with Cups Task) and preadolescents' involvement as a bully/victim. On the one hand, a higher number of risky decisions, which means poorer executive function was related to higher HCC. On the other hand, those preadolescents involved in bullying as a bully/victim showed higher HCC (Table 2).

Structural equation model for victims

The results of the model testing the way HCC changes depending on involvement in bullying as a victim and other individual and school-related factors are shown in Figure 2. The final structural equation model showed a good fit between the model and data ($\chi^2(3) = 5.876; p = .118; CFI = 0.912; RMSEA = 0.038; p = .596$). Results indicated that only one of the studied variables was associated with HCC, specifically, higher HCC was associated with taking more risky decisions, which means poorer executive function ($b = 0.113; p = .004$). Besides, in this model, we observed associations between some predictor variables: having problems with peers ($b = 0.130; p = .001$) was related to being more involved as a victim, while perceiving a good environment was related negatively to be a victim ($b = -0.132; p = .001$).

Structural equation model for bullies

The results of the model testing the way in which HCC changes depending on preadolescents' involvement in bullying as a bully and other individual factors related to school are shown in Figure 3. The final structural equation model showed a good fit between the model and data ($\chi^2(1) = 1.151; p = .283; CFI = 0.900; RMSEA = 0.015; p = .592$). Results indicate that only one of the studied associations was statistically significant, higher HCC were related to a higher number of risky choices ($b = 0.112; p = .004$).

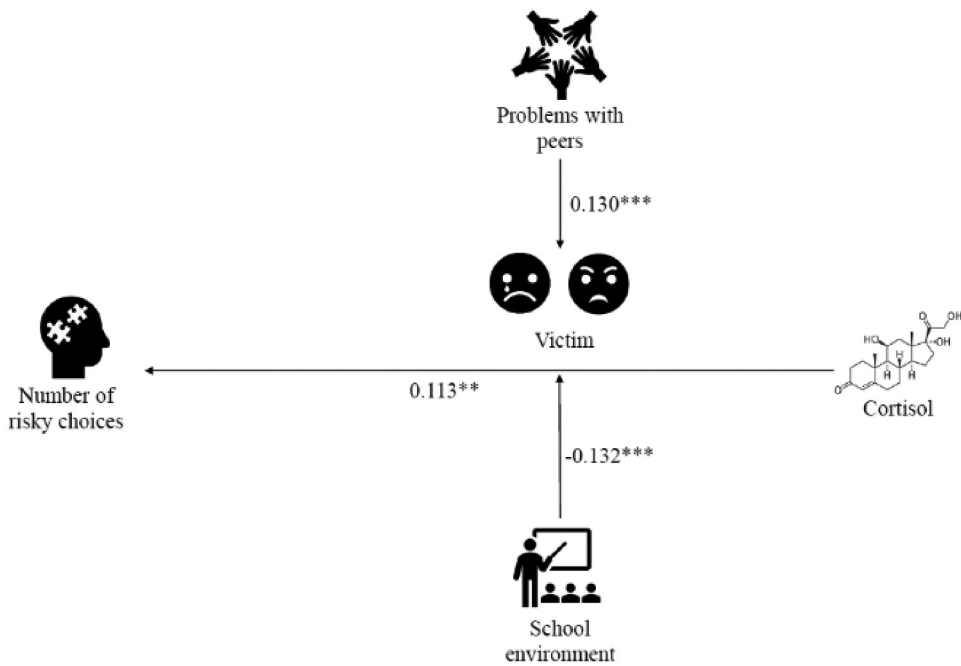


Figure 2. Final structural equation model taking into account victim role. Numbers are the standardized coefficients of the corresponding relationship. * $p < .05$; ** $p < .01$; *** $p < .001$, $p < .10$.

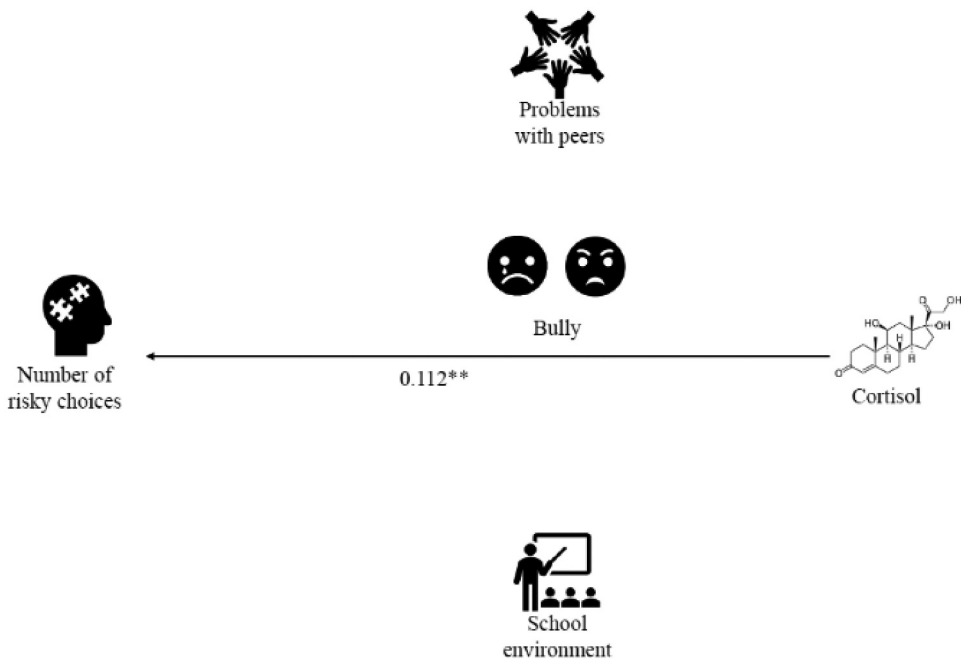


Figure 3. Final structural equation model taking into account bully role. Numbers are the standardized coefficients of the corresponding relationship. * $p < .05$; ** $p < .01$; *** $p < .001$, $p < .10$.

Structural equation model for bully/victim

The results of a model that test how HCC is affected by your involvement in bullying as a bully/victim and other school-related factors are shown in Figure 4. The final structural equation model showed a good fit between the model and data ($\chi^2(1) = 1.153$; $p = .283$; CFI = 0.978; RMSEA = 0.015; $p = .592$). Results indicate that only one of the studied variables seems to be related to hair cortisol levels, showing a marginally significant association. Particularly, being more involved as a bully/victim was associated with higher hair cortisol levels ($b = 0.075$; $p = .056$). Moreover, we observe that higher cortisol levels were related to a higher number of risky choices ($b = 0.112$; $p = .004$), which means that having higher cortisol levels was associated with lower inhibitory control. Lastly, we found that reporting a worse school environment showed a marginally significant association with being involved in bullying as a bully/victim ($b = -0.068$; $p = .079$).

Sensitivity analysis

In order to analyze the effect of academic achievement, a sensitivity analyses was conducted, only for participants from Gipuzkoa. The results of the model testing the way HCC changes in response of involvement in bullying as a victim and other individual and school-related factors showed good fit between the model and the data ($\chi^2(2) = 5.991$; $p = .200$; CFI = 0.916; RMSEA = 0.039; $p = .543$). The results showed that HCC was related to making more risky decisions, what means having poorer executive function ($b = 0.105$; $p = .056$). A worse a perception of school environment ($b = -0.108$; $p = .045$) and having more problems with peers ($b = 0.119$; $p = .028$) were related to being a victim.

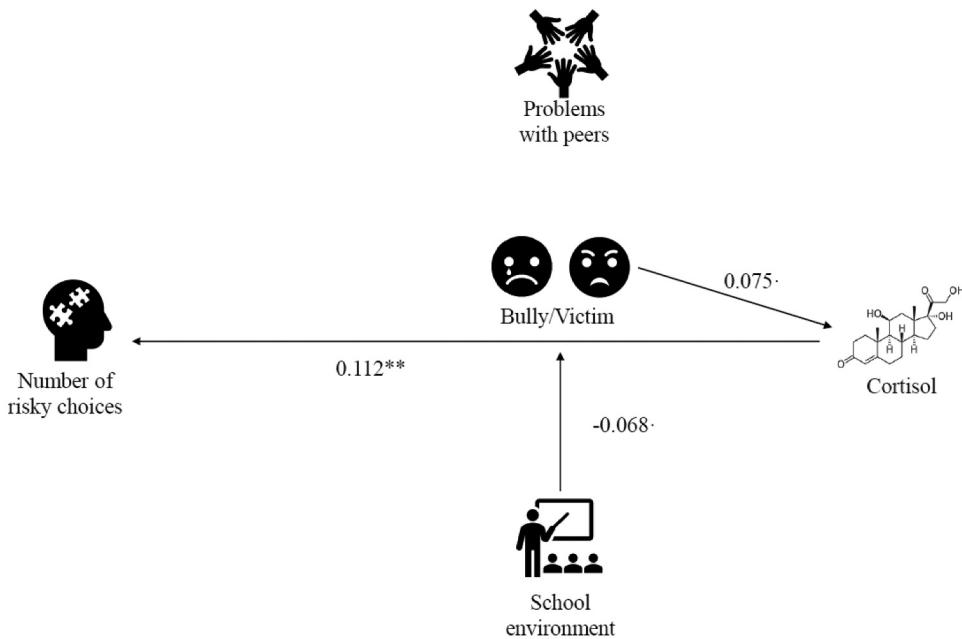


Figure 4. Final structural equation model taking into account bully/victim role. Numbers are the standardized coefficients of the corresponding relationship. *= $p < .05$; **= $p < .01$; ***= $p < .001$; $p < .10$.

Apart from these associations, this model showed that being involved as a victim ($b = -0.148$; $p = .006$) and having peer problems ($b = -0.121$; $p = .024$) was related to having lower academic achievement. Moreover, taking more risky decisions, that is, having poorer executive function, was related to having poorer academic achievement ($b = -0.093$; $p = .082$). Finally, the models for bullies and for bully/victims did not fit the data.

Discussion

The main objective of this work was to study the influence that school environment in general and bullying, in particular, have on HCC, examining the effect of executive function and sex. Regarding the association of the school-related stressors and HCC, we only found a trend-level association: being involved as a bully/victim was positively related to higher HCC. To the best of our knowledge, this is the first study analyzing the association of different roles that children may take in bullying with HCC. As far as we know only two previous study has examined the association between bullying and cortisol levels measured hair samples. One study found that although cumulative psychosocial adversities were associated with cortisol levels, peer victimization was not statistically significant (Ouellet-Morin et al., 2021). Other study concluded that HCC interacted with sex, finding that boys exposed to high victimization had higher HCC (Ouellet-Morin et al., 2021). However, the vast majority of studies investigating bullying and cortisol levels have used saliva samples and produced inconsistent results. Our results goes in line of what González-Cabrera et al. (2017) found in their study that HPA axis was over activated in cyberbully/victims. The heterogeneity of results in the studies examining the association between bullying and cortisol may be due to measurement of cortisol and bullying, research design, or sampling. But the physiology of the stress response may also explain differences in cortisol levels. According to previous research, stressors can induce a hyper- or hypo-response to the HPA axis. Different environments and experiences may not trigger the same response because they are not equally threatening. In response to recent or short-term stressors, the physiological stress response is often hyperactive; however, as time passes and the stressor becomes chronic and when this is temporary distant or is no longer present, the cortisol levels tend to lower (Liu & Doan, 2019; Miller et al., 2007). These findings are consistent with the hypothesis that when chronic stress first begins there is an activation of the axis, which result in elevated concentrations of cortisol and as time passes the activity diminished and cortisol levels are below normal. This hypothesis can explain the association found in the present study. We observed a positive correlation between bullying/victimization and having higher levels of HCC. This could be due to the fact that the time when the source of stress, i.e., bullying, is relatively close to the date when the hair was collected or even still present.

On the other hand, none of the other studied school-related variables, such as problems with peers, school environment perception, or academic achievement were related to HCC. Previous literature found that, students in non-supportive classes and with conflictive relationships with teachers and peers showed a flattered response of cortisol (Ahnert et al., 2012; Bai et al., 2017), whereas spending more

time than usual doing school homework was linked to more cortisol secretion on the day (McHale et al., 2012). However, a recent review concluded that the published research provided inconsistent and limited evidence of the association between social adversity and hair cortisol in children (Bryson et al., 2021).

We also studied the effect that risk seeking as indicator of executive function could have due to its relationship with the stressors and also with cortisol levels. We found a statistically significant association between high HCC and a greater number of risky decisions, and therefore, with poorer executive function. Although acute stress has a significant impact on executive functions, the role that chronic stress has on executive functions is not well understood. As far as we are aware, only three studies have examined the association between HCC and executive functions, and of those, only one previous study analyzed this association in children which found that a negative relationship between HCC and working memory even though it was not statistically significant (Pyle Hennessey et al., 2020).

Finally, the sensitivity analysis showed that, when added information about academic achievement, we only obtained a valid model for victims. Although all the previous associations were still present, no association was observed between academic achievement and HCC. Previous literature showed that even if academic problems were related to having higher cortisol wake-up responses, there was no association between academic problems and the diurnal cortisol profile (Bai et al., 2017). Additionally, in this model we observed that having poorer executive functions, having more peer problems and being involved as a victim was related to having worse academic achievement. Our results are in line with previous studies which have shown that executive functions, peer problems and bullying experiences are associated with school-related difficulties, such as academic achievement or school attendance (Gomes et al., 2020; Pascual et al., 2019).

This study is not, however, without limitations. First, this study has a cross-sectional design. Second, Bullying was assessed using only a self-report questionnaire. The use of peer nominations or a teacher questionnaire may be considered for future studies. Additionally, the scale we used to assess bullying is composed of two subscales assessing victimization and perpetration specifically, and the bully/victim category was created by the researchers of this work. Third, we did not take into account other possible confounding school-related factors, such as relationships with teachers or time preadolescents spent doing their homework, and we neither assessed perceived stress or other social factors that could be having influence on HCC out of the school context. Finally, it is also possible that the sample is a limitation. Some participants in our study were excluded because they did not have information about the variables of interest, as we explained earlier. When comparing the differences in the study variables between participants included and not included, we found that those who were excluded experienced more peer difficulties, the school environment was perceived as less friendly, academic scores were lower, and more people experienced bullying. These differences may be showing that perhaps the participants included in our study (those who had complete information) score better on the variables related to the school context and bullying and therefore we may be missing the effect of these variables on stress levels.

Implications and contributions

Our study allows to gain a deeper understanding of the relationship between school stressors and neurophysiological function. Besides, this study offers results that may be relevant for designing programs for prevention and intervention. These could modify individual physiological responses to stress and reduce the effects of stress on the health.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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ORCID

Izaro Babarro  <http://orcid.org/0000-0002-3626-7036>

Jesus Ibarluzea  <http://orcid.org/0000-0002-6933-5373>

Monica Guxens  <http://orcid.org/0000-0002-8624-0333>

Ainara Andiarena  <http://orcid.org/0000-0002-8535-7118>

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Appendix A. Differences in the variables between included and non-included participants

Table A1. Differences in the variables between included and non-included participants.

Variable	Included	Non-included	Statistic
Cortisol (pg/mg)	2.21 (0.46)	2.22 (0.42)	$t(697) = 0.112; p = .911$
Number of risky decisions (Cups Task)	30.47 (9.11)	30.61 (9.55)	$t(853) = -0.180; p = .857$
Problems with peers (SDQ)	0.72 (0.75)	0.94 (0.79)	$t(859) = -3.515; p = .000$
School environment (Kidscreen-27)	1.64 (0.42)	1.54 (0.41)	$t(843) = -2.834; p = .005$
Academic achievement	4.29 (0.88)	3.96 (1.02)	$t(376) = 2.277; p = .023$
Victim total (OBVQ)	604 (91.9)	171 (86.4)	$Chi(1) = 5.56; p = .018$
	Not involved	53 (8.1)	
	Frequent	27 (13.6)	
Bully total (OBVQ)	Not involved	197 (99.0)	$Chi(2) = 0.457; p = .499$
	Frequent	2 (1)	
Bully_victim total (OBVQ)	Not involved	264 (40.8)	$Chi(2) = 0.021; p = .886$
	Frequent	3 (1.5)	

M = mean; SD = Standard Deviation; F = frequency.