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Do prepubertal hormones, 2D:4D index and psychosocial context jointly explain 11-year-old preadolescents' involvement in bullying?

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ABSTRACT

Background: Bullying is a type of aggressive behavior that occurs repeatedly and intentionally in school environments and where there is a power imbalance. The main objective of this study was to analyze the association that hormones and the psychosocial context jointly have with bullying behavior.
Method: Participants were 302 11-year-old preadolescents from the Gipuzkoan cohort of the INMA Project. Bullying was assessed using the Olweus Bully/victim Questionnaire. Prenatal sexual hormones were assessed by calculating 2D:4D ratio and in order to measure prepubertal testosterone and cortisol levels saliva samples were collected within a week of each other. Additionally, various psychosocial factors were evaluated: executive function, family context, school environment and social context. To analyze our complex hypothesis, six meta-models were tested using structural equation modeling.
Results: In relation to victims, results showed that victimization was related to worse school environment' perception in boys, and higher stress and conflict in the family in girls. In the case of their involvement in bullying as a bully, lower salivary cortisol levels, worse school environment' perception and lower peers and social support was related to being more frequently involved as a bully in boys, while having more family stress and conflict was related with being a bully in girls.
Conclusions: This approach makes it possible not only to explore the different biological and psychosocial factors affect bullying behavior, but also to explore associations between the predictor variables.

In general terms, human aggression is defined as any type of behavior intended to harm another person (Bushman & Anderson, 2001) and sometimes it is used in order to obtain status in the social group. One type of aggressive behavior that occurs typically during childhood and adolescence and tends to emerge in school environments is bullying, defined as a repetitive and intentional use of coercion, force, hurtful teasing or threats, to abuse, aggressively dominate or intimidate that tends to emerge in school environments and where there is a real or perceived power imbalance (Olweus, 1996).

In a survey carried out with children from different countries of Europe, the USA and Canada, the World Health Organization (WHO) concluded that prevalence of bullying victimization ranged between 2% and 32%, while perpetration varied from 1% to 26% (Currie et al., 2012). Data from a systematic review showed that in Spain prevalence of bullying victimization ranged between 2.2% and 29% (García-García et al., 2017) and particularly in the Basque country, a study showed that 13.2% of the participants were victims of bullying, 1.65% bullies and 2% were bullies/victims (Machimbarrena & Garaigordobil, 2018). Due to the high prevalence and the impact that bullying may have in peoples' life, it is now recognized as a public health problem (Craig & Harel, 2004).

Recent research indicates that the origin of aggressive behavior is multicausal and that biologic, social and cultural factors are continually interacting in it (Popova et al., 2018). Regarding psychosocial factors, some reviews and meta-analyses have found that individual-, school-, family- and community-related factors may act as risk or protective

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factors for bullying (Cook et al., 2010; Kljakovic & Hunt, 2016; Saarento et al., 2015; Suárez-García et al., 2019; Zych et al., 2019). Evidence has shown that those having a neurodevelopmental disorder, poor cognitive or executive function or emotional or behavioral problems are more likely to engage in bullying situations (Kljakovic & Hunt, 2016; Suárez-García et al., 2019). Certain family characteristics as low socio-economic level, punitive parenting style and family conflict or violence have also been associated with children having higher rates of bullying involvement (Cook et al., 2010; Suárez-García et al., 2019; Zych et al., 2019). Finally, having a good school environment and a supportive social environment reduces the risk of being involved in bullying situations (Cook et al., 2010; Kljakovic & Hunt, 2016; Suárez-García et al., 2019; Zych et al., 2019).

As regards the biological factors that influence behavior, previous research have focused on the role that hormones, specifically testosterone and cortisol, play in two developmental periods. The prenatal and the pubertal are hormone-sensitive periods for the development of the nervous system in which hormones trigger structural changes that may influence behavior (Auyeung et al., 2013; Schulz et al., 2009; Vigil et al., 2016). Decades of behavioral endocrinology have demonstrated an association of the activity of the hypothalamic-pituitary adrenal axis (HPA) and the hypothalamic-pituitary-gonadal axis (HPG) with the behavior that underlies social interactions and relationships. A large literature has indicate that aggressive behavior is influenced by high levels of testosterone and low levels of cortisol (Popma et al., 2007; Terburg et al., 2009).

Testosterone is important for its role in sexual differentiation and reproductive activity in animals and humans; and it has been linked to aggression (Teisl, 2008). The role of testosterone during prenatal development is vitally important for early sexual differentiation of the brain (Romeo et al., 2002). One way to study prenatal androgen levels in general population using a noninvasive technique is using the 2D:4D ratio. The 2D:4D ratio is calculated by dividing the length of the second (index) finger by that of the fourth (ring) finger and it becomes relatively stable in early life. This ratio has been considered a prenatal indicator of sex hormones (Mikac et al., 2016), lower ratios reflecting higher androgens levels *in utero*.

Lutchmaya et al. (2004) first demonstrated a relationship between fetal testosterone and estradiol concentrations in amniotic fluid and the 2D:4D ratio, the association reaching significance when ratios were measured in the right hand. Years later, Ventura et al. (2013) also found an association between amniotic testosterone and digit ratio, being only statistically significant for girls. 2D:4D ratio has also been studied in relation to congenital adrenal hyperplasia (CAH) as this condition provides an opportunity to examine the effects of elevated androgen exposure during gestation. Hönekopp and Watson (2010) in their meta-analysis found that those subjects with CAH had lower ratios than their normal development counterparts. Recently, there has been some replication of these findings, but, the effect size was smaller than that found in previous meta-analyses (Richards et al., 2020). Finally, some other researchers failed to found association between the 2D:4D ratio and prenatal levels of sex hormones (Hollier et al., 2015; Nave et al., 2021; Richards et al., 2021). Despite the mixed results, the 2D:4D ratio is still used as an indicator of prenatal androgen exposure and it has been studied in relation to different behaviors, including aggressiveness. One meta-analysis found that the average effect size between the ratio and aggressive behavior was really small and only significant for males (Hönekopp & Watson, 2011). Whereas other authors did not find association between these prenatal testosterone and aggressive behavior (Hilgard et al., 2019; Joyner & Beaver, 2021).

Puberty is another period of important development, characterized by changes in individuals' biology, psychology and behavior. From a neurobiological perspective, it is a sensitive periods when the biological system develops and the vulnerability to stressful events increases (Lupien et al., 2009) and from a psychosocial perspective, is a period of interpersonal relationships where peer dynamics are of particular importance (Rodkin & Ryan, 2011). Pubertal testosterone has been studied in relation to aggressive behavior, showing mixed results. Some authors have found that higher testosterone levels are associated with higher levels of aggressiveness (Grotzinger et al., 2018; Sánchez-Martín et al., 2011), while others have found no association (Popova et al., 2018). Regarding the association between testosterone and bullying, one study found that in a sample of 12 years old preadolescents, bullied girls produced less testosterone than their non-bullied counterparts whereas bullied boys produced more testosterone than their non-bullied counterparts (Vaillancourt et al., 2009).

Other hormone studied in relation to aggressive behavior is cortisol, the final product of the HPA axis and which regulates the acute stress response (Teisl, 2008). A recent systematic review concluded that victimization was consistently associated with cortisol (Kliewer et al., 2019). Most of the studies found that children involved in bullying or cyberbullying showed lower levels or blunted pattern of cortisol (González-Cabrera et al., 2017; Östberg et al., 2018; Peters et al., 2011), however, some studies did not found any association (Vaillancourt & Sunderani, 2011; Williams et al., 2017). Further, Vaillancourt (2009) concluded that this association was sex dependent, being occasionally bullied was associated with higher cortisol levels in boys, but with lower cortisol levels in girls.

Although testosterone and cortisol have independently shown associations with human behavior, the HPA and HPG axes have shown to be related. The dual-hormone hypothesis posits that testosterone is related to aggressive behavior when cortisol levels are low (Dabbs et al., 1991; Mehta & Josephs, 2010). A review and a meta-analysis concluded that the association between testosterone and cortisol on status-relevant behavior was statistically significant, but that its effect size was small (Dekkers et al., 2019; Grebe et al., 2019).

Apart from being puberty a period of many biological changes, early adolescence is a period in which children develop new interests and characteristics and it is expected that they develop new friendships (Georgiou, Ionnou, & Stavrinides, 2017). In general, peer dynamics are positive during this period but sometimes children and adolescents become involved in bullying situations. Considering bullying is a form of aggression whose peak occurs between the ages of 11 and 13 (Eslea & Rees, 2001), and that this coincides with first stages of puberty (Vigil et al., 2016), we are interested in analyzing testosterone and cortisol levels in relation to bullying, taking into account other psychological factors.

Therefore the main objectives of the present study were (1) to establish an association between prenatal and pubertal hormone levels (testosterone and cortisol) with the different roles that preadolescents take in bullying (victim, bully, bully/victim); (2) to study the relationship between the different psychosocial predictors (impulse control, family context, peer support, school environment) and the involvement that preadolescents have in bullying and (3) to explore the relationships that the different predictors show between each other.

1. Method

1.1. Participants

The study participants were 302 preadolescents from the Gipuzkoan (Basque Country) cohort of the INMA Project (Children and the Environment, www.proyectoinma.org). This project gathers data on children and their families in seven cohorts across Spain with the goal of analyzing the association between early environmental exposure and children's health and development (Guxens et al., 2012). The participants' mothers were informed about the INMA project and 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. The ethics committees of the hospitals in the region involved approved the project and informant consent was obtained for all participants in each of the phases. In this study, we used

data from the 8-year and 11-year follow-up phases. In the 8-year follow-up we visited 397 families and 379 in the 11-year follow-up. Of these, 77 were excluded due to missing data for one or more important variables. Complete information for all variables included in the meta-model was available for 302 cases (144 boys and 158 girls).

1.2. Instruments

1.2.1. Bullying

Bullying was assessed using a short version of the Olweus Bully Victim Questionnaire (OBVQ) (Olweus, 1996) at the 11-year follow-up. In this study, we used a version consisting of a standardized definition of bullying and 16 questions to which preadolescents were asked to respond thinking of the last 2 months. The first eight items refer to experiences of victimization and the second eight to the context of bullying others. Items are rated on a 5-point Likert scale (0 "it hasn't happened [to me]", 4 "it happens [to me] several times a week").

Following the recommendations of Solberg and Olweus (2003) those preadolescents who scored two or more in the Likert scale at least in one of the 8 questions of the first subscale were identified as victims, and those who scored higher than two in at least one of the 8 questions that conformed the second subscale, were identified as bullies. A third role (bully/victim) was created for those preadolescents who presented scores higher than two in both subscales. After being preadolescents classified into one of these roles, three categories were created based on bullying frequency: never involved, occasionally involved, and frequently involved (Vaillancourt et al., 2008). The OBVQ showed adequate internal consistency in the sample of the INMA project: $\alpha = 0.81$ for the whole questionnaire, $\alpha = 0.81$ for the victim scale and $\alpha = 0.67$ for the bully scale.

1.2.2. 2D:4D ratio

We measured the 2D:4D ratio at the children's school at the time of their 11-year follow-up. One trained researcher obtained images of all the preadolescents' hands following an ad hoc protocol based on the recommendations given by Mikac et al. (2016) and using a portable scanner (Epson Perfection V39). Once the images were obtained, these were measured using the AutoMetric computer program. We collected data of both right and left 2D:4D index and the correlation for data of both hands was substantial and statistically significant ($r = 0.652$; $p = 0.0001$) (Appendix 1). In light of this correlation, we only used data from one hand based on a meta-analysis of Hönekop & Watson (2010), which indicated that sex differences in 2D:4D were greater in the right hand than in the left.

1.2.3. Prepubertal hormone levels: Testosterone and Cortisol

At the 11-year follow-up, to assess testosterone and cortisol levels, two saliva samples were collected from each child within a week of each other and between 8 and 10 am, in order to avoid changes in hormone levels due to diurnal fluctuations. Preadolescents and their parents were asked to collect these saliva samples at home; they received a study pack with standardized written instructions and a kit with clean containers for collecting the samples by passive drool. Preadolescents were asked to avoid eating, drinking or brushing their teeth for 1 h prior to sample collection and not to attend the dentist in the 24 h before taking the samples.

Samples were analyzed in the Psychobiology Laboratory (Faculty of Psychology - University of the Basque Country). Saliva samples were centrifuged at 3000 rpm for 15 min to remove mucins and were stored at − 80ºC until analysis. All samples were assayed in duplicate using an enzyme immunoassay kit (Salimetrics, State College, PA, USA). Plates were read at 450 nm for both hormones using a Synergy™ HT plate reader (Bio-Tek Instruments, Inc., Winooski, VE, USA). The average inter-assay coefficient of variation (CV) was less than 5% for both hormones measured, and the intra-assay CV was less than 10% for cortisol and 12% for testosterone levels, relative to control samples. Samples with a CV> 10% were reanalyzed. Samples were excluded if the hormone levels were below the limit of detection or were above or below three standard deviations from the mean. The sensitivity of the kit was < 0.007 μg/dL for cortisol and < 1.0 pg/mL for testosterone. For the statistical analyses, the mean of both measures was calculated for each hormone.

1.2.4. Risky decision making: cups task roulette version

At the 11-year follow-up preadolescents' decision making was evaluated using this computer task, an adapted version of the Cups Task (Levin et al., 2007). It consists of 54 trials that assess decision making by observing the number of risky choices a child makes. In this task, the participant is presented with two wheels divided into segments of equal size and each associated with an amount of money. On each trial, the participant is asked to choose which wheel to spin, in order to gain or avoid losing money. After the response, the wheel selected is spun for 2 s, then ends on the amount of money to be won or lost. One wheel is riskless: each segment has the same small amount of money associated with it ($ ± 1.00). The other wheel represents a risky choice: only one segment has an amount associated with it ($ ± 2.00, $ ± 3.00, or $ ± 5.00) while the other segments have $0.00. Both wheels have the same number of segments, which vary between 2, 3 and 5; thus, when selecting the risky wheel, chances were either 50%, 33%, or 20% that the wheel will stop on the segment associated with an amount of money. Half trials are gains trials (i.e., with a positive amount of money), the other half are loss trials (with a negative amount of money). The entire task comprises 54 trials. In each condition (gain and loss), there is an equal number of risk- advantageous, risk-disadvantageous, and equal expected value (EV) trials. For this study, we took into account the overall score of total number of risky choices made by each child, a higher number indicating poorer executive function.

1.2.5. Quality of family interactions: Haezi-Etxadi Family Assessment Scale (HEFAS) (Barreto-Zarza et al., 2021)

At the 8-year follow-up parents completed this instrument which assess the quality of the family context. It consists of 85 items divided into 5 subscales, namely: Promotion of cognitive and linguistic development, Promotion of socio-emotional development, Organization of the physical environment and social context, Parental stress and conflict, and parental profile fostering child development. A higher score on the scale indicates a high quality of interactions in a family context. In this study, we only used the parental stress and conflict subscale, which we considered the most useful among the five subscales. One reason is that previous literature has demonstrated that family conflict or violence is associated with bullying behavior. Additionally, the family context can cause stress and, as a result, lead to elevated cortisol levels in children. The psychometric properties of this subscale are adequate, the internal consistency for each of the five subscales, being $\alpha = 0.75$ for the stress and conflict subscale used in this study.

1.2.6. Social context: Kidscreen-27 questionnaire (Kidscreen-27)

At the 11-years follow-up preadolescents completed this self-reported questionnaire which assesses health-related quality of life in children and adolescents. The scale consists of 27 items divided into 5 subscales, assessing physical well-being, psychological well-being, peers and social support, autonomy and parent relations, and school environment. Each item is rated on a 5-point Likert scale (1 "never/not at all"– 5 "always/extremely"). In all cases, a higher score means a better quality of life in the dimension measured. The Spanish version of the Kidscreen-27 was validated showing adequate psychometric properties (Quintero et al., 2011). The questionnaire showed acceptable internal consistency in the present sample for each of the subscales ($\alpha > 0.70$). For this study, we used two of the five subscales, namely, peers and social support and school environment.

Note: the resulting dataset is available for interested researchers, upon reasonable request to the corresponding author.

1.3. Data analysis

R software v. 4.0.0 (R Core Team, 2020) was used to conduct all statistical analyses. We used structural equation modeling (SEM) to test specified model based in our theoretical framework (Fig. 1) as this type of modeling allows the analysis of complex causal hypotheses (Duncan, 1975; Heise, 1975). SEM assumes linearity in the relationships between continuous variables and Gaussian error terms. For this reason, as suggested by Tukey (1977), we square root-transformed pubertal testosterone and cortisol levels. This was sufficient to ensure the linearity of relationships and, therefore, the suitability of the global estimation method.

After making descriptive analysis, we carried out bivariate analysis. On the one hand, independent t-test was used to assess differences by sex. On the other hand, Pearson correlation was used to test the association between the independent variables, as all were continuous variables. Kendall rank correlation was used to test the association between the independent variables and the dependent ones, because these last were ordinal in nature. Finally, we carried out the structural equation modeling and obtained global estimates via the maximum likelihood method. Specifically, the said metamodel was fitted and tested using the sem() function in the R package *lavaan*, with the <ordered> argument in this function to specify that the tested responses are ordinal. Data-to-model consistency is evaluated using a chi-square test and two goodness-of-fit measures were used: the comparative fit index (CFI) and the root mean square error of approximation (RMSEA) (Kenny & Kaniskan, 2015). The final models were accepted only when all three of the following conditions were met: chi-square test p-value > 0.05, CFI > 0.95, RMSEA p-value > 0.05.

2. Results

2.1. Bullying prevalence

The descriptive analyses showed that in terms of severity, the prevalence of bullying was as follows: 9.6% were considered victims of bullying (girls: 8.9%; boys: 10.4%) as they were frequently bullied, and a further 13.9% of the participants were occasionally bullied (girls: 17.1%; boys: 10.4%). On the other hand, 1.7% were classified as frequent bullies (girls: 2.5%; boys: 0.7%) and 7.6% as occasional bullies (girls: 7%; boys: 8.3%). Lastly, 1.7% of the sample were considered frequent bully/victims (girls: 1.3%; boys: 2.1%) and 8.3% occasional bully/victims (girls: 4.4%; boys: 12.5%).

2.2. Descriptive analysis

Table 1 reports the descriptive analysis of the independent variables and sex differences in each. Regarding hormone levels, results showed that boys had lower 2D:4D ratios than girls, indicating higher prenatal exposure to androgen levels. On the other hand, girls showed higher prepubertal levels of cortisol. Considering individual factors, boys scored higher in making risky choices. Finally, regarding social factors, girls scored higher in school environment than boys. No sex differences were found in peers and social support, or family stress and conflict.

2.3. Bivariate findings

Pearson correlations were performed to explore the relationship between the predictor variables (Table 2), while Kendall rank correlation coefficient test was used to analyze the associations between each predictor and the dependent variables (Table 3). Concerning associations between the predictor variables, results showed that in boys, 2D:4D ratio was associated negatively with family stress and conflict scores, that is, higher prenatal androgens levels in boys were related to less parental stress and conflict. Moreover, in girls, 2D:4D ratio was positively associated with cortisol levels, that is, less prenatal androgen' exposure was related to higher prepubertal cortisol levels and prepubertal testosterone was negatively related to school environment. For both sexes, we found a positive association between salivary testosterone and salivary cortisol and a positive association between the two subscales of the Kidscreen-27: school environment and peers and social support.

Bivariate analysis between the predictor variables and the dependent variables showed that only one predictor was significantly associated with victim propensity in girls: family stress and conflict. In boys, school environment was the only significant predictor related to victimization. In the case of bully propensity, none of the predictors was significantly associated with the dependent variable in girls, but peers and social support was related to being a bully in boys. Lastly, in girls 2D:4D ratio and peers and social support were related with bully/victim propensity.

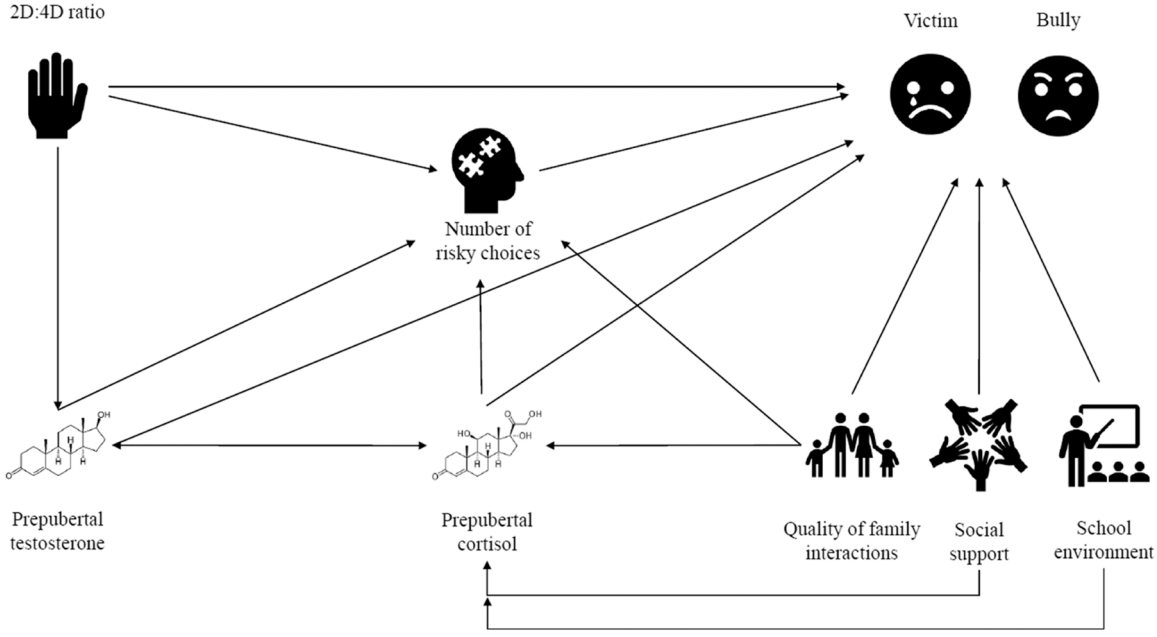

Fig. 1. Metamodel summarizing the hypothesized relationships among <biological> and <psychosocial> variables.

Table 1
Descriptive statistics of the seven quantitative independent variables for the whole sample, together with Welch two sample t-test for the difference between boys and girls.

Variable	Sex	*n*	Min	Max	Mean	SD	Difference	95% C.I. L. B.	95% C.I. U. B.	*t*-value	df	*p*-value	*Cohen's d*
2D:4D ratio	B	144	0.864	1.054	0.956	0.038	–	–	–	–	–	–	
	G	158	0.867	1.088	0.970	0.036	–	–	–	–	–	–	
	–	302	0.864	1.088	0.963	0.038	-0.01	-0.02	-0.01	-3.42	300.0	0.001	0.38
Salivary testosterone (pg/mL)	B	144	7.64	93.94	29.12	14.43	–	–	–	–	–	–	
	G	158	6.69	94.46	32.08	16.15	–	–	–	–	–	–	
	–	302	6.69	94.46	30.67	15.40	-0.25	-0.55	0.05	-1.62	300.0	0.106	0.19
Salivary cortisol (μg/dl)	B	144	0.06	0.90	0.28	0.13	–	–	–	–	–	–	
	G	158	0.05	0.90	0.33	0.16	–	–	–	–	–	–	
	–	302	0.05	0.90	0.31	0.15	-0.04	-0.07	-0.01	-2.54	300.0	0.011	0.34
Number of risky choices (Cups Task)	B	144	5.0	52.0	33.2	8.5	–	–	–	–	–	–	0.36
	G	158	10.0	51.0	29.9	9.6	–	–	–	–	–	–	
	–	302	5.0	52.0	31.48	9.2	3.29	1.23	5.35	3.29	300.0	0.002	
Peers and social support (KidScreen-27)	B	144	-0.05	4.23	2.52	1.10	–	–	–	–	–	–	
	G	158	-0.05	4.23	2.64	1.17	–	–	–	–	–	–	
	–	302	-0.05	4.23	2.58	1.13	-0.13	-0.37	0.14	-0.87	300.0	0.386	0.11
School environment (KidScreen-27)	B	144	-1.16	4.65	2.55	1.40	–	–	–	–	–	–	
	G	158	-0.54	4.65	2.91	1.32	–	–	–	–	–	–	
	–	302	-1.16	4.65	2.74	1.37	-0.36	0.16	-0.66	-2.26	300.0	0.024	0.26
Family stress and conflict (HEFAS 7–11)	B	144	44.44	98.61	77.47	9.14	–	–	–	–	–	–	
	G	158	55.56	98.61	76.85	9.72	–	–	–	–	–	–	
	–	302	44.44	98.61	77.15	9.44	0.61	-1.52	2.75	0.56	300.0	0.572	0.06

Note: B = boys; G = girls; Min = minimum; Max = maximum; SD = Standard Deviation; 95% CI LB = 95% confidence interval lower bound; 95% CI UB = 95% confidence interval upper bound; df = degrees of freedom.

Table 2
Associations between the six structural equation model quantitative variables considered in the structural equation metamodel.

Boys		Number of risky choices	Peers and social support	School environment	Family stress and conflict	Salivary testosterone	Salivary cortisol
	2D:4D ratio	0.104	0.055	0.039	**-0.312**	0.059	-0.076
	Number of risky choices		-0.059	-0.105	-0.018	0.067	0.130
	Peers and social support			**0.225**	-0.005	0.127	0.126
	School environment				-0.103	-0.064	-0.086
	Family stress and conflict					0.003	-0.030
	Salivary testosterone						**0.530**
Girls		Number of risky choices	Peers and social support	School environment	Family stress and conflict	Salivary testosterone	Salivary cortisol
	2D:4D ratio	-0.004	-0.065	-0.074	-0.049	0.101	**0.152***
	Number of risky choices		-0.016	-0.120	0.061	0.085	0.024
	Peers and social support			**0.410**	-0.046	-0.092	-0.107
	School environment				0.053	**-0.187***	-0.096
	Family stress and conflict					0.083	0.074
	Salivary testosterone						**0.498**

* Note:= $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3
Associations between the six structural equation model and quantitative variables considered in the structural equation metamodel and the dependent variables.

Boys		2D:4D ratio	Number of risky choices	Peers and social support	School environment	Family stress and conflict	Salivary testosterone	Salivary cortisol
	Victim propensity	0.097	-0.004	-0.071	**-0.146** *	-0.085	-0.048	-0.035
	Bully propensity	0.092	-0.058	**-0.174** *	-0.115	-0.043	-0.104	-0.105
	Bully/victim propensity	-0.023	-0.029	**-0.183** *	-0.126	-0.021	-0.065	-0.091
Girls		2D:4D ratio	Number of risky choices	Peers and social support	School environment	Family stress and conflict	Salivary testosterone	Salivary cortisol
	Victim propensity	-0.046	0.037	-0.074	-0.041	**-0.128** *	-0.007	-0.010
	Bully propensity	-0.046	-0.073	0.000	0.039	-0.100	-0.065	-0.067
	Bully/victim propensity	**0.129** *	0.044	**-0.178** *	**-0.205** * *	-0.052	0.050	0.116

Note: *= $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

While in boys, peers and social support was the only predictor associated with bully/victim propensity.

2.4. Structural equation models

We hypothesized that preadolescents' bullying involvement was related to biological factors (2D:4D ratio and prepubertal hormone levels) and their psychosocial context (i.e. decision making, quality of family interactions, peers and social support, school environment). When examining the relationship that hormone levels have with social behaviors, such as aggressive behavior, a number of individual differences may be of particular importance due to their relationship with both hormone levels and behavior. Executive functioning in general and decision making in particular, has shown correlation not only with hormone levels but also with various psychopathological conditions such as aggressive behavior.

With this objective and based on previous literature, we designed the following metamodel (Fig. 1).

The proposed metamodel is very ambitious because it was designed based on several empirical studies that have shown some associations between independent variables and aggressiveness in general, or involvement in bullying in particular. Nonetheless, to our knowledge, no previous study has analyzed the influence of biological and psychosocial factors on the roles that children take in bullying situations.

Structural equation model for victims (Fig. 2)**.** The results of the model testing boys' propensity to be a victim showed a good fit between the model and data (X^2 (5)= 5.018; $p = 0.414$; CFI= 1.00; RMSEA= 0.005; $p = 0.604$). Results indicate that a poor school environment' perception was associated with boys' victimization frequency (b=−0.25; $p = 0.035$), explaining 6.1% of the variance. Moreover, we observed that greater peers and social support was associated with higher levels of cortisol (b=0.18; $p = 0.045$) explaining 3.3% of salivary cortisol variance. Apart from these results, a positive correlation was found between prepubertal testosterone and cortisol ($r = 0.53$; $p = 0.001$).

The model for girls' propensity to be a victim (Fig. 3), showed a good fit between the model and the data (X^2 (3) = 0.997; p-value = 0.802; CFI = 1.00; RMSEA = 0.00; $p = 0.877$). Lower score in family stress and conflict scale, indicating lower quality of family context, was related with being more frequently involved as a victim in girls (b=−0.22; $p = 0.042$), explaining 4.7% of the variance. Moreover, results also suggested that higher salivary testosterone was related with worse perceived school environment (b=-0.19; $p = 0.007$), explaining 3.8% of the variance. Apart from these results, a positive correlation was found between prepubertal testosterone and cortisol ($r = 0.50$; $p = 0.001$).

2.4.1. Structural equation models for bullies

Results of the model testing boys' propensity to be a bully (Fig. 4) showed a good fit between the model and data for the final structural equation model (X^2 (3)= 3.518; $p = 0.318$; CFI= 0.988; RMSEA= 0.035; $p = 0.467$) and support the main idea that propensity to be a bully is associated with some of the studied biological and psychosocial variables. Lower salivary cortisol levels (b=−0.23; $p = 0.051$), worse perceived school environment (b=-0.22; $p = 0.120$), and less peers and social support (b=-0.30; $p = 0.048$) were associated with being more frequently involved as a bully, explaining a large amount of the variance (R^2=25.5). Results also suggest that peers and social support was associated with cortisol levels (b=0.18; $p = 0.045$) explaining 3.3% of the variance. Finally apart from these results, we found a positive correlation prepubertal testosterone and cortisol ($r = 0.53$; $p = 0.001$).

The results of the model testing girls' propensity to be a bully (Fig. 5), showed a good fit between the model and data for the final structural equation model (X^2 (7)= 6.140; $p = 0.499$; CFI= 1; RMSEA = 0.00; $p = 0.744$). Results showed that a worse quality of family interaction was associated with being more frequently involved as a bully (b=−0.22; $p = 0.040$), explaining 4.8% of the variance. Results also suggest 2D:4D ratio was positively related to prepubertal testosterone levels (b=0.15; p = 0.077) explaining 2.3% of the variance and that higher prepubertal testosterone levels were related to worse school environment' perception (b=-0.20; $p = 0.006$), explaining 3.9% of the variance. Apart from these results, a positive correlation was found between prepubertal testosterone and cortisol ($r = 0.51$; $p = 0.001$).

2.4.2. Structural equation models for bully/victims

In the case of the bully/victim role and for both boys and girls, we were unable to fit any valid model (all candidate models presented chi-square test p-value <0.05; CFI values <0.95, and RMSEA p-value

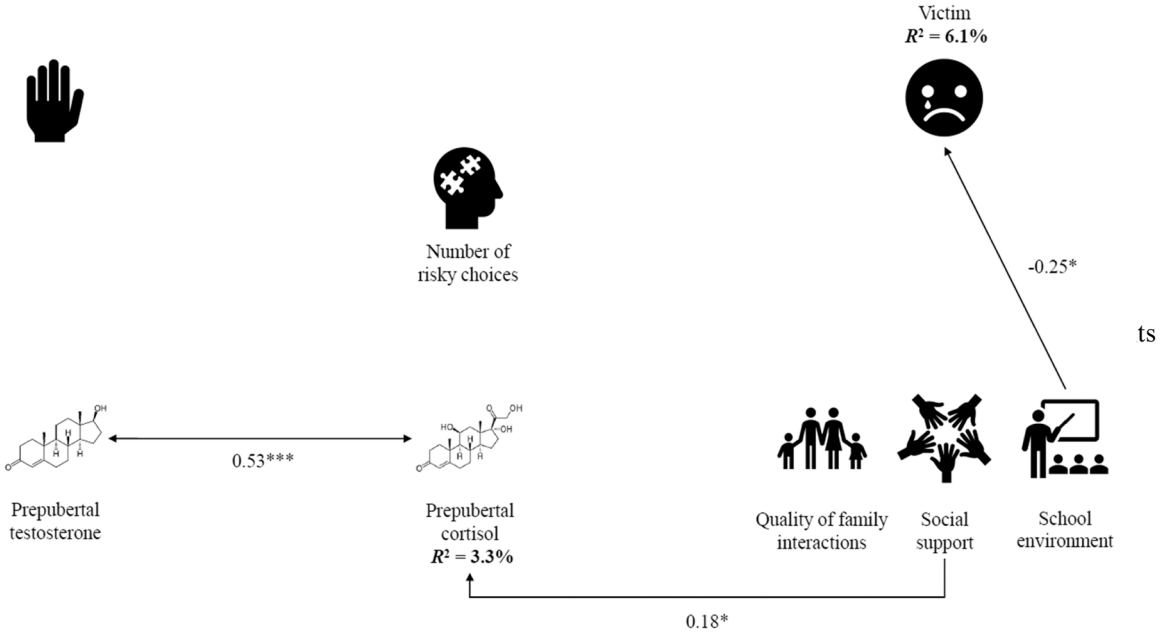

Fig. 2. Final structural equation model for boy's propensity to be a victim. Note: R^2 values indicate the percentage of variance explained. Numbers are the standardized coefficients of the corresponding relationship. *=p < *0.05;* **p < *0.01;* * **p < *0.001*.

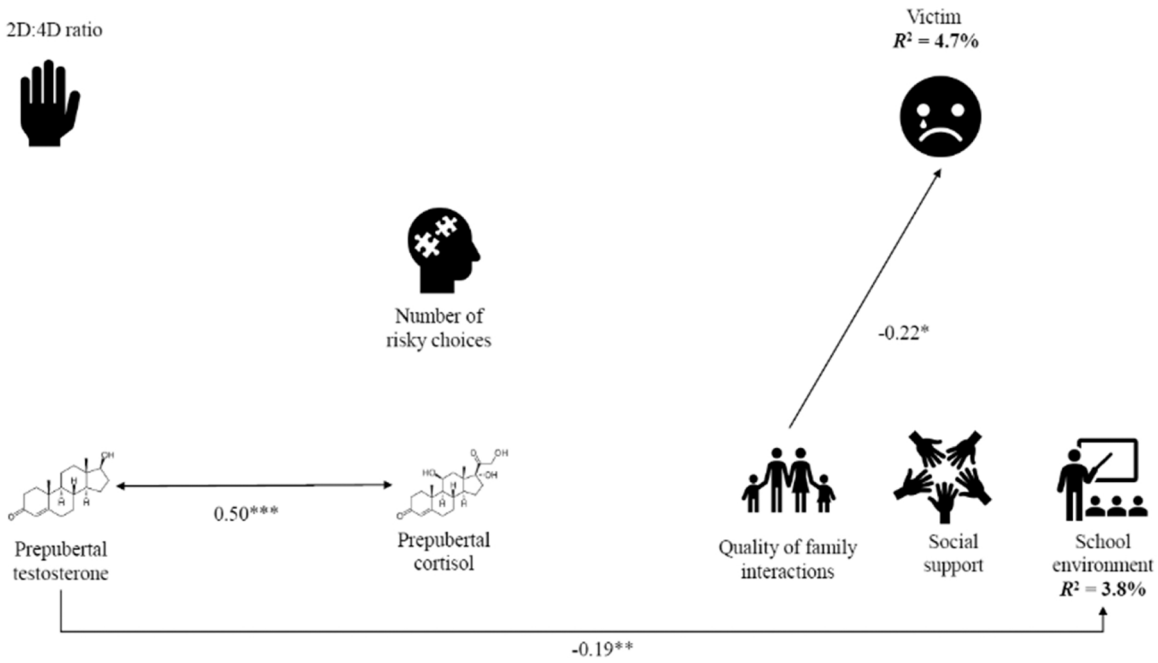

Fig. 3. Final structural equation model for girl's propensity to be a victim. Note: R^2 values indicate the percentage of variance explained. Numbers are the standardized coefficients of the corresponding relationship. *=p < 0.05; **p < 0.01; ***p < 0.001.

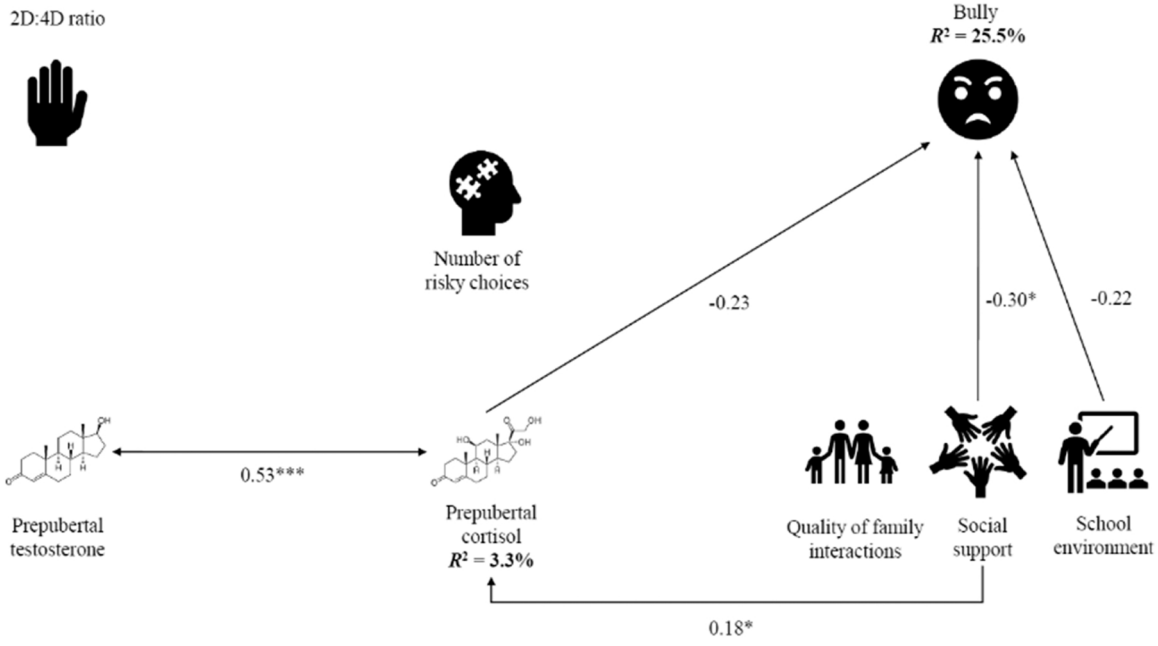

Fig. 4. Final structural equation model for boys' propensity to be a bully. Note: R^2 values indicate the percentage of variance explained. Numbers are the standardized coefficients of the corresponding relationship. * = $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

<0.05).

3. Discussion

The objective of this work was to study the association that biological and psychosocial factors have with three different roles that preadolescents may take in bullying situations.

First, it should be highlighted that the prevalence of bullying, as well as the preadolescent hormone levels in our sample correspond to values found in other populations of similar ages. Concerning bullying prevalence, our rates are similar to those found in Spanish population in general (García-García et al., 2017) and in population of the Basque Country in particular (Machimbarrena & Garaigordobil, 2018). Likewise, 2D:4D ratio values (Butovskaya et al., 2019; Shaw et al., 2012; Voracek & Offenmüller, 2007) and prepubertal hormone levels in saliva in our study do not differ from those found by other authors (Ostatníková et al., 2002; Pascual-Sagastizabal et al., 2019). Regarding our sample's social characteristics, it is a Spanish sample composed of non-clinical preadolescents and quite homogeneous regarding the sociodemographic factors. As far as school characteristics, half of the sample attended public schools and the other half private schools.

Our main objective was to explore the association that hormone

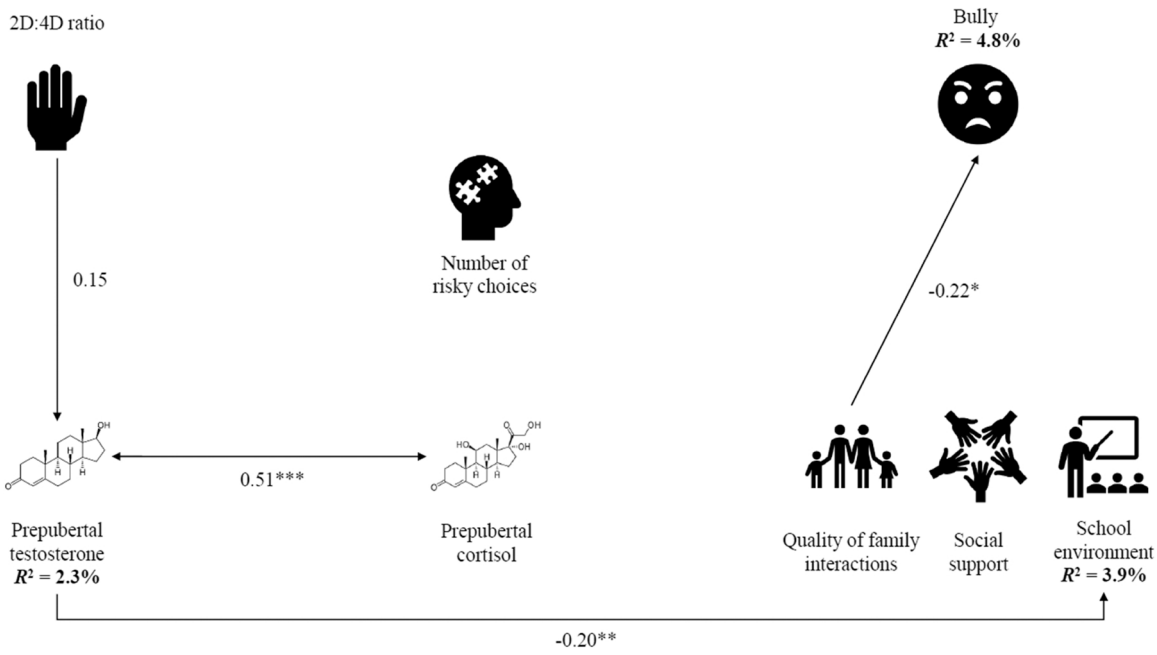

Fig. 5. Final structural equation model for girls' propensity to be a bully. Note: R^2 values indicate the percentage of variance explained. Numbers are the standardized coefficients of the corresponding relationship. *= $p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

levels and psychosocial factors have with bullying involvement. In relation to the victims, in the model for boys, we found that having a poor school environment' perception was related to being victimized more frequently. It can be concluded that the association between school environment and bullying remains stable cross-culturally, as other authors have shown similar results in populations from China (Han et al., 2017), Hong Kong (Chan & Wong, 2015), Thailand (Pengpid & Peltzer, 2013), United States (Gower et al., 2015), United Kingdom (Muijs, 2017), Iceland (Mann et al., 2015), the Netherlands (Jansen et al., 2012) and Colombia (Moratto Vásquez et al., 2017).

Regarding girls, the score on family stress and conflict was consistently related to victimization, showing that those girls with higher family stress and conflict are more frequently victimized. This result goes in line with other authors confirming that having good connectivity and good communication between parents and children are protective factors of victimization (Pengpid & Peltzer, 2013; Shetgiri et al., 2013), whereas family conflict increases the risk of being victimized (Hemphill et al., 2012, 2015). Additionally, Garaigordobil and Machimbarrena (2017) showed in a study carried out in the Basque Country that parents of severe victims, cyber-victims and aggressors had higher stress levels related to their parenting role.

In the case of bullies, in boys, the obtained model supported the main idea that biological and psychosocial factors were related to being more frequently involved as a bully. Lower levels of prepubertal cortisol, worse perceived school environment and less peers and social support were related to being more frequently involved. González-Cabrera et al. (2017) in their study found that cyberbullies showed flattered cortisol secretion curves. In addition, one recent systematic review reached the conclusion that in children and adolescents, bullying was consistently related cortisol (Kliewer et al., 2019), those bullied children showed lower cortisol levels or blunted pattern of cortisol (Östberg et al., 2018; Peters et al., 2011). Regarding psychosocial factors, having a good perceived school environment and higher scores in peers and social support was related to being less frequently involved as a bully. Previous research concluded that having trust in school (Muijs, 2017); good relationships with classmates (Han et al., 2017) and stronger social support (Pengpid & Peltzer, 2013) decreased the risk of being involved in bullying situations.

In the model about girls' propensity to be a bully, higher family stress and conflict was related to being involved as a bully. In this line, one previous study carried out in the Basque Country found that parents of cyber-aggressors showed higher parental stress (Garaigordobil & Machimbarrena, 2017).

Finally, in the case of bully/victim propensity, we did not obtain any valid model. There may be different two possible explanations for this. First, even if we calculated bully/victim involvement based on scores of victim and bully subscale, the OBVQ questionnaire we used does not have a specific subscale to assess this role. Second, the number of participants taking this role in our sample was relatively small, which decreases the probability of detecting the effects, if any, of the studied variables. To the best of our knowledge, few studies analyzed the bully/victim role and therefore, we consider it important to comment on the results obtained in the bivariate analysis. For both sexes, lower score on peers and social support was correlated with being bully/victim involvement. A previous meta-analysis confirm that higher score on social support and good relationship with peers protects against the risk of being involved in bullying situations (Cook et al., 2010). In addition, in girls, the school environment and the 2D:4D ratio were found to be related to this role. Regarding school environment, our results go in line with what other studies found, that is, a worse school environment perception is related to being more frequently involved in bullying situations (Han et al., 2017; Muijs, 2017). In terms of hormone levels, we found that the 2D:4D ratio, i.e. lower prenatal androgens levels, was associated with more frequently involved as bully/victim. To the best of our knowledge there are no previous studies analyzing the association between 2D:4D ratio and bullying. Previous studies analyzed the association between 2D:4D ratio and aggressive behavior, founding a negative association (Burton et al., 2009; Shaw et al., 2012). On the other hand, Vaillancourt, deCatanzaro, Duku, & Muir (2009) in their study found that bullied girl had lower testosterone levels, measured in saliva. Further studies would be necessary to draw conclusions and to understand the mechanisms underlying the relationships between 2D:4D and bullying behavior.

Apart from studying the factors directly associated with bullying, this work aimed to establish possible associations between the different predictor variables. Taking into account boys' models, we observed an interesting positive association between two of the predictor variables: peers and social support and cortisol levels. Previous evidence showed

mixed results concerning the direction of this association, finding one study also a positive association between long-term social support and cortisol measured in saliva. This study concluded that these differences may be explained by the type of social support being assessed, the duration of this social support and the method used to measure cortisol levels (Rosal et al., 2004).

Considering girls' models, we observed a negative association between testosterone and school environment perception. One previous study found that higher testosterone levels were associated with lower sociability in prepubertal boys and girls (Strong & Dabbs, 2000). Additionally, girls with higher testosterone may be exhibiting more typically masculine characteristics and they might be displaced in their peer group and so have more problems at school. This is an interesting result because although testosterone was not directly related to bullying, it showed an association with school environment, which is the context where children and adolescents develop their social relations. Further investigation would be needed to find out the effects that testosterone has in the school environment in general and in bullying in particular.

In addition, taking into account all the models, a positive and statistically significant correlation was found between prepubertal testosterone and cortisol levels. Traditionally, researchers have argued that cortisol and testosterone are mutually inhibitory (Dekkers et al., 2019; Mehta & Josephs, 2010). Interestingly, as our results showed, a study with incarcerated male adolescents found a positive association between testosterone and cortisol (Dismukes et al., 2015). In order to explain this discrepancy, some authors suggested a developmental hypothesis, describing that this positive association may be unique to early stages of puberty when activity in both HPG and HPA axes is growing following childhood (Dahl & Gunnar, 2009; Marceau et al., 2013; Matchock et al., 2007).

We did not find some of the expected associations, we believe that this may be due to the fact that our work is not without limitations. First, the sample size was relatively small considering not only the complexity of the model but also that these were made separated by sex. Second, bullying was assessed using a self-report questionnaire which Basque version was not validated. This scale is composed of two subscales assessing victimization and perpetration specifically, and the bully/victim category was created by the researchers of this work. The reason of assessing this role is that nowadays, bullying is seen as an important role that children can play. The questionnaire allows for differentiation between bullying children and bullying victims, allowing the conclusion to be made that those who have identified themselves in both roles are assigned the role of bullies/victims. Third, most of the measures were transversal and it would be interesting to analyze the association of some longitudinal effects. Despite these limitations, to the best of our knowledge, this is the first study that analyses the association of biological and psychosocial factors with three bullying roles preadolescents may take, analyzing sex differences. This is interesting because although we are not able to directly change the biological factors studied (namely, hormone levels), it would be possible to develop preventive programs that influence the other psychosocial variables that are risk factors themselves but that are related to cortisol levels too. For future research, it would be desirable to continue studying bullying from a biopsychosocial perspective, analyzing the potential mediation and moderation effects of these independent variables using a larger sample size and study population from other countries.

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CRediT authorship contribution statement

Izaro Babarro: Conceptualization, Investigation, Data collection, Writing – original draft, Supervision. **Ainara Andiarena:** Conceptualization, Investigation, Supervision, Writing – original draft. **Eduardo Fano:** Conceptualization, Investigation, Visualization. **Gonzalo García-Baquero:** Methodology, Data curation, Visualization. **Andrea Lebeña:** Data curation, Visualization. **Enrique B. Arranz-Freijo:** Visualization, Funding acquisition. **Jesus Ibarluzea:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

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