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Empathy at school project: Effects of didactics of emotions® on emotional competence, cortisol secretion and inflammatory profile in primary school children. A controlled longitudinal psychobiological study

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ABSTRACT

Background: There is mounting evidence of the presence of chronic stress among children during primary school: girls and boys under the age of 15 years often experience anxiety, irritability and sleeping problems with negative consequences on scholastic climate and the spread of bullying and dropping out of school. The promotion of emotion regulation within school environment through innovative didactic methodologies represents a valuable tool for teachers and parents to reduce emotional distress and associated risk behaviours and to promote wellbeing.

Aim: Our research aims to explore the psychological and biological consequences of teaching emotional training in an experimental group of Italian Primary School children.

Methods: A sample of pupils (81 children aged between 6 and 8) was divided into an experimental group (33 subjects) and a control group (30 subjects). A further advanced group of 18 subjects, who have experienced the method in the previous school year, was also included. The experimental study lasted one school year (from October 2021 to May 2022). The following psychological tests were administered to all groups: TEC (Test of Emotion Comprehension) to measure the children's different emotional abilities and the Projective test (PT) 'A person in the rain', to identify the coping skills of children in a stressful condition. Morning salivary cortisol, IL-6 and TNF-alpha assays were conducted in all three groups. Psychological and biological tests were administered at the beginning of the study and at the end of the study.

Results: The MR-Anova model for TEC score showed that there was not a significant group effect [Fgroup = 2.24, p = 0.114]. Pairwise comparisons showed that mean score significantly increased only in the Experimental group (pB < 0.001) and at the end of the project there was a significant difference between Experimental group and Control group (pB = 0.012). The mean score of PT test increased significantly from baseline to the end of the project for the Experimental group (pB < 0.001) and for the Advanced group (pB = 0.004). At the end of the project, there were significant differences between the Experimental group and the Control group (pB = 0.004) and between the Advanced group and the Control group (pB < 0.001). Salivary cortisol analysis revealed a significant effect between subjects [Fgroup = 9.66; p < 0.001] and significant effects within subjects with the main effect of the time [Ftime = 35.41; p < 0.001] and the significant interaction "time x group" [Ftimexgroup = 3.38; p = 0.040]. Pairwise comparisons showed that cortisol levels decreased significantly over time only in the Experimental group (pB < 0.001). Regarding to IL-6 levels, there was not a significant effect between subjects [Fgroups = 0.0481; p = 0.953]. The mean level decreased significantly for each group from baseline to post project (pB < 0.001). With respect to TNF-alpha levels, the mean levels decreased over time for all groups (pB = 0.006 for Experimental group; pB < 0.001 either for the Advanced or Control group).

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Conclusion: the results documented in the experimental groups who experienced didactics of emotion for at least one school year show a significant increase in children's ability to cope with reality, stress and anxiety, and an improvement of their emotional competence. Meanwhile, a significant reduction in the amount of salivary cortisol was observed in the experimental group at the end of the scholastic year; meantime a stable reduced amount of salivary cortisol in advanced group throughout the project was also observed. These findings show that an intervention through an emotional education program is able to regulate interpersonal skills and the stress axis response.

1. Introduction

Worldwide, UNICEF estimates that more than 13% of adolescents aged 10–19 live with a diagnosed mental disorder: 80 million aged 10–14 and 86 million aged 15–19 [1]. In the United Kingdom, one in eight young people experiences mental health problems, and emotional disturbances such as anxiety and depression are the most common issues experienced by young people [2]. The international report Health Behavior in School-aged Children (HBSC), a World Health Organization collaborative cross-national study on health, well-being, social environment and health behavior of 11-, 13- and 15-year-old boys and girls, reports increased prevalence of multiple stress-related health gripes since 2014: nervousness, irritability and sleep difficulties [3]. Mental well-being among young people decreased in recent years.

An Italian survey that examined the period 2010–2018, showed a remarkable worsening trend of teenagers' psychological well-being, with high school work pressure and poor social support emerging as the main issues [4]. School access restrictions and implementation of distance education due to Covid-19 pandemic has further worsened this condition increasing mental health problems such as depression, anxiety and emotional dysregulation [5]. In Italy, the lockdown and the restrictive measures enacted to counter the SARS-CoV-2 pandemic in 2020-21 caused a drastic drop in psychological well-being, particularly affecting younger age groups, also due to the spread of depressive and anxious symptoms among adults with the consequent worsening of family climate [6,7]. The exacerbation of psychological distress in young people has had obvious repercussions on the school climate.

Previous research has established that 52.7% of persons between the age of 11–17 years-old have suffered from abusive and/or violent actions from peers throughout the year. During their careers, 75.8% of head teachers had to manage a growing number of reports of cases of bullying, of which 52.8% cyberbullying [8]. In Italy, UNICEF has recently documented in a survey that the vast majority of students believe that the school should strongly promote mental health [1,9]. In fact, schools represent the main setting to promote children's mental health with active educational programs, even in socially disadvantaged conditions [10,11]. Despite all that, several barriers still hinder the dissemination and effectiveness of health promotion interventions at school [12], including school organizational deficiencies, teachers' unpreparedness in dealing with emotions and youth stress-related externalizations, and, last but not least, the age of the students. Most interventions are in fact implemented among pre-adolescents and adolescents, when it is more difficult to organize effective programs and obtain the necessary compliance. Although there have been interesting findings related to teens [13,14], our opinion is that the promotion of emotional education would be more effective and useful if started in primary school and delivered directly by teachers, as focused in a recent review [15].

Evidences from neuroscience research and clinical developmental psychology confirm that the first years of school, through the essential learning of reading and writing acquisitions, represent an exceptional phase of development when psycho-neural pathways begin to take shape [16,17] Furthermore, "written language learning is one of many social and cultural imprints acquired during the human brain's development" [18]. In this fundamental phase of human mental and cerebral development, the acquisition of cognitive skills and the elaboration of emotional and relational patterns take place simultaneously. Emotions and cognition are not rigidly separated phenomena and cerebral processes: "we are feeling creatures that think and thinking creatures that feel" [19].

Recent research shows that cognitive abilities may be heavily influenced by the affective context, documenting that young school-aged children are sensitive to the value of emotions and that this facilitates the acquisition of abstract words [20]. Furthermore, research suggests that adolescent vulnerability to the emergence of psychopathology (i.e. mental disorders, suicidality and addiction) can have its developmental roots in childhood [21]. Early life and childhood stress can have effects on brain development with consequences [22,23] on adult mental and physic health [24,25]. Individual stress increases feelings of anxiety and creates a negative scholastic climate, leading to the spread of violent attitudes and bullying, with consequences on the quality of teaching and on the number of students who drop out of school. Negative scholastic climate worsens individual stress in children with poor familiar support and eventually leads to what some authors define as toxic stress for brain development, with persistent detrimental consequences for health in adulthood [26]. Cortisol, the main stress hormone, as conceptualized by Robert Sapolsky, works as an inverted-U shape system: in the mild-to-moderate range it has beneficial and salutary effects; conversely, in high persistent concentration it has detrimental effects particularly on the hippocampus, the major brain area involved in memory processes and circadian regulation of the stress axis [27]. Neuroinflammation, induced by cortisol, represents a mechanistic hypothesis of hippocampus damage, the severity of which is well understood in the developing brains [28].

As demonstrated in experimental research, several educational interventions have been implemented to test whether a particular classroom training can change the school climate and help students to cope with stress by improving learning capabilities and emotion management skills [29,30]. These studies have documented an improvement in students' learning skills and social communication, with some limitations: 1) the target audience is mostly represented by groups of adolescents or near teens; 2) interventions are only student-based and not addressed to teachers; 3) to our knowledge, no biological markers related to the stress system have been so far measured in this setting. Only one research used a medical device to record autonomic nervous system activity and heart rate variability in a group of preschool students [31]. Our research aims to explore these unrecognized aspects. Therefore, we investigated the effects of a teacher-led educational model of emotion management (Didactics of Emotions®, see below) on Primary School children of 7 years old, assessed by both psychological and biological tests, particularly cortisol as the main stress hormone and IL-6 and TNF-α as inflammatory markers.

2. Materials and methods

2.1. *Didactics of Emotions®*

The method [32,33] was developed by Ulisse Mariani and Rosanna Schiralli and has been applied with good results to specific measurement tests on very large samples of students in Italy and in some European Union countries. The Didactics of Emotions® consists of a set of strategies, techniques, teaching units and very simple interventions, used by

teachers in the classroom, aimed at early building of the best skills in pupils to modulate and manage their own emotional world. Training with continuity and using different techniques according to age groups, results in an appropriate decoding both of the pupils' own emotions and of the others' mood swings, at the same time activating deep communication channels and an internal sensitivity of the children that work together to build empathy. When students activate empathic behaviours and gradually learn to manage impulses, by transforming them into more modular sensations, they also learn to develop better interpersonal skills, to increase their autonomy, self-esteem and self-efficacy.

2.2. Sample

The project was carried out in two Primary Schools in the province of Viterbo, Italy. For this experiment, a total of 5 second-year primary school classes were recruited before the start of the school year. This is a quasi-experimental sample study; in fact the sample was constructed on the basis of the voluntary adherence of the classes to the project without randomization. The sample of pupils (a total of 81 children) was divided into an experimental group (32 subjects) and a control group (30 subjects). Alongside these two groups, another so-called *advanced* group was recruited. The pupils in this group (19 subjects) had already used the Didactics of Emotions® method during the entire previous school year and continued the experience the same method throughout the 2021–2022 school year. The mean age of children is 7. Although there are classes with a considerable presence of children of different nationalities, no problems with language and comprehension were noted (except for one girl during the pre-test). The children's names were always associated with an alphanumeric code.

2.3. Brief analysis of the territory

The Italian municipalities of Viterbo and Vitorchiano are adjacent, just a few kilometres apart and connected by a convenient motorway. Viterbo, the provincial capital, has just over 66,000 inhabitants, while Vitorchiano is a small medieval town of just 5,300, with a steadily growing population. The economy of the two centres is mainly driven by the *non-advanced* tertiary sector and the presence of agricultural activities with *valuable produce* (olives and hazelnuts). The presence of industrial production is scarce and craft businesses, except for rare excellences, are not adequately developed. The municipality of Viterbo also has many military installations of the Army and Air Force. The area is full of well-preserved and accessible historical artefacts, a prestigious spa centre and a remarkable landscape. Nevertheless, tourism is not yet adequately developed. It suffers from historically conservative and not very expansive tourism policies. In recent years, however, the municipality of Vitorchiano has heavily invested in this sector, achieving good results and tangible returns. Viterbo is home to a well-established university centre attended by about 10,000 students, driving a fair amount of related activities. The crime index puts the Viterbo area in the 68th place out of 106 provinces, in Italy. In the city of Viterbo, the presence of foreign citizens is almost 10%, with a clear prevalence of subjects from Romania. In Vitorchiano, the share is 5% with a prevalence of subjects from Romania and North Macedonia. In the two municipalities, the activities offered by public and private childcare facilities are substantially good, accessible to all and numerically sufficient.

2.4. First stage

2.4.1. Teacher training

At the beginning of the school year, the teachers of the classes in the experimental group were trained. The training took place on two successive days (September 24–25, 2021) for a total of 8 h. Through discussion in a group chat and several meetings held throughout the school year, the training was implemented, questions answered, and critical issues addressed. The training focused on the theoretical foundations of the method and practical operations to be proposed in the classroom. Didactics of emotion methodology was the same one both for experimental and for advanced group. Teachers of the experimental group was trained before the start of the experimental phase as specified above. Teachers of the advanced group have already been trained in the previous scholastic year. Experimental measures were conducted by the same research group members in each group.

2.4.2. Informed consents

Informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed. The informed consents signed by the parents of each participating child were obtained on September 24, 2021 after the meaning of the research in all its phases had been explained to everyone (22 and 23 September in two separate meetings). This first phase ended on October 4, 2021.

2.5. Second stage

The study started on October 11, 2021 and ended on May 25, 2022, according to the following schedule.

- October 11,12, 2021 administration of battery of tests by psychologists;
- October 14, 2021 first saliva sampling;
- October 22, 2021 start of activities in the experimental classes;
- May, 23–24, 2022 administration of test battery by psychologists;
- May 25, 2022 s (final) saliva sampling.

The study formally concluded with the implementation of the second saliva sampling (end of May). At present, since the protocol indications for psychological tests do not allow multiple uses of samplings within a six-months timeframe, two test administrations were carried out.

2.5.1. Psychological tests

During the research programme, a battery of tests, comprising a test to measure the children's different emotional abilities (*TEC*) and a projective test *(PT)* to identify the relationship between children and stress management, were administered to the pupils of the two experimental groups plus the control group at the beginning and at the end of the school year. The administration, as already indicated, took place in the days immediately preceding the first and the third saliva sampling.

2.5.2. Projective test (PT)

'A person in the rain' by various authors and with specific indications (obtained personally) from Prof. Castellazzi, Italy's leading expert on this test [34]. The test of the *paper and pencil* series, consists of inviting each child to draw a *person in the rain*. Although it does not provide a coding system with scores, the test allows formal, graphological and content elements to be identified, grading them on a *Likert scale* (1–5). This scale measures: the presence of adequate defences; the level of stress and frustration; ways of coping with stress; the ability to cope with reality; the capacity to adapt; the level of anxiety. At the end there is a total score that defines the level of each child's ability to cope with reality, stress and anxiety mainly in the family dimension. The test was administered immediately after finishing the TEC (see below).

2.5.3. Test of Emotion Comprehension (TEC)- Italian version

The Test of Emotion Comprehension, by Pons and Harris [35], is the most reliable test available to date for measuring various traits of the emotional world of children. In our study the TEC adapted Italian version by Paola Molina and Ottavia Albanese was administered by specialized personnel. It involves showing 23 boards to each child (the test includes 23 boards for boys and 23 boards for girls). Each child, from time to time, after listening to a short story, has to identify the expression of a face, among four possibilities, congruent with that story. The test is able to measure, with excellent reliability, several skills and

dimensions of the subject's emotional sphere: recognition of emotions; recognition of the causes of emotions; ability to provide conflicting emotional responses to the same object; knowledge of one's own worldview and that of others (theory of mind); understanding of the link between memory and emotions; ability to regulate emotions through behavioural and mental strategies; management of emotions in contrast to stimuli; simultaneous management of two conflicting emotions; ability to grasp the emotional dimension of moral choices. The test was not administered inside the classroom, but in *neutral* spaces. After coding the raw scores and transforming them according to a table provided in the manual, standard deviations (*z-scores*) and percentile scores were obtained ready for comparison.

2.5.4. Saliva samples

Saliva samples were collected without any problem at three successive times: October 14, 2021, February 28 and 25 May 25, 2022. Small *Salivette swabs* were used (see below).

2.6. Monitoring

The monitoring of the Didactics of Emotions® activities carried out in the classroom during the school year by the teachers proved to be an important moment. Rather than optimising the teachers' interventions, the meetings and comparisons held during the school year at the reference schools was functional to reassuring the teachers. Indeed, feeling invested with great and unprecedented responsibilities, they had to reinforce what they were doing on several occasions (4 meetings during the period). Positive reinforcements, meetings at the schools, a 24/7 chat support for participants, *ad personam comparisons* as well as the invitation to feel part of an important research project proved to be decisive for the best progress of the programme.

2.7. Classroom activities

Each teacher, after the training course, tried to create the right atmosphere in the classroom and the best conditions for their pupils to train effectively in managing emotions without feelings being judged or evaluated. The teachers chose some techniques to apply according to the age of the pupils, their own educational style and priority needs. Moreover, teachers were encouraged to modify techniques or even invent new ones more suited to the reality of their class group, without ever distorting objectives and aims. The Covid-19 pandemic slightly affected the course of the programme without, however, altering its development and execution. Throughout the entire programme, each group of pupils was able to make use of three or four techniques. These were applied with adequate continuity, albeit with varying intensity from class to class and from teacher to teacher. In fact, no matter how homogeneous and uniform the training was, and no matter how numerous and pressing the indications and recommendations were, it was not possible to isolate or make the 'teacher-emotional coach' variable *homogeneously effective*: the use of techniques, in itself, constitutes the best expedient for minimising the effectiveness gap between the teachers' educational styles and for effectively inducing them to relate with their pupils, activating emotionally deep channels of communication. The range of techniques and strategies chosen by the teachers (all of which were presented during the training) made it possible to plan a longitudinal educational programme, structuring the activities according to needs and times and monitoring every change, every problem, every evolution and every group dynamic, through versatile, easy-to-use and immediately readable tools. The Didactics of Emotions® techniques and activities were an immediate success in the classes in which they were proposed: pupils often urged their teachers to adopt the emotional education techniques even beyond the scheduled times and spaces, proving how, for many of them, it was vitally important to have time and space to reflect on their own about each others' emotions. Institutionalised within school life, certain strategies, in particular the *call of emotions*, became an elective channel of expression of affective and emotional dimensions finally considered important by the adult world. The most frequently used techniques in this research were: the *emotion card*; the *collage of emotions*; the *call of emotions*; the *emotion box*; *guess how you feel*. Regarding the conduct of the activities, there were no problems whatsoever; each phase of the work was extremely simple, pleasant, interesting, and even relaxing. The programme needed support from the teachers at regular intervals (monitoring): giving feedback on their work, encouraged each one of the teachers to continue the activities with passion and enthusiasm. Further detailed contents and examples of the training programme and the learning objectives achieved are described in the Supplementary Appendix.

2.8. Salivary biological tests

2.8.1. Saliva collection

For the collection of saliva the Salivette® device (SARSTEDT AG & Co. KG Nümbrecht Germany) was used, since it provides an optimal method for the hygienic collection of saliva. The Salivette is designed to obtain accurate analytical values from small volumes and samples with very low metabolite levels. It consists of an absorbent chewable swab to collect the saliva, a plastic tube with a hole in the bottom, to house the swab, and an external tube to protect the sample. The Salivette is available with several swab options: a simple cotton swab, a cotton swab dipped in a citric acid preparation to stimulate salivation and a specially designed synthetic swab for the determination of cortisol. The version with synthetic swab for cortisol was used in our work.

2.8.2. Conditions for salivary sampling

The study subjects were required, starting 1 h before the saliva collection: not to take food or drinks (except water); not to chew gum or candy. 10 min before salivary collection: rinse their mouth with tap water; finally, drink a glass of water. At the time of saliva collection (a few minutes after drinking water): use a Salivette device.

Saliva collection is performed by chewing the swab for about a minute and placing it in its dedicated tube. The study personnel finally collected the used Salivette tubes, maintained them at 4 °C until processing (no longer than 3 h later in the lab), when the Salivette tubes were centrifuged at 1000 g for 2 min, to obtain a clear saliva sample in the conical tube. Residual particles and mucus collected in the specially designed tip of the Salivette. The portion containing the swab was then discarded, while the clear sample of collected saliva was aliquoted and used for analysis. The recovery rate in Salivette for Cortisol has been shown to be nearly 100%, regardless of the metabolite concentration, the saliva volume or the measuring method applied. Moreover, the dimensionally stable synthetic swab offers superior absorption quality and virtually complete saliva recovery under the recommended centrifugation conditions.

2.9. Cortisol assay

To measure salivary cortisol The DetectX® Cortisol Immunoassay kit Arbor Assay (Ann Arbor, Michigan USA) has been used. This ELISA (Enzyme-Linked-Immunosorbent Assay) kit uses the Competitive-ELISA principle with mouse anti-cortisol antibodies and is designed to quantitatively measure total cortisol in extracted samples and in serum and plasma and free cortisol in saliva and urine. A cortisol standard is provided to be diluted in serial dilutions to generate a standard curve for the assay and all samples should be read off in relation to the standard curve. Saliva samples should be diluted $\geq$1:4 or greater with the supplied Assay Buffer prior running in the assay. The microtiter ELISA plate provided in this kit has been pre-coated with an antibody specific to capture mouse antibodies. 50 µl aliquots of Standards or of diluted samples are pipetted into wells in the plate. 75 µl of Assay buffer are pipetted into the nonspecific binding (NSB) wells. 50 µl of assay buffer are pipetted into the maximum binding wells. A cortisol-peroxidase conjugate is added to

the standards and samples in the wells using a repeater pipette. The binding reaction is initiated by the addition of a monoclonal mouse antibody to cortisol to each well except the NSB wells. The Fc portion of the antibody to cortisol will bind to the antibody adhered to the bottom of the well. After a 1-h incubation the plate is washed, and substrate is added. The substrate reacts with the bound cortisol-peroxidase conjugate. After a short incubation, the reaction is stopped, and the intensity of the generated color is detected in a microtiter plate reader capable of measuring light absorbance at 450 nm wavelength. The concentration of cortisol in the sample is then calculated, after making suitable corrections for the dilution of the sample, using a dedicated software of the plate reader utilized (HiPo Biosan, Riga, Latvia). The higher the concentration of cortisol in the sample, the lower the amount of labeled cortisol that will bind to the antibody, therefore the less intense the staining will be. If the amount of cortisol in the sample is low, the antibody binding site will be mainly occupied by the labeled cortisol and the staining will be more intense, with the corresponding absorbance reading.

The detection limit of the salivary cortisol measurement kit adopted here is 45,4 pg/ml. Its sensitivity is 27,6 pg/ml (according to the producer). The calibration curve we realized included a range between 50 and 3200 pg/ml. For each series of samples and for each sample collection date, a calibration curve was drawn and all the experimental data measured were falling inside the range of corresponding calibration curve. Cortisol concentrations are expressed as nM units. The conversion factor between nM and pg/ml is as follows: 100 pg/mg are equivalent to 275,9 pM, i.e. 0,2759 nM (according to the kit datasheet). The within- and between-assays variations have been very limited (<1%) and however considered thanks to the calibration curves repeated at each assay session.

2.10. Interleukin-6 and TNF-alpha assays

To measure salivary Interleukin-6 and Salivary TNF-alpha Human IL-6 Elisa Kit and Human TNF-alpha, ELISA kits by Elabscience (Houston, Texas, USA) have been used. These ELISA kits apply to the in vitro quantitative determination of Human IL-6 or TNF-alpha concentrations in serum, plasma, saliva and other biological fluids. Both these ELISA kits use the Sandwich-ELISA principle. The micro-ELISA plates provided in these kits have been pre-coated with an antibody specific to Human IL-6 and TNF-alpha respectively. Human Il-6 or TNF-alpha standards are provided to be diluted in serial dilutions to generate a standard curve for the assay and all samples should be read in relation to the standard curve. Samples (or Standards) are added to the micro-ELISA plate wells and incubated overnight at 4 °C to interact with the specific antibody. Then a biotinylated detection antibody specific for Human IL-6 (or TNF-alpha) is added to each micro plate well and incubated for 1 h at 37 °C. Avidin-Horseradish Peroxidase (HRP) conjugate is added successively to each micro plate well and incubated for 30 min. Free components are washed away. The substrate solution is added to each well. Only wells containing salivary IL-6 or TNF-alpha will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The absorbance is measured in a microtiter plate reader at a wavelength of 450 nm, as described above. The concentration of IL-6 (or TNF-alpha) in the sample is then calculated, after making suitable corrections for the dilution of the sample, using a dedicated software of the plate reader utilized (HiPo Biosan, Riga, Latvia). The absorbance value is proportional to the concentration of human IL-6 or TNF-alpha. The concentration of these cytokines in the samples can be calculated by comparing the absorbance of the samples to the standard curve. IL-6 and TNF-alpha concentrations are expressed as pg/mL units.

2.11. Statistical analysis

All variables were analyzed and reported as mean and standard deviation (SD) or percentages if they were quantitative or qualitative data. The Shapiro-Wilk test was performed to test the normality for continuous data. To compare baseline data among groups (Advanced, Experimental and Control groups), a Kruskal Wallis test was used. For all psychological and biological measures, Anova model for repeated measures (MR-Anova) was performed, to examine the differences between the measures investigated and the groups, taking into account the effect of the time and the interaction term involving time x group. A Natural log transformation ln(xi) or ln(xi+100) for psychological data (TEC and PT tests) and for biological data (cortisol, IL-6, TNF-alpha levels) was applied before performing MR-Anova model. Greenhouse-Geisser corrections were applied to F-values that violated Mauchly's test of sphericity. Multiple comparisons using Bonferroni methods correction were performed (pB), when appropriate. The analyses were performed using STATA 14/MP and Jamovi software. Uncompleted data were excluded from the analyses, as missing data and for all analyses, group size was reported as nE, nA, nC for the three Experimental, Advanced and Control groups respectively. All tests were two-tailed, and $p < 0.05$ was considered statistically significant.

3. Results

Eighty-one students have enrolled for the study from two different schools: forty-two were females and thirty-nine were males. Eighteen participants were part of the advanced group, thirty-three of the experimental group, and thirty were controls; their characteristics are detailed in Table 1.

Table 2 reports baseline data with respect to the psychometric tests and Table 3 the biological evaluations. For the analysis 2 data for TEC and PT were excluded because of uncompleted data. There were no differences among groups at baseline for all measures investigated. For The TEC and PT tests, the advanced group showed a higher score on average: 0.272 (SD = 1.03) and 15.1 (SD = 5.14) respectively. With respect to biological evaluations, 3 data for cortisol and 36 data for IL-6 and TNF-α respectively were excluded because of insufficient amount of biological samples. The baseline cortisol level, on average, was higher in the control group: 5.60 (4.50).

3.1. Psychological measures

Regardless of TEC score, the analysis evidenced that there were significant effects within subjects, in fact, there was a main effect of the time [$F = 44.28$, $p < 0.001$], and the interaction "*time x group*" was significant [$F = 4.83$, $p = 0.011$], showing that the change in scores over time was different depending on group. The model showed that there was not a significant group effect [$Fgroup = 2.24$, $p = 0.114$]. Pairwise comparisons showed that mean score significantly increased only in the Experimental group ($pB < 0.001$) and at the end of the project, there was a significant difference between Experimental group and Control group ($pB = 0.012$) (see Fig. 1).

The MR-Anova model reported that for PT test, there was a main significant effect of the time [$Ftime = 35.42$; $p < 0.001$], a statistically

Table 1
Characteristics of the participants.

Characteristics	N (%)
Gender	
F	42 (51.9)
M	39 (48.1)
School	
Viterbo	52 (64.2)
Vitorchiano	29 (35.8)
Group	
Experimental	33 (40.7)
Advanced	18 (22.2)
Control	30 (37.0)

Table 2
Psychological baseline measures by groups.

Psychological measures		
	mean (SD)	mean (SD)
Groups	TEC (n = 79)*	PT (n = 79)*
Advanced	0.272 (1.03)	15.1 (5.14)
Experimental	−0.352 (1.24)	13.1 (5.11)
Control	0.002 (4.38)	12.4 (4.38)
	P = 0.1386	P = 0.1066

Note: * total is not 81 because of uncompleted data.

Table 3
Biological baseline measures by groups.

Biological evaluation			
	mean (SD)	mean (SD)	mean (SD)
Groups	CORTISOL nM (n = 78)*	IL-6 pg/mL (n = 45)*	TNF pg/mL (n = 45)*
Advanced	4.57 (2.94)	12.7 (1.37)	12.1 (1.62)
Experimental	5.00 (3.50)	14.5 (4.82)	12.1 (4.29)
Control	5.60 (4.50)	12.6 (3.95)	12.5 (2.86)
	P = 0.9868	P = 0.1041	P = 0.6597

Note: * total is not 81 because of uncompleted data.

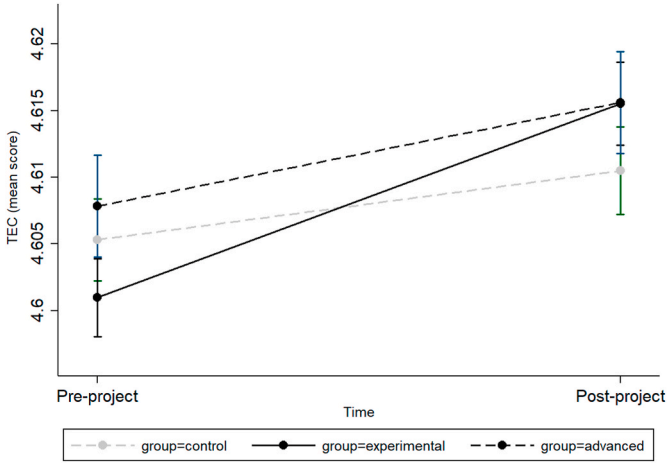

Fig. 1. TEC score by group and time. Data (log data) represented as estimated marginal means. Bars are confidential intervals (95%CI). nE = 29; nA = 18; nC = 26. The analysis denotes a significant increase from baseline to the end of the project in the Experimental group; at post-project, a significant difference between Experimental and Control group (pB < 0.05).

significant interaction "*time x group*" [Ftimexgroup = 3.38, p = 0.040], then the change in scores over time was different depending on group. The model also, showed the significant effect between groups [Fgroup = 9.66, p < 0.001]. The mean score increased significantly from baseline to the end of the project for the Experimental group (pB < 0.001) and for the Advanced group (pB = 0.004). At the end of the project, there were significant differences between Experimental group and Control group (pB = 0.004) and between Advanced group and Control group (pB < 0.001), (Fig. 2).

3.2. Biological evaluations

Our analysis revealed a significant effect between subjects [Fgroup = 9.66; p < 0.001] and significant effects within subjects with the main effect of the time [Ftime = 35.41; p < 0.001] and the significant interaction *"time x group"* [Ftimexgroup = 3.38; p = 0.040]. Pairwise comparisons showed that cortisol levels decreased significantly over time

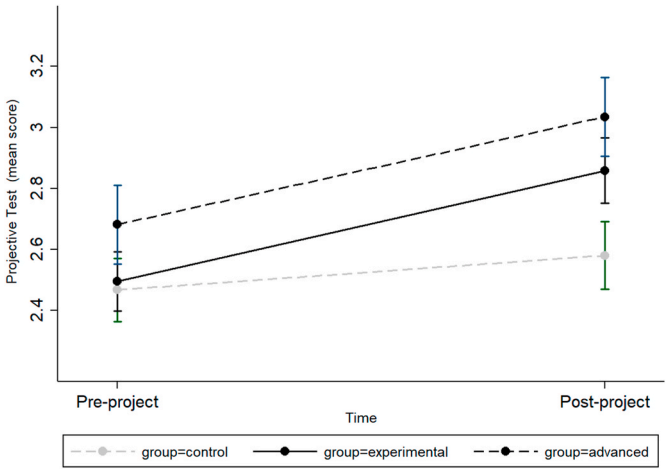

Fig. 2. Projective Test score by group and time. Data (log data) represented as estimated marginal means. Bars are confidential intervals (95%CI). nE = 29; nA = 18; nC = 26. Analysis denotes: i) a significant increase from baseline to the end of the project for Experimental group (pB < 0.001) and for the Advanced group (pB = 0.004); ii) at post-project time, a significant difference between Experimental and Control group (pB = 0.004) and between Advanced and Control group (pB=<0.001).

only in the Experimental group (pB < 0.001). The cortisol level in the Control group gained not significantly from baseline to the end but at the end of the project it was significantly higher than the levels of Experimental and Advanced groups (pB < 0.001), (Fig. 3).

The MR-ANOVA of IL-6, reported the significant main effect of the time [Ftime = 89.661, p < 0.001] and a non-significant interaction "time x group" [Ftimexgroup = 0.108; p = 0.898]. There was not a significant effect between subjects [Fgroups = 0.0481; p = 0.953]. The mean level of IL-6 decreased significantly for each group from baseline to post project (pB < 0.001) (Fig. 4).

With respect to TNF-alpha levels, our analysis found a significant effect of the time [Ftime = 75.96; p < 0.001] and the group [Fgroup = 4.54; p = 0.019], the interaction "time x group" was not significant [Ftimexgroup = 1.26; p = 0.298]. The mean levels decreased over time for all groups (pB = 0.006 for Experimental group; pB < 0.001 either for the Advanced or Control group) (Fig. 5).

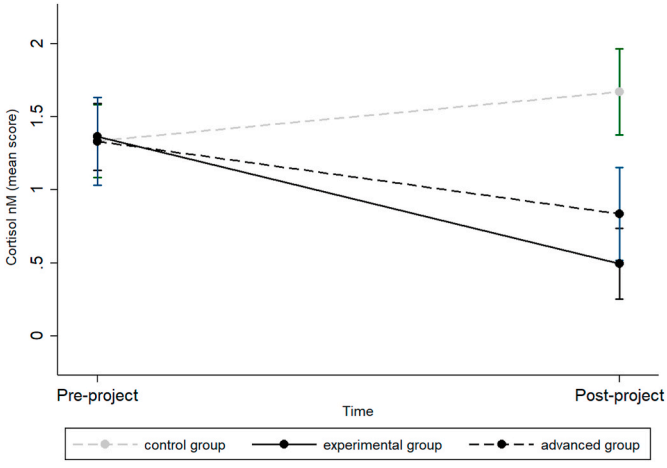

Fig. 3. Cortisol levels by group and time. Data (log data) represented as estimated marginal means. Bars are confidential intervals (95%CI). nE = 29; nA = 17; nC = 21. Analysis denotes: i) a significant difference between pre and post project for the Experimental group (pB < 0.05); ii) a significant difference at the post-project between Experimental and Control group and Advanced and Control group (pB < 0.05).

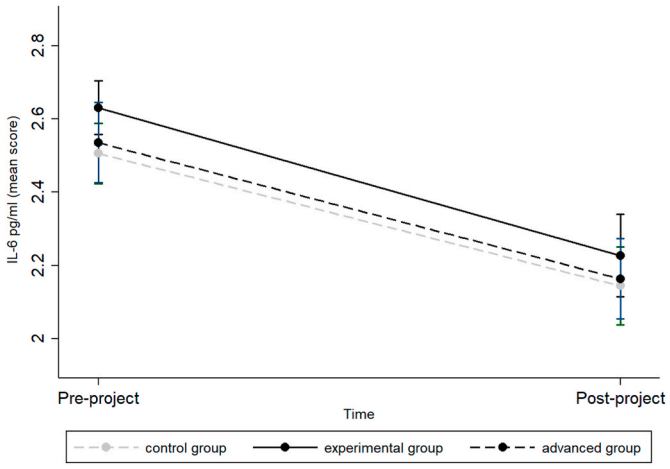

Fig. 4. IL-6 levels by group and time. Data (log data) represented as estimated marginal means. Bars are confidential intervals (95%CI). nE = 12; nA = 9; nC = 12. Analysis denotes significant difference between pre and post project for both the Experimental group (pB < 0.001) and the Control group group (pB < 0.001) and the Advanced group (pB < 0.001).

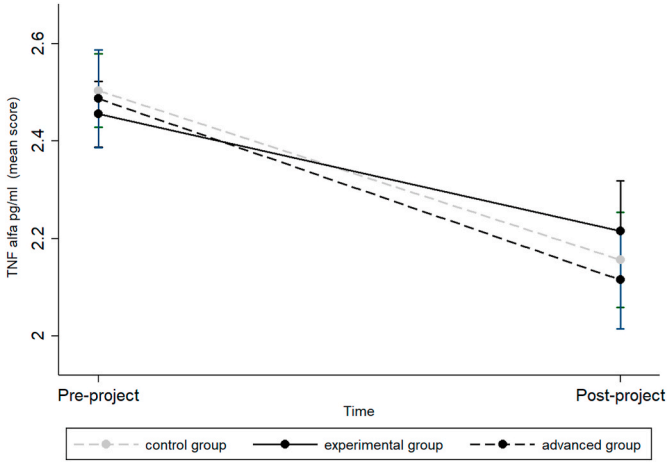

Fig. 5. TNF-alpha levels by group and time. Data (log data) represented as estimated marginal means. Bars are confidential intervals (95%CI). nE = 12; nA = 9; nC = 12. Analysis denotes significant difference between pre and post project for all three groups: Experimental group (pB = 0.006); Control group group (pB < 0.001); Advanced group (pB < 0.001).

4. Discussion

We investigated the effects of a teacher-led educational model of emotion management (Didactics of Emotions®) on primary school children (7 years old), assessed by both psychological and biological tests over a whole school year. In the experimental group, in contrast to the control group, we documented a significant increase both in the children's ability to cope with reality, stress and anxiety, especially in family dimension (according to the Projective Test 'A Person in the Rain'), and in their emotional competence, as measured by the TEC (Test of Emotion Comprehension). In parallel with the psychological results, the children in the experimental group, if compared to those in the control group, showed a statistically significant reduction in the amount of salivary cortisol at the end of the emotional didactic program. The Projective test 'A person in the rain', and the Test of Emotion Comprehension (TEC) were administered by specialized personnel (school psychologists), who processed the results blindly and homogeneously. Particularly, TEC is the most reliable test available to date for measuring various traits of the emotional world of children, including recognition of emotions, knowledge of one's own worldview and that of others (theory of mind), empathy, and more (see above).

The connection between improved interpersonal and emotion management skills and cortisol reduction only in children who were educated to better feel and regulate their own emotions, suggests that the two phenomena are closely interdependent. Managing emotions lowered individual stress and improved the school climate. In turn, a lower activation of the stress system favoured the implementation of skills related to empathy and friendly cooperation among the children and between them and the teachers. According to Psychoneuroendocrineimmunology (PNEI) research, emotions and feelings influence organic functions through bidirectional connections between biologic and psychic dimensions [36].The physiological stress response, which starts in the hypothalamic nucleus, passes through pituitary gland and culminates with the secretion of cortisol by adrenal cortex, is activated by environmental factors (such as heat and cold, humidity and drought, wind, noise, and pollutants), as well as by endogenous factors (such as a significant reduction in blood pressure and volume of circulating blood, body hydration and nutrition, acute infection or hemorrhage), but also by emotional or cognitive factors [37–39] (see Fig. 6).

Our research takes place in a context of few published studies of school-based interventions; in particular, there is a lack of long-term studies on wellbeing and mental health of students in school settings [40]. Furthermore, to our knowledge, there are very few studies carried out on Primary School students, the object of our research [41,42]. Recently, a study on 45 pre-school children was published. The intervention, called "Pythagorean Self-Awareness", was carried out on 3-6 year-old children by specialized personnel over a period of 9 weeks [43] and showed a reduction in perceived stress and global hearth rate mean index reduction, with improvement in anger, guilt, emotion management and social relationships.

For the first time, to our knowledge, we have documented that emotion management-based teaching, carried out by the Didactics of Emotions® method, simultaneously improves the emotional and relational competence of Primary School children and reduces over time salivary cortisol concentration, as the main biological marker of the stress system reactivity. The positive results achieved also in Advanced group in terms of psychological wellbeing and lower cortisol secretion confirm its efficacy throughout the observation period.

In our research, cortisol variation is not correlated to immunological indicators, like IL-6 and TNF-α, that have shown however a homogeneous decline in all groups, plausibly related to seasonal trends [44,45], and spread of viral infections during the initial measurement, although we cannot rule out the paucity of salivary samples as concurrent cause.

5. Limits

The results should be used with caution because of the limitations related to the fact that this is a quasi-experimental longitudinal study with control group without randomization.

The characteristics of the psychological intervention (didactics of emotions®) didn't allow us to carry out the study under conditions of blindness; however, specialized personnel who blindly assessed psychological evaluations was different from those who performed the didactics of emotions® in the classrooms.

Since the baseline cortisol level in the Control group was higher than in the Experimental and Advance groups at the beginning of the study, one could put forward the hypothesis that it is the result of selection bias due to the absence of randomization; nevertheless, the significative reduction of salivary cortisol was observed only in the experimental group.

6. Conclusions and perspectives

Our research shows that it is possible to moderate the concentration of basal morning cortisol in 7-year-old children, through an emotional

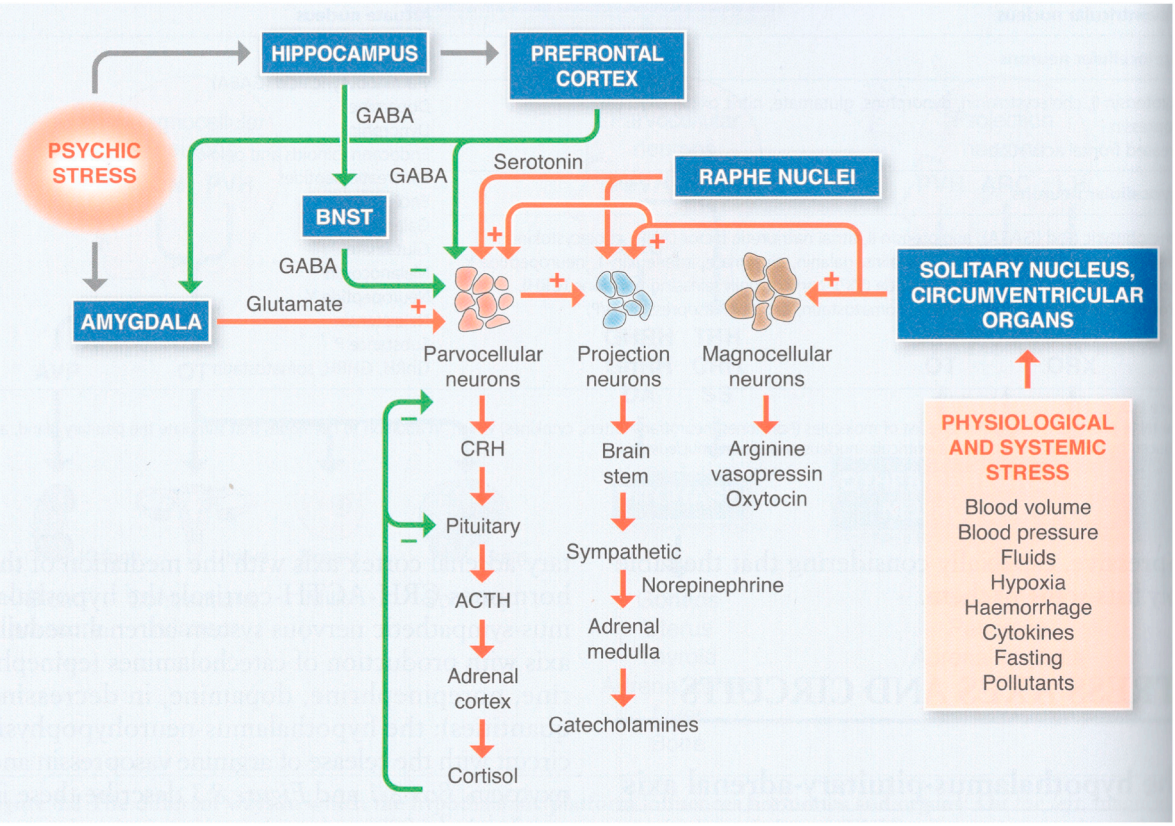

Fig. 6. The paraventricular hypothalamic nucleus is the cerebral structure that activates the stress response. It is divided into three sectors: magnocellular, which releases oxytocin and arginine vasopressin; parvocellular, which releases CRH; the neurons projecting towards the brain stem, where the neurovegetative nuclei are located, in particular in the locus coeruleus and in the rostral ventromedial medulla, where the control nuclei of the sympathetic nervous system are located. Activation of neural pathways afferent to CRH neurons in the PVN causes a rapid calcium influx that stimulates the fusion of CRH-containing vesicles to the cell membrane and subsequent release CRH. Depending on the type of stressor, individual or even all areas of the paraventricular nucleus (PVN) are activated. For example, a life-threatening stress event, like hemorrhage, activates all PVN neurons. The magnocellular sector receives inputs related to volume, blood tonicity and fluid state. In the parvocellular sector, on the other hand, physiological and systemic inputs arrive at the parvocellular sector through the solitary nucleus and some circumventricular organs, but also emotional ones, which come from the cortical, limbic or sensory and nociceptive areas. The subcortical areas, which are emotionally activated, send projections to the bed nucleus of the stria terminalis (BNST), which then acts as a centre for the projection of emotional signals from the limbic to the PVN. The other fundamental route that brings emotional stress to the paraventricular nucleus is the one coming from the amygdala. The end result of the stress response is the release of cortisol and catecholamines; cortisol, through negative feedback on the pituitary and hypothalamus, regulates the activity of the axis. Reprinted with permission from Ref. Bottaccioli & Bottaccioli 2020, p. 136, Copyright 2020, Edra

education programme (Didactics of Emotions®), carried out directly by teachers at school. At the same time, emotional didactic programme increases the emotional and relationship skills of young students also with benefits on scholastic climate and performance.

Present results showed a simultaneous improvement in psychological, emotional and biological outcomes, and add evidences to limited previous literature on children under 10 years old in school context and in general on the effects of managing emotions and behaviours on mental health, particularly in developmental age [46,47]. The significant reduction of salivary cortisol in the group of primary school children who experienced one year of emotion management didactic lessons, reliably shows that educative interventions are able to improve emotional and interpersonal skills and can directly affect biological regulation pathways of stress response. Although further, larger and more rigorous studies are necessary in the future, present findings provide insight for possible applications in the current educational system with the aim to improve the ability to manage emotions, relationships between students and between scholars and teachers; with this strategy it is possible to implement a new comprehensive, emotional and cognitive education model.

All authors have made substantial contributions to the conception and design of the study, acquisition, analysis and interpretation of data, drafting and final approval. This research received funds from not-for-profit Emotional Training Center ONLUS, Viterbo, Italy. Declarations of interest: none.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2023.100183.

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