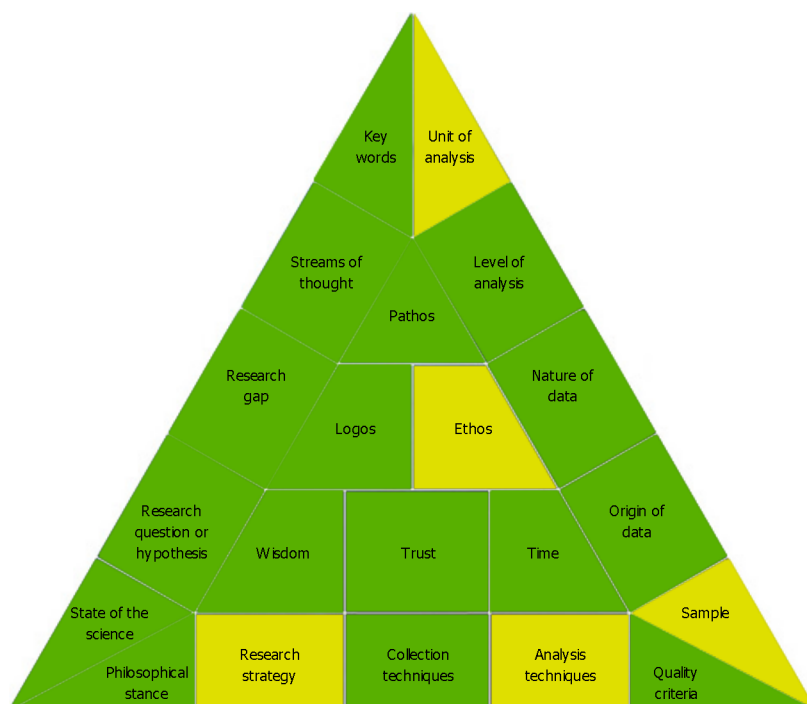


## Biological Consequences of Exposure to Social Adversity in Childhood

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**Self-evaluation:** 88%



### Key words

#### 1/21. Theoretical question: which theoretical keywords are implicit in your research?

This investigation is grounded on the following keywords: early life; adverse childhood experiences; bullying and biological pathways.

Early life is usually defined as the time period between birth and the age of twelve years, covering all the period of infancy until pre-adolescence [1]. Early childhood has been increasingly recognized as the most crucial period of development during the life span and is highly sensitive to external influences [2]. During this period, the foundations are laid for every individual's physical and mental capacities, influencing their subsequent growth and health throughout the life course.

Adverse Childhood Experiences (ACEs) can be defined as a set of traumatic and stressful psychosocial conditions and/or circumstances that are out of child's control and often persist over time. ACEs have been related with life-time biological effects [3,4] and, consequently with the onset of chronic health problems in adult life, including maternal outcomes, cancer, liver diseases, skeletal fractures, chronic lung disease, and ischemic heart disease [5-7]. The ACEs typically considered in studies include household poverty, separation from a parent, death of a parent, neglect, abuse (including emotional, psychological, physical, and sexual), and peer bullying.

Bullying behaviour may take different forms and is usually defined as an intentional aggressive and negative behaviour, repeated in time that involves an imbalance of power favouring the author. Meaning that the victim of bullying does not have the means to defend himself or herself in physical and/or psychological strength [8,9]. The most common forms are name-calling, teasing, making threats, spreading rumours, taking of personal belongings, and rejection by excluding someone from a group on purpose [10].

There is evidence showing the association between social environment and biological pathways. Socioeconomic status, household dysfunction and traumatic experiences might influence biological pathways [5, 11-15].

## References:

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**Self-evaluation:** 100%

## Streams of thought

### 2/21. Theoretical question: which streams of thought are implicit in your research?

Literature has been relating Adverse Childhood Experiences (ACEs) with life-time biological effects [1,2] and, consequently with the onset of chronic health problems [3-5]. The link between ACEs and adult health outcomes may be explained by an indirect and cumulative effect, through health behaviours, or by a direct effect of stress in children%u2013s biology [6-9].

Although the biological mechanisms are still not very clear to explain the short-term effects of social adversity already in childhood, it is hypothesized that stressful adverse experiences shape subsequent responsivity of the hypothalamus-pituitary-adrenal axis, and this response will persist over the life course [10]. Few studies were conducted among children in order to assess the extent to which such effects can be seen early in development. However, the existing evidence showed that children when exposed to stressful events such as low parental socioeconomic status [11], maltreatment and bullying tend to present high levels of cortisol dysregulation and of C-reactive protein [12].

## References:

1. Hertzman, C. and T. Boyce, How Experience Gets Under the Skin to Create Gradients in Developmental Health. *Annual Review of Public Health*, 2010. 31(1): p. 329-347.
2. Rutter, M., Achievements and challenges in the biology of environmental effects. *Proceedings of the National Academy of Sciences of the United States of America*, 2012. 109(Suppl 2): p. 17149-17153.
3. Felitti M, F.V.J., et al., Relationship of Childhood Abuse and Household Dysfunction to Many of the Leading Causes of Death in Adults: The Adverse Childhood Experiences (ACE) Study. *American Journal of Preventive Medicine*, 1998. 14(4): p. 245-258.
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**Self-evaluation:** 100%

## Research gap

### 3/21. Theoretical question: which research gap is implicit in your research?

The empirical gap behind this research project is the short-term effects of social adversity on biological mechanisms. Particularly, we want to assess if exposure to bullying is already expressed in gene and biological markers during childhood.

What we know is that both human and animal studies have identified several interrelated processes through which the social environment could be embedded, including towards inflammatory processes and, eventually, epigenetic mechanisms [1, 2]. The existing evidence showed that children when exposed to stressful experiences such as low parental socioeconomic status [3], maltreatment or bullying, tend to present high levels of cortisol deregulation and of C-reactive protein [4,5]. However, few studies were conducted among children in order to assess the extent to which such effects can be seen early in development.

References:

1. Rutter, M., Achievements and challenges in the biology of environmental effects. *Proceedings of the National Academy of Sciences of the United States of America*, 2012. 109(Suppl 2): p. 17149-17153.
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**Self-evaluation:** 100%

## Research question or hypothesis

### 4/21. Theoretical question: which research question or hypothesis is implicit in your research?

Theoretical models show that adversity, in particular cumulative adverse experiences, is early shaped on biological mechanisms. A study among 10 years-old children shows that variations in the corticotrophin releasing hormone receptor-1 is highly associated with cortisol deregulation among mistreated children. It is suggested that monocytes/macrophages will develop response tendencies that give rise to a chronic pro-inflammatory state in children early exposed to environments of harsh parenting, conflict and violence, and lacking material resources [1]. The inflammatory response is particularly affected by adverse experiences, thus this biological system may be one potential mechanism to explain the association of early social adversity with disease onset in young adults [1,2,3]. Also, it has been shown that exposure to bullying was associated with high levels of inflammation in the short term within childhood or long term into young adulthood [4, 5].

References:

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**Self-evaluation:** 100%

## State of the science

### 5/21. Theoretical question: which is the current answer to your research question or hypothesis?

Theoretical models show that adversity, in particular cumulative adverse experiences, is early shaped on biological mechanisms. A study among 10 years-old children shows that variations in the corticotrophin releasing hormone receptor-1 is highly associated with cortisol deregulation

among mistreated children. It is suggested that monocytes/macrophages will develop response tendencies that give rise to a chronic pro-inflammatory state in children early exposed to environments of harsh parenting, conflict and violence, and lacking material resources [1]. The inflammatory response is particularly affected by adverse experiences, thus this biological system may be one potential mechanism to explain the association of early social adversity with disease onset in young adults [1,2,3]. Also, it has been shown that exposure to bullying was associated with high levels of inflammation in the short term within childhood or long term into young adulthood [4, 5].

#### References:

1. Cicchetti, D., F.A. Rogosch, and A. Oshri, Interactive effects of CRHR1, 5-HTTLPR, and child maltreatment on diurnal cortisol regulation and internalizing symptomatology. *Development and psychopathology*, 2011. 23(4): p. 1125-1138.
2. Danese, A., et al., Adverse Childhood Experiences and Adult Risk Factors for Age-Related Disease: Depression, Inflammation, and Clustering of Metabolic Risk Markers. *Archives of pediatrics & adolescent medicine*, 2009. 163(12): p. 1135-1143.
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5. Takizawa R., Danese A., Maughan B., Arseneault L. Bullying victimization in childhood predicts inflammation and obesity at mid-life: a five-decade birth cohort study. *Psychol Med.* Oct 2015;45(13):2705-2715.

**Self-evaluation:** 100%

## Philosophical stance

### 6/21. Methodological question: which philosophical stance is implicit in your research?

Philosophically, this research project methodology stands on positivism, of August Comte. Positivism is a study of society that specifically uses scientific evidence like experiments, statistics and qualitative results to reveal a truth about the way society operates and functions. The main assumption regarding positivism is that it is possible to observe social life and establish reliable, valid knowledge about how it works. In this research project, we will use statistical methods to presume assumptions about the effects of bullying in inflammatory markers, adiposity and allostatic load on 10 years-old children.

#### References:

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**Self-evaluation:** 100%

## Research strategy

### 7/21. Methodological question: which research strategy is implicit in your research?

The research strategy beyond this research project is a cross-sectional study [1] using real data from a well-defined epidemiological research structure from the Institute of Public Health%u2013University of Porto. Generation 21 is an ongoing birth cohort established in 2005 in Porto, Portugal.

#### References:

1. Bernd Röhrig, et al. Types of Study in Medical Research: Part 3 of a Series on Evaluation of Scientific Publications. *Deutsches Arzteblatt International*, 2009. 106(15): p. 262268. doi: 10.3238/arztebl.2009.0262

**Self-evaluation:** 50%

## Collection techniques

### 8/21. Methodological question: which data collection techniques are implicit in your research?

This research project is integrated in the Generation 21 cohort, and all data used is from all study waves (birth, 4, 7 and 10 years of age). Information was collected by means of structured questionnaires (self-administered and administered by trained interviewers) [1], physical examinations and blood collection.

Parents were asked about socioeconomic characteristics and household conditions, child%u2019s health care use and disease diagnosis. The physical examination collected information on anthropometrics (weight, height, and waist, hip, arm, thigh and thoracic circumferences), blood pressure levels (diastolic and systolic), body composition (using tetra-polar bioelectric impedance from RJL Systems®), body weight (measured to the nearest tenth of a kilogram using a digital scale from SECA®), and height (measured to the nearest centimetre using a wall stadiometer from SECA®). Blood pressure was evaluated using an aneroid sphygmomanometer (Erka®). A fasting venous blood sample was collected from all participants to perform measurements of glucose, insulin and lipids%u2019 profile, inflammatory markers and DNA methylation.

#### References:

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**Self-evaluation:** 100%

## Analysis techniques

### 9/21. Methodological question: which data analysis techniques are implicit in your research?

This research project intends to answer to different questions that will demand different statistical approaches. Transversal to all questions, we will describe children's characteristics by bullying involvement, using means and standard errors for continuous variables and proportions for categorical variables. We will use the Chi-square test to compare proportions and logistic regression models to estimate the association of bullying involvement as dependent variable with CRP levels, and adiposity, adjusting for the potential confounders such as gender, age and parental socioeconomic status. General linear models, controlling for relevant covariates, will be used to test for effects of bullying on DNA methylation. We expect to use sequential Cox proportional hazard regressions to analyze the association between the level of bullying involvement and allostatic load.

All analyses will be two-tailed with  $\alpha=0.05$  and data conformed to the assumptions of the statistical tests, and will be conducted with STATA 9.2, SAS Institute Inc.

References:

1. Campbell, M.; Machin, D.; Walters, S.; Medical Statistics: A textbook for the Health Sciences. Fourth Edition. 2007, Wiley Editions; ISBN: 978-0-470-02519-2
2. Bland, M.; An Introduction to Medical Statistics, 4th Edition; 2015, Oxford University Press; ISBN: 978-0-19-958992-0

**Self-evaluation:** 50%

## Quality criteria

### 10/21. Methodological question: which scientific quality criteria are implicit in your research?

The quality criteria that will be adopted during this research project are external validity, internal validity, convergent validity and reliability.

The work uses tactics to increase external validity (by using data from Generation 21 cohort, that already counts on 4 evaluations, and a response proportion of 80%), internal validity (inductive reasoning for theory development and use of appropriate and validated statistical methods to achieve results), convergent validity (triangulation of thought currents comparing the results of this research project with those obtained in studies conducted in adult populations, data collection techniques and origin of data) and reliability (validation of questionnaires and scales used and software for analysis of quantitative data).

**Self-evaluation:** 100%

## Unit of analysis

### 11/21. Empirical question: which unit of analysis is implicit in your research?

In this research project we will use individual data from Generation 21 cohort and statistical tests. We will analyse the effect of involvement in bullying (using indicators of bullying scale answers [1]) in the inflammatory markers, adiposity and allostatic load (using this indicators as tertiles - last vs. other) during childhood,

References:

1. Hamburger ME, Basile KC, Vivolo AM. Measuring Bulling Victimization, perpetration and Bystander Experiences: A Compendium of Assessment Tools. Atlanta 2011.

**Self-evaluation:** 50%

## Level of analysis

### 12/21. Empirical question: which level of analysis is implicit in your research?

The level of analysis implicit in this research project is individual (children).

**Self-evaluation:** 100%

## Nature of data

### 13/21. Empirical question: which nature of data is implicit in your research?

The answer to the research questions of this project is based on statistical analysis of quantitative data, using continuous (e.g.: age) and categorical variables (e.g.: gender).

References:

1. Campbell, M.; Machin, D.; Walters, S.; Medical Statistics: A textbook for the Health Sciences. Fourth Edition. 2007, Wiley Editions; ISBN: 978-0-470-02519-2
2. Bland, M.; An Introduction to Medical Statistics, 4th Edition; 2015, Oxford University Press; ISBN: 978-0-19-958992-0

**Self-evaluation:** 100%

## Origin of data

### 14/21. Empirical question: which origin of data is implicit in your research?

The data used in this research project is secondary data. We will be using data that was collected in the Generation 21 evaluation at children's 10 year-old. Briefly, this cohort was assembled between 2005 and 2006 and includes 8647 babies born in Porto, Portugal. The recruitment was conducted in five level III maternity units by inviting all mothers who delivered a live born baby during assembling period. At recruitment, data were collected 24 to 72 hours after delivery [1]. At child's age of 4, 7 and 10 years all the cohort was invited for a new study wave (80% participation rate).

References:

1. Alves E, Correia S, Barros H, Azevedo A. Prevalence of self-reported cardiovascular risk factors in Portuguese women: a survey after delivery. International journal of public health. 2012;57(5):837-47.

**Self-evaluation:** 100%

## Sample

### 15/21. Empirical question: which sample is implicit in your research?

For this research project we will be using data from the last wave of the ongoing population based cohort, Generation 21. The cohort included 8647 children at recruitment (baseline), and we expect a sample of 4500 children in the last wave (children with 10 years of age).

**Self-evaluation:** 50%

## Pathos

### 16/21. Rhetorical question: which scientific, commercial, and public interest is implicit in your research?

The positive emotions associated with streams of thought in this research topic include a new and improved understanding of the role of adverse childhood experiences, namely bullying, on different biological mechanisms such as inflammatory markers (C-Reactive Protein %u2013 CRP, levels), DNA methylation, adiposity and allostatic load.

Contextualizing these data within a wider array of biological systems may be crucial in identifying why some individuals go on to develop physical or psychiatric disorders, whereas other remain resilient in the face of exposure to trauma or adversity. These findings may also have clinical relevance. Besides the potential impact of the increased inflammation on metabolic outcomes and physical illness, the assessment of inflammatory markers may also aid the development of prevention and treatment strategies. For example, a meta-analysis has demonstrated that higher CRP levels seem to precede the development of depressive disorders [1], and patients with increased inflammation seem less likely to respond to conventional antidepressants [2], and more likely to respond to adjunctive anti-inflammatory treatment [3].

Also, attaining the maximum functional capacity in adulthood is highly dependent on the functional capacity achieved in the early stages of life. Thus, healthy aging is dependent on healthy growth and development. The comprehension of lifelong health trajectories will allow the definition of effective interventions so that the effects of adverse childhood experiences can be reversed. Therefore, improving the health and developmental outcomes in early childhood is being suggested as a top priority in research from the Horizon 2020, focusing on Ageing and Active Life, as potential interventions are expected to have a stronger impact on individual's life course health, achieving higher returns than later interventions [4].

Thus, assessment of bullying together with inflammatory markers, adiposity and allostatic load may prove to be crucial in developing more effective prevention strategies and treatments, affecting long-term health outcomes.

Additionally, we aim to share our findings through the expected publication of several scientific papers, the attendance at national and international scientific meetings and conferences to release our work.

We believe we do not have negative emotions associated with this research project. In Generation 21, it was designed an information sheet and informed consent form, to notify all participants of the implications of their participation in the study. After parents informed consent is signed, children's are informed and consent is also asked to them. The children's will be always respected regardless parents authorization. Moreover, the present investigation complies with current national legislation and has been approved by the Ethics Committee of S. João Hospital Center/Faculty of Medicine-University of Porto and by the Portuguese Data Protection Authority.



## References:

1. Valkanova V., Ebmeier K.P. Vascular risk factors and depression in later life: a systematic review and meta-analysis. *Biol. Psychiatry* 2013; 73: 406%u2013413.
2. Cattaneo A., Gennarelli M., Uher R., Breen G., Farmer A., Aitchison K.J. et al. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline %u2018predictors%u2018 and longitudinal %u2018targets%u2018. *Neuropsychopharmacology* 2013; 38: 377%u2013385.
3. Raison C., Rutherford R.. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *JAMA Psychiatry* 2013; 70: 31%u201341.
5. Doyle O, Harmon CP, Heckman JJ, Tremblay RE. Investing in early human development: timing and economic efficiency. *Econ Hum Biol.* Mar 2009;7(1):1-6.

**Self-evaluation:** 100%

## Logos

### 17/21. Rhetorical question: which scientific logic is implicit in your research?

The scientific logic behind this research project is inductive reasoning. Theoretically, inductive reasoning occurs when the premises are viewed as supplying strong evidence for the truth of the conclusion. The truth of the conclusion is probable, based upon the evidence given [1]. So, based on previous evidence, adverse experiences during childhood have a proven effect on later health outcomes in the adult life. With this work and using quantitative methodology (statistical methods), we will be able to obtain more complete information on how soon those adverse experiences are already embedded biologically for the Generation 21 participants. We will use our data to show if bullying impacts on biological markers. We expect to inductively build a theory that supports that adverse experiences occurred during the early years, such as bullying, are already biologically embedded into multiple bodily systems, namely inflammatory markers, adiposity and allostatic load. We will then be able to move from the participants in Generation 21 to the general population.

## References:

1. Cook, Roy T. (2009). *A Dictionary of Philosophical Logic*. Edinburgh: Edinburgh University Press. p. 273. ISBN 978-0-7486-2559-8

**Self-evaluation:** 100%

## Ethos

### 18/21. Rhetorical question: which limitations are implicit in your research?

There are several limitations associated to this research project. The first is related to the few publication of opposing thought theories regarding the effect of adverse childhood experiences on biological markers, already seen in children. The main methodological limitations of this research project is the cross-sectional design, and the expectation that severe experiences must be less frequent which may result in less statistical power to show some of the relations.

**Self-evaluation:** 50%

## Wisdom

### 19/21. Authorial question: which education and experience are implicit in your research?

I have a 3-year degree in Biomedical Sciences, a Master in Pharmaceutical Medicine and 6 years of experience in several national and international research projects. Furthermore I am currently in my second year of a full-time PhD scholarship from Fundação para a Ciência e Tecnologia (FCT). My academic and scientific experience has provided me the tools, practice and knowledge to successfully conduct and finish my research. I participated in several national and international conferences where I have presented my work, and was recently part of the selected group to present at the "Young Researchers Forum", where I was awarded with the 3rd prize. As part of my daily routine I attend different meetings as part of the Epidemiology Research Unit (EPIUnit) group and the Health and Society group, and also attend weekly seminars at the Institute of Public Health of the University of Porto - ISPUP.

**Self-evaluation:** 100%

## Trust

### 20/21. Authorial question: which partners are implicit in your research?

All my work is supported by my supervisor Silvia Fraga and co-supervisor Henrique Barros, both with large experience on epidemiological studies, perinatal health and social determinants. My attendance to several meetings within my research group and department are also extremely important to have feedback, share knowledge and have different views on the ongoing project tasks. My research team is part of an international H2020 project %u2013LIFEPATH- (grant 633666), and the also partner HuGeF Foundation from Torino, Italy, that will provide us with specific laboratory analysis.

**Self-evaluation:** 100%

## Time

### **21/21. Authorial question: which availability of time and resources is implicit in your research?**

I have a full-time scholarship (SFRH/BD/108742/2015) from FCT, in Portugal. At institutional level I am team member of a FCT research project (PTDC/DTP-EPI/1687/2014), (POCI-01-0145-FEDER-016838) that partially supports my research and attendance to conferences. My research team is part of the Health and Society group and member of Social Epidemiology group of the Epidemiology Research Unit (EPIUnit) (UID/DTP/04750/2013). As part of the scholarship from FCT I have my progress monitored by yearly reports, as well as the support and monitoring from my supervisor and co-supervisor and regular meetings within the research unit and research group.

**Self-evaluation:** 100%