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À minha avó, sempre.

Ó sino da minha aldeia,
Dolente na tarde calma,
Cada tua badalada
Soa dentro da minha alma.

E é tão lento o teu soar,
Tão como triste da vida,
Que já a primeira pancada
Tem o som de repetida.

Por mais que me tanjas perto
Quando passo, sempre errante,
És para mim como um sonho.
Soas-me na alma distante.

A cada pancada tua
Vibrante no céu aberto,
Sinto mais longe o passado,
Sinto a saudade mais perto.

Fernando Pessoa

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“In a sense, the body is telling the story”

Nancy Krieger

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ABBREVIATIONS

ACEs: Adverse Childhood Experiences

AOR: Adjusted Odds Ratio

BMI: Body Mass Index

CDC: Centers for Disease Control and Prevention

CI: Confidence Interval

CRP: C-Reactive Protein

CVD: Cardiovascular Disease

HPA: Hypothalamic-Pituitary-Adrenal

Hs: High sensitivity

IL: Interleukin

IPV: Intimate Partner Violence

OR: Odds Ratio

SEP: Socioeconomic Position

SES: Socioeconomic Status

UNICEF: United Nations Children's Fund

WHO: World Health Organization

ABSTRACT

Background

Childhood is the most important period of development during life-course, highly sensitive to external influences and with a profound impact on children's lives. During this period the foundations for every individual's physical and mental health capacities and attainment are laid, influencing growth, development and well-being in adolescence and adulthood. Therefore, examining social adversity in childhood, which may include material deprivation or psychosocial stressful events, is of relevance in life-course epidemiology to understand the production of health inequalities. Social adversity during the first years of life has been associated with a variety of poor health outcomes later in life and premature death, but the short-term embodiment of social experiences already during childhood has not been widely explored. To fill this gap in the literature, we sought to ascertain the relationship between exposure to adversity and childhood biological consequences in the first ten years of life. This thesis intends to answer the following specific questions: "Can social differences in health biomarkers be found in early ages?" and "What is the influence of adverse childhood experiences on children's health?".

Methods

We used cross-sectional and longitudinal data from the prospective population-based birth cohort Generation XXI. Data on socioeconomic indicators such as maternal and paternal education, occupation and income were collected at baseline using standardized structured questionnaires. Information regarding exposure to parental disciplinary practices (Parent-Child Conflict Tactics Scales (CTS-PC)) was measured at the age of seven years, bullying (Bully Scale Survey developed by the Centers for Disease Control and Prevention) and Adverse Childhood Experiences (ACEs) were measured at the age of ten years, and information on these exposures was provided by the child. Anthropometric measurements and blood pressure were taken at four, seven and ten years of age. Also, after an overnight fast, a venous blood sample was collected, and measurements of the different blood markers on fresh blood samples were performed.

Results

We investigated whether early socioeconomic circumstances impact cardiometabolic health and inflammatory markers already in childhood. We found that children from low educated mothers presented higher body mass index, higher waist circumference, and increased blood pressure at the age of seven years. At the age of ten years, social differences were already observed in other cardiometabolic biomarkers, and remained significant, even after accounting for cumulative measures (Paper I). Also, the higher the mother's education and disposable household income,

the lower the minimum value of the log high sensitivity C-Reactive Protein (hs-CRP) observed throughout childhood. Children from less advantaged socioeconomic circumstances presented increased CRP levels from the age of four until the age of ten years (Paper II).

A systematic review allowed us to identify the most frequently studied type of adversity, and the most commonly studied biological consequences. This review showed that adverse childhood experiences were associated with biological risk already at early ages, and violence, particularly physical and sexual abuse were the experiences with the strongest association with biological markers (Paper III). Therefore, using data from Generation XXI, we were able to identify that exposure to parental extreme physical violence was biologically imprinted, and children presented higher levels of CRP as early as at the age of seven years (Paper IV). Furthermore, and grounded in the hypothesis that body mass index can be the biomarker that partially mediates the association between adversity and inflammatory markers, we conducted a mediation analysis. With this study, we observed that there might be different pathways involved in the biological embedding of childhood experiences. On one hand, the impact of exposure to ACEs on CRP seems to occur via effect of these experiences on stress mechanisms, and consequently low-grade inflammation. On the other hand, body mass index seems to mediate a great part of the association between exposure to bullying victimization and CRP levels at the age of ten years (Paper V).

Conclusion

With this thesis we provided evidence that the biological consequences of social adversity can be observed already in the first ten years of life, and as early as at the age of four years. Specifically, our results showed that differences in health biomarkers can be observed at very early ages. Adverse childhood events, particularly violence, may also be biologically imprinted in childhood. Thus, there is a potential impact of early life social adversity on physiology and metabolic dysregulation, supporting a detrimental effect of disadvantaged early life circumstances with origin in childhood. Our findings emphasize the importance of investing in social policies and families' support to provide children with a better start for better health. "Upstream" factors identified during childhood seem to represent meaningful opportunities to prevent adversity and improve health, since they are modifiable risk factors that should be targeted in local and global public health interventions to attenuate or reduce inequalities and its effects in health, starting at early ages. That way, society will be providing children with better health and wellbeing, since it is already recognized as a priority and a human right.

RESUMO

Introdução

A infância é um período fundamental no desenvolvimento da criança. É nos primeiros anos de vida que se estabelecem as bases para um crescimento saudável e consequente saúde física e mental ao longo da vida. Desta forma, estudar os efeitos da exposição a situações de stresse na infância, seja a adversidade económica ou adversidade psicossocial, é crucial para compreender a origem das desigualdades em saúde. Alguns estudos já mostram que a adversidade social vivenciada no período da infância está associada a maior probabilidade de apresentar vários problemas de saúde na vida adulta, e está até mesmo associada com maior risco de morte prematura. No entanto, a incorporação biológica destas experiências já durante a infância e o seu impacto na saúde não foi ainda muito explorado. Assim, esta tese pretende identificar as consequências biológicas da adversidade social nos primeiros dez anos de vida. Em particular, esta tese tem como objetivo responder às seguintes questões: "Podemos já observar diferenças sociais em marcadores biológicos já durante infância?" e "Qual a influência da exposição a eventos adversos de vida durante a infância na saúde das crianças?".

Métodos

Foi usada informação proveniente da coorte de nascimentos portuguesa Geração XXI. A informação sobre os indicadores socioeconómicos, incluindo a educação e a profissão da mãe e do pai, e o rendimento do agregado familiar, foi recolhida na primeira avaliação da coorte. As práticas de disciplina parental foram avaliadas aos 7 anos de idade, usando a Escala de Táticas de Conflito entre Pais e Crianças (CTSPC); o envolvimento em comportamentos de bullying foi medido usando o instrumento Bully Scale Survey (desenvolvido pelo Centers for Disease Control and Prevention); a história de exposição a experiências traumáticas de vida foi avaliada aos dez anos de idade. Esta informação foi reportada pela própria criança. A avaliação antropométrica e a medição da pressão arterial foram realizadas aos quatro, sete e dez anos de idade da criança. Nas mesmas avaliações foram recolhidas amostras de sangue, após um período de jejum de 12 horas, e no próprio dia quantificaram-se os diferentes marcadores biológicos.

Resultados

Os resultados mostraram que as condições socioeconómicas, ao nascimento da criança, tinham efeito na saúde cardiometabólica e nos marcadores inflamatórios durante os primeiros dez anos de vida. Verificou-se ainda que os filhos de mães com baixa escolaridade apresentaram maior índice de massa corporal, maior circunferência da cintura e maior pressão arterial aos sete anos de idade. Aos dez anos, as diferenças sociais observadas aos sete anos permaneceram

significativas, e surgem noutros marcadores cardiometabólicos, mesmo após ter em conta medidas repetidas (Artigo I). Também se observou que quanto mais alta a educação da mãe e o rendimento do agregado familiar, menor o valor observado da proteína C-Reativa (PCR) medida durante a infância, enquanto que crianças de contextos socioeconómicos menos favorecidos apresentavam maiores níveis de PCR entre os quatro e os dez anos de idade (Artigo II).

A revisão sistemática permitiu identificar os eventos adversos de vida e as consequências biológicas mais frequentemente estudadas na literatura. Esta revisão mostrou que os eventos adversos de vida ocorridos na infância estavam já associados a maior risco de alterações biológicas em idades precoces. A violência, e em particular o abuso físico e sexual, foram as experiências com maior associação com os marcadores biológicos (Artigo III). Assim, usando dados da Geração XXI, conseguimos observar que a exposição à violência física grave perpetrada pelos pais, foi incorporada biologicamente, e as crianças vítimas de violência apresentavam níveis mais altos de PCR, já aos sete anos de idade (Artigo IV). Além disso, e fundamentados na hipótese de que o índice de massa corporal pode ser o marcador biológico que pode mediar a associação entre a adversidade e os marcadores inflamatórios, observamos que diferentes mecanismos poderão estar envolvidos na incorporação biológica de eventos adversos na infância. Por um lado, o impacto da exposição a eventos adversos de vida na PCR parece ocorrer via mecanismos de stress, levando à ativação do eixo hipotálamo-pituitária-adrenal e, consequentemente, à inflamação de baixo grau. Por outro lado, o índice de massa corporal parece mediar grande parte da associação entre a exposição à vitimização por bullying e os níveis de PCR aos dez anos de idade (Artigo V).

Conclusão

Estes resultados parecem mostrar que as consequências biológicas da adversidade social podem ser observadas já nos primeiros dez anos de vida. Existe assim um impacto potencial da adversidade social na fisiologia e desregulação metabólica da criança, mostrando um efeito prejudicial de ambientes menos favorecidos na infância.

Enfatiza-se assim a importância de investir em políticas sociais e de apoio às famílias para que se proporcione às crianças um melhor começo de vida para uma melhor saúde a longo prazo. Fatores identificados durante a infância parecem representar oportunidades para evitar a exposição à adversidade e melhorar a saúde. Ao serem fatores de risco modificáveis, as intervenções locais e globais de saúde pública devem ser direcionadas para atenuar ou reduzir as desigualdades e os seus efeitos na saúde, começando em idades mais precoces.

1. INTRODUCTION

Childhood is the most important period of development during life-course, highly sensitive to external influences and with a profound impact on children's lives (1, 2). During this period, the foundations for every individual's physical and mental health capacities and attainment are laid, influencing growth, development and well-being in adolescence and adulthood.

Retrospective studies have demonstrated that children exposed to social adversity, which may include material deprivation or psychosocial stressful events, are likely to experience negative long-term outcomes in the adult life, including chronic illness (3-6) and premature death (7, 8). Therefore, examining these stressors in childhood is of particular relevance in life-course epidemiology for understanding the production of health inequalities. Even though, experiences of violence, maltreatment, or intra-familiar events that may be deviated from societal norms should be distinguished from events or conditions linked to the socio-economic and material environment.

Over the past century, there has been a remarkable progress in understanding the consequences of adverse childhood experiences (ACEs) (9). In the '80s, Michael Rutter set the ground for the study of stressful conditions in childhood (10), but the "ACEs study" was the first to use the term "adverse childhood experiences" and to describe a graded relationship of exposure to several stressful events and conditions occurred during childhood with several causes of death later in life (3). The hypothesis raised by these studies lies in the assumption of the physiological embedding of stress, i.e., these experiences cause chronic or acute stress responses that may alter fundamental biological functions.

Thus, two main biological pathways are proposed to explain how these experiences may "get under the skin" and be associated with later negative health outcomes: it might be explained either through the adoption of unhealthy behaviours (e.g., poor diet, sedentary behaviour, smoking) or through a direct physiological disruption of regulatory pathways responsive to stress caused by deprivation. ACEs increase activation of neurobiological systems, such as the hypothalamic-pituitary-adrenal axis (HPA) or the sympathetic nervous system (11, 12).

Social adversity during the first years of life has been associated with a variety of poor health outcomes later in life, but the short-term embodiment of social experience already during childhood has not been widely explored. However, studies using animal models have identified several interrelated processes through which the social environment could be embedded biologically (13). Also, it has been described that animals exposed to lower maternal care in the

first days of life showed that variations in the quality of maternal care shape subsequent responsivity of the HPA axis to stress (14), that will persist over the life-course (15). These findings are suggestive of childhood being a sensitive period for the effects of stress to become "embedded" in some physiological systems for the long term. Although studies in children are scarce, there is some evidence that allows hypothesizing that social adversity, in particular cumulative ACEs, is already shaped on biological mechanisms at early ages (2, 16, 17). These mechanisms, in interaction with socioeconomic and material environment, may ultimately lead to social inequalities in health.

Thus, childhood stands as a central stage to lay a base for optimizing opportunities for good health and an independent and high quality of life. As a source of stress that affects children's well-being, bodily processes, and production of health inequalities, social adversity is a key modifiable risk factor. Therefore, a focus on "equity from start" is crucial to define childhood as an important period on life that deserves special attention, and when strategies of intervention may work to prevent or make transformative modifications in the life of children exposed to social adversity. This investment would improve social and economic environments in which children grow up, live, and learn, leading to reductions in health and developmental inequalities which span the entire life-course (18). As governments and societies understood that the period between conception and the first years of life is of paramount importance to achieve a healthy adult life, also investments in education and social skills will be crucial to have informed and intervenient citizens that will participate in the development of strong and competitive policies and participate in the economy of the countries (19). These investments, that are not only economic and financial, should include citizen participation and community action, be transversal within the society, but with a special emphasis on poorer families and marginalized populations, with governments providing care for these children across the life-course (19). By providing high-quality services, including universal health care coverage, free education and good nutrition and food security for all, thoughtful urban planning, safe and affordable housing and transport, clean energy for all and equitable social welfare policies, will be possible to help those children to become engaged and productive adult citizens (19). Therefore, to guarantee a solid and steady economic and human development, each country must use all tools available to diminish the existent gap between the poorest and the richest proportion of the children population (19), by providing a better start for a better life of those in the bottom end of the society, and consequently to achieve a future with a more equal and just society.

1.1. A life-course approach to health: the importance of early exposures

Life-course epidemiology is *"the study of long term effects on later health or disease risk of physical or social exposures during gestation, childhood, adolescence, young adulthood and later adult life"* (20). Also, accumulating evidence from life-course epidemiology indicates that many diseases typically diagnosed in adulthood have social and physiologic antecedents much earlier in life. Thus, life-course epidemiology has been particularly focused on studying the long term effects of childhood and adolescent risk factors on later disease and mortality, namely on how socially patterned experiences during childhood, adolescence, and in early adult life influence risk of disease and socioeconomic circumstances in adulthood (20).

Childhood is defined by the World Health Organization (WHO) as the period between prenatal development to the age of ten years and has been recognized as the most critical period of development in the life-course (21), as well as the most highly sensitive to external influences (22). Thus, studies of the biological embedding of early life experiences have focused largely on prenatal or childhood life, and have led to the understanding that periods of rapid organ system development during these phases of life are critical to adult health (20).

The concept of sensitive periods in life-course epidemiology is borrowed from concepts originally used by neurobiology and physiology (23). According to the life-course approach, a sensitive period is a time window during which exposures can lead to lasting physiological changes in the organism. In its stricter form, no excess risk would be observed if the exposure occurred in periods outside the window (20, 24). Also, sensitive periods are times of rapid individual change, but there is an opportunity to modify or even reverse those changes outside the time window (20). These periods of human development hold a central place in policy discussion, drawing attention to "windows of opportunity" for prevention and intervention. The roots for physical and mental capacities are laid in these first years of life, influencing growth, health and development throughout life.

Acknowledging the importance of the early years, the Commission on Social Determinants of Health's *Closing the Gap in a Generation* report proposes that *"equity from the start"* is a crucial factor of any attempt to improve health outcomes overall and, in particular, to address health inequalities (18). Also, the WHO Europe's Review of Social Determinants and the Health Divide supports this message and defends that *"the strongest instruments to break the vicious circles of disadvantage lie in the start of life"* (25). Understanding a child's development through the life-

course requires a broader framework, including the social processes affecting households, like these, in turn, impact children's lives (26).

Epidemiological approaches typically use statistical techniques drawing on individual-level data to identify associations between early circumstances and later health outcomes. Later health outcomes can either be an accumulation of consequences due to health-damaging behaviours acquired over time or as the result of sensitive periods with long-term consequences. Nevertheless, the foetal origins hypothesis recognises that critical periods of deprivation can occur even before birth (27, 28). A study focusing on the health of children conceived during the Dutch famine at the time of World War II, identified a link between foetal malnutrition, caused by the famine, and worse adult health, with the degree of impact depending on the stage of pregnancy in which the famine was experienced (29).

The interplay of brain and biological development with the environment is the driving force of growth. The human brain develops and changes throughout life, but the period of fastest brain growth and the period of highest plasticity occurs in the last pregnancy trimester and the first two years of life, the so-called "first 1000 days of life" (30). In the early years, the child is highly exposed to the influences of the external environment, with sensitive periods during growth influencing brain development (31), characterized by rapid rates of neuronal proliferation (number of cells), growth and differentiation (complexity), myelination, and synaptogenesis (connectivity) (30). The way early experiences interact by shaping brain and biological development over the life-course are known as biological embedding (31). Children grow up and develop in specific physical, social, cultural, economic and historical circumstances (their socio-cultural context and consequently the social determinants of health) (32), all of which will influence their childhood and growth into adulthood. Children's socio-cultural context can have a large influence on their development that will be different depending on the cultural environment surrounding them (33).

Thus, children are social beings shaped by the environment in which they are growing. Children will achieve their highest development in a warm, responsive and safe environments that protect them from inappropriate disapproval and punishment, where they can find opportunities to explore their world, to play, and to learn how to interact with others (34).

1.2. Social inequalities in children's health

According to the World Health Statistics 2011, there is a 36-year gap in life expectancy between countries: while a child born in Japan could live for as long as 83 years, a child born in Malawi could expect to live for only 47 years (35); in Chad, one in every five children dies before they reach the age of 5, while in the WHO European Region, the under-five mortality rate is 13 out of 1000 (35). Also, in the European Region, the country with the highest life expectancy is San Marino (83 years), and the country with lowest life expectancy is Ukraine (68 years). A child born in Portugal has a life expectancy of 79 years, four more years than the life expectancy for the all European region (75 years) (35). There is no biological or genetic reason for these disturbing differences in health and life opportunity to occur across countries. However, the economic and political circumstances of countries vary largely, and these two dimensions are determinant in mitigating or enhancing health inequalities. Besides the differences in health observed across countries, there are substantial gaps in health outcomes that are observed within a country, with differences in social status, income, ethnicity, gender, disability and/or sexual orientation being translated in health differences in the various group populations. In the United States of America for instance, infants born to African-American women are 1.5 to 3 times more likely to die than infants born to women of other ethnicities (36). In Europe, differences in income are associated with lower life expectancy, increased risk of mental illness, obesity, infant mortality, teenage births, homicides, imprisonment, educational attainment, and lower levels of social mobility (37). As depicted in Figure 1 income inequalities are linked to worse health and social outcomes between countries.

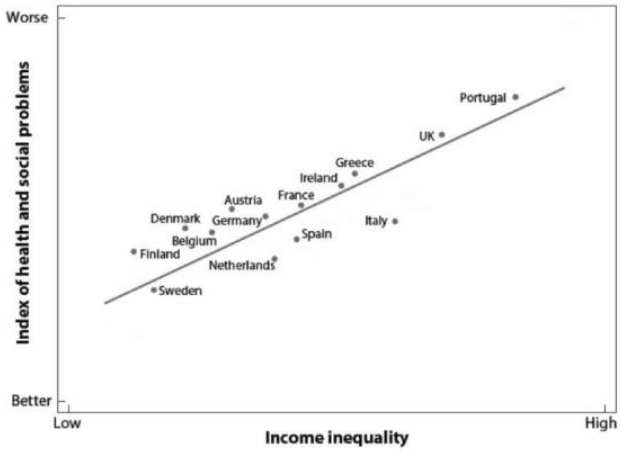

Figure 1. Adverse consequences of income inequality. Reproduced from Pickett & Wilkinson (2015, p. 317), including countries of the European Union and the United Kingdom *(Note: Index of health and social problems in relation to income inequality in selected European Union countries* and the *United Kingdom. Income inequality is measured by the ratio of incomes among the top to the bottom income quintile in each country. The index combines data on life expectancy, mental illness, obesity, infant mortality, teenage births, homicides, imprisonment, educational attainment, distrust, and social mobility.).*

Recent evidence suggests that socially patterned health differences may be widening (38-40), calling for consistent attention to the issues of health inequalities. And, although countless resources and outcomes are unevenly distributed across nations and social groups, health differences can be viewed as particularly intolerable and inconceivable from a human rights perspective (41, 42). Researchers are now challenged to understand how socioeconomic circumstances "get under the skin" to produce these inequalities.

1.2.1. Pathways to childhood inequalities

In adulthood, health inequalities are usually captured according to the socioeconomic position. However, as children do not have their socioeconomic position they are categorised according to a set of characteristics of their parents (education, occupation), household (income) and neighbourhood (area deprivation), this is, their health is the result of their circumstances. Therefore, throughout this scientific work, the term socioeconomic circumstances is employed to describe childhood inequalities.

Early life socioeconomic circumstances have been shown to predict risk factors and manifest disease later in life (43-45). The link between disadvantaged socioeconomic circumstances and cardiometabolic conditions, including cardiovascular disease (CVD) (46, 47), diabetes (48, 49) and obesity (50) is well established. Moreover, exposure to disadvantaged socioeconomic circumstances during the first years of life, until adolescence, has been shown to be predictors of CVD (51), indicating that socioeconomic circumstances are modifiers of health in childhood, and not only in adulthood.

Several studies have found that the more socially disadvantaged are at greater biological risk of developing disease. Furthermore, a persistent socioeconomic gradient in the biological health score was observed (52, 53), with those living in poorer socioeconomic conditions presenting worse health outcomes. Other studies showed an increased risk of coronary heart disease and CVD in participants with low adulthood socioeconomic position (SEP) (54), exposed to childhood adversities, including financial hardship, with a dose-response association where the more adversities reported, the greater the risk (55). In a biracial community-based study, it was found that worse SEP in early life was associated with an increased risk of cardiovascular disease mortality (56) and incident heart failure (46). However, when accounting for adulthood SEP the association was attenuated (46). Most of this evidence comes from studies using retrospective data collection. In addition, cross-sectional studies showed that low childhood socioeconomic status, measured by parental education and occupation, was associated with an increase in inflammatory levels in adulthood (57).

Socioeconomic inequalities are observed in nearly all aspects of children's physical and mental health (58). Children living in more socioeconomically disadvantaged circumstances experience worse health than their more advantaged counterparts (2, 58). Figure 2 displays the degree of inequality for several important mental and physical health outcomes in the United Kingdom. Children in the highest income quintile have better health indicators than children in the lowest income quintile.

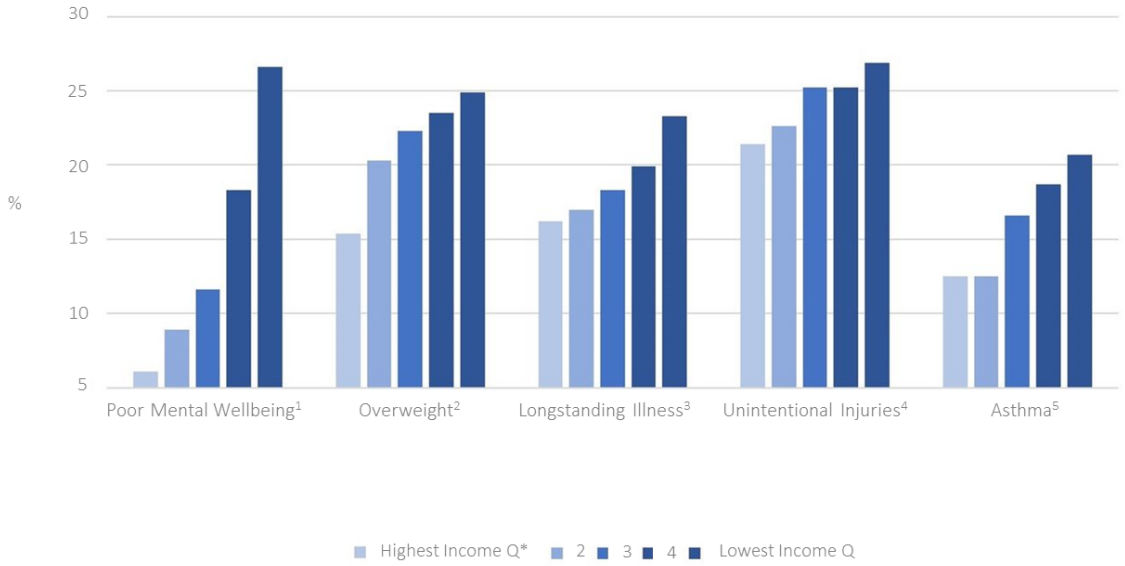

Figure 2. Child health inequalities, UK Millennium Cohort; Reproduced from the University of London. Institute of Education (2008, 7th Edition, http://doi.org/10.5255/UKDA-SN-6411-7) (*Note:[1]Borderline—abnormal total difficulties score, using the parent-reported Strengths and Difficulties Questionnaire. [2]Including obese, applying International Obesity Task Force cut-offs to measured body mass index. [3]Parent report of conditions that have troubled or are likely to trouble the child for a period of time. [4]Medical opinion sought for one or more unintentional injuries occurring since the last survey (~5 years). [5]Parent report of the child has ever had asthma. *Quintiles, based on Organisation for Economic Co-operation and Development equivalised household income.).*

In a Portuguese cohort, it was observed that the prevalence of chronic low-grade inflammation during adolescence was significantly higher among participants with low childhood socioeconomic conditions (59). In children, some authors have shown associations between lower parental income, educational attainment and occupational social class with a wide range of poor health and developmental outcomes in early childhood (2). Other study indicated that both low parental education and household income were associated with higher C-Reactive Protein (CRP) in childhood, with body mass index (BMI) partially mediating these associations (17).

A model proposed by Anna Pearce (32) and colleagues, based on Bronfenbrenner's Ecological Systems Theory of Child Development (60) and Social Model of Health by Dahlgren and Whitehead (61) is depicted in Figure 3, representing the social determinants of health and their proximity with the child. The child inner characteristics (such as age, sex and ethnicity) is

represented in the centre of the semi-circle and is surrounded by concentric layers of potentially modifiable social determinants. The innermost layer includes the determinants that are most proximal to individual health, such as health behaviours and lifestyle factors. And, the more distal, the less influential, until the outer layer, where the macro-level political, cultural, commercial and economic conditions can be observed. All of the social determinants of health are inter-related, both within and between the layers, as depicted by the arrows (32).

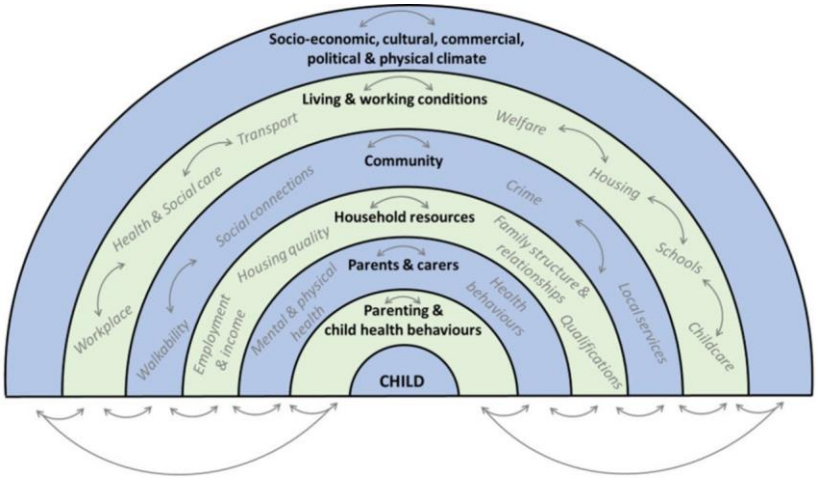

Figure 3. Social determinants of child health. Reproduced from Anna Pearce (2019,http://dx.doi.org/10.1136/ archdischild-2018-314808).

One theoretically key pathway linking low socioeconomic circumstances to poor children's health is through exposure to chronic stress and related biological changes (62-64). Biological research evidence proposes that exposure to chronic stress may result in dysregulation of the neuroendocrine and immune systems and these systems may be particularly sensitive during childhood (65, 66).

Although such changes do not always lead to disease, the underlying atherosclerotic process has a long asymptomatic phase of development that often starts during early childhood (67-70), tracks over time and can predict the onset of disease several years later (71).

1.3. Adverse childhood experiences and health outcomes

Adverse childhood experiences are potentially traumatic events that occur in childhood and adolescence, until the age of 18 and encompass various aspects of family dysfunction such as experiences of sexual abuse, physical or emotional abuse, and physical neglect, witnessing parental intimate partner violence (IPV) or experiencing other types of home violence (e.g. parental disciplinary practices). Additionally, having a family member attempt or die by suicide, growing up in a household witnessing substance abuse, mental health problems and instability due to parental separation, divorce or incarceration (3) are also considered ACEs. Factors such as peer victimization (bullying), single-parent household, and low socioeconomic status have also been included as other possible examples of ACEs (72-74).

Since the publication of the ACEs Study by the Centers for Disease Control and Prevention (CDC) and Kaiser Permanente in 1998, and over the past years, there has been tremendous progress in understanding ACEs and well documenting their association with health and well-being across the life span (3, 4, 75). Thus, exposure to ACEs is preventable and a key modifiable risk factor for several health outcomes (75) (Figure 4). Despite this progress and the increasing bulk of literature, victims of ACEs still have poorer long-term outcomes, low educational achievements and employment potential (3, 75-77). Victims of ACEs are key to study these experiences and its consequences as they can describe their experience and provide insights on the factors that may buffer or accentuate the risk of negative outcomes. However, many of these experiences (e.g. IPV, abuse) may remain hidden since the perpetrators have an interest in hindering reports and detection.

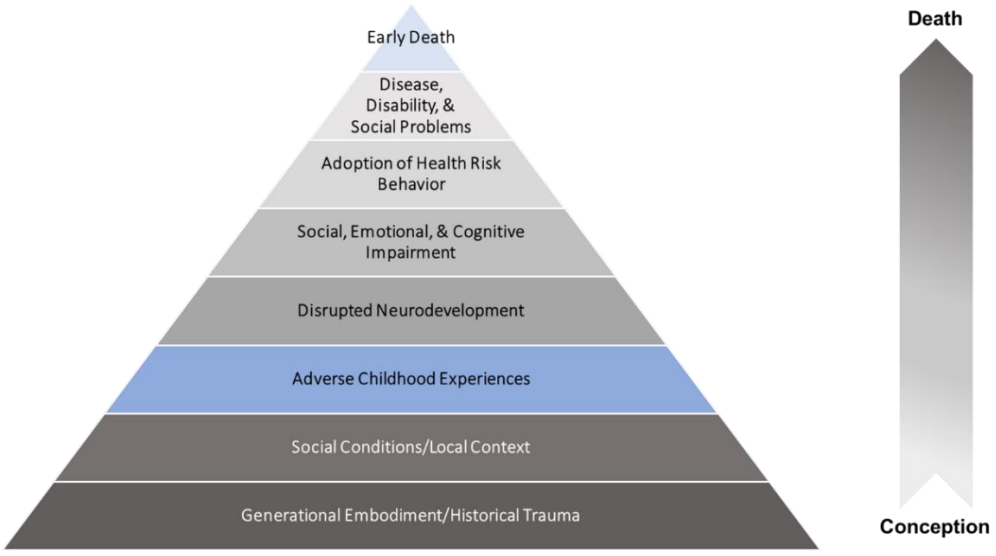

Figure 4. ACE Study Pyramid of ACEs across the Lifespan. Reproduced from CDC-Kaiser ACE Study.

The European status report on preventing child maltreatment documented a high prevalence of child maltreatment: 9.6% for sexual abuse, 16.3% for physical neglect, 18.4% for emotional neglect, 22.9% for physical abuse, and 29.6% for emotional abuse (78). More recently, a systematic review of evidence published between 1990 and 2015 estimated prevalence of ACEs in school-aged youth ranging from 41% to 97% (79). This variation might be explained by the operational definition of ACEs, the assessment, the recall period and the setting. In Portugal, few studies assessed the self-report of ACEs (80, 81). Among a sample of students from a Portuguese college, parental substance abuse was the most commonly reported form of ACES (21.9%), followed by mental illness and suicide of a family member (20.0%), and parents' divorce (18.1%) (80). According to the WHO, one in four adults reports having been physically abused as a child by their parents or other caregivers (82). Corporal punishment during childhood in the form of hitting, punching, kicking or beating, is a common form of parental discipline towards their children is socially and legally accepted in some countries (83). However, corporal punishment is responsible for thousands of deaths during childhood each year and for survivors, it has been associated with other problems in childhood and later in life (83).

Additionally, a systematic review reported increased odds of depressive disorders in victims of physical abuse (Odds Ratio (OR) = 1.54; 95% Confidence Interval (CI) 1.16–2.04), emotional abuse (OR = 3.06; 95% CI 2.43–3.85), and neglect (OR = 2.11; 95% CI 1.61–2.77); of drug use in victims of physical abuse (OR = 1.92; 95% CI 1.67–2.20), emotional abuse (OR = 1.41; 95% CI 1.11–1.79), and neglect (OR = 1.36; 95% CI 1.21–1.54); and of suicide attempts in victims of physical abuse (OR = 3.40; 95% CI 2.17–5.32), emotional abuse (OR = 3.37; 95% CI 2.44–4.67), and neglect (OR = 1.95; 95% CI 1.13–3.37) (84).

Stressful events, namely the ones within the familial context, occurring from conception until adolescence cause a cascade of physiological responses that may lead to an adaptive biological response during sensitive periods of development. This may alter a persons' biology in a way that may deviate them to a trajectory of vulnerability and increased risk of chronic disease over their life-course. Studies using animal models, where rat pups were firstly separated from their mothers and then reunited, identified epigenetic alterations on gene receptors involved in the stress response and functional changes in physiological systems in the rat mothers (15). Changes were also found in stress reactivity of the offspring, with female offspring of maternal high licking/grooming and arched-back nursing being behaviourally less fearful and showing more modest HPA axis responses to stress than do the offspring of low licking/grooming and arched-back nursing mothers (85). Even though strong scientific cautions are needed when examining biological literature of animal models, these have the advantage of demonstrating causal

relationships between exposures and outcomes and allow to experimentally manipulate the environment by randomly assigning animals to early-life stress conditions, regardless of the pre-existing characteristics.

As it can be difficult to study childhood experiences during the first years of life, much of what is currently being studied is based on studies of adults who recall their experiences. Thus, most of the research has been based on retrospective measures, self-reported by adults.

According to the CDC, in a recent report including data from 144 017 adults, 60.9% of participants had experienced at least one type of ACE, and 15.6% reported experiencing four or more types of ACEs (77). In the same report, a graded dose-response association was found between the number of ACEs and a range of negative health outcomes. Other studies showed an association between the number of ACEs and later health conditions, including mental health, cancer and cardiovascular disease (4, 86, 87). Evidence suggests that ACEs affect neurological, hormonal, and immunological body systems (12, 88). Exposure to adverse experiences during childhood has also been linked to increased risk of engaging in unhealthy behaviours, including smoking, harmful alcohol consumption, and drug use, in adult life (4, 86, 88, 89).

It was observed that adults reporting more ACEs had higher odds of having chronic health conditions (Adjusted OR (AOR)=1.2; 95% CI = 1.1–1.3 for overweight or obesity; AOR= 2.8; 95% CI = 2.5–3.1 for chronic obstructive pulmonary disease), compared with adults reporting no ACE exposure. After adjusting for age, sex, and ethnicity, odds of depression (AOR = 5.3; 95% CI = 4.9–5.7), being a current smoker (AOR = 3.1; 95% CI = 2.8–3.3) or heavy drinker (AOR = 1.8; 95% CI = 1.6–2.0), and socioeconomic challenges including current unemployment (AOR = 1.7; 95% CI = 1.5–2.0) were also higher among adults with the highest levels of ACEs, compared with those reporting no exposure to ACEs (77). A prospective study also showed a dose-response association between childhood maltreatment experiences during the first decade of life and high inflammation levels at age 32, although authors recognize that some residual confounding may be taken into account for this long-term association (5). Thus, ACEs are associated with increased levels of biomarkers for inflammation (90, 91), shortened telomeres (92), DNA methylation (93), neurobiological alterations (94), among others, which is consistent with direct effects of ACEs on chronic diseases such as cancer (95), cardiovascular disease (88), and respiratory disease (96).

A growing body of research has confirmed that childhood adversity is associated with increased risk of adult chronic disease and premature death. Thus, early detection and intervention can have a positive, lifelong impact on an individual's health and well-being. However, a smaller

number of studies have attempted to collect data on actual experiences of childhood adversities using information prospectively gathered during childhood through official records, parental reports and, self-reports from children, and have then followed up participants over time. Exposure to childhood adversity has been linked to the dysregulation of the neuroendocrine-immune circuitry, through a mechanism that is influenced by genetic, social, and biological factors, which results in alterations of brain architecture and other organ systems during sensitive periods of development (97, 98).

Evidence shows that children exposed to adversity have an increased likelihood of paediatric health outcomes, including physical and developmental health (99, 100). Also, children victims of neglect and abuse presented greater amygdala volume (101, 102) and smaller anterior cingulate cortex and orbital frontal cortex volume (103-105) than children who did not report abuse nor neglect. Also, children with histories of exposure to ACEs, especially those with parental substance abuse, may have higher levels of inflammation (106).

A recent systematic review, using data from longitudinal studies, shows an association between childhood adversity and increased risk of cognitive delays, asthma, infections, somatic complaints and sleep disruptions (107). These health outcomes represent a range of conditions that reflect the multiple systems impacted by a chronically dysregulated stress response in childhood. Thus, these results provide insight into the association between early manifestations of a dysregulated stress response and biological health outcomes.

While most of the studies of ACEs include adverse experiences mainly occurring in a familial context, bullying experiences at school cannot be discarded. Although bullying in childhood has been mostly related with psychosocial outcomes, these experiences might also be associated with adverse physical health functioning, namely sleep problems, abdominal pain, appetite suppression, headaches, and increased frequency of illnesses (108-111). Evidence shows that during childhood and adolescence, the number of waves at which the child was bullied predicted increasing levels of CRP (16). However bullying has its specificities depending on the role of the involvement, and although CRP levels rose for all participants from childhood into adulthood, being bullied predicted greater increases in CRP levels, whereas bullying others predicted lower increases in CRP compared with those uninvolved in bullying (16).

Though some evidence focuses in the association of ACEs with biomarkers, the growth of research and technology in this domain will help to explain the biological mechanisms underlying the relationship between adversity and health outcomes already in childhood.

Socioeconomic circumstances are important to the definition of individual experiences, exposures, and behaviours. Thus, socioeconomic circumstances are a major determinant of wellbeing and physical health. Lower socioeconomic circumstances are usually associated with concurrent exposure to other stressors, as daily hassles, ACEs, and perceived burdens (112-115). Also, those in lower socioeconomic circumstances generally have fewer psychosocial resources (116, 117), and might be more prone to interpret ambiguous situations as threatening, replicating beliefs shaped by more adverse exposures during lifetime (118).

1.4. The embodiment of social adversity: biological pathways

"How do we live is a profoundly social and biological question because, as implied by the concept of embodiment, what we manifest in our bodies is simultaneously an expression of our experiences in the world and their literal incorporation within us.". Based on this assumption, the social, material, and ecological circumstances into which we are born, grow, and live are responsible for the health patterns of the society, including social disparities in health (119).

Since developmental processes occur at different periods throughout childhood, they vary in their biological and behavioural complexity and they yield more plastic and adaptive physiological and cognitive functions. According to the Strachan-Sheikh revised model of life-course, ageing is a broad concept of the two-stages process: build-up and decline (120). The build-up stage extends from early intrauterine life to late adolescence and is characterised by different phases. The build-up is also characterized by a rapid development where a biological system can be more sensitive to environmental exposures and deviations from "normal" exposures (121). Although the mechanisms explaining the involvement in the biological embodiment of social adversity are poorly understood in early ages, accumulating evidence suggests that adversity may become programmed molecularly, leaving behind biological memories that can persistently translate into an increased susceptibility of disease later in life (13-15).

Exposure to social adversity during childhood may result in early life stress that has the potential to alter physiological systems. This association may occur through a direct or an indirect pathway. A more direct pathway occurs via physiological disruption of regulatory pathways responsive to stress caused by adversity. Indirectly, it can be explained by the adoption of unhealthy behaviours (e.g., poor diet, sedentary behaviour, smoking), that may contribute to explain social differences in inflammation, with those from less advantaged socio-economic circumstances being more prone to engage in more unhealthy risk behaviours (122, 123). Even though in adult's, behaviours are fully established, it is not expected the same contribution of health-related behaviours in children.

ACEs result in a variety of physiological changes in children (12), including epigenetic mechanisms (13, 15), alteration of neural function and structure (13-15), increased activation of neurobiological systems, such as the HPA axis or the sympathetic nervous system (11, 12). Increased activation of these systems leads to a cascade of physiological processes (11, 12), which in adults, has been linked with the development of central fat, dysregulated carbohydrate

metabolism and the accumulation of blood lipids in the arterial lining, all of which accelerate chronic disease development (124) (Figure 5).

Some evidence also suggests that this may occur earlier in life namely during childhood. In fact, maltreated children present structural and functional differences in a number of neural regions, namely the corpus callosum, amygdala, hippocampus, prefrontal cortex, and cerebellum, when compared to non-maltreated children, and those differences can be observed during childhood and maintained until adulthood (125-127).

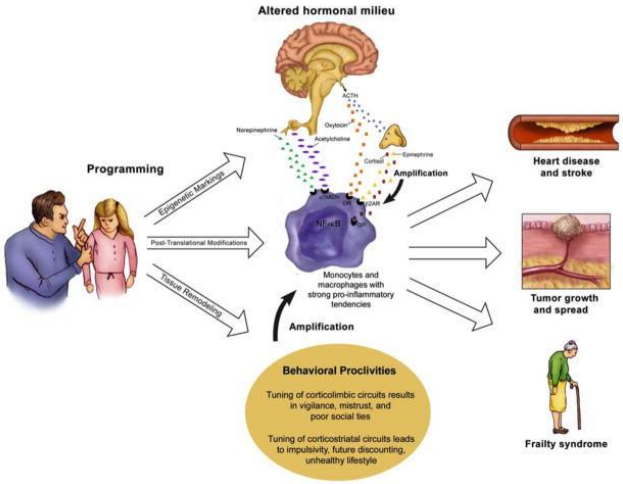

Figure 5. The Biological Embedding of Childhood Adversity Model. Reproduced from Gregory E. Miller (2011, p. 959–997.doi: 10.1037/a0024768) (*Note: ACTH = adrenocorticotropic hormone; α7nAChr = α7 subunit of nicotinic acetylcholine receptor; β2AR = β2 adrenergic receptor; GR = glucocorticoid receptor; OR = oxytocin receptor).*

Researchers have focused on the underlying mechanisms that explain how ACEs can lead to the onset of disease and inflammation has been pointed out as one of the potential mechanisms linking adversity to health outcomes (11, 128). Inflammation is a natural and early response of the immune system to pathogens and injured tissue. In response to damaged tissue or infection, immune communication molecules known as pro-inflammatory cytokines, facilitate the clearance of the pathogen and injured tissue from the body and promote repair. Thus, inflammation is essential to healing and survival.

However, prolonged inflammatory states can also be harmful. Inflammation has been implicated in a number of chronic diseases, such as atherosclerosis (129, 130), diabetes (131), hypertension (132), depression (133) and some cancers (134, 135). Also, CRP, a marker of inflammation, is considered a robust and reliable predictor of cardiovascular disease with clinical cut-off points indicative of risk (136).

The immune system interacts with the central nervous system, via the HPA axis and the autonomic nervous system (137, 138). Thus, early adversity induces changes in neural development that may give origin to downstream inflammatory processes and consequently impact health.

Exposure to ACEs has been related to changes in neural regions that belong to a network that regulates the HPA axis and the autonomic nervous system activity in the presence of a stressor. In turn, the inflammatory response is regulated by the HPA axis and the autonomic nervous system. Thus, altered neural development in these regions can lead to more frequent, prolonged, and/or exaggerated HPA axis and autonomic nervous system responses to subsequent exposure to ACEs. Such responses can lead to dysregulation of the HPA axis and autonomic nervous system, and, as both regulate the inflammatory response, their dysregulation over long periods can promote inflammation, thereby increasing the risk of later health outcomes.

1.5. The Public Health approach to childhood social adversity

The Universal Declaration of Human Rights, in its Article 1 states: *"All human beings are born free and equal in dignity and rights"* (139). However, all over the world economic and financial crises, war, public health emergencies and climate change have threatened this right. In fact, these factors have contributed to a more unequal global society, and inequalities regarding income and wealth have emerged as an important area of interest and to intervene (140). Thus, the 2030 Agenda for Sustainable Development, adopted by all United Nations Member States in 2015 in its article 10 indicated that the Member States should *"Reduce inequality within and among countries"*. It is well documented that richer households are more likely to have better social and health outcomes than poorer households. Also, health and social problems are worse in more unequal countries (37). Moreover, countless resources and outcomes are unevenly distributed across nations and social groups, that are translated in health differences and are a particularly intolerable form of social discrimination from a human rights perspective (41, 42). Inequalities produce differences in access to health, education, housing and inhibits people's equal access to justice and political participation (141). Thus, health inequalities refer to any measurable aspect of health that varies across individuals (142). On the contrary, health inequities are a specific type of health inequality that denotes an unjust social difference in health. Health inequities are systematic health disparities that can be preventable and are unnecessary and avoidable by reasonable means (143, 144).

Also, the Convention on the Rights of the Child, an important agreement by countries of the United Nations, compromised to protect children's rights, and in its article 19 states *"Freedom from abuse"*, where the member states are bound to *"protect the child from all forms of maltreatment by parents or others responsible for the child's care and shall establish appropriate social programmes for the prevention of abuse and the treatment of victims"* (145). However, statistics still reveal that children experience violence across all stages of childhood, in various settings, and frequently perpetrated by family or friends, with whom they interact daily. According to the United Nations Children's Fund (UNICEF), three-quarters of children aged 2 to 4 are regularly victims of violent parental disciplinary practices (physical punishment and/or psychological) (146). When in school, relationships with friends and peers have the potential to contribute to a child's sense of well-being and social competence (147) but are also associated with exposure to other forms of victimization, like bullying. Worldwide, more than 1 in 3 students between the ages of 13 and 15 experience bullying (146). A study found a proportion around 30% of bullying victimization for a sample of children aged 7–8 years (148), while others reported a

proportion of victimization at the age of 10 years between 14 to 16% (149). The harmful effects of being maltreated or abused by an adult, to be a victim of bullying or to witness serious domestic violence are well-defined. Many children exposed to violence will develop behavioural, emotional and/or learning problems (150). But less is known if adverse experiences like exposure to violence can lead to hidden biological alterations which may have detrimental effects on short-term health.

Provide children with better health and wellbeing is a priority and a human right. Child wellbeing is anchored in rights and equity across their life-course, to enhance protective factors and mitigate vulnerability.

Childhood is a special time of vulnerability but is also a special time for opportunity. Successful societies invest in their children and protect their rights. Intervening in childhood does not only achieve child health goals but also derive key benefits for future generations. Putting child wellbeing at the centre of the Sustainable Development Goals agenda is one of the main objectives of the WHO–UNICEF–Lancet Commission on Child Health and Wellbeing (19, 151).

Some children are born and growing in disadvantaged circumstances, and by consequence are at-risk of not attaining a healthy and fulfilled adult life. Exposure to socioeconomic hardship, violence or other types of adversity may prevent children from achieving their full potential in adult life, and potentially become dysfunctional adults, and with increased risk of developing chronic disease and even premature death. However, some strategies have been suggested to help societies mitigate the effects of social adversity, such as integration of behavioural healthcare in families with children, offer of support to parents, availability of peer-based education and identification of community resources to help boost resilience and moderate the effects of adversity. In fact, some children seem to surpass histories of adversity, and prosper against the odds, as they adapt strengths and take advantage from protective factors to overcome adverse experiences and thrive, becoming resilient (152). An investment in strategies for resilience will provide children with tools to develop good mental and physical health turning them better prepared to bear, adapt to, and thrive after exposure to adversity (152).

To provide people with tools to promote health, public health authorities, advocates, governments, societies and communities are urged to promote health policies, tackle health inequities and develop investments to achieve equity. Thus, public health policymaking will be urged to develop transformative resilience capacities.

2. STUDY RATIONALE AND OBJECTIVES

The biological imprinting of social adversity increases disease risk later in life. However, while the disease emerges in adulthood, the embodiment of these experiences is likely to begin in early childhood. Identifying the biological consequences of exposure to social adversity during childhood may be essential to the understanding and reducing of health inequalities. However, little focus has been given to the effect of these exposures in the first years of life.

To fill this gap in the literature, we sought to ascertain the relationship between exposure to adversity and childhood biological consequences in the first ten years of life. Using cross-sectional and longitudinal data from the Portuguese birth-cohort Generation XXI, this thesis intends to answer the following specific questions:

1. Can social differences in health biomarkers be found in early ages?

    a. Do children born in less advantaged socio-economic circumstances grow and develop in a worst cardiometabolic milieu since early life? (Paper I).

    b. Do different socioeconomic circumstances at birth influence trajectories of C-Reactive Protein, since the age of four years? (Paper II).

2. What is the influence of adverse childhood experiences on children's health?

    a. Which adverse childhood experiences may have a potential immediate impact on physiological systems? (Paper III).

    b. Is there an association between exposure to violence and high-sensitivity C-reactive protein in children at the age of seven years? (Paper IV).

    c. Is the association between ACEs, and bullying victimization, with high-sensitivity CRP, mediated by BMI? (Paper V).

3. PARTICIPANTS AND METHODS

The objectives of this thesis were accomplished through the analysis of data obtained from Generation XXI birth cohort study, since baseline through follow-up evaluations at four, seven and ten years of age. Specifically, socioeconomic indicators such as maternal and paternal education, occupation and income, were collected at baseline. Information regarding exposure to social adversity, such as exposure to parental disciplinary practices, bullying and information on cardiometabolic and inflammatory biomarkers, was retrieved at the ages of four, seven and ten years. A general description of Generation XXI participants and data collection procedures is provided below. Additional details about the study have been published elsewhere (153, 154). The selection of participants eligible for each paper analysis depends on the specific objectives of the research paper and is described in detail in the methods sections of each chapter.

3.1. The Generation XXI cohort study

Generation XXI is the first Portuguese population-based birth cohort study and was assembled during 2005 and 2006 in the Porto Metropolitan Area. It was established as a multi-purpose prospective population-based cohort that aims to characterize prenatal and postnatal growth and development and to identify health determinants. That is, it aims at better understanding health and its determinants using a prospective life-course approach, which will allow generating knowledge that will contribute to health gains among the population.

3.1.1. Participants

Recruitment was conducted between April 2005 and August 2006 at the five public maternity units of Porto Metropolitan Area providing obstetrical and neonatal care covering, at the time: Centro Hospitalar de Vila Nova de Gaia, Centro Hospitalar do Porto - Maternidade de Júlio Dinis, Hospital de São João, Centro Hospitalar do Porto - Hospital de Santo António and Unidade Local de Saúde de Matosinhos - Hospital Pedro Hispano. All maternities were level III units, with differentiated perinatal support, and in 2004, were responsible for 91.6% of the deliveries in the whole catchment population, with the remaining occurring in private hospitals/clinics.

All women living in the recruitment area - one of the six municipalities of the metropolitan area of Porto (Figure 6) - and who delivered a live-born child with more than 23 weeks of gestation in one of the five maternity units during the recruitment period, were eligible to participate. Seventy per cent of the eligible mothers were invited and, of these, 91.4% accepted to participate. The cohort study enrolled a total of 8647 infants and their mothers (n=8495).

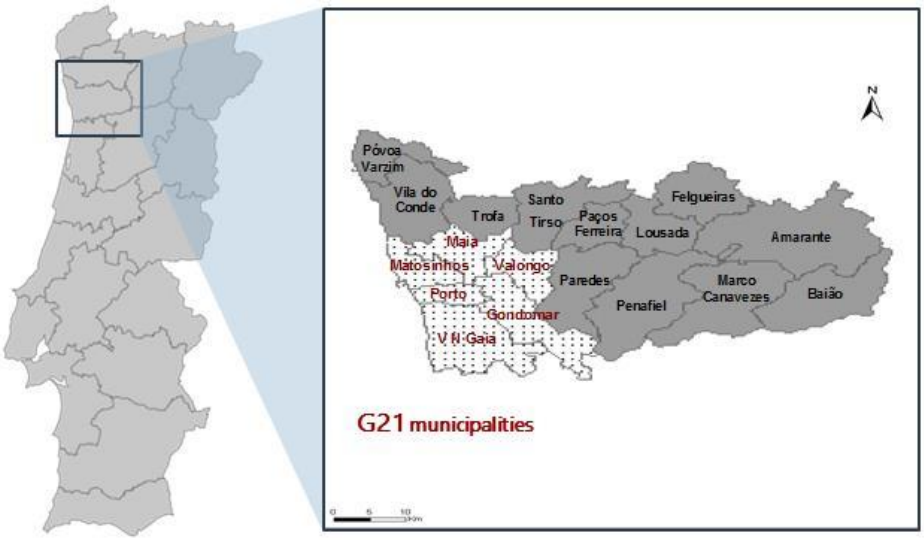

Figure 6. Representation of the six municipalities of the metropolitan area of Porto.

3.1.2. Ethical considerations

Generation XXI study protocol complies with the Ethical Principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Hospital São João and the University of Porto Medical School. The study protocol also follows the national legislation and is registered with the Portuguese Data Protection Authority. Procedures were developed to guarantee data confidentiality and protection. All participants were informed about the purposes and design of the study. Signed informed consent was obtained from all parents or legal guardians, and oral assent was obtained from children at each evaluation.

3.1.3. Data collection

At baseline, in the first 24 to 72 hours after delivery during the hospital stay, trained interviewers placed at the five hospitals were responsible for presenting the Generation XXI study and for inviting mothers to participate. Data were collected in a face-to-face interview and clinical records at birth were reviewed by the trained interviewers.

Subsamples of the cohort were evaluated at the ages of 6 months (n=1555), 15 months (n=1043) and 2 years (n=855). Four years after birth, between April 2009 and July 2011, a follow-up evaluation of the entire cohort was performed and 7459 children (86.3% of the cohort) were re-evaluated. Of these, 5987 children (69.2% of the cohort) were evaluated in a face-to-face interview while for 1472 children (17.0% of the cohort), the ones unable to attend an in-person

evaluation, a shorter version of the questionnaire was completed by their legal guardian via telephone. From April 2012 to April 2014, all families were invited to attend the 7-year follow-up evaluation of the cohort. Overall, 6889 children were reassessed (79.7% of the entire cohort), of which 5849 children (67.6% of the cohort) were evaluated in person and 1040 (12.0% of the cohort) provided information by telephone interview.

A subsequent evaluation of the cohort took place between July 2015 and July 2017, when children were ten years old, and 6397 children (76.0% of the cohort) were re-evaluated (Figure 7). The 13-year- old evaluation started in August 2018 and is still ongoing.

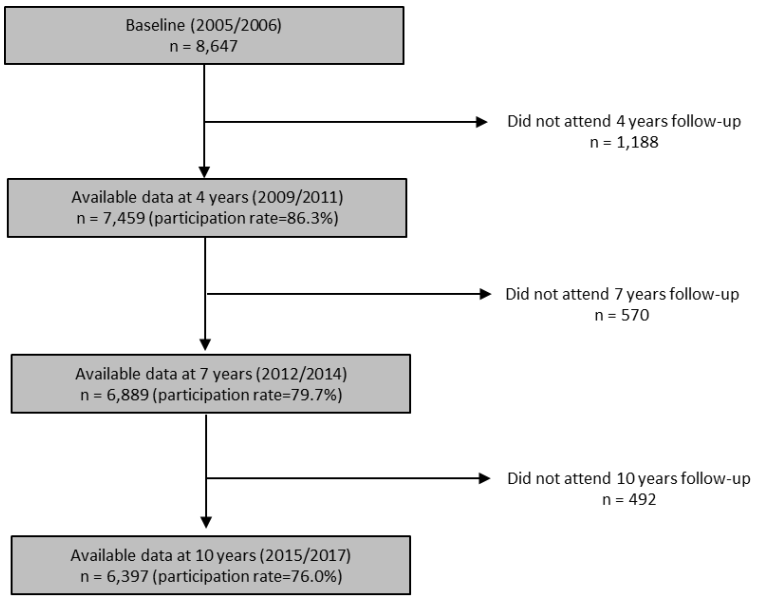

Figure 7. Flowchart of Generation XXI participants.

Follow-up evaluations of the cohort take place at *Departamento de Ciências da Saúde Pública e Forenses, e Educação Médica* in the University of Porto Medical School. Standard procedures were established and adopted in all cohort evaluations. A multidisciplinary team of interviewers and health professionals, that is periodically trained and supervised by Generation XXI administration team, is responsible for the application of structured questionnaires, performing the physical examination of children and parents, and extracting information from clinical records and National Health Service official health books.

The following description of procedures refers specifically to data collection sections from the baseline, and four, seven and ten-year-old follow-up evaluations. These were cohort evaluation waves from which data to answer to the objectives of this work were obtained.

a) Questionnaires

Face-to-face interviews using structured questionnaires designed and applied by a multidisciplinary team were conducted to obtain information on the following areas:

- Family demographic, socioeconomic, psychosocial circumstances and lifestyles: including caregivers date of birth, educational level, working conditions and smoking habits, household income and other socioeconomic indicators of psychosocial adversity.
- Child and family medical history: the existence of chronic diseases, healthcare use - including prenatal care - and maternal gynecologic and obstetric history.
- Child behaviours: including food intake and sedentary time, exposure to parental disciplinary tactics, bullying and other ACEs.

Data on sensitive topics such as household financial support needs and intimate personal behaviours (e.g. parental disciplinary tactics, exposure to bullying and other ACEs) were gathered through self-administered questionnaires to parents or legal guardians and children, filled in at the study site or home.

a) Physical examination

Physical examination of the child included anthropometric, cardiovascular (including blood pressure assessments), respiratory and dental evaluation. Measurement devices were carefully standardized and regularly calibrated.

b) Anthropometric assessment

Measurements of length/height and weight using stadiometers and scales were conducted. Waist, hip, arm and leg circumferences were measured using flexible and non-distensible tapes and body composition was assessed by bioelectric impedance (TANITA, Arlington Heights, Illinois, USA). All measurements were obtained while the child stood barefoot in light indoor clothing.

c) Blood sample collection

All blood evaluations were performed at the Clinical Pathology Service, Hospital de São João, Porto, Portugal. In each evaluation, trained nurses collected child and mother blood samples drawn from an antecubital vein after overnight fasting. All children were offered local dermal analgesia with lidocaine/prilocaine (EMLA®). Samples were centrifuged and sera were stored frozen at -80ºC in the biobank at the University of Porto Medical School until analyses.

d) Review of clinical records and National Health Service official pregnancy and children's health book

At the baseline evaluation, obstetric clinical records held at the maternity units and the National Health Service official pregnancy booklet - a record of check-ups, ultrasounds, tests and medical notes provided as part of routine primary care to all pregnant women - were reviewed. These records were reviewed to recover only data missing from the baseline questionnaire, including data on prenatal care, pregnancy complications, pregnancy anthropometrics and delivery as well as neonatal characteristics such as childbirth weight, length and gestational age.

At each follow-up evaluation, legal guardians were asked to bring their children's National Health Service official health and vaccination books to abstract data on the child's development. Specifically, from the children's health book, which is a record of data obtained as part of children's routine health care, the research team abstracted all the length/height and weight measurements performed at every medical visit to a health professional.

4. RESULTS

1. Soares, S.; Santos, A.C.; Soares Peres, F.; Barros, H.; Fraga, S.; 2020; Early life socioeconomic circumstances and cardiometabolic health biomarkers in childhood: evidence from the Generation XXI cohort; Preventive Medicine; https://doi.org/10.1016/j.ypmed.2020.106002

2. Soares, S.; López-Chéda, A.; Santos, A.C.; Barros, H.; Fraga, S.; 2020; How do early socioeconomic circumstances impact inflammatory trajectories? Findings from Generation XXI; Psychoneuroendocrinology; https://doi.org/10.1016/j.psyneuen.2020.104755

3. Soares, S.; Peres, F.S.; Rocha, V.; Kelly-Irving, M.; Stringhini, S.; Fraga, S.; The biological consequences of exposure to violence during childhood: a systematic review [submitted]

4. Fraga, S.; Soares, S.; Santos, A.C.; Barros, H.; Fraga, S., Soares, S.; Santos, A.C.; Barros, H.; Parents' use of extreme physical violence is associated with elevated high-sensitivity C-reactive protein in children [submitted]

5. Soares, S.; Santos, A.C.; Fraga, S.; From adverse childhood experiences and bullying towards inflammation in children: the role of BMI [submitted]

4.1 Early life socioeconomic circumstances and cardiometabolic health biomarkers in childhood: evidence from the Generation XXI cohort

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Early life socioeconomic circumstances and cardiometabolic health in childhood: Evidence from the Generation XXI cohort

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ABSTRACT

Social adversity is thought to become biologically embedded during sensitive periods of development which could set children on a trajectory of increased risk for later diseases. This study estimated the association between early socioeconomic circumstances and cardiometabolic biomarkers during childhood.

We analyzed data from 2962 participants in the birth cohort Generation XXI. Early socioeconomic circumstances included parental education and occupation and household income measured at the child's birth; cardiometabolic biomarkers included a set of parameters that were determined at seven and 10 years old. The association between early socioeconomic circumstances and cardiometabolic biomarkers in children aged seven and 10 years old was estimated using generalized estimating equations.

We observed, after adjustment for birth weight, sex, five-a-day fruit and vegetable intake and sedentary activity, that children with low educated mothers presented higher body mass index z-score (β = 0.22; 95%CI: 0.12, 0.33), higher waist circumference (β = 1.14; 95%CI: 0.55, 1.73) and increased systolic blood pressure z-score (β = 0.15; 95%CI: 0.08, 0.22) at the age of seven. At 10 years, children with mothers with low education, presented higher body mass index z-score (β = 0.32; 95%CI: 0.21, 0.43), higher waist circumference (β = 2.79; 95%CI: 1.94, 3.64), increased diastolic blood pressure z-score (β = 0.11; 95%CI: 0.06, 0.17) and increased systolic blood pressure s-score (β = 0.20; 95%CI: 0.12, 0.28). When repeated measures of cardiometabolic biomarkers were taken into account, the association between socioeconomic circumstances and cardiometabolic biomarkers remained significant.

Low socioeconomic circumstances have a possible detrimental effect on children's cardiometabolic health. Thus, socioeconomic adversity might impact health outcomes already in the first decade of life, emphasizing the early social patterning of cardiometabolic health and the need of social policies targeting children and families to modify or reverse its negative impact on health.

1. Introduction

Obesity, insulin resistance, dyslipidaemia and hypertension, are major drivers of the global burden of morbidity and mortality due to the increased risk of developing cardiovascular disease and type 2 diabetes in adults (Morrison et al., 2007; Morrison et al., 2008). Prior studies conducted in samples of adolescents have also observed that area-level socioeconomic status at ages 12–19 predicted cardiometabolic dysfunction in adult life (Wickrama et al., 2015), and victims of childhood adversity are more likely to be overweight or obese (Isohookana et al., 2016), present higher blood pressure (Alastalo et al., 2013), and increased risk of type 2 diabetes (Huang et al., 2015). Also, the occurrence of these diseases is higher among individuals living in poor socioeconomic circumstances (Castagne et al., 2016; Non et al., 2014; Taylor et al., 2011).

A disadvantaged socioeconomic position during childhood, such as family economic hardship or low parental education, has been linked to the development of chronic diseases and premature death (Galobardes et al., 2004; Galobardes et al., 2008; Parsons et al., 1999; Power et al., 2007; Stringhini et al., 2017), supporting socioeconomic health differentials origin in early life (Castagne et al., 2016; Non et al., 2014). Childhood social adversity is thought to become biologically embedded during sensitive periods of development, setting children on a trajectory of increased risk of chronic diseases in adulthood (Felitti et al., 1998;

Abbreviations: BMI, body mass index; FFM, fat-free mass; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein-cholesterol; GEE, generalized estimating equations

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Miller et al., 2011; Taylor et al., 2011).

The association between childhood social adversity and later life adverse health outcomes might be explained through the adoption of unhealthy behaviours (e.g., poor diet, sedentary behaviour, smoking), or direct physiological disruption of regulatory pathways responsive to stress caused by deprivation. Adverse childhood exposures result in a variety of physiological changes in children (Danese and McEwen, 2012), including increased activation of neurobiological systems, such as the hypothalamic-pituitary-adrenal axis or the sympathetic nervous system (Danese and McEwen, 2012; Miller et al., 2011). Increased activation of these systems leads to a cascade of physiological processes (Danese and McEwen, 2012; Miller et al., 2011), which in adults, has been linked with the development of central fat, dysregulated carbohydrate metabolism and the accumulation of blood lipids in the arterial lining, all of which accelerate chronic disease development (McEwen and Gianaros, 2010).

Although such changes do not always lead to disease, the underlying atherosclerotic process has a long asymptomatic phase of development that often starts during early childhood (Brambilla et al., 2007; Chen et al., 2000; Hostinar et al., 2017; Stoner et al., 2017), tracks over time and can predict the onset of cardiometabolic disease several years later (Magnussen et al., 2016).

There is scarce research on the role of social differences in cardiometabolic biomarkers during childhood (Iguacel et al., 2018). Thus, we hypothesized that children born and living in families from less advantaged socioeconomic circumstances grow and develop in a worst cardiometabolic milieu since early life.

2. Methods

2.1. Study design and participants

The study sample consisted of children participating in Generation XXI, a prospective Portuguese population-based birth cohort. As previously reported (Alves et al., 2012; Larsen et al., 2013), recruitment occurred during 2005–2006. Mothers and children (n = 8647) were recruited in public maternity units in Porto, Portugal. The entire cohort was invited to attend the second (2009–2011), third (2012–2014) and fourth (2016–2017) study waves, when children were aged four, seven and 10 years old, respectively. Anthropometric measures and blood samples were collected in all study waves, following the same standardized procedures. Data on demographic and socioeconomic characteristics, obstetric history, personal history of disease and health-related behaviours were collected by trained interviewers through structured questionnaires. Data on delivery and newborn characteristics, including birth weight, were additionally abstracted from clinical records, as previously described (Alves et al., 2012; Larsen et al., 2013). Generation XXI was approved by the Portuguese Data Protection Authority and by the Ethics Committee of Hospital São João, and data confidentiality and protection were guaranteed in all procedures according to the Declaration of Helsinki. Informed consent was obtained for all participants, signed by their legal guardian at every study wave (Alves et al., 2012).

Fig. 1 shows participation attrition of cohort participants. The present investigation includes data from participants with complete information on cardiometabolic factors at the age of seven (n = 3015) and ten (n = 3631). Thus, the analyses are based on 2962 participants with complete information both from third and fourth Generation XXI study waves.

A comparative analysis was conducted between the group of participants included and not included in the present study. Data indicated that participants who were not included belonged to families with a lower monthly disposable household income (n = 2126, $p < 0.001$), whose mothers (n = 2988, $p < 0.001$) and fathers (n = 1426, $p < 0.001$) had lower levels of education, and whose mothers (n = 1268, $p < 0.001$) and fathers (n = 2255, $p < 0.001$) had lower occupational positions.

2.2. Measures

2.2.1. Early socioeconomic circumstances

Information on monthly disposable household income, maternal and paternal education and occupation, was provided by the mother at baseline.

Monthly disposable household income included salaries and other sources of income, such as financial assistance, rent, monetary allowances for the whole household. A low monthly disposable household income was defined as 1000€ per month or less; intermediate if between 1001€ and 2000€; high if higher than 2000€. The categories were defined to guarantee more uniform distribution of the participants across the classes, with the lower class including the situation where both parents received at least the minimum national wage (374.70€ in 2005 and 385.90€ in 2006 (PORDATA, 2019)).

Education was measured as the number of years of formal schooling successfully completed and classified according to the International Standard Classification of Education 2011 classes (UNESCO Institute of Statistics, 2012). Low education corresponded to 9 years or less of formal schooling; intermediate education to 12 years of formal education; and high education to > 12 years.

Occupations were classified by major professional groups, according to the National Classification of Occupations and grouped in three categories: low (blue-collar: farmers, skilled and unskilled workers, craftsmen, machine operators and assembly workers); intermediate (lower white-collar: administrative and related workers, service, and sales workers); and high (upper white-collar: executive civil servants, industrial directors, scientists, middle management and technicians) (Oakes and Kaufman, 2006).

2.2.2. Anthropometric measures and blood sample collection

At seven and 10 years of age, trained researchers performed anthropometric measurements according to standardized procedures (Alves et al., 2012). In brief, weight and height were measured with the child in underwear and in bare feet. Weight was measured to the nearest one-tenth of a kilogram with the use of a digital scale (Tanita), and height was measured to the nearest one-tenth of a centimetre with the use of a wall stadiometer (seca®).

Following an overnight fast, a venous blood sample was obtained by trained nurses, before 11 a.m., after applying a topical analgesic cream (EMLA cream).

2.2.3. Cardiometabolic biomarkers

Body mass index (BMI) was calculated as the value of weight (kg) over squared height (m), and computed as an age- and sex-specific BMI standard deviation (SD) score (z-score), according to the World Health Organization Child Growth Standards (5–19 years) (WHO, 2018). Waist circumference measurements were taken with an inextensible tape measure to the nearest 0.1 cm, at the umbilicus level, with the child in a standing position, the abdomen relaxed, arms at the sides and feet positioned together (Gibson, 2005). Tetrapolar bioelectric impedance analysis was performed (BIA 101 Anniversary; Akern, Florence, Italy). Fat-free mass (FFM) was determined by using the Schaefer et al. equation, and fat mass was derived accordingly (Schaefer et al., 1994).

Blood pressure (BP) was measured with a sphygmomanometer (Omron®), using a stethoscope placed over the brachial artery pulse, proximal and medial to the cubital fossa, and below the bottom edge of the cuff (i.e., 2 cm above the cubital fossa) (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Two measurements of systolic (SBP) and diastolic (DBP) BP were taken, separated by at least 5 min, after a 10-min rest. If the difference between the measurements was < 5 mmHg for SBP or DBP, the mean was calculated. If the difference was larger than 5 mmHg, a third measurement was taken and the mean of

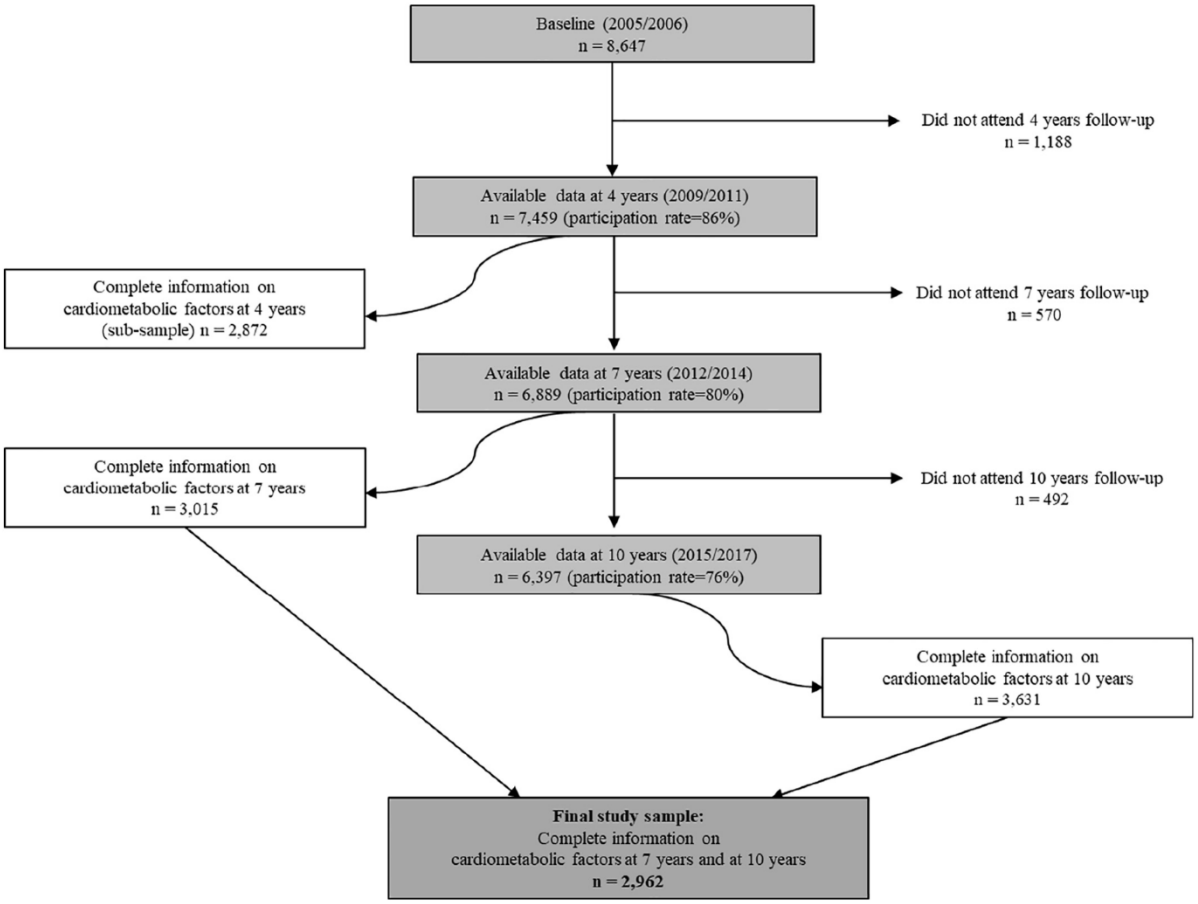

Fig. 1. Flow chart of participation in all Generation 21 study waves.

the two closest values was used. A new variable was computed as an age-, sex- and height-specific BP SD score (z-score) according to the American Academy of Pediatrics (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children, 2004).

All blood evaluations were performed at the Clinical Pathology Department, of the Hospital of São João, Porto, Portugal. Glucose was measured using a UV enzymatic assay (hexokinase method); triglycerides and high-density lipoprotein-cholesterol (HDL-c) were measured using an enzymatic colorimetric assay.

2.2.4. Covariates

The consumption of at least five portions of fruits and vegetables each day (five-a-day fruit and vegetable intake) was used as a proxy to measure healthy eating habits (Subar et al., 1995). The variable was computed using the report of daily intake of fruit, soup, boiled and raw vegetables, and dichotomized in less than five or five or more portions per day.

Duration, in minutes, of sedentary activities, was defined as the total time spent on on-screen activities (e.g. television, computer, video games, etc.), reading, painting, studying or doing homework (not accounting for classes hours), considering, and weekends.

2.3. Statistical analyses

Descriptive statistical analyses were conducted. Mean and standard deviation of cardiometabolic health biomarkers were calculated by categories of socioeconomic circumstances. Linear regression models and generalized estimating equations (GEE) were fitted to estimate the association between early socioeconomic indicators and cardiometabolic biomarkers, adjusted for birth weight, sex, five-a-day fruit and vegetable intake, and duration of sedentary activities. Population-averaged means in each cardiometabolic indicator during childhood were estimated using GEE, considering the correlation of repeated measurements from the same children, and statistical adjustments were made for birth weight, sex, five-a-day fruit and vegetable intake, and duration of sedentary activities. A sex interaction in the associations was tested, however, as no significant differences were found, all analyses were performed with both sexes together. Analyses were carried out in Stata/SE 15.1 (StataCorp LP, Texas, USA).

3. Results

The sample was composed of 52.1% of boys. Parents presented more frequently low educational levels (mothers: 42.1% and fathers: 48.6%). Regarding occupational position, mothers were more frequently in the intermediate category (48.3%) while fathers were more frequently in the highest category (41.2%). Also, disposable household income was more frequently in the intermediate category (48.6%). At both ages, participants were more likely to eat < 5 portions of fruits and vegetables per day (7 years: 59.3% and 10 years: 67.0%) (Table 1).

We observed a dose-response relationship between socioeconomic

Table 1
Descriptive statistics of selected sociodemographic characteristics and health-related outcomes in the Generation XXI cohort (n = 2962).

	Total
	n (%)
Sex	
Girl	1419 (47.9)
Boy	1543 (52.1)
Maternal education	
High	879 (29.8)
Intermediate	828 (28.1)
Low	1239 (42.1)
Maternal occupation	
High	849 (30.4)
Intermediate	1348 (48.3)
Low	595 (21.3)
Paternal education	
High	393 (23.4)
Intermediate	471 (28.0)
Low	817 (48.6)
Paternal occupation	
High	1088 (41.2)
Intermediate	530 (20.1)
Low	1021 (38.7)
Monthly disposable household income	
High	440 (16.9)
Intermediate	1262 (48.6)
Low	898 (34.5)
7 years	
Cardiometabolic parameters	
BMI[a] [mean (SD)]	0.70 (1.17)
WC (cm) [mean (SD)]	58.88 (6.68)
FFM (%) [mean (SD)]	83.88 (10.30)
Glycaemia (mg/dl) [mean (SD)]	82.97 (6.97)
Triglycerides (mg/dl) [mean (SD)]	67.70 (33.15)
HDL (mg/dl) [mean (SD)]	59.19 (10.62)
DBP[b] [mean (SD)]	0.99 (0.66)
SBP[b] [mean (SD)]	0.66 (0.80)
Five-a-day fruit and vegetable intake	
Less than 5 portions a day	1751 (59.3)
5 or more portions a day	1200 (40.7)
Sedentary activities	
Mean (SD) of time in minutes on sedentary activities per week	211.9 (122.1)
10 years	
Cardiometabolic parameters	
BMI[a] [mean (SD)]	0.72 (1.24)
WC (cm) [mean (SD)]	67.87 (9.68)
FFM (%) [mean (SD)]	83.85 (7.74)
Glycaemia (mg/dl) [mean (SD)]	86.87 (7.74)
Triglycerides (mg/dl) [mean (SD)]	67.15 (35.28)
HDL (mg/dl) [mean (SD)]	55.44 (10.43)
DBP[b] [mean (SD)]	0.67 (0.62)
SBP[b] [mean (SD)]	0.61 (0.86)
Five-a-day fruit and vegetable intake	
Less than 5 portions a day	1964 (67.0)
5 or more portions a day	968 (33.0)
Sedentary activities	
Mean (SD) of time in minutes on sedentary activities per week	366.8 (155.7)

BMI: body mass index; WC: waist circumference; FFM: fat-free mass; HDL: high-density lipoprotein-cholesterol; DBP: diastolic blood pressure; SBP: systolic blood pressure.

[a] According to World Health Organization z-score.

[b] Age-, sex- and height-specific blood pressure z-score according to the American Academy of Pediatrics.

circumstances and cardiometabolic indicators. Children with mothers and fathers with low educational level, low occupational position and low disposable household income presented a higher mean of BMI z-score, a higher mean of waist circumference and an increased mean of SBP z-score at the ages of seven and 10 when compared with those from more advantaged socioeconomic circumstances (intermediate and high). At the age of 10, participants born in low socioeconomic circumstances also presented increased means of DBP z-score (Table 2). Furthermore, at the age of 10 it was observed that higher FFM (%) and increased HDL cholesterol concentrations were observed among those children with mothers or fathers with intermediate and high education, and intermediate and high paternal occupation, and also in families with intermediate and high household income when compared with those from low socioeconomic circumstances (Table 2).

After adjustment for birthweight, sex, and behavioural factors such as five-a-day fruit and vegetable intake and sedentary activity, it was observed that less advantaged socioeconomic circumstances, namely, low maternal education, paternal occupation and disposable household income were associated with higher BMI z-score, higher waist circumference and increased z-score of SBP at the age of seven. In particular, children with mothers with low education, presented higher BMI z-score (β = 0.22; 95% CI: 0.12, 0.33), higher waist circumference (β = 1.14; 95% CI: 0.55, 1.73) and increased SBP z-score (β = 0.15; 95% CI: 0.08, 0.22). Children with fathers in low occupations also presented higher BMI z-score (β = 0.15; 95% CI: 0.05, 0.25), higher waist circumference (β = 0.73; 95% CI: 0.16, 1.30) and increased SBP z-score (β = 0.10; 95% CI:0.03, 0.17). On the contrary, children presented lower FFM(%) if maternal education and occupation was low (β = −1.12; 95% CI: −2.03, −0.21; β = −1.53; 95% CI: −2.62, −0.44, respectively) and if paternal education and occupation was also low (β = −1.65; 95% CI: −2.87, −0.43; β = −0.97; 95% CI: −1.84, −0.09, respectively) (Table 3). Social differences in biomarkers found at the age of seven remained at the age of 10. Children with mothers with low education, presented higher BMI z-score (β = 0.32; 95% CI: 0.21, 0.43), higher waist circumference (β = 2.79; 95% CI: 1.94, 3.64), increased DBP z-score (β = 0.11; 95% CI: 0.06, 0.17) and increased SBP z-score (β = 0.20; 95% CI: 0.12, 0.28). Children with fathers with low education also presented higher BMI z-score (β = 0.43; 95% CI: 0.28, 0.58), higher waist circumference (β = 3.52; 95% CI: 2.37, 4.68) and increased DBP z-score (β = 0.11; 95% CI: 0.04, 0.19). Similarly with the age of seven, at the age of 10, FFM(%) was inversely correlated with maternal education (β = −1.56; 95% CI: −2.41, −0.70) and paternal education (β = −2.34; 95% CI: −3.52, −1.17).

When repeated measures of cardiometabolic biomarkers were taken into account, the association between socioeconomic circumstances and cardiometabolic biomarkers remained (Table 4).

4. Discussion

This study showed that low socioeconomic circumstances have a possible detrimental effect on children's cardiometabolic health. At the age of seven, children presented differences in cardiometabolic biomarkers depending on the socioeconomic circumstances of the family, that remained at the age of 10. Additionally, these differences seem to be more evident at the age of 10, even though more studies are needed.

According to the biology of social adversity theory, the exposure to adversity is translated into changes in biological markers that might be precursors of disease later in life, and those changes may be tracked over the life-course, already from very early ages. Our results showed that children from less advantaged socioeconomic circumstances might be set in poorest health trajectories when compared to those in more advantaged socioeconomic circumstances, even though results might not have clinical implications they identify a potential trajectory of health disadvantage in the early life. In addition, the Life Course Health Development model suggests that numerous biological, psychological and cultural factors interact simultaneously in a transactional manner to influence an individual's life-course during each stage to determine a "health developmental" trajectory on multiple levels (Halfon and Forrest, 2018). These experiences are thought to become biologically embedded during sensitive periods of development, when exposure occurrence coincides with the time period of greatest maturation or plasticity of most of the organs and biological mechanisms (Bailey Jr

Table 2
Means and standard deviation of cardiometabolic health biomarkers by early socioeconomic circumstances.

	BMI[a]		WC (cm)		FFM (%)		Glycaemia (mg/dl)		Triglycerides (mg/dl)		HDL (mg/dl)		DBP[b]		SBP[b]	
7 years																
Maternal education																
High	0.53 (1.08)	< 0.001	58.04 (5.74)	0.005	84.67 (9.39)	0.024	82.82 (6.77)	0.338	67.61 (30.50)	0.718	56.54 (10.35)	0.439	0.94 (0.66)	0.073	0.55 (0.80)	< 0.001
Intermediate	0.76 (1.15)		58.99 (6.64)		83.85 (10.44)		82.90 (6.72)		68.23 (37.52)		56.20 (10.73)		0.99 (0.65)		0.69 (0.78)	
Low	0.78 (1.23)		59.34 (7.19)		83.31 (11.00)		83.05 (7.27)		67.50 (32.11)		55.95 (10.80)		1.02 (0.67)		0.72 (0.81)	
Maternal occupation																
High	0.54 (1.10)	< 0.001	58.07 (5.93)	0.005	84.84 (9.64)	0.007	82.94 (6.69)	0.708	67.99 (31.33)	0.742	55.69 (10.71)	0.279	0.95 (0.66)	0.064	0.71 (0.80)	< 0.001
Intermediate	0.75 (1.16)		58.99 (6.62)		83.75 (10.53)		82.86 (6.56)		67.43 (33.79)		56.38 (10.83)		0.99 (0.64)		0.69 (0.81)	
Low	0.76 (1.25)		59.38 (7.33)		83.09 (10.73)		83.04 (8.37)		67.35 (34.66)		56.06 (10.32)		1.03 (0.69)		0.56 (0.79)	
Paternal education																
High	0.48 (0.99)	0.015	57.77 (5.69)	< 0.001	82.85 (9.45)	0.108	82.71 (6.91)	0.076	68.20 (30.45)	0.640	57.57 (10.27)	0.021	0.88 (0.70)	0.005	0.72 (0.81)	< 0.001
Intermediate	0.66 (1.16)		58.27 (6.13)		84.24 (9.92)		82.86 (6.25)		68.30 (29.21)		55.83 (10.57)		0.98 (0.65)		0.61 (0.78)	
Low	0.77 (1.20)		59.37 (6.96)		83.41 (10.60)		83.38 (7.87)		68.84 (37.58)		55.87 (10.81)		1.03 (0.68)		0.52 (0.79)	
Paternal occupation																
High	0.60 (1.10)	0.004	58.27 (6.19)	0.031	84.56 (9.83)	0.052	82.85 (6.75)	0.199	66.97 (29.25)	0.149	56.95 (10.54)	0.044	0.99 (0.67)	0.395	0.62 (0.81)	0.006
Intermediate	0.75 (1.19)		59.06 (6.64)		83.35 (10.83)		83.09 (6.38)		65.64 (33.42)		55.79 (10.81)		0.97 (0.66)		0.64 (0.75)	
Low	0.76 (1.19)		59.12 (6.89)		83.48 (10.47)		83.25 (7.64)		69.20 (36.86)		55.77 (10.53)		1.03 (0.66)		0.73 (0.81)	
Monthly disposable household income																
High	0.55 (1.06)	0.010	58.15 (5.90)	0.160	84.93 (9.09)	0.080	82.78 (6.57)	0.236	68.92 (38.55)	0.960	56.65 (10.44)	0.410	0.95 (0.69)	0.077	0.59 (0.82)	0.011
Intermediate	0.69 (1.17)		58.80 (6.58)		83.79 (10.34)		83.14 (6.93)		67.58 (31.49)		56.44 (10.61)		0.98 (0.63)		0.65 (0.80)	
Low	0.77 (1.19)		59.17 (6.94)		83.49 (10.83)		82.96 (7.32)		67.99 (34.29)		55.98 (10.81)		1.03 (0.66)		0.71 (0.78)	
10 years																
Maternal education																
High	0.50 (1.15)	< 0.001	66.05 (8.53)	< 0.001	84.86 (9.06)	< 0.001	86.83 (5.92)	0.308	67.08 (35.38)	0.883	56.15 (10.69)	0.016	0.59 (0.59)	< 0.001	0.49 (0.81)	< 0.001
Intermediate	0.77 (1.21)		67.92 (9.38)		83.83 (9.93)		86.74 (5.90)		66.69 (34.38)		55.77 (10.56)		0.67 (0.61)		0.64 (0.89)	
Low	0.82 (1.29)		69.02 (10.37)		83.20 (9.98)		87.05 (10.91)		67.74 (36.13)		54.70 (10.21)		0.73 (0.64)		0.69 (0.86)	
Maternal occupation																
High	0.52 (1.17)	< 0.001	66.14 (8.62)	< 0.001	84.95 (9.27)	0.002	87.15 (10.93)	0.180	66.46 (35.88)	0.157	55.85 (10.66)	0.333	0.59 (0.59)	< 0.001	0.50 (0.83)	< 0.001
Intermediate	0.76 (1.23)		68.11 (9.68)		83.53 (9.81)		86.70 (5.98)		67.79 (34.27)		55.40 (10.23)		0.70 (0.64)		0.65 (0.86)	
Low	0.82 (1.30)		69.10 (10.35)		83.45 (10.11)		87.04 (6.05)		66.00 (33.18)		54.84 (10.58)		0.73 (0.62)		0.70 (0.85)	
Paternal education																

(continued on next page)

Table 2 (*continued*)

	BMI[a]		WC (cm)		FFM (%)		Glycaemia (mg/dl)		Triglycerides (mg/dl)		HDL (mg/dl)		DBP[b]		SBP[b]	
High	0.40 (1.15)	< 0.001	65.25 (8.23)	< 0.001	85.59 (8.99)	0.001	86.46 (6.10)	0.146	67.61 (35.51)	0.558	56.77 (10.37)	0.012	0.57 (0.57)	0.006	0.52 (0.81)	0.109
Intermediate	0.64 (1.19)		66.97 (8.89)		83.98 (9.40)		87.57 (13.65)		65.40 (36.76)		55.40 (10.52)		0.61 (0.62)		0.57 (0.81)	
Low	0.82 (1.24)		68.78 (10.03)		83.39 (10.02)		87.04 (5.63)		66.94 (32.31)		54.89 (10.29)		0.69 (0.63)		0.64 (0.88)	
Paternal occupation																
High	0.56 (1.19)	< 0.001	66.48 (8.87)	< 0.001	84.70 (9.39)	0.006	86.69 (8.42)	0.078	67.46 (39.43)	0.278	56.30 (10.59)	0.014	0.62 (0.61)	< 0.001	0.56 (0.82)	0.014
Intermediate	0.81 (1.23)		68.34 (9.74)		83.28 (10.05)		86.99 (8.02)		65.73 (33.38)		55.16 (10.06)		0.71 (0.60)		0.65 (0.86)	
Low	0.80 (1.24)		68.52 (9.99)		83.76 (10.00)		87.14 (7.43)		66.96 (29.39)		54.89 (10.33)		0.73 (0.63)		0.68 (0.88)	
Monthly disposable household income																
High	0.48 (1.18)	< 0.001	65.80 (8.48)	< 0.001	85.17 (9.41)	0.018	87.05 (11.06)	0.486	67.73 (38.44)	0.246	55.90 (10.57)	0.011	0.58 (0.62)	< 0.001	0.52 (0.85)	< 0.001
Intermediate	0.71 (1.24)		67.71 (9.55)		83.76 (9.74)		86.99 (7.72)		65.99 (33.79)		56.13 (10.61)		0.67 (0.60)		0.59 (0.84)	
Low	0.80 (1.25)		68.75 (10.03)		83.55 (10.05)		86.73 (5.97)		68.10 (33.13)		54.68 (10.29)		0.73 (0.63)		0.69 (0.85)	

BMI: body mass index; WC: waist circumference; FFM: fat-free mass; HDL: high-density lipoprotein-cholesterol; DBP: diastolic blood pressure; SBP: systolic blood pressure.

[a] According to World Health Organization z-score.

[b] Age-, sex- and height-specific blood pressure z-score according to the American Academy of Pediatrics.

Table 3
Linear regression models to test the association between early socioeconomic circumstances and cardiometabolic health biomarkers (β (95% CI)) at the age of seven and 10, adjusted for birthweight, sex, five-a-day fruit and vegetable intake, and duration of sedentary activities.

	BMI[a]	WC (cm)	FFM (%)	Glycaemia (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	DBP[b]	SBP[b]
7 years								
Maternal education								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.21 (0.10; 0.33)	0.88 (0.24; 1.52)	−0.80 (−1.78; 0.18)	−0.03 (−0.70; 0.63)	0.73 (−2.48; 3.95)	−0.34 (−1.36; 0.69)	0.04 (−0.025; 0.10)	0.13 (0.05; 0.21)
Low	0.22 (0.12; 0.33)	1.14 (0.55; 1.73)	−1.12 (−2.03; −0.21)	0.19 (−0.43; 0.81)	−0.11 (−3.08; 2.87)	−0.48 (−1.43; 0.46)	0.07 (0.010; 0.13)	0.15 (0.08; 0.22)
Maternal occupation								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.19 (0.09; 0.30)	0.80 (0.22; 1.37)	−0.98 (−1.86; −0.09)	−0.13 (−0.74; 0.48)	−0.64 (−3.56; 2.27)	0.35 (−0.58; 1.28)	0.04 (−0.02; 0.10)	0.12 (0.05; 0.19)
Low	0.19 (0.07; 0.32)	1.10 (0.39; 1.81)	−1.53 (−2.62; −0.44)	0.03 (−0.72; 0.78)	−0.68 (−4.28; 2.91)	−0.23 (−1.38; 0.92)	0.08 (0.01; 0.15)	0.14 (0.05; 0.22)
Paternal education								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.14 (0.01; 0.30)	0.36 (−0.51; 1.24)	−0.92 (−2.28; 0.43)	−0.07 (−1.04; 0.91)	0.22 (−4.37; 4.80)	−1.74 (−0.32; −0.30)	0.09 (0.03; 0.19)	0.09 (−0.02; 0.20)
Low	0.27 (0.13; 0.41)	1.51 (0.72; 2.30)	−1.65 (−2.87; −0.43)	0.53 (−0.35; 1.41)	0.60 (−3.54; 4.75)	−1.66 (−2.96; −0.37)	0.13 (0.04; 0.21)	0.18 (0.08; 0.28)
Paternal occupation								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.14 (0.02; 0.26)	0.64 (−0.06; 1.33)	−1.07 (−2.13; 0.00)	0.10 (−0.64; 0.83)	−1.33 (−4.84; 2.19)	−1.10 (−2.21; 0.18)	−0.02 (−0.09; 0.05)	0.01 (−0.08; 0.09)
Low	0.15 (0.05; 0.25)	0.73 (0.16; 1.30)	−0.97 (−1.84; −0.09)	0.30 (−0.31; 0.91)	2.31 (−0.59; 5.20)	−1.10 (−2.02; −0.19)	0.04 (−0.02; 0.09)	0.10 (0.03; 0.17)
Monthly disposable household income								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.13 (0.01, 0.26)	0.52 (−0.20, 1.25)	−1.10 (−2.22, 0.01)	0.24 (−0.53, 1.01)	−1.11 (−4.84, 2.62)	−0.18 (−1.35, 1.00)	0.03 (−0.04, 0.10)	0.06 (−0.03, 0.15)
Low	0.19 (0.05, 0.33)	0.77 (0.00, 1.54)	−1.16 (−2.35, 0.02)	−0.02 (−0.84, 0.79)	−0.81 (−4.77, 3.15)	−0.66 (−1.90, 0.59)	0.07 (0.00, 0.15)	0.10 (0.01, 0.20)
10 years								
Maternal education								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.25 (0.13, 0.37)	1.70 (0.78, 2.62)	−1.02 (−1.94, −0.09)	−0.35 (−1.10, 0.40)	−0.83 (−4.23, 2.57)	−0.39 (−1.40, 0.61)	0.06 (0.00, 0.12)	0.15 (0.07, 0.23)
Low	0.32 (0.21, 0.43)	2.79 (1.94, 3.64)	−1.56 (−2.41, −0.70)	−0.22 (−0.91, 0.47)	−0.34 (−3.48, 2.81)	−1.39 (−2.32, −0.46)	0.11 (0.06, 0.17)	0.20 (0.12, 0.28)
Maternal occupation								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.23 (0.12, 0.33)	1.78 (0.96, 3.61)	−1.34 (−2.17, −0.50)	−0.50 (−1.18, 0.19)	0.70 (−2.30, 3.70)	−0.35 (−1.26, 0.56)	0.09 (0.04, 0.15)	0.15 (0.08, 0.22)
Low	0.28 (0.15, 0.42)	2.71 (1.69, 3.74)	−1.36 (−2.40, −0.32)	−0.14 (−0.99, 0.71)	−1.32 (−5.04, 2.39)	−0.95 (−2.08, 0.17)	0.12 (0.05, 0.18)	0.20 (0.11, 0.29)
Paternal education								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.24 (0.07, 0.40)	1.68 (0.41, 2.95)	−1.81 (−3.10, −0.51)	1.03 (−0.16, 2.23)	−1.79 (−6.44, 2.85)	−1.57 (−2.98, −0.16)	0.04 (−0.05, 0.12)	0.08 (−0.04, 0.19)
Low	0.43 (0.28, 0.58)	3.52 (2.37, 4.68)	−2.34 (−3.52, −1.17)	0.61 (−0.48, 1.69)	−0.64 (−4.85, 3.58)	−2.00 (−3.28, −0.73)	0.11 (0.04, 0.19)	0.14 (0.03, 0.24)
Paternal occupation								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.24 (0.11, 0.37)	1.77 (0.77, 2.76)	−1.39 (−2.41, −0.37)	0.31 (−0.54, 1.15)	−2.02 (−5.66, 1.62)	−1.08 (−2.18, 0.01)	0.08 (0.01, 0.14)	0.09 (−0.002, 0.18)
Low	0.23 (0.12, 0.34)	1.84 (1.02, 2.67)	−0.81 (−1.65, 0.03)	0.48 (−0.22, 1.17)	−0.93 (−3.94, 2.08)	−1.40 (−2.31,-0.50)	0.09 (0.04, 0.14)	0.12 (0.05, 0.20)
Monthly disposable household income								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.23 (0.09, 0.36)	1.81 (0.76, 2.85)	−1.47 (−2.53, −0.40)	−0.18 (−1.05, 0.69)	−2.08 (−5.86, 1.71)	0.22 (−0.93, 1.38)	0.09 (0.02, 0.15)	0.08 (−0.02, 0.17)
Low	0.30 (0.16, 0.44)	2.53 (1.43, 3.64)	−1.38 (−2.51, −0.25)	−0.39 (−1.31, 0.53)	−0.63 (−4.64, 3.38)	−1.06 (−2.28, 0.17)	0.13 (0.06, 0.21)	0.16 (0.06, 0.26)

[a] According to World Health Organization z-score.
[b] Age-, sex- and height-specific blood pressure z-score according to the American Academy of Pediatrics.

Table 4
Generalized Estimating Equation Regressions of early socioeconomic circumstances and cumulative measures of cardiometabolic health biomarkers, adjusted for birthweight, sex, five-a-day fruit and vegetable intake, and duration of sedentary activities (coefficient (95%CI)).

	BMI[a]	WC (cm)	FFM (%)	Glycaemia (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	DBP[b]	SBP[b]
Maternal education								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.04 (−0.01, 0.09)	0.61 (0.13, 1.10)	−0.45 (−1.14, 0.23)	−0.34 (−1.08, 0.39)	−0.91 (−4.09, 0.41)	−0.16 (−0.89, 0.58)	0.04 (−0.01, 0.10)	0.08 (0.00, 0.16)
Low	0.07 (0.02, 0.12)	1.19 (0.74, 1.65)	−0.65 (−1.29, −0.19)	−0.28 (−0.96, 0.40)	−0.08 (−3.02, 2.86)	−1.03 (−1.71, 0.35)	0.09 (0.04, 0.14)	0.12 (0.05, 0.19)
Maternal occupation								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.03 (−0.02, 0.08)	0.74 (0.30, 1.18)	−0.67 (−1.29, −0.04)	−0.46 (−1.13, 0.21)	1.03 (−1.78, 3.84)	−0.62 (−1.28, 0.05)	0.08 (0.03, 0.13)	0.09 (0.02, 0.21)
Low	0.06 (0.00, 0.12)	1.13 (0.58, 1.68)	−0.12 (−0.90, 0.65)	−0.15 (−0.98, 0.69)	−1.13 (−4.61, 2.35)	−0.67 (1.49, 0.15)	0.09 (0.02, 0.15)	0.12 (0.04, 0.21)
Paternal education								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.08 (0.00, 0.15)	1.07 (0.38, 1.75)	−1.05 (−2.03, −0.08)	1.01 (−0.16, 2.19)	−1.66 (−6.02, 2.70)	−0.32 (−1.34, 0.70)	0.00 (−0.07, 0.08)	0.03 (−0.07, 0.14)
Low	0.15 (0.08, 0.22)	1.50 (0.87, 2.12)	−1.08 (−1.97, −0.20)	0.49 (−0.58, 1.56)	−0.70 (−4.66, 3.26)	−0.78 (−1.70, 0.15)	0.07 (−0.00, 0.14)	0.04 (−0.05, 0.14)
Paternal occupation								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.09 (0.03, 0.14)	0.81 (0.28, 1.35)	−0.56 (−1.32, 0.20)	0.25 (−0.58, 1.08)	−1.52 (−4.95, 1.89)	−0.39 (−1.19, 0.41)	0.08 (0.01, 0.14)	0.07 (0.00, 0.15)
Low	0.07 (0.02, 0.12)	0.79 (0.34, 1.23)	−0.04 (−0.67, 0.59)	0.39 (−0.29, 1.07)	−0.16 (−4.56, 1.10)	−0.60 (−1.26, 0.06)	0.08 (0.03, 0.13)	0.07 (0.00, 0.14)
Monthly disposable household income								
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Intermediate	0.08 (0.02, 0.14)	1.07 (0.52, 1.63)	−0.64 (−1.44, 0.15)	−0.21 (−1.07, 0.64)	−1.55 (−5.05, 1.95)	0.37 (−0.48, 1.22)	0.07 (0.01, 0.14)	0.04 (−0.04, 0.12)
Low	0.09 (0.02, 0.14)	1.43 (0.85, 2.02)	−0.49 (−1.34, 0.35)	−0.39 (−1.30, 0.52)	−0.16 (−3.88, 3.56)	−0.72 (−0.48, 1.22)	0.10 (0.04, 0.17)	0.10 (0.01, 0.19)

BMI: body mass index; WC: waist circumference; FFM: fat-free mass; HDL: high-density lipoprotein-cholesterol; DBP: diastolic blood pressure; SBP: systolic blood pressure.

[a] According to World Health Organization z-score.

[b] Age-, sex- and height-specific blood pressure z-score according to the American Academy of Pediatrics.

et al., 2001; Knudsen, 2004), setting children on a trajectory of increased risk for the development of chronic diseases in adulthood (Felitti et al., 1998; Miller et al., 2011; Taylor et al., 2011).

So far, the literature shows that early stressful experiences "get under the skin" and seem to have a negative impact on later health (Danese and McEwen, 2012; Miller et al., 2011). Our results, corroborating previous studies (Power et al., 2007; Stringhini et al., 2017), show that early disadvantageous socioeconomic circumstances seem to be incorporated and their potentially negative effects are already expressed during the first years of life, even though this does not necessarily mean increased risk of later health outcomes. Little is known about the moment when childhood socioeconomic circumstances matter the most or for how long they need to last, but these results evidenced the effect of family socioeconomic circumstances at childbirth. It was also conducted a sensitivity analysis in a subsample of children when they were four years old (second wave of Generation XXI cohort), and at this age, we found that the means of z-scores of BMI, waist circumference and SBP were already higher in those children from early low socioeconomic circumstances (Supplementary Table 1). These results show that socioeconomic inequalities found at the ages of seven and 10, might be identified as early as four years of age. These findings of a potential tracking of cardiometabolic alterations throughout childhood until adolescence has been observed elsewhere, with data from IDEFICS/I.Family cohort, showing that prevalence of abdominal obesity and other cardiometabolic indicators increased with age, and these alterations were less likely to be reversed to the metabolically healthy status throughout the years (Börnhorst et al., 2019). We believe that very early exposures carry the potential to have a strong impact on adult health, and social differences are expected to increase, regardless of the socioeconomic circumstances achieved during adulthood (Galobardes et al., 2004; Galobardes et al., 2008), which is the usual prediction of timing models (Dal Canto et al., 2018).

We used socioeconomic circumstances measures from both parents and found that both maternal and paternal characteristics impact children's cardiometabolic health. However, different socioeconomic measures might be capturing different effects on a child's care and consequently on a child's health (Galobardes et al., 2006). Knowledge and skills attained through formal education may affect a person's cognitive functioning (Galobardes et al., 2006) with maternal education, in particular, being associated with child health outcomes as it seems to contribute through factors more closely associated with mothers' literacy, thus reflecting on children's care and choices (Erola et al., 2016). While paternal occupation, another indicator commonly used to measure early socioeconomic circumstances, might be more related to financial availability and material assets (Erola et al., 2016; Galobardes et al., 2006). In fact, some studies have reported that paternal occupation has a significant direct impact on an individual's health and that the effect of the father's occupation exceeds that of mother's (Pinilla et al., 2017). As self-reported measures, both education and occupation are reliable sources of information about families' socioeconomic circumstances, since they are easy to measure (Galobardes et al., 2006). Income, however, has the potential for being underestimated (Moore et al., 2000). Also, literature refers that the differences between reported income and tax reported income is bigger in the highest income participants, and therefore, if any bias, it would be in the direction of increasing the inequalities (Medeiros et al., 2015). Moreover, as income is categorized into three categories, we are certain that this potential bias on income reporting would not affect our results. In the present study we found an association between both maternal and paternal low education and occupation, and higher means in several cardiometabolic biomarkers. Thus, it is expected that all socioeconomic indicators influence together with the child's health and help distinguishing social differences in children at these ages. Changes in socioeconomic conditions throughout this period of life, i.e. since birth to 10 years of age were not expected to have a strong impact on the health trajectory already established.

The association of socioeconomic circumstances with cardiometabolic biomarkers might be explained via stress. Likewise, exposure to chronic stress can act as a risk factor that triggers, exacerbates, or causes weight gain (Sinha and Jastreboff, 2013). The hypothalamic–pituitary–adrenal (HPA) axis activation, one of the regulation mechanisms pointed as a response to stress exposure, leads to altered insulin sensitivity, increased blood pressure, and inflated central adiposity (Danese and McEwen, 2012). In fact, our results show higher mean of waist circumference and z-scores means of BMI and blood pressure among children from less advantaged socioeconomic circumstances. This can be explained by the role of adiposity on the onset of cardiometabolic alterations, which is reflected through early alterations of biomarkers such as blood pressure and BMI (Aggoun, 2007). Additionally, it is expected that BMI trigger some effect on all cardiometabolic biomarkers (e.g. triglycerides and glycaemia), via vascular damage caused by the adiposity (Aggoun, 2007). This process is complex (Bays and Ballantyne, 2006) and may take time to be observed, which could explain the lack of statistical power to observe it in this population of 10-year-old children.

Also, the early development of obesity favours the onset of several metabolic dysfunctions in childhood and adolescence (Simmonds et al., 2015), and is associated with poor diet and physical inactivity (Ambrosini et al., 2010; Segal and Opie, 2015). It is known that sedentary and dietary behaviours are socioeconomically patterned, i.e., those in lower socioeconomic position have lower consumption of fruits and vegetables, and more sedentary activities (Pulsford et al., 2013; Strike and Steptoe, 2004). Thus, we used five-a-day fruit and vegetable intake and sedentary activity, as proxies of healthy choices. Additionally, as the link between adverse childhood exposures and health outcomes later in life might be explained through the adoption of health-risky behaviours, such as unhealthy diet and inactivity, or via direct physiological changes resulting from disruption of regulatory pathways, we believe that by using these models we excluded to a certain extent, the effect of behaviours already potentially established at early ages. Nevertheless, we are aware of the effect that BMI might have on the other cardiometabolic indicators, especially at the age of 10. This might be occurring due to the fact that some of these children are already in or at the onset of puberty, which affects their BMI and consequently their cardiometabolic health (Fonseca et al., 2019).

Currently, no consensus in the definition of an adverse metabolic profile in the paediatric population exists (Magge et al., 2017) and there is a lack of reference values for the different cardiometabolic indicators during childhood (Mellerio et al., 2012). However, although it is rare for adolescents to meet diagnostic criteria for cardiometabolic diseases, adolescents with cardiometabolic functioning deviating from population-level norms can be considered at risk of developing cardiometabolic disease during adulthood (Magnussen et al., 2016; Morrison et al., 2007; Morrison et al., 2008). Thus, children's cardiometabolic health may contribute to the earlier establishment of an unfavourable profile in early life stages (Hostinar et al., 2017; Stoner et al., 2017). And, even though cardiovascular events during childhood are not expected to occur, it can be hypothesized that the underlying atherosclerotic process might already be in course (Brambilla et al., 2007; Chen et al., 2000; Hostinar et al., 2017; Stoner et al., 2017). Therefore, our study is a step forward showing that disadvantageous early socioeconomic circumstances seem to be associated with higher means of cardiometabolic biomarkers at the very early stages of life.

4.1. Strengths and limitations

The key strengths of this study are its large sample size, the use of repeated cardiometabolic biomarkers measurements during childhood and the use of different socioeconomic circumstances indicators. The use of Generation XXI cohort data allowed us to observe and analyse different stages of children's metabolic profile, and to establish a potential causal relationship between the exposure, early socioeconomic

circumstances, and the outcome, i.e., cardiometabolic biomarkers at the age of seven and 10. However, as it is common in a prospective birth cohort, there has been attrition over time, leading to a reduction in the sample size and a more socioeconomically advantaged group of participants at older ages. Nevertheless, we believe that the inclusion of the more disadvantaged group would have widened the differences, which may be underestimating the effect of socioeconomic circumstances on children's cardiometabolic biomarkers. Moreover, generalized estimating equations were used to account for the longitudinal study design and correlation of repeated measures.

Additionally, information on socioeconomic circumstances indicators was collected at childbirth, thus decreasing the risk of recall bias, and the collection of data about both the mother and the father, allowed us to have a more comprehensive assessment of early family socioeconomic circumstances. In fact, we observed that parental education seemed to play an important role in different cardiometabolic biomarkers of both girls and boys at the ages of seven and 10. Moreover, the cardiometabolic biomarkers were standardized according to age and sex. For SBP and DBP, the z-scores were based on sex and height, classifying in this way the blood pressure according to the body size and according to a reference population.

In the analyses, it was not considered the family history of cardiometabolic risk, even though we are aware of some genetic predisposition in some of the children. Nevertheless, we expect that at the population level, in a "healthy-apparent" sample, this effect should be minimal. Although we have tested some confounding variables, it is not possible to discard some potential residual confounding. Also, African Americans and South-East Asians populations appear to be disproportionately affected by cardiovascular risk factors, and this higher incidence is likely to represent the complex interactions from several innate and environmental factors (Dal Canto et al., 2018). As our sample was exclusively Caucasian, there is no ethnic variability to account for. Thus, ethnicity is not to consider in the observed inequalities, and the associations we found are mainly due to socioeconomic circumstances differences.

We found that social differences in the cardiometabolic biomarkers can be already observed at the age of seven and 10, and as the cohort participants grow older, we will be able to explore if the inequalities identified in the first 10 years of life are maintained or widened throughout adolescence and adult life. Additionally, working with data from a currently ongoing birth cohort allowed to identify differences in means of cardiometabolic biomarkers according to socioeconomic circumstances, and probable sensitive periods of development, that can be translated in population-targeted interventions potentially more effective and efficient in preventing social and health inequalities in the general population.

5. Conclusions

Our results showed that there is a potential impact of early life socioeconomic environment on physiology and metabolic dysregulation, supporting a detrimental effect of disadvantaged socioeconomic circumstances with origin in childhood. Our findings emphasize the importance of researching the effect of more investments in social policies and families' support to provide children with a better start for better health. Thus, "upstream" factors identified during childhood seem to represent meaningful opportunities for improving health and reducing health disparities, since they pose as modifiable risk factors that should be targeted in local and global public health interventions to attenuate or reduce inequalities in health, starting at early ages.

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Declaration of competing interest

None.

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4.2 How do early socioeconomic circumstances impact inflammatory trajectories? Findings from Generation XXI

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How do early socioeconomic circumstances impact inflammatory trajectories? Findings from Generation XXI

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Declarations of Interest: None.

Abstract

Background: The association between socioeconomic position and markers of inflammation in adults, including C-reactive protein (CRP), is well-established. We hypothesized that children from families of less-advantaged socioeconomic circumstances may be at higher inflammatory risk during childhood and, consequently, throughout their life course. Thus, we aimed to investigate whether early socioeconomic circumstances impact CRP trajectories using repeated measures of data from a population-based birth cohort.

Methods: Data from 2510 participants of Generation XXI, a prospective Portuguese population-based birth cohort, were included in this study. Early socioeconomic circumstances comprised maternal education and occupation, paternal education and occupation, and household income at the child's birth. Venous blood samples were collected from the children at ages four, seven, and ten years, and high-sensitivity CRP (hs-CRP) was quantified. Hs-CRP trajectories were computed using a linear mixed-model approach.

Results: Participants from less-advantaged socioeconomic circumstances presented higher levels of hs-CRP by age of ten years. The higher the mother´s education and disposable household income, the lower the minimum value of the log hs-CRP observed throughout childhood. Further, the age at which that minimum log hs-CRP value was reached occurs later, meaning that children born in more-advantaged socioeconomic circumstances had lower levels of log hs-CRP compared with children from less-advantaged families.

Conclusions: Poor socioeconomic circumstances early in life are associated with increased inflammation levels throughout the first decade of life. This study demonstrates that social inequalities may impact population health beginning at very early ages.

Keywords: Socioeconomic circumstances; C-Reactive Protein; childhood; trajectories

Introduction

Social adversity during childhood is thought to become biologically embedded during sensitive periods of development, setting children on a trajectory of increased risk for chronic diseases in adulthood (1-3). Inflammatory processes have been suggested as a mechanism that explains the association between socioeconomic circumstances and later health outcomes (4-6). Life-course models hypothesize that exposure to social adversity is related to prolonged low-grade activation of the immune system and, consequently, elevated levels of inflammatory markers (7, 8). High-sensitivity (Hs) C-reactive protein (CRP) has been shown to be associated with future cardiovascular events, with both innate and adaptive immune responses leading to clinical manifestations of cardiovascular disease (9).

There is evidence of the temporal dynamics through which socioeconomic status (SES) relates to physiological biomarkers of age-related health mechanisms at different phases of the life course (10). Literature shows that low childhood SES is associated with elevated concentrations of inflammatory markers, such as circulating levels of CRP and proinflammatory cytokines, in adulthood (11, 12). Similarly, although scarce, evidence shows that adolescents from families with less-advantaged SES already have higher levels of inflammatory markers (13, 14). To our knowledge, few studies have investigated the effect of socioeconomic circumstances on inflammatory markers during childhood. Nevertheless, cross-sectional studies have shown that children living in neighbourhoods with high levels of poverty or crime had elevated CRP levels compared with children from other neighbourhoods (15), that children born to a parent with less than a high school degree have a higher CRP than those born to a parent with a college degree, and that children from low-income families also had higher CRP levels than those from a higher-income family (16).

Thus, if social differences in inflammatory markers exist at early ages, then children born to families from less-advantaged socioeconomic circumstances may be on a trajectory toward higher inflammatory risk throughout childhood and, consequently, later in life.

Understanding the influence of socioeconomic circumstances in inflammatory markers during childhood may help identify social health inequalities early in life. Additionally, there are still gaps in our knowledge of inflammatory processes in childhood. Namely, few existing studies have prospectively examined childhood adversity and inflammation using repeated measures to study the impact of different socioeconomic measures that might capture different effects on children's care and, consequently, their health (17). Furthermore, few studies have considered the variation of CRP levels, in light of adiposity rebound, after which it is expected a consistent increase of these levels can be

observed throughout life (18, 19). Thus, our study aimed to add to the literature evidence of the impact of different SES measures in longitudinal hs-CRP trajectories in the first ten years of life.

In this study, we investigated the impact of early socioeconomic circumstances on hs-CRP trajectories over childhood, using repeated measures of data from a population-based birth cohort at ages four, seven, and ten years old.

2. Methods

2.1. Study design and participants

The study sample consisted of children who participated in Generation XXI, a prospective Portuguese population-based birth cohort. Briefly, recruitment occurred during 2005-2006 (20, 21), with mothers and children (n=8647) being recruited in public maternity units in Porto, Portugal. The entire cohort was invited to attend the second (2009-2011), third (2012-2014), and fourth (2016-2017) study waves, when children were aged four, seven, and ten years old, respectively. Anthropometric measures and blood samples were collected in all study waves, following the same standardized procedures. Data on demographic and socioeconomic characteristics, personal history of disease, and health-related behaviours were collected by trained interviewers through structured questionnaires.

Generation XXI was approved by the National Data Protection Authority and by the ethics committee of Hospital de São João. Data confidentiality and protection were guaranteed in all procedures according to the Declaration of Helsinki. Written informed consent was obtained for all participating children, signed by their legal guardian at every study wave (20).

Figure 1 shows the participation attrition of cohort participants. The present study uses data from participants with complete information on hs-CRP levels for at least two of the three included study waves. Thus, the analyses were based on data from 2510 participants (1174 girls and 1336 boys), and the sample characteristics are presented in Table 1.

A comparative analysis was conducted between the group of participants who met inclusion criteria and those who did not for the present study. Data indicated that participants who were not included in the study belonged to families with a lower monthly disposable household income (n = 1988, $p < 0.001$), whose mothers (n = 2783, $p < 0.001$) and fathers (n = 1314, $p < 0.001$) had lower levels of education, and whose mothers (n = 1179, $p < 0.001$) and fathers (n = 1050, $p < 0.001$) had lower occupational positions.

2.2. Measures

2.2.1. Early socioeconomic factors

Information on maternal and paternal education and occupation and disposable household income per month was provided by the mother at baseline.

Education was measured as the number of years of formal schooling successfully completed and classified according to the International Standard Classification of Education 2011 classes (22). The low educational level corresponded to 9 years or less of formal schooling; intermediate education to 12 years of formal education; and high education to more than 12 years of formal education.

Occupation was classified by major professional groups, according to the National Classification of Occupations (23), and grouped into three categories: low (blue collar: farmers, skilled and unskilled workers, craftsmen, machine operators, and assembly workers); intermediate (lower white collar: administrative and related workers, service and sales workers); and high (upper white collar: executive civil servants, industrial directors, scientists, middle management, and technicians) (24).
Current disposable household income per month included salaries and other sources of income, such as financial assistance, rent, and monetary allowances for the whole household. It was collected using the following question*:* "Looking at this scale, choose from the following ranges or total the monthly net income (including earnings and other sources of income such as subsidies, agendas, monetary aids, food) of all the people living in your house". A low disposable household income was defined as 1000€ per month or less and in which both parents received at least the minimum national wage (374.70€ in 2005 and 385.90€ in 2006 [(25)]). The intermediate category was defined as between 1001€ and 2000€ per month, and the highest category was defined as higher than 2000€ per month.

2.2.2. Hs-CRP

Following an overnight fast, a venous blood sample was collected before 11 a.m. by trained nurses in our research center after applying a topical analgesic cream (EMLA cream). The samples were centrifuged at 3500 rpm for 10 minutes and plasma was aliquoted. Biomarkers were assayed in fresh blood samples. Hs-CRP was assayed using CardioPhase hsCRP Flex and the Dimension Vista System from Siemens. Samples of 1.37µL were placed in the cuvette and used nondiluted. Reactant (27.3µL) was added and the processing of samples was conducted at 37ºC for 5 minutes and 50 seconds, at a wavelength of 840nm. During each test day, two separate analyses were performed with two test samples for each material tested for 20 days. Coefficient of variation for low control was 5.4% at a concentration of 0.06 mg/L and for high control was 4.4% at a concentration of 0.15 mg/L. All blood evaluations were performed at the Clinical Pathology Service, Hospital de São João, Porto, Portugal.

Due to a highly skewed distribution, and for statistical purposes, hs-CRP was log-transformed. The minimum detectable values were recoded as 0.2 mg/L for all study waves. Further, because high levels of hs-CRP could represent an acute condition instead of a chronic inflammatory state (26), the analyses excluded participants with hs-CRP levels higher than 10 mg/L to overcome this issue.

2.3. Covariates

In the models, we included body mass index (BMI) as a covariate. For children at four, seven, and ten years of age, trained researchers performed anthropometric measurements according to standardized procedures. In brief, weight and height were measured with the child in underwear and in bare feet. Weight was measured to the nearest one-tenth of a kilogram with the use of a digital scale (Tanita), and height was measured to the nearest one-tenth of a centimetre with the use of a wall stadiometer (seca®). BMI was calculated as the value of weight (kg) over squared height (m^2). For statistical purposes, BMI was computed as an age- and sex-specific BMI standard deviation (SD) score (*z* score), according to the World Health Organization Child Growth Standards (5-19 years) (27).

2.4. Data analysis

To evaluate the association between socioeconomic position and hs-CRP levels, we used linear mixed-effects models (LMMs), calculating linear regression coefficients (β_2) and respective 95% confidence intervals (CIs). The assessment of nadir (the lowest point in a curve) related to log hs-CRP throughout childhood, together with the age at which it was observed, were assessed using trajectories of log hs-CRP levels by socioeconomic circumstances. LMMs are frequently used to examine changes in human behaviour over time, are very flexible, and estimate model parameters (28, 29). To explain and interpret the values of β_2, Supplementary Figure 1 shows three simulated trajectories. Specifically, β_2, β_1, and β_0 represent the coefficients of equation $\beta_2*x^2 + \beta_1*x + \beta_0=0$. Focusing on the x-axis, h is the distance from the origin to the point x, where the curve reaches its minimum, that is, $h=-\beta_1/(2*\beta_2)$. Focusing on the y-axis, k is the distance from Y=0 to the point where the curve reaches its minimum, that is, $k= \beta_2*h^2 + \beta_1*h + \beta_0$. Then, curve 3 presents a higher value of h than curves 1 and 2 because the distance between the origin and the point at which the function reaches its minimum is larger than in the other curves. Additionally, curve 1 has the lowest value of k because the minimum point of this function is reached around 0.5. In contrast, the values of k for curves 2 and 3 are around 1.5 and 2.5, respectively.

All coding was implemented using R language (30). Specifically, the analysis was conducted using the R package nlme (31) uploaded in the Comprehensive R Archive Network (CRAN). Supplementary Figures 2a and 2b correspond to a preliminary analysis using local polynomial regression fitting methods. This allowed us to verify that the LMM approach used in this study fitted the data. Specifically, we applied the locally weighted scatter-plot smoother (LOWESS) from the ggplot2 (32) R package. The LOWESS method provided a nonparametric estimation of the predicted trajectories, with the advantage of not having to assume a parametric model for the data. For all the socioeconomic indicators, the quadratic shape was generally similar for the three categories (low, intermediate, and high). Then, the LMM approach (considering a quadratic shape) was suitable for the data.

Thus, for the present analyses, we considered the role of BMI and underlying chronic conditions, such as asthma, because they may account for the studied association in children. Because this sample was not heterogeneous in terms of ethnicity, we were not able to consider this factor in the statistical models.

The interaction term between socioeconomic indicators and sex was fitted in the regression models, and no significant interaction was found (mother's education: $p > 0.764$; mother's occupation: $p > 0.980$; father's education: $p > 0.123$; father's occupation: $p > 0.910$; disposable household income: $p > 0.995$). However, to control for increased exposure to sex hormones, puberty-related shifts in body structure with significant changes in body composition—where girls tend to accumulate more fat than boys—and consequently hs-CRP levels (33, 34) were stratified.

2.5. Sensitivity analysis

Information on disease history was obtained from caregivers. Although the prevalence of medical conditions is low in the study population, asthma is the most common chronic disease in children (35) and may play a role in the reported association between socioeconomic circumstances and hs-CRP (36). Because asthma is an inflammatory disease, some patients with asthma have higher CRP levels than their healthy counterparts (37-39). Furthermore, because formal analysis of interaction between socioeconomic indicators and asthma was found with paternal occupation ($p = 0.024$), a stratified analysis was performed to assess differences in log hs-CRP trajectories in participants with and without an asthma diagnosis.

3. Results

3.1. General results description

Sample characteristics are shown in Table 1. At baseline, around 20% of mothers had a low occupational position, while almost 40% had less than nine years of formal education. Among fathers, almost 50% had a low level of formal education, and close to 40% had blue-collar occupations. For household income levels, the proportions were very similar for both girls and boys: less than 35% and around 50% of families had low and intermediate income, respectively.

Compared with children from families with more socioeconomic-advantaged circumstances, the median of the log hs-CRP was higher among seven-year-old girls with fathers with a low occupational position and among seven-year-old boys with mothers with a low level of education and a low occupational position and from families with a low disposable household income. At the age of ten years, the median hs-CRP was higher in girls and boys with mothers and fathers with a low level of formal education, and in girls with fathers with a low occupational level. Boys with mothers with a low occupational position and from families with a low disposable household income also had higher median hs-CRP levels when compared with those from more socioeconomically advantaged families (Supplementary Table 1).

Figure 2 shows inflammation trajectories according to socioeconomic categories. In general, log hs-CRP levels increased throughout childhood, and both girls and boys from less-advantaged socioeconomic circumstances presented high levels of hs-CRP at the age of ten years. Table 2 includes the estimated results considering a linear mixed-effects model. Nadir and the age at which it occurs differs with socioeconomic circumstances. Specifically, log hs-CRP levels were higher, and nadir occurred earlier in participants from less-advantaged families.

3.2. Parental education

Girls whose mothers had high educational levels had a log hs-CRP level 0.15 lower (95% CI: -0.27 to -0.03) than girls whose mothers had a low education level (Table 2). With regard to the age at which nadir occurred, among girls with highly educated mothers, nadir occurred 16.13 months later than in girls whose mothers had intermediate and low educational levels. Girls whose fathers had high educational levels had a log hs-CRP level 0.21 lower (95% CI: -0.39 to -0.03) than girls whose fathers had a low education level (Table 2). Among girls with fathers with high educational levels, age at which nadir occurred was 8.43 months later than in girls with fathers with low educational levels.

In boys, the same tendency was observed. For instance, boys with mothers with intermediate and high educational levels had a log hs-CRP level 0.14 and 0.08 lower, respectively, than boys with mothers with a low level of education. In regard to paternal education, boys with fathers with intermediate and high educational levels presented a log hs-CRP level 0.05 and 0.14 lower, respectively, than boys with fathers with a low level of education. The same trend regarding age at nadir observed among girls was seen among boys. Boys with mothers with high levels of education had nadir 12.31 months later than boys with mothers with a low level of education. Boys with fathers with intermediate and high levels of education reached age at nadir 14.94 and 26.83 months later, respectively, than boys with fathers with a low level of education.

3.3. Parental occupation

Girls whose mothers were in the highest category of occupation had a log hs-CRP level 0.02 lower (95% CI: -0.19 to -0.14) than girls whose mothers were in the lowest category (Table 2). It was observed that among girls with mothers in the highest category of occupation, age at nadir occurred 24.43 months later than in girls with mothers in the lowest category of occupation. Girls whose fathers had high occupational levels had a log hs-CRP level 0.16 lower (95% CI: -0.29 to -0.04) than girls whose fathers had low occupational levels (Table 2). Age at which nadir occurred was 4.47 months later than among girls whose fathers had low occupational levels.

In boys, the same trend was observed. For instance, boys with mothers with intermediate and high occupational levels had a log hs-CRP level 0.10 and 0.04 lower, respectively, than boys with fathers with a low level of occupation. Regarding paternal occupation, boys whose fathers had intermediate and high occupational levels had a log hs-CRP level 0.03 and 0.09 lower, respectively, than boys whose fathers had a low level of occupation. The same trend regarding age at nadir observed among girls was also seen among boys. Boys whose mothers had high levels of occupation reached nadir 17.73 months later than boys whose mothers had a low level of occupation. Boys whose fathers had intermediate and high levels of occupation had age at nadir 0.87 and 7.17 months later, respectively, than boys with fathers with a low level of occupation.

3.4. Household income

Girls from families with a high disposable household income had a log hs-CRP level 0.19 lower (95% CI: -0.34 to -0.03) than those from families with a low disposable household income (Table 2). Age at nadir occurred 5.33 and 12.82 months later in girls from families with an intermediate and high disposable household income, respectively, compared with girls from families with a low disposable household income (Table 2).

Among boys from families with intermediate and high disposable household income, the log hs-CRP level was 0.04 and 0.12 lower, respectively, than for boys from families with low disposable household income. Boys from families with intermediate and high household disposable income reached age at nadir 2.11 and 10.28 months later, respectively, than boys from families with a low disposable household income.

3.5. Sensitivity analyses

A sensitivity analysis was conducted by adjusting for children's BMI, and a similar trend of the log hs-CRP trajectories was found according to socioeconomic indicators, with nadir among participants from less-advantaged socioeconomic circumstances being higher than among more-advantaged participants. It also occurred earlier, as observed in the nonadjusted trajectories (Supplementary Table 2).

Stratified analysis was performed between participants with (5.3%) and without an asthma diagnosis. Children diagnosed with asthma presented a log hs-CRP level at nadir lower than those without an asthma diagnosis (Supplementary Table 3a and 3b). The exception was found among boys when using the father´s occupation as the socioeconomic indicator (p = 0.024). Boys with asthma and whose father had a high occupational level had a log hs-CRP level of -0.23 (95% CI: -0.83 to 0.36), while those without asthma had a log hs-CRP level of -0.08 (95% CI: -0.18 to 0.01), which was very similar to the results found among the whole sample (log hs-CRP level, -0.09 (95% CI: -0.20 to 0.02).

4. Discussion

The results of the present study show that children from less-advantaged socioeconomic circumstances had higher levels of hs-CRP throughout childhood and began an increasing trajectory of hs-CRP, after nadir, earlier than those from intermediate or high socioeconomic groups.

Inflammation is hypothesized to play a role in the link between socioeconomic circumstances and cardiometabolic health outcomes (4) and cancer (5), as well as an increased risk of premature death (6). The onset of this process of disease due to chronic low-grade inflammation seems to begin during childhood (40, 41). In fact, cross-sectional studies have been reporting associations between lower socioeconomic position and low-grade inflammation already during childhood (16). Thus, our results support the evidence that social differences may start early in life, with the potential to increase over the life course.

The pathways by which socioeconomic circumstances seem to impact inflammatory processes can occur via stress sensitization, by activation of the hypothalamic-pituitary-adrenal axis (42), leading to altered insulin sensitivity, increased blood pressure, and inflated central adiposity, and consequently to elevated inflammation (1, 42). The adoption of harmful health habits, such as sedentary lifestyles, poor diet, and smoking (43), might also be mediating the association between low early socioeconomic circumstances and later disease development. In adult studies, health-related behaviours, such as smoking or sedentarism, may partly explain social differences in inflammation, with those from less-advantaged socioeconomic circumstances being more prone to engage in more unhealthy risk behaviours (44, 45). In fact, it has been described that higher CRP levels are observed among participants from low SES when compared with participants in higher SES groups, after adjusting for health behaviours (46). Additionally, people living in poverty were more likely to be obese and less likely to exercise, contributing to a higher risk of very high CRP levels (47). Yet, the same study has shown that controlling for acute and chronic conditions and health behaviours did not fully account for the effect of poverty on CRP levels (47). Another study showed that low parental socioeconomic position was associated with chronic low-grade inflammation in adolescence, after adjustment for sex, perinatal and physical environment factors, health-related behaviours, and health status (48). Although we do not expect the same contribution of health-related behaviours in children, because they may not be fully established at these ages, they also seem to not fully explain the association between disadvantaged socioeconomic circumstances and elevated CRP levels. Thus, observing social differences in inflammatory markers in childhood leads us to hypothesize that exposure to adverse socioeconomic conditions during this sensitive developmental period may be explained by the stress pathway caused by deprivation, which may lead to chronic activation of the hypothalamic-pituitary-

adrenocortical axis and, consequently, to the establishment of chronic low-grade inflammation (42, 49), in the first years of life.

The results were stratified by sex, but an overall hs-CRP trajectory throughout childhood was added as supplementary material (Supplementary Figure 4). The rationale for stratifying is due to the biological differences found between girls and boys, and in accordance with published literature finding significantly greater mean CRP levels in women compared with men (50-52). Although published literature shows sex differences in adults, our results showed that these differences can be found in early ages, as levels of hs-CRP are higher across all childhood in girls when compared with boys. However, although we observe some sex differences in childhood, this does not necessarily mean an increased risk of developing disease later in life. In fact, CRP has been shown to independently predict cardiovascular events in both men and women (51, 53). And although women have higher CRP levels, they are at lower risk for cardiovascular events compared with men (54), and some discussion has been raised on optimal sex-specific CRP cut-offs to be defined that most accurately predict cardiovascular risk (51). There are several factors that can potentially contribute to sex-differentiated trajectories of CRP. Among those, adiposity/obesity/BMI, which is one of the factors most strongly associated with CRP (52, 55, 56) (see Supplementary Figure 3). Although CRP levels generally increase throughout childhood, as previously described (18), a decrease in the levels of hs-CRP from the age of four years to the age of seven years was observed in the trajectories (nadir). This decline may be explained by the close association between CRP levels and BMI (57). BMI rapidly increases during the first year of life, then subsequently declines and reaches a minimum at around the age of six years (as the adiposity rebound starts), before it begins to increase up to the end of adolescence (58, 59). Thus, one may expect that hs-CRP levels follow BMI patterns and shift from decreasing to increasing from late childhood until the end of the growth period. We conducted a sensitivity analysis adjusting for BMI and found similar trends in the hs-CRP trajectories and age at nadir (Supplementary Table 2). Also, physical/sexual maturation might contribute to sex-differentiated trajectories of CRP by increasing exposure to sex hormones, with potentially different effects on girls and boys (Shanahan et al., 2013; Williams et al., 2004). Because some of these children are already in or at the onset of puberty, there is increased exposure to sex hormones and puberty-related shifts in body structure with significant changes in body composition, where girls tend to accumulate more fat than boys and consequently hs-CRP levels (Fonseca et al., 2019; Nemet et al., 2003). This is supported by a previous study conducted in Generation XXI that showed that girls were more sexually mature than boys (Tanner≥2) and, independently of previous BMI, preteens with early puberty had more adiposity at the age of ten years (Fonseca et al., 2019).

In the present study, we found an association between early socioeconomic circumstances and higher levels of log hs-CRP levels. We used three socioeconomic indicators to include different aspects of the early socioeconomic conditions that might influence educational and health-related choices regarding the child. However, different socioeconomic measures may capture different effects on the child's care and consequently on the child's health (17). Knowledge and skills attained through formal education may affect a person's cognitive functioning (17), with mother's education, in particular, contributing through factors more closely associated with mothers' literacy and consequently with mothers' care and choices regarding their children (60). Further, paternal occupation being more associated with financial availability and material assets (60) may have a significant direct impact on children´s health (61). As easy and self-reported measures, both education and occupation are reliable sources of information about families' socioeconomic circumstances (Galobardes et al., 2006a). On the other hand, there is potential for underestimation in regard to income, with differences between reported income and tax-reported income, in particular, among the highest income participants. Although asking for family income might potentially lead to bias through underestimation (62), existence of bias would lead to an increasing of inequalities (63), therefore, not affecting our results. Nevertheless, the observed association between different indicators and low-grade inflammation during childhood emphasizes the role of early socioeconomic conditions on the onset and establishment of inflammatory processes during the first years of life.

4.1. Strengths and limitations

The main strength of this study lies in the use of longitudinal data from the well-established population-based birth cohort Generation XXI. The use of cohort data allowed us to observe and analyse different stages of children's growth and to establish a causal relationship between the exposure, family socioeconomic circumstances at birth, and the outcome of low-grade inflammation throughout childhood. Also, this study explored the onset of a trajectory of inflammation at very early ages. The assessment of three aspects of children's socioeconomic circumstances (parental education and occupation and household income), collected at the time of the children's birth, decreased the potential for recall bias. The collection of data about both parents also allowed us to have a more comprehensive assessment of the family´s socioeconomic circumstances. We used the above-mentioned measures of socioeconomic circumstances because we believe they allow us to characterize a family´s socioeconomic position. Socioeconomic circumstances were used as a proxy of exposure to adverse conditions because they have also been demonstrated to be significant predictors of health in adult life.

Nevertheless, this study also has potential limitations. The association between increasing inflammatory levels with low parental socioeconomic circumstances throughout childhood may be explained by the fact that these children may be more exposed to environmental and physical risk factors, and thus be more susceptible to infections (14). Trying to minimize the effect of acute infections, we excluded participants with hs-CRP levels higher than 10 mg/L (26) from the analysis. However, the number of exclusions was low and thus not having an impact in the trajectory definition (Supplementary Table 4). Moreover, this study only comprised one measure of inflammation, but several population studies, also using CRP levels, have been successful in establishing an association between being born in less-advantaged socioeconomic circumstances with prolonged low-grade activation of the immune system and consequently higher inflammatory levels (13, 64). Other studies have shown an inverse association of basal circulating levels of interleukin (IL)-6 with indicators of parental SES during the first two years of life, but not later in childhood. These associations were independent of adult SES, suggesting that SES in early childhood has a unique role in adult inflammation (65). Also, in general, behavioural and psychosocial health risk factors, such as smoking, lower physical fitness, poorer sleep quality, lower self-compassion, and loneliness, are associated with larger increases in circulating IL-6 (66). Although data on IL-6 could possibly reinforce our results, we only have information on this biomarker for a subsample of participants at the age of ten years and, while CRP is broadly known as a marker of chronic inflammation (67), IL-6 is mainly an acute-phase protein, synthesized in a local lesion in the initial stage of inflammation (68). Additionally, we did not expect behavioural risks to be present during the first years of life and we would not expect to observe a perceptible impact on this biomarker.

The use of Generation XXI cohort data allowed us to observe and analyse levels of hs-CRP across the first ten years of life. However, because it is common in prospective birth cohorts, there has been attrition over time, leading to a reduction in the sample size and a more socioeconomically advantaged group of participants throughout childhood. Nevertheless, we believe that the inclusion of the more-disadvantaged group would have widened the differences found in the inflammatory profile, which may be underestimating the effect of socioeconomic circumstances on children's CRP levels.

Research indicates that family structure at birth seems to influence children's health outcomes, with children raised in stable married families presenting better overall health (69). Also, children living in single-parent households may be affected from not having both parents living in the same house, thus relying on the parent that is more present in all life stages and having fewer resources available at home to face expenses. Even a single parent supporting the family with an above-average salary may increase

the burden and impact a child's health. However, by using different socioeconomic indicators we believe that we overcame this issue.

Despite these limitations, these results show a relevant role of early socioeconomic circumstances shaping inflammatory processes over the period of early to late childhood. These results also increase our understanding of socioeconomic circumstances as an aspect of children's family contexts that may induce inflammation related to chronic stress exposure.

5. Conclusion

Our results suggest that socioeconomic circumstances at birth are associated with increased inflammation levels throughout the first decade of life. This study demonstrates that the impact of social inequalities in population health seems to have its onset at very early ages. We hypothesize that early childhood may be a sensitive developmental period that reflects the embodiment of family socioeconomic characteristics. This might be reflecting a pathway for the onset of low-grade inflammation, rather than the accumulation of risk that will only be more evident in later stages of life, such as the end of adolescence and during adult life. Interventions in early childhood and strategies for ensuring that every child has an optimal start in life are crucial to reducing the burden of inflammation and, consequently, health inequalities.

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Table 1. Descriptive statistics of selected sociodemographic characteristics and anthropometric measures in the Generation XXI cohort

	Girls	Boys
Children´s current characteristics		
Age, months (median [25th-75 percentile])		
2nd wave	50.0 (48.0-53.0)	50.0 (48.0-53.0)
3rd wave	85.0 (84.0-85.0)	85.0 (84.0-85.0)
4th wave	123.1 (122.0-124.2)	123.1 (122.2-124.1)
Body Mass Index		
2nd wave (4 years)		
Underweight/normal	712 (65.8)	889 (72.0)
Overweight	246 (22.7)	249 (20.2)
Obese	124 (11.5)	97 (7.8)
3rd wave (7 years)		
Underweight/normal	737 (62.8)	878 (65.7)
Overweight	262 (22.3)	278 (20.8)
Obese	175 (14.9)	180 (13.5)
4th wave (10 years)		
Underweight/normal	650 (57.5)	740 (57.4)
Overweight	307 (27.1)	327 (25.4)
Obese	174 (15.4)	221 (17.2)
Family´s characteristics at baseline		
Maternal education		
Low	446 (38.2)	499 (37.6)
Intermediate	358 (30.6)	417 (31.3)
High	365 (31.2)	414 (31.1)
Maternal occupation		
Low	230 (20.9)	260 (20.4)
Intermediate	531 (48.3)	622 (48.9)
High	339 (30.8)	390 (30.7)
Paternal education		
Low	315 (49.5)	370 (47.9)
Intermediate	165 (25.9)	226 (29.3)
High	157 (24.6)	176 (22.8)
Paternal occupation		
Low	387 (37.0)	460 (38.5)
Intermediate	219 (21.0)	245 (20.6)
High	439 (42.0)	489 (40.9)
Household income		
Low	361 (34.8)	389 (32.6)
Intermediate	492 (47.5)	596 (50.0)
High	183 (17.7)	207 (17.4)

Table 2. Predicted values of minimum log hs-CRP (nadir) and calendar age at nadir (months), by socioeconomic indicators using a mixed-effects model, in girls and boys.

		Girls				Boys			
Socioeconomic indicator		β_2[a]	Nadir (95% CI)[b]	*p*	Age at nadir (months) (95% CI)	*p*	β_2[a]	Nadir (95% CI)[b]	*p*	Age at nadir (months) (95% CI)	*p*
Maternal Education	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.08 (-0.21; 0.06)	0.259	-0.56 (-16.65; 15.52)	0.945		-0.14 (0.25; -0.02)	0.027	0.12 (-8.37; 8.61)	0.978
	High		-0.15 (-0.27; -0.03)	0.017	16.13 (-2.28; 34.53)	0.086		-0.08 (-0.19; 0.03)	0.169	12.31 (2.96; 21.66)	0.010
Maternal Occupation	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.08 (-0.08; 0.24)	0.338	11.30 (-7.38; 29.99)	0.236		-0.10 (-0.24; 0.04)	0.153	2.93 (-7.14; 13.00)	0.569
	High		-0.02 (-0.19; -0.14)	0.770	24.43 (-0.61; 48.24)	0.045		-0.04 (-0.18; -0.11)	0.616	17.73 (5.35; 30.11)	0.005
Paternal Education	Low	0.0001	Reference		Reference		0.0001	Reference		Reference	
	Intermediate		-0.02 (-0.19; 0.15)	0.813	13.14 (-11.66; 37.95)	0.300		-0.05 (-0.11; -0.21)	0.526	14.94 (0.03; 29.86)	0.050
	High		-0.21 (-0.39; -0.03)	0.023	8.43 (-15.34; 32.20)	0.488		-0.14 (-0.30; 0.02)	0.096	26.83 (7.78; 45.88)	0.006
Paternal Occupation	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.09 (-0.23; 0.06)	0.246	8.29 (-10.58; 27.15)	0.390		-0.03 (-0.11; 0.17)	0.644	0.87 (-9.00; 10.74)	0.864
	High		-0.16 (-0.29; -0.04)	0.010	4.47 (-10.77; 19.72)	0.566		-0.09 (-0.20; 0.02)	0.128	7.17 (-1.18; 15.53)	0.093
Household Income	Low	0.0001	Reference		Reference		0.0001	Reference		Reference	
	Intermediate		-0.01 (-0.14; 0.11)	0.844	5.33 (-11.82; 22.47)	0.543		-0.04 (-0.16; 0.08)	0.499	2.11 (-7.25; 11.47)	0.659
	High		-0.19 (-0.34; -0.03)	0.020	12.82 (-10.32; 35.95)	0.278		-0.12 (-0.27; 0.02)	0.096	10.28 (-2.45; 23.01)	0.114

[a] β_2: linear regression coefficient.

[b] Nadir: minimum value of log hs-CRP throughout childhood.

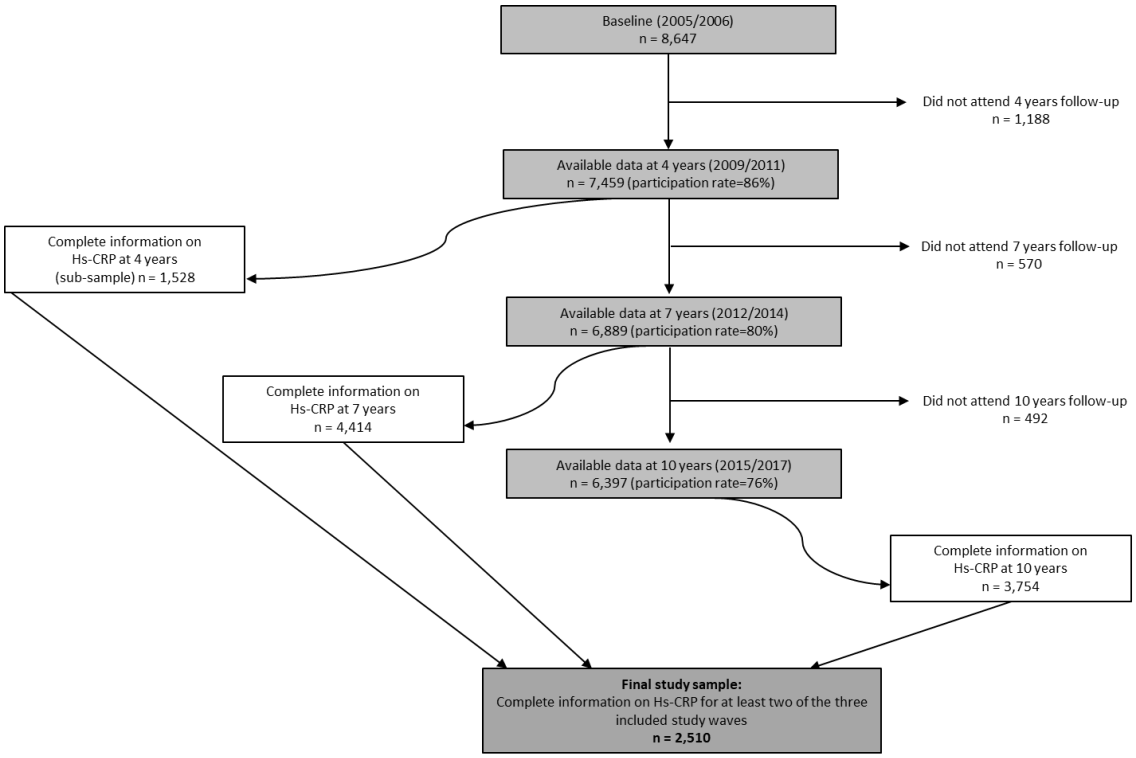
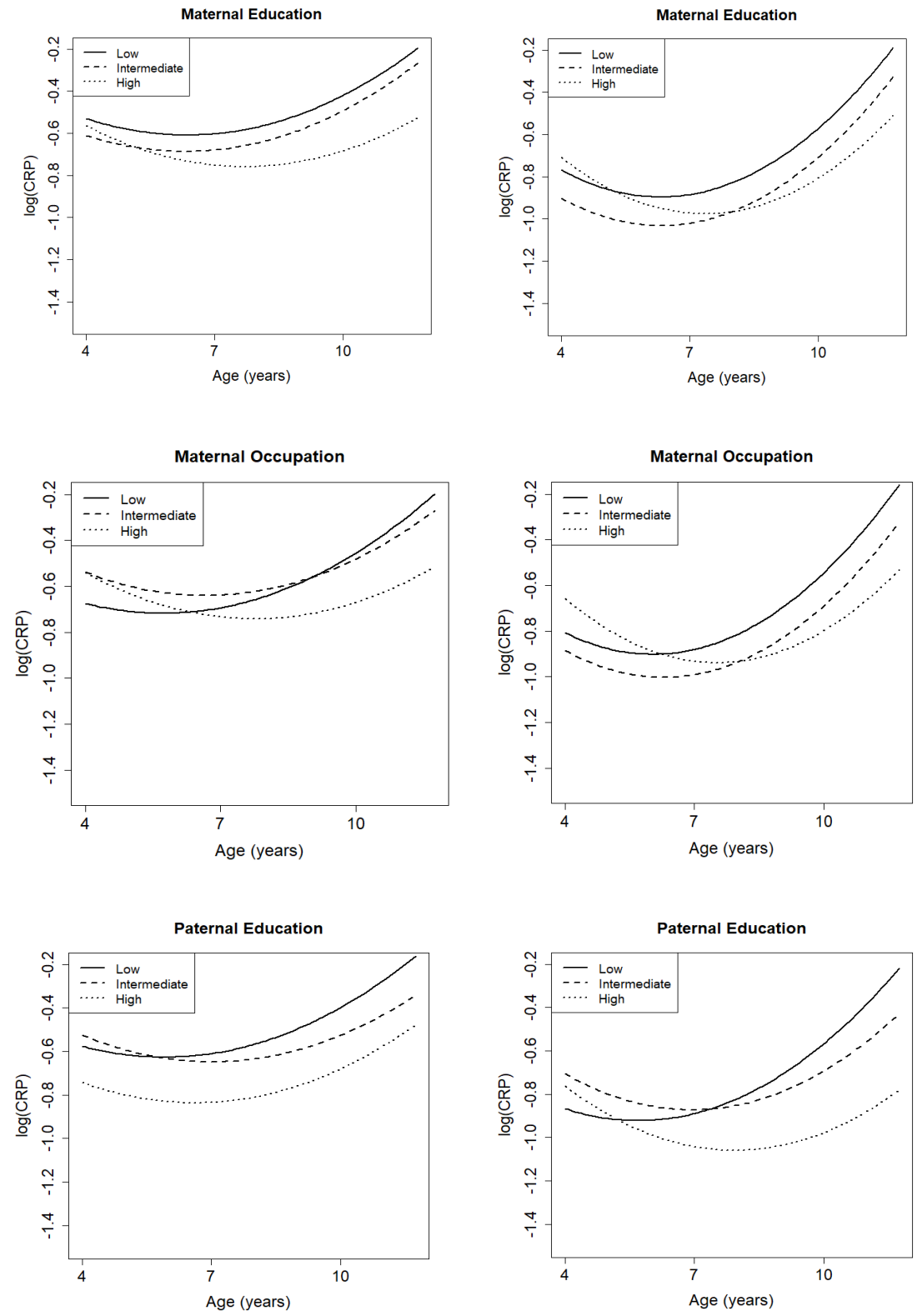
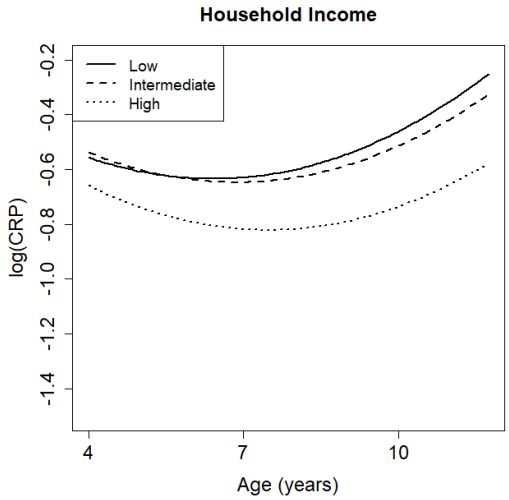
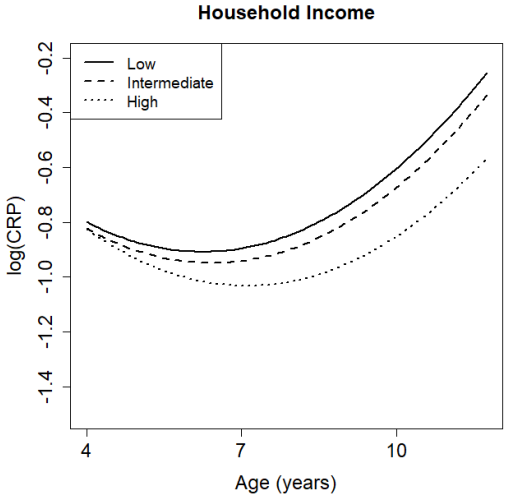


Figure 1. Flow chart of participation in all Generation 21 study waves

Figure 2. Predicted fixed effects of log hs-CRP and child age, by socioeconomic indicators, in girls and boys.



Girls	Boys
 Paternal Occupation Low Intermediate High log(CRP) -0.2 -0.4 -0.6 -0.8 -1.0 -1.2 -1.4 4 7 10 Age (years)	 Paternal Occupation Low Intermediate High log(CRP) -0.2 -0.4 -0.6 -0.8 -1.0 -1.2 -1.4 4 7 10 Age (years)
 Household Income Low Intermediate High log(CRP) -0.2 -0.4 -0.6 -0.8 -1.0 -1.2 -1.4 4 7 10 Age (years)	 Household Income Low Intermediate High log(CRP) -0.2 -0.4 -0.6 -0.8 -1.0 -1.2 -1.4 4 7 10 Age (years)

Supplementary Table 1. Distribution of Hs-C-reactive protein (median (P25-P75)) by sociodemographic characteristics, in participants, enrolled in each study wave.

	hs-CRP (4 years) Median (P25-P75)		hs-CRP (7 years) Median (P25-P75)		hs-CRP (10 years) Median (P25-P75)	
Family´s characteristics at baseline	Girls	Boys	Girls	Boys	Girls	Boys
Maternal education						
Low	0.5 (0.2-1.0)	0.3 (0.2-0.8)	0.4 (0.2-1.1)	0.3 (0.2-0.6)	0.6 (0.2-1.5)	0.5 (0.2-1.2)
Intermediate	0.4 (0.2-0.9)	0.3 (0.2-0.7)	0.4 (0.2-1.0)	0.2 (0.2-0.5)	0.5 (0.2-1.2)	0.4 (0.2-0.9)
High	0.5 (0.2-1.5)	0.3 (0.2-0.9)	0.3 (0.2-0.7)	0.2 (0.2-0.5)	0.4 (0.2-0.9)	0.3 (0.2-0.9)
Maternal occupation						
Low	0.3 (0.2-0.9)	0.4 (0.2-0.6)	0.4 (0.2-1.0)	0.3 (0.2-0.7)	0.5 (0.2-1.5)	0.5 (0.2-1.3)
Intermediate	0.5 (0.2-1.1)	0.3 (0.2-0.7)	0.4 (0.2-0.9)	0.2 (0.2-0.5)	0.6 (0.2-1.3)	0.4 (0.2-1.0)
High	0.5 (0.2-1.6)	0.3 (0.2-1.0)	0.3 (0.2-0.7)	0.2 (0.2-0.6)	0.5 (0.2-0.9)	0.3 (0.2-0.9)
Paternal education						
Low	0.5 (0.2-1.0)	0.3 (0.2-0.8)	0.4 (0.2-1.0)	0.2 (0.2-0.6)	0.7 (0.3-1.5)	0.5 (0.2-1.2)
Intermediate	0.5 (0.2-1.0)	0.4 (0.2-1.2)	0.4 (0.2-1.0)	0.2 (0.2-0.6)	0.6 (0.2-0.9)	0.4 (0.2-1.0)
High	0.4 (0.2-1.0)	0.3 (0.2-0.5)	0.3 (0.2-0.7)	0.2 (0.2-0.5)	0.4 (0.2-0.9)	0.3 (0.2-0.6)
Paternal occupation						
Low	0.5 (0.2-1.3)	0.3 (0.2-0.7)	0.5 (0.2-1.0)	0.2 (0.2-0.6)	0.7 (0.3-1.5)	0.5 (0.2-1.1)
Intermediate	0.4 (0.2-0.9)	0.3 (0.2-0.8)	0.4 (0.2-1.1)	0.2 (0.2-0.6)	0.5 (0.2-1.0)	0.5 (0.1-1.1)
High	0.5 (0.2-1.1)	0.3 (0.2-0.7)	0.4 (0.2-0.7)	0.2 (0.2-0.5)	0.5 (0.2-1.0)	0.3 (0.2-0.9)
Household income						
Low	0.5 (0.2-1.3)	0.3 (0.2-0.7)	0.4 (0.2-1.0)	0.3 (0.2-0.6)	0.6 (0.2-1.4)	0.5 (0.2-1.1)
Intermediate	0.5 (0.2-1.0)	0.3 (0.2-0.8)	0.4 (0.2-1.0)	0.2 (0.2-0.6)	0.6 (0.2-1.1)	0.4 (0.2-1.0)
High	0.4 (0.2-1.5)	0.3 (0.2-0.5)	0.3 (0.2-0.7)	0.2 (0.2-0.5)	0.4 (0.2-0.9)	0.3 (0.2-0.8)

Supplementary Table 2. Predicted values of minimum log hs-CRP (nadir) and calendar age at nadir (months), by socioeconomic indicators, using a mixed-effects model, adjusted for body mass index, in girls and boys

Socioeconomic indicator		Girls				Boys			
		β_2	Nadir (95%CI), adjusted for BMI	p	Age at nadir (months) (95%CI), adjusted for BMI	p	β_2	Nadir (95%CI), adjusted for BMI	p	Age at nadir (months) (95%CI), adjusted for BMI	p
Maternal Education	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.05 (-0.17; 0.06)	0.372	-1.43 (-14.44; 11.57)	0.829		-0.12 (-0.23; -0.02)	0.022	0.63 (-7.41; 8.67)	0.878
	High		-0.08 (-0.19; 0.03)	0.178	12.33 (-1.64; 26.30)	0.084		-0.04 (-0.14; 0.06)	0.481	11.79 (3.02; 20.56)	0.009
Maternal Occupation	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.07 (-0.07; 0.21)	0.311	7.01 (-7.44; 21.47)	0.342		-0.12 (-0.24; 0.00)	0.058	2.42 (-7.07; 11.92)	0.617
	High		-0.03 (-0.11; 0.17)	0.675	17.72 (0.44; 35.00)	0.045		-0.02 (-0.14; 0.11)	0.790	15.10 (3.84; 26.35)	0.009
Paternal Education	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.01 (-0.14; 0.16)	0.883	8.95 (-12.16; 30.06)	0.407		0.05 (-0.09; 0.18)	0.495	13.66 (0.55; 26.77)	0.042
	High		-0.14 (-0.31; 0.02)	0.090	1.37 (-19.07; 21.81)	0.896		-0.09 (-0.23; 0.05)	0.219	22.24 (6.25; 38.23)	0.007
Paternal Occupation	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.04 (-0.17; 0.09)	0.536	8.70 (-7.49; 24.90)	0.293		-0.01 (-0.13; 0.11)	0.852	1.99 (-7.27; 11.25)	0.674
	High		-0.08 (-0.19; 0.04)	0.188	3.38 (-9.57; 16.34)	0.609		-0.08 (-0.18; 0.01)	0.092	6.08 (-1.74; 13.89)	0.128
Household Income	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		0.01 (-0.10; 0.12)	0.695	1.87 (-11.72; 15.46)	0.676		-0.06 (-0.16; 0.04)	0.249	4.28 (-4.68; 13.23)	0.224
	High		-0.13 (-0.27; 0.02)	0.366	5.16 (-12.50; 22.82)	0.931		-0.21 (-0.08; 0.05)	0.167	9.94 (-2.14; 22.02)	0.289

β_2: linear regression coefficient; nadir: minimum value of log hs-CRP throughout childhood

Supplementary Table 3a. Predicted values of minimum log hs-CRP (nadir) and calendar age at nadir (months), by socioeconomic indicators, using a mixed-effects model, in girls and boys with asthma

Socioeconomic indicator		Girls					Boys				
		β_2	Nadir (95%CI), adjusted for BMI	p	Age at nadir (months) (95%CI), adjusted for BMI	p	β_2	Nadir (95%CI), adjusted for BMI	p	Age at nadir (months) (95%CI), adjusted for BMI	p
Maternal Education	Low	0.0003	Reference		Reference		0.0001	Reference		Reference	
	Intermediate		-0.12 (-0.63; 0.39)	0.652	8.07 (-10.32; 26.47)	0.398		0.24 (-0.32; 0.81)	0.405	24.24 (-19.97; 68.44)	0.289
	High		0.11 (-0.48; 0.71)	0.713	20.12 (-5.38; 45.62)	0.131		0.26 (-0.28; 0.80)	0.354	30.08 (-18.68; 78.84)	0.233
Maternal Occupation	Low	0.0004	Reference		Reference		0.0001	-0.97 (-1.40; -0.55)		Reference	
	Intermediate		-0.37 (-0.96; 0.21)	0.220	12.72 (-7.70; 33.13)	0.233		0.07 (-0.49; 0.63)	0.631	16.24 (-8.67; 41.16)	0.256
	High		0.04 (-0.55; 0.63)	0.885	22.61 (-1.84; 47.07)	0.079		0.04 (-0.74; 0.81)	0.280	34.17 (-8.97; 77.30)	0.217
Paternal Education	Low	0.0003	Reference		Reference		0.0003	Reference		Reference	
	Intermediate		0.46 (-0.43; 1.35)	0.328	30.96 (-7.70; 69.63)	0.133		-0.04 (-0.87; 0.78)	0.921	-30.23 (-77.59; 17.12)	0.225
	High		0.22 (-0.55; 1.00)	0.586	14.43 (-18.44; 47.30)	0.405		-0.57 (-1.28; 0.13)	0.126	7.29 (-28.51; 43.08)	0.696
Paternal Occupation	Low	0.0003	Reference		Reference		0.0001	Reference		Reference	
	Intermediate		0.10 (-0.61; 0.80)	0.791	23.73 (-9.19; 56.65)	0.168		-0.07 (-0.76; 0.62)	0.853	10.83 (-36.02; 57.68)	0.654
	High		0.21 (-0.31; 0.74)	0.432	30.44 (0.82; 60.06)	0.051		-0.23 (-0.83; 0.36)	0.443	29.77 (-26.77; 86.30)	0.309
Household Income	Low	0.0003	Reference		Reference		0.0001	Reference		Reference	
	Intermediate		0.07 (-0.49; 0.63)	0.810	16.24 (-8.67; 41.16)	0.213		0.43 (-0.73; 1.60)	0.472	40.83 (-79.38; 161.03)	0.511
	High		0.04 (-0.74; 0.81)	0.927	34.17 (-8.97; 77.30)	0.132		0.27 (-1.06; 1.60)	0.696	42.63 (-98.91; 184.18)	0.560

β_2: linear regression coefficient; nadir: minimum value of log hs-CRP throughout childhood

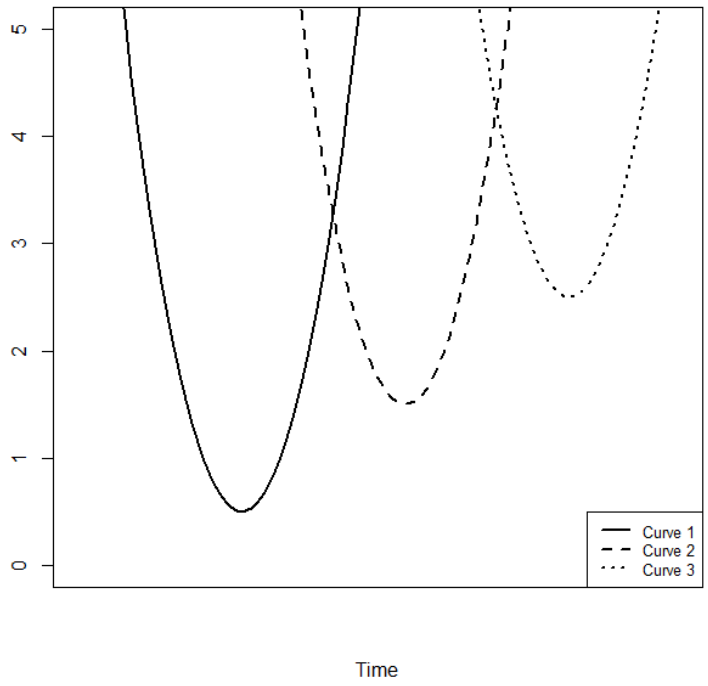
Supplementary Table 3b. Predicted values of minimum log hs-CRP (nadir) and calendar age at nadir (months), by socioeconomic indicators, using a mixed effects model, in girls and boys without asthma

Socioeconomic indicator		Girls					Boys				
		β_2	Nadir (95%CI), adjusted for BMI	p	Age at nadir (months) (95%CI), adjusted for BMI	p	β_2	Nadir (95%CI), adjusted for BMI	p	Age at nadir (months) (95%CI), adjusted for BMI	p
Maternal Education	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.09 (-0.23; 0.05)	0.224	-2.15 (-21.22; 16.93)	0.826		-0.15 (-0.28; -0.03)	0.014	-0.73 (-9.47; 8.01)	0.869
	High		-0.17 (-0.29; -0.04)	0.010	17.61 (-4.48; 39.71)	0.119		-0.10 (-0.22; 0.01)	0.087	11.11 (-1.61; 20.60)	0.022
Maternal Occupation	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.09 (-0.08; 0.26)	0.304	11.64 (-10.79; 34.07)	0.310		-0.12 (-0.238; 0.004)	0.112	0.34 (-10.01; 10.70)	0.949
	High		-0.03 (-0.20; 0.14)	0.737	26.86 (-2.63; 56.35)	0.075		-0.02 (-0.143; 0.108)	0.365	15.04 (2.65; 27.43)	0.018
Paternal Education	Low	0.0001	Reference		Reference		0.0001	Reference		Reference	
	Intermediate		-0.06 (-0.24; 0.13)	0.554	12.43 (-15.32; 40.17)	0.381		0.06 (-0.10; 0.22)	0.494	20.00 (2.95; 37.06)	0.022
	High		-0.23 (-0.42; 0.04)	0.017	10.46 (-16.96; 37.88)	0.455		-0.11 (-0.28; 0.06)	0.202	27.84 (7.43; 48.25)	0.008
Paternal Occupation	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.12 (-0.27; 0.03)	0.102	6.00 (-15.46; 27.46)	0.584		-0.01 (-0.13; 0.11)	0.538	1.10 (-9.14; 11.34)	0.833
	High		-0.20 (-0.33; -0.075)	0.002	0.85 (-16.59; 18.29)	0.924		-0.08 (-0.18; 0.01)	0.235	6.21 (-2.36; 14.79)	0.156
Household Income	Low	0.0001	Reference		Reference		0.0002	Reference		Reference	
	Intermediate		-0.02 (-0.14; 0.11)	0.818	3.80 (-15.60; 23.21)	0.701		-0.06 (-0.18; 0.06)	0.338	0.52 (-8.99; 10.04)	0.915
	High		-0.20 (-0.36; 0.04)	0.015	11.95 (-14.26; 38.15)	0.372		-0.13 (-0.28; 0.01)	0.075	9.16 (-3.65; 21.96)	0.161

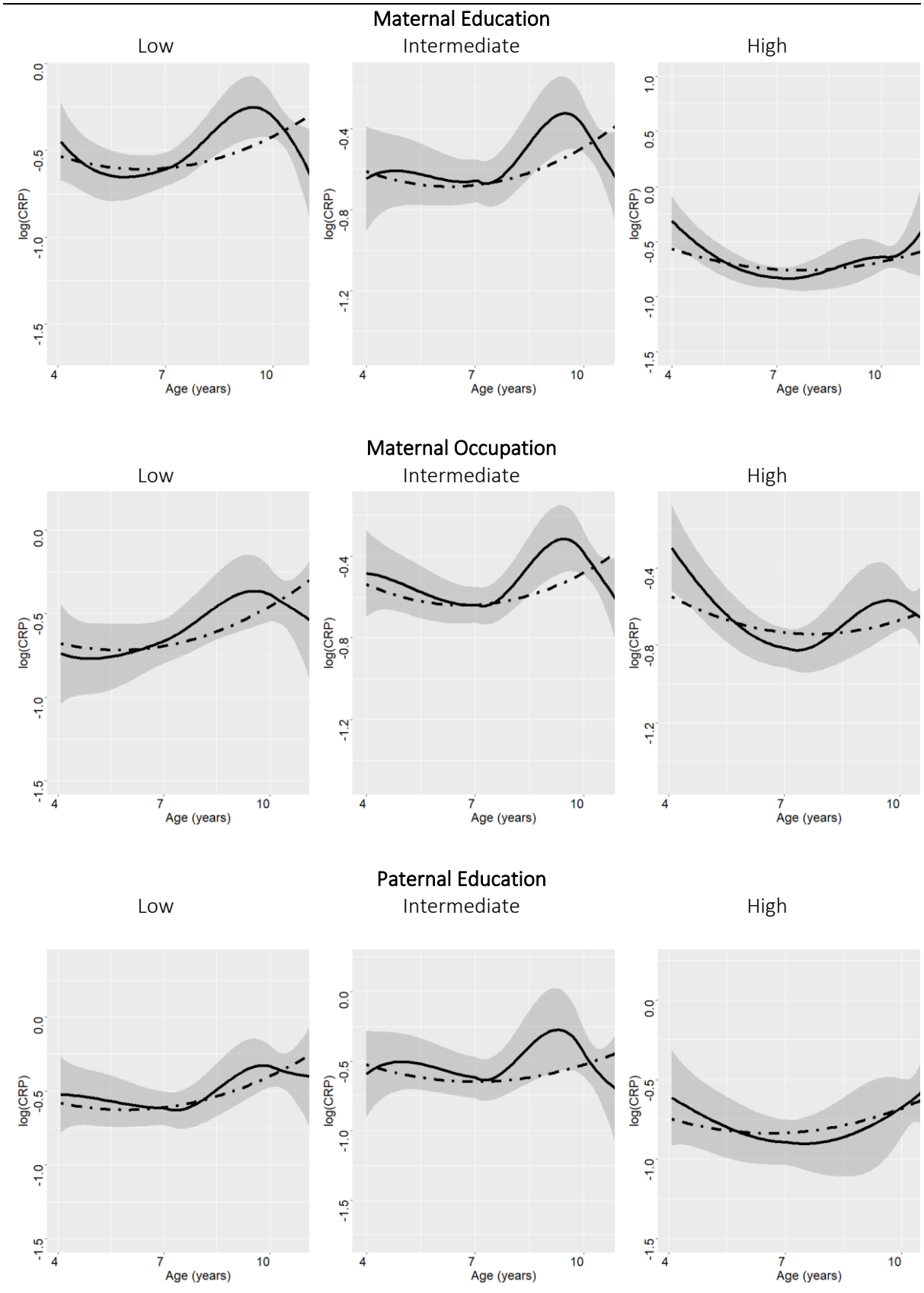
β_2: linear regression coefficient; nadir: minimum value of log hs-CRP throughout childhood

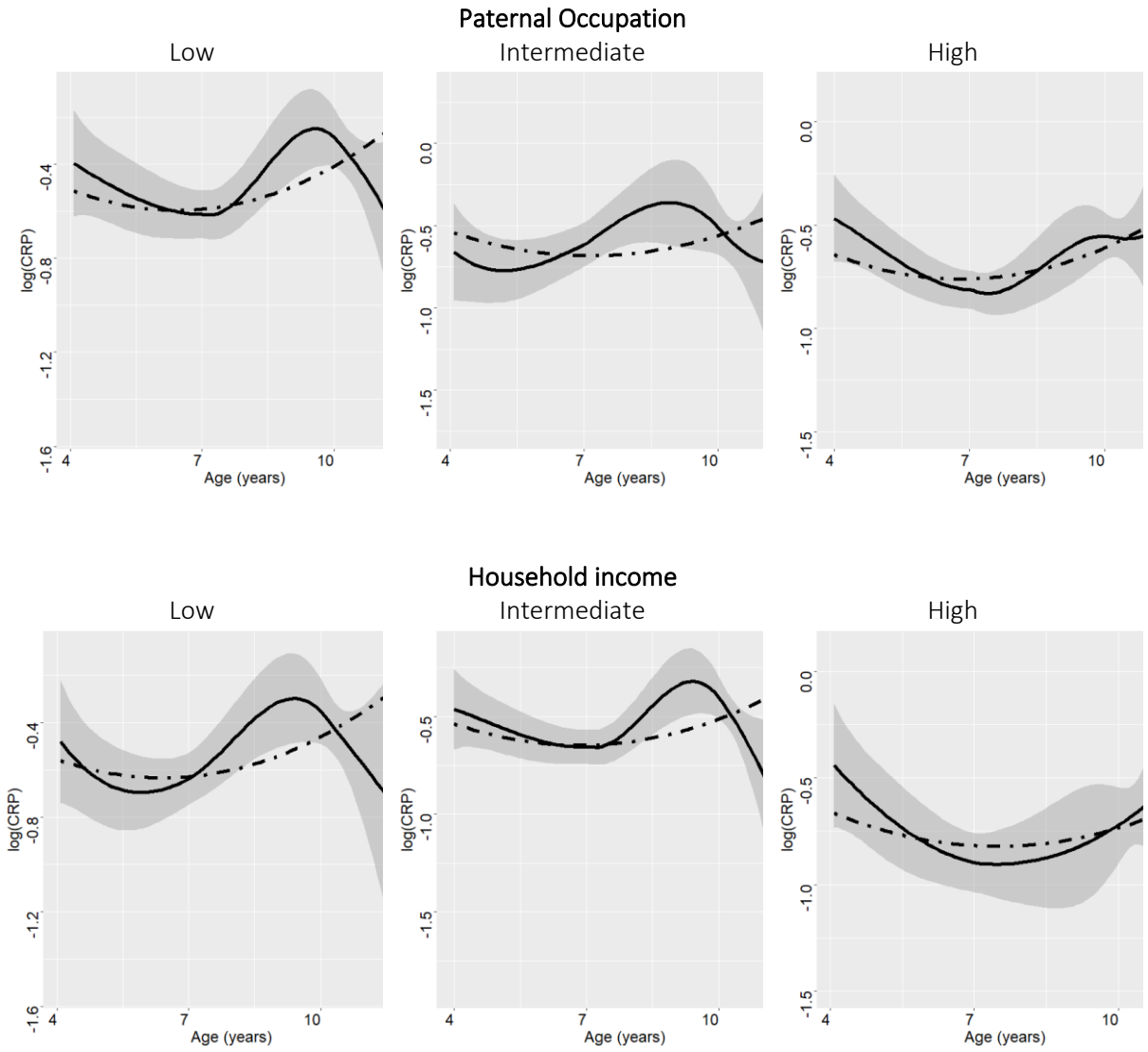
Supplementary Table 4. Descriptive statistics of hs-CRP in the Generation XXI cohort

	Girls	Boys
hs-CRP [median(P25-P75)]		
2nd wave (4 years)	0.5 (0.2; 1.1)	0.3 (0.2; 0.8)
3rd wave (7 years)	0.4 (0.2; 0.9)	0.2 (0.2; 0.6)
4th wave (10 years)	0.5 (0.2; 1.2)	0.4 (0.2; 1)
hs-CRP <0.2 mg/L		
2nd wave (4 years)	205 (27.9)	319 (40.2)
3rd wave (7 years)	718 (33.2)	1128 (49.2)
4th wave (10 years)	466 (26.0)	657 (33.5)
hs-CRP >10 mg/l		
2nd wave (4 years)	31 (4.2)	35 (4.4)
3rd wave (7 years)	85 (3.9)	48 (2.1)
4th wave (10 years)	50 (2.8)	35 (1.8)

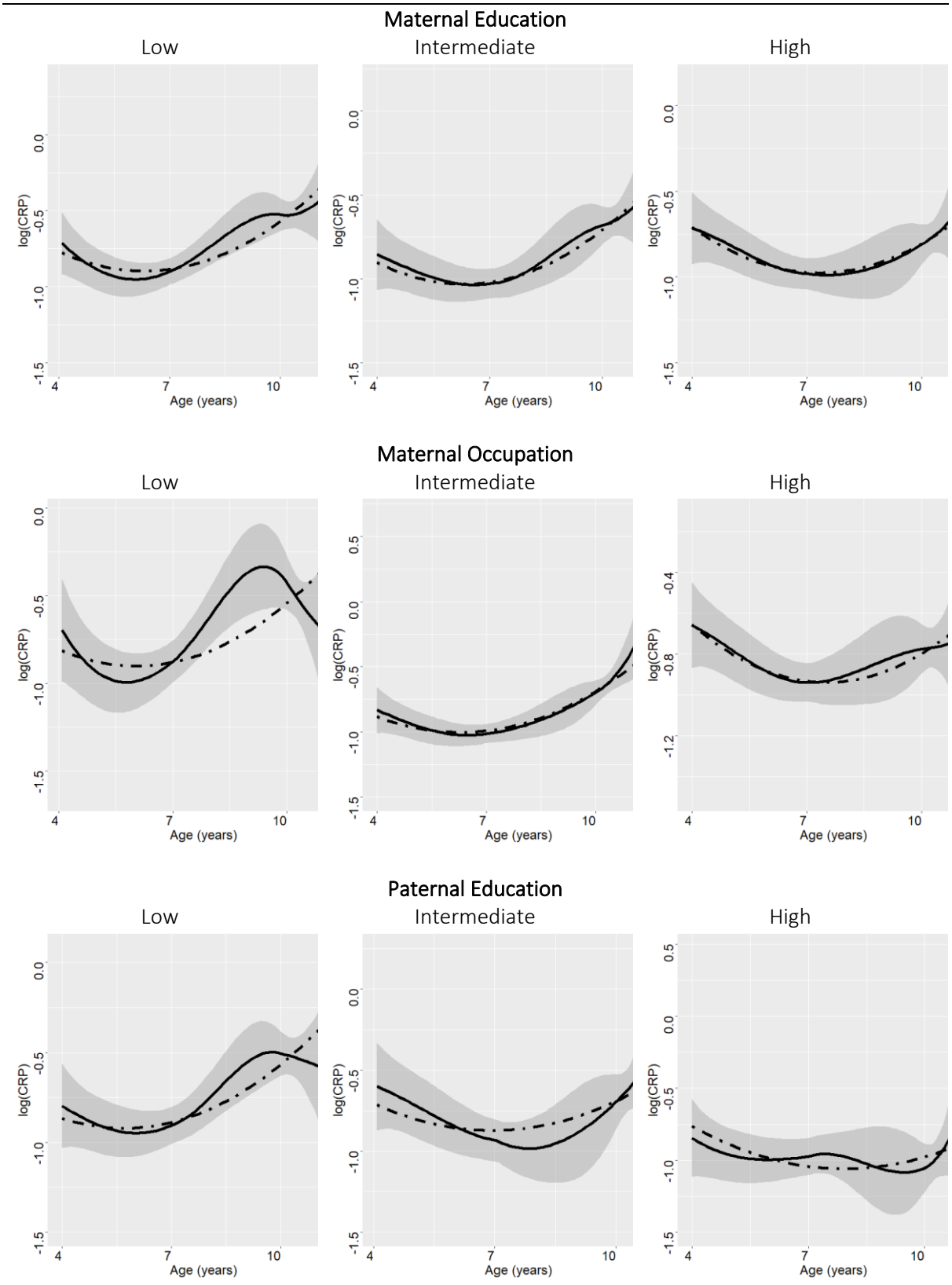


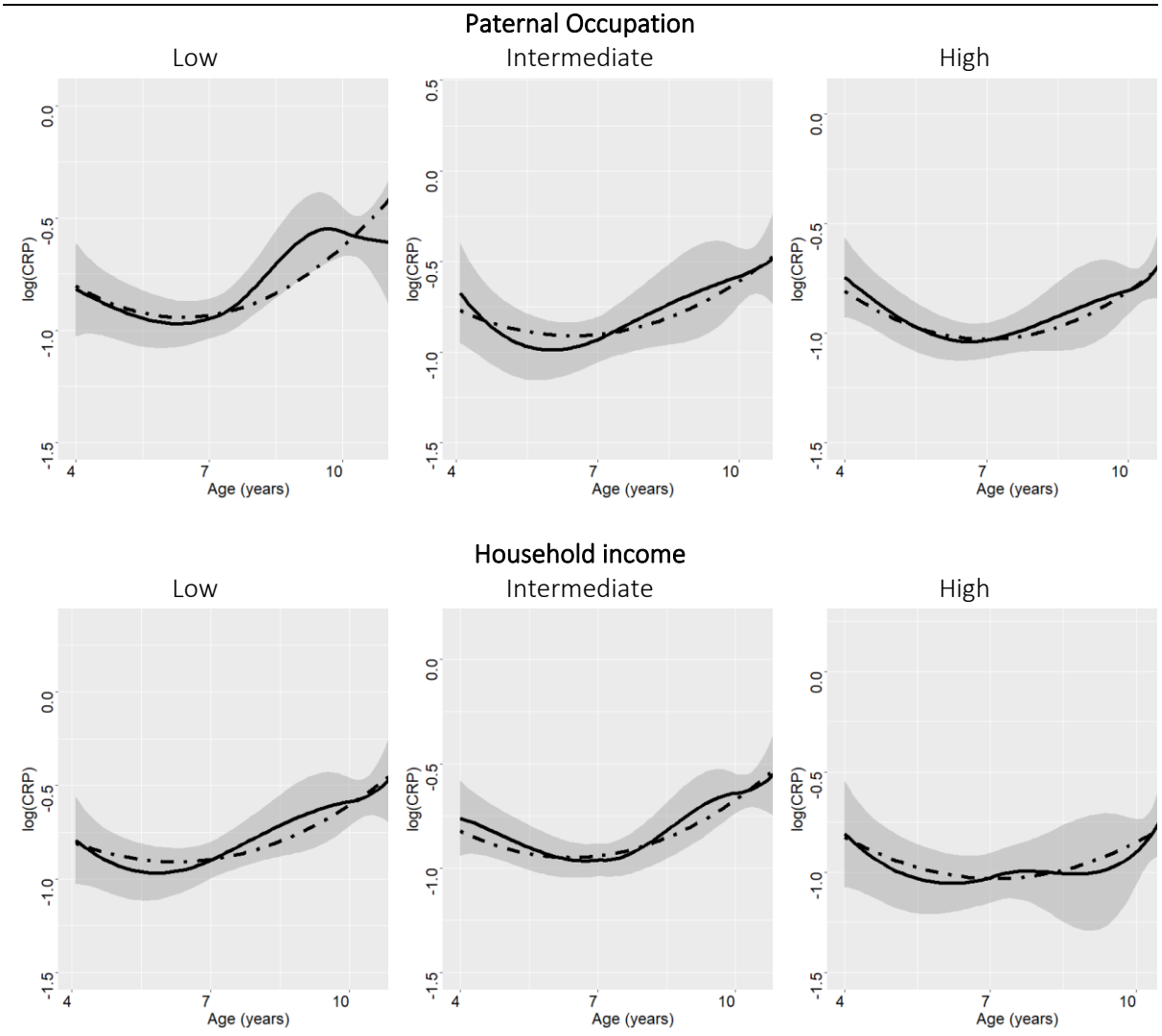
Supplementary Figure 1. Simulated trajectories using Linear Mixed-Effects Models, to calculate linear regression coefficients (β_2).


Maternal Education
Low
Intermediate
High
log(CRP)
Age (years)
Maternal Occupation
Low
Intermediate
High
log(CRP)
Age (years)
Paternal Education
Low
Intermediate
High
log(CRP)
Age (years)



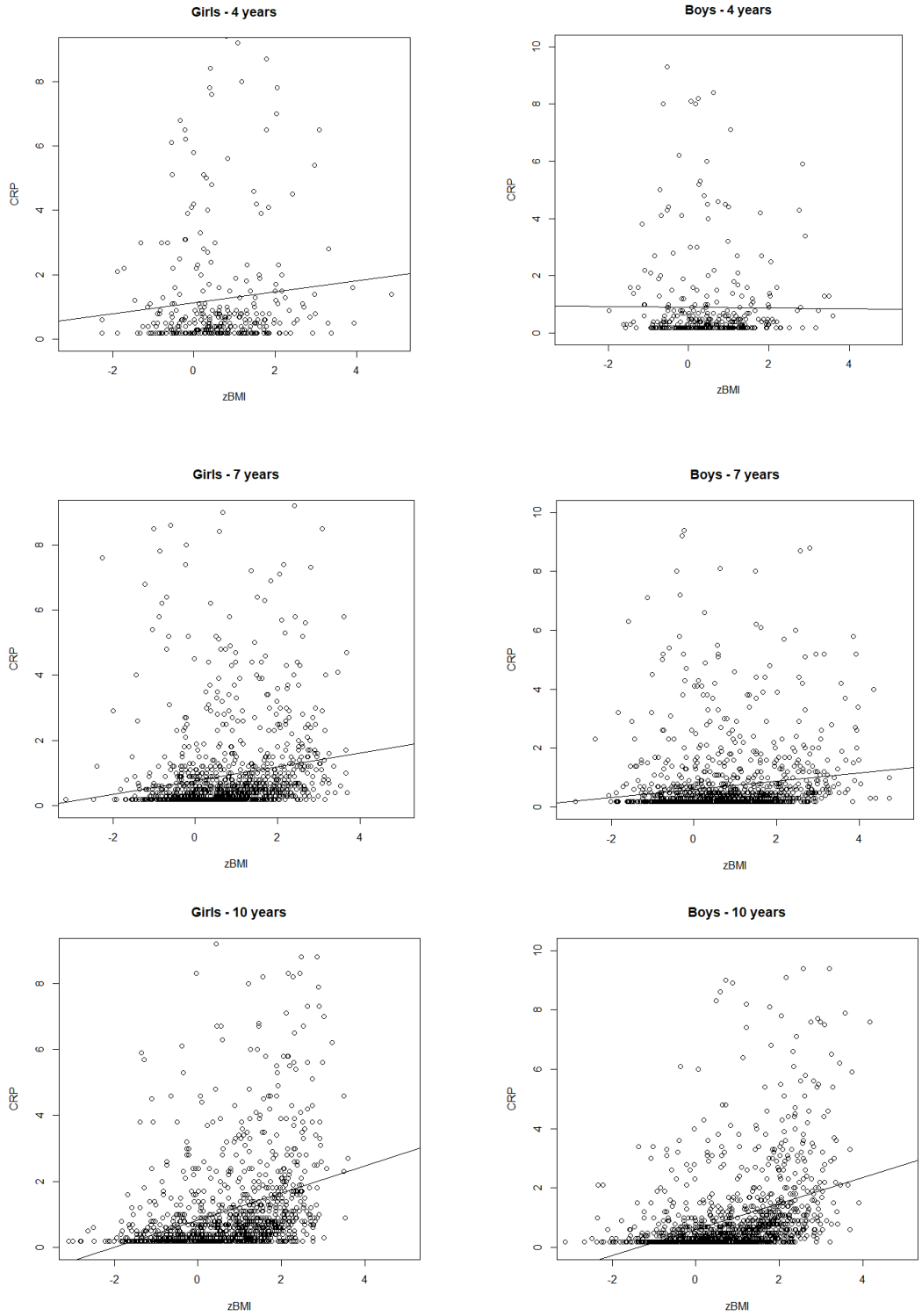
Supplementary Figure 2a. Predicted and observed trajectories of hs-CRP vs. age. Solid black lines correspond to the predicted values of the log hs-CRP throughout time, in each socioeconomic indicator, and the corresponding 95% confidence intervals for the predictions are also drawn (grey shaded area). The dot-dash lines represent the observed trajectories of this dataset, in girls.


Maternal Education
Low
Intermediate
High
Maternal Occupation
Low
Intermediate
High
Paternal Education
Low
Intermediate
High
log(CRP)
Age (years)

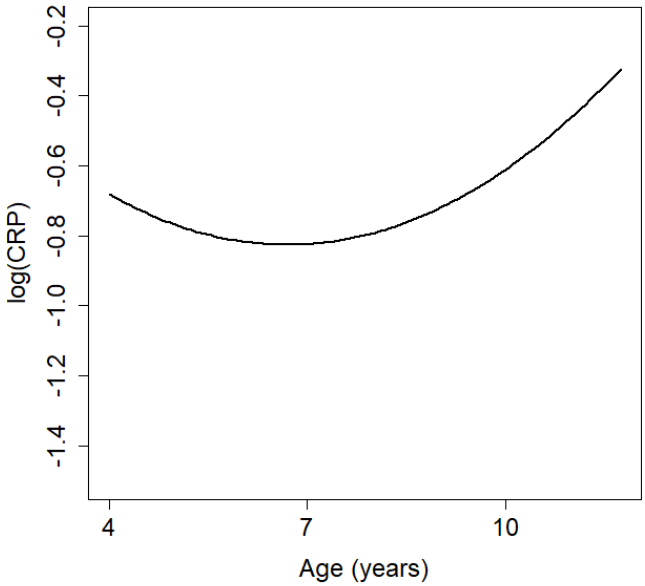


Supplementary Figure 2b. Predicted and observed trajectories of hs-CRP vs. age. Solid black lines correspond to the predicted values of the log hs-CRP throughout time, in each socioeconomic indicator, and the corresponding 95% confidence intervals for the predictions are also drawn (grey shaded area). The dot-dash lines represent the observed trajectories of this dataset, in boys.

Girls - BMI	Boys - BMI



Supplementary Figure 3. Scatterplots of the association between hs-CRP and BMI across childhood.



Supplementary Figure 4. Main effects of age/time on hs-CRP trajectories across childhood.

4.3 The biological consequences of exposure to violence during childhood: a systematic review

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The following is a pre-peer-review version of an article currently under review.

Introduction

Adverse childhood events (ACEs), including maltreatment, abuse or neglect, and other traumatic events, have been compellingly associated with a life-long increased risk for psychopathology and stress-related chronic health problems (1, 2). Evidence shows that exposure to ACEs during childhood is strongly associated with a higher likelihood of developing ischemic heart disease, cancer, stroke, chronic bronchitis, emphysema or diabetes later in life and even with premature death (3-5). Thus, exposure to adversity has already been associated with later negative outcomes, posing as a long-lasting or long-term effect of these exposures on the individuals' health. However, the potential mechanisms involved in the biological embodiment of social adversity in early ages that would be translated into an increased risk of disease later in life are still not fully understood (6-8).

This association may occur through a direct or an indirect pathway. Indirectly, it can be explained by the adoption of unhealthy behaviours (e.g., poor diet, sedentary behaviour, smoking), or more directly, via physiological disruption of regulatory pathways responsive to stress caused by adversity. Adverse childhood exposures result in a variety of physiological changes in children (9), including epigenetic mechanisms (6, 8), alteration of neural function and structure (6-8), increased activation of neurobiological systems, such as the hypothalamic-pituitary-adrenal (HPA) axis or the sympathetic nervous system (9, 10). Increased activation of these systems leads to a cascade of physiological processes (9, 10), which in adults, was linked with the development of central fat, dysregulated carbohydrate metabolism and the accumulation of blood lipids in the arterial lining, all of which accelerate chronic disease development (11).

Additionally, the exposure to violence and adversity in early life has been related to behavioural, emotional and learning problems during childhood and adolescence (12), but may also lead to hidden biological alterations that have lifelong effects on health (3, 10). The scientific literature shows that exposure to adversity may have a long-lasting and cumulative health effect, however, some work shows that alterations in biological systems may occur in a shorter period and be measured before adult-life. Also, exposure to social adversity during childhood may result in early life stress that has the potential to alter physiological systems, thus accounting for a more immediate effect of these exposures, including all the effects occurred during childhood and adolescence, before adulthood but that may not necessarily lead to disease.

Evidence allows us to hypothesise that exposure to adversity during the first years of life might already be biologically embedded well before adult life, independently of the effects of behaviours in this association. Whether these embodied biological characteristics impact later health depends on the

characteristic's expression and relevance to health outcomes, which can include disease (13). Also, exposure to stressful circumstances between conception into adolescence causes a cascade of physiological responses that may modify an individual's biology in the long term in a way that makes them vulnerable to develop disease later in life (14). Little is known about our ability to predict which children will do well and endure resilience and which children will develop disease after experiencing adversity. These gaps in the literature obstruct the ability to decide the best ways to develop effective interventions targeting victimised children (15). Thus, identify the effect of exposure to adversity on biological markers at early ages would allow understanding the pathways by which adversity may impact later development of disease, and consequently define interventions to protect children in a trajectory of increased risk of poor health or to mitigate the effects already in place to avoid the development of disease in the adult life.

As a biomarker or a biological marker is a measurable indicator of some biological state or condition and is often measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention, in this work we aimed to identify biomarkers that are part of biological/physiological systems and therefore can be changed as a result of exposure to adversity. Therefore, this review aims to summarize evidence reporting physiological embedding of exposure to adverse events in children. Specifically, it aims to describe which ACEs have been associated with a shorter period until measurement of biological consequences, to identify which physiological systems have been investigated, and finally, to describe the impact of ACEs on the physiological systems.

Methods

Search strategy

PsicINFO®, PubMed®, Isi Web of Knowledge and Scopus were searched from inception to July 2019, to identify published papers reporting biological effects of exposure to ACEs before the age of 18 years. The keywords were chosen based on the literature and previously published theoretical reviews (16) and systematic reviews (17, 18), according to the usually used markers to measure biological alterations, adapted to each database and included the following terms: child maltreatment, child trauma, child adversity, early life stress, child abuse, child neglect, emotional stress, violence, bullying, and C-reactive Protein, CRP, Tumour Necrosis Factor, TNF-α, cytokine, interleukin, IL-6, inflammatory, inflammation, fibrinogen, white blood cell, methylation, DNA, DNA methylation, nervous system, amygdala, amygdala volume, hippocampus, hippocampal volume, prefrontal cortex volume, endocrine system, HPA axis, cortisol.

Selection of studies

The list of references retrieved was screened independently by two reviewers (SS and FSP), following predefined criteria, to determine the eligibility of each article (Figure 1). Inclusion criteria are as following: case-control and cohort studies; original research; studies evaluating adverse childhood events; studies reporting biomarker measures in adulthood (≤18 years-old); studies reporting an association between ACEs and biomarkers. The criteria for exclusion of studies were the following: (1) research not involving humans (e.g., in vitro or animal research); (2) non-eligible publication types (reviews, editorials, comments, guidelines, conference abstracts); (3) studies in disease setting samples; (4) studies reporting biomarker measures in adulthood (>18 years-old); (5) studies not reporting an association between ACEs and biomarkers; (6) other (studies evaluating allostatic load, adverse events during pregnancy, post-traumatic stress disorder, laboratory procedures to induce stress).

ACEs were defined considering Felitti exposure categories (3), namely psychological, physical and sexual abuse, and household dysfunction. Specifically, any adversity involving relationships (caregivers, family and peers) was included in the review. Adverse events were categorized into: sexual abuse (includes any type of sexual abuse reported), life stressors (includes the death of a family member, trouble with a teacher, exposure to community violence, …), physical abuse (includes abuse perpetrated by parents, caregivers or other relatives and by teachers) and physical neglect (includes physical neglect by parents or other caregivers). Biomarkers were defined according to the definition from the International Programme on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labor Organization, as *"any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or*

disease" (19). Biological markers were then divided by the biological mechanism with which they fitted better: "immune system" (including CRP - an acute-phase protein of hepatic origin whose circulating concentrations rise in response to inflammation; IL-6 - an important mediator of fever and of the acute phase response; TNF-α – a cytokine involved in systemic inflammation and one of the cytokines that make up the acute phase reaction; IL-1b – a cytokine and important mediator of the inflammatory response, involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis; IL-10 - a cytokine with multiple, pleiotropic, effects in immunoregulation and inflammation; IL-12p70 - an interleukin naturally produced by dendritic cells, macrophages and neutrophils, that stimulates the production of interferon-gamma (IFN-γ) and TNF-α from T cells and natural killer (NK) cells; IL-8 - also known as neutrophil chemotactic factor, induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection, also stimulates phagocytosis once they have arrived; and cortisol - prevents the release of substances in the body that cause inflammation); "structural & functional brain changes" (BDNF - acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support survival of existing neurons, and encouraging growth and differentiation of new neurons and synapses; hippocampal volume - chronic stress resulting in elevated levels of cortisol, is seen to be a cause of neuronal atrophy in the hippocampus; this atrophy results in a smaller hippocampal volume; amygdala volume and amygdala functional connectivity (the amygdala is a key region of the brain and plays a crucial role in processing fear, mediates the ability to associate emotional significance to a formerly neutral stimulus, triggers a host of adaptive responses to threatening stimuli, for example, by regulating the magnitude and duration of serotonergic responses), grey matter - contains most of the brain's neuronal cell bodies; includes regions of the brain involved in muscle control, and sensory perception such as seeing and hearing, memory, emotions, speech, decision making, and self-control, neurologic abnormalities - Structural, biochemical or electrical abnormalities in the brain, spinal cord or other nerves, pituitary gland volume - is important for mediating the stress response, via the hypothalamic–pituitary–adrenal axis (HPA axis) and can be adversely affected by an over- or under-production of associated hormones, voxel-based morphometry –a technic that allows the detection of focal microstructural differences in brain anatomy in vivo between groups of individuals without requiring any a priori decision concerning which structure to evaluate), "genetic & epigenetic" (including methylation - the addition of a methyl group on a substrate, or the substitution of an atom (or group) by a methyl group and telomere length – telomeres are regions of repetitive nucleotide sequences at each end of a chromosome, which protects the end of the chromosome from deterioration or from fusion with neighbouring chromosomes) and "others" (including copeptin - is actively released from the hypothalamus and is of clinical for being closely linked to the pathophysiological pathways of heart failure and acute coronary

syndrome , leptin - is the "energy expenditure hormone" and reduces appetite as a circulating signal; and dehydroepiandosterone (DHEA) - an endogenous steroid hormone, produced in the adrenal glands, the gonads, and the brain, functioning as a metabolic intermediate in the biosynthesis of the androgen and estrogen sex steroids both in the gonads and in various other tissues).

The decisions taken independently by the reviewers in each step were compared, and discrepancies were solved by consensus or after discussion with a third researcher (SF) (Figure 1).

Data extraction

Two investigators (SS and FSP) independently extracted data from 58 studies regarding the year of publication, country and region where the study was conducted, sample characteristics (sample, sample size, participant's age, female proportion, type of ACEs, the instrument used to measure adverse events, age at event exposure and biological marker assessed).

Differences in the data extracted by the two investigators were discussed until consensus and involving a third researcher (SF), whenever necessary.

Data synthesis and analysis

Two summary tables of results were created, compiling the extracted information (Table 1 and Table 2). Studies were divided according to the different development phases of growth using the age at which ACEs occurred, as following: toddlerhood (0-2 years); childhood (3-12 years and further classification into play from 3 to 5 years and middle childhood from 6 to 12 years); and adolescence (13-18 years and divided in mid-adolescence from 13 to 15 years and late adolescence from 15 to 18 years). Due to heterogeneity of ACEs measures, analytic methods and in the biomarkers, a qualitative description of the association and the strength of the reported association were assigned based on the magnitude of the reported effect measures (20), defined according to author's results description, as strong or weak, and statistical significance of the provided results. Results were then summarized in a table presenting positive and inverse associations, and the strength of association (Table 3).

Methodological quality of studies

The quality of reporting of the included studies was assessed using the Strengthening Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies (21). All studies scoring higher than the median in the STROBE checklist for cohort, case-control, and cross-sectional studies (combined) and thus revealing a satisfactory to good quality were included (Table 1).

Results

The characteristics of the fifty-eight included publications are described in Table 1 and Table 2.

Twelve studies were conducted in Europe (5 countries), thirty-six in the Americas (4 countries), six in Asia (4 countries), three in Australia and one in Africa (Tanzania). Most studies were conducted in the United States of America (USA) (thirty-one studies), and the sample size varied from 21 to 5802 participants. Studies were divided according to time at which ACEs occurred. The distribution of papers is as follows: two papers during toddlerhood, thirty-four studies during childhood (seven from 3 to 12 years, five from play - 3 to 5 years- and twenty-two from middle childhood - 6 to 12 years), fifteen studies during adolescence (twelve studies in adolescence – 13 to 18 years, one in mid-adolescence – 13-15 years and two in late adolescence – 15 to 18 years) and seven studies that present ACEs measured from an overall period – comprising experiences occurred before 18 years (Table 1 and Table 2). In childhood, most publications (fifteen) are in the "immune system" and "genetic & epigenetic" categories, while "structural & functional brain changes" has three publications. During adolescence there are six publications with biomarkers from the "immune system", nine from the "structural & functional brain changes", and seven from "genetic & epigenetic" category.

Publication of studies increased over time, with most of the studies being published after 2012. The first study using DNA methylation as a biomarker of exposure to adversity was published in 2012, and after that, the number of papers studying the association with genetic and epigenetic biomarkers has been consistently increasing (Figure 2).

We observed that ACEs were mostly assessed by standardized instruments, although some authors used non-validated questions (seventeen studies). The most frequently used instrument was the Childhood Trauma Questionnaire (nine studies), followed by the Maltreatment Classification System (eight studies). High heterogeneity was found among studies both in the exposure measurement and in the outcome summary measures. Regarding exposure, the most frequent adverse event measured in these studies was sexual abuse (twenty-six studies, sixteen studies in childhood and ten studies in adolescence), followed by the life stressors category, that includes the death of a family member, trouble with a teacher, exposure to community violence, among others (twenty studies, ten in childhood and ten in adolescence), by physical abuse (eighteen studies, eleven studies in childhood and seven in adolescence) and physical neglect (fifteen studies, nine studies in childhood and six in adolescence). The minimum time from exposure to ACEs and measurement of biomarkers was 72 hours and maximum 18 years. In toddlerhood the average time between exposure to ACEs and measurement of biomarkers was 2 years, in childhood was 7.2 years and during adolescence was 5.5 years (Table 1).

We categorized papers according to the outcome measured, i.e., referring to the biological marker used to assess the effect of ACEs on biological mechanisms. Biological markers were then divided by the

biological mechanism with which they fitted better: “immune system” (including CRP, IL-6, TNF-α, IL-1b, IL-10, IL-12p70, IL-8 and cortisol), “structural & functional brain changes” (BDNF, hippocampal volume, amygdala volume, amygdala functional connectivity, grey matter, neurologic abnormalities, pituitary gland volume, voxel-based morphometry), “genetic & epigenetic” (including methylation and telomere length) and others (including copeptin, leptin and dehydroepiandosterone (DHEA)).

In almost all studies, exposure to ACEs was associated with biomarker alterations already during childhood, while six found no evidence of effect modification (Table 3).

Mainly due to the nature and type of biomarkers, associations found can be expressed through increases or decreases in respective biomarkers. Thirty-nine studies presented a positive association, meaning that participants exposed to adverse events presented higher levels of biological markers, and twenty-nine studies showed inverse associations, corresponding to a decrease in the biological markers when adversity was reported. We observed that most authors study the association of ACEs with biomarkers of the immune system followed by genetic and epigenetic biomarkers and then structural and functional brain changes.

Immune system

Of the studies that addressed the biological consequences of ACEs on the immune system, sixteen focused on cortisol, five on CRP, three on IL-6, two on IL-10, one on TNF-α, IL-1b, IL-12p70 and IL-8. Of these, the majority (seventeen studies) showed a positive association between ACEs exposure and biomarkers of the immune system, meaning that those exposed to adverse experiences presented higher levels of biomarkers of the immune system. Other studies showed an inverse association (five studies), with exposure to ACEs being associated with lower levels of biomarkers, or no association (five studies). Majority of studies presented strong associations, while four publications reported weak associations between exposure to ACEs and biomarkers. Regarding the type of ACEs more associated with biomarkers of the immune system, we saw that the categories sexual abuse, life stressors and physical abuse, neglect, maltreatment were the more prevalent (Figure 3). Changes in cortisol levels can be observed as early as between 3 to 6 years. Also, analysing the distribution of publications by age, eleven studies on the immune system were conducted between the ages of 6 and 12 years. Four studies were conducted between 13 and 18 years and three between 3 to 12 years.

Structural & functional brain changes

Authors measured the impact of ACEs in the structural and functional brain changes, using several types of outcomes. Hippocampal volume was measured in five studies, and amygdala functional connectivity in three, BDNF, amygdala volume and grey matter in two, neurologic abnormalities, pituitary gland volume and voxel-based morphometry in one study each. Of these, three studies showed a moderate or weak association between exposure to ACEs and the outcomes measured, while all the others

presented a strong association. Most studies showed an inverse association of ACEs, namely when reporting the association between sexual abuse, life stressors and physical abuse, neglect, maltreatment with structural and functional brain changes (Figure 3). Amygdala functional connectivity was the biomarker of the group "Structural & functional brain changes", that presented changes measured earlier (mean=6.1 years). Examining the distribution of publications by age, majority of studies in this category (seven studies) were conducted between the ages of 13 and 18 years, and three studies were conducted between 6 to 12 years.

Genetic & epigenetic

DNA methylation was assessed in twenty studies, with eighteen showing more methylation in participants exposed to ACEs, four showing less methylation and one reporting no association. The effect of ACEs on telomere length was presented in two studies and showed that exposure was associated with shorter telomere length. Majority of associations found was regarding the association with sexual abuse, life stressors and physical abuse (Table 3). DNA methylation is altered as early as between 3 to 5 years, and changes in telomere length can be observed at 10.2 years. Also, analysing the distribution of publications by age, eight studies of the "genetic and epigenetic" category were conducted between 3 to 12 years and seven studies were conducted between the ages of 6 and 12 years.

Others

One study evaluated copeptin, and other DHEA, and both showed a positive association with ACEs exposure and were conducted in middle childhood, i.e. between 6 to 12 years old. A study on leptin showed no association with ACEs.

Discussion

This review shows that exposure to ACEs might impact the immune system, structural & functional brain changes and genetic & epigenetic changes, and these changes can be observed as early as childhood. However, a high heterogeneity is observed between included studies in ACEs measures, analytic methods and heterogeneity in the biomarkers.

Assessment of ACEs among children

In these studies, ACEs were assessed through different methods of inquiry and instruments. The development and testing of measures of retrospective adult recall of ACEs have been a fruitful area of research for the past few decades with several measures being developed and field-tested. Thus, the majority of studies used retrospective measures to identify exposure to ACEs. The major issue raised is that several critical aspects of the measurement systems are inconsistent across measures, making it difficult to synthesize knowledge generated to date (22). In this review, by focusing on studies that assess exposure and outcome measured in the first 18 years of life, we see that biological alterations caused by exposure to traumatic events can be observed in the first years of life. In fact, majority of included publications studies the effect of adversity in toddlerhood and childhood, i.e. before the age of 12 years (thirty-six) while fifteen studies evaluated adverse experiences between 13 to 18 years of age.

The heterogeneity on measurement instruments used gives rise to another assessment inconsistency, in particular, the fact that not all types of victimization are alike. Some involve physical injury (sexual or physical abuse), whereas others involve psychological insult (emotional abuse or neglect). In this review, we observed that sexual abuse was, among the categories of ACEs studied, the type of adversity that most studies presented in association with different biomarkers. This might be explained by the fact that the biological embedding of social experiences occurs sooner when the experience is very traumatic or repeated over time (23).

Potential biological mechanisms to the embodiment of ACEs

The impact of ACEs in the immune system, structural and functional brain, as well as the genetic and epigenetic changes, was explored in the reviewed studies including samples of children. Overall, the associations observed followed the hypothesis that ACEs are associated with biological risk, which can be expressed through increases or decreases in respective biomarkers, depending on the nature and type of biomarker.

Immune and non-immune cells produce cytokines, messenger proteins such as TNF-α, IL-1β, IL-8, IL-6, IL-10 and IL-12p70, whose role is to regulate immune responses and interplay between pro and anti-inflammatory mediators (24, 25). CRP is an acute-phase protein synthesized by the liver in response to

systemic effects of inflammation (26) and may intervene in the biological chain that embeds exposure to ACEs. Cortisol is the end product of the HPA axis and has been widely used as a stress biomarker. All of these biomarkers play a role in the regulation of the immune responses and interplay between pro and anti-inflammatory mediators (24, 25) indicating an interrelated activation of the entire inflammatory cascade (27). More recently, evidence has reviewed the effect of early exposure to adversity on the chronic inflammatory state (18, 28) and concluded that early adversity is likely to increase inflammation (17, 18, 28) and risk for poor health outcomes in adulthood (28, 29), independent of clinical comorbidities (17, 18). This review shows that these biomarkers seem to show alterations in the first 18 years of life, and thus the effect of exposure to childhood adversity in the immune system, in particular in the inflammatory biomarkers, where alterations were reported as early as between 3 to 6 years.

Several papers included in this review assessed methylation in a multiplicity of genes or focused on specific genes, such as NR3C1, SLC6A4 and FKBP5. In particular, these three genes seem to play an active role in the biological embodiment of exposure to ACEs and we hypothesized that the effect of adversities would be observed on alterations already at early ages. On one hand, NR3C1 is a gene known to encode glucocorticoid receptor, involved in inflammatory responses (30), and it's a higher level of methylation has been associated with childhood violence (31); and the SLC6A4 gene that encodes an integral membrane protein and seems to play a role in depression-susceptibility in people experiencing emotional trauma (32). FKBP5 encodes to a protein member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes. Genetic studies have identified a role of this gene in posttraumatic stress disorder, depression and anxiety (33) and have been found to interact with childhood trauma to predict severity of adult posttraumatic stress disorder (34).

Although multiple types of epigenetic modifications have already been identified (35), all involve chemical modifications that regulate chromatin structure and/or DNA accessibility. Methylation, corresponding to the covalent modification of DNA whereby methyl groups are coupled to cytosine residues at CpG sites, is perhaps the best studied of these epigenetic mechanisms, due in part to its tractability to study (36). In this review, we identified several studies evaluating DNA methylation after exposure to ACEs. As dynamic molecular markers that have been shown to change with age (37) and experience (38), epigenetic signatures are attractive candidates for elucidating the underlying mechanisms of complex diseases (39).

Emerging evidence shows that environmental signals give rise to epigenetic changes, affecting phenotypic trajectories by altering the expression of genes (40). Thus, changes in epigenetic regulation of gene expression seem to be responsible for an increased immune activation via modifications of the

HPA axis. Neuroplasticity-related methylation patterns (8, 41) may be a possible mechanism through which the association between early adverse events and long-term alterations in human stress response and immune systems are mediated.

Also, although not very conclusive, some structural and functional brain changes after exposure to adversity have been identified by the studies explored in the review. Six studies concluded that hippocampal and amygdala volume and grey matter decreased after participants experienced adverse events. However, more evidence is needed to have a comprehensive view of the effect of adversity in these systems.

Impact of ACEs on the physiological systems

Most of the included studies showed a significant impact of ACEs on the different physiological systems. Nevertheless, some studies showed increases in biomarker levels, while others presented decreases in those levels, depending on the nature and type of biomarker. Regarding telomere length, amygdala and hippocampal volume, the direction of the observed associations was consistent with our hypotheses. Telomere length decline is a normal consequence of cellular division, ageing, differentiation, and senescence. Accelerated telomere shortening in adults has been associated with a history of childhood maltreatment and early adversity (42, 43). DNA methylation also can occur via hypermethylation, i.e. increased methylation, that was found in the promoter region of SLC6A4 in adult men after early and recent life stress (44), or hypomethylation, i.e. decreasing methylation, observed at intron 7 of FKBP5 in adults exposed to childhood trauma (45). Thus, the direction of methylation may depend on the gene, promoter and/or region studied. However, we did not expect to find different directions of association for biomarkers such as cortisol. But, there is some evidence of the attenuation hypothesis (46), suggesting that exposure to early and severe stress leads to an initial heightened stress response, that may be suppressed over time. This suppression may be suggestive of an adaptive response. Cortisol levels increase immediately after exposure to ACEs, and attenuate after a certain period, but continue to reflect the effects of severe trauma. Evidence from primates showed that early life stressors, when not tremendously severe, were associated to the subsequent development of biologic and social resilience suggesting that ACEs represent a challenge that, when overcome, bring about functional adaptations (47). In regards to amygdala functional connectivity, some inconsistencies might be explained by within-subject variability and fluctuations in large-scale network patterns, including connectivity between a limbic and default mode network, results that seem to suggest that bi-nodal functional connectivity, may generally reflect larger-scale network patterns.

Additionally, our review shows that age at exposure is very different across publications, varying from less than 6 months to under 18 years old. Although there is great variability across studies, it has been defended that given the vast array of developmental processes occurring between conception and

adolescence, every developmental window is in fact characterized by a different susceptibility depending on various environmental factors (48).

With this review, we cannot assess if the experiences reported are single episodes or if they are related to several experiences throughout childhood and adolescence resulting in cumulative exposures during these maturation periods. The exception is one study that specifically states that adversity must last for at least six months (49). In fact, there is evidence showing that cumulative exposures seem to have stronger associations with later health outcomes (3). This means that we could be looking at an interplay between biological functions and the environment across the life course which we cannot disentangle them from the mechanism of accumulation. For example, an individual most at risk of developing cancer or ischemic heart disease after childhood exposure to violence or adversity is also more likely to have accumulated further negative experiences over time and to adopt risky health behaviours as a stress-reducing escape. However, by restricting the search to studies with participants 18-year-old or younger, the time for accumulation of risk-taking behaviours is sufficiently limited to avoid an impact on the studied association. Moreover, when compared to adult life, neurodevelopment during childhood and adolescence is more plastic and susceptible to programming influences from stressful environmental and social contexts (50).

Biological consequences of bullying involvement

It is not consensual to include bullying as an adverse experience in childhood. However, the awareness of this problem has widely increased, and it was shown to compromise the child's health. Literature settles on the conviction that social and psychological effects of bullying involvement may be independent of other childhood experiences (51), but the biological mechanisms of the embodiment of these experiences are still not fully elucidated. Although some authors agree that one potential mechanism is related to the chronic systemic low-grade inflammation (52), once inflammation is activated similarly by a diverse range of health risky behaviours (poor diet, sedentary life) and environmental challenges (low socioeconomic status, psychosocial stress) (53), others support the hypothesis of embodiment throughout HPA axis activation or autonomic nervous system (ANS) activation. Bullying has also some specificity as the type of involvement, as a victim or as an aggressor or both simultaneously might have a different biological impact. Evidence has shown that although being bullied predicted higher increases in CRP levels, bullying others predicted lower increases in CRP compared with those uninvolved in bullying, even when controlling for potential confounders (54). This review identified six studies evaluating bullying as an experience of adversity. One studied the impact of bullying on CRP, and found that CRP levels were higher in victims of bullying and lower in aggressors (54); two studies found that DNA methylation was higher among victims of bullying (55, 56), and other found that telomere length was shorter (57). Two other studies evaluated cortisol and one described

lower levels of cortisol among victims of bullying (58) while other study found no association (59). Nevertheless, we believe that further investigation is needed to explore the impact of children's type of involvement in bullying on different biological markers.

Nowadays, another important and prevalent form of bullying is by using technologies and social media, named cyberbullying. Due to the potential of widespread accessibility of victims and an infinite audience by using communication technologies (60), cyberbullying is another important source of stress and consequently to biological alterations that can later lead to disease. This is another important issue that deserves attention in future studies.

Strengths and limitations

We believe that this review is comprehensive and robust enough to show the studied association. Even though there is always the possibility of residual confounding when exploring the association between childhood exposure and biological markers, we believe that studying these biomarkers already during childhood is an important step to eliminate the effect of health-risk behaviours that may confound this association. We must acknowledge that there are different biological, psychological and social aspects that may contribute to the changes in the biomarkers studied, which are difficult to control for. However, our results are in line with previously reported associations (18, 40, 61, 62), and allow us to retrieve important conclusions on the effect of early exposure to ACEs and alterations in human stress response and biological systems, already during childhood. The reported biomarkers were also chosen based on previously published literature, and others emerged from the search, showing that several systems may be affected by adverse experiences in childhood. Even though we cannot exclude the hypothesis that more biomarkers might be affected by these experiences, we believe that our comprehensive search allowed us to catch most studies. Nevertheless, excluding allostatic load from our search might be considered a limitation. We did not include the allostatic load in our search because these indices are diverse across studies and are frequently assessed differently, using different biomarkers included in different physiological systems and different methods of assembling; besides due to cumulative nature of the allostatic load, identifying which biological system would suffer the most the impact of exposure to adversity would be more difficult. Moreover, to our knowledge, only one publication assessed the effect of maltreatment on allostatic load in children (63). In this study (63), participants were aged 8 to 10 years, included maltreated or non-maltreated low-income children that attended a summer research day camp. Authors found that maltreatment did not independently predict differences in allostatic load levels. Additionally, due to the diversity of ACEs measures, analytic methods and heterogeneity in the biomarkers we were not able to calculate a summary measure of association between ACEs and biological markers, and thus we were unable to conduct a meta-analysis.

Instead, a qualitative description of the strength of association was assigned based on the magnitude of effect measures.

Moreover, none of the exclusion criteria chosen to conduct this review is related with any aspect of human differences such as socioeconomic status, race, ethnicity, language, nationality, sex, gender identity, sexual orientation, religion, geography, ability, age, or culture. Thus, this review holds diversity as a core value and all papers were included based on the criteria defined and no other.

Conclusion

Despite the considerable inconsistency in ACEs assessment, most articles reviewed found an association between exposure to ACEs and biological markers, where the increase or decrease in the biomarker is associated with heightened risk to subsequent health. Experiences of violence in childhood appear to "get under the skin" and induce physiological changes, such as increases in immune, structural, and functional brain changes, and genetic and epigenetic markers, from childhood. Thus, supporting evidence of a more immediate biological impact of these exposures and alterations might be strongly associated with later development of disease. These results allow us to argue that the population's burden of disease could be reduced if all violence towards children was successfully prevented (64) and when it occurs, appropriately treated to mitigate the consequences (65). Exposure to adverse childhood experiences should be prevented as a question of human rights, and children should be protected against all types of abuse by law enforcement & providing nurturing childhood environments. Moreover, as adverse experiences seem to impact children's biology and children may be growing in a trajectory of worse health throughout life, beginning at early ages, when exposure to adversity cannot be prevented, clinicians may have an important role in helping identify any biological alterations related with adversity victimization and intervene to mitigate their impact on health.

Practice and Policy Implications

The jumping-off point for the current systematic literature review was the understanding that the biological consequences of adversity could be occurring already during childhood. As exposure to adversity has been associated with several poor health outcomes later in life, the early identification of the biological systems that may be more susceptible to suffer alterations is important to prevent and avoid future development of disease.

Indeed, enormous resources are dedicated to prevention programs to preclude maltreatment or to help children to cope with the consequences of adversity. However, the current literature review emphasizes the need to identify at very early ages children that are exposed to adversity and to monitor their health to mitigate, minimize or avoid the later health consequences that will come from the embodiment of those experiences. Although prevention of adversity is a societal and community challenge, health workers, and in particular clinicians may have an important role in helping identify and prevent adverse childhood experiences and in mitigating their impact on health. Clinicians may be able to ask or screen for those experiences, or to question altered results in routine clinical exams. And by thus helping in the implementation of strategies for the prevention and care of children exposed to adversity.

The current systematic literature review pointed to several key conclusions that need to be taken into consideration in future studies and efforts in the field of adversity and its biological consequences. Although prevention of adversity is of central importance, there is a lack of research using longitudinal studies on the importance of the effects of these exposures at very early ages. This identified gap in the literature represents an urgent need to promote studies in this area to better adapt to both prevention and intervention efforts in the field exposure to adversity.

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Table 1. Descriptive characteristics of all included studies (n=58)

Author (Year)	Sample size	Participants' age (years) (range/mean)	Female proportion (%)	Type of ACE	Instrument to assess ACE	Age at the ACE (years)	Age at measure of biomarker	Time between exposure and biomarker measure [a]	Biomarker	Quality[b]
Toddlerhood: 0-3 years										
(Bhopal et al., 2019)	T: 436	12.4	n.m.	Life stressors (e.g., death of a family member, trouble with teacher)	n.m.	12 months	1	0-1	Cortisol	22
(Dahmen et al., 2018)	T: 51 ACE+: 25	ACE+: 10.6 ACE-: 10.4	ACE+:50.0 ACE-: 44.0	Maltreatment	German self-report questionnaire	0-3	10.6	7.6-10.6	Hippocampal volume	19
Childhood: 3-12 years										
(Bucker et al., 2015)	T: 62 ACE+:36	ACE+:9.44 ACE-: 8.96	ACE+:38.9 ACE-: 42.3	Sexual abuse, maltreatment, and/or neglect	n.m.	3-12	3-12	0-9	IL-12p70, IL-6, IL-8, IL-10, IL1β, TNF-α and BDNF	19
(Chen et al., 2013)	T: 516 ACE+: 271	ACE+: 10 ACE-: 11	ACE+:40.2 ACE-: 50.6	Life stressors (e.g., death of a family member, trouble with teacher)	Exposure to Violence Scale questionnaire	Lifetime	0-9	0-9	DNA methylation (ADCYAP1R1)	21
(Cicchetti & Handley, 2017)	T: 534 ACE+: 285	T: 9.41 ACE+: 9.45 ACE-: 9.97	48.5	Abuse and neglect	Maltreatment classification system	Lifetime	9.4	9.4	DNA methylation (NR3C1)	21
(Cicchetti, Handley, & Rogosch, 2015)	T: 489 ACE+: 267	8-12 (M= 9.72)	ACE+:42.7 ACE-: 53.7	Abuse and neglect	Maltreatment Classification System	0-9	0-9	0-9	CRP	21
(Fujisawa et al., 2019)	T: 85 ACE+: 44	M=12.9	35.3	Physical, emotional and sexual abuse, physical and emotional neglect	n.m.	Early in life	12.9	-	DNA methylation	19
(Shalev et al., 2013)	T: 236	T1: 5 T2: 10	49.2	Life stressors (e.g., death of a family member, trouble with teacher), bullying and physical maltreatment	n.m.	5-10	5-10	0-5	Telomere length	21
(Slopen, Kubzansky, McLaughlin, & Koenen, 2013)	T: 5802	IL-6: 10 and 15 CRP: 10	49.8	Life stressors (e.g., death of a family member, trouble with teacher) and sexual abuse	n.m.	0-8	10-15	0-15	IL-6; CRP	22
Play: 3-5 years										
(Bruce, Fisher, Pears, & Levine, 2009)	T: 177 ACE+: 117	3-6	ACE+:46.0 ACE-:47.0	Physical and sexual abuse, physical neglect, and emotional maltreatment	Maltreatment Classification System	Lifetime	3-6	3-6	Cortisol	15

(Parade et al., 2017)	T: 231 ACE+: 123	51.2 months	52.4	Physical and sexual abuse, physical neglect and emotional maltreatment	System for coding subtype and severity of maltreatment in child protective records	3 -5	3-5	0-2	DNA methylation	20
(Parent et al., 2017)	T: 260 ACE+: 134	3-5 ACE+: 8.1 ACE-: 8.4	53.8	Physical and sexual abuse, physical neglect and emotional maltreatment	The Diagnostic Infant and Preschool Assessment	Past 6 months	3-5	0.5	DNA methylation	21
(Tyrka, Parade, et al., 2015)	T: 184	3-5	51.1	Physical and sexual abuse, physical neglect and emotional maltreatment	Diagnostic Infant and Preschool Assessment	Past 6 months	3-5	0.5	DNA methylation (NR3C1)	20
(Tyrka, Ridout, et al., 2015)	T: 174	3-5	51.7	Physical and sexual abuse, physical neglect and emotional maltreatment	Diagnostic Infant and Preschool Assessment	Past 6 months	3-5	0.5	DNA methylation (FKBP5 and NR3C1)	19
Middle childhood (6-12 years)										
(Baldwin et al., 2018)	T: 1732	18.4	51.3	Several types of victimization	n.m.	5, 7, 10, 12	18	6-13	CRP	21
(Bevans, Cerbone, & Overstreet, 2008)	T: 68	7.6- 13.8 (M= 10.7)	56.0	Life stressors (e.g., death of a family member, trouble with teacher)	The Life Events Checklist and UCLA PTSD Index for DSM-IV Child- and Parent-Report versions	Lifetime	10.7	10.7	Cortisol	14
(Buchweitz et al., 2019)	33	10–14 (M=11.45)	42.4	Life stressors (e.g., death of a family member, trouble with teacher) and sexual abuse	Juvenile Victimization Questionnaire (reduced version)	Lifetime	11.4	11.4	Cortisol	17
(Bush et al., 2018)	T: 178	9-11 (M=10.92)	47.0	Life stressors (e.g., death of a family member, trouble with teacher)	n.m.	Lifetime	10.9	10.9	DNA methylation	21
(Cicchetti, Hetzel, Rogosch, Handley, & Toth, 2016)	T: 548 ACE+: 298	M= 9.40	47.8	Abuse and neglect	Maltreatment Classification System	Lifetime	9.4	9.4	DNA methylation	21
(Cicchetti, Rogosch, & Cox Kearns, 2001)	T: 384	M= 9.25	39.5	Abuse and neglect	Maltreatment Classification System	Lifetime	9.25	9.25	Cortisol	22
(Coelho et al., 2016)	T: 136 ACE+: 65	ACE+: 9.44 ACE-: 8.99	ACE+:47.8 ACE-: 52.2	Physical, emotional and sexual abuse, physical and emotional neglect	Childhood Trauma Questionnaire	Lifetime	9.4	9.4	Copeptin	20
(Danese et al., 2014)	T: 172 ACE+: 81	12	n.m.	Physical maltreatment	Childhood Trauma Questionnaire	5-12	12	0-7	Leptin and CRP	19
(Doom, Cicchetti, & Rogosch, 2014)	T: 341 ACE+: 187	M=8.4	49.6	Physical, emotional and sexual abuse, physical and emotional neglect	Maltreatment Classification System	Lifetime	8.4	8.4	Cortisol	18
(Doom, Cicchetti, Rogosch, & Dackis, 2013)	T: 247 ACE+: 137	7.9–10.9 (M=9.42)	47.8	Abuse and neglect	Maltreatment Classification System	Lifetime	9.42	7.9-10.9	Cortisol and DHEA	18
(Drury et al., 2014)	T: 80 ACE+: 46	5-15 (M= 10.2) ACE+: M= 0.4 ACE-: M=9.9	T: 49.0 ACE+:57.0 ACE-:38.0	Life stressors (e.g., death of a family member, trouble with teacher)	Part of Preschool Age Psychiatric Assessment	Lifetime	10.2	10.2	Telomere length	18

(Huang, Gundapuneedi, & Rao, 2012)	T: 32 ACE+=19	ACE+= 16.0 ACE-: 15.9	ACE+:53.8 ACE-:73.7	Physical and sexual abuse, and/or witnessed domestic violence	Childhood Adversity Interview	<10 (persistent for ≥6 months)	15.89	0-10	Voxel-based morphometry	21
(Naumova et al., 2012)	T: 28	7-10 ACE+: M=8.14 ACE-: M=8.35	32.1	Foster care	n.m.	Lifetime	8.14	8.14	DNA methylation	20
(Non et al., 2016)	T: 136 ACE+: 82	12.5	ACE+:48.0 ACE-:51.0	Foster care	n.m.	Lifetime	12	12	DNA methylation	21
(Park et al., 2018)	T: 79	4.0-8.0 (M=6.1)	50.6	Life stressors (e.g., death of a family member, trouble with teacher)	Life Events Scale for Young Children	Past 12 months	6.06	1	Amygdala functional connectivity	20
(Romens, McDonald, Svaren, & Pollak, 2015)	T: 56 ACE+: 18	11–14 (M= 12.1)	46.4	Physical maltreatment	Child Protective Services records	Lifetime	12.1	12.1	DNA methylation (NR3C1)	20
(Simsek, Kaplan, Uysal, Yuksel, & Alaca, 2016)	T: 76 ACE+: 38	ACE+: M=13.4 ACE-: M=13.5	ACE+: 28.0 ACE-: 28.0	Sexual abuse	n.m.	11.7	13.4	1.7	Cortisol	21
(Stroud, Chen, Doane, & Granger, 2019)	T: 113	12.3	100	Life stressors (e.g., death of a family member, trouble with teacher)	Youth Life Stress Interview	Lifetime	12.3	12.3	Cortisol	21
(Trickett, Noll, Susman, Shenk, & Putnam, 2010)	T: 173 ACE+: 84	6–16 (M=11)	100	Sexual abuse	n.m.	7.8	6-16	6-16	Cortisol	21
(Vaillancourt et al., 2008)	T: 154	147 months	51.9	Bullying	Adapted from Olweus (1986)	Past 3 months	12.2	0.25	Cortisol	19
(Whittle et al., 2013)	T: 117	12.7	48.7	Physical and sexual abuse, physical neglect and emotional maltreatment	Childhood Trauma Questionnaire	<12	12.7	12.7	Hippocampal and amygdala volumes	21
(Yang et al., 2013)	T: 192 ACE+: 96	5 - 14 (M =10.2)	58.0	Physical, sexual, emotional abuse and witnessed domestic violence	n.m.	Past 6 months	10.2	0.5	DNA methylation	21
Adolescence: 13-18 years										
(Cicchetti, Hecker, et al., 2016)	T: 60 ACE+: 35	ACE+: 9–15 (M= 11.31) ACE-: 10–14 (M=11.76)	ACE+:60.0 ACE-:56.0	Abuse	Maltreatment and Abuse Chronology of Exposure (pediatric version)	Lifetime	9-15	9-15	DNA methylation	21
(Cisler, 2017)	T: 56 ACE+: 26	11-17 ACE+: 15.2 ACE-: 14.7	100	Physical, emotional and sexual abuse, physical and emotional neglect	National Survey of Adolescents and Childhood Trauma Questionnaire	Lifetime	11-17	11-17	Amygdala functional connectivity	16
(Copeland et al., 2014)	T: 1309	9-16	52.5	Bullying	Bullying part of CAPA	9-16	9-16	0-7	CRP	21
(Humphreys et al., 2019)	T: 178	9.1-14.0 (M= 11.4)	57.0	Life stressors (e.g., death of a family member, trouble with teacher), physical and sexual abuse	Traumatic Events Screening Inventory for Children	Lifetime	9.1-14.0	9.1-14.0	Hippocampal volume	17

(Ito et al., 1993)	T: 104	13.0	49.0	Physical, emotional and sexual abuse	Medical records and Department of Social Services records	Lifetime	13	13	Neurological abnormalities	15
(Kaess, Whittle, O'Brien-Simpson, Allen, & Simmons, 2018)	T: 69	12.62	30.0	Physical, emotional and sexual abuse, physical and emotional neglect	Childhood Trauma Questionnaire	Lifetime	14-16	14-16	Pituitary gland volume	20
(Malhi et al., 2019)	T: 201	12–17	100	Emotional abuse and/or neglect	Childhood Trauma Questionnaire	Lifetime	12-17	12-17	Hippocampal volume	20
(Östberg, Låftman, Modin, & Lindfors, 2018)	T: 198	14-16	59.2	Bullying	Pressure and Activation Stress Scale	Lifetime	14-16	14-16	Cortisol	19
(Pagliaccio et al., 2015)	T: 120	9-14 (M=11.2)	48.3	Life stressors (e.g., death of a family member, trouble with teacher)	Preschool-Age Psychiatric Assessment and Childhood and Adolescent Psychiatric Assessment	Lifetime	9-14	9-14	Amygdala functional connectivity	22
(Ruttle, Armstrong, Klein, & Essex, 2014)	T: 330	14.5-19.2	n.m.	Life stressors (e.g., death of a family member, trouble with teacher)	Adolescent Perceived Events Scale and the Life Events Survey	9-18	14.5-19.2	1.2-10.2	Cortisol	20
(Saxbe et al., 2018)	T: 21	M=16.9	43.0	Life stressors (e.g., death of a family member, trouble with teacher)	Survey of Children's Exposure to Community Violence, Domestic Conflict Index and Conflict Tactics Scale–Parent/Child	11.79-13.93	16.92	2.99-5.13	Amygdala and hippocampal volume	21
(Simsek, Yuksel, Kaplan, Uysal, & Alaca, 2015)	T: 86 ACE+: 44	8-17 ACE+: 13.1 ACE-: 13.8	ACE+:72.7 ACE-:71.4	Sexual abuse	n.m.	22.72 months before examination	8-17	1.9	Cortisol, BDNF	18
Mid Adolescence: 13-15 years										
(Efstathopoulos et al., 2018)	T: 1149	13–14	54.4	Bullying and other life stressors (e.g., death of a family member, trouble with teacher)	n.m.	Lifetime	13-14	13-14	DNA rmethylation (NR3C1)	20
Late Adolescence: 15-18 years										
(Edmiston et al., 2011)	T: 42	12-17 (M= 15.3)	50.0	Physical, emotional and sexual abuse, physical and emotional neglect	Childhood Trauma Questionnaire	Lifetime	15.33	15.33	Grey Matter	18
(Esposito et al., 2016)	T: 83 ACE+:50	ACE+: 12.7–18.7 (M=15.7) ACE-: 13.0–17.2 (M=15.4)	ACE+:50.0 ACE-: 54.5	Life stressors (e.g., death of a family member, trouble with teacher)	The Life Events Checklist (child/adolescent version)	Past year	15	1	DNA methylation	19
0-18 years										
(Marzi et al., 2018)	T: 1468	18	n.m.	Domestic violence, bullying, physical maltreatment, sexual	Juvenile Victimization Questionnaire and	5, 7, 10, and 12 and 12-18	18	0-6	DNA methylation (NR3C1)	21

				abuse, emotional abuse and neglect, and physical neglect	Childhood Trauma Questionnaire					
(Radtke et al., 2015)	T: 46	M=15	60.9	Life stressors (e.g., death of a family member, trouble with teacher), physical, emotional and sexual abuse, physical and emotional neglect	KERF-I	<18	11-18	0-18	DNA methylation (NR3C1)	19
(Serbulent, Ozlem, & Murat, 2017)	T: 27 ACE+: 17	ACE+: 3-16 (M=15) ACE-: 6-16 (M=10.4)	74.0	Sexual abuse	n.m.	72 hours before the examination	0-18	72 hours	IL6, IL10, cortisol	22
(Tyborowska et al., 2018)	T: 37	M=14.6 and M=17.1	22.0	Life stressors (e.g., death of a family member, trouble with teacher)	Life events questionnaire and Coddington's Life Events Scale for Children	<5 and 14-17	0-17	0-17	Grey matter volume	20
(van der Knaap et al., 2014)	T: 468	14-18 (M=16.1)	50.4	Life stressors (e.g., death of a family member, trouble with teacher)	n.m.	0-15	16.1	1.1-16.1	DNA methylation	20
(van der Knaap et al., 2015)	T: 939	M=16.2	n.m.	Life stressors (e.g., death of a family member, trouble with teacher)	Childhood Trauma Questionnaire (based)	0-15	16.2	1.2-16.2	DNA methylation (SLC6A4)	22
(White et al., 2017)	T: 537	3–16 ACE+: M=9.86 ACE-: M=10.08	50.6 ACE+:46.1 ACE-:54.5	Physical and sexual abuse, physical neglect and emotional maltreatment	Maltreatment Classification System	Lifetime	3-16	3-16	Cortisol	22

a. Time between exposure to ACEs and measure of biomarker; b. Quality of reporting of the included studies was assessed using the Strengthening Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. All studies scoring higher than the median in the STROBE checklist for cohort, case-control, and cross-sectional studies (combined) and thus revealing a satisfactory to good quality were included

Table 2. Descriptive characteristics of all included studies (n=58)

Author (Year)	Country	Study design	Sample	Year of the survey	Prevalence of ACEs (%)
Toddlerhood: 0-3 years					
(Bhopal et al., 2019)	India	Longitudinal	SPRING-ELS	2015	n.m.
(Dahmen et al., 2018)	Germany	Case-control	Community	2006-2007	Amongst cases: 51.0
Childhood: 3-12 years					
(Bucker et al., 2015)	Brazil	Case-control	Multi-cohort	n.m.	Amongst cases: Neglect: 91.75 Physical abuse: 52.8 Sexual abuse: 19.4
(Chen et al., 2013)	Puerto Rico	Case-control	Neighborhood clusters	2009-2010	1.20
(Cicchetti & Handley, 2017)	USA	Case-control	Research summer camp program	n.m.	Amongst cases: Emotional maltreatment: 62.5 Neglect: 75.4 Physical abuse: 28.4 Sexual abuse: 8.8
(Cicchetti, Handley, & Rogosch, 2015)	USA	Case-control	Research summer camp program	n.m.	Amongst cases: 54.6
(Fujisawa et al., 2019)	Japan	Case-control	Community	n.m.	Amongst cases: 52.4
(Shalev et al., 2013)	United Kingdom	Longitudinal	Environmental-Risk Study	1995 2000	Overall: 45.8 Bullying: 24.1 Domestic IPV: 16.9 Physical maltreatment: 26.7
(Slopen, Kubzansky, McLaughlin, & Koenen, 2013)	USA	Longitudinal	Avon Longitudinal Study of Parents and Children	n.m.	n.m.
Play: 3-5 years					
(Bruce, Fisher, Pears, & Levine, 2009)	USA	Case-control	Community	n.m.	Amongst cases: 68.8
(Parade et al., 2017)	USA	Case-control	Community	n.m.	53.0
(Parent et al., 2017)	USA	Longitudinal	Community	n.m.	51.5
(Tyrka, Parade, et al., 2015)	USA	Cross-sectional	Community	n.m.	Amongst cases: Emotional maltreatment: 66.2 Lack of supervision: 27.0 Neglect: 12.2 Physical abuse: 12.2 Sexual abuse: 21.6
(Tyrka, Ridout, et al., 2015)	USA	Cross-sectional	Community	n.m.	Amongst cases: Emotional maltreatment: 68.1 Lack of supervision: 30.4 Neglect: 11.6 Physical abuse: 11.6 Sexual abuse: 18.8

Middle childhood (6-12 years)					
(Baldwin et al., 2018)	United Kingdom	Longitudinal	Environmental Risk Longitudinal Twin Study	1994-1996 to 2012- 2014	26.5
(Bevans, Cerbone, & Overstreet, 2008)	USA	Cross-sectional	Community	n.m.	n.m.
(Buchweitz et al., 2019)	Brazil	Cross-sectional	Community	n.m.	Lifetime: 82.5 Last year: 72.5
(Bush et al., 2018)	USA	Longitudinal	Peers and Wellness Study	2003- 2005; 2010	n.m.
(Cicchetti, Hetzel, Rogosch, Handley, & Toth, 2016)	USA	Case-control	Research summer camp program	n.m.	Amongst cases: Emotional abuse: 59.4 Neglect: 71.2 Physical abuse: 27.2 Sexual abuse: 8.7
(Cicchetti, Rogosch, & Cox Kearns, 2001)	USA	Case-control	Research summer camp program	n.m.	Amongst cases: Emotional maltreatment: 74.3 Neglect: 79.4 Physical abuse: 37.1 Sexual abuse: 16.6
(Coelho et al., 2016)	Brazil	Cross-sectional	High Risk Cohort Study for Psychiatric Disorder	n.m.	Amongst cases: 47.8
(Danese et al., 2014)	USA	Case-control	Environmental-Risk Longitudinal Twin Study	n.m.	n.m.
(Doom, Cicchetti, & Rogosch, 2014)	USA	Case-control	Multi-cohort	n.m.	Amongst cases: Emotional maltreatment: 49.7 Neglect: 66.3 Physical abuse: 29.9 Sexual abuse: 6.4
(Doom, Cicchetti, Rogosch, & Dackis, 2013)	USA	Case-control	Summer camp program	n.m.	Amongst cases: Emotional abuse: 49.7 Neglect: 66.3 Physical abuse: 29.9 Sexual abuse: 6.4
(Drury et al., 2014)	USA	Case-control	Community	n.m.	57.0
(Huang, Gundapuneedi, & Rao, 2012)	USA	Case-control	Part of a larger study	n.m.	14.7
(Naumova et al., 2012)	Russia	Case-control	Community	n.m.	Amongst cases: 50.0
(Non et al., 2016)	Romania	Case-control	Bucharest early intervention project	n.m.	Amongst cases: 50.0
(Park et al., 2018)	USA	Cross-sectional	Part of two larger studies	n.m.	n.m.
(Romens, McDonald, Svaren, & Pollak, 2015)	USA	Case-control	Community	n.m.	32.0
(Simsek, Kaplan, Uysal, Yuksel, & Alaca, 2016)	Turkey	Case-control	Department of Child Psychiatry at Dicle University Hospital	May - November 2012	n.m.
(Stroud, Chen, Doane, & Granger, 2019)	USA	Case-control	Part of a larger study	n.m.	n.m.
(Trickett, Noll, Susman, Shenk, & Putnam, 2010)	USA	Case-control	Community	n.m.	n.m.
(Vaillancourt et al., 2008)	Canada	Cross-sectional	Community	n.m.	Physical bullying: 20.8 Social bullying: 43.5 Verbal bullying: 58.4
(Whittle et al., 2013)	Australia	Longitudinal	Orygen Adolescent Development Study	n.m.	n.m.

(Yang et al., 2013)	USA	Case-control	Community	2011	Amongst cases: Emotional abuse: 65.0 Neglect: 83.0 Physical abuse: 65.0 Sexual abuse: 24.0 Witness domestic violence: 70.0
Adolescence: 13-18 years					
(Cicchetti, Hecker, et al., 2016)	Tanzania	Case-control	Community	n.m.	n.m.
(Cisler, 2017)	USA	Case-control	Community	n.m.	Amongst cases: 46.4
(Copeland et al., 2014)	USA	Longitudinal	Great Smoky Mountains Study	n.m.	n.m.
(Humphreys et al., 2019)	USA	Cross-sectional	Part of a larger study	n.m.	98.0 (at least 1 event > 6years)
(Ito et al., 1993)	USA	Cross-sectional	Medical records	n.m.	66.9
(Kaess, Whittle, O'Brien-Simpson, Allen, & Simmons, 2018)	Australia	Cross-sectional	Orygen Adolescent Development Study	n.m.	19.0 (CTQ > 35)
(Malhi et al., 2019)	Australia	Cross-sectional	Community	n.m.	37.8
(Östberg, Låftman, Modin, & Lindfors, 2018)	Sweden	Cross-sectional	School Stress and Support Study	2010	13.5
(Pagliaccio et al., 2015)	USA	Cross-sectional	Preschool Depression Study	n.m.	n.m.
(Ruttle, Armstrong, Klein, & Essex, 2014)	USA	Longitudinal	Wisconsin Study of Families and Work	n.m.	n.m.
(Saxbe et al., 2018)	USA	Longitudinal	Urban sample Longitudinal study of youth	n.m.	n.m.
(Simsek, Yuksel, Kaplan, Uysal, & Alaca, 2015)	Turkey	Case-control	Department of Child Psychiatry at Dicle University Hospital	December 2011 and April 2012	n.m.
Mid Adolescence: 13-15 years					
(Efstathopoulos et al., 2018)	Sweden	Cross-sectional	KUPOL project	2013-2014 2014-2015	n.m.
Late Adolescence: 15-18 years					
(Edmiston et al., 2011)	USA	Cross-sectional	Community	n.m.	85.7
(Esposito et al., 2016)	USA	Case-control	Community	n.m.	n.m.
0-18 years					
(Marzi et al., 2018)	United Kingdom	Longitudinal	Environmental Risk Longitudinal Study	1999-2000; 2001-2002; 2006-2007; 2012-2013	28.1
(Radtke et al., 2015)	Germany	Cross-sectional	Community	n.m.	n.m.
(Serbulent, Ozlem, & Murat, 2017)	Turkey	Case-control	Department of Child Protective Service	May 2016 - July 2016	Amongst cases: 63.0
(Tyborowska et al., 2018)	Netherlands	Longitudinal	Nijmegen Longitudinal Study on Child and Infant Development	n.m.	n.m.

(van der Knaap et al., 2014)	Netherlands	Longitudinal	Tracking Adolescents' Individual Lives Survey	2001-2002 2003-2004 2005-2007 2008-2010	Physical abuse: 38.7 Sexual abuse: 7.1 Other trauma: 24.8
(van der Knaap et al., 2015)	Netherlands	Longitudinal	Tracking Adolescents' Individual Lives Survey	2001-2002 2003-2004 2005-2007 2008-2010	Physical abuse: 35.5 Sexual abuse: 7.0 Other trauma: 22.6
(White et al., 2017)	Germany	Case-control	Community	n.m.	n.m.

Table 3. Direction and strength of association between exposure to ACEs and biomarker by biological mechanism (positive associations indicate that biomarker increases with ACEs exposure and/or frequency; inverse associations indicate that biomarker decreases with ACEs exposure and/or frequency).

Author, year	Biomarker	Type of ACEs	Direction of association	Strength of association
Genetic & Epigenetic				
(Bush et al., 2018)	DNA methylation	Life stressors (e.g., death of a family member, trouble with teacher)	Positive	Weak to moderate
(Cicchetti, Hecker, et al., 2016)	DNA methylation	Abuse	Positive	Strong
(Cicchetti, Hetzel, Rogosch, Handley, & Toth, 2016)	DNA methylation	Abuse and neglect	Positive	Strong
(Fujisawa et al., 2019)	DNA methylation	Physical, emotional and sexual abuse, physical and emotional neglect	Positive	Strong
(Naumova et al., 2012)	DNA methylation	Foster care	Positive	Strong
(Non et al., 2016)	DNA methylation	Foster care	Inverse	Strong
(Parade et al., 2017)	DNA methylation	Physical and sexual abuse, physical neglect and emotional maltreatment	Inverse	Strong
(Parent et al., 2017)	DNA methylation	Physical and sexual abuse, physical neglect and emotional maltreatment	Positive	Strong
(Tyrka, Parade, et al., 2015)	DNA methylation	Physical and sexual abuse, physical neglect and emotional maltreatment	Positive	Strong
(van der Knaap et al., 2014)	DNA methylation (NR3C1 CpG1) DNA methylation (NR3C1 CpG2) DNA methylation (NR3C1 CpG3)	Life stressors (e.g., death of a family member, trouble with teacher)	Positive Positive Inverse	Strong Strong Strong
(Yang et al., 2013)	DNA methylation	Physical, sexual, emotional abuse and witnessed domestic violence	Positive	Strong
(Esposito et al., 2016)	DNA methylation	Life stressors (e.g., death of a family member, trouble with teacher)	Positive	Weak
(van der Knaap et al., 2015)	DNA methylation (SLC6A4)	Life stressors (e.g., death of a family member, trouble with teacher)	Positive	Strong
(Chen et al., 2013)	DNA methylation (ADCYAP1R1)	Life stressors (e.g., death of a family member, trouble with teacher)	Positive	Weak
(Cicchetti & Handley, 2017)	DNA methylation (NR3C1)	Abuse and neglect	Positive	Strong
(Marzi et al., 2018)	DNA methylation (NR3C1)	Domestic violence, bullying, physical maltreatment, sexual abuse, emotional abuse and neglect, and physical neglect	Positive	Weak
(Radtke et al., 2015)	DNA methylation (NR3C1)	Life stressors (e.g., death of a family member, trouble with teacher), physical, emotional and sexual abuse, physical and emotional neglect	Positive	Strong
(Romens, McDonald, Svaren, & Pollak, 2015)	DNA methylation (NR3C1)	Physical maltreatment	Positive	Strong
(Tyrka, Ridout, et al., 2015)	DNA methylation (FKBP5)	Physical and sexual abuse, physical neglect and emotional maltreatment (adversity composite) Physical and sexual abuse, physical neglect and emotional maltreatment (lifetime contextual stress) Physical and sexual abuse, physical neglect and emotional maltreatment (past-month contextual stress and the number of traumatic life events)	Inverse Positive No association	Strong Strong -
(Efstathopoulos et al., 2018)	DNA methylation (NR3C1)	Bullying and other life stressors (e.g., death of a family member, trouble with teacher)	Positive	Strong
(Shalev et al., 2013)	Telomere length	Life stressors (e.g., death of a family member, trouble with teacher), bullying and physical maltreatment	Inverse	Strong
(Drury et al., 2014)	Telomere length	Life stressors (e.g., death of a family member, trouble with teacher)	Inverse	Strong
Immune system				
(Bevans, Cerbone, & Overstreet, 2008)	Cortisol	Life stressors (e.g., death of a family member, trouble with teacher) (within the past 12 months, recent & frequent trauma and afternoon cortisol) Life stressors (e.g., death of a family member, trouble with teacher) (within the past 12 months, recent & frequent trauma and morning cortisol)	Positive No association	Strong -
(Bhopal et al., 2019)	Cortisol	Life stressors (e.g., death of a family member, trouble with teacher)	Positive	Strong

(Bruce, Fisher, Pears, & Levine, 2009)	Cortisol	Physical and sexual abuse, physical neglect, and emotional maltreatment (emotional maltreatment)	Positive	Strong
		Physical and sexual abuse, physical neglect, and emotional maltreatment (severity of physical neglect)	Inverse	Strong
(Buchweitz et al., 2019)	Cortisol	Life stressors (e.g., death of a family member, trouble with teacher) and sexual abuse	Positive	Strong
(Cicchetti, Rogosch, & Cox Kearns, 2001)	Cortisol	Abuse and neglect	Positive	Strong
(Doom, Cicchetti, & Rogosch, 2014)	Cortisol	Physical, emotional and sexual abuse, physical and emotional neglect	Positive	Strong
(Doom, Cicchetti, Rogosch, & Dackis, 2013)	Cortisol	Abuse and neglect	Positive	Strong
(Östberg, Låftman, Modin, & Lindfors, 2018)	Cortisol	Bullying (girls)	Inverse	Weak
		Bullying (boys)	Inverse	Strong
(Ruttle, Armstrong, Klein, & Essex, 2014)	Cortisol	Life stressors (e.g., death of a family member, trouble with teacher)	No association	-
(Simsek, Kaplan, Uysal, Yuksel, & Alaca, 2016)	Cortisol	Sexual abuse	Positive	Strong
(Simsek, Yuksel, Kaplan, Uysal, & Alaca, 2015)	Cortisol	Sexual abuse	Positive	Strong
(Stroud, Chen, Doane, & Granger, 2019)	Cortisol	Life stressors (e.g., death of a family member, trouble with teacher)	Inverse	Strong
(Trickett, Noll, Susman, Shenk, & Putnam, 2010)	Cortisol	Sexual abuse	Inverse	Strong
(Vaillancourt et al., 2008)	Cortisol	Bullying	No association	-
(White et al., 2017)	Cortisol	Physical and sexual abuse, physical neglect and emotional maltreatment	Inverse	Strong
(Serbulent, Ozlem, & Murat, 2017)	Cortisol	Sexual abuse	No association	-
	IL-6		Positive	Strong
	IL-10		No association	-
(Baldwin et al., 2018)	CRP	Several types of victimization (only for girls)	Positive	Strong
(Cicchetti, Handley, & Rogosch, 2015)	CRP	Abuse and neglect (only for those with at least one A allele)	Positive	Weak
(Copeland et al., 2014)	CRP (childhood)	Bullying	No association	-
	CRP (late adolescence)	Bullying (victims)	Positive	Strong
	CRP (late adolescence)	Bullying (bullies)	Inverse	Strong
	CRP (late adolescence)	Bullying (bully-victims)	No association	-
(Danese et al., 2014)	CRP	Physical maltreatment	No association	-
(Bucker et al., 2015)	IL-12p70	Sexual abuse, maltreatment, and/or neglect	No association	-
	IL-6		No association	-
	IL-8		No association	-
	IL-10		No association	-
	IL1β		No association	-
	TNF-α		Positive	Strong
	BDNF		Positive	Strong
(Slopen, Kubzansky, McLaughlin, & Koenen, 2013)	IL-6	Life stressors (e.g., death of a family member, trouble with teacher) and sexual abuse	Positive	Strong
	CRP		Positive	Strong
Structural & functional brain changes				
(Cisler, 2017)	Amygdala functional connectivity	Physical, emotional and sexual abuse, physical and emotional neglect	Inverse	Strong
(Pagliaccio et al., 2015)	Amygdala functional connectivity	Life stressors (e.g., death of a family member, trouble with teacher)	Positive	Strong
(Park et al., 2018)	Amygdala functional connectivity	Life stressors (e.g., death of a family member, trouble with teacher)	Inverse	Strong
(Dahmen et al., 2018)	Hippocampal volume	Maltreatment	Inverse	Strong
(Edmiston et al., 2011)	Grey matter	Physical, emotional and sexual abuse, physical and emotional neglect	Inverse	Strong
(Tyborowska et al., 2018)	Grey matter	Life stressors (e.g., death of a family member, trouble with teacher)	Inverse	Strong

(Humphreys et al., 2019)	Hippocampal volume	Life stressors (e.g., death of a family member, trouble with teacher), physical and sexual abuse (early exposure)	Inverse	Moderate
(Kaess, Whittle, O'Brien-Simpson, Allen, & Simmons, 2018)	Pituitary gland volume	Physical, emotional and sexual abuse, physical and emotional neglect	Positive	Weak
(Whittle et al., 2013)	Hippocampal volume	Physical and sexual abuse, physical neglect and emotional maltreatment	Positive	Strong
	Amygdala volume		Inverse	Strong
(Malhi et al., 2019)	Hippocampal volume	Emotional abuse and/or neglect	Inverse	Strong
(Saxbe et al., 2018)	Hippocampal volume	Life stressors (e.g., death of a family member, trouble with teacher)	Inverse	Strong
	Amygdala volume		Inverse	Weak
(Simsek et al., 2015)	BDNF	Sexual abuse	Inverse	Strong
(Ito et al., 1993)	Neurological abnormalities	Physical, emotional and sexual abuse	No association	-
(Huang, Gundapuneedi, & Rao, 2012)	Voxel-based morphometry	Physical abuse, sexual abuse, and/or witnessed domestic violence	Inverse	Strong
Other				
(Coelho et al., 2016)	Copeptin	Physical, emotional and sexual abuse, physical and emotional neglect	Positive	Strong
(Doom et al., 2013)	DHEA	Abuse and neglect (only for boys)	Inverse	Strong
(Danese et al., 2014)	Leptin	Physical maltreatment	Positive	Strong

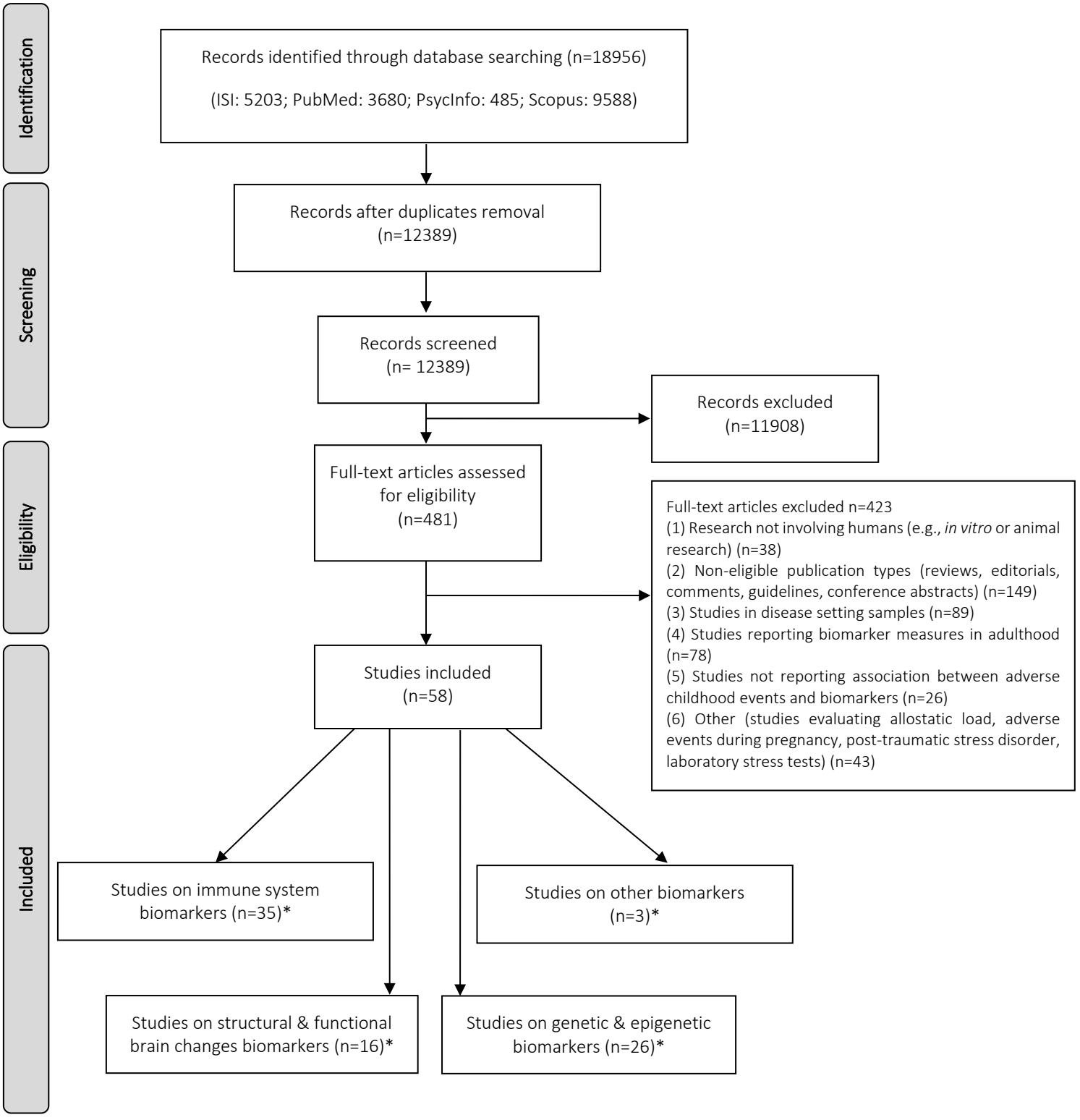

Figure 1. *PRISMA flow diagram of the literature search*
*some papers evaluated more than one biomarker, within or not the same biological mechanism

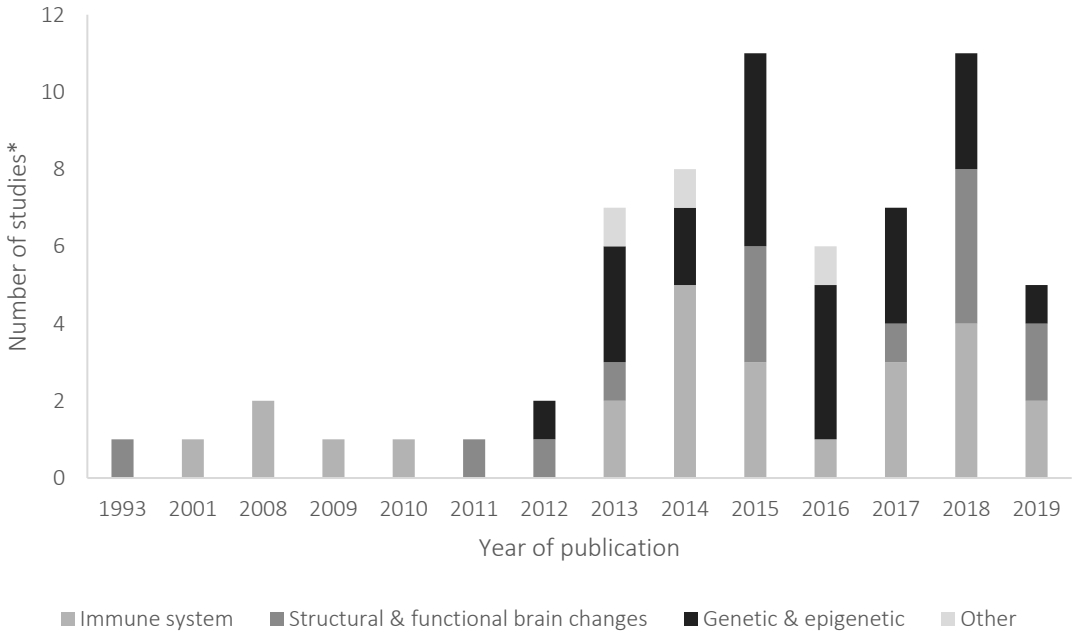

Figure 2. Frequency (number of studies*) by biological mechanism, published per year.
*some papers evaluated more than one biomarker, within or not the same biological mechanism. Year of 2019 includes papers published online until july

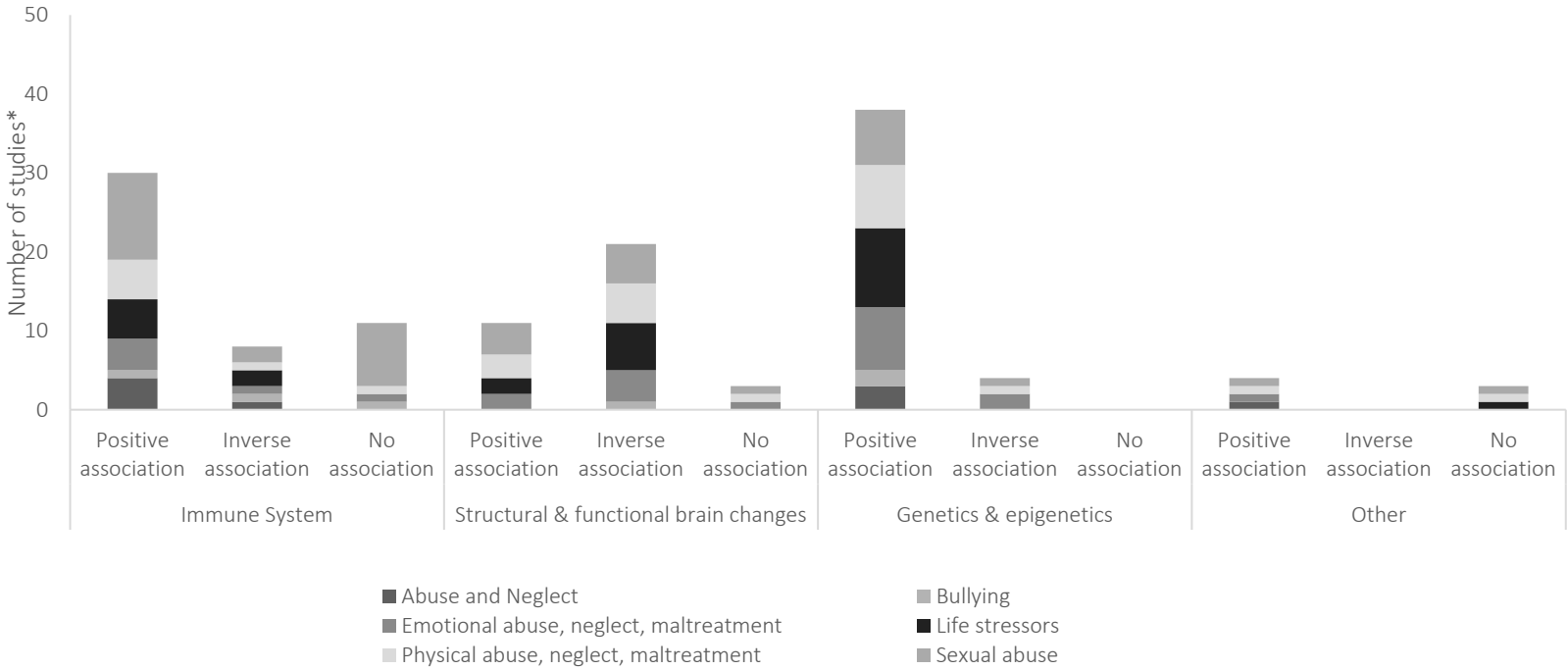

Figure 3. Frequency (number of studies*) categorized by presence of association (positive, negative) or absence of association (no association) found between exposure to ACEs and biomarker, by biological mechanism and type of ACE.

*some papers evaluated more than one biomarker, within or not the same biological mechanism, and more than one type of ACE.

4.4 Parents' use of extreme physical violence is associated with elevated high-sensitivity C-reactive protein in children

Sílvia Fraga; Sara Soares; Ana Cristina Santos; Henrique Barros

The following is a pre-peer-review version of an article currently submitted.

Parents' use of extreme physical violence is associated with elevated high-sensitivity C-reactive protein in children

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Introduction

Exposure to stressful and traumatic experiences during sensitive periods of neurological and cognitive development may have lasting implications in mental and physical health (1), being a significant early determinant of disease and premature mortality (1, 2). Theoretical models have been proposed to explain the embodiment of social adversity (2-4). In particular, a model stated that when stressful events occur, it gets embedded in the regulation mechanisms of the inflammatory response. Thus to the extent that children spend their early years exposed to adversity their monocytes/macrophages will tend to develop response promoting a chronic pro-inflammatory state (2), namely through the hypothalamic-pituitary-adrenal (HPA) axis activation (4). The HPA axis activation leads to altered insulin sensitivity, increased blood pressure, inflated central adiposity, and consequently, more inflammation (2, 4). Therefore, we hypothesize that experiencing abuse may trigger an increase in the child high sensitivity C-reactive protein (hs-CRP). This study builds on the aforementioned research, examining the effect of parental physical violence on low-grade inflammation in early childhood.

Methods

This study includes children from Generation XXI, a prospective Portuguese population-based birth cohort that was first assembled between 2005 and 2006 (5). Responses to the *Parent-Child Conflict Tactics Scales* (CTSPC) and measurements of hs-CRP were available for 4175 children when they were seven years old. Children reported parents' disciplinary practices through the CTSPC administered by trained interviewers, in a private setting, and after the specific parents' and children's own consent. A four-category variable of physical violence based on the occurrence and severity was defined: I=low frequency and no severe acts; II= frequent but no severe acts; III= frequent and severe acts; IV= frequent and extreme acts.

Venous blood samples were obtained after 8 to 12 hours of overnight fast and hs-CRP levels were determined by immunonephelometric assays with CardioPhase. Due to the presence of floor effects (high number of values below the 0.2 detection threshold) and skewed distribution, hs-CRP was dichotomized using tertiles of distribution (top tertile vs others). The study protocol was approved by the Ethics Committee of Hospital de São João and registered with the Portuguese Authority of Data Protection and was carried out in accordance with the principles of the Declaration of Helsinki. In all study waves, written informed consent was obtained from parents or legal guardians and oral assent from children. Severe cases were reported by the interviewers to the Cohort Coordination that followed a specific protocol established according to the national guidelines for each particular situation.

Results

Overall, 44.0% of children reported low frequency of physical violence, 50.1% reported frequent but not severe physical violence, 5.3% reported frequent and severe physical violence, and 0.6% of children reported parental extreme physical violence. Significantly higher levels of hs-CRP were observed among children who reported the highest grade of violence severity (58.3%). Table 1 shows that after adjustment for child's sex, age and parental education, with the increasing grade of violence severity increased the odds of higher hs-CRP levels, with children reporting the highest grade of violence severity (IV) presenting a threefold greater risk of being in the upper CRP tertile (95%CI: 1.36-7.12) than children reporting less severe grades of violence severity. When exposure to smoking or body mass index were included to the model, the main results remained statistically significant, with children in the highest grade of violence severity being three times more likely to be in the upper CRP tertile.

Discussion

Higher hs-CRP levels were found among children reporting extreme violence, including "grab the children by the neck and chock them" or "burn the children or scold them on purpose". Although little is known if alterations in inflammatory markers after experiencing abuse at early ages may be reversed, our results seem to support evidence for biological imprinting and short-term physiological effect of adverse childhood experiences. No significant increase in the levels of hs-CRP was found in children exposed to less severe violence. However, that does not mean that those forms of violence have no impact on health, as retrospective studies showed that childhood exposure to violence later impacts in major adverse health outcomes (6, 7). On the contrary, we might not be able to see its impact on hs-CRP in a shorter period, but such experiences might be biologically embodied during this sensitive period of development when exposure coincides with the period of greatest maturation or plasticity of most of the organs and biological mechanisms which might have the potential to be setting children on a trajectory of increased risk for the development of chronic diseases in adulthood. The underlying atherosclerotic process might already be ongoing under a long asymptomatic phase of development, tracking over time and triggering the onset of disease several years later (8). Furthermore, exposure to parental violence is expected to have a great impact in child development with the familial dynamics triggering a cascade of psychosocial vulnerabilities, including deficits in social competence and emotional regulation, and a susceptibility to compensate with health-risk behaviours over the life course, increasing the risk of later poorer social and health outcomes (2).

The use of children reports of violence and the availability of biological markers in this sample are the major strengths of this study. Statistical analyses were conducted by excluding participants with hs-CRP levels higher than 10 mg/L to remove the presence of an acute infection.

The cross-sectional nature of the study prevents us from concluding the directionality of the effects, although we provided theoretical arguments for the proposed pathway. Child abuse should be prevented, and therefore more investment in policies and programs that effectively identify and reduce child maltreatment and improve child well-being should be a priority. Considering this biological marker in the child's clinical evaluation, and if a child had not an acute infection, it can be useful to signal possible situations of threat for the child that may warrant further investigation.

Declarations:

Funding: This work was supported by the European Regional Development Fund (ERDF) through the Operational Programme Competitiveness and Internationalization and national funding from the Foundation for Science and Technology (FCT), Portuguese Ministry of Science, Technology and Higher Education under the projects "BioAdversity: How childhood social adversity shapes health: The biology of social adversity" (POCI-01- 0145-FEDER-016838; Reference FCT PTDC/DTP-EPI/1687/2014), "HIneC: When do health inequalities start? Understanding the impact of childhood social adversity on health trajectories from birth to early adolescence" (POCI-01-0145-FEDER-029567; Reference: PTDC/SAU-PUB/29567/2017). It is also supported by the Unidade de Investigação em Epidemiologia - Instituto de Saúde Pública da Universidade do Porto (EPIUnit) (Reference UIDB/04750/2020), Administração Regional de Saúde Norte (Regional Department of Ministry of Health) and Fundação Calouste Gulbenkian; PhD Grant SFRH/BD/108742/2015 (to SS) co-funded by FCT and the Human Capital Operational Programme (POCH/FSE Program); FCT Investigator contracts CEECIND/01516/2017/CP1406/CT0001 (to SF) and IF/01060/2015 (to ACS).

Conflicts of interest/Competing interests: None to declare.

Availability of data and material: Not applicable

Code availability: Not applicable

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Figure 1. Proportion of hs-CRP levels in the upper tertile according to the severity of parental physical violence (p=0.048)

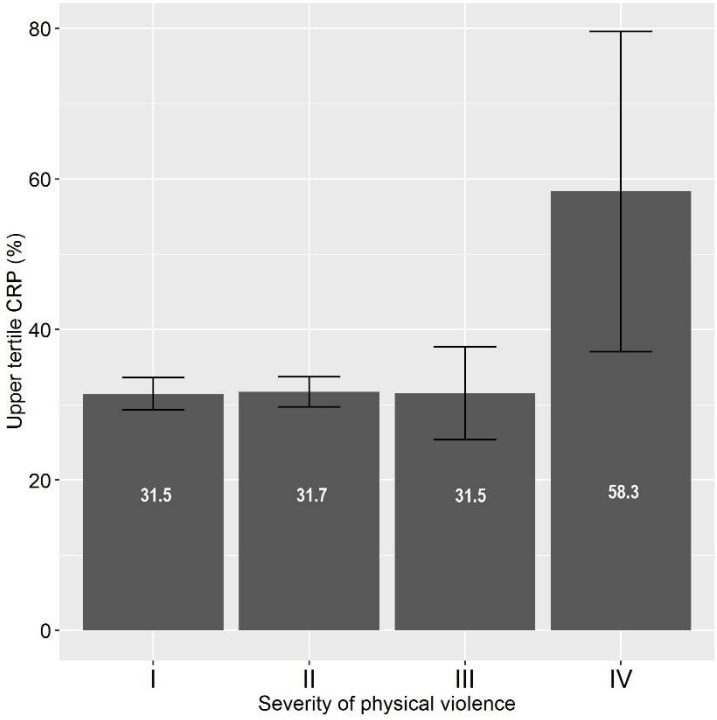

Table 1. Association of exposure to physical violence with the upper hs-CRP tertile (N=4175).

		Upper hs-CRP tertile			
		OR (95%CI)			
Severity of physical violence (grades)	N	Adjusted for sex and age	Adjusted for sex, age, and parental education	Adjusted for sex, age, parental education and exposure to smoking	Adjusted for sex, age, parental education and BMI
I	1837	Reference	Reference	Reference	Reference
II	2092	1.03 (0.90-1.17)	1.03 (0.90-1.18)	1.04 (0.91-1.19)	1.10 (0.88,1.17)
III	222	1.11 (0.82-1.50)	1.08 (0.80-1.47)	1.10 (0.81-1.49)	1.06 (0.77-1.44)
IV	24	**3.42 (1.50-7.80)**	**3.11 (1.36-7.12)**	**3.10 (1.35-7.11)**	**3.13 (1.34-7.31)**

I=low frequency and no severe acts; II= frequent but no severe acts; III= frequent and severe acts; IV= frequent and extreme acts; Odds ratio (OR) was considered significant at the 5% level (95% confidence interval) (shown in bold).

4.5 From adverse childhood experiences and bullying towards inflammation in children: the role of BMI

Sara Soares; Ana Cristina Santos; Sílvia Fraga

The following is a pre-peer-review version.

From adverse childhood experiences and bullying towards inflammation in children: the role of BMI

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Potential Conflicts of Interest: The authors have no conflicts of interest relevant to this article to disclose.

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Abstract

Background: Exposure to Adverse Childhood Experiences (ACEs) and bullying victimization has been associated with a life-long increased risk of developing chronic diseases later in life and with premature death. The association between ACEs and bullying victimization and circulating C-reactive protein (CRP) might be driven by body mass index (BMI), with the link between adversity and elevated distress being associated with the adoption of risky behaviours. Thus, evidence allows us to assume that exposure to adversity during the first years of life might already be biologically embedded well before adult life, that BMI might be a mediator in the association of ACEs, and bullying victimization, with inflammatory levels measured using high-sensitivity (hs-) CRP.

Methods: Children from the Portuguese population-based birth cohort Generation XXI were included (n=3738). At the age of 10 years, children were asked about exposure to a lifetime experience of ACEs, assessed by a questionnaire adapted from the original ACEs study, and an adaptation of The Bully Scale Survey, was used to assess frequency (never, rarely, sometimes, often, and always) of bullying victimization, through self-administered questionnaires. Regression coefficients and respective 95% CI [β(95%CI)] were computed using path analysis.

Results: ACEs had a positive total effect on hs-CRP levels at the age of 10 years (β=0.14; 95%CI: 0; 0.30). Direct effect (β=0.09; 95%CI: -0.05; 0.23) accounted for 64.3% of the association, while the indirect effect through BMI (β=0.05; 95%CI: 0; 0.11) explained 35.7% of the pathway between ACEs and hs-CRP. A positive total effect of bullying victimization on hs-CRP levels (β=0.20; 95%CI: 0.06; 0.34) was observed. A direct effect (β=0.08; 95%CI: -0.05; 0.21) accounted for 40% of the association, while an indirect effect through BMI (β=0.12; 95%CI: 0.06; 0.18) explained 60.0% of the pathway between bullying victimization and Hs-CRP.

Conclusions: There might be different mechanisms involved in the biological embedding of childhood experiences. On one hand, the impact of exposure to ACEs on hs-CRP levels seems to be less mediated by BMI, but instead, these experiences might cause an HPA axis activation, and consequently low-grade inflammation. On the other hand, BMI seems to mediate a great part of the association between exposure to bullying victimization and hs-CRP levels at the age of 10 years.

Introduction

Adverse childhood experiences (ACEs) are defined as stressful and/or traumatic experiences that occur during childhood and include emotional, physical, and sexual abuse, emotional and physical neglect, and household dysfunction (1). ACEs have been associated with a life-long increased risk of developing cardiovascular disease, among others, and premature death (1-3). Even though exposure to adversity may have a long-lasting and cumulative health effect, these experiences seem to have an immediate impact on a child's health (unpublished data).

While ACEs include adverse experiences occurring in the household, recently, bullying experiences, more common at school, have been recognized as an ACE (4-6). Bullying in childhood has also a great impact on children's health, being associated with adverse physical health functioning and increased frequency of illnesses (7-10). Also, victims of bullying have worse health outcomes (11), poorer perceived quality of life (12) and increased risk of psychiatric disorders (13) and suicidality (12) in adult life.

The link between exposure to ACEs or bullying and health outcomes later in life may be explained by the inflammatory process (14, 15). And repeated exposures to adversity are likely to affect the human stress regulatory system, followed by an increase in inflammation (16-18). Once the detrimental effects of adversity on the human stress response system are in place, they will result in chronic inflammation over the life course (19, 20). However, so far, what it is known is that the association between adversity and disease might be explained through the adoption of unhealthy behaviours (e.g., poor diet, sedentary behaviour, smoking) (21-24), or via stress sensitization, in particular through the hypothalamic-pituitary-adrenal (HPA) axis (18). The HPA axis activation leads to altered insulin sensitivity, increased blood pressure, and inflated central adiposity, consequently leading to elevated low-grade inflammation (18, 25, 26) and high levels of C-Reactive protein levels (CRP) (27).

Even though the biological mechanisms mediating these associations remains unclear, childhood adversity seems to contribute to a pro-inflammatory state in adulthood (28, 29). However, the biological consequences of bullying in childhood have been poorly studied (30), although victims present increased levels of CRP in adolescence (30) and during mid-life (31). This gap in literature might be due to some specificity regarding the type of involvement in this type of violence since the involvement as a victim or as a bully could be related to different effects on biological markers (30).

It is known that those who are living in a context of adversity are more able to engage in sedentary behaviours (32) or poor diet (33), leading to elevated BMI, and consequently increased CRP levels (34),

a marker of systemic inflammation. Few studies showed that BMI mediates the association between early life adversity and in adolescence (35, 36).

Therefore, using data from a Portuguese birth cohort Generation XXI, we aimed to examine the potential mediating role of BMI on the association between adversity (ACEs and bullying) and hs-CRP levels in 10-year-old children.

Methods

2.1. Study Design and Participants

The study sample consisted of children who participated in Generation XXI, a prospective Portuguese-population-based birth cohort. As previously reported (37, 38), recruitment occurred during 2005-2006. Briefly, mothers and children (n=8647) were recruited in public maternity units in Porto, Portugal. The entire cohort was invited to attend the second (2009-2011), the third (2012-2014) and the fourth (2016-2017) study waves, when children were aged four, seven and ten years old, respectively. Anthropometric measures and blood samples were collected in all study waves, following the same standardized procedures. Data on demographic and socioeconomic characteristics, personal history of disease and health-related behaviours were collected by trained interviewers through structured questionnaires. Generation XXI was approved by the Portuguese Data Protection Authority and by the Ethics Committee of Hospital de São João, and data confidentiality and protection were guaranteed in all procedures according to the Declaration of Helsinki. Signed informed consent was obtained for all adults and children participants had it signed by their legal guardian at every study waves (37).

The present investigation includes data on participants with complete information on ACEs and bullying victimization reports and on hs-CRP levels at the age of 10 years. Thus, the analyses were based on data from 3738 participants from the study's fourth wave, and the sample description is presented in Table 1.

2.2. Measures

2.2.1. ACEs

Conventional ACEs questions adapted from the original CDC-Kaiser ACE study (1) were used to collect information through self-administered questionnaires and children were helped by a trained interviewer whenever requested. Children were asked on a lifetime experience of moving from a house, school or neighbourhood against their will; learning problems at school; the death of a family member; injury or serious illness in the family; child hospitalization due to a disease or an accident; parents called to school because the child was in trouble; parental divorce or separation; financial issues in the household; a family member with a drug or alcohol addiction; incarceration of a household member; witnessing parents arguing or fighting; experiencing someone in the household shouting, yelling or screaming; insulting or humiliating the child; and finally, being hit, kick or punched by someone at home. For each positive answer, children were also asked about how they felt when the experience occurred by picking one of the three options: "*I didn't get angry or sad", "I got angry or sad"* or *"I got very angry or very sad"*. Since individuals may respond differently to situations of potential stress (39, 40), the exposure to ACEs was only considered when children answered positively to at least one

situation of the ACEs scale, but reported having been negatively affected by that experience, answering that they have felt "angry or sad" or "very angry or very sad". The score of ACEs considered the sum of the number of exposures and was defined as having up to 4 experiences and having 5 or more experiences, corresponding to the highest quintile.

2.2.2. Bullying

The Bully Scale Survey, a structured questionnaire created by the Centers for Disease Control and Prevention (41) was used to assess bullying involvement. In a private setting, and after the specific parents' consent on information collection, children were asked to report the frequency (never, rarely, sometimes, often, and always) in which they were involved in bullying either as a victim and/or as an aggressor.

Victims of bullying were defined as those who answered "always" to one or more situations in the frequencies: How did you get bullied? (Check how often this happened) *"Called me names", "Made fun of me", "Said they will do bad things to me", "Played jokes on me", "Won't let me be a part of their group", "Broke my things", "Attacked me", "Nobody would talk to me", "Wrote bad things about me", "Said mean things behind my back" "Pushed or shoved me*"; and mentioned never have been aggressors.

2.2.3. Hs-CRP

Following an overnight fast, a venous blood sample was collected before 11 a.m. by trained nurses in our research center, after applying a topical analgesic cream (EMLA cream). The samples were centrifuged at 3500 rpm for 10 min and plasma was aliquoted. Biomarkers were assayed in fresh blood samples. Hs-CRP was assayed using CardioPhase®hsCRP Flex®, Dimension Vista® System, from Siemens. During each test day, two separate analyses were performed with two test samples for each material tested for 20 days. Coefficient of variation for low control was 5.4% at a concentration of 0.06 mg/L and for high control was 4.4% at a concentration of 0.15 mg/L. All blood evaluations were performed at the Clinical Pathology Service, Hospital de São João, Porto, Portugal.

Due to a highly skewed distribution, and for statistical purposes, hs-CRP was log-transformed. The minimum detectable values were recoded into 0.2 mg/L for all study waves. Also, as high levels of hs-CRP could represent an acute condition instead of a chronic inflammatory state (42), the analyses excluded participants with hs-CRP levels higher than 10 mg/L.

2.2.4. BMI

Body mass index (BMI) was calculated as the value of weight (kg) over squared height (m), and computed as an age- and sex-specific BMI standard deviation (SD) score (z-score), according to the

World Health Organization Child Growth Standards (5-19 years) (43), and recoded into underweight or normal weight (BMI <2 SD) and overweight/obese (BMI >2 SD).

Data Analysis

Pearson correlations between age, sex, ACEs exposure, bullying victimization, BMI and hs-CRP were estimated.

Path analysis was used to estimate the regression coefficients (β) and 95% confidence intervals (95% CI), which represent the increase in mg/L of log hs-CRP levels with exposure to ACEs and bullying victimization. Path analysis was conducted according to current knowledge, based on the theoretical model depicted in Figure 1. The presence of multicollinearity was assessed by calculating the Variance Inflation Factor (VIF), which was lower than 5, indicating no multicollinearity (44). Bootstrapping was used for estimation of the 95% CI for the direct and indirect effects. The Comparative Fit Index (CFI) (45), the Tucker–Lewis Index (TLI) (46), and the Root Mean Square Error of Approximation (RMSEA) were used to assess the general fit of the models (47). A CFI and TLI equal to or higher than 0.90 (48) and a RMSEA lower than 0.05 indicate a good model fit (49). Analyses were performed using STATA version 15.1.

The formal analysis found no interaction between sex and ACEs and bullying victimization.

Results

Table 1 shows the participants' characteristics. In this sample, more than half were male (52.2%) and their mean (SD) age at Generation XXI 4th wave was 10.1 (0.33) years. Their mean (SD) weight was 37.7 (8.74) kg and the majority were classified as having a normal BMI (57.4%). The median of hs-CRP levels was 0.79 (P25-75: 0.58-1.09) mg/L. Bullying victimization was reported by 26.8% of participants and 30.1% of the children were in the 5th quintile of ACEs.

Correlations between all study variables are presented in Table 2. Positive, but poor correlations are observed for ACEs and BMI (p=0.02) and hs-CRP (p=0.04). Bullying victimization is positively correlated with BMI (p=0.07) and hs-CRP (p=0.04).

A positive total effect of ACEs on hs-CRP levels (β=0.14; 95%CI: 0; 0.30) was estimated. In this model, and independently of the child's sex, a direct effect (β=0.09; 95%CI: -0.05; 0.23) and an indirect and statistically significant effect through BMI (β=0.05; 95%CI: 0; 0.11) were observed. The indirect effect explains 35.7% of the pathway between ACEs and hs-CRP. Additionally, a positive total effect of bullying victimization on hs-CRP levels (β=0.20; 95%CI: 0.06; 0.34) was estimated. In this model, and independently of sex, a direct effect (β=0.08; 95%CI: -0.05; 0.21) and an indirect and statistically significant effect through BMI (β=0.12; 95%CI: 0.06; 0.18) was observed. The indirect effect explains 60.0% of the pathway between bullying victimization and hs-CRP (Table 3).

Discussion

In a sample of participants of Generation XXI birth cohort, we observed that childhood BMI played a pivotal role in the pathway from bullying victimization to inflammation, while adverse experiences in the family context seem to be less mediated by BMI. Despite well-documented associations between early life adversity and later inflammation, less is known about how early life adversity exerts effects on inflammation in childhood and if increased BMI plays a role in this association. The present study focused on early life adversity within a family context and peer-related victimization and their relationships to BMI to investigate the links between adversity in infancy and low-grade, chronic inflammation also at early ages.

Identifying which mechanism is responsible for the increase in inflammation levels and consequently by the establishment of low-grade inflammation is of utmost importance to define effective strategies to prevent and mitigate the health-related consequences of ACEs later in life. Also, as it has been described that living in an environment of exposure to ACEs may also be closely related to growing with diminished or unpredictable household availability of sufficient, adequate and nutritious food (50) In the model with ACEs, it was observed that the direct effect accounts for most of the association with hs-CRP. The indirect effect of a high number of adverse experiences in the household on the hs-CRP levels through BMI accounts for 35.7% of the association studied. Thus, the direct effect seems to account for most of this association, meaning that the effect might be more related to the HPA axis dysregulation than with the adoption of health-risk behaviours. As the majority of association occurs directly on hs-CRP, this might be explained by the variation in the HPA axis activity commonly associated with the neurobiology of stress sensitization (18, 51), leading to altered insulin sensitivity, increased blood pressure, inflated central adiposity, and consequently to elevated low-grade inflammation (18, 25) and high levels of CRP (18, 25, 27).

On the contrary, the direct effect of bullying victimization on hs-CRP levels is lower than the BMI-mediated effect, accounting for 60.0% of the association. Thus, most of the association between bullying victimization and hs-CRP is likely to be driven by BMI. Even though some discussion remains on the mechanisms that might be involved in the biological embodiment of these experiences (51, 52), that is potentially different from the ones involved in ACEs, our results on mediation effects of BMI show that health behaviours seem to be important in the establishment of chronic low-grade inflammation, at least, at a shorter-term.

These results may be reflecting the existence of different pathways that explain adversity embodiment, whether it is caused by victimization through exposure to adverse experiences more related with the familial environment, or victimization by bullying, more related with peer relationships and school environment. The observed results reinforce the importance of health risk behaviours on the embodiment of adversity. We may speculate that exposure to bullying might lead to the adoption of negative health behaviours (53, 54), as a way of trying to reduce tension or stress with a potential contribution for later disease development (1, 55) or premature death (3). Higher inflammation levels have been observed in participants reporting bullying victimization when compared with participants non-victims, and with aggressors (30). Even though we do not expect the same contribution for all health-related behaviours, such as smoking and alcohol consumption in children, as they are not expected to be established at these ages, sedentary behaviour and/or poor diet may lead to increased BMI, explaining some of the association between bullying and hs-CRP, via adiposity. Especially when recent literature has established that food is sometimes used as a coping mechanism in response to stress, commonly known as emotional eating, overeating (56) or selecting high-calorie foods, when facing stressful circumstances (57), behaviours that will increase per cent body fat, overweight, and obesity.

In two studies it is shown that BMI mediates the association between early life adversity and inflammation in adolescence (35, 36), our results add to these demonstrating, to a certain degree, a role of adiposity on the studied association. One of the studies found that BMI mediates the association between serious interpersonal conflict stress and increased hs-CRP, but no association was observed regarding serious financial stress or maternal depression (35), while the other study showed that BMI attenuated the associations between cumulative adverse events and immediate events, that included being taken into foster care, being a victim of physical or sexual abuse or separated from mother or father, and inflammation (36). However, neither quantified the indirect effect of BMI in the studied associations.

It is important to emphasize that high levels of inflammation do not necessarily mean risk, even though children's inflammatory profile may contribute to the earlier establishment of an unfavourable profile in early life stages. However, we cannot assume a deterministic view and conclude that these children will develop disease later in life. But it can be hypothesized that the underlying atherosclerotic process might already be in course and high levels of inflammatory markers at such early ages can be a precursor of later development of disease (18, 26). And the results seem to show a direct and indirect effect of traumatic experiences on hs-CRP levels, that may have the potential of accumulation by continuous exposure (58).

Strengths and limitations

The use of data from the well-established population-based birth cohort Generation XXI is one of the main strengths of this study. Generation XXI' allowed us to establish a potential causal relationship between the exposure, ACEs and bullying victimization during childhood, and the outcome, hs-CRP levels at the age of 10 years. Also, by using data on ACEs and bullying victimization collected at the age of 10 years, allow us to overcome one of the main limitations of many papers published exploring the association between exposure to traumatic experiences and adult inflammation, that are highly dependent on retrospectively-collected data, and thus limited by recall bias. Recent studies have described discordance between prospective and retrospective reporting of ACEs (59, 60). We explored exposure to ACEs and how the child felt when the exposure occurred, involvement in bullying as a victim and the frequency by which the victimization occurred during the lifetime, all reported by the child. Also, we use data from solely victims, excluding participants that may have been involved in bullying also as aggressors. This decision is supported by data reporting that being bullied predicted greater increases in CRP levels, whereas bullying others predicted lower increases in CRP compared with those uninvolved in bullying (30). As exposures are collected close to the occurrence, children can describe their own experiences in a safe and protective environment. Children exposed to ACES are more frequently from less socioeconomic advantaged families (61), and, for that reason may be more exposed to environmental and physical risk factors, and consequently being more susceptible to infections (62). Trying to minimize the effect of acute infections, we excluded participants with hs-CRP levels higher than 10 mg/L (42) from the analyses.

Nevertheless, this study also has potential limitations. This study only comprised one measure of inflammation, widely used as a marker of systemic inflammation, and in several population studies. CRP levels have been successful in establishing an association between exposure to adverse events with prolonged low-grade activation of the immune system and consequently higher inflammatory levels (18, 26). Moreover, the measure of inflammation was performed when children answered the questions on ACEs and bullying victimization. The cross-sectional nature of the analyses follows the theoretical claim of temporal precedence of adversity (36, 63), and thus claims and does not limit conclusions.

However, despite these limitations, our results show a relevant role of exposure to traumatic experiences into shaping inflammatory processes during childhood.

Conclusion

This study suggests that there might be different mechanisms involved in the biological embedding of childhood experiences. On one hand, the impact of exposure to ACEs on hs-CRP levels seems to be less mediated by BMI, and on the other hand, BMI seems to mediate most of the association between exposure to bullying victimization and hs-CRP levels at the age of 10 years.

Even though further research is still needed to better understand the mechanisms explaining the emergence and persistence of health poorer outcomes later in life for victims of abuse, efforts focusing on preventing, identifying and stopping ACEs exposure and bullying victimization should be in place, to protect children of becoming the target and to provide them with a better start for better health.

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Table 1. Child's, family and parental characteristics according to children's CRP levels and bullying victimization (3738)

	Total, n (%)
Child's characteristics	
Sex	
Female	1785 (47.8)
Male	1953 (52.2)
Age (years)	
Mean (SD)	10.1 (0.33)
Weight (Kg)	
Mean (SD)	37.7 (8.74)
Height (m)	
Mean (SD)	1.41 (6.55)
BMI	
Underweight/ Normal	2145 (57.4)
Overweight/ Obese	1593 (42.6)
CRP	
Median (P25-75)	0.79 (0.58-1.09)
Exposure to adverse childhood experiences	
Bullying victimization	
No	2737 (73.2)
Yes	1001 (26.8)
ACEs	
0-5	2612 (69.9)
6 or more	1126 (30.1)

BMI z-score, age and sex-specific BMI standard deviation scores according to the World Health Organization (WHO, 2006)

Table 2. Pearson correlations between all the variables included in the study.

Variables	Age	Sex	ACEs	BMI z-score	Log CRP
Age	1		-	-	-
Sex	-0.03	1			
ACEs	0.03	0.07*	1	-	-
BMI z-score	-0.01	0	0.04	1	-
Log CRP	-0.02	-0.09*	0.04	0.40*	1

*p<0.05; ACEs: Adverse childhood experiences; BMI: body mass index; CRP: C-Reactive Protein

Variables	Age	Sex	Bullying victimization	BMI z-score	Log CRP
Age	1		-	-	-
Sex	-0.02	1			
Bullying victimization	-0.03	0.08*	1	-	-
BMI z-score	-0.01	0.01	0.07*	1	-
Log CRP	-0.02	-0.09*	0.04*	0.4*	1

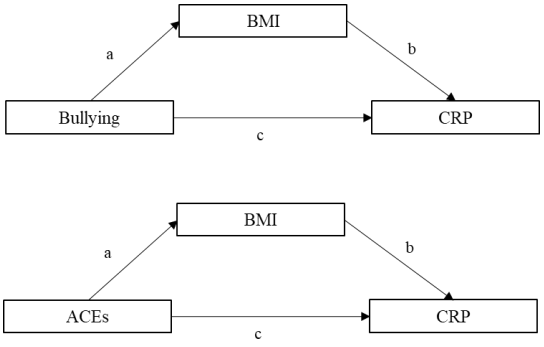
*p<0.05; ACEs: Adverse childhood experiences; BMI: body mass index; CRP: C-Reactive Protein

Table 3. Total, direct and indirect effects derived from the path analysis model for the association between ACEs, bullying and BMI and CRP

	CRP β(95%CI)	% of the total effect
ACEs[1]		
Total effect on CRP	0.14 (0; 0.30)	
Direct effect on CRP	0.09 (-0.05; 0.23)	64.3
Indirect effect through BMI	0.05 (0; 0.11)	35.7
Bullying[1]		
Total effect on CRP	0.20 (0.06; 0.34)	
Direct effect on CRP	0.08 (-0.05; 0.21)	40.0
Indirect effect through BMI	0.12 (0.06; 0.18)	60.0

[1]adjusted for sex; β(95%CI): Beta and corresponding 95% confidence interval;
ACEs: Adverse childhood experiences; BMI: body mass index; CRP: C-Reactive Protein

Figure 1. Conceptual framework of the mediation models for the present study. Indirect effect = ab, direct effect = c, total effect = ab + c



5. OVERALL DISCUSSION

This thesis shows that children growing up in socially disadvantaged circumstances and that are exposed to ACEs will be in a worse milieu of health and development and may consequently be in a trajectory of disadvantageous health into the life-course (20).

Literature, although scarce, shows that social adversity is already shaped on biological mechanisms (2, 16, 17), thus having biological consequences and alterations in biomarkers at early ages. These results highlight the complex process of embodiment, clustering and cumulative nature of disadvantage and inequalities in early life.

According to the biology of social adversity theory, the exposure to social adversity is translated into changes in biological markers that might be precursors of disease later in life, and those changes may be tracked over the life-course, already since very early ages. Our results show that children experiencing social adversity, either economic or through exposure to ACEs, namely violence, might be set in worst health trajectories when compared to those non-exposed to social adversity. Additionally, the Life-course Health Development model suggests that numerous biological, psychological and cultural factors interact simultaneously in a transactional manner to influence an individual's life-course during each stage to determine a "health developmental" trajectory on multiple levels (155). These experiences are thought to become biologically embedded during sensitive periods of development, when exposure coincides with the period of greatest maturation or plasticity of most of the organs and biological mechanisms (156, 157), setting children on a trajectory of increased risk for the development of chronic diseases in adulthood (3, 11, 158).

Data from Generation XXI birth cohort allowed us to observe that disadvantaged socioeconomic circumstances in early life are associated with poor cardiometabolic health and increased levels of inflammation throughout childhood. However, we cannot support that the differences found in the first ten years of life will be maintained during adolescence and adulthood. Also, such changes do not always lead to disease but the underlying atherosclerotic process has a long asymptomatic phase of development that often starts during early childhood (67-70), tracks over time, and can predict the onset of chronic disease several years later (70). Even though we cannot discuss which are the clinical implications of our findings, we observe a potential trajectory of health disadvantage in early life in children exposed to less advantaged circumstances. Thus, from a public health viewpoint we should not ignore that even though diseases in the adult life are not programmed, the predisposition towards a disease might be programmed early in life (159).

Socioeconomic circumstances appear to be strong determinants of an individual's maximum attained health (160, 161). Although some of the views on the long-term consequences of early life may be very deterministic, a better understanding of sensitive periods may explain mechanisms that contribute to the onset of health inequalities (162). The identification of such sensitive periods, and determining when socioeconomic circumstances matter the most, as well as when their health consequences start, are still under discussion (163). In fact, our results show that at very early ages there are alterations in both cardiometabolic and inflammatory markers after exposure to social adversity, while other studies found no association between childhood socioeconomic position and inflammation, after controlling for adulthood socioeconomic position (164). The same study showed that inflammation was associated with the socio-economic position in all stages of the life-course, not just with childhood socioeconomic position, being more aligned with the "chain of risk" hypothesis. This hypothesis suggests that the exposure to a low socioeconomic position in early life is important since more frequently leads to lower adulthood socioeconomic position (164) and the establishment of health risk behaviours (165). However, and in line with others, our results seem to provide evidence of the existence of sensitive periods of exposure to early adversity that may be imprinted biologically for a long time (163, 166, 167). But another hypothesis that must not be overlooked is the existence of a cumulative risk pathway, indicating that exposure to low socioeconomic circumstances at different stages of life is closely associated with exposure to other ACEs, accumulate to promote the adoption of risky health behaviours, and consequently, the increased inflammatory state. Another study also described a decrease in the prevalence of cardiovascular and metabolic disease risk factors by mid-30s in children from low-income families who have received an educationally enhancing and risk-reducing intervention between the ages of 3 to 5 years. These results seem to indicate a potential for modifying risk-related health development trajectories when appropriately timed and targeted interventions are in place (168). Thus several targets should be taken into account for potential interventions to be effective, supporting previous work which identified that advantage and risks are embedded within families and communities (2).

Exposure to ACEs is of particular importance in life-course epidemiology since it allows to understand the production of health inequalities. Exposure to violence seems to be the most significant experience with serious impact on child health and development. In the case of physical abuse perpetrated by parents or other close adults (82) and victimization by corporal punishment during childhood, these are still socially accepted behaviours and the prevalence of corporal punishment found in Generation XXI is high, even though these practices are fully prohibited by the Portuguese law since 2007 (169). Parental beliefs as well as the cultural

acceptance that corporal punishment is a way to discipline and educate children, contribute to these forms of discipline not being yet abandoned. However, corporal punishment is responsible for thousands of deaths during childhood each year and regarding its survivors, it has been associated with health problems in childhood and later in life (83). In Generation XXI, we observed increased inflammation levels in children exposed to parental extreme physical violence, even though no increase in the levels of hs-CRP was found in children exposed to less severe violence. These results do not mean that less severe forms of violence have no impact on health, but we might not be able to see an immediate impact on hs-CRP (170). Nevertheless, many of these experiences may remain hidden since perpetrators have an interest in hindering reports and detection. And none of the evidence on ACEs nor parental disciplinary practices should be used to incriminate parents, but rather to reveal the circumstances, particularly social conditions, in which parents and children live and how they cope (171).

Thus, having prospective data collected throughout childhood about exposure to ACEs is key to study these experiences and its consequences as the victims can describe their experience in a secure and protected environment. ACEs are evitable and preventable (77). As ACEs are responsible for engagement in health-risky behaviours, poor health outcomes and premature death, preventing them is crucial to address public health and social challenges, and consequently to improve the lives of children, families and communities. Within ACEs, violence is among the most prominent public health problem in the world (172, 173). Besides being one of the leading causes of mortality, especially among children and young adults, the ones that are non-fatal injuries will result in life-long disabilities and health consequences (172, 174). Growing up in a context of violence mostly perpetrated by the ones that should be the main protectors of the child by providing them with a healthy and a safe environment may trigger a cascade of psychosocial vulnerabilities, including deficits in social competence and emotion regulation (175), and a propensity to compensate for it with health-compromising behaviours over the life-course (176, 177). Involvement in bullying is also an adverse experience associated with negative mental health effects, such as feelings of sadness, loneliness, and isolation, and consequences for the physical health (sleep disturbance, heart disease, eating disorders), being a risk factor for youth suicidality, that can last into adulthood (72). According to data from the 2018 National Survey of Children's Health in the United States of America, in 2017-2018, one in three children under the age of 18 years reported to have suffered at least one ACE in their lifetime and 14% experienced two or more ACEs. And the most prevalent ACEs reported was having parents or caregivers divorced or separated (23.4%), living with anyone with alcohol and/or drug problem (8.0%), and

living with a parent or caregiver that served time in jail (7.4%) (178), all of these previously related with health outcomes in adult life (179-181).

So, and according to the CDC, five strategies can be put in place within communities to prevent ACEs: strengthening economic and financial support to families; changing social norms to support parents and positive parenting; providing quality care and education in early life; enhancing parenting skills to promote healthy child development; intervening to reduce harms and avoid future risk (182). The American Academy of Pediatrics also suggested the following strategies: integration of behavioural healthcare into the household with children, offer of support to parents, availability of peer-based education and identification of community resources to help boost resilience and moderate the effects of adversity (107). A resilient child will have good mental and physical health despite exposure to early adversity, and thus, will be better prepared to withstand, adapt to, and recover from adversities (152). Resilience acts as a buffer at the time of adversity by reducing its impact, their negative chain reactions, and supporting opportunities for recovery (183). Also, several interventions have shown results in enhancing resilience in children exposed to adversity (184, 185). And randomized controlled and matched-group trials have achieved between 48 to 52% in reduction of child abuse and neglect rates, associated with preschool improvement and early childhood home visitation programs (77). Interventions should be designed towards reducing social inequalities and must tackle macroenvironmental factors (income and education), physical and social environment, risky-health behaviours and access to health care (186).

Nowadays we must pay attention to the expansion of the definition of ACEs and that might include measures of parental separation, parental education, parental unemployment and child poverty. This extension in the type of adversity, as defended by some authors, might create some confusion since the family structure and socioeconomic conditions can be associated with other risk factors for poor health (187). However, efforts to improve health outcomes should always focus on reducing modifiable situations, that consistently include socioeconomic circumstances and ACES, or their early identification, that consequently will prevent or at least decrease health inequalities and its effects, since early ages.

It is currently debated as to whether poverty itself should be considered an “adverse childhood experience”. This debate is very relevant to a better discussion regarding the relationship between socioeconomic circumstances and adverse childhood experiences in the context of causal pathways between health-related exposures and outcomes. Childhood socioeconomic circumstances are proposed by some authors as one adverse experience (188) but it is dismissed

by others as conceptually muddled and potentially resulting in the importance of key socioeconomic determinants of health being overlooked (187). A systematic review demonstrated a clear relationship between socioeconomic conditions in childhood and ACEs/maltreatment, suggesting that low childhood socioeconomic circumstances is a determinant of such adversity, and the longitudinal nature of many studies supports a causal association (189). Actually, this fits with the strong international evidence of the "fundamental causes" of health inequalities being socioeconomic (190), including the evidence of the importance of childhood socioeconomic conditions in explaining variation in outcomes across the life-course (7, 32, 191). Whereas other studies have shown that the relationship between ACEs and health outcomes persists even after adjustment for measures of socioeconomic circumstances (192). This suggests either residual confounding or that the relationship between socioeconomic circumstances and ACES is much more complex and requires further research to fully unpick. A previous study showed that children from low-income families were more likely to report ACES, however, when children from high-income families experience ACEs, a greater effect of ACEs is observed on their health, suggesting that higher income does not act as a protective factor against its potential harmful effect (193).

There are at least two known pathways by which social adversity seem to impact inflammatory processes. On one hand, via stress sensitization, by activation of the HPA axis (194), that leads to altered insulin sensitivity, increased blood pressure and inflated central adiposity, and consequently to elevated inflammation (11, 194). On the other hand, the adoption of harmful health habits, such as sedentary lifestyles, poor diet and smoking (195), that might be mediating the association between social adversity and later disease development. In adult studies, health-related behaviours, such as smoking or sedentary behaviour, may contribute to explain social differences in inflammation, with those from less advantaged socio-economic circumstances being more prone to engage in more unhealthy risk behaviours (122, 123). Even though we do not expect the same contribution of health-related behaviours in children, as they may not be fully established at these ages, they also seem to not fully explain the association between social adversity and biological markers. However, the early development of obesity favours the onset of several metabolic dysfunctions in childhood and adolescence (196), increases inflammation (197, 198) and is a result of poor diet and physical inactivity (199, 200). It is known that sedentary and dietary behaviours are socioeconomically patterned, i.e., those in lower socioeconomic position have lower consumption of fruits and vegetables, and more sedentary activities (195, 201). Thus, we used five-a-day fruit and vegetable intake and sedentary activity, as proxies of healthy choices. Additionally, as the link between exposure to social adversity and health

outcomes later in life might be explained through either the adoption of health-risk behaviours or via direct physiological changes resulting from disruption of regulatory pathways, we believe that by using these variables we excluded to a certain extent, the effect of behaviours already potentially established at early ages. Nevertheless, we are aware of the effect that BMI might have on the cardiometabolic and inflammatory indicators, especially at the age of ten years. This may be due to the fact that some of these children are already in or at the onset of puberty, which affects their BMI and consequently their health (202). Also, it is important to note that children are a result of the environment in which they are inserted, the communities in which they are living and the families in which they are growing. That said, they cannot change and are dependent and rely on the adults responsible by them, at a governmental level, local authorities, school officials and family and parents to make the best choices possible, for them to thrive, prosper and succeed as healthy and devoted adults that will have a valuable contribution to the society.

Therefore, according to the pathways that explain the biological embodiment of adversity, our results show that we must have an immediate effect of adversity on the children's biology. However, from what we know from literature, if the child keeps on the path of adversity, they will acquire risk-health behaviours, and thus maintain a trajectory of adversity and a cumulative effect of behaviours, which in turn will most likely increase the gap in health inequalities throughout life.

Strengths and limitations

One of the key strengths of this thesis is the use of data from the Portuguese population-based birth cohort Generation XXI. With these data, we could observe and analyse different stages of children's inflammatory and cardiometabolic profile, and to establish a potential causal relationship between the exposure, social adversity, and the outcome, biological consequences in the first ten years of life. However, as it is common in a prospective birth cohort, there has been attrition over time, leading to a reduction in the sample size and a more socioeconomically advantaged group of participants at older ages. Nevertheless, we believe that the inclusion of the more disadvantaged group would have widened the differences observed.

Additionally, information on socioeconomic indicators was collected at childbirth, thus decreasing the risk of recall bias, and the collection of data about both the mother and the father allowed us to have a more comprehensive assessment of family early socioeconomic circumstances. However, different socioeconomic measures might be capturing different effects on a child's care

and consequently on a child's health (203). Knowledge and skills attained through formal education may affect a person's cognitive functioning (203) with maternal education, in particular, is associated with child health outcomes as it seems to contribute through factors more closely associated with mothers' literacy, thus reflecting on children's care and choices (204). Paternal occupation, another indicator commonly used to measure early socioeconomic circumstances, on the other hand, might be more related to financial availability and material assets (203, 204). In fact, some studies have reported that paternal occupation has a significant direct impact on an individual´s health and that the effect of the father's occupation exceeds that of the mother's (205). As self-reported measures, both education and occupation are reliable sources of information about families' socioeconomic circumstances, since they are easy to measure (203). Income, however, has the potential for being underestimated (206). Also, literature refers that the differences between reported income and tax reported income is bigger in the highest income participants, and therefore if there is any bias, it would be in the direction of increasing the inequalities (207). Moreover, as income is categorized into three categories, we believe that this potential bias on income reporting would not affect our results. Our findings show an association between parental socioeconomic circumstances and children's health. Thus, it is expected that all socioeconomic indicators influence the child's health together and help distinguishing social differences in children at these ages. Even though little is known about the moment when childhood socioeconomic circumstances matter the most or for how long they need to last, these results evidenced the effect of family socioeconomic circumstances at childbirth on different health indicators in the first years of life. Thus, very early exposures carry the potential to have a strong impact on adult health.

Another strength of this work is the prospective measure of adversity, namely parental physical violence, bullying and ACEs reported by the child. As the exposure is collected close to the occurrence, children can describe their own experience in a safe environment. Moreover, recent studies have described discordance between prospective and retrospective reporting of ACEs (208, 209). Whereas some ACEs are relatively common in Generation XXI, like experiencing the death of a family member or someone in the household shouting, yelling or screaming to the child, others are less common (e.g., someone in the household abusing alcohol or being a drug addict or being or having been in jail) and different exposures might have a different meaning and effect in the life of a child (193). Also, variation in severity and duration of stressors and in methods for assessing biological markers may help explain some of the differences found across studies. In this particular work, we explored exposure to ACEs and how the child felt when the exposure occurred, involvement in bullying as a victim and as an aggressor or both as a victim

and as an aggressor simultaneously, exposure to violent disciplinary tactics and the frequency by which the victimization occurred during the lifetime, all reported by the child.

In the analyses, it was not considered the family history of cardiovascular disease, even though we are aware of some genetic predisposition in some of the children. Nevertheless, we expect that at the population level, in a "healthy-apparent" sample, this effect should be minimal. Also, African Americans and South-East Asians populations appear to be disproportionately affected by cardiovascular risk factors, and this higher incidence is likely to represent the complex interactions from several innate and environmental factors (210). As our sample was exclusively Caucasian, there is no ethnic variability to account for. Thus, ethnicity is not to consider in the observed inequalities, and the associations we observed are mainly due to exposure to social adversity.

In regards to the studies on CRP, and trying to minimize the effect of acute infections, we excluded participants with hs-CRP levels higher than 10 mg/L (211) from the analysis. Even though we acknowledge that some discussion may arise, the proposals to overcome this issue are not suitable for our sample (212) and the number of children excluded by these criteria in each study was low, thus not affecting the results found. Moreover, this study only comprised one measure of inflammation. However, several population studies, which also used CRP levels, were successful in establishing an association between exposure to social adversity and prolonged low-grade activation of the immune system and consequently higher inflammatory levels (213, 214). Other studies showed an inverse association of basal circulating levels of interleukin (IL)-6 with indicators of parental SES during the first two years of life, but not later in childhood. These associations were independent of adult SES, suggesting that SES in early childhood have a unique role in adult inflammation (215). Also, in general, behavioural and psychosocial health risk factors, such as smoking, lower physical activity, poorer sleep quality, lower self-compassion, and loneliness are associated with larger increases in circulating IL-6 (216). Although data on IL-6 could possibly reinforce our results, we only have information on this biomarker for a subsample of participants at the age of ten years. Additionally, since we do not expect behavioural risks to be already present during the first years of life, we would not anticipate a perceptible impact on this biomarker.

Although we have tested some confounding variables, it is not possible to discard some potential residual confounding. But, so far, the literature shows that early stressful experiences "get under the skin" and seem to have a negative impact on later health (11, 12). Thus, our results, in line with previous studies (7, 217), show that exposure to social adversity might be incorporated and

its potentially negative effects are already expressed during the first years of life, even though this does not necessarily mean an increased risk of later health outcomes or being deterministic of development of disease in the adult life. Therefore, and by using data from a prospective ongoing birth-cohort, in future studies, we will be able to assess if the social and health inequalities found at these ages will be maintained, increase or even decrease throughout life.

6. CONCLUSIONS

This investigation aimed to approach the biological consequences of social adversity in childhood, focusing in the exposure to poor socioeconomic circumstances, exposure to ACEs, parental physical violence and bullying, and its impact on cardiometabolic and inflammatory markers.

Two studies were conducted to evaluate the effect of socioeconomic circumstances at birth on cardiometabolic and inflammatory indicators. We observed that at birth exposures already impact the health trajectories of children since early ages. Even though we cannot infer about long-term effects, we can hypothesize that children born in disadvantaged circumstances will grow and develop in worse health than those from more advantaged circumstances.

A systematic review was conducted to identify published literature on the immediate biological consequences of exposure to ACEs. This study allowed us to identify the most frequently studied type of adversity, and the most commonly studied biological consequences. Then, using data from Generation XXI, we were able to identify that exposure to parental extreme physical violence was biologically imprinted, and children presented higher levels of hs-CRP as early as at the age of seven years. Furthermore, and grounded in the hypothesis that BMI can be the biomarker that partially mediates the association between adversity and inflammatory markers, we conducted a mediation analysis. With this study, we observed that there might be different pathways involved in the biological embedding of childhood experiences. On one hand, the impact of exposure to ACEs on hs-CRP seems to be by the effect of these experiences on stress mechanisms, causing the HPA axis activation, and consequently low-grade inflammation. On the other hand, BMI seems to mediate a great part of the association between exposure to bullying victimization and hs-CRP levels at the age of ten years.

To sum up, the results of this thesis provided evidence that the biological consequences of social adversity can be observed already in the first ten years of life, and as early as at the age of four years. Specifically, our results showed that there is a potential impact of early life social adversity on physiology and metabolic dysregulation, supporting a detrimental effect of disadvantaged early life circumstances with origin in childhood. The findings of this thesis carry important implications for scientific knowledge, policy, and practice:

(i) social adversity, namely at the household level, including socioeconomic circumstances and exposure to ACEs, appear to influence children's health;

(ii) this influence extends across a wide range of health outcomes in early life, particularly biological markers of inflammation and childhood cardiometabolic health;

(iii) it spans the entire continuum of early life, at least until the age of ten years.

Thus, it is possible that early stressful experiences *"get under the skin"* already at early ages. The embodiment of adversity during childhood might be putting children in a trajectory of worse health for a longer period and thus making even more difficult to revert these biological alterations. As they enter adolescence they will be at increased risk of starting smoking and alcohol consumption. However, identifying these children can also be a window of opportunity to intervene.

Our findings emphasize the importance of investments in social policies and families' support to provide children with a better start for a better health. "Upstream" factors identified during childhood seem to represent meaningful opportunities to prevent adversity and improve health, since they are modifiable risk factors that should be targeted in local and global public health interventions to attenuate or reduce inequalities and its effects in health, starting at early ages. That way, society will be providing children with better health and wellbeing, since it is already recognized as a priority and a human right.

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