

# **under the skin**

**Early-life stress exposure and the co-occurrence of mental and physical health problems**

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# PREFACE

## Note

This is a work in progress. Stay tuned :)

Dear reader,

Let me begin this book the only way that feels right: with a little note of honesty. Writing this was hard, much harder than I anticipated. Perhaps out of pride, or because of this irresistible greed I feel to dissect and point out limitations - that, I am told, makes for a good scientist as much as a horrible friend - I kept wondering whether any of this would be worth your time.

I guess 5-years-ago me hoped, at this point, they could promise you sensational scientific breakthroughs and game-changing statistical tricks ahead. Well, it looks like we may both have to settle for a somewhat less glamourous, but perhaps more truthful, process of scientific discovery.

So here is a collection of small steps toward understanding, many little increments of knowledge, a couple of false starts, a few frustratingly inconclusive results, and a handful of insights (some grim, some hopeful).

Still here? Didn't think so... let's talk about early-life stress, shall we?

*“In the depth of winter, I found there was, within me, an invincible summer.”*  
Albert Camus – Summer (1954)

# 1 General Introduction

## 1.1 Early-life stress (ELS)

When my mother was pregnant with me, my older brother died of a congenital condition. I never met the little guy, never experienced any grief myself. Yet, for the longest time I thought that, somehow, his death had cast a sort of shadow over the rest of my life. And I was not the only one. The idea that exposure to stressful experiences during “*critical*” periods of early development wields profound, long-term impact on emotional and physiological regulation, is nothing new. Sigmund Freud, among others, made quite a sensational career out of it, and - perhaps partly because of the success of psychoanalytic theories - the scientific literature on the topic today is almost intimidatingly vast.

So vast in fact, that it requires a pinch of terminology clarification, before we can dive into it. For the remainder of this book, I will use the term “Early-Life Stress” (ELS) to refer to a host of adverse life events or conditions, that are experienced during the first years of life, e.g., in childhood, and, even earlier, during gestation. These experiences (sometimes also referred to as “childhood trauma”, “maltreatment”, “early adversity”, or “adverse childhood experiences”) encompass a wide range of stressors, including neglect and abuse, bereavement, bullying but also poverty, material deprivation and/or parental psychopathology (Heim, 2020).

ELS is a widely studied concept, partly because it is surprisingly common: more than half the population experiences at least one form of ELS before the age of 18 years (Madigan et al., 2024). But more importantly, ELS is one of strongest, most consistent predictors of mental health problems later in life, particularly depression (LeMoult et al., 2020; Li et al., 2016; Li et al., 2023).

Systematic scientific investigations into ELS and its consequences, really gained momentum in the late 90s, when large-scale epidemiological studies, began to also highlight a relationship between ELS and an increased risk of chronic diseases and premature mortality (e.g. (Felitti et al., 1998)). This line of evidence also proved very robust over time, so ELS is now a well-recognised risk factor for cardio-metabolic health problems, including obesity (Danese & Tan, 2014; Wiss & Brewerton, 2020), type 2 diabetes (Zhu et al., 2022), hypertension and cardio-vascular disease (Jacquet-Smailovic et al., 2022; Jakubowski et al., 2018).

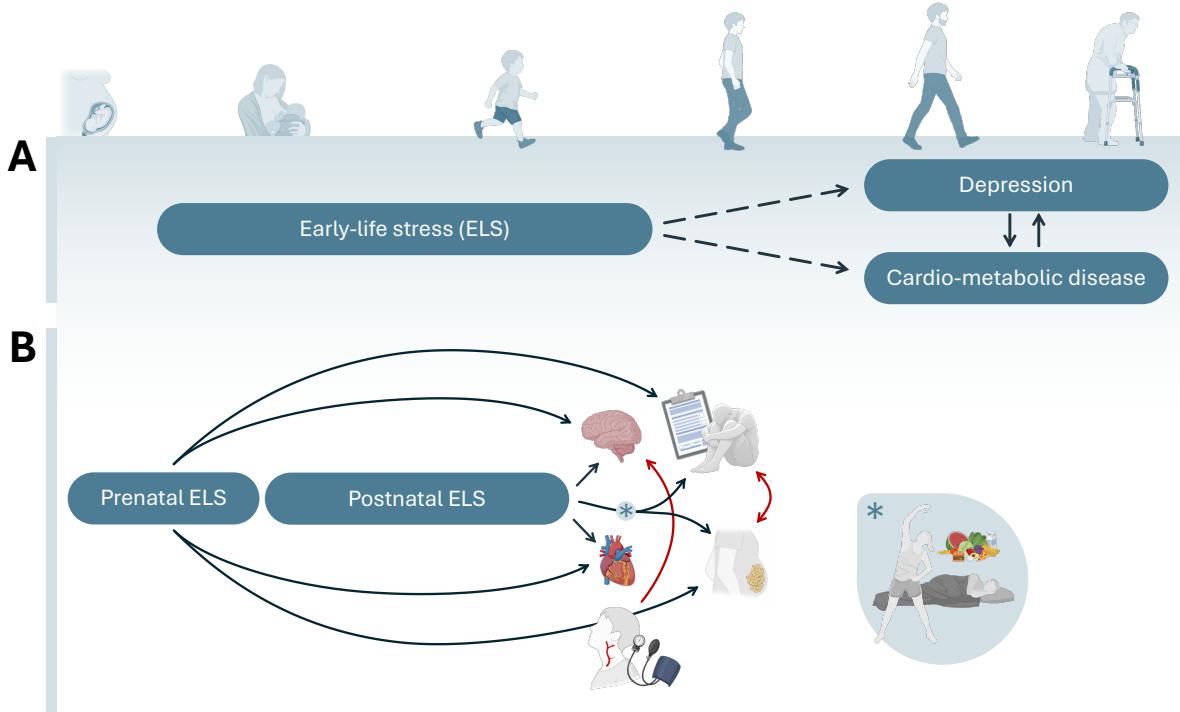
## **1.2 Mental & physical health: a comorbidity paradigm**

Interestingly, parallel to this growing interest in the effects of stress and psychosocial factors on physical health, the early 2000s also witnessed a surge of epidemiological studies uncovering the relationship between depression and cardio-metabolic health problems(Luppino et al., 2010; Penninx et al., 2001; Van der Kooy et al., 2007). Historically, these two conditions had been largely investigated as separate entities, each with distinct risk factors and treatment approaches. Their connection was not entirely overlooked (see for example early theoretical work by Engel (1977)), but it remained peripheral to mainstream medical research until relatively recently.

As more evidence accumulated that depression and cardio-metabolic conditions tend to co-occur (Anwar et al., 2018; Blasco et al., 2020; Gutiérrez-Rojas et al., 2020), several potential mechanisms have been proposed to explain this observed comorbidity (Milaneschi et al., 2019). One possibility is that these conditions share common risk factors, such as genetic liability for instance, but also ELS exposure, as discussed above and supported by a recent large scale meta-analysis (Souama et al., 2023). It is also possible that experiencing depression may directly increase the risk of developing cardio-metabolic health problems later in life. For example, in two meta-analyses based on longitudinal data, depression was found to be risk factor for obesity and diabetes (Ditmars et al., 2022; Mannan et al., 2016). Other longitudinal meta-analyses however, have also shown, in turn, that poor cardio-metabolic health was a robust predictor of later depression (Zhou et al., 2024), supporting the hypothesis of a bi-directional relationship.

## **1.3 ELS and psycho-physical health: towards an integrated approach**

So far, we have described a triangle of relationships - i.e., between *a*) ELS and mental health, *b*) ELS and cardio-metabolic health, and *c*) mental and cardio-metabolic health (Figure 1.1 A), which received considerable attention in the medical literature. This isn't surprising. Depression and cardio-metabolic conditions are among the leading causes of death and disability worldwide, shaping an enormous public health burden which is largely preventable (Anwar et al., 2018). ELS is a promising shared risk factor, which can be measured years - and even decades - before clinical symptoms emerge, making it an ideal candidate for informing early detection and improving the integrated prevention of psycho-physical health problems.



**Figure 1.1: Schematic representation of this thesis, in the context of previous literature**

(A) A schematic summary of the existing evidence, on the relationships under study. This is largely based on adult outcomes and retrospectively measured ELS exposure (indicated by the dashed line).

(B) A Schematic summary of the relationships examined in this thesis.

However, there are three key challenges that need to be addressed, before these findings could be effectively translated into clinical practice.

Firstly, as briefly noted above, the literature is very heterogeneous in its definition of ELS, with many studies focusing on specific types of adversity (e.g., childhood neglect and abuse). However, there is convincing evidence that exposure to multiple stressors - i.e., different sources of stress, as well as repeated or continued exposure over time - has much worse consequences for psycho-physical health, compared to individual stressors (Evans et al., 2013; Hughes et al., 2017). This underscores the importance of identifying children who face multiple forms of ELS, as they may represent a particularly vulnerable population, who should be prioritized in intervention programs.

Secondly, existing studies typically only address one “side” of this triad of relationships. For instance, while ELS has been linked to both depression and obesity independently, its role in determining their co-occurrence remains heavily understudied. Consequently, it is hard to establish the relative importance of ELS as a risk factor for each disorder in isolation

vs. their comorbidity, which poses an obstacle to the development of integrated prevention and intervention (Anwar et al., 2018).

Lastly, the overwhelming majority of studies investigating either the role of ELS, or the(bidirectional) relationships between mental and physical health, has been conducted in adult or aging populations.

On one hand, this brings about important measurement issues. Namely, the proposed relationship between ELS and adult psycho-physical health relies heavily on retrospective reports of ELS exposure, which may have introduced recall bias, unrealistically inflating the association estimates reported by the literature (Reuben et al., 2016).

On the other hand, both depression and cardio-metabolic health problems very often find their onset well before adulthood. A growing number of adolescents, for instance, experience depressive symptoms before the age of 20 years (Keeley, 2021; Patalay & Gage, 2019). Concurrently, while cardio-metabolic endpoints such as myocardial infarction and diabetes are traditionally associated with later life stages, their prodromal forms, including hypertension, dyslipidemia, and obesity, manifest more and more frequently between childhood and adolescence (NCD-RisC, 2017; WHO, 2022). Intervening during this early developmental window could be crucial to mitigate the progression of such prodromal psycho-physical symptoms into chronic mental and physical health conditions later in life.

In this context, prospective cohort studies that begin at conception and capture a broader spectrum of ELS exposures and psycho-physical symptoms, are essential to map causal pathways and inform on optimal intervention windows.

## 1.4 This thesis: one step back in time

This thesis (Figure 1.1 B) aims to address some of these limitations and open questions.

**Part 1** will focus on characterizing the prospective relationship between ELS and adolescent psycho-physical health. First, in Chapter 2, I quantify the relationship between ELS exposure during two key developmental periods (i.e. pregnancy and childhood) and adolescent *a)* internalizing symptoms, *b)* adiposity, and *c)* their comorbidity. Chapter 3 further explores whether three common lifestyle factors (i.e., physical activity, sleep duration, and diet quality) may moderate the associations identified in Chapter 2. I will then take a closer look at the potential “biological scars” that pre- and postnatal ELS exposure may leave behind on children’s brains (Chapter 4) and on their hearts (Chapter 5).

In **Part 2** I will then characterize the reciprocal relationship between mental and cardio-metabolic health across childhood and adolescence. Chapter 6 examines the longitudinal co-development of depressive symptoms and several markers of cardio-metabolic health from childhood to early adulthood. In Chapter 7, I assess the relationship between early cardiovascular health markers (i.e., arterial health and blood pressure) and the developing brain.

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# **PART 1: The role of ELS in the development of mental and physical health**

## 2 ELS and adolescent psycho-physical health

Adapted from:

**Differential effects of pre- and postnatal early-life stress on internalizing, adiposity and their comorbidity**

**Defina, S.**, Woofenden, T., Baltramaityte, V., Pariante, C. M., Lekadir, K., Jaddoe, V. W., Serdarevic, F., Tiemeier, H., Walton, E., Felix, J. F., & Cecil, C. A. M., \*on behalf of the EarlyCause Consortium. (2023). *JAACAP*. DOI [DOI](#)

### Abstract

Objective: Depression and obesity are two highly prevalent and often comorbid conditions. Exposure to early-life stress (ELS) has been associated with both depression and obesity in adulthood, as well as their preclinical manifestations during development. However, it remains unclear whether: *(i)* associations differ depending on the timing of stress exposure (prenatal vs postnatal) and *(ii)* ELS is a shared risk factor underlying the comorbidity between the two conditions.

Methods: Leveraging data from two large population-based birth cohorts (ALSPAC: n=8428 (52% male participants); Generation R: n=4268 (48% male participants)), we constructed comprehensive cumulative measures of prenatal (in utero) and postnatal (from birth to 10 years) ELS. At age 13.5 years we assessed: a) internalizing symptoms (using maternal reports); b) fat mass percentage (using dual-energy X-ray absorptiometry); c) their comorbidity, defined as the co-occurrence of high internalizing and high adiposity.

Results: Both prenatal (*total effect* [95%CI] = 0.20 [0.16; 0.22]) and postnatal stress ( $\beta$  [95%CI] = 0.22 [0.17; 0.25]) were associated with higher internalizing symptoms, with evidence of a more prominent role of postnatal stress. A weaker association (primarily driven by prenatal stress) was observed between stress and adiposity (prenatal: 0.07 [0.05; 0.09]; postnatal: 0.04 [0.01; 0.07]). Both pre- (OR [95%CI] = 1.70 [1.47; 1.97]) and postnatal stress (1.87 [1.61; 2.17]) were associated with an increased risk of developing comorbidity.

Conclusions: We found evidence of *(i)* timing and *(ii)* shared causal effects of ELS on psychocardiometabolic health in adolescence, but future research is warranted to clarify how these associations may unfold over time.

## **Links**

**Supplementary materials:** <https://osf.io/s7f9h/files/osfstorage>



**Project's code:** <https://github.com/SereDef/association-ELS-PCM-project>



**ELS score:** <https://github.com/SereDef/cumulative-ELS-score>



## **Keywords**

Early-life stress; Internalizing symptoms; Adiposity; Comorbidity; Generation R; ALSPAC.

## **Abbreviations**

Avon Longitudinal Study of Parents and Children (ALSPAC), Body Mass Index (BMI), Child Behavior Checklist (CBCL), Confidence Interval (CI), Dual-Energy X-ray Absorptiometry (DXA), Early-Life Stress (ELS), False Discovery Rate (FDR), Generation R Study (GenR), Natural Direct Effect (NDE), Natural Indirect Effect (NIE), Odds Ratio (OR), Strengths and Difficulties Questionnaire (SDQ), Total Effect (TE).

## 2.1 Introduction

The co-occurrence of depression and obesity is a rising public health concern, affecting increasingly younger populations (Sutaria et al., 2019). Individuals with obesity are ~30-40% more likely to develop depression compared to the general population (Pereira-Miranda et al., 2017). In turn, depression also increases the risk of developing obesity (Pratt & Brody, 2014) and related cardiometabolic disease (Hare et al., 2014). While the relationship between depression and adiposity is likely multifactorial and complex, the observed comorbidity between the two may be partially explained by shared environmental risk factors, such as exposure to stressful experiences early in life (Shonkoff et al., 2012).

Indeed, early-life stress (ELS) is a well-established risk factor for both adult depression (Li et al., 2016) and obesity (Danese & Tan, 2014). In children and adolescents, ELS exposure in utero and postnatally (e.g., adverse childhood experiences) have been separately linked to preclinical manifestations of depression, such as internalizing problems (Cecil et al., 2017; Van den Bergh et al., 2020), and several adiposity measures (Burgueño et al., 2020; Elsenburg et al., 2017).

Identifying critical exposure windows (i.e., prenatal vs postnatal) can provide important insights into the best timing for prevention and intervention programs, and shed light on the mechanisms through which stress may lead to disease (Hartman & Belsky, 2018). However, very few studies prospectively investigated the influence of both pre- and postnatal stress on these outcomes, and, because stress shows continuity over time, it is unclear whether *(a)* previously reported postnatal associations may partly reflect preceding prenatal exposures (i.e., prenatal ELS as confounder), and *(b)* observed prenatal associations may be partly mediated by postnatal ELS (i.e., postnatal ELS as mediator).

Further, existing studies have examined ELS associations with internalizing and adiposity either separately (Slopen et al., 2014) or as part of a broader “multisystemic” disease constructs (Juster et al., 2016). It remains unknown whether ELS represents a shared risk factor for comorbid emotional problems and adiposity. Establishing such association is important, since protocols for the integrated detection and management of these health conditions are lacking (Anwar et al., 2018), and differential patterns of ELS exposure may help identify subgroups of adolescents at higher risk for comorbidity.

To address these gaps, we leveraged longitudinal data from two population-based prospective birth cohorts to examine *(i)* how pre- and postnatal ELS (up to age 10 years) associate to internalizing symptoms and adiposity in early adolescence (i.e., at age 13 years), taking into account potential confounding and mediation effects; and *(ii)* whether ELS accounts for comorbidity between internalizing problems and excess adiposity, above its contribution to each health outcome individually. Based on previous findings, we expect that both pre- and postnatal ELS prospectively associate with internalizing symptoms and adiposity, as well as their comorbidity. No a priori hypotheses were specified regarding the relative importance of pre- vs postnatal ELS.

## 2.2 Methods

This manuscript follows STROBE guidelines (Elm et al., 2008).

### 2.2.1 Participants

Our sample was drawn from two population-based cohorts: the Generation R Study (GenR), including 9,778 pregnant women in Rotterdam (the Netherlands), who delivered their babies between April 2002 and January 2006 (Kooijman et al., 2016); and the Avon Longitudinal Study of Parents and Children (ALSPAC) involving 14,541 pregnant women in Avon (UK), with delivery dates between April 1991 and December 1992 (Boyd et al., 2013; Fraser et al., 2013). The [ALSPAC website](#) contains details of all the data that is available through a fully searchable data dictionary and variable search tool.

Response rates at the 13 years follow-up were 64% in GenR and 61% in ALSPAC. Participants with > 50% missing ELS variables in the pre- or postnatal period were excluded, as were all twins. In the case of non-twin siblings, only one was selected (see Figure S1, available online). The final sample included 4268 (GenR) and 8428 (ALSPAC) children.

### Ethical standards

Ethical approval was obtained from the medical ethical committee of Erasmus MC, University Medical Center Rotterdam and from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Written informed consent was obtained for all participants and both studies conform with the World Medical Association Declaration of Helsinki (2013).

### 2.2.2 Measures

#### Prenatal and postnatal ELS

The prenatal (i.e., maternal exposure during pregnancy) and postnatal (i.e. from birth to 10 years) cumulative ELS scores comprise information about five stress domains in line with previous literature (Cecil et al., 2014; Rijlaarsdam et al., 2016): life events (e.g., death of a parent), contextual risk (e.g., financial difficulties), parental risk (e.g., parental psychopathology), interpersonal risk (e.g., family conflicts) and direct victimization (only postnatally, e.g. maltreatment or bullying). Note that, consistent with other work using this measure (Schuurmans et al., 2022), we use the term “postnatal” (in contrast to “prenatal”) to encompass stressors experienced across childhood (i.e., until the age of 10 years), rather than immediately following birth. A detailed description of the ELS scores is provided in online [Supplement 1](#) (see also the score’s [GitHub repository](#) for further details and scripts). Briefly, ~100 stress items were selected from each cohort, dichotomized into no risk (=0) or risk (=1), and assigned to a

domain based on expert knowledge (see Figure 2.1 for an overview of included items). Within each domain, dichotomized risks were summed and divided by the number of items in the domain. Finally, domain scores were summed within periods to obtain prenatal and postnatal stress scores.

### **Internalizing symptoms**

Internalizing symptoms were measured at an average age of 13.5 years (range: 12.5-16.8 years) using the Child behavior checklist (CBCL 6-18) (Achenbach, 1999) in GenR and the Strengths and difficulties questionnaire (SDQ) (Goodman et al., 2000) in ALSPAC. Both instruments are well-validated parental reports of emotional and behavioral functioning referring to the past 6 months, and have been shown to be comparable (Goodman & Scott, 1999). The CBCL internalizing subscale consists of 32 items rated on a 3-point scale, e.g., “*my child feels worthless or inferior*”. The SDQ emotional problems subscale contains 5 items rated on a 3-point scale, e.g., “*often unhappy, down-hearted or tearful*”.

### **Adiposity (fat mass)**

Body composition was measured using a dual-energy X-ray absorptiometry (DXA) scanner at an average age of 13.5 years (range: 12.5-16.6 years). Technical details of these measurements are provided elsewhere (Boyd et al., 2013; Voortman et al., 2016). Fat mass percentage was calculated by dividing the total body fat mass (kg) by the weight (kg) and multiplying by 100. To explore the importance of body fat distribution, measurements of android fat mass were also extracted from DXA scans.

### **Comorbidity**

To compute psycho-cardiometabolic comorbidity, internalizing symptoms and fat mass percentage were first dichotomized into high versus low-moderate, based on a cohort-specific 80th percentile cut-off value. The dichotomized values were then used to assign children to four groups: “healthy” (both outcomes <80th percentile); “high internalizing” (internalizing >80th & fat mass percentage 80th); “high adiposity” (internalizing 80th & fat mass percentage >80th); and “comorbid” (both outcomes >80th percentile). For additional information see Table 2.1 and online [Supplement 2](#).

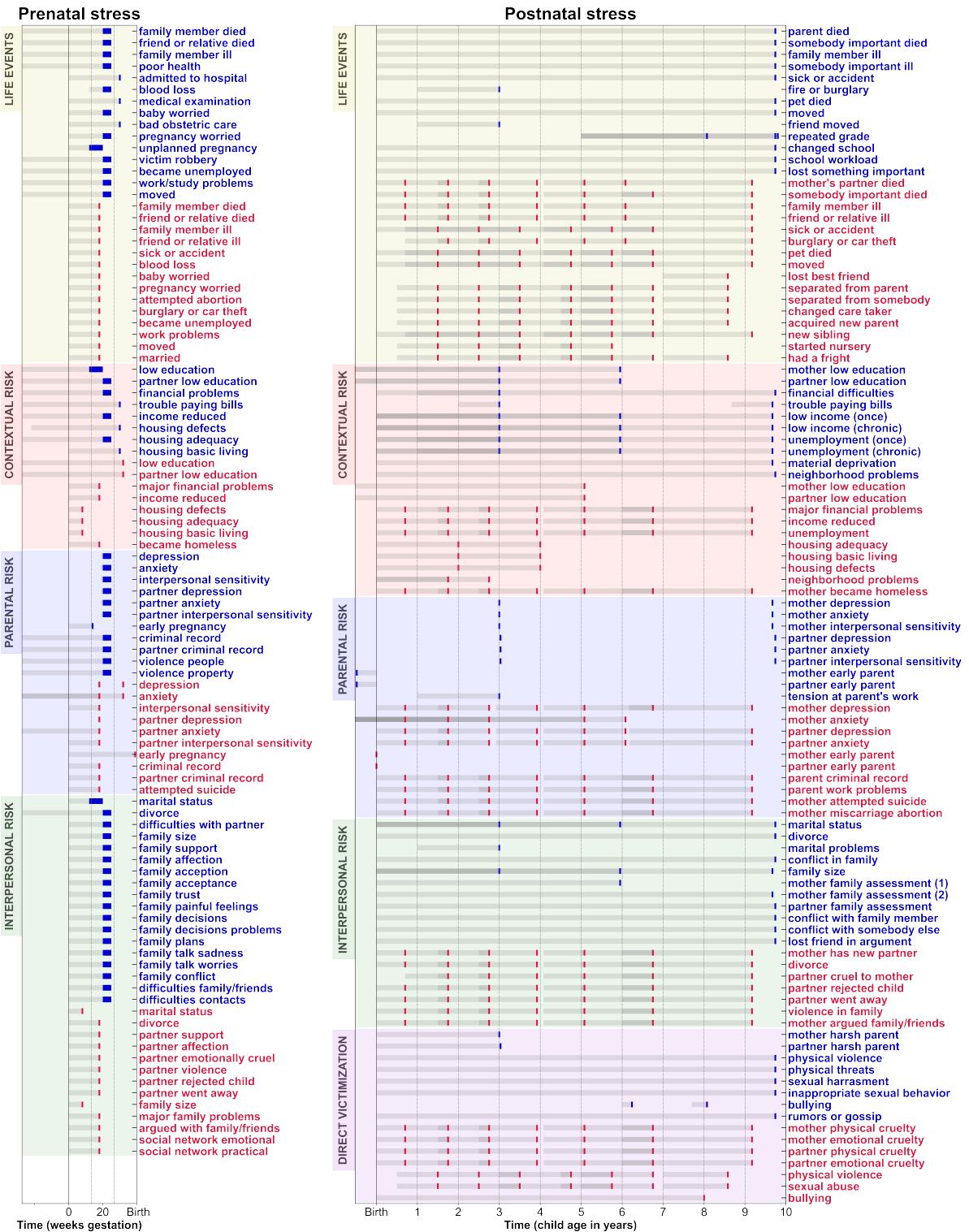


Figure 2.1: Temporal structure of the prenatal and postnatal early-life stress (ELS) score

The two graphs summarize the complex temporal structure of the prenatal (on the left) and postnatal (on the right) ELS scores. Time is depicted on the x-axis on the scale of weeks (gestation) for prenatal and years for postnatal items. For each question/item on the y-axis, a coloured dot represents the timepoint(s) at which the item was measured, and a grey shadow indicates the time period the question refers to (e.g., "since pregnancy" or "in the last year"). Red dots and labels refer to ALSPAC items and blue dots/labels refer to Generation R items. Items are grouped by domain, as indicated by the background color (yellow for life events, red for contextual risk, blue for parental risk, green for interpersonal risk and purple for direct victimization). The solid black vertical line indicates the beginning of the exposure period of interest, i.e., conception (or start of pregnancy) for prenatal items and birth for postnatal items. The dashed grey lines additionally provide temporal markers, i.e., trimesters in the prenatal period and a span of 1 year in the postnatal period.

## Covariates

During pregnancy, mothers reported on their smoking status, alcohol consumption, and pre-pregnancy body mass index (BMI). Information about child sex and date of birth was extracted from registries. Ethnic background (only available for GenR children) was determined by questionnaire-based assessment of the country of origin of participants' parents. Following Statistics Netherlands' guidelines (Alders, 2001), if one of the parents was born abroad, the child's ethnicity was determined according to that parent. If both parents were born abroad, the child was classified according to the mother's birthplace. Six large national groups were identified (i.e., Cape Verdean, Dutch, Dutch Antillean, Moroccan, Surinamese, and Turkish). Smaller national groups were aggregated into five additional categories: "Africa and Middle East", "Asia and Oceania", "Europe", "Latin America", and "North America" ([Figure S5](#)). See Table 2.1 and online [Supplement 3](#) for additional information on covariates measurement and distribution.

### 2.2.3 Statistical analysis

Analyses were run separately in the two cohorts, using R version 4.0.3 (R Core Team, 2021) All scripts are available on the project [GitHub repository](#). Missing values in the exposure, covariate and outcome variables were imputed by conditional multiple imputation (Van Buuren, 2018) using 60 iterations and 30 imputed datasets (for a complete assessment of missing values and detailed imputation strategy see [Supplement 4](#) and [Table S1](#), available online). Model parameters were fit in each imputed dataset and then pooled according to Rubin's rules. Pre- and postnatal stress, internalizing and adiposity were standardized using a z transformation. All statistical tests were two-sided and interpreted at a p-value significance threshold of 0.05. To account for multiple comparisons, false discovery rate (FDR) correction was applied.

### **Association of prenatal ELS with internalizing symptoms and adiposity**

For each continuous outcome (i.e., internalizing and adiposity), we performed a causal mediation analysis featuring prenatal stress as the exposure and postnatal stress as mediator (Wang & Arah, 2015). The method is described in detail in online *Supplement 5*. In summary, the “total” effect of prenatal ELS on each outcome was decomposed into a direct (i.e., not due to postnatal ELS) and indirect pathway (i.e., acting through postnatal ELS), allowing us to quantify the direct and mediated contribution of prenatal stress.

### **Association of postnatal ELS with internalizing symptoms and adiposity**

For each continuous outcome, four multiple linear regression models were run: 1) baseline (covariate only) model; 2) prenatal stress + covariates model; 3) postnatal stress + covariates model; and 4) prenatal + postnatal stress + covariates model. The baseline model served as reference for the computation of  $R_{inc}^2$ ; the prenatal model was used to ensure comparability of estimates between approaches.

### **Association of prenatal and postnatal ELS with comorbidity**

For the combined comorbidity outcome, two multinomial logistic regression models were performed, using the “healthy” group as reference, and pre-/postnatal stress as independent predictors. The odds ratios (OR) and 95% confidence intervals (CI) of developing comorbidity were visually compared to those of developing high internalizing and high adiposity only, to determine whether pre-/postnatal stress influence comorbidity beyond either health problem alone.

### **Follow-up and sensitivity analyses**

We examined effect modification by sex and by ethnic background - in GenR only, given its multi-ethnical composition (Kooijman et al., 2016). Additionally, to explore the relative contribution of different types of stress, three regression models (for internalizing, adiposity and comorbidity) were run including all 9 domain scores (4 prenatal and 5 postnatal) as independent predictors.

To assess the impact of the imputation procedure on our results, we ran the analyses in the subsample with complete outcome data (i.e., both internalizing and adiposity). Finally, we tested the stability of our results using android fat mass as an alternative measure of adiposity.

## 2.3 Results

### 2.3.1 Sample descriptives

Sample characteristics were pooled across imputed datasets and summarized in Table 2.1. Briefly, the ALSPAC sample included 8428 (48% male) children, whose mothers were 30% highly educated (i.e., held a college or university degree). The GenR sample included 4268 (52% male) participants, 62% of which were “Dutch” and 14% had highly educated (i.e., “higher, phase 2”) mothers. Pre- and postnatal ELS were moderately correlated ( $r = \text{GenR}: 0.56$ ; ALSPAC: 0.48; see online Supplement 1), whereas the correlation between internalizing symptoms and adiposity was weak ( $r = \text{GenR}: 0.15$ ; ALSPAC: 0.11).

Table 2.1

<b>Sample descriptives</b>	<b>GenR</b> <i>(n = 4268)</i>	<b>ALSPAC</b> <i>(n = 8428)</i>
<b>Prenatal stress</b> , median (range)		
Total score	0.42 (0–2.60)	0.48 (0–2.34)
Life events	0.13 (0–0.67)	0.07 (0–0.57)
Contextual risk	0.25 (0–1.00)	0.25 (0–0.88)
Parental risk	0.00 (0–0.71)	0.10 (0–0.83)
Interpersonal risk	0.06 (0–0.95)	0.00 (0–0.84)
<b>Postnatal stress</b> , median (range)		
Total score	0.64 (0–3.59)	2.69 (0.17–16.43)
Life events	0.23 (0–0.82)	1.07 (0–3.50)
Contextual risk	0.20 (0–1.00)	0.50 (0–2.90)
Parental risk	0.00 (0–0.79)	0.57 (0–3.62)
Interpersonal risk	0.00 (0–0.79)	0.29 (0–5.49)
Direct victimization	0.13 (0–0.86)	0.00 (0–3.10)
<b>Internalizing score</b> , median (range)	4.00 (0–41)	1.00 (0–10)
<b>Fat mass percentage</b> , median (range)	24.7 (8.5–54.6)	23.9 (4.9–56.3)
<b>Outcome groups</b> , n (%)		
Healthy	2791 (65)	5916 (70)
High internalizing	623 (15)	795 (9)
High adiposity	631 (15)	1476 (18)
Comorbid	223 (5)	241 (3)
<b>Sex</b> , n (%)		
Male participants	2087 (48)	4370 (52)
Female participants	2181 (52)	4058 (48)
<b>Ethnic background</b> , n (%)		

Table 2.1

**Sample descriptives**

Generation R (GenR) and ALSPAC cohorts

Africa and Middle East <sup>a</sup>	115 (2.7)	
Asia and Oceania <sup>a</sup>	100 (2.3)	
Cape Verdean	100 (2.3)	
Dutch	2673 (62.6)	
Dutch Antillean	118 (2.8)	
Europe <sup>a</sup>	334 (7.8)	
Latin America <sup>a</sup>	72 (1.7)	
Moroccan	176 (4.1)	
North America <sup>a</sup>	25 (0.6)	
Surinamese	296 (6.9)	
Turkish	247 (5.8)	
<b>Age of the child</b> , median (range), years	13.5 (12.6–16.6)	13.5 (12.8–15.0)
<b>Pre-pregnancy BMI</b> , median (range), kg/m <sup>2</sup>	22.6 (14.4–50.2)	22.1 (12.5–48.6)
<b>Maternal smoking</b> , n (%)		
Never	3228 (76)	4412 (52)
Until (early) pregnancy	390 (9)	2524 (30)
During pregnancy	650 (15)	1492 (18)
<b>Maternal alcohol consumption</b> , GenR: n (%); ALSPAC: median (range)		
Never	1694 (40)	
Until early pregnancy	596 (14)	
Continued occasionally	1570 (37)	0.50 (0 – 3.5)
Continued frequently	407 (10)	
<b>Maternal education</b> , n (%) <sup>b</sup>		
Low	1716 (40.2)	4216 (50.0)
Medium	1278 (29.9)	3001 (35.6)
High	1274 (29.9)	1212 (14.4)
<b>Household income</b> , n (%) <sup>c</sup>		
Low	702 (16.4)	1318 (15.6)
Medium	2070 (48.5)	4324 (51.3)
High	1497 (35.1)	2786 (33.1)

Table 2.1

---

**Sample descriptives**

Generation R (GenR) and ALSPAC cohorts

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Note: Sample descriptives pooled across 30 imputed datasets. BMI = Body-mass index.

**a Ethnic background grouping:**

Africa and Middle East = Iran (n=11); Iraq (10); South Africa (8); Angola (7); Eritrea (7); Israel (6); Cameroon (5); Egypt (5); Nigeria (5); Ethiopia (4); Algeria (3); Ghana (3); Lebanon (3); Liberia (3); Syria (3); Tanzania (3); Côte d'Ivoire (2); Guinea (2); Mozambique (2); Saudi Arabia (2); Senegal (2); Zimbabwe (2); Africa (1); Armenia (1); Burundi (1); Congo (1); French Congo (1); Gambia (1); Kenya (1); Mali (1); Mauritania (1); Palestine (1); Sierra Leone (1); Somalia (1); Sudan (1); Togo (1); Tunisia (1); Uganda (1); Yemen (1).

Asia and Oceania = Indonesia (n=23); Pakistan (9); Australia (6); China (6); Japan (6); Philippines (6); Thailand (6); India (5); Afghanistan (4); Hongkong (4); South Korea (4); Vietnam (4); Bangladesh (3); Korea (3); Taiwan (3); Kazakhstan (2); New Zealand (2); Dutch New Guinea (1); East Timor (1); Singapore (1); Sri Lanka (1).

Europe = Germany (n=55); Belgium (35); United Kingdom (30); France (29); Portugal (22); Spain (18); Yugoslavia (18); Poland (16); Italy (12); Bosnia-Herzegovina (11); Russia (10); Croatia (7); Czech Republic (7); Switzerland (7); Hungary (6); North Macedonia (6); Serbia-Montenegro (5); Denmark (4); Ireland (4); Norway (4); Sweden (4); Greece (3); Lithuania (3); Romania (3); Austria (2); Kosovo (2); Ukraine (2); Canary Islands (1); Estonia (1); Finland (1); Luxembourg (1); Madeira Islands (1); Moldova (1); Monaco (1); Slovakia (1); Slovenia (1).

Latin America = Colombia (n=18); Brazil (11); Dominican Republic (8); Chile (6); Venezuela (6); Cuba (4); Mexico (4); Argentina (3); Peru (3); Ecuador (2); Guyana (2); Belize (1); Bolivia (1); Haiti (1); Paraguay (1); Trinidad and Tobago (1).

North America = United States of America (n=16); Canada (9).

**b Maternal education:** low = “secondary, phase 2” or lower in GenR, “None”, “CSE”, “Vocational” or “O level” in ALSPAC; medium = “higher, phase 1” in GenR, “A level” in ALSPAC; high = “higher, phase 2” in GenR, “(College or university) degree” in ALSPAC. Categorization based on ISCED 2011.

**c Household income:** low = < €1600 /month in GenR, < £200 /week in ALSPAC; medium = between €1600 and € 4000 /month in GenR, between £200 and £400 /week in ALSPAC; high = > € 4000 /month in GenR, > £400 /week in ALSPAC.

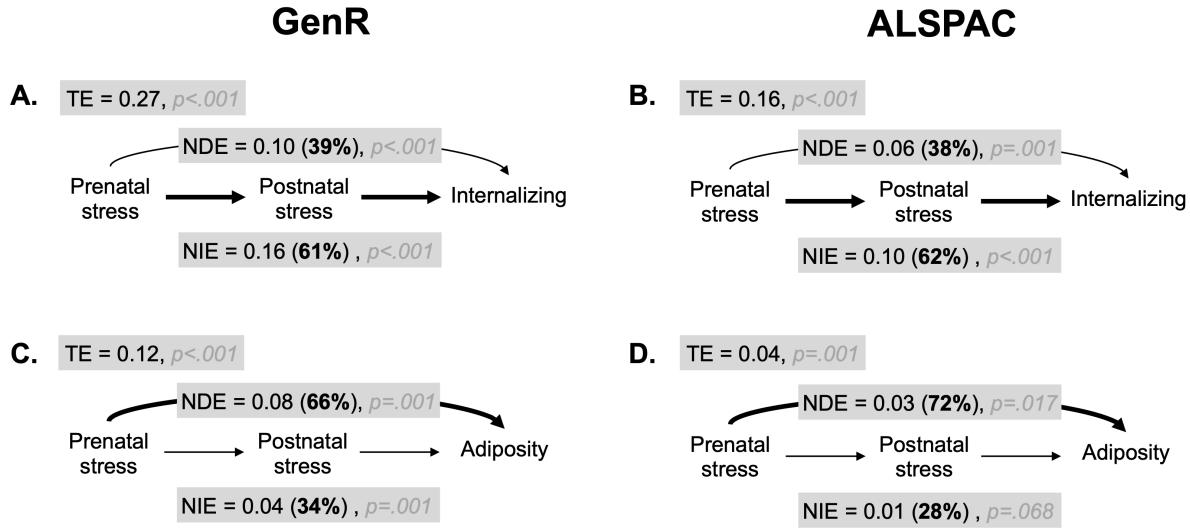
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### 2.3.2 Associations of prenatal ELS with internalizing symptoms and adiposity

Results of the mediation analysis linking prenatal stress to internalizing and adiposity were highly consistent across cohorts (see Figure 2.2 and online [Table S2](#)).

About ~60% of the total effect (TE) of prenatal stress on internalizing symptoms (TE [95%CI]

= GenR: 0.27 [0.23;0.30]; ALSPAC: 0.16 [0.13;0.18]) was mediated through postnatal stress (GenR: 0.16 [0.14;0.19]; ALSPAC: 0.10 [0.08;0.11]). The TE of prenatal stress on adiposity (GenR: 0.12 [0.09;0.15]; ALSPAC: 0.04 [0.03;0.06]) was smaller compared to internalizing and largely (~70%) operating via the direct pathway (GenR: 0.08 [0.04;0.12]; ALSPAC: 0.03 [0.01;0.05]).



*Figure 2.2: Prenatal early-life stress (ELS) contribution (causal mediation analysis results)*

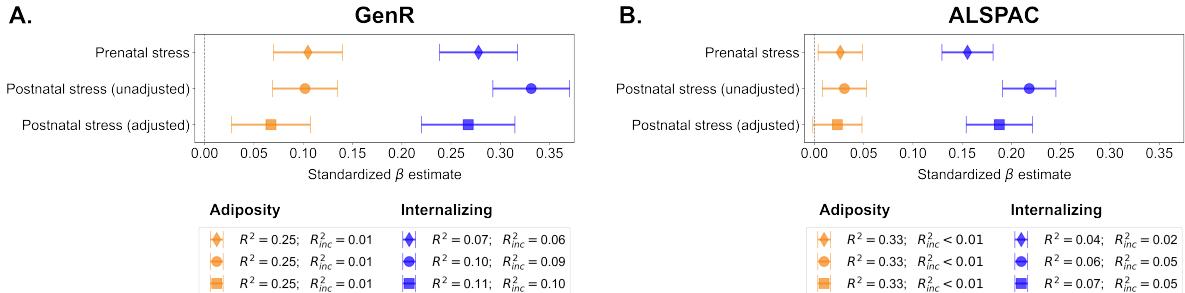
(A-D) The causal estimates for the total effect (TE), natural direct (NDE) and natural indirect effect (NIE) of prenatal stress on internalizing symptoms (A. Generation R and B. ALSPAC) and adiposity (C. Generation R and D. ALSPAC) are displayed in the grey boxes. The percentage of the total effect due to direct and indirect pathway is reported between brackets and the respective p-values are marked in grey. The predominant path is highlighted with a thicker arrow. NDE = natural direct effect; NIE = natural indirect effect; TE = total effect.

### **2.3.3 Association of postnatal ELS with internalizing symptoms and adiposity**

Results of the hierarchical regressions examining the association of postnatal stress with internalizing and adiposity were also largely similar across cohorts (see Figure 2.3; online *Tables S3 and S4*).

Higher postnatal stress associated with increased internalizing symptoms both before ( $\beta$  [95%CI] = GenR: 0.33 [0.29;0.37]; ALSPAC: 0.22 [0.19;0.25]) and after adjustment for prenatal stress (GenR: 0.27 [0.22;0.31]; ALSPAC: 0.19 [0.15;0.22]).

Higher postnatal stress also associated with increased adiposity (GenR: 0.10 [0.07;0.13]; ALSPAC: 0.03 [0.01;0.05]). The association remained after prenatal stress adjustment in GenR (0.07 [0.03;0.11]), but not in ALSPAC (0.02 [0.00;0.05]).

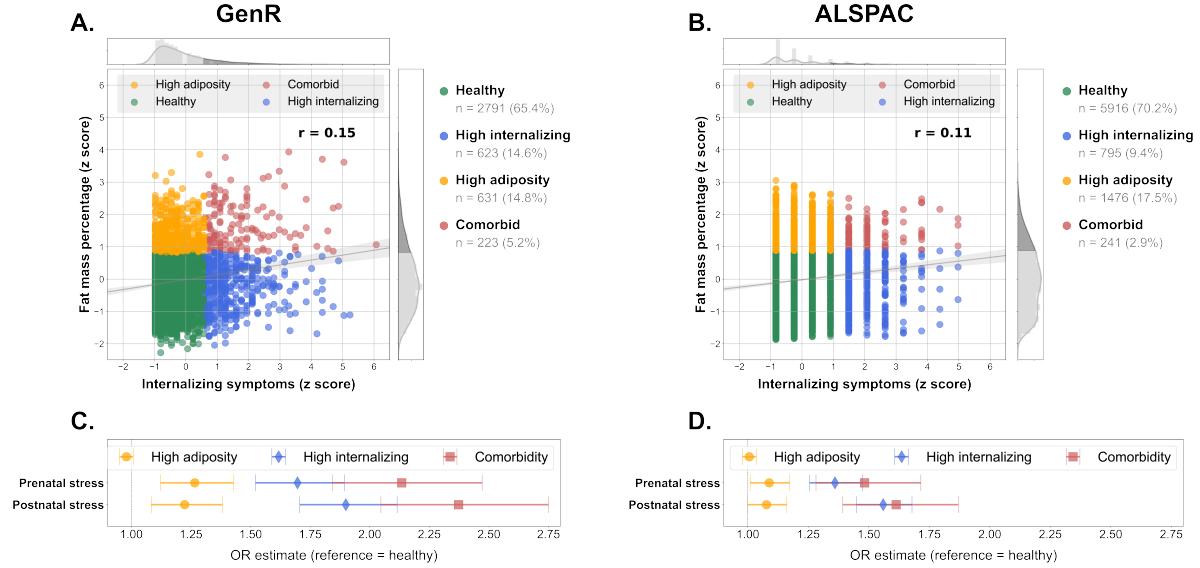


**Figure 2.3: Postnatal early-life stress (ELS) contribution (hierarchical regression results)**  
(A, B) In each cohort (A. Generation R and B. ALSPAC), the standardized beta estimates of pre- and postnatal ELS (and their 95% confidence intervals) are represented along the x-axis, using different color sets for internalizing symptoms (light and dark blue) and adiposity (yellow and orange). Estimates generated by the prenatal only model are presented on the first row and marked in lighter colours (i.e., light blue and yellow); these correspond to the TE displayed in Figure 2. Postnatal ELS beta estimates, before (round marker) and after (square marker) prenatal adjustment, are displayed in darker colors (blue and orange). For each model, the total and incremental  $R^2$  is reported in the legend below the graphs. The first number provides an indication of total model fit; the latter quantifies the increase in variance explained due to the introduction of the predictor (compared to the covariate only model).

### 2.3.4 Association of prenatal and postnatal ELS with comorbidity

Higher stress in the prenatal (OR [95%CI] = GenR: 2.13 [1.84;2.47]; ALSPAC: 1.48 [1.28;1.71]) and postnatal periods (GenR: 2.37 [2.05;2.75]; ALSPAC: 1.61 [1.39;1.87]) was associated with higher odds of belonging to the comorbidity group compared to the healthy group (see Figure 2.4 and online *Table S5*). This association was the strongest compared to all other (single-

outcome) groups. However, the CIs of the comorbidity estimates did overlap with those of high internalizing only (Figure 2.4 C and D).



**Figure 2.4: Pre- and postnatal early-life stress (ELS) and psycho-cardiometabolic comorbidity**

(A, B) Scatterplots of internalizing symptoms (on the x axis) and fat mass percentage (on the y axis), for the Generation R (A) and ALSPAC (B) cohorts. The univariate distributions of both primary outcomes are shown on the respective axes, with darker shadow indicating the 80th percentile cut-off used in construction of the comorbidity variable. Colour indicates the assigned group (green = healthy; blue = high internalizing; yellow = high adiposity; red = comorbid). Group sizes (i.e., n and percent of the total cohort sample) were pooled across imputed datasets and reported on the right of each scatterplot. (C, D) Effect estimates for pre- and postnatal stress (and their 95% CIs) on the odds ratio (OR) scale are represented along the x-axis, with different colours depending on the comparison they refer to (yellow = healthy vs. high adiposity; blue = healthy vs. high internalizing; red = healthy vs. comorbid), in Generation R (C) and ALSPAC (D) children.

### **2.3.5 Follow-up analyses**

#### **Interaction with sex and ethnic background**

After stratifying by sex, in GenR, the association between prenatal ELS and adiposity was larger in girls than in boys ( $Z=1.89$ ,  $p=.029$ ), whereas that of postnatal ELS was slightly larger in boys ( $Z=-1.38$ ,  $p=.083$ ). A similar pattern of associations was found in ALSPAC but with smaller magnitudes (see *Figures S2-S4* and *Tables S6-S9*, available online).

In GenR, Cape Verdian and Dutch Antillean children experienced the highest cumulative prenatal and postnatal stress followed by Turkish, Surinamese and Moroccan children (*Figure S5-B*). We did not find evidence for a significant interaction between pre- or postnatal ELS and the examined ethnic background groups on any outcome of interest (i.e., internalizing symptoms, adiposity or comorbidity; see *Table S10* and *Figure S5-C*, available online). Note however that the association between pre-/postnatal ELS and comorbidity in the “North American” group could not be estimated due to insufficient number of observations (i.e., comorbidity group size = 5).

#### **Contribution of specific stress domains**

Across cohorts, internalizing symptoms were consistently associated with higher prenatal and postnatal parental risk (e.g., parental psychopathology), postnatal life events and direct victimization (see *Figure S6* and *Table S11*, available online). We found no consistent associations for adiposity. Only postnatal parental risk was consistently associated comorbidity status (vs. healthy) across cohorts (see *Figure S7* and *Table S12*, available online).

#### **Sensitivity analyses**

Restricting the analyses to participants with complete outcome data ( $n =$  GenR: 2749; ALSPAC: 4096) did not substantively change the reported findings (see *Figure S8* and *Tables S13-S15*, available online), nor did the use of android fat mass rather than fat mass percentage as a proxy of adiposity (see *Figure S9* and *Table S16*, available online). None of the main conclusions was impacted by FDR correction.

## **2.4 Discussion**

Our aim was to elucidate the role of ELS on adolescent internalizing problems and adiposity, as well as their comorbidity, based on prospective data from two population birth cohorts. We highlight two key findings. Firstly, exposure to cumulative stress is strongly associated with internalizing symptoms (especially postnatal ELS) and, to a lesser extent, with adiposity

(especially prenatal ELS). Secondly, both pre- and postnatal stress associate with psycho-cardiometabolic comorbidity more strongly than to individual health outcomes.

Our first objective was to disentangle the relative contribution of prenatal and postnatal stress exposure to adolescent internalizing symptoms and adiposity.

We found that, although both pre- and postnatal ELS contribute to internalizing symptoms, the impact of postnatal stress is larger and it is not explained by prenatal confounding, while ~60% of the prenatal effect was mediated through postnatal stress. This finding aligns with previous studies investigating the contribution of prenatal and postnatal exposure to specific stressors (Clayborne et al., 2021; Plant et al., 2015), and holds promising clinical implications given that several aspects of the postnatal environment may be modifiable (Yap et al., 2016). In particular, parental risk factors (such as psychopathology), direct victimization (e.g., maltreatment) and life events emerged as independent predictors of internalizing symptoms in our exploratory analyses, indicating that these may represent important targets for intervention.

To our knowledge, no study to date has explored such timing effects on adiposity or related outcomes. Here, we found that ~70% of the effect of prenatal stress on adiposity was “direct” (i.e., not mediated by postnatal stress); the effect of postnatal stress, both as mediator and as predictor in the adjusted models, was smaller and resulted statistically significant only in GenR. While it is important to note that the effect sizes observed in the adiposity models were markedly smaller than for internalizing symptoms, these findings provide some indication that fat accumulation processes could be particularly vulnerable to (stress-induced) alterations of the prenatal environment. This is in line with previous theoretical (Barker, 1998; Gluckman et al., 2008) and empirical (Entringer et al., 2012; Entringer, 2013) accounts showcasing the impact of stress and stress hormones during prenatal life on the programming of metabolic function and obesity risk. In our exploratory follow-up analyses, we additionally found some evidence that adiposity may be more strongly associated with prenatal stress in girls, versus postnatal stress in boys. However previous accounts of these sex differences are mixed (Murphy & Loria, 2017; Paternain et al., 2013), and differences in pubertal development may be an important confounding factor that was not accounted for in our analysis.

It is also possible that stronger associations between postnatal ELS and adiposity will emerge later in development. Indeed, accumulating postnatal risks may influence biological (e.g., inflammatory and neuro-endocrine) and behavioral factors (e.g., diet and exercise) that in turn increase physical health burden, but this might become evident only later in life (Danese & Tan, 2014; Elsenburg et al., 2017).

Our second aim was to examine whether ELS relates to psycho-cardiometabolic comorbidity, as suggested by some theoretical accounts (Juster et al., 2016), but never explicitly investigated before. If comorbidity was a discrete stress-related pathophysiological process, then the effect of ELS on comorbidity would differ from the effect of ELS on mental and physical health separately. This expectation was partially confirmed by our data: ELS increased the risk of developing comorbidity compared to being healthy and this estimate was highest relative to

all other groups. However, the overlap between CIs of the comorbidity and the internalizing-only estimate indicates that neither pre- nor postnatal stress levels were sufficient to predict whether a child will develop comorbidity vs. internalizing problems alone. Notably, cross-sectional correlations between internalizing and adiposity at age 13 were small (and so were the comorbidity group sizes), which may partly explain these findings. However, comorbidity is known to increase with age (Barnett et al., 2012) and it is possible that pre- / postnatal stress may serve as better discriminators between comorbidity and internalizing problems in older samples, with higher comorbidity rates.

This study has several important strengths. We analysed data from two large population-based cohorts with remarkably consistent results, which adds confidence to the robustness and generalizability of our findings. We used a longitudinal and comprehensive assessment of ELS, enabling us to quantify the relative contribution of pre- and postnatal exposure to a broad spectrum of stressors. We focused on two pre-clinical health markers which manifest in adolescence and may be important targets for early prevention. Also, the challenge of incomplete data and possible selection bias was thoroughly addressed by multiple imputation and sensitivity analyses. However, it is important to note that our measures of ELS and internalizing symptoms rely primarily on parent reports, which might have introduced information bias. Further, although several important confounders were taken into account, it will be important in the future to examine the role of other potential contributors, including (epi-)genetic influences (Inoue et al., 2022), pubertal status, disability/functional impairment and other behavioral factors (e.g., sleep, exercise, diet).

In conclusion, current approaches to the prevention and management of depression and obesity have yielded limited success. We believe the adoption of an integrated, developmental framework is necessary to improve our understanding and set the stage for better detection and prevention of these disorders, both in isolation and in their comorbid form. We provide evidence that both pre- and postnatal ELS associate with adolescent internalizing symptoms (with prenatal < postnatal stress), adiposity (with prenatal > postnatal stress), and their comorbidity at age 13. While recommendations for how to best intervene when a higher psychosocial stress burden is identified are still at embryonic stage, one novel suggestion emerging from our findings is that prenatal stress may be an underrecognized factor for identifying children at higher risk of overweight. We would therefore advise clinicians to enquire about prenatal stress exposure as part of routine pediatric assessments, so that adequate monitoring and lifestyle preventative measures can be introduced as early as infancy.

Finally, as we follow these children, it will be informative to see how these associations evolve over time. For instance, the association between ELS exposure and adiposity-related outcomes may not emerge fully until adulthood and it is possible that the nature of the relation between ELS and comorbidity also differs as a function of developmental stage.

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### 3 Lifestyle factors, ELS and adolescent psycho-physical health

Adapted from:

**The role of lifestyle factors in the association between early-life stress and adolescent psycho-physical health: Moderation analysis in two European birth cohorts**

**Defina, S., Woofenden, T., Baltramonaityte, V., Tiemeier, H., Fairchild, G., Felix, J. F., Cecil, C.A.M., & Walton, E.** (2024). *Preventive Medicine*. [DOI](#)

#### Abstract

**Objective:** Early-life stress (ELS) is an established risk factor for a host of adult mental and physical health problems, including both depression and obesity. Recent studies additionally showed that ELS was associated with an increased risk of comorbidity between mental and physical health problems, already in adolescence. Healthy lifestyle factors, including physical activity, sleep and diet have also been robustly linked to both emotional and physical wellbeing. However, it is yet unclear whether these lifestyle factors may moderate the association between ELS and psycho-physical comorbidity.

**Methods:** We investigated whether (a) participation in physical activity, (b) sleep duration, and (c) adherence to a Mediterranean diet, moderated the relationship between cumulative ELS exposure over the first 10 years of life and psycho-physical comorbidity at the age of 13.5 years. Analyses were conducted in 2022-2023, using data from two large adolescent samples based in the UK (ALSPAC; n=8428) and The Netherlands (Generation R; n=4268).

**Results:** Exposure to ELS was significantly associated with a higher risk of developing comorbidity, however this association was not modified by any of the three lifestyle factors investigated. Only physical activity was significantly associated with a reduced risk of comorbidity in one cohort ( $OR_{ALSPAC}$  [95%CI] = 0.73 [0.59; 0.89]).

**Conclusions:** In conclusion, while we found some evidence that more frequent physical activity may be associated with a reduction in psycho-physical comorbidity, we did not find evidence in support of the hypothesised moderation effects. However, more research is warranted to examine how these associations may evolve over time.

## Links

**Supplementary materials:** <https://osf.io/2wz4u/files/osfstorage>



**Project's code:** <https://github.com/SereDef/lifestyle-moderators-project>



**ELS score:** <https://github.com/SereDef/cumulative-ELS-score>



## Keywords

Adverse Childhood Experiences; Comorbidity; Physical Activity; Sleep; Diet, Mediterranean; Depression; Adiposity; Moderation analysis.

## Abbreviations

Avon Longitudinal Study of Parents and Children (ALSPAC), Body Mass Index (BMI), Confidence Interval (CI), Child Behavior Checklist (CBCL), Dual-energy X-ray absorptiometry (DXA), Early Life Stress (ELS), Food Frequency Questionnaire (FFQ), Generation R (GenR), Odds Ratio (OR), Strengths and Difficulties Questionnaire (SDQ), Variance Inflation Factor (VIF), United Kingdom (UK).

### **3.1 Introduction**

Over the past few decades, the prevalence of comorbid mental and physical diseases has risen dramatically, posing a major challenge for health services across the world (Launders et al., 2022; Ronaldson et al., 2021). In particular, multiple large studies have shown a substantial degree of comorbidity between common mood disorders, such as depression and anxiety, and cardio-metabolic conditions, including diabetes, obesity and cardiovascular disease (Fisher et al., 2014; Gold et al., 2020; Souama et al., 2023). Mounting evidence is further suggesting an early origin of these psycho-physical comorbidity patterns, involving shared risk factors and pathophysiological pathways that begin already in utero (Milaneschi et al., 2019). One of such risk factors is psychosocial stress – including for instance family conflict, financial difficulties or victimization, experienced in the first years of life – here collectively defined as early-life stress (ELS). For example, a recent study conducted in two independent population-based samples reported that exposure to ELS during gestation and throughout the first 10 years of life prospectively associated with increased internalizing symptoms<sup>1</sup>, adiposity, as well as their co-occurrence in adolescence (Defina et al., 2023). The study focused on broad, pre-clinical measures of depression/anxiety (i.e. internalizing symptoms) and cardio-metabolic risk (i.e. adiposity) respectively. However, these associations were also shown to persist into mid- and late adulthood and manifest into clinical diagnoses (Bright et al., 2016; Milaneschi et al., 2019; Souama et al., 2023), highlighting the early developmental origins of these risk pathways.

While these findings certainly support the importance of primary prevention programmes aimed at reducing the incidence of ELS, preventing ELS may not always be possible. As such, there is a need to identify alternative modifiable factors that could mitigate the negative impact of ELS on later health, and inform the development of complementary intervention strategies.

Healthy lifestyle factors, including physical activity, sleep and diet have been robustly linked to both emotional and physical wellbeing (Briguglio et al., 2020; Firth et al., 2020). For example, regular physical activity was associated with reduced internalizing (Wheatley et al., 2020) and depressive symptoms (Oberste et al., 2020), as well as with lower body mass index (BMI) (Schwarzfischer et al., 2017) in children and adolescents. Adolescents reporting longer sleep durations have also been shown to be at lower odds of developing depression and obesity (Owens et al., 2014). Moreover, diet quality, particularly adherence to a Mediterranean diet, was associated with reduced internalizing symptoms (Orlando et al., 2022) and risk of depression (Shafiei et al., 2023), as well as with lower adiposity (Tognon et al., 2014) in childhood.

However, it remains unclear whether any of these lifestyle factors could effectively attenuate the association between ELS exposure and the risk of comorbidity between mental and physical

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<sup>1</sup>Internalizing symptoms (Achenbach, 1966) refer to a host of problems, including feelings of sadness, withdrawal, anxiety, loneliness or somatic complaints that are typical of early manifestations of depression and anxiety during childhood and adolescence.

health problems, in early adolescence.

To address this gap, we replicated and extended Defina et al.'s (2023) approach, by investigating the interaction between cumulative ELS exposure and (a) participation in physical activity, (b) sleep duration, and (c) adherence to a Mediterranean diet, on adolescent psycho-physical comorbidity, defined as the co-occurrence of high internalizing symptoms and high adiposity.

## 3.2 Methods

This study follows STROBE guidelines (Elm et al., 2008).

### 3.2.1 Participants

Our sample was drawn from two ongoing population-based longitudinal birth cohorts: the Avon Longitudinal Study of Parents and Children (ALSPAC) involving a total of 14,833 pregnant women whose children were born in Avon (UK) between 1991 and 1992 (Boyd et al., 2012; Fraser et al., 2013); and the Generation R (GenR) Study, involving 9,778 pregnant women, with children born in Rotterdam (the Netherlands) between 2002 and 2006 (Kooijman et al., 2016). Please note that the [ALSPAC website](#) contains details of all the data that is available through a fully searchable data dictionary and variable search tool. More detailed information about ALSPAC study numbers can be found in [Supplementary Text S1](#), available online.

Response rates at the 13 years follow-up were 61% in ALSPAC and 64% in GenR. Participant selection criteria have been previously described (Defina et al., 2023). In summary, children with more than 50% missing ELS items in either the pre- or postnatal period were excluded. All twins were further excluded and, in the case of non-twin siblings, only one was retained in the sample. The final sample included 8,428 and 4,268 children in ALSPAC and GenR respectively.

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee, the University of Bath Psychology Research Ethics Committee (reference number: 20-195), and from the Medical Ethical Committee of Erasmus MC, University Medical Center Rotterdam (MEC-198.782.2001.31).

### 3.2.2 Measures

#### Early Life Stress (ELS) exposure

A detailed description of the ELS exposure score can be found elsewhere [Defina et al. (2023); [ELS score repository](#)]. Briefly, a prenatal (i.e., maternal exposure during pregnancy) and

postnatal (i.e. from birth to 10 years) cumulative ELS score was constructed in each cohort by combining ~100 stress-related items (e.g., death of a relative; financial difficulties; parental psychopathology; maltreatment or bullying). Prenatal and postnatal stress scores were then summed to obtain a total ELS exposure score, spanning from pregnancy to age 10 years.

### **Physical and mental health outcomes**

Following the same approach as Defina et al. (2023), we defined psycho-physical comorbidity as the co-occurrence of high internalizing symptoms and high adiposity.

Internalizing symptoms were assessed by parental reports when children were on average 13.5 years old (range: 12.5-16.8 years) using the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997) in ALSPAC and the Child Behavior Checklist (CBCL 6-18) (Achenbach, 1999) in GenR. Both instruments are well-validated reports of emotional and behavioural functioning referring to the past 6 months, and have been shown to be comparable ( $r=0.74$ ) (Goodman & Scott, 1999).

Adiposity was measured using total body fat mass percentage estimated via dual-energy X-ray absorptiometry (DXA) scanner (ALSPAC: Lunar Prodigy DXA scanner, GE Healthcare; GenR: iDXA scanner, GE Healthcare, Madison, WI) at the average age of 13.5 years (range: 12.5-16.6 years).

Finally, to obtain psycho-physical comorbidity, internalizing symptoms and fat mass percentage were first dichotomized into high versus low-moderate, based on a cohort-specific 80th percentile cut-off value. The dichotomized values were then used to assign children to 4 groups: “Healthy” (both outcomes <80th percentile); “High internalizing” (internalizing >80th & fat mass percentage 80th); “High adiposity” (internalizing 80th & fat mass percentage >80th); and “Comorbid” (both outcomes >80th percentile). Note that the 80th percentile cut-off was based on previous validation studies (Achenbach & Rescorla, 2001; Bourdon et al., 2005; Flegal et al., 2010; Weber et al., 2014) and the resulting size of the comorbidity group was larger than expected by chance (permutation test  $p < 0.01$ ; see *Supplement 2* in Defina et al. (2023)).

### **Lifestyle factors**

**Physical activity.** Frequency of physical activity was measured via questionnaires when children were on average 10.7 years old (range: 10.6-14.7) in ALSPAC and 9.7 years (range: 8.9-12.4) in GenR. ALSPAC mothers were asked about the “*Average number of times their child participated in vigorous physical activity in past month*” (“none”, “less than once a week”, “1-3 times a week”, “4-6 times a week” and “daily”), while GenR children reported “*How often did they play sports at a sports club or team?*” (“once a week”, “2 times a week”, “3 times a week”, “4 times a week”, or “5 or more times a week”).

**Sleep.** In the ALSPAC sample, sleep duration (hours) was calculated using maternal reports of the “*Time their child usually goes to sleep and wakes up on normal school days*”, collected at the mean age of 11.7 years (range: 11.4-13.8).

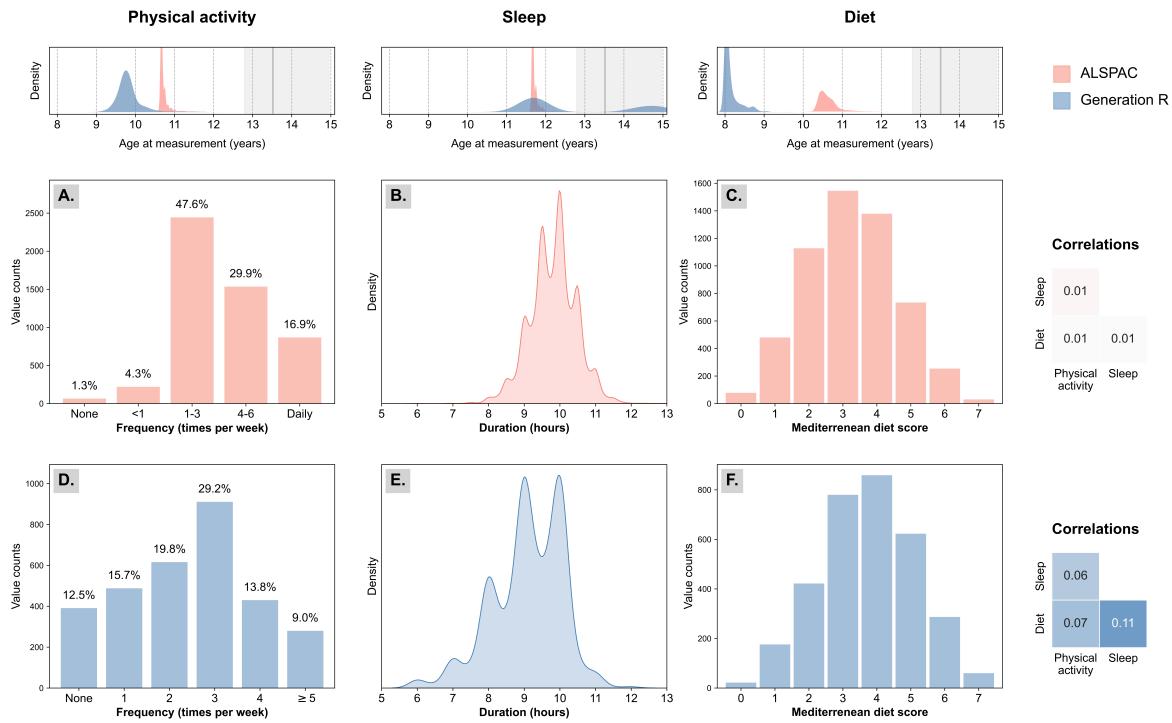
In GenR, a selected subsample of children [N=1,483; Koopman-Verhoeff et al. (2019)] completed a sleep diary including questions about the time they went to bed and woke up, on nine consecutive days (i.e., 5 weekdays and 4 weekend days). These self-reports were used to calculate average sleep duration across the nine days. Mean age of measurement was 12.7 years (range: 10.4-15.6), however, due to logistical reasons, data collection was split into two waves, resulting in a 3-years age difference between the first and second group (i.e., 11 and 14 years; see Figure 3.1 B). Sleep duration values measured after either outcome of interest were set to missing (N=519).

**Diet.** Nutritional intake was assessed at 10.6 years (range: 9.8-12.2) in ALSPAC and at 8.2 years (range: 7.5-10.8) in GenR, using a 3-day child-reported food diary (Cribb et al., 2011), and the 4-week parent-reported food frequency questionnaire [FFQ; Dutman et al. (2011)], respectively.

ALSPAC participants were asked to record all foods and drinks consumed over three individual days (preferably one weekend and two weekdays, not necessarily consecutive), using household measures. The structured diary was designed for the child to complete with the help of their parents. They would then bring the diary to the clinic visit where, if possible, any uncertainties were clarified by a member of the nutrition team. Food records were transformed into food codes and associated weights using the DIDO software (Price et al., 1995).

The FFQ consists of 71 food items relevant for the energy intake of Dutch children (Netherlands Nutrition Centre, 1998). Information on frequencies, types, and portion sizes was converted into grams of individual food items per day based on standard Dutch portion sizes (Velde et al., 2019).

A Mediterranean diet adherence score (ranging from 0 to 7) (Trichopoulou et al., 2003) was constructed by assigning 1 point for the elevated (i.e., median) consumption of five beneficial food groups (i.e., vegetables, legumes, fruits and nuts, cereal, and fish), and 1 point for the restricted (i.e., < median) consumption of two detrimental food groups (i.e., meat and dairy products). Details of specific items included in the food groups in each cohort can be found in *Supplementary Text S2*.



**Figure 3.1: Lifestyle factors characteristics in ALSPAC and Generation R children (UK, 2002-2005; The Netherlands, 2010-2016)**

(A-F) Characteristics of physical activity, sleep and diet measurements in the ALSPAC (A-C; in pink) and GenR cohorts (D-F; in blue). In the top panel, the distributions of child age at moderator measurement are depicted for both cohorts. A grey line and shadow also indicate the average and range of child age at outcome measurement. The central and bottom panel depict the distribution and correlation between the three lifestyle factors in ALSPAC and GenR respectively.

### 3.2.3 Statistical analysis

Analyses were run separately in the two cohorts, using R version 4.1.0 (R Core Team, 2021) (scripts publicly available [here](#)).

Missing values in all variables of interest (i.e., exposures, outcomes, moderators and covariates) were imputed by conditional multiple imputation (Buuren & Groothuis-Oudshoorn, 2011) using 60 iterations and 30 imputed datasets (for a complete assessment of missing values and detailed imputation strategy see *Supplementary Text S3* and *Supplementary Table S1*, available online). Model parameters were fit in each imputed dataset and then pooled according to Rubin's Rules. To account for multiple comparisons, false discovery rate correction was applied.

## Main analyses

To address our primary hypotheses, three multinomial logistic regressions (i.e., one for each lifestyle factor) were performed, with psycho-physical risk group as a dependent variable. The reference level was set to “healthy”. These models included: *a*) the main effect of total ELS, *b*) that of each lifestyle factor (separately; i.e., physical activity, sleep and diet) and *c*) their interaction, as well as the full set of covariates. In order to generate meaningful and comparable estimates for the main effects of interest, both ELS scores and lifestyle factor variables were standardized using a z transformation before entering the models. The covariate set included child sex, age at outcome measure, ethnicity (dichotomized into “White” and “non-White”), maternal smoking and alcohol consumption during pregnancy and maternal pre-pregnancy BMI. To diagnose multicollinearity between independent variables we computed generalized variance inflation factors (VIF) for each predictor (James et al., 2013).

## Follow-up analyses

Several follow-up analyses were conducted, whereby the three main models (i.e., for physical activity, sleep and diet) were modified to:

- a) Feature (*i*) internalizing symptoms and (*ii*) adiposity (rather than their comorbidity) as dependent variables (i.e., in two separate linear regression models);
- b) Examine pre-/postnatal ELS (rather than the total ELS score) as main stress exposure;
- c) Assess the effect of adhering to international guidelines regarding weekly physical activity, sleep duration and diet, by using a dichotomized version of each moderator. We dichotomised the physical activity variable according to the WHO recommended guideline for vigorous physical activity of “at least 3 times a week” for children aged 5-17 (Bull et al., 2020). Applied to available response options in both cohorts, participation in physical activity was deemed “infrequent” (0-3 times a week) or “frequent” (4+ times a week). The sleep variable was dichotomized based on the recommendations of the ‘American Academy of Sleep Medicine’ for children aged 6-12 years (Paruthi et al., 2016). Children who slept between 9-12 hours were categorised as sleeping a “recommended” duration, in comparison to “insufficient/excessive” sleep. Finally, we used a median-split approach to dichotomise the diet variable. Children were categorised as having lower ( median), or higher adherence (> median) in each sample.

We additionally explored potential non-linear associations between each lifestyle factor and the main outcomes of interest by including second- and third-degree polynomial terms in the models. To assess the extent to which maternal health and lifestyle behaviour may have moderated the relationships of interest (Epstein et al., 2023; Heslehurst et al., 2019), we further investigated the interaction between ELS and maternal BMI on child comorbidity. Finally, to assess the impact of the imputation procedure on our results, we ran the analyses in the subsample with complete moderator and outcome data.

### 3.3 Results

#### 3.3.1 Sample characteristics

Sample characteristics were pooled across imputed datasets and summarized in Table 3.1. Briefly, the ALSPAC sample included 8,428 (48% male) children, whose mothers were 96% White and 30% highly educated (i.e., held a college or university degree). The GenR sample included 4,268 (52% male) participants, 71% of which were encoded as “White” (i.e., European, North American, Japanese or Indonesian) and 14% had highly educated (i.e., “higher, phase 2”) mothers. The distribution of each lifestyle factor and their correlations are displayed in Figure 3.1. The proportion of children categorized as comorbid was small (2.9% in ALSPAC and 5.1% in GenR), as also reflected by the weak correlations between internalizing scores and adiposity ( $r = \text{ALSPAC: } 0.10; \text{ GenR: } 0.12$ ; see *Supplementary Table S2*).

Table 3.1

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#### Sample descriptives

Generation R (GenR) and ALSPAC cohorts

	<b>ALSPAC (n=8428)</b> mean (range) / n (%)	<b>Generation R (n=4268)</b> mean (range) / n (%)		
<b>Age at outcome</b>	13.49 (12.79-14.96)		13.56 (12.59-16.63)	
<b>Sex</b>	Male 4370 (51.9)	Male 2091 (49.0)		
<b>Ethnic background</b>	White 8103 (96.1)	White 3061 (71.7)		
<b>Early life stress</b>	3.57 (0.43-18.50)		1.28 (0.00-6.00)	
<b>Frequency of participation in physical activity</b>	None <1 week 1-3 week 4-6 week Daily	128 (1.5) 377 (4.5) 3946 (46.8) 2408 (28.6) 1566 (18.6)	None Once a week Twice a week Three times a week Four times a week 5 or more times a week	568 (13.3) 626 (14.7) 728 (17.1) 1138 (26.7) 643 (15.1) 563 (13.2)
<b>Sleep duration (hours)</b>	9.81 (6.00-13.00)		8.99 (6.00-12.00)	
<b>Mediterranean diet score</b>	3.24 (0.00-7.00)		3.83 (0.00-7.00)	
<b>Internalizing symptoms</b>	1.43 (0.00-10.00)		5.75 (0.00-41.00)	

Table 3.1

**Sample descriptives**

Generation R (GenR) and ALSPAC cohorts

<b>Fat mass percentage</b>		24.36 (4.92-56.25)		25.53 (8.49-54.62)
<b>Comorbidity risk group</b>	Healthy	5916 (70.2)	Healthy	2789 (65.4)
	High	795 (9.4)	High	622 (14.6)
	internalizing		internalizing	
	High adiposity	1476 (17.5)	High adiposity	637 (14.9)
	Comorbidity	241 (2.9)	Comorbidity	219 (5.1)
<b>Maternal BMI (kg/m<sup>2</sup>)</b>		22.79 (12.49-48.62)		23.46 (14.38-50.21)
<b>Maternal smoking</b>	Never	4412 (52.3)	Never	3234 (75.8)
	Until pregnancy	2524 (30.0)	Until pregnancy	386 (9.0)
	During	1492 (17.7)	During	648 (15.2)
	pregnancy		pregnancy	
<b>Maternal alcohol consumption</b>	Never		Never	1694 (39.7)
	continuous score <sup>1</sup>	0.73 (0.00-3.50)	Until pregnancy	599 (14.0)
			Continued occasionally	1567 (36.7)
			Continued frequently	405 (9.5)
<b>Maternal education<sup>2</sup></b>	Low	4216 (50.0)	Low	1716 (40.2)
	Medium	3001 (35.6)	Medium	1278 (29.9)
	High	1212 (14.4)	High	1274 (29.9)

Note: Sample characteristics of each cohort, pooled across 30 imputed datasets. We report mean (range) for continuous variables and n (percentage) of each group for categorical variables.

<sup>1</sup> higher scores correspond to higher alcohol use frequencies throughout pregnancy.

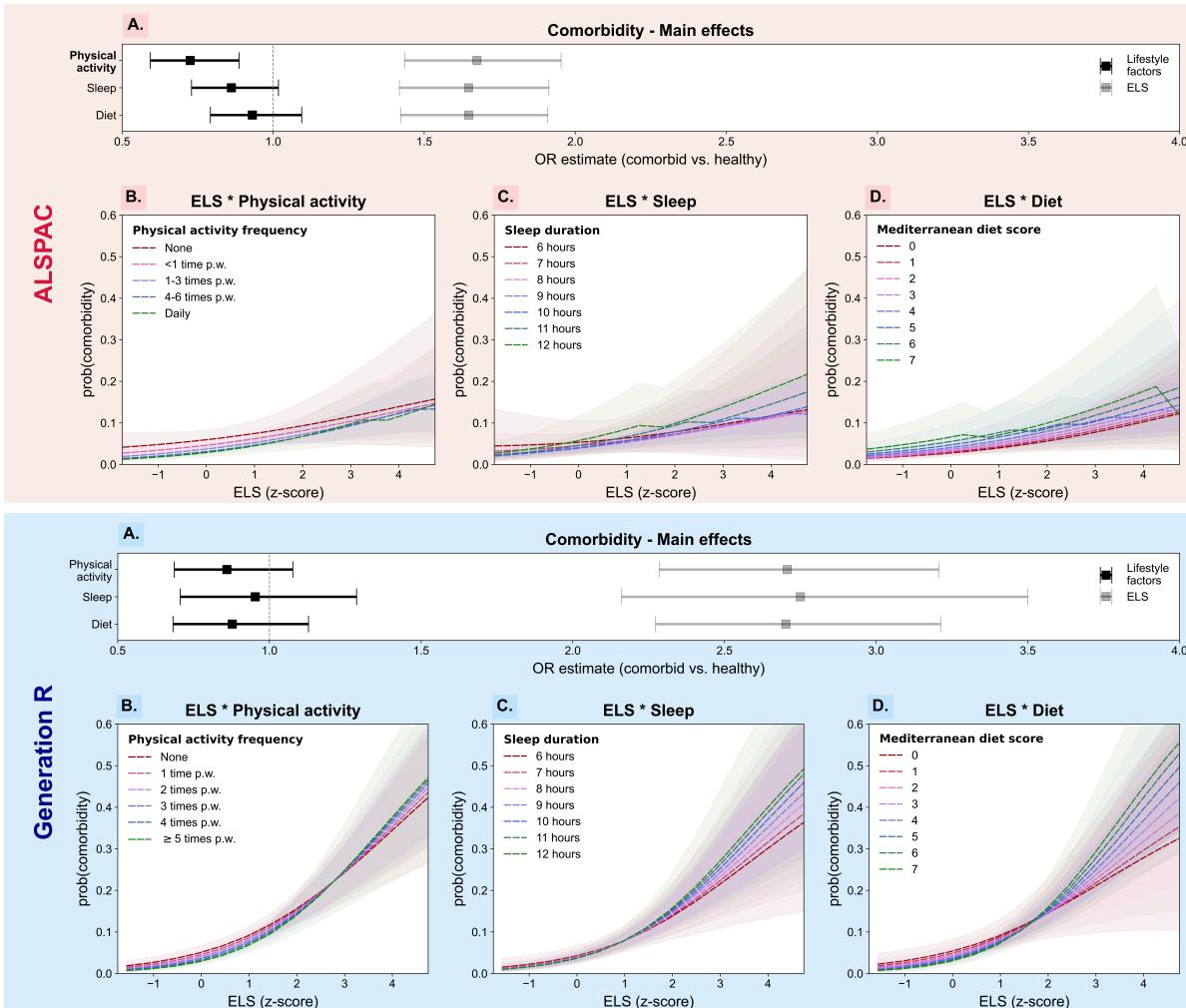
<sup>2</sup> Maternal education: low = 'None", "Certificate of Secondary Education", "Vocational" or "O level" in ALSPAC, "secondary, phase 2" or lower in GenR; medium = "A level" in ALSPAC, "higher, phase 1" in GenR; high = "(College or university) degree" in ALSPAC, "higher, phase 2" in GenR. Categorization based on the International Standard Classification of Education 2011.

### 3.3.2 Main analyses

We did not find evidence in support of any of the hypothesised moderation effects (*Supplementary Table S3*). Increased ELS was significantly associated with higher risk of developing comorbidity (vs. being healthy; Figure 3.2 A; ORs: 1.65-1.67 in ALSPAC and 2.70-2.75 in

GenR), but this association was not modified by any of the three lifestyle factors we investigated (Figure 3.2 B-D).

Conversely, while comorbidity risk was generally lower in children who engaged in favourable lifestyle behaviours (Figure 3.2 A), this effect was only significant for physical activity and only in one of the two cohorts ( $OR_{ALSPAC}$  [95%CI] = 0.73 [0.59;0.89]). The magnitude of multicollinearity was low in all the models (VIF 1.17; see *Supplementary Table S3*).



**Figure 3.2: Main effects and interactions between lifestyle factors and early-life stress on comorbidity risk in ALSPAC and Generation R children (UK, 1992-2006; The Netherlands, 2002-2016)**

(A) Odd ratios (OR) and their 95% confidence intervals (CI) are represented along the x-axis for the main effects of physical activity, sleep and diet (in black) and for that of ELS (in grey). Statistically significant terms are highlighted in bold.

(B-D) The predicted probability of developing comorbidity (y-axis) against ELS exposure levels (x-axis), stratified by lifestyle factor levels (red to green coloured lines). The 95% CIs around the predicted probabilities are also shown for each line (i.e., lifestyle factor level) and their overlap is an indication for the lack of interaction between ELS and physical activity, sleep or diet.

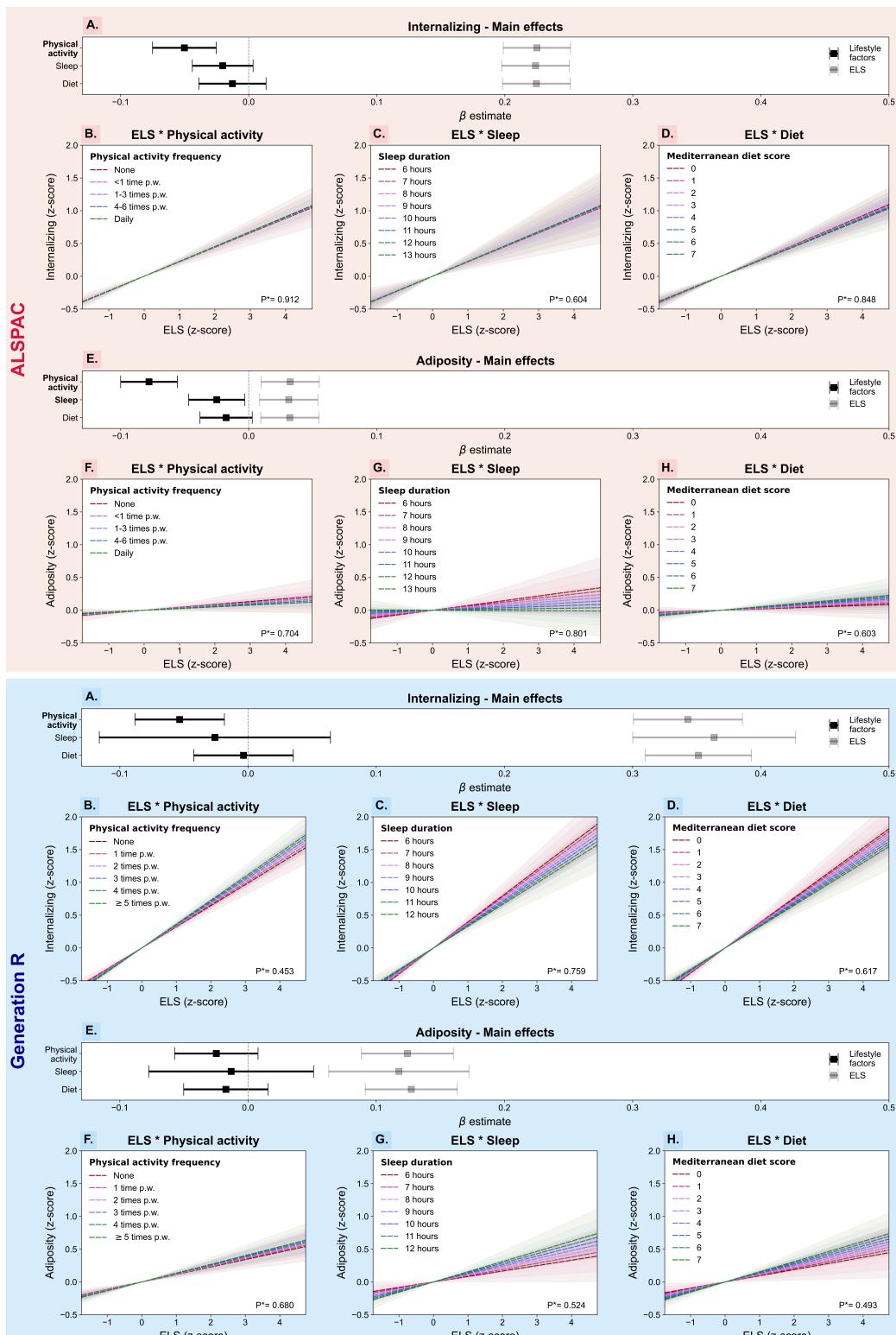
### 3.3.3 Follow-up analyses

When internalizing symptoms and adiposity were examined as two separate outcomes, a similar pattern of results emerged (*Supplementary Table S4*; Figure 3.3). ELS was significantly associated with increased internalizing symptoms and adiposity, but neither association was modified by physical activity, sleep or diet.

Only the main effect of physical activity on internalizing symptoms was statistically significant in both cohorts (*ALSPAC* [95%CI] = -0.05 [-0.08;-0.03]; *GenR* [95%CI] = -0.05 [-0.09;-0.02]).

Engagement in two of the three favourable lifestyle behaviours was linked to lower adiposity levels, but only in one of the two cohorts (*ALSPAC* [95%CI]: physical activity = -0.08 [-0.10;-0.06]; sleep = -0.02 [-0.05;0.01]).

Findings did not change substantially when ELS exposure was restricted to the prenatal or postnatal periods only (*Supplementary Table S5*), nor when lifestyle factors were dichotomized into adherence and non-adherence to international guidelines (*Supplementary Table S6*). The non-linear relationships between each lifestyle factor and the main outcomes of interest are represented in *Supplementary Figure S1*. Only for the relationship between sleep and internalizing symptoms did we find any evidence for a non-linear (inverse logarithmic) trend. We did not find a statistically significant interaction between ELS exposure and maternal BMI on child comorbidity (*Supplementary Table S7*). The sensitivity analysis conducted in the subsample with complete outcome and moderator data (sample size = ALSPAC: 3680-4237; GenR: 961-2369) also did not impact our main conclusions (*Supplementary Table S8*).



**Figure 3.3: Main effects and interactions between lifestyle factors and early-life stress on internalizing symptoms and adiposity in ALSPAC and Generation R children (UK, 1992-2006; The Netherlands, 2002-2016)**

(A-E). For each outcome (A. Internalizing symptoms; E. Adiposity), the standardized effect estimates and their 95% confidence intervals (CI) are represented along the x-axis for the main effects of physical activity, sleep and diet (in black) and for that of ELS (in grey). Statistically significant terms are highlighted in bold.

(B-D) and (F-H). Linear association between ELS exposure on the x-axis and internalizing symptoms (B-D) or adiposity (F-H) on the y-axis, stratified by lifestyle factor levels (red to green coloured lines). The 95% CIs around each slope (i.e., lifestyle factor level) are also shown and their overlap is indication of the lack of interaction between ELS and physical

### **3.4 Discussion**

In this study based on data from two independent population-based cohorts, we did not find evidence that either physical activity, sleep or dietary behaviour may attenuate the association between ELS exposure and adolescent psycho-physical comorbidity.

However, at average levels of stress exposure, engaging in more frequent physical activity (more so than sleeping adequately or following a Mediterranean diet) was significantly associated with a reduced risk of developing comorbidity, as well as with lower levels of both internalizing symptoms and adiposity, in one of the cohorts investigated. While the direction of effects was generally consistent across cohorts, only the association between physical activity and internalizing symptoms resulted statistically significant in both ALSPAC and GenR children. Overall, it is important to note that the association between lifestyle behaviours and psycho-physical comorbidity was considerably smaller compared to that between ELS and comorbidity.

In line with these findings, previous studies conducted in adult populations also showed how the association between retrospectively reported childhood maltreatment and higher odds of psycho-physical comorbidity, was still present after additional adjustment for concurrent lifestyle factors such as smoking, alcohol use, sleep and physical activity (Souama et al., 2023; Tomasdottir et al., 2015), although none of these previous studies formally tested for ELS-by-lifestyle interactions.

Our results also align with the previously reported protective effects of physical activity (e.g., swimming or cycling) for reducing the risk of depression (Choi et al., 2019, 2020; Firth et al., 2020; He et al., 2021; Schuch et al., 2018), although this was not specific to children exposed to higher levels of stress (Choi et al., 2020).

In contrast, we could not find convincing evidence that either longer sleep duration or improved diet quality would alleviate the risk for depression or cardiometabolic problems (or their combination), as suggested by some previous studies (He et al., 2021; Katikireddi et al., 2017; Lassale et al., 2019; Zhai et al., 2015). Developmental timing and / or differences in outcome measurement may have played a role in explaining this discrepancy. It is possible, for example, that engaging in healthy lifestyle behaviours later in life may be more beneficial, or that the protective effects of childhood lifestyle behaviours may manifest only later in adulthood. It is also possible that the associations reported in the adult literature may be biased through reverse causation [e.g., depression being a causal risk factor for poor diet and sleep; Choi et al. (2020)].

One further insight emerging from these analyses was that of a stronger magnitude of association between ELS and comorbidity compared to that between each of the three lifestyle factors and comorbidity. While this result could be interpreted as an indication of the more severe, long-lasting impact of ELS, as discussed elsewhere (Henchoz et al., 2019; Souama et al., 2023; Tomasdottir et al., 2015), we would like to highlight that differential measurement error could have played a role in explaining this finding. Indeed, our ELS measure was considerably

more comprehensive (i.e., comprised of many more items and covering a longer time period) compared to each of the lifestyle factors, which were only assessed at a single time point and based on fewer indicators. It is possible, for example, that the hypothesized moderation effects may emerge when a more long-term engagement in physical activity is considered.

In addition to this point, a few other study limitations need to be considered when interpreting these results. First, our measurement of ELS, lifestyle behaviours and internalizing symptoms rely primarily on parent reports, which might have introduced information bias. ELS exposure was assessed over a large (~10 years) temporal window, but the measure was inevitably constrained by data availability. Additionally, cohort differences in the reporting of physical activity (i.e., “vigorous physical activity” in ALSPAC, vs. “sports” in GenR) may have contributed to heterogeneous findings for the main effect of physical activity on comorbidity. For example, “sports” can include non-vigorous activities, which are potentially less effective in reducing depressive symptoms (Oberste et al., 2020). Second, while we focused here on three lifestyle markers that are most relevant to the developmental period of interest and may constitute important targets for early prevention, other potentially relevant factors such as smoking, alcohol use or psychological coping strategies, are also likely to be of relevance (Hanlon et al., 2020; Skou et al., 2022) and should be taken into account in future studies. Further, although we adjusted our analyses for several important confounders and we addressed the challenge of selection bias thoroughly by multiple imputation and sensitivity analyses, we cannot exclude the possibility that residual sampling bias and/or other unmeasured factors (e.g., pubertal status or (epi-)genetic influences) may have influenced our results. For instance, the majority of the children included in our samples were of European descent, and these findings may not be generalizable to different populations. Furthermore, shared biological risk factors for depression and obesity (e.g. dysregulation of the hypothalamic-pituitary-adrenal axis, chronic inflammation, or microbiome dysbiosis; (Milaneschi et al., 2019) could potentially mediate the effect of ELS on psycho-physical comorbidity. Finally, our study focused on one aspect of adolescent mental health, i.e. internalizing symptoms, that is most predictive of future depression/anxiety problems. However, other measures of adolescent behaviour, e.g., externalizing problems, could represent interesting targets for future studies in the field.

In conclusion, several international guidelines and policies already acknowledge the importance of lifestyle interventions not only for the obesity and poor cardiovascular health crisis, but also with respect to their protective effects against poor mental health (Australian Department of Health, 2014; D. of Health & Social Care, 2011; N. I. for Health & Care Excellence, 2015; Stubbs et al., 2018; Tremblay et al., 2016). Although the current study reports a lack of influence from lifestyle factors, a non-significant interaction should not be interpreted as evidence that adopting a healthy lifestyle is ineffective in mitigating the detrimental consequences of ELS on comorbid internalizing and adiposity in childhood. Instead, our study further emphasizes the need for the investigation of early comorbidity prevention strategies that target children who have experienced ELS, and that more research is warranted to examine the potential moderating effects of a wider range of lifestyle behaviours.

### 3.4.1 Conclusions

Our study could not detect convincing evidence in support of the hypothesis that the detrimental effects of ELS on psycho-physical comorbidity risk may be attenuated by adopting healthier lifestyle habits in childhood. We found some suggestive evidence that engaging in more frequent physical activity may reduce the risk for comorbidity, irrespective of stress exposure levels. If confirmed in future studies, this set of findings could highlight the need for more comorbidity prevention efforts focused on reducing ELS, in addition to current intervention programmes that focus on lifestyle behaviours such as physical activity.

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## 4 ELS and intracortical myelination

Adapted from:

**Early-life stress exposure and intracortical myelination in childhood: a population-based neuroimaging study**

**Defina, S., Manzoni, D., Tiemeier, H., Brouwer, R.M., Cecil, C.A.M., & Muetzel R.L.** (*in preparation*)

### **Abstract**

Objective:

Method:

Results:

Conclusion:

**4.1 Introduction**

**4.2 Methods**

**4.3 Discussion**

**References**

## 5 ELS and cardiac morphology

Adapted from:

Early-life stress exposure and heart morphology in childhood: a prospective population-based study

Defina, S., Kamphuis, A., Gaillard, R., & Felix, J. F. (*in preparation*)

### Abstract

Objective:

Method:

Results:

Conclusion:

## **5.1 Introduction**

In summary, this book has no content whatsoever (Boyd et al., 2012; Fraser et al., 2013). But I shall write it anyway cause. Cause I kind of do have no choice so, checking justification works

## **5.2 Methods**

## **5.3 Discussion**

## **References**

# PART 2: Reciprocal relationships between mental and physical health problems

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## 6 Longitudinal psycho-physical co-development

Adapted from:

**Longitudinal co-development of depressive symptoms and cardio-metabolic risk factors from childhood to young adulthood**

Defina, S., Cecil, C.A.M., Felix, J.F., Walton, E., & Tiemeier, H. (*in press*) *Journal of Child Psychology and Psychiatry* DOI [DOI](#)

### Abstract

Background: Depressive symptoms and cardio-metabolic risk factors often co-occur. However, our understanding of the potential mechanisms and temporal dynamics underlying their co-development remains elusive.

Methods: This population-based cohort study examined bidirectional longitudinal associations between depressive symptoms and cardio-metabolic risk factors from age 10 to 25 years, using prospective data from the ALSPAC Study. Participants with at least one (of six) follow-up measurement for each outcome were included in the analyses. We measured depressive symptoms through self- as well as parent-reports, and assessed several cardio-metabolic risk factors (including adiposity measures, lipid profiles and inflammation).

Results: Among our 7970 (47% male, 96% White) participants, we found bidirectional, within-person associations between self-reported depressive symptoms and adiposity (i.e., fat/lean mass index, but not body mass index), across the study period. Adiposity was more stable over time ([range] = 0.75 [0.54; 0.84]), compared to depressive symptoms (0.26 [0.12; 0.38]), and it had a stronger prospective (i.e., cross-lagged) association with future depressive symptoms (0.07 [0.03, 0.13]) compared to that between depressive symptom and future adiposity (0.04 [0.03, 0.06]). The magnitude of these associations reached its peak between 14 and 16 years. We did not find evidence of cross-lagged associations in either direction between depressive symptoms and waist circumference, insulin, triglycerides, LDL cholesterol or C-reactive protein.

Conclusions: These findings suggest a bidirectional relationship between depressive symptoms and cardio-metabolic risk factors, particularly adiposity (i.e., fat/lean mass). Adiposity showed a stronger prospective association with future depressive symptoms, than vice versa, however their relationship revealed more reciprocal than previously thought.

## Links

**Web application:** <https://longit-comorbidity.onrender.com>



**Supplementary materials:** <https://osf.io/wyzd8>



**Project's code:** <https://github.com/SereDef/comorb-longit-project>



**Pre-print:** <https://www.medrxiv.org/content/10.1101/2024.10.01.24314697v1>

## Keywords

Depressive symptoms; Cardio-metabolic risk; Comorbidity; Longitudinal; ALSPAC.

## Abbreviations

Avon Longitudinal Study of Adults and Children (ALSPAC), Autoregressive Latent Trajectory Model with Structured Residuals (ALT-SR), Body Mass Index (BMI), C-reactive protein (CRP), Fat Mass Index (FMI), High- / Low-Density Lipoprotein (HDL / LDL [cholesterol]),

Lean Mass Index (LMI), Random-Intercept Cross-Lag Panel Model (RI-CLPM), Short Mood and Feelings Questionnaire (SMFQ).

## 6.1 Introduction

Over 20% of the general population faces at least one depressive episode in their lifetime (Gutiérrez-Rojas et al., 2020) and a growing number of adolescents experience depressive symptoms before the age of 20 years (Keeley, 2021; Patalay & Gage, 2019). Concurrently, the prevalence of child obesity and related cardio-metabolic risk factors is alarmingly high, affecting one in three children and almost half of young adults (NCD-RisC, 2017; WHO, 2022). Moreover, depressive symptoms and cardio-metabolic risk factors often co-occur (Anwar et al., 2018; Blasco et al., 2020; Gutiérrez-Rojas et al., 2020). For example, a large meta-analysis showed that people suffering from depression had a 58% higher risk of developing obesity, while individuals with obesity had a 55% elevated risk of developing depression, compared to the general population (Luppino et al., 2010).

Several potential mechanisms have been proposed to explain this observed comorbidity, both in adults (Carey et al., 2014; Milaneschi et al., 2019) and children (Defina et al., 2023; Sutaria et al., 2019). However, scientific efforts to empirically model the co-developmental processes that may underlie this comorbidity (i.e., temporal precedence and/or bidirectional relationships) have been sparse, inconsistent, and mostly focused on adult or aging populations (Forman-Hoffman et al., 2007; Konttinen et al., 2014) and/or genetic liabilities (Chen et al., 2023; Jokela & Laakasuo, 2023).

In the pediatric literature, a small number of studies have investigated longitudinal relationships between body mass index (BMI) and internalizing / emotional problems (an early marker of depressive symptoms) (Bradley et al., 2008; Jansen et al., 2013; Patalay & Gage, 2019; Zhou et al., 2022). The majority of these studies found higher BMI to precede increases in internalizing symptoms, but not the other way around (Bradley et al., 2008; Jansen et al., 2013; Patalay & Gage, 2019), in line with some (but not all, e.g. Chen et al. (2023)) Mendelian randomization studies investigating the causal effect of obesity on depression (Jokela & Laakasuo, 2023). A more recent investigation however, did identify reciprocal relationships between BMI and internalizing symptoms, by employing more advanced modeling frameworks, capable of decomposing between- and within-person variances over time (Zhou et al., 2022).

Importantly, while changes in fat mass are hypothesized to be a key mechanism in these studies, they rely exclusively on BMI measures, which cannot discriminate between fat mass and lean (e.g. muscle) mass, and are thus a suboptimal measure of cardio-metabolic risk (Dencker et al., 2007; Liu et al., 2013; Vanderwall et al., 2017). Moreover, existing evidence largely relies on parental reports of depressive symptoms, which may be less sensitive compared to self-reports (Cohen et al., 2019). Finally, these studies only investigated relatively short follow-up periods and early developmental windows (i.e., 1 to 5 years, from childhood to early adolescence),

leaving the period between adolescence to young adulthood, which is when these conditions typically find their onset, largely unexplored.

To address these gaps, we aimed to characterize the temporal dynamics underlying the (co-)development of depressive symptoms and cardio-metabolic risk as they unfold jointly across 15 years, from the age of 10 to 25 years. We investigated several cardio-metabolic risk factors (including total fat and lean mass) and multi-informant reports on depressive symptoms.

In the spirit of open science, we also provide an open-source interactive web-application that can be used, alongside this article, to flexibly explore our results and verify their robustness across multiple outcomes and analytical choices.

## 6.2 Methods

### 6.2.1 Sample and measures

This study is based on data from the Avon Longitudinal Study of Parents and Children (ALSPAC). Pregnant women resident in Avon (UK) with expected delivery dates between 1st April 1991 and 31st December 1992 were invited to take part in the study. The initial number of pregnancies enrolled was 14,541, with 13,988 children who were alive at 1 year of age. When children were approximately 7 years old, additional eligible cases were re-invited, resulting in a total sample of 15,447 pregnancies and 14,901 children who were alive at 1 year of age (Boyd et al., 2012; Fraser et al., 2013; Northstone et al., 2019). Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Bristol (Harris et al., 2009). Please note that the ALSPAC website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (<http://www.bristol.ac.uk/alspac/researchers/our-data/>).

### Ethical considerations

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Informed consent was obtained from participants' parents.

### Depressive symptoms

Depressive symptoms were repeatedly measured using the Short Mood and Feelings Questionnaire (SMFQ) (Angold et al., 1995; Kwong, 2019). The instrument includes 13 items referred to the past two weeks and scored between 0–2 (i.e., “*not true*” / “*sometimes true*” / “*true*”). A summary score ranging between 0–26 was computed at each occasion, with higher scores indicating greater depressive symptoms (Figure 6.1 A). The SMFQ was administered to the

child/young person on six occasions between the ages of 10 and 25 years (at the median ages of 10.6, 12.8, 13.8, 16.6, 17.8, and 23.8 years). The questionnaire was also completed by participants' parents (most commonly mothers) on four additional occasions (when children were on average 9.6, 11.7, 31.1, and 16.7 years old) which were used in secondary analyses.

### **Cardio-metabolic risk markers**

The primary cardio-metabolic measure examined in this study was fat mass index (FMI; Figure 6.1 B), computed as participants' total body fat mass divided by their squared height (kg/m<sup>2</sup>). Total body fat mass was derived from whole body dual energy X-ray absorptiometry (DXA) scans at six occasions (at median ages of 9.8, 11.8, 13.8, 15.4, 17.8, and 24.5 years) (Dangardt et al., 2019).

Ten other cardio-metabolic risk factors were further examined in secondary analyses, including lean mass index (LMI), body mass index (BMI), waist circumference, android fat mass, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels, triglycerides, insulin, and C-reactive protein (CRP) (see [Appendix S1](<https://osf.io/wyzd8>, *Figure S1*).

### **Sample demographics**

Participant sex was measured at birth. Participants' parents further reported on their ethnical identity (at recruitment) and their educational attainment (when children were 5 years old).

#### **6.2.2 Statistical analyses**

Analyses were conducted using R version 4.2.2 (R Core Team, 2021); model specification and fit was implemented in lavaan (version 0.6–16) (Rosseel, 2012). All scripts are publicly available [here](#).

#### **Data pre-processing**

To ensure optimal model conversion, we first performed data cleaning by setting extreme outlier values (i.e., > 5 interquartile ranges above the third quartile or below the first quartile) to missing. We then transformed the data to reduce skewness, using a square root transformation (for depressive symptoms scores; see Figure 6.1 A) or a ln transformation (for cardio-metabolic risk markers; see Figure 6.1 B). Finally, we performed min-max normalization (see Equation 6.1) to rescale of all variables to a [0, 1] range.

$$x_{norm} = \frac{x - \min(x)}{\max(x) - \min(x)} \quad (6.1)$$

This procedure allowed us to obtain comparable estimates across different units and improve model convergence, while preserving relative differences within each variable over time (e.g. mean variations).

## Main analyses

We fit a lag-1 random-intercept cross-lag panel model (RI-CLPM) (Hamaker et al., 2015) to characterize the relationship between self-reported depressive symptoms and fat mass index across 6 time points (~10, 12, 14, 16, 18 and 24 years). The model was specified as a structural equation model composed of four parts (see also [Appendix S2](#)

1. A between-person part, consisting of the “random intercepts” ( $\eta_{dep}$  and  $\eta_{FMI}$ ). These are latent variables that have each measurement occasion as indicator and factor loadings set to 1. They reflect stable (i.e., “time-invariant”) between-person differences (e.g., some children may have systematically higher fat mass over time compared to others).
2. A within-person part, consisting of “within-unit fluctuations”: time-specific residual terms specified as latent variables with factor loading set to 1, and (measurement error) variances set to 0. They represent random changes that make observations unique, allowing individuals to differ (from themselves) at each occasion. For example, these could reflect a life event that raises/lowers a person’s depression at a given time  $t$ .
3. The (lag-1) regressions between the within-unit components: i.e. the auto-regressive and cross-lagged terms.
  - a) auto-regressive terms quantify the persistence (or “inertia”) of a construct, i.e. its tendency to retain its state over time. For example,  $AR_{\sim}^* dep_{\sim}^*$  captures the proportion of past depression that persists directly to the next measurement occasion.
  - b) In contrast, cross-lagged relations measure the proportion of past variance in one variable that is reflected in the other variable at the next measurement occasion, and are therefore used to infer (Granger) causality. For example,  $CL_{\sim}^* dep_{\sim}^*$  indicates how much within-person variance in depression at time  $t$  is uniquely explained by FMI at time point  $t - 1$  (controlling for the persistence of past values of depression).
4. Covariances in the between- and within- person part.
  - a) To control for between-person trends that may confound the (within-person) system dynamics reflected by auto-regressive and cross-lagged terms, the covariance between  $\eta_{dep}$  and  $\eta_{FMI}$  is freely estimated.
  - b) Similarly, because within-unit fluctuations may be non-independent (e.g., when a random change affects both variables simultaneously) this is modelled by estimating their covariance within each wave.

Full information maximum likelihood (FIML) estimation was used to account for missing patterns that may not conform to MCAR. Coefficients were standardized and conventional robust standard errors were used to compute 95% confidence intervals (95%CI).

Model fit was evaluated using the Root Mean Square Error of Approximation (RMSEA), the Comparative Fit Index (CFI), the Tucker Lewis Index (TLI), and the Standardized Root Mean Square Residual (SRMR). We considered model fit to be adequate when: RMSEA < 0.05, CFI and TLI > 0.95, and SRMR < 0.08 (Kline, 2016).

## Exploratory analyses

We further conducted three sets of exploratory follow-up analyses.

- a) We replaced FMI with each of 10 alternative cardio-metabolic risk markers, including measures of adiposity (i.e., BMI, LMI, android fat mass and waist circumference), lipid profiles (i.e., HDL and LDL cholesterol levels, and triglycerides), insulin levels and inflammation (i.e., CRP).
- b) We replaced self-reported depressive symptoms scores with maternal reports (at the available time points).
- c) Estimated an Autoregressive Latent Trajectory Model with Structured Residuals (ALT-SR) with linear latent growth as an alternative to the RI-CLPM. Briefly, the ALT-SR is a more flexible modelling approach, which allows to explicitly model developmental trends (i.e. latent random slopes), in addition to the stable trait-like between-person differences (i.e., latent random intercepts) (Curran et al., 2014).

Note that the project [web-application](#) offers the opportunity for researchers to interact with model settings and examine the robustness of findings against violations of modelling assumptions, such as the temporal stability of the between- and within-person components ([Appendix S2](#)).

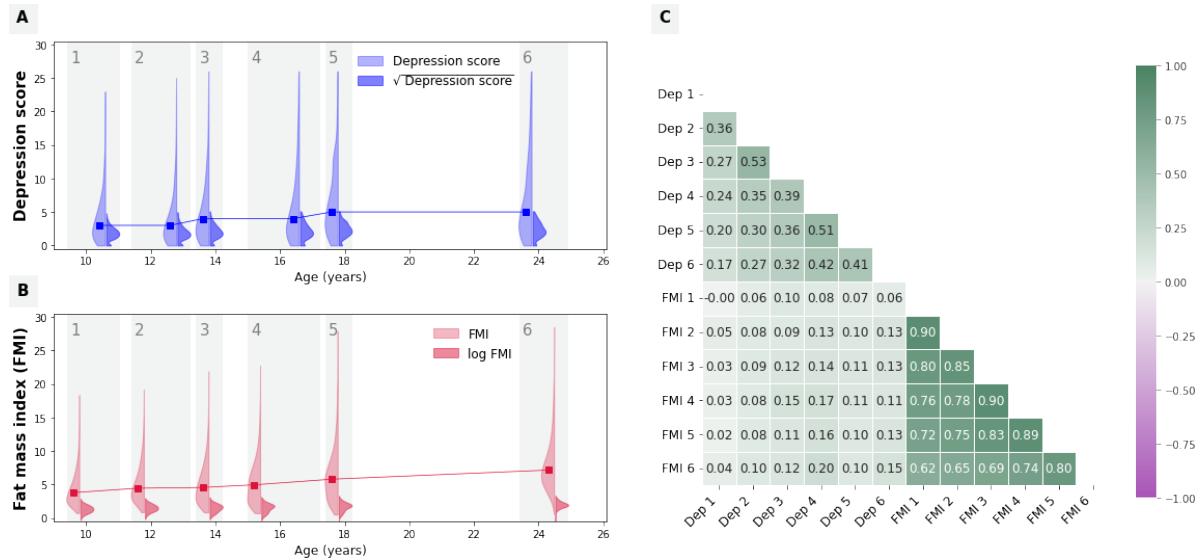
## 6.3 Results

### 6.3.1 Descriptive statistics

Sample descriptives are presented in Table 6.1 and Figure 6.1. The main analytical sample consisted of 7970 (47% male) participants, who had at least one measurement of depressive symptoms and FMI; 96% of participants' parents identified as ethnically "White".

Both depressive symptoms and FMI increased slightly with age (see Figure 6.1 A-B); their cross-sectional correlations ranged from 0.00 to 0.17 ( $r_{\text{mean}} = 0.10$ ; Figure 6.1 C).

Girls had systematically higher FMI compared to boys across time points, and they reported higher depressive symptoms scores from the ages of 14 years onwards.



**Figure 6.1: Main outcomes descriptives**

(A, B) The distribution of observed values for SMFQ depressive scores (A) and fat mass index (B) is presented on the y-axis against measurement time (x-axis). In the violin plots, lighter colors are used to represent the original value distributions, while darker colors represent the same variable distributions and after data transformation was applied (i.e., square root for depressive symptoms scores and log transformation for FMI). The line graph connects the median points (in the original data scale) at each timepoint. (C) The univariate, pairwise Pearson correlation coefficients between each repeated measure of depressive symptoms (Dep) and fat mass index (FMI).

Table 6.1

### Sample descriptives

ALSPAC cohort

	Total sample (n=7970)	Male participants (n=3769)	Female participants (n=4185)
<b>SMFQ depressive symptom score</b> , median (range) [% missing values]			
10 years	3 (0-23) [15%]	3 (0-23) [14%]	3 (0-21) [17%]
12 years	3 (0-25) [19%]	3 (0-25) [17%]	3 (0-24) [21%]
14 years	4 (0-26) [24%]	3 (0-26) [21%]	4 (0-26) [26%]
16 years	4 (0-26) [43%]	3 (0-26) [51%]	5 (0-26) [36%]
18 years	5 (0-26) [45%]	4 (0-26) [51%]	6 (0-26) [40%]
24 years	5 (0-26) [54%]	4 (0-26) [66%]	5 (0-26) [42%]
<b>Fat mass index</b> , median (range) [% missing values]			
10 years	3.8 (0.7-18.4) [50%]	3.0 (0.7-15.4) [49%]	4.4 (1.0-18.4) [51%]
12 years	4.4 (0.9-19.1) [51%]	3.8 (0.9-16.8) [50%]	5.0 (1.1-19.1) [51%]

Table 6.1

**Sample descriptives**

ALSPAC cohort

14 years	4.6 (0.8-21.9) [24%]	3.1 (0.8-17.7) [22%]	5.7 (1.5-21.9) [27%]
16 years	5.0 (0.7-22.8) [36%]	2.8 (0.7-20.0) [36%]	6.4 (1.5-22.8) [36%]
18 years	5.8 (0.5-27.9) [40%]	3.4 (0.5-24.1) [44%]	7.1 (0.5-27.9) [36%]
24 years	7.2 (0.6-28.5) [53%]	5.7 (1.8-23.2) [63%]	8.0 (0.6-28.5) [44%]
<b>Sex, n (%)</b>			
Male	3769 (47%)		
Female	4185 (53%)		
<b>Ethnic background, n (%)<sup>a</sup></b>			
Non-white	280 (4%)	134 (4%)	146 (4%)
White	6718 (96%)	3215 (96%)	3503 (96%)
<b>Maternal education, n (%)<sup>b</sup></b>			
No education	210 (3%)	89 (3%)	121 (4%)
Medium	4836 (78%)	2374 (78%)	2462 (77%)
High	1164 (19%)	561 (19%)	603 (19%)
<b>Paternal education, n (%)<sup>b</sup></b>			
No education	349 (7%)	165 (6%)	184 (7%)
Medium	3475 (66%)	1676 (65%)	1799 (66%)
High	1469 (28%)	730 (28%)	739 (27%)

<sup>a</sup>**Ethnic background:**

"White" if both parents identified as "White"; "Non-white" if either parent identified as "Black Caribbean", "I

**6.3.2 Main results**

Results of the main analyses, examining the co-development of depressive symptoms and FMI, are summarized in Table 6.2 and Figure 6.2. The model showed good fit ( $\chi^2(37) = 356.77$ ,  $p < 0.001$ ; RMSEA [95%CI] = 0.033 [0.030-0.036]; CFI = 0.991; TLI = 0.983; SRMR = 0.027).

We found positive auto-regressive associations, indicating substantial within-person stability over time in FMI (mean  $\beta$  [range] = 0.75 [0.54; 0.84], SE = 0.016) and, to a lower extent, in depressive symptoms ( $\beta$  [range] = 0.26 [0.12; 0.38], SE = 0.022).

After accounting for within-person (i.e., autoregressive associations) and between-person stability (i.e., random intercepts), the following within-person cross-lag dynamics emerged from our models (Table 6.2): higher FMI was associated with increased subsequent depressive symptoms across the study period, except between 12 and 14 years ( $\beta$  [range] = 0.07 [0.03, 0.13],

$SE = 0.030$ ); higher depressive symptoms were associated with increased subsequent FMI, although these associations were weaker on average, compared to those between FMI and future depressive symptoms ( $\beta$  [range] = 0.04 [0.03, 0.06],  $SE = 0.01$ ).

Table 6.2

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**Auto-regressive and cross-lag associations  
between depressive symptoms and FMI**

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Term		Estimate [95%CI]	Median age (years)	Time lag (years)	Yearly estimate [95%CI]
<b>Auto-regressive associations</b>	1	<b>0.84</b> [0.82; <b>0.85</b> ]	9.8 → 11.8	2.0	0.42 [0.41; 0.43]
	2	<b>0.73</b> [0.71; <b>0.76</b> ]	11.8 → 13.8	2.0	0.37 [0.36; 0.38]
	3	<b>0.84</b> [0.82; <b>0.85</b> ]	13.8 → 15.4	1.6	0.52 [0.51; 0.53]
	4	<b>0.82</b> [0.80; <b>0.84</b> ]	15.4 → 17.8	2.4	0.34 [0.33; 0.35]
	5	<b>0.54</b> [0.47; <b>0.60</b> ]	17.8 → 24.5	6.7	0.08 [0.07; 0.09]
Depressive symptoms	1	<b>0.12</b> [0.08; <b>0.16</b> ]	10.6 → 12.8	2.2	0.05 [0.04; 0.07]
	2	<b>0.32</b> [0.28; <b>0.35</b> ]	12.8 → 13.8	1.0	0.32 [0.28; 0.35]
	3	<b>0.21</b> [0.18; <b>0.25</b> ]	13.8 → 16.6	2.8	0.08 [0.06; 0.09]
	4	<b>0.38</b> [0.34; <b>0.42</b> ]	16.6 → 17.8	1.2	0.31 [0.28; 0.35]
	5	<b>0.26</b> [0.21; <b>0.30</b> ]	17.8 → 23.8	6.0	0.04 [0.03; 0.05]

Table 6.2

**Auto-regressive and cross-lag associations  
between depressive symptoms and FMI**

	1	<b>0.04</b> [0.00; <b>0.09</b> ]	9.8 → 12.8	3.0	0.01 [0.00; 0.03]
<b>Cross-lag associations</b>	2	0.03 [-0.01; 0.06]	11.8 → 13.8	2.0	0.01 [-0.01; 0.03]
	3	<b>0.13</b> [0.09; <b>0.17</b> ]	13.8 → 16.6	2.8	0.05 [0.03; 0.06]
	4	<b>0.08</b> [0.03; <b>0.12</b> ]	15.4 → 17.8	2.4	0.03 [0.01; 0.05]
	5	<b>0.08</b> [0.03; <b>0.13</b> ]	17.8 → 23.8	6.0	0.01 [0.01; 0.02]
	1	<b>0.03</b> [0.01; <b>0.05</b> ]	10.6 → 11.8	1.2	0.03 [0.01; 0.05]
<b>Dep→FMI</b>	2	<b>0.04</b> [0.01; <b>0.06</b> ]	12.8 → 13.8	1.0	0.04 [0.01; 0.06]
	3	<b>0.05</b> [0.03; <b>0.07</b> ]	13.8 → 15.4	1.6	0.03 [0.02; 0.04]
	4	<b>0.03</b> [0.00; <b>0.05</b> ]	16.6 → 17.8	1.2	0.02 [0.00; 0.04]
	5	<b>0.06</b> [0.00; <b>0.12</b> ]	17.8 → 24.5	6.7	0.01 [0.00; 0.02]
	1	<b>-0.08</b> [-0.12; <b>-0.04</b> ]	9.8 ~ 10.6	0.8	
<b>Cross- sectional correla- tions</b>	FMI ~ Dep	2	0.02 [-0.02; 0.06]	11.8 ~ 12.8	1.0
		3	<b>0.10</b> [0.07; <b>0.13</b> ]	13.8 ~ 13.8	0.0

Table 6.2

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**Auto-regressive and cross-lag associations  
between depressive symptoms and FMI**

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4	<b>0.11</b> [ <b>0.07;</b> <b>0.14</b> ]	15.4 ~ 16.6	1.2
5	-0.02 [-0.06; 0.01]	17.8 ~ 17.8	0.0
6	<b>0.10</b> [ <b>0.03;</b> <b>0.17</b> ]	23.8 ~ 24.5	0.7

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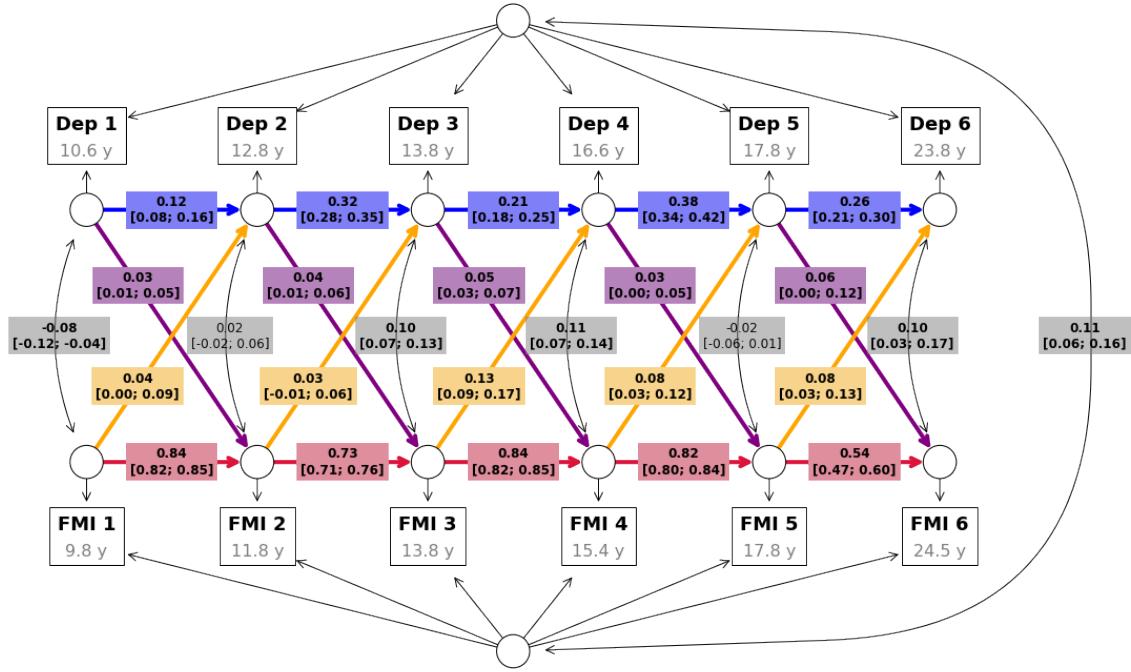


Figure 6.2: RI-CLPM of depressive symptoms and FMI

(A) Results of the main analysis (*i.e.*, beta values and their 95% confidence intervals) are displayed within their implied directed graph. Autoregressive associations are shown in red for FMI and in blue for depressive symptoms (Dep), while cross-lag effects are shown in yellow for FMI to depressive symptoms paths and in purple for depressive symptoms to FMI paths. Grey boxes enclose the correlation coefficients (and their 95% confidence intervals) between the two random intercepts, as well as the cross-sectional correlations between the two outcomes at each measurement wave.

We additionally found a positive correlation between the random intercepts ( $r$  [95%CI] = 0.11 [0.06-0.16]), suggesting some stability of between-person associations between depressive symptoms and FMI.

### 6.3.3 Exploratory analysis results

## 6.4 Discussion

### References

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## 7 Arterial health and brain development

Adapted from:

**Associations of arterial thickness, stiffness and blood pressure with brain morphology in early adolescence: A prospective population-based study**

Defina, S., Silva, C.C.V., Cecil, C.A.M., Tiemeier, H., Felix, J.F., Mutzel, R.L., & Jaddoe, V.W.V. (2023). *Hypertension* DOI [DOI](#)

### Abstract

**Background:** Arterial wall thickness and stiffness, and high blood pressure have been repeatedly associated with poorer brain health. However, previous studies largely focused on mid- or late-life stages. It is unknown whether any arterial health-related brain changes may be observable already in adolescence.

**Methods:** We examined whether (1) carotid intima-media thickness, (2) carotid distensibility, and (3) systolic blood pressure and diastolic blood pressure, measured at the age of 10 years, were associated with brain volumes and white matter microstructure (i.e., fractional anisotropy and mean diffusivity) at the age of 14 years. In addition to cross-sectional analyses, we explored associations with longitudinal change in each brain outcome from 10 to 14 years. Analyses were based on 5341 children from the Generation R Study.

**Results:** Higher diastolic blood pressure was associated with lower total brain volume ( $[95\% \text{ CI}] = -0.04 [-0.07; -0.01]$ ) and gray matter volume ( $[95\% \text{ CI}] = -0.04 [-0.07; -0.01]$ ) at the age of 14 years, with stronger associations in higher diastolic blood pressure ranges. Similar associations emerged between systolic blood pressure and brain volumes, but these were no longer significant after adjusting for birth weight. No associations were observed between blood pressure and white matter microstructure or between carotid intima-media thickness or distensibility and brain morphology.

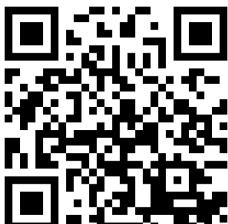
**Conclusions:** Arterial blood pressure, but not intima-media thickness and distensibility, is associated with structural neuroimaging markers in early adolescence. Volumetric measures may be more sensitive to these early arterial health differences compared to microstructural properties of the white matter, but further studies are needed to confirm these results and assess potential causal mechanisms.

## Links

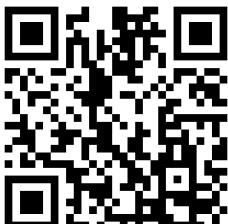
**Supplementary materials:** <https://osf.io/2f4sg>



**Project's code:** <https://github.com/SereDef/arterial-health-brain>



**OSF Pre-registration:** <https://osf.io/ryc7e>



## Keywords

Blood pressure; Carotid intima-media thickness; Carotid distensibility; Neuro-imaging; Adolescence.

## Abbreviations

Body Mass Index (BMI), Carotid intima–media thickness (cIMT), Coronary Artery Risk Development in Young Adults (CARDIA), Diffusion tensor imaging (DTI), False discovery rate (FDR), Fractional anisotropy (FA), Framingham Third-Generation Cohort Study (FHS-G3), Grey matter volume (GMV), Magnetic Resonance Images (MRI), Mean diffusivity (MD), Systolic / diastolic blood pressure (SBP / DBP), Total brain volume (TBV).

## 7.1 Introduction

While cardiovascular disease remains the leading cause of death worldwide, the global burden of brain disease is rapidly increasing and is often associated with the same risk factors, as highlighted by the latest statistical update from the American Heart Association (Tsao et al., 2022). Indeed, known cardiovascular risk factors such as hypertension (Alateeq et al., 2022; Beauchet et al., 2013), arterial stiffness (Badji et al., 2019; Baradaran & Gupta, 2020) and atherosclerosis (Baradaran & Gupta, 2020; Wang et al., 2022), have been consistently associated with the pathogenesis of cerebrovascular disease (e.g., stroke and white matter lesions) and cognitive decline in the elderly.

Accumulating evidence further suggests that more subtle vascular health-related brain changes may occur in younger adults, far before evident injury events, and even when the overall vascular burden is low. For example, the Framingham Third-Generation Cohort Study (FHS-G3) investigators reported cross-sectional associations of arterial stiffness with lower grey-matter density and white-matter microstructural integrity, assessed with diffusion tensor imaging (DTI), in the fifth decade of life (Maillard et al., 2016, 2017). Higher arterial stiffness was also associated with poorer processing speed and executive function, and with larger lateral ventricular volumes in younger adults (30-45 years) from the FHS-G3 (Pase et al., 2016). Moreover, in a group of 45-year-old adults from the Coronary Artery Risk Development in Young Adults (CARDIA) Study, higher carotid intima-media thickness (cIMT), a subclinical marker of atherosclerosis, was associated with reduced cerebral blood flow and with poorer cognitive function five years later (Al Hazzouri et al., 2015; Cermakova et al., 2020). In the same cohort, midlife hypertension (Launer et al., 2015) and increasing blood pressure trajectories from young adulthood to middle age (Hu et al., 2022) were associated with lower cerebral perfusion and volumetric markers of poor white- and grey- matter health at age 50. Blood pressure was also linearly associated with reduced grey-matter volumes and white matter microstructural integrity at age 40 in the FHS-G3 sample (Maillard et al., 2012) and with grey matter and white matter lesion volume before the age of 45 in the UK Biobank (Alateeq et al., 2022).

Although analogous neuroimaging studies in children are lacking, there is evidence linking elevated blood pressure with cognitive performance already in childhood (Lamballais et al., 2018; Lande & Kupferman, 2019). Further, related cardiovascular risk factors, such as adiposity (Silva et al., 2021; Steegers et al., 2021) and diabetes (Redel et al., 2022) have been shown to associate with brain structural changes in children and adolescents. However, thus far very little attention has been paid to the potential impact of vascular health on the brain during development, despite evidence that arterial stiffening and thickening, and blood pressure dysregulation begin very early in life (Kruger et al., 2021; Rosner et al., 2013; Song et al., 2020). From a clinical standpoint, characterizing these associations is a fundamental step in the development of early intervention strategies that may prevent abnormal brain development before irreversible structural damage occurs.

Using data from the Generation R Study, a large population-based birth cohort, we investigated how arterial thickness and distensibility, as well as systolic / diastolic blood pressure (SBP

/ DBP), measured at age 10 associate with brain morphology and microstructure extracted from MRI scans at age 10 and 14 years.

This study was [pre-registered](#). Briefly, we hypothesized that: 1) higher cIMT, 2) lower distensibility and 3) higher SBP / DBP would associate with *a*) lower total brain volume, *b*) lower grey-matter volume, *c*) lower global fractional anisotropy and/or *d*) higher mean diffusivity.

## 7.2 Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### 7.2.1 Study population

The study uses data from the Generation R Study, an ongoing population-based prospective cohort study based in the city of Rotterdam, the Netherlands. A detailed cohort description is provided elsewhere (Kooijman et al., 2016). In summary, the cohort included 9749 children born between April 2002 and January 2006. The analytical sample consisted of 5341 singletons who participated in the baseline (i.e., 10-year) visit and had no siblings in the sample (see [Figure S1](#)). Of these children, 2054 had complete structural MRI data and 2308 had complete DTI data. Missing values in all variables of interest were imputed by random-forest multiple imputation. Ethical standards. The study conforms with the World Medical Association Declaration of Helsinki (2013). Written informed consent was obtained from parents. The medical ethical committee of Erasmus MC, University Medical Center Rotterdam approved the study.

### 7.2.2 Arterial health

At the age of 10 years, carotid artery ultrasound was performed using the Logiq E9 device (GE Medical Systems, Wauwatosa, Wisconsin) while blood pressure was simultaneously assessed at the right brachial artery. Children were in supine position, with their head tilted slightly away from the transducer. The common carotid artery was identified in a longitudinal plane, ~10 mm proximal from the carotid bifurcation. Each common carotid artery was measured three times, resulting in six recordings that ideally included multiple heart cycles.

**Carotid intima–media thickness.** For each ultrasound recording, and at all R waves of the simultaneous electrocardiogram, cIMT was computed at the far wall as the average distance between lumen-intima and media-adventitia borders. Average cIMT of all frames of the acquired image sequence was then computed. Analyses were performed offline and semiautomatically, using the application Carotid Studio (Cardiovascular Suite; Quipu srl, Pisa,

Italy). Overall mean cIMT (millimeters) was standardized using a z-transformation - i.e., (value – sample mean) / sample SD.

**Carotid distensibility.** The c coefficient, i.e., the relative change in lumen area during systole for a given peripheral pressure change was calculated as the difference between the maximal (diastolic) and minimal (systolic) lumen diameter of the carotid artery. Lumen diameter was computed as the average distance between the far and near media-adventitia interfaces for each frame of the acquired image sequence. Per recording, average distension and diameter values were used to compute the average carotid distensibility. Overall mean carotid distensibility ( $\text{kPa}^{-1} \times 10^{-3}$ ) was standardized using a z-transformation.

**Blood pressure.** SBP and DBP were measured at the right brachial artery, four times with 1-min intervals, using the validated automatic sphygmomanometer Datascope Accutorr PlusTM (Paramus, New Jersey, USA). SBP and DBP (mm Hg) were determined by excluding the first measurement and averaging the other measurements, and were standardized using a z-transformation.

### 7.2.3 Brain imaging

At the ages of 10 and 14 years, participants visited the Generation R research center at Erasmus MC – Sophia Children’s Hospital, where brain Magnetic Resonance Images (MRI) were acquired using a single, dedicated 3-Tesla scanner (General Electric MR750w, Milwaukee, WI, USA) with an eight-channel head coil. To minimize head motion, participants were familiarized with the scanner environment using a mock scanner.

**Brain volume.** High resolution T1-weighted images were obtained with an inversion recovery fast-spoiled gradient recalled sequence (parameters: TR = 8.77 ms, TE = 3.4 ms, TI = 600 ms, flip angle = 10°, FOV = 220×220mm, acquisition matrix = 220×220, slice thickness = 1mm, number of slices = 230, voxel size = 1×1×1mm, ARC Acceleration = 2). Images were processed using FreeSurfer 6.0 (Fischl, 2012). The technical details of these procedures are described elsewhere (Muetzel et al., 2019). In brief, this included removal of the non-brain tissue, segmentation of white and grey matter structures, tessellation of the grey-white matter boundary, topology correction and surface deformation to identify the cortical grey-white matter and the grey-cerebrospinal fluid boundary. Reconstructions were visually inspected and those with insufficient quality were further excluded (Muetzel et al., 2019).

Global metrics of volume, i.e., total brain (TBV) and total grey matter volume (GMV), as well as specific subcortical structures’ volumes were extracted. Brain metrics were be standardized using a z-transformation.

**White matter microstructure.** DTI data were obtained using an echo-planar sequence with three  $b = 0$  scans and 35 diffusion-weighted images ( $b = 1000 \text{ s/mm}^2$ ). The following parameters were used: TR = 12.5 ms, TE = 72.8 ms, FOV = 240×240 mm, acquisition matrix = 120×120, slice thickness = 2 mm, number of slices = 65. Images were pre-processed using

**FSL 6.0.1.** Briefly, non-brain tissue was removed, images were corrected for eddy current-induced distortions and minor head motion using ‘eddy’, and the diffusion gradient table was rotated accordingly. A diffusion tensor was fit at each voxel using a weighted least squares method, and common scalar metrics including global fractional anisotropy (FA) and mean diffusivity (MD) were computed. FA describes the degree to which water diffuses preferentially along one direction (e.g., along a bundle of myelinated axons) and is sensitive to microstructural changes. MD describes the average diffusion in all directions. White matter tracts, were also delineated using fully-automated probabilistic fiber tractography as implemented in FSL AutoPtx (De Groot et al., 2013). Average FA and MD were calculated for each tract. Global and tract-specific FA and MD values were standardized using a z-transformation. Image quality was assessed by manual and automated inspection (Muettzel et al., 2018).

#### 7.2.4 Covariates

Information on the maternal age and child ethnic background (based on parental country of origin and dichotomized into European vs. non-European) was collected by questionnaire at enrollment. Date of birth and child sex, weight and gestational age were recorded at birth. Both caregivers reported on their highest completed educational level when children were 6 years old, and these reports were combined into a single “parental education” score. Child height (in m) and weight (in kg) were measured during the 10-years visit, and used to compute body mass index (BMI) z-scores.

#### 7.2.5 Statistical analysis

Analyses were conducted using R version 4.2.0(R Core Team, 2021). All scripts are [publicly available](#).

**Imputation.** Missing data in all exposures, outcomes and covariates were imputed by random-forest multiple imputation (Shah et al., 2014), using 20 imputed datasets, 10 trees and 40 iterations, as implemented by the mice R package (Buuren & Groothuis-Oudshoorn, 2011). Details of the imputation model and quality are provided in Supplementary materials ([Methods S1](#) and [Table S1](#)).

**Main analyses.** All models were fit in each imputed dataset, and pooled across imputations using Rubin’s rules (Rubin, 1987). For each exposure of interest (i.e., carotid IMT and distensibility, SBP and DBP), four multiple linear regressions were performed including *a*) TBV, *b*) GMV, *c*) global FA and *d*) global MD, measured at age 14, as dependent variable. We ran a “base model” adjusting for child sex, height, age at MRI assessment and age gap between clinical and MRI assessments, and a “confounder model”, which additionally included child ethnicity, BMI z-score, parental education and maternal age. Covariates were identified based on the graphical criteria for confounding ([Figure S2](#)).

To minimize false positive findings due to multiple testing ( $k=16$ ), false discovery rate (FDR) correction (Benjamini & Yekutieli, 2001) was applied to all p-values.

Non-linear terms for each arterial health exposure (i.e., natural splines) were retained in the model when they significantly improved its fit (see [Methods S2](#)).

**Exploratory follow-up analyses.** We further assessed associations between each exposure and the longitudinal change in each neuroimaging marker from age 10 to 14 years, using linear mixed-effects models with a random intercept per subject (see [Methods S3](#); (Bates et al., 2015)). Note that brain outcomes were not standardized for these analyses to prevent incorrect estimation of the correlation structure.

To further characterize the regional specificity of these effects, we assessed associations with: total intracranial, cerebro-spinal fluid, white matter, cortical and subcortical grey matter volumes, as well as subcortical regional volumes (Accumbens, Amygdala, Caudate, Hippocampus, Pallidum, Putamen, Thalamus); white-matter tracts FA and MD (Cingulate gyrus, Cortico-spinal tract, Uncinate fasciculus, Inferior & Superior longitudinal fasciculus, Major & Minor forceps), and vertex-wise cortical thickness (see [Methods S4](#)) at age 14. These latter analyses were further adjusted for total intracranial volume.

Finally, since sex differences and premature birth have been implicated in the associations of interest (Gluckman et al., 2008; Pasha et al., 2018), we *a*) investigated effect modification by sex and *b*) additionally adjusted the main models for birthweight and gestational age at birth.

**Sensitivity analyses.** To assess the impact and adequacy of our sample selection and imputation procedure, we ran all analyses in the full cohort ( $n=9749$ ) and in the subsample with complete outcome data ( $n=2054-2308$ ).

## 7.3 Results

Participants characteristics. Sample descriptives are displayed in Table 7.1 (see also [Tables S1-S2](#)).

Table 7.1

Sample descriptives		
Generation R (GenR)		
Measure	Time point	Descriptives
Sex, n (%)	Birth	
Male		2647 (49.6%)
Female		2694 (50.4%)
Ethnic background, n (%)	Birth	

Table 7.1

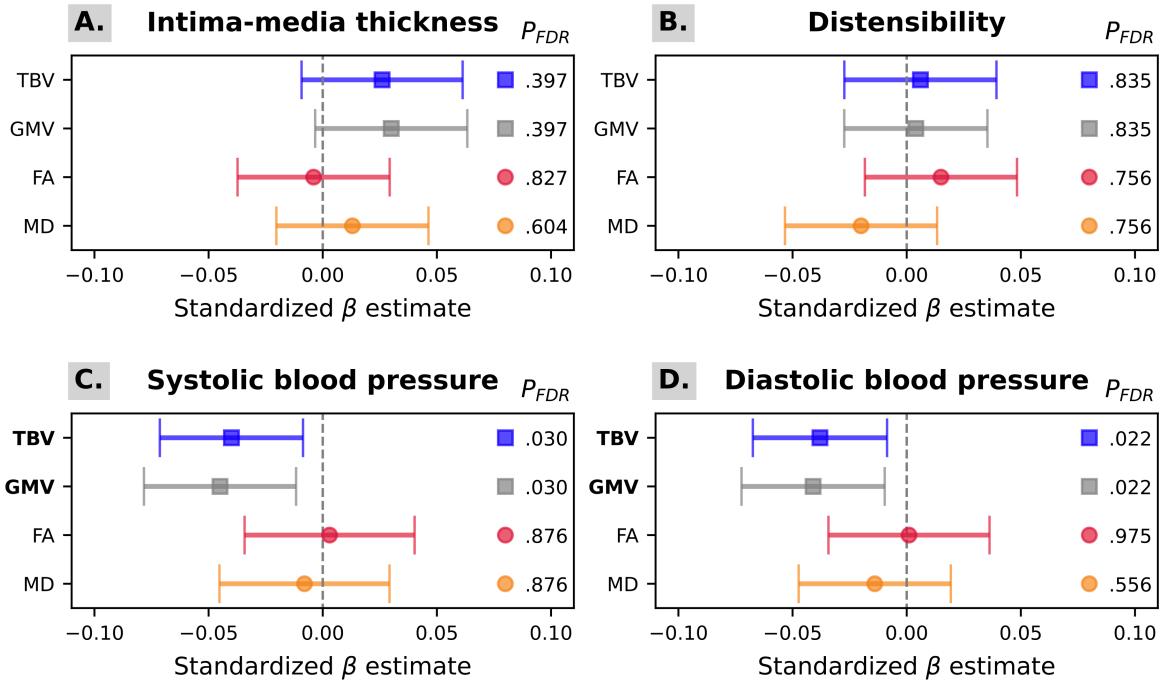
<b>Sample descriptives</b>		
Generation R (GenR)		
European		3448 (64.6%)
Non-European		1893 (35.4%)
Maternal age, years, mean (SD)	Birth	30.9 (5.0)
Maternal education <sup>a</sup> , n (%)	6y	
Low		2447 (45.8%)
Medium		1424 (26.7%)
High		1470 (27.5%)
Height, cm, mean (SD)	10y	141.5 (6.7)
Body mass index (BMI), kg/m <sup>2</sup> , median [range]	10y	17.01 [12.02-35.41]
Carotid intima-media thickness (IMT), mm, mean (SD)	10y	0.46 (0.04)
Carotid distensibility, kPa <sup>-1</sup> × 10 <sup>-3</sup> , mean (SD)	10y	57.2 (12.4)
Systolic blood pressure (SBP), mm Hg, mean (SD)	10y	103 (8)
Diastolic blood pressure (DBP), mm Hg, mean (SD)	10y	59 (6)
Age (at MRI visit), years, median [range]	10y 14y	9.98 [8.55-13.00] 13.82 [12.59-16.68]
Total brain volume (TBV), cm <sup>3</sup> , mean (SD)	10y 14y	1196.8 (97.5) 1223.8 (95.5)
Grey matter volume (GMV), cm <sup>3</sup> , mean (SD)	10y 14y	750.7 (58.6) 748.5 (56.3)
Global fractional anisotropy (FA), mean (SD)	10y 14y	0.54 (0.02) 0.55 (0.01)
Global mean diffusivity (MD), mm <sup>2</sup> /sec × 10 <sup>3</sup> , mean (SD)	10y 14y	0.82 (0.02) 0.80 (0.02)

<sup>a</sup>Maternal education: low = “secondary, phase 2” or lower; medium = “higher, phase 1”; high = “higher, phase 2”. Categorization based on ISCED 2011.

### 7.3.1 Main analyses

An overview of the main results is presented in Figure 7.1. Complete model outputs are reported in supplementary materials ([Table S3](#)). Neither carotid IMT nor distensibility were

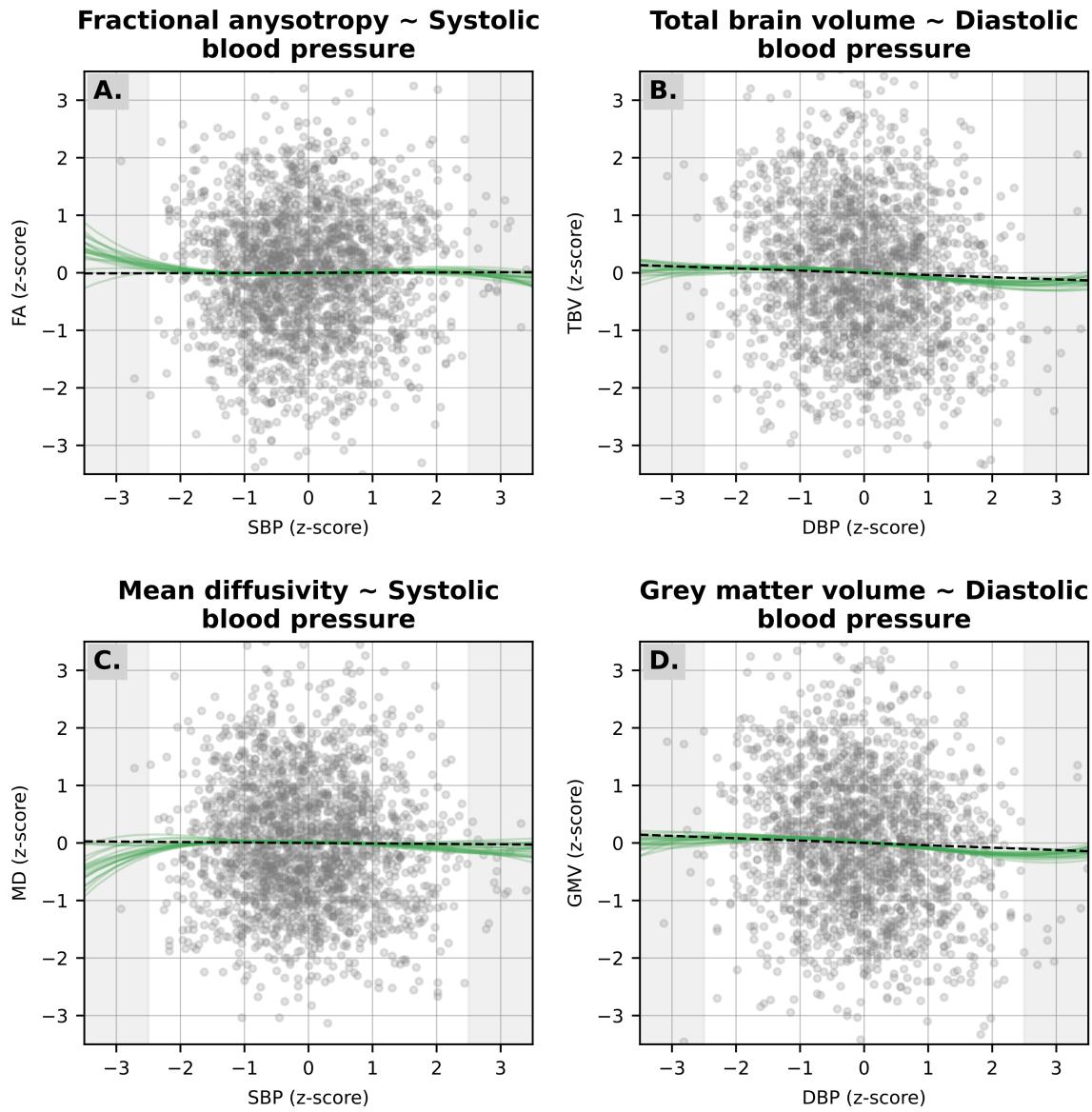
significantly associated with any brain outcome (Figure 7.1 A-B, [Table S3](#)). After adjustment for potential confounders, SBP was (cross-sectionally) associated with lower TBV ( [95%CI] = -0.04 [-0.07;-0.01],  $P_{FDR} = .030$ ) and GMV ( [95%CI] = -0.05 [-0.08;-0.01],  $P_{FDR} = .030$ ) but not with either FA or MD (Figure 7.1 C, [Table S3](#)). Analogously, DBP was (cross-sectionally) associated with TBV ( [95%CI] = -0.04 [-0.07;-0.01],  $P_{FDR} = .022$ ) and GMV ( [95%CI] = -0.04 [-0.07;-0.01],  $P_{FDR} = .022$ ), but not FA or MD (Figure 7.1 D, [Table S3](#)).



*Figure 7.1: Arterial thickness, stiffness and blood pressure and brain morphology at 14 years.*

(A-D) For each exposure of interest: **A.** intima-media thickness (IMT); **B.** carotid distensibility; **C.** Systolic blood pressure (SBP); and **D.** Diastolic blood pressure (DBP), the standardized association estimates and their 95% confidence intervals are displayed on the x-axis for each outcome (total brain volume (TBV) in blue; grey matter volume (GMV) in grey; global fractional anisotropy (FA) in red; and mean diffusivity (MD) in orange). The corresponding FDR-corrected P-values are also reported.

We found evidence of a non-linear relationship between SBP and white matter microstructure ( $P=.032$  for FA;  $P=.023$  for MD) and between DBP and brain volumes ( $P=.012$  for TBV;  $P=.006$  for GMV). However, these departures from linearity were small, as shown in Figure 7.2 and [Figure S3](#).

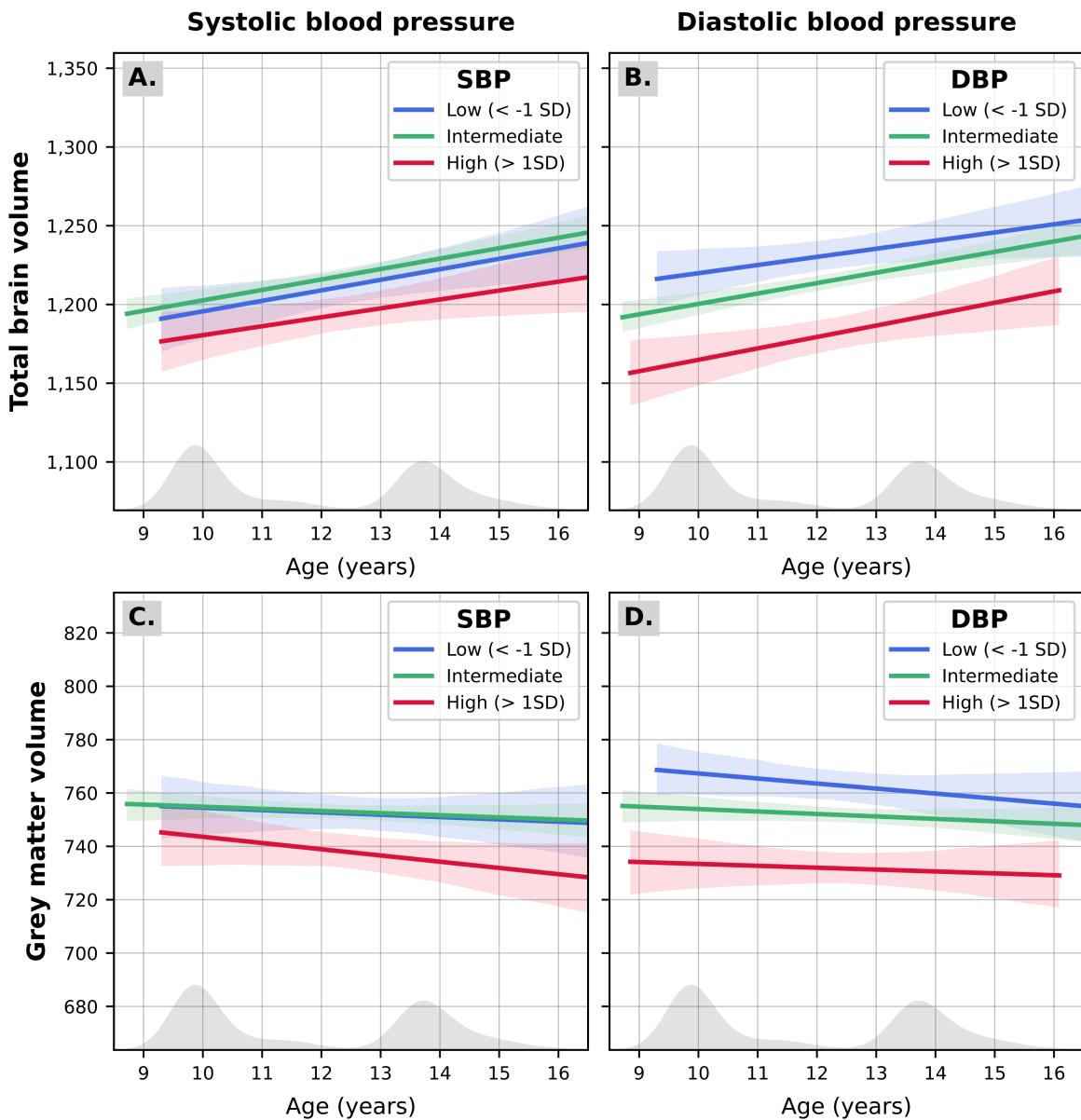


*Figure 7.2: Non-linear relationships between blood pressure and brain morphology at 14 years.*

(A-D) The linear and non-linear relationship between SBP and A. FA; and C. MD; and between DBP and B. TBV; and D. GMV. Linear associations (black dashed line) were pooled across datasets (and correspond to the estimates presented in Figure 1), while non-linear associations (green continuous lines) were fit in each imputed dataset individually using natural splines with 4 knots. The gray vertical shadows also mark the  $-2.5$  and  $+2.5$  SD cutoffs of each exposure distribution.

### 7.3.2 Exploratory follow-up analyses

In our exploratory longitudinal models, we did not find any significant interaction between arterial health markers and age at MRI measurement ([Table S4](#); Figure 3). However, when interaction terms were excluded from the models, we could further confirm a negative main effect of SBP and DBP on total brain and grey matter volumes measured at 10 and 14 years ([Table S5](#)). Specifically, while TBV increased with age ( $b$  [95%CI] = 7.0 [6.4; 7.7]  $\text{cm}^3$  per year) each SD increase in SBP was associated with -3.6 [-6.4; -0.8]  $\text{cm}^3$  ( $P_{FDR} = .048$ ) and each SD increase in DBP was associated with -3.4 [-6.1; -0.7]  $\text{cm}^3$  ( $P_{FDR} = .040$ ) TBV. Conversely, GMV showed a slight decline over our age range ( $b$  [95%CI] = -0.6 [-1.0; -0.2]  $\text{cm}^3$  per year) while each SD increase in SBP was associated with -2.4 [-4.1; -0.7]  $\text{cm}^3$  ( $P_{FDR} = .042$ ) and each SD increase in DBP was associated with -2.2 [-3.8; -0.6]  $\text{cm}^3$  ( $P_{FDR} = .039$ ) GMV.



*Figure 7.3: Structural brain changes from 10 to 14 years.*

(A-D) The longitudinal change in total brain (A, B) and grey matter volume (C, D) is represented for children with “high” levels of exposure (i.e.,  $> 1$  SD above the mean in systolic or diastolic blood pressure; in red), exposure values in the intermediate range (i.e., between  $-1$  and  $1$  SD around the mean; in green) or low levels of the exposure (i.e.,  $< -1$  SD below the mean; in blue). The distribution of age at MRI measurement is also depicted in grey on the bottom of each graph.

Associations were largely homogenous between sexes (Figure S4, Table S6) and did not seem to be explained by differences in cranium size, nor by a significant increase in cerebro-spinal fluid volumes (Figure S5, Table S7). There were no significant associations between either exposure and total or region-specific subcortical volumes (Figure S5, Table S8) nor with white matter tracts FA and MD (Figures S6-S7, Tables S9-S10). SBP was significantly associated with total cortical volume ( $[95\%CI] = -0.05 [-0.08;-0.01]$ ,  $P_{FDR} = .036$ ; see Figure S5 and Table S7), but no local associations with cortical thickness emerged from the vertex-wise analyses (Table S11).

Notably, the associations between SBP and total/grey matter volumes were significantly attenuated after adjusting for birth-related covariates (TBV:  $[95\%CI] = -0.03 [-0.06; 0.01]$ ,  $P_{FDR} = .230$ ; GMV:  $[95\%CI] = -0.03 [-0.06; 0.00]$ ,  $P_{FDR} = .229$ ; see Table S12).

### 7.3.3 Sensitivity analyses

Restricting the analyses to participants with complete outcome data ( $n = 2054$  for structural MRI and 2308 for DTI) did not substantively change the reported findings, besides a slight increase in effect sizes for both SBP (TBV:  $[95\%CI] = -0.05 [-0.09;-0.01]$ ,  $P_{FDR} = .016$ ; GMV:  $[95\%CI] = -0.06 [-0.09;-0.02]$ ,  $P_{FDR} = .013$ ) and DBP (TBV and GMV:  $[95\%CI] = -0.05 [-0.09;-0.02]$ ,  $P_{FDR} = .012$ ; see Tables S13-S14).

## 7.4 Discussion

In this population-based prospective cohort study, we observed that systolic and diastolic blood pressure at age 10 years were associated with lower total and grey matter volumes at age 10 and 14 years. The associations between systolic blood pressure and brain volumes were partially explained by birth-related confounders (particularly birthweight). No significant associations were observed for carotid intima-media thickness or distensibility, nor for white matter microstructural markers.

### 7.4.1 Interpretation of main findings

To the best of our knowledge, this is the first study to establish a link between high blood pressure and brain volume reduction in the general pediatric population. Together with previous pediatric neuroimaging studies focusing mainly on adiposity (Brain Development Cooperative Group, 2012; Silva et al., 2021; Steegers et al., 2021), our findings confirm the idea that, already at school age and within subclinical ranges, an adverse cardiovascular profile may negatively impact brain development.

We could not find evidence for a significant role of arterial wall thickness or stiffness on the adolescent brain, as we had expected based on adult reports (Cermakova et al., 2020; Maillard

et al., 2016). This might have been due to insufficient sensitivity of ultrasound measures and/or lack of variability in these markers in healthy pediatric population.

In line with our findings, several previous studies reported a negative association between (diastolic) blood pressure and cognitive function in children and adolescents (Dawson et al., 2021; Lamballais et al., 2018; Lande & Kupferman, 2019; Lucas et al., 2022). The biological substrate underlying these associations, however, is less studied. Interestingly, a few transcranial Doppler ultrasound studies demonstrated blunted cerebrovascular reactivity in hypertensive children (Lande & Kupferman, 2019; Wong et al., 2011), however, these were conducted in small samples and suffer from wide inter-observer measurement variability. We corroborated and extended these findings using more stable structural MRI markers, and found that morphological brain outcomes (volumetric measures) may be already sensitive to early increase in arterial blood pressure. This was not true for microstructural properties of the white matter, which was unexpected, as adult studies typically point to impaired white matter integrity as an early marker of neurovascular pathology (Maillard et al., 2012). However, it is important to note that both macroscopic and microstructural changes reported in older populations may reflect different processes (e.g., cellular atrophy and white matter lesions) compared to earlier developmental windows, where the same neuroimaging markers may underlie, for instance, grey and white matter maturation. Indeed, while grey matter maturation tends to peak already in early adolescence, white matter growth typically doesn't peak until mid-adulthood, which could partly explain the observed pattern of results (Groeschel et al., 2010).

#### **7.4.2 Biological mechanisms**

Several potential mechanisms could explain the reported associations. For example, it was proposed that high blood pressure could cause damage to the neurovasculature and result in reduced cerebral blood perfusion, leading to suboptimal oxygen and nutritional supplies, and potentially altering ongoing neurodevelopmental processes (Lucas et al., 2022). Additionally, alterations in immune and hypothalamic-pituitary-adrenal axis functioning resulting from chronically high blood pressure could trigger neuroinflammation, further impairing brain development (Perrotta et al., 2018).

#### **7.4.3 Clinical relevance**

Over the past decades, the prevalence of high blood pressure among children and adolescents has increased dramatically, in concert with the global epidemic of obesity (Riley et al., 2018). Elevated blood pressure in childhood is known to track into adulthood and increase the risk of cardiovascular disease (Yang et al., 2020). In our 10-year-old sample, each 6 mmHg increase in diastolic blood pressure (i.e., 1 SD) was followed by a 3.6 cm<sup>3</sup> reduction in total brain volume, and a 2.3 cm<sup>3</sup> reduction in grey matter volume four years later. In our longitudinal follow-up analysis, we could further show how this corresponded to about one third of the estimated yearly change in TBV. These results are important from a developmental perspective, since

they suggest that early detection and prevention of elevated blood pressure may be relevant not only for long-term cardiovascular health but also for structural brain development. However, whether these associations are clinically relevant (e.g., in terms of cognitive performance or mental health symptomatology) or whether they will evolve into long-term alteration of brain health, needs to be further studied. Nevertheless, pediatric screening for high blood pressure may be a relatively easy measure to implement to monitor and potentially decrease the risk of future brain disease and cognitive impairment.

#### **7.4.4 Strengths and limitations**

This study used prospectively measured data from a large population-based sample. Detailed and objective measures of both arterial and brain health were available and selection bias due to non-response was addressed thoroughly using multiple imputation and sensitivity analyses. However, it is important to note that, although we adjusted the analyses for several socio-demographic and lifestyle factors known to influence the associations, residual confounding, for example by genetic predisposition, exposure to stress, nutritional intake or physical activity, may still be present. Additionally, note that, for practical reasons, blood pressure was measured in supine position in this study, as opposed to more conventional sitting measures. Further studies are undoubtedly needed to replicate and validate these findings, and to further investigate potential underlying mechanisms and/or modifiable factors that could explain or attenuate these associations.

#### **7.4.5 Conclusions**

High blood pressure in childhood was associated with suboptimal brain development already in early adolescence, particularly with respect to reduced total brain and grey matter volumes. Pediatric screening for high blood pressure may thus be relevant not only for the prevention of long-term cardiovascular health problems, but also for structural brain development. Identifying such early biomarkers of neurovascular health entails important implications for future clinical and public health priorities, especially considering the established relationship between these markers and later prognosis of cerebrovascular disease and dementia.

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# 8 General discussion

In this dissertation we observed that systolic and diastolic blood pressure at age 10 years were associated with lower total and grey matter volumes at age 10 and 14 years. We focus on adolescence, a critical developmental period, when many mental health problems first emerge, and early signs of cardio-metabolic dysregulation begin to manifest. By examining these relationships prospectively, this thesis' goal was to provide a clearer understanding of (a) how ELS impacts psycho-physical health and (b) how mental and physical health influence each other, during this critical, formative stage of life.

From a clinical standpoint, characterizing these associations is a fundamental step in the development of early intervention strategies that may prevent mental and physical health problems before the onset of more severe symptoms and irreversible structural changes (e.g. to the brain, the heart or the arteries).

Now, I started this book with a confession: I absolutely hated writing this thesis, down to almost every paragraph. But, because you made it this far - or anyway you happened to open the book on this doomed last chapter - you won the dubious reward of getting to know why. Here is a collection of things I would have done differently, followed by a few things I wish we all did differently.

## 8.1 Modelling ELS

In the four chapters enclosed in Part I of this thesis, I have tried to map the influence of ELS on physical and mental health, including some biomarkers. I say “influence” with cognition, mind you. I saw you raising your eyebrow. “Causal wording, rephrase” is among the most common “co-author comments” I’ve seen across the years, which should have surprised me after 3 university degrees, in three different fields, all dominated by the same mantra: “correlation is not causation”.

### 8.1.1 Causal(-ish) inferences

This comes from a good place of course, because it is so true and also so frequently i want to shout it at

### **8.1.2 Parental reports**

Like most previous literatures, children's stress exposure information was obtained via parental report and thus likely reflect a combination of parents' own stress experiences, psychological state, and personality.

### **8.1.3 Missing piece: resilience**

### **8.1.4 Information bias?**

Our measurement of ELS, lifestyle behaviours and internalizing symptoms rely primarily on parent reports, which might have introduced information bias. Does that generalise to self reports? Other measures of adolescent behaviour, e.g., externalizing problems, could represent interesting targets for future studies in the field.

## **8.2 Alternative modelling methods**

A cumulative risk approach examines the number of stressors experienced rather than the intensity or the pattern of stress exposure. We define stressors dichotomously and then sum across multiple dichotomous exposures. As an additive model, this technique is a straightforward, way of identifying children at increased odds for developing a range of maladaptive outcomes, including cognitive deficits, behavioral adjustment problems, and poor physical health.

Multiple stressors exposures can sometimes overlap (e.g., harsh and unresponsive parenting) or be independent (e.g., life events), but in each case prediction is enhanced by combining multiple risks in the model. Multiple stressors exposures can sometimes overlap (e.g., material deprivation and overcrowding) or be independent (e.g., death of a parent and overcrowding), but in each case prediction is enhanced by combining multiple risks in the model. There are several advantages of formulating indices of multiple stress exposures into a composite score. Measurement error is reduced, validity is enhanced because no one singular measure adequately captures the full meaning of the construct of interest, avoids the issue of collinear predictors in the same general linear model, which may lead to unstable estimates and diminish statistical power. For example, many low-income families live in substandard housing, located in high crime neighborhoods; their children may attend schools with inadequate facilities, staffed by less experienced teachers; and many live in single parent households.

It does not take into account interaction between the individual stressors. Singling out the effects of school noise exposure on reading quality without taking into account these overlapping risk factors for reading deficits could overestimate the harmful impacts of noise on reading deficits. On the other hand, noise by itself may have negligible impact on reading deficits except when accompanied by household disadvantage or only if the exposure happens within the context

of a school with inadequate facilities and staff. In the latter case, by isolating the singular impact of noise exposure as a risk factor for reading deficits, we might erroneously conclude that noise does not matter. It may in fact be a contributing risk factor for reading deficits but only when considered within the natural ecological context wherein high noise schools typically operate. Another way to think about this is that perhaps there is no main effect of noise on reading deficits but an interaction or moderator effect. Noise matters but only in the presence of certain other variables. As we explain below however, the use of an interactive, nonadditive model of multiple risk exposure is often not possible when a large number of risk factors are under consideration. Use of additive models of multiple risk factors is a common approach for dealing with this dilemma.

## **8.3 Modelling comorbidity**

“Together with previous pediatric neuroimaging studies focusing mainly on adiposity (Brain Development Cooperative Group, 2012; Silva et al., 2021; Steegers et al., 2021), our findings confirm the idea that, already at school age and within subclinical ranges, an adverse cardiovascular profile may negatively impact brain development.”

Additionally, alterations in immune and hypothalamic-pituitary-adrenal axis functioning resulting from chronically high blood pressure could trigger neuroinflammation, further impairing brain development

### **8.3.1 old data, old insights: mental health**

How do we measure mental health? Badly In this thesis, we relied on standardized questionnaires, such as the Strengths and Difficulties Questionnaire (SDQ), which provide a composite score of emotional and behavioral difficulties. While these tools are widely used and validated, they are not without limitations. Self-reports and parent-reports are subject to biases, including social desirability and recall bias. Furthermore, these measures often fail to capture the dynamic and context-dependent nature of mental health, which can fluctuate over time and across environments.

Future research should aim to integrate multi-method approaches, combining self-reports, observational data, and physiological markers, to provide a more comprehensive assessment of mental health.

### **8.3.2 old data, old insights: BMI**

Measuring mental health is an inherently hard task: it requires coming up with a tangible (or better, measurable) quantity to stand in for an abstract, multifaceted and frustratingly complex construct. You would think the job is considerably easier when the object of study is

something like adiposity, which enjoys a much longer, solid tradition of objective measurement and clinical usefulness. Well, let's talk about BMI.

In this thesis, we made out best effort to stir away from BMI when we measured adiposity and here is a few thoughts on why i'd defend this choice.

The concept of BMI was introduced in 1835 by Adolphe Quetelet, a Belgian astronomer. Quetelet became increasing interested in defining the characteristics of the “average man” (*l'homme moyen*, to use his words)... among French and Scottish conscripts. (a concept than was about to become very popular among Eugenics enthusiasts)

BMI has since become a widely used tool in public health and clinical settings, although it was never intended to be a diagnostic measure of individual health or adiposity. Its simplicity and ease of use have contributed to its popularity, so much so that, today, virtually everyone has heard of BMI.

but it has significant limitations, particularly in distinguishing between muscle and fat mass, and in accounting for variations in body composition across different populations.

### **8.3.3 Alternative modelling methods**

network

#### **the role of lifestyle and puberty**

“while we focused here on three lifestyle markers that are most relevant to the developmental period of interest and may constitute important targets for early prevention, other potentially relevant factors such as smoking, alcohol use or psychological coping strategies, are also likely to be of relevance (Hanlon et al., 2020; Skou et al., 2022) and should be taken into account in future studies.”

## **8.4 So what was this all for?**

So what was this all for? Excellent question. Kept me up a few nights I argued prevention of comorbidity was key, but am I actually any closer, even purely theoretically to a useful insight?

### **8.4.1 Preventing ELS**

In Chapter 3 you can find me saying: “*While these findings certainly support the importance of primary prevention programmes aimed at reducing the incidence of ELS, preventing ELS may not always be possible. As such, there is a need to identify alternative modifiable factors that could mitigate the negative impact of ELS on later health, and inform the development of complementary intervention strategies.*”

#### **Lifestyle**

In the same chapter I did not find evidence of such promising “modifiable factors”. Based on data from two independent population-based cohorts, either physical activity, sleep or dietary behaviour did not attenuate the association between ELS exposure and adolescent psycho-physical comorbidity. That was surprising to me, but also, as I later came to find out not so rare in the research on lifestyle factors. “Developmental timing and / or differences in outcome measurement may have played a role in explaining this discrepancy. It is possible, for example, that engaging in healthy lifestyle behaviours later in life may be more beneficial, or that the protective effects of childhood lifestyle behaviours may manifest only later in adulthood. It is also possible that the associations reported in the adult literature may be biased through reverse causation (e.g., depression being a causal risk factor for poor diet and sleep; (Choi et al., 2020).” “differential measurement error could have played a role in explaining this finding. Indeed, our ELS measure was considerably more comprehensive (i.e., comprised of many more items and covering a longer time period) compared to each of the lifestyle factors, which were only assessed at a single time point and based on fewer indicators. It is possible, for example, that the hypothesized moderation effects may emerge when a more long-term engagement in physical activity is considered.”

#### **SES**

What seems to emerge from the studies is that the key factor across physical and mental health was the contextual risk component of the ELS score. Let’s unpack that. The key stress indicators within contextual risk are low parental education, financial and neighborhood problems. These are constructs commonly referred to as low socio-economic status (SES).

## **8.5 Ethnicity**

In some chapters, ethnicity was reduced to “White” vs. “non-White” (most often because of limitation of the data), however this is a major limitation.

## **8.6 Science is dead, and we killed it**

Before I let you go, dear reader, there is one more thing I need to get off my chest, if you'll let me. I began this book with a confession: writing this was so much harder than I expected, and in all honesty this is a big part of why. potential publication bias may limit the generalizability of our conclusions. Studies with null findings are less likely to be published than those with statistically significant results. journals should not gatekeep knowledge.

The academic publishing dream: You do the research - You write the paper - You review someone else's paper (for free) - Then you pay to publish your own - And your university pays again so others can read it. Meanwhile, publishers sit back and make huge profits — built on public funding, unpaid labor, and a prestige system they didn't create but fully exploit. And we keep playing along. Maybe it's time we stop pretending this is normal. There are alternatives. Fair, open, non-profit models exist — run by researchers for researchers. Think community-owned journals, preprints, and platforms that don't turn scientific knowledge into a paywall business. We don't need to burn down the system — we just need to stop feeding it. It is about time academics and academic leaders learn the craft of negotiating. Had they had it, things might have ended differently and they (and the tax payer) would not have been exploited in this ridiculous way. Perhaps it is not too late yet: get organised and bargain. Publishers are not invincible! If there is such a sincere agreement in academic community that the current model is not sustainable, then where are lawsuits? And why such lawsuits are not brought up by national-level funding watchdogs, as after all it is mostly public funding that's being extracted via scientific publication process to... where?

<https://news.justia.com/antitrust-lawsuit-brought-against-academic-publishers-for-peer-review-and-submission-restrictions/>

code is the scientific product

## **References**