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Exploring the Influence of Early-Life Diet and Other Environmental Exposures on Pubertal Timing

MARTA FILIPA PINTO DA COSTA

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Throughout the preparation of this PhD thesis, I actively collaborated in defining the hypotheses under study and objectives to be answered in each of the articles, as well as in the collection, curation, analysis and interpretation of the data. I participated in the process of handling data and checking databases related to breastfeeding, dietary intake and puberty in G21 and GENOBOX studies, as well in the harmonization of puberty data for the ATHLETE project. I also contributed to the dissemination of results in both national and international scientific conferences. I have supervised a bachelor's thesis. I was responsible for writing the first draft of all the manuscripts and first authored and actively collaborated in preparing the final versions of all publications.

This research was conducted within the framework of the Doctoral Program in Public Health at the Faculty of Medicine of University of Porto, at the Epidemiology Research Unit (EPIUnit) and the Laboratory for Integrative and Translational Research in Population Health (ITR) of the Institute of Public Health of the University of Porto (ISPUP), under the supervision of Professor Sofia Vilela (*EPIUnit, ITR*), Professor Joana Araújo (*Departamento de Ciências da Saúde Pública e Forenses e Educação Médica da Faculdade de Medicina da Universidade do Porto, EPIUnit* and *ITR*), and Professor Augusto Anguita-Ruiz (*Barcelona Institute for Global Health*).

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Para o meu filho, João Duarte.
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Abstract

Background

Puberty is a critical biological transition between childhood and adolescence, characterized by physical, hormonal, and reproductive changes. Recent evidence shows a worldwide declining trend in age at menarche and pubertal onset, particularly in girls. Early pubertal development has been associated with an increased risk of cardiometabolic and psychosocial disorders, as well as hormone-related cancers. While genetic factors account for a substantial proportion of inter-individual variability in pubertal timing, non-genetic factors, such as body weight status, early-life feeding practices (including breastfeeding and childhood dietary intake), physical activity, sleep, and urban environmental exposures may also influence pubertal timing. However, most studies on puberty have focused on girls, with findings that are not entirely consistent and, in some cases, are limited.

Objectives

The main aim of this thesis was to explore how early-life eating habits, lifestyle, and environmental exposures, from childhood to adolescence, are associated with pubertal timing using longitudinal data from two population-based cohorts, as well as one case-control study, studying the effect beyond obesity. The following specific objectives were defined:

1. To evaluate the tracking of diet quality from pre-school age (4 years) to adolescence (13 years) and its associated factors (Generation XXI) (Paper I);

2. To determine whether the predicted Maturity Offset is a good proxy of puberty timing, in comparison with the Tanner stage evaluation (Generation XXI and GENOBOX) (Paper II);

3. To specifically examine whether breastfeeding and its duration are associated with pubertal timing (Generation XXI) (Paper III);

4. To understand the role of diet quality and other lifestyle behaviours across childhood on pubertal timing (Generation XXI) (Paper IV);

5. To evaluate the effects of early-life urban environmental exposures on pubertal timing, within an exposome framework (Generation XXI and INMA) (Paper V).

Participants and Methods

This thesis included data from three European children's populations: the Portuguese population-based birth cohort Generation XXI (G21) (n=8647), the Spanish multicentre case-control GENOBOX study (n=1699), and the Spanish prospective INMA cohort (n=3768).

In the G21 cohort, a subsample of children was evaluated at 6-, 15-, and 24-months, and the entire cohort was re-evaluated at 4, 7, 10, 13 and 18 years. In the GENOBOX study, participants aged 2 to 18 years were cross-sectional evaluated, but for this thesis, only participants aged 8-18 years were considered. In the INMA cohort, follow-up assessments were conducted from pregnancy through childhood (birth, 6 months, 1–1.5, 2–2.5, 3, 4–5, 6–7, 9–10, and 12–13 years). In all studies, trained personnel conducted face-to-face interviews and collected information on sociodemographic, clinical, and lifestyle characteristics. In addition, anthropometric measurements, pubertal development evaluations, biological samples, and environmental exposure data were obtained.

The outcomes examined in this thesis included indicators of pubertal development (Tanner stages, Puberty score and Pubertal Development Scale (PDS)), age at menarche, and trajectories of diet quality. Pubertal development was performed by trained professionals through the Tanner stage at G21, GENOBOX and one INMA-subcohort (Valencia). Additionaly, in G21, a Pubertal score was developed by summing Tanner stages for genital (in males) or breast (in females) and pubic hair (in both), ranging from 2 to 10. The PDS was used in INMA, and was parent-reported. Age at menarche was self-reported by girls at 9- (INMA), 10-, 13- and 18-years follow-up (G21). Diet quality was assessed in G21 using a Healthy Eating Index (HEI) at ages 4, 7, 10 and 13 years, ranging from 8 to 32 points, based on a Food Frequency Questionnaire validated and calibrated in this sample.

Exposures included sociodemographic, maternal, and child factors. Maternal exposures comprised age, education, pre-pregnancy Body Mass Index (BMI), and age at menarche as a proxy for genetic heritability. Child characteristics included sex, exact age at

assessment, birth weight, BMI, dietary intake, sleep duration, sedentary time, and practice of physical activity. Dietary exposures included HEI, intake of ultra-processed foods (UPF), and human milk breastfeeding duration and exclusivity, categorized as never, non-exclusive, or exclusive. Environmental exposures during pregnancy, at birth, 1 year, and 4–5 years were also assessed in G21 and INMA using Geographic Information System (GIS)-based models.

Statistical analyses varied according to the research questions and outcomes of each paper. For Paper I, the tracking of HEI across ages 4 to 13 was assessed using intraclass correlation coefficients (ICC), and linear mixed-effects models were used to examine associations between child and maternal characteristics and HEI trajectories over time. In Paper II, early normal puberty was defined using sex-specific Tanner stage cut-offs, and predicted Maturity Offset (pMO) was estimated using modified Moore equations. Paper III and IV used multinomial logistic regression, Cox proportional hazards models, and ordinal logistic regression to estimate associations of early lifestyle behaviours with Puberty score and age at menarche, adjusting for several sociodemographic and child characteristics. Paper V used an Exposome-Wide Association Study (ExWAS) framework and hierarchical clustering to evaluate single and combined environmental exposures in relation to pubertal development (PDS, Tanner stages, and age at menarche), with ordinal logistic regression used to estimate associations while adjusting for relevant covariates and applying correction for multiple testing.

Results

Paper I

Overall diet quality decreased from early childhood [mean (SD): 22.2 (3.6)] to adolescence [18.2 (3.9)], with moderate tracking over time (ICC = 0.53). Higher maternal age (β = 0.079, 95% CI: 0.061; 0.097), maternal education (β = 0.203, 95% CI: 0.178; 0.229), and household income at baseline (β = 0.024, 95% CI: 0.007; 0.041) were positively associated with diet quality over time. In contrast, greater time spent in sedentary activities (β = -0.009, 95% CI: -0.014; -0.003) was negatively associated with diet quality. Being female (β = -0.094, 95% CI: -0.132; -0.056) and having longer sleep duration (β = 0.039, 95% CI: 0.015; 0.064) were also linked to higher diet quality throughout childhood and adolescence.

Paper II

In G21, at the 10-year follow-up, the mean ages were 11.7 (1.6) years for girls and 12.5 (1.4) years for boys, with most participants at Tanner Stages 2-3. In GENOBOX, mean ages were 12.9 (2.0) years for girls and 13.4 (1.7) years for boys, with most boys at Tanner Stage 2 and most girls at Stage 5. In both studies, peak height velocity occurred at Tanner Stage 3 in girls and at later stages in boys. Statistically significant differences in pMO between studies were observed at several Tanner Stages, with higher values in GENOBOX. Overall, children classified as early normal developers had consistently lower pMO values than normal developers at the same Tanner stage in both studies.

Paper III

After adjusting for confounders, females who were breastfed (exclusive or non-exclusive) had lower Puberty scores at 10 years compared to those never breastfed. Exclusive breastfeeding was also associated with later age at menarche. In boys, no significant associations were observed, except for those receiving non-exclusive breastfeeding, who had lower odds of being in the highest Puberty score (≥5).

Paper IV

Higher diet quality at age 4 was associated with lower Puberty scores (OR=0.976; 95% CI: 0.955; 0.997) and a later age at menarche (β=0.967; 95% CI: 0.935; 0.999) in females, although the latter association was attenuated after adjustment for maternal characteristics. Higher consumption of energy-dense foods at 4 years of age and meat and meat products at 7 years of age was associated with earlier menarche, particularly among girls with normal weight. Positive associations between the 'Fish and eggs' group, fatty acids, vitamin D, zinc and calcium and Puberty scores were observed mainly in boys. No consistent associations were found for sleep duration or sedentary behaviour, whereas physical activity was associated with age at menarche only among girls with overweight or obesity.

Paper V

In the G21 cohort, living in more unfavourable urban environments during childhood, characterised by higher building density, traffic, and air pollution and lower access to natural spaces, was associated with an earlier age at menarche (β=-0.181; 95% CI: -0.339; -0.024). In the INMA cohort, boys exposed to more unfavourable environmental profiles

during childhood showed more advanced pubertal development (β=0.437; 95% CI: 0.049; 0.825). Among environmental domains, air pollution and traffic were the factors most consistently associated with pubertal timing, at birth (G21) and during childhood (INMA and G21). Longer duration of exclusive breastfeeding was associated with a lower Tanner stage among girls in G21 (β=-0.120; 95% CI: -0.195; -0.045). No significant interactions between breastfeeding duration and environmental exposure clusters were observed.

Conclusions

These findings indicate that pubertal timing is influenced by modifiable early-life exposures, including breastfeeding practices, childhood diet quality, and urban environmental factors, with some associations independent of adiposity. The results highlight the importance of considering the cumulative effects of multiple behavioural and environmental exposures throughout childhood and adolescence, supporting the use of a comprehensive exposome perspective. From a public health perspective, these findings underscore the importance of developing and implementing interventions that promote breastfeeding, encourage healthy dietary patterns from early childhood, and create urban environments that foster healthy growth and development, with the potential to positively impact long-term health trajectories.

Resumo

Introdução

A puberdade é uma transição biológica crítica entre a infância e a adolescência, caracterizada por alterações físicas, hormonais e reprodutivas. Evidências recentes indicam uma tendência mundial de declínio na idade de menarca e no início da puberdade, particularmente em meninas. O desenvolvimento puberal precoce tem sido associado a um risco aumentado de distúrbios cardiometabólicos e psicossociais, bem como de cancros hormonais. Embora fatores genéticos expliquem uma proporção substancial da variabilidade interindividual no desenvolvimento pubertário, fatores não genéticos, como o peso corporal, práticas alimentares na infância (incluindo aleitamento materno e alimentação), atividade física, sono e exposições ambientais também podem influenciar o momento em que ocorre a puberdade. No entanto, a maioria dos estudos tem-se concentrado apenas em raparigas, apresentando resultados que nem sempre são consistentes e que, em alguns casos, são ainda limitados.

Objetivos

O objetivo principal desta tese foi explorar de que forma é que os hábitos alimentares, estilo de vida e exposições ambientais, desde a infância até à adolescência, estão associados à altura em que ocorre a puberdade, utilizando dados longitudinais de duas coortes populacionais e de um estudo caso-controlo, avaliando o efeito para além da obesidade. Foram definidos os seguintes objetivos especificos:

1. Avaliar a estabilidade (tracking) da qualidade da dieta desde a idade pré-escolar (4 anos) até à adolescência (13 anos) e identificar os seus fatores associados (Geração XXI) (Artigo I);
2. Determinar se o *Maturity Offset* previsto é um bom indicador do *timing* pubertário, em comparação com a avaliação por estádios de Tanner (Geração XXI e GENOBOX) (Artigo II);

3. Examinar se o aleitamento materno e a sua duração se associam ao *timing* pubertário (Geração XXI) (Artigo III);

4. Avaliar o papel da qualidade da alimentação e de outros comportamentos de estilo de vida ao longo da infância no *timing* pubertário (Geração XXI) (Artigo IV);

5. Avaliar os efeitos das exposições ambientais urbanas precoces no momento da puberdade, usando uma análise de exposoma (Geração XXI e INMA) (Artigo V).

Participantes e Métodos

Esta tese incluiu dados de três populações europeias de crianças: a coorte de nascimento portuguesa de base populacional Geração XXI (G21) (n=8647), o estudo multicêntrico caso-controlo espanhol GENOBOX (n=1699) e a coorte prospetiva espanhola INMA (Infancia y Medio Ambiente) (n=3768).

Na coorte G21, uma subamostra de crianças foi avaliada aos 6, 15 e 24 meses, e toda a coorte foi reavaliada aos 4, 7, 10, 13 e 18 anos de idade. No estudo GENOBOX, os participantes com idades entre os 2 e os 18 anos foram avaliados transversalmente, tendo sido consideradas nesta tese apenas crianças e adolescentes com idades entre os 8 e os 18 anos. Na coorte INMA, as avaliações foram realizadas desde a gravidez até à adolescência (nascimento, 6 meses, 1–1,5 anos, 2–2,5 anos, 3 anos, 4–5 anos, 6–7 anos, 9–10 anos e 12–13 anos).

Em todos os estudos, profissionais treinados realizaram entrevistas presenciais e recolheram informação sobre características sociodemográficas, clínicas e comportamentais. Foram também obtidas medições antropométricas, avaliações do desenvolvimento pubertário, amostras biológicas e dados de exposições ambientais.

Os outcomes analisados nesta tese incluíram indicadores de desenvolvimento pubertário (estadios de Tanner, *Puberty Score* e *Pubertal Development Scale* (PDS)), idade da menarca e trajetórias da qualidade da alimentação. O desenvolvimento pubertário foi avaliado através dos estadios de Tanner na G21, no GENOBOX e numa subcoorte do INMA (Valência), sendo a avaliação realizada por profissionais treinados. Na G21 foi desenvolvido um *Puberty Score* através da soma dos estadios de Tanner para desenvolvimento genital (nos rapazes) ou mamário (nas raparigas) e pelos púbicos (em ambos), variando entre 2 e 10. No INMA foi utilizada o PDS, reportado pelos pais. A idade da menarca foi autorreportada pelas raparigas aos 9 anos no INMA e aos 10, 13 e 18 anos na G21. A qualidade da dieta foi avaliada na G21 através de um *Healthy Eating*

Index (HEI) aos 4, 7, 10 e 13 anos (intervalo: 8–32 pontos), baseado num questionário de frequência alimentar validado e calibrado nesta população.

As exposições incluíram fatores sociodemográficos, maternos, e da criança. As exposições maternas incluíram idade, escolaridade, índice de massa corporal pré-gestacional e idade da menarca, utilizada como *proxy* de hereditariedade genética. Os fatores ao nível da criança incluíram sexo, idade exata no momento da avaliação, peso ao nascimento, índice de massa corporal, ingestão alimentar, duração do sono, tempo sedentário e prática de atividade física. As exposições alimentares incluíram o HEI, a ingestão de alimentos ultraprocessados (UPF) e a duração e exclusividade do aleitamento materno, categorizadas como nunca amamentado, amamentado não exclusivamente ou exclusivamente. As exposições ambientais durante a gravidez, ao nascimento, ao 1º ano e aos 4–5 anos foram também avaliadas nas coortes G21 e INMA utilizando modelos de Sistemas de Informação Geográfica (GIS).

As análises estatísticas variaram de acordo com as questões de investigação e os outcomes de cada artigo. No Artigo I, a estabilidade da qualidade da alimentação entre os 4 e os 13 anos foi avaliada através do coeficiente de correlação intraclasse (ICC), e modelos lineares mistos foram utilizados para examinar as associações entre características maternas e da criança e as trajetórias do HEI ao longo do tempo. No Artigo II, a puberdade normal foi definida utilizando pontos de corte específicos por sexo dos estádios de Tanner, e o *predicted Maturity Offset* (pMO) foi estimado utilizando equações modificadas de Moore. Nos Artigos III e IV foram utilizados modelos de regressão logística multinomial, modelos de riscos proporcionais de Cox e regressão logística ordinal para estimar associações entre aleitamento materno, dieta, sono, atividade física e comportamento sedentário com o Puberty Score e a idade da menarca, ajustando para várias características sociodemográficas e da criança. No Artigo V foi utilizado um enquadramento de Exposome-Wide Association Study (ExWAS) e análise de clustering hierárquico para avaliar exposições ambientais individuais e combinadas em relação ao desenvolvimento pubertário (PDS, estádios de Tanner e idade da menarca), sendo aplicada regressão logística ordinal com ajuste para covariáveis relevantes e correção para testes múltiplos.

Resultados

Artigo I

A qualidade geral da alimentação diminuiu da infância [média (DP): 22,2 (3,6)] para a adolescência [18,2 (3,9)], com estabilidade moderada ao longo do tempo (ICC = 0,53). Uma maior idade materna (β = 0,079; IC 95%: 0,061;0,097), maior escolaridade materna (β = 0,203; IC 95%: 0,178;0,229) e maior rendimento familiar ao nascimento (β = 0,024; IC 95%: 0,007;0,041) associaram-se positivamente à qualidade da alimentação ao longo do tempo. Em contraste, maior tempo em atividades sedentárias (β = -0,009; IC 95%: -0,014; -0,003) apresentou uma associação negativa. Ser do sexo feminino (β = -0,094; IC 95%: -0,132 ;-0,056) e ter maior duração do sono (β = 0,039; IC 95%: 0,015;0,064) associaram-se a maior qualidade da alimentação durante a infância e adolescência.

Artigo II

Na G21, no seguimento dos 10 anos, as idades médias foram 11,7 (1,6) para as raparigas e 12,5 (1,4) anos para os rapazes, com a maioria dos participantes em estadios de Tanner 2–3. No GENOBOX, as idades médias foram 12,9 (2,0) anos para as raparigas e 13,4(1,7) anos para os rapazes, com a maioria dos rapazes no estadio Tanner 2 e das raparigas no estadio Tanner 5. Foram observadas diferenças estatisticamente significativas no pMO entre estudos em vários estádios de Tanner, com valores mais elevados no GENOBOX. De forma geral, crianças classificadas como tendo um desenvolvimento precoce apresentaram valores de pMO consistentemente mais baixos do que as crianças que apresentavam um desenvolvimento normal no mesmo estádio de Tanner em ambos os estudos.

Artigo III

Após ajuste para confundidores, meninas que foram amamentadas (de forma exclusiva ou não) apresentaram menor Puberty Score aos 10 anos, em comparação com aquelas que nunca foram amamentadas. O aleitamento materno exclusivo esteve associado a uma idade mais tardia de menarca. Nos rapazes, não foram observadas associações significativas, exceto entre aqueles que receberam aleitamento materno não exclusivo, apresentando menor probabilidade de se encontrarem no Puberty Score mais elevado (≥5).

Artigo IV

Uma maior qualidade da alimentação aos 4 anos associou-se a um menor Pubertal Score (OR=0,976; IC 95%:0,955; 0,997) e menarca mais tardia (β=0,967; IC 95%:0,935; 0,999)

em raparigas, embora esta última associação tenha sido atenuada após ajuste pelas características maternas. Um maior consumo de alimentos de elevada densidade energética aos 4 anos e de carne e produtos cárneos aos 7 anos associou-se a uma menarca mais precoce, particularmente entre raparigas com peso normal. Foram observadas associações positivas entre o grupo alimentar 'Peixe e ovos', e nutrientes como ácidos gordos, vitamina D, zinco e cálcio e o Puberty Score, sobretudo nos rapazes. Não foram encontradas associações consistentes para a duração do sono ou comportamento sedentário, enquanto a prática de atividade física esteve associada à idade da menarca apenas entre raparigas com excesso de peso ou obesidade.

Artigo V

Na coorte G21, viver em ambientes urbanos mais desfavoráveis durante a infância, caracterizados por maior densidade de edificios, tráfego e poluição do ar, e menor acesso a espaços naturais, associou-se a uma menarca mais precoce (β=-0,181; IC 95%: -0,339; -0,024). Na coorte INMA, rapazes expostos a perfis ambientais mais desfavoráveis durante a infância apresentaram desenvolvimento puberal mais avançado (β=0,437; IC 95%: 0,049; 0,825). Entre os domínios ambientais avaliados, a poluição do ar e tráfego foram os fatores mais consistentemente associados ao timing pubertário. Uma maior duração do aleitamento materno exclusivo associou-se a menor estadio de Tanner entre meninas da G21. Não se observaram interações significativas entre a duração da amamentação e clusters de exposições ambientais.

Conclusões principais

Estes resultados indicam que o timing pubertário é influenciado por exposições precoces modificáveis, incluindo práticas de aleitamento materno, qualidade da alimentação na infância e fatores ambientais urbanos, sendo algumas associações independentes da adiposidade. Os resultados destacam a importância de considerar os efeitos cumulativos de múltiplas exposições comportamentais e ambientais ao longo da infância e adolescência, apoiando uma perspetiva abrangente de exposoma. Do ponto de vista da saúde pública, estes resultados reforçam a necessidade de desenvolver e implementar estratégias que promovam o aleitamento materno, incentivem padrões alimentares saudáveis desde a primeira infância e criem ambientes urbanos que favoreçam o

crescimento e desenvolvimento saudáveis, com potencial para impactar positivamente as trajectórias de saúde a longo prazo.

Abbreviations

BPA - Bisphenol A

BMI - Body Mass Index

EDCs - Endocrine-Disrupting

EDF – Energy Dense Food

FFQ - Food Frequency Questionnaire

FSH - Follicle-Stimulating Hormone

G21 - Generation XXI birth cohort

GnRH - Gonadotropin-Releasing Hormone

GPR54 - G-Protein Coupled Receptor 54

HDL - High-Density Lipoprotein

HEI - Healthy Eating Index

HPA - Hypothalamic-Pituitary-Adrenal axis

HPG - Hypothalamic-Pituitary-Gonadal axis

IGF-1 - Insulin-Like Growth Factor 1

INMA - Infancia y Medio Ambiente

Kcal - kilocalories

KNDy neuron - Kisspeptin, Neurokinin B, Dynorphin neuron

KOR - Kappa-Opioid Receptor

LH - Luteinizing Hormone

NDVI - Normalized Difference Vegetation Index

NK3R - Neurokinin B Receptor

NO_2 - Nitrogen Dioxide

NPY - Neuropeptide Y

PCS - Pubertal Category Score

PDS - Pubertal Development Scale

PHV - Peak Height Velocity

PM - Particulate Matter

$PM_{2.5}$ - Particulate Matter ≤2.5 μm

UPF - Ultra-Processed Food

WHO - World Health Organization

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INTRODUCTION

1. Puberty: Biological background and epidemiology

1.1. Definition of puberty

Puberty is a complex biological process that typically occurs during early adolescence and is marked by rapid growth in height and weight, the development of primary (sex hormones) and secondary sexual characteristics, and the attainment of reproductive capacity (1-4). Puberty usually begins between 8 and 13 years of age in females, and between 9 and 14 years of age in males (5). It is initiated by a brain-neuroendocrine process, involving activation of the hypothalamic–pituitary–gonadal (HPG) axis and subsequent changes in hormone secretion, which trigger the physical and psychosocial changes that culminate in reproductive maturity (1, 6). These physical changes include growth acceleration and the development of secondary sexual characteristics, such as pubic hair development, voice deepening and genital changes in males, as well as breast development and the onset of menstruation in females (5).

1.2. Physiological and hormonal mechanisms of puberty

Puberty activates both the HPG and the hypothalamic-pituitary adrenal axis (HPA) (7). The HPG axis, which coordinates the endocrine function of the hypothalamus, pituitary gland and gonads (8), becomes relatively quisecient a few months after birth and is subsequently reactivated at the onset of puberty (9). This reactivation is characterized by the pulsatile secretion of gonadotropin-releising hormones (GnRH) (10). Pulsatile GnRH is generated by the activity of kisspeptin neurons in the arcuate nucleus, which release neurokinin B and dynorphin (11). These neurons regulate the activity of GnRH neurons and, thereby, stimulate the pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary, stimulating the production of gonadal sex steroids (2, 12-14). In males, LH stimulates the Leydig cells in the testicles to produce and secrete testosterone, which is responsible for testicular development and voice changes (15), whereas FSH acts on Sertoli cells and stimulates the continuous production of spermatozoa (9). In females, LH stimulates ovarian theca cells to produce androgens, mainly testosterone, while FSH promotes granulosa cells proliferation, increases aromatase enzyme activity, and upregulates LH receptor expression in these cells. The androgens produced by theca cells then diffuse into the granulosa cells and are converted

into estradiol, which influences breast development and menarche (15). Progesterone, which is also produced by the ovary, is synthesized early in the steroidogenic pathway and represents another key gonadal hormone during pubertal maturation (Figure 1) (9).

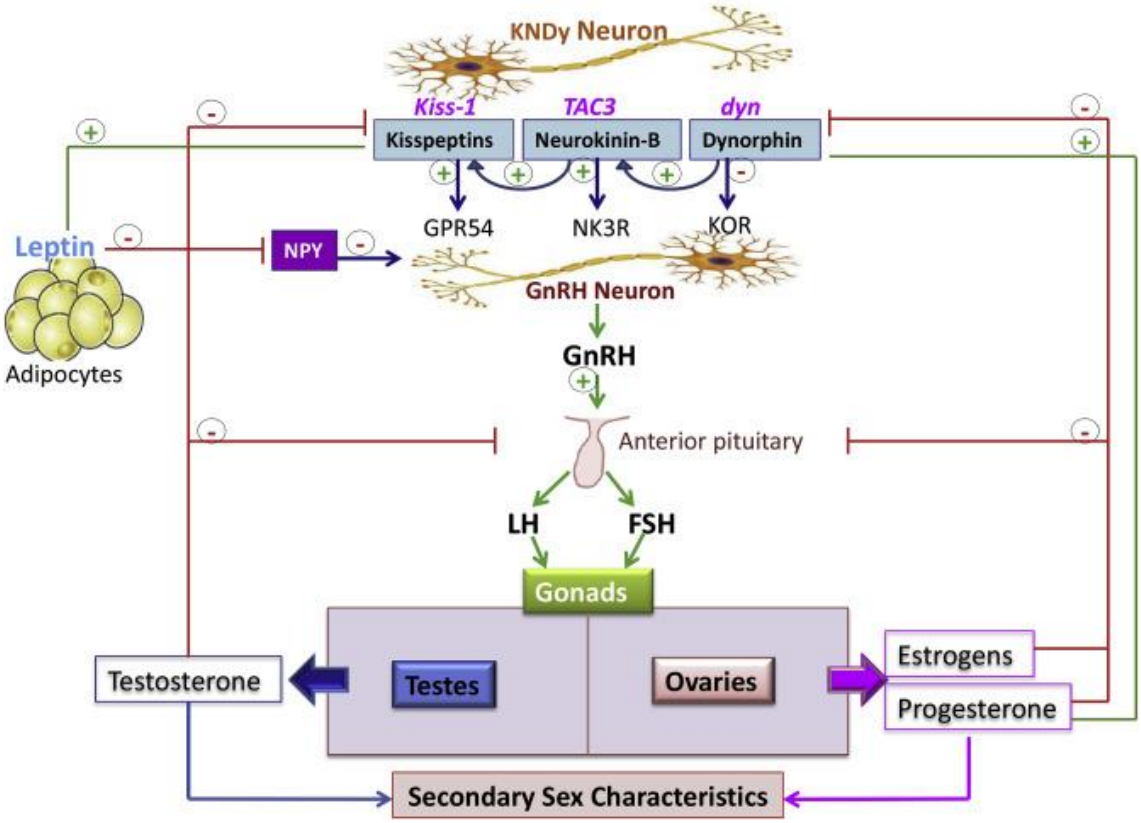


Figure 1. Neuroendocrine regulation of GnRH secretion and control of the HPG axis. Abreviations: KNDy neuron – kisspeptin, neurokinin B, dynorphin neuron; GPR54 - G-protein coupled receptor 54; NK3R – neurokinin B receptor; KOR – kappa-opioid receptor; NPY – neuropeptide Y; GnRH – gonadotropin releasing hormone; LH - luteinizing hormone; FSH - follicle stimulating hormone; + stimulatory, - inhibitory. [source: adapted from reference (9), with permission from the Publisher Elsevier].

In addition to GnRH,the hormone leptin also plays an important role in the regulation of pubertal onset (Figure 1). Leptin is an adipocytokine mainly secreted by adipose tissue and plays a central role in the control of food intake, body weight and energy balance by suppressing appetite through inhibition of the hypothalamic orexigenic neuropeptide Y (NPY) (16). Experimental studies have shown that central administration of leptin suppresses NPY expression in the arcuate nucleus and reduces food intake in rats (17). Moreover, NPY exerts an inhibitory effect on GnRH secretion and acts as a

neurobiological brake on pubertal activation during juvenile animals' development (18). Leptin is therefore recognized as a key permissive factor for pubertal onset in both boys and girls (19).

Beyond the leptin-NYP interaction, accumulating evidence also indicates that leptin influences pubertal onset and reproductive function through the kisspeptin (KISS-1gene) system (20). Although GnRH neurons do not express leptin receptors (21), KISS-1gene neurons do and, consequently, leptin can stimulate kisspeptin production and indirectly promote pulsatile GnRH secretion (20) (Figure 1).

1.3. Assessment of pubertal development in epidemiological studies

In epidemiological research, pubertal development is commonly assessed using a range of clinical methods, such as Tanner staging and hormonal biomarkers, as well as self- or parent-reported indicators like the Pubertal Development Scale (PDS) (22-24). Tanner staging, based on the assessment of secondary sexual characteristics, is the most widely used method to assess pubertal development (i.e. sexual maturation) in population-based studies (25-27). The Tanner staging system, also known as the Sexual Maturity Rating, was introduced in 1969 by James Tanner, a British pediatrician, after a two-decade-long study following the physical changes in girls undergoing puberty (22, 23). This study led to the development of separate scales for males and females to classify the progression of external genitalia development. Tanner staging classifies pubertal development into five stages according to breast and pubic hair development in females, and genital and pubic hair development in males. The stages are defined as follows: stage 1 (prepubertal), stage 2 (early pubertal), stage 3 (mid-pubertal), stage 4 (late pubertal), and stage 5 (post-pubertal) (22, 23). Table 1 describes in detail the characteristics of each Tanner stage.

Table 1. Detailed description of the characteristics of each Tanner stage according to breast and pubir hair development in girls and genital and pubic hair in boys (22, 23, 28).

Tanner stages	Pubic hair (both males and females)	Female breast development	Male external genitalia development
Stage 1	No hair.	No glandular breast tissue palpable. Elevation of the papilla only.	Testes, scrotum, and penis are approximately the same size and proportion as in early childhood. Testicular volume <4 ml.
Stage 2	Growth of long, slightly pigmented, downy hair. Straight or only slightly curled.	Breast bud plapable under the areola. Enlargement of areolar diameter (first pubertal sign).	Enlargement of the scrotum and testes with changes in the texture of the scrotal skin. Tescticular volume 4 ml-8 ml (first pubertal sign).
Stage 3	Hair becomes darker, coarser and more curled.	Breast tissue palpable beyond the areola, with no separation of their countourns.	Growth of the penis, testes and scrotum. Testicular volume 9 ml-12 ml.
Stage 4	Adult-type hair, but covering a smaller area than in most adults.	Projection of the areola above the countour of the breast, forming a secondary mound .	Penis enlarged in length with development of the glans. Testes and scrotum further enlarged, with further darkening of the scrotal skin. Testicular volume 15-20 ml.
Stage 5	Adult in quantity and type, extending beyond the inguinal crease onto the thigh.	Projection of papilla only, with areolar hyperpigmentation and nipple protrusion.	Genitalia adult in size and shape. Testicular volume >20 ml.

Tanner staging should be performed by a trained professional (29), ideally a paediatrician or a nurse, and requires the child/adolescent to be examined without clothing. In girls, breast development is assessed by visual inspection and palpation to distinguish the glandular tissue from adipose tissue (22). In boys, testicular volume is assessed by visual inspection and palpation using a Prader orchidometer (23). The Tanner scale is still the gold standard procedure for assessing pubertal status. However, the clinical assessment of Tanner stages is often perceived as invasive (26) and, in some cases, children/adolescents and their parents may be reluctant to agree with a physical examination (30). For this reason, many epidemiological studies rely on self- or parent-assessment of pubertal development using standardized photographs (31) or line drawings (32) representing the different Tanner stages (33).

The Pubertal Development Scale (PDS) is a widely used tool in epidemiological studies for self- or parent-reports of pubertal development. The PDS was developed by Petersen and collaborators in 1988 (34), as a non-invasive alternative to Tanner staging. This tool asks adolescents or their parents to answer less invasive questions about puberty, without mapping directly onto Tanner stages, avoiding the need for physical examination, and therefore being more ethically suitable for research scenarios (32, 35, 36).

The PDS is based on five physical characteristics: breast development, pubic hair, genital development, skin changes, and deepening voice. Each characteristic is scored from 1 (not yet started) to 4 (seems completed) (36). For girls, an additional question about the menarche is applied. If the girl has already reached her first menstrual period, a score of 4 points is assigned; If not, only 1 point is given. Point values are averaged for all items to provide a PDS, done according to Carskadon et al (37). The Pubertal Category Score (PCS) can then be estimated using Carskadon's criteria (37), based on the PDS response items, allowing classification equivalent to Tanner stages. Five categories were considered: 1 - Prepubertal, 2 - Early Pubertal, 3 - Midpubertal, 4 - Late pubertal and 5 - Postpubertal. To be assigned to a category, the following criteria must be followed:

• PCS for boys considers information on body hair growth, voice change, and facial hair growth (scored as presented in Table 2) (37);

• PCS for girls used body hair growth, breast development, and menarche (scored as presented in Table 3) (37).

Table 2. Attribution of categories according to Pubertal Development Scale (PDS) punctuation in males.

PDS score	**Pubertal Category scores**
3	Prepubertal
4 or 5 (no 3-points responses)	Early pubertal
6, 7 or 8 (no 4-points responses)	Midpubertal
9-11	Late pubertal
12	Postpubertal

Table 3. Attribution of categories according to Pubertal Development Scale (PDS) punctuation in females.

PDS score	Pubertal Category scores
3	Prepubertal
3 and no menarche	Early pubertal
4 and no menarche	Midpubertal
≤ 7 and menarche	Late pubertal
8 and menarche	Postpubertal

However, despite pubertal development self-assessment through images and the PDS being widely used in epidemiological studies, its reliability compared to physical examination (i.e. Tanner staging) has been questioned. Previous studies report different levels of agreement, ranging from moderate to good, fair, and even slight, between clinical assessment and self-assessment using the PDS (38-42). Although PDS categories cannot be directly mapped onto the five-point Tanner scale, they seem to be effective in distinguishing pre-, mid-, and post-pubertal stages (43).

1.4. Secular trends in pubertal timing

A growing body of evidence has documented changes in pubertal timing over the past decades, particularly a worldwide declining trend in age at menarche and age at pubertal onset in many high- and middle-income countries, including Portugal (44-52). This trend has been reported more consistently in females, especially for age at menarche (49, 50), although several studies have also described earlier breast development (46, 47). In addition, emerging evidence suggests that pubertal timing may also be shifting towards earlier ages in boys (53, 54).

Data from the Copenhagen Puberty Study showed that girls from a more recent birth cohort reached pubertal milestones approximately one year earlier than girls from a comparable cohort born 15–16 years earlier (47). A study including three Portuguese population-based cohorts further demonstrated a decreasing trend in age at menarche across cohorts, with women born before 1930 presenting a significantly higher age at menarche than those born after 1990 (44). Similarly, a longitudinal study conducted in

the United States reported earlier breast development among girls born in the late 1990s (53).

Several studies have also reported earlier pubertal maturation in boys, including a decline in the age at onset of the pubertal growth spurt in Danish boys (54) and younger ages at attainment of pubertal testicular volumes among boys in the United States (53).

The underlying drivers of these secular changes in pubertal timing in both females and males will be discussed later in this thesis. Briefly, proposed determinants include genetic factors (55, 56), improvements in early-life nutrition and health (57, 58), increasing childhood adiposity (59), lifestyle-related factors (60) and exposure to environmental contaminants (7, 61).

1.5. Consequences of early puberty and early age at menarche

Early pubertal development has important public health implications, as it has been consistently associated with an increased risk of both short- and long-term adverse health outcomes. Previous epidemiological studies have shown that early puberty is associated with an increased risk of several chronic diseases in adulthood, including endocrine (62, 63) and cardiometabolic (64) disorders and hormone-related cancers (65, 66).

One of the most consistent associations is between earlier age at menarche (62, 67) and pubertal timing (in both males and females) and an elevated risk of metabolic diseases, including type 2 diabetes (62) and metabolic syndrome (63) in adulthood. Earlier pubertal timing has been associated with higher adult Body Mass Index (BMI), diastolic blood pressure, and lower high-density lipoprotein (HDL) cholesterol in both sexes (63). Earlier pubertal timing has also been linked to an increased risk of developing cardiovascular diseases in adulthood in both sexes, particularly a higher risk of angina and hypertension (64). In addition, the association between earlier pubertal age and hormone-related cancers is a well-established finding in the literature. In females, an earlier age at menarche increases the cumulative lifetime exposure to endogenous estrogens, which play a central role in breast tissue and mammary gland proliferation and are recognised risk factors for the development of breast cancer in adulthood (65, 68-70). In males, although the available evidence is more limited, some studies suggest that pubertal timing may also influence the risk of prostate cancer (66, 71-73). Beyond these adverse health

outcomes, early menarche has been associated with a higher risk of all-cause mortality in a meta-analytic study (74).

Moreover, earlier pubertal onset may also adversely affect mental health and psychosocial development, and has been associated with a higher prevalence of emotional and behavioural problems, depressive symptoms (75, 76), lower psychosocial well-being, and increased engagement in risk-taking behaviours during adolescence and later life (77, 78). These behaviours include substance use (79), such as cigarette smoking, alcohol consumption and marijuana (80, 81). Early developers are also more likely to engage in risky sexual behaviours (82), to report a higher number of sexual partners (81), and to experience adolescent pregnancy (82).

2. Factors influencing pubertal timing

2.1. Genetic versus non-genetic factors

Pubertal timing is a complex and multifactorial trait, influenced by both genetic and non-genetic factors, as well as by interactions between these factors (83). Genetic predisposition has been shown to explain a substantial proportion of the inter-individual variability in the timing of pubertal onset (56, 59, 83). However, accumulating evidence over recent years indicates that non-genetic factors also appear to contribute greatly to the variability in pubertal timing. In particular, early-life and childhood nutrition (57, 84), as well as childhood adiposity (59, 85), are among the most consistently reported modifiable factors influencing pubertal development, acting through metabolic and neuroendocrine pathways involved in the activation of the hypothalamic–pituitary–gonadal axis (59). In parallel, growing evidence has highlighted the role of environmental exposures (83), particularly endocrine-disrupting chemicals (EDCs) (86), in pubertal development. Several studies suggest that these exposures may interfere with hormonal signalling pathways and the regulation of the hypothalamic–pituitary–gonadal axis, thereby contributing to shifts in the timing of pubertal maturation (87, 88). Besides these factors, socioeconomic status and lifestyle behaviours have also been consistently associated with variations in pubertal timing, possibly reflecting differences in early-life stress, nutrition, health-related behaviours and access to health care (89-91).

These factors will be explored individually in the following sections, after which a comprehensive approach will be proposed.

2.2. Body weight status

Body weight status has been consistently identified as one of the most important modifiable factors influencing pubertal timing (92-96). The association between higher BMI and earlier pubertal development is well established in girls, with several studies reporting that females with overweight or obesity during childhood have a higher likelihood of earlier breast development and earlier age at menarche compared with their normal-weight counterparts (93, 95-98). In boys, however, this association is less clear, as results from existing studies are inconsistent. While some studies have reported delayed pubertal onset (93, 99), others have found earlier onset or no significant association (94, 96, 100).

Several mechanisms have been proposed to explain the relationship between body weight status and pubertal timing. One potential link between adiposity and the activation of the HPG axis may be explained by leptin. Adipose tissue functions as an endocrine organ that produces adipokines, particularly leptin, which acts on the HPG axis and stimulates the release of GnRH, leading to the initiation of puberty (92, 101, 102). However, in boys the role of leptin may be more complex, as elevated leptin levels in males with obesity appear to suppress testosterone production indirectly by increasing the conversion of androgens into estrogens, which may delay pubertal onset (103, 104).

Another potential pathway may involve insulin resistance. Elevated insulin levels can increase estrogen production and interact with leptin signaling pathways. Insulin may also act directly on the HPG axis and promote earlier activation of reproductive hormonal pathways (101, 105). Childhood obesity is frequently associated with hyperinsulinemia, which may therefore contribute to alterations in pubertal timing (106).

2.3. Early life nutrition and feeding practices

2.3.1. Breastfeeding

Breastfeeding and the nutritional status during childhood have been shown to play an important role in pubertal timing (58, 90, 107). Although the influence of nutrition on pubertal onset can be explained through other mechanisms, such as nutrient deficiencies affecting hormonal regulation (58), it is most commonly explained by overfeeding and the subsequent development of overweight and obesity (108).

Breastfeeding has several well-documented health benefits (109), including a lower risk of rapid weight gain and obesity (110-112). In addition, some authors have suggested that breastfeeding may also significantly influence the timing of puberty (58, 84, 113). While findings on the relationship between breastfeeding and pubertal onset have been inconsistent, with some studies reporting no association (114, 115), most research has focused on girls, and a growing body of evidence suggest that breastfeeding and its duration may act as a protective factor against early puberty timing, particularly in girls (116-118). More recently, one study suggested that the absence of breastfeeding may be associated with earlier pubertal timing, but only in boys (119).

Beyond the direct influence of childhood body weight on the relationship between breastfeeding and pubertal timing, human milk also provides bioactive components, including regulatory hormones such as leptin (120). Leptin not only promotes early satiety and helps regulate energy balance during childhood (120), but it has also been linked to the onset and development of puberty (120-122). Serving as a signal of the body's nutritional status (123), leptin affects the hypothalamic-pituitary-gonadal axis (124), and in girls, low prepubertal leptin levels have been associated with subsequent increases in body fat (125), which may in turn influence the timing of pubertal onset (126, 127).

2.3.2. Diet quality and food groups

Previous studies have suggested an association between childhood dietary intake and pubertal onset (107, 128, 129). However, most of this evidence focused on individual food items and nutrients rather than overall dietary quality. Among the most frequently

investigated food groups are dairy products (130-132), red meat (133, 134), and energy-dense foods (135-137). Previous studies showed that dairy milk consumption during childhood is associated with increased risk for early menarche. Similarly, a higher frequency of red meat intake (133, 134) and a greater consumption of energy-dense foods (135-137) during childhood have also been associated with an earlier age at menarche. These associations have been examined predominantly in females, with age at menarche being the main outcome assessed.

To date, only a small number of studies have specifically examined the association between ultra-processed food (UPF) consumption and pubertal timing, with findings indicating a potential association between higher intake of UPF and an earlier age at menarche. (137, 138). The mechanisms proposed to explain this relationship include increased adiposity, higher insulin resistance and hormonal dysregulation, which may, in turn, accelerate activation of the HPG axis (139, 140).

Tracking of diet quality refers to the stability or maintenance of overall dietary patterns over time. This continuity can have positive or negative implications, depending on the quality of the diet being tracked. A change from a higher-quality dietary pattern to a lower-quality one represents a lack of stability in diet quality (141). Understanding how diet quality is maintained throughout childhood allows for the identification of patterns that are more or less consistent, providing valuable information for the development of nutritional interventions and the identification of critical windows for promoting health. Moreover, the few studies that have examined overall diet quality suggest that higher dietary quality during the pre-pubertal period is associated with a later age at menarche (138) and slower progression through pubertal stages (129, 142), highlighting the potential role of consistent, high-quality dietary intake in modulating pubertal timing. Although most research on tracking of eating habits has been assessed on adults or the transition from adolescence to adulthood, few studies have examined have evaluated the stability of diet quality during childhood and from childhood to adolescence (141, 143, 144), a critical period for sexual maturation and the establishment of long-term eating habits.

2.3.3. Macro- and micronutrient intake

Energy and macronutrient intake during childhood have been proposed as potential determinants of pubertal development. However, the available evidence remains, in some cases, inconsistent. One study reported a significant association between higher energy intake during childhood and earlier pubertal onset among girls with higher BMI (128). Nevertheless, several previous studies did not find consistent associations between total energy intake and pubertal timing (107, 145-148).

Overall, studies examining the association between total fat intake during childhood and pubertal onset have also produced inconsistent results (149-153). More recent evidence, however, has begun to support the hypothesis that high-fat diets may promote earlier pubertal development, independently of BMI (58). With regard to specific types of dietary fat, the evidence for polyunsaturated fatty acids (PUFA) appears to be more consistent, with several studies reporting that higher PUFA intake is associated with an increased risk of earlier age at menarche (128, 153). In contrast, studies conducted have generally not found an association between saturated fatty acid intake and age at pubertal onset (150, 154-156). Evidence regarding monounsaturated fatty acids (MUFA) is also conflicting. While Nguyen et al. (128) reported that higher prepubertal MUFA intake was inversely associated with age at menarche, suggesting a delaying effect, a preliminary study suggested that higher MUFA intake may be associated with earlier pubertal onset (157). In contrast to dietary fat, findings regarding protein intake show greater consistency. Most studies indicate that higher prepubertal intake of animal protein is associated with earlier pubertal development (158, 159). A plausible biological mechanism underlying this association is that higher consumption of animal protein may stimulate the hypothalamic–pituitary–gonadal axis through increased secretion of insulin-like growth factor-1 (IGF-1) (128, 160). Prepubertal energy intake derived from carbohydrates has not been consistently reported as a marker of pubertal development in cohort studies (150, 151, 161).

Evidence regarding the potential effect of micronutrients on pubertal timing is scarce, and the existing findings remain inconsistent. Some studies suggest that the requirements for micronutrients such as zinc, iron, calcium, and vitamin D increase during the pubertal growth spurt (84), reflecting their role in supporting rapid somatic and sexual maturation. Regarding vitamin D, the literature provides no consistent evidence to support a role in

pubertal timing (57, 162). However, a previous study reported that girls with vitamin D deficiency at baseline were twice as likely to experience menarche during follow-up compared with those with adequate levels (163). Similarly, high dietary iron intake (164) and zinc-rich diets (153) have been associated with earlier menarche onset in two separate populations. In addition, a small randomized trial involving only 17 children indicated that those supplemented with zinc experienced earlier initiation of puberty compared with children in the placebo group (165).

Overall, these findings highlight potential associations but are limited by small sample sizes, heterogeneous study designs, and inconsistent measurement of micronutrient status. Future studies are needed to elucidate the role of micronutrients in pubertal development in children.

2.4. Physical activity and sedentary behaviours

The usual practice of physical activity during the prepubertal period may influence pubertal onset. Studies suggest that higher levels of physical activity and lower levels of sedentary behaviours are associated with a lower risk of early puberty (90, 166). This effect may be mediated by a reduction of body fat, which can lead to lower leptin levels, since low leptin levels can delay puberty onset (167, 168), and by the regulation of hormones such as estrogen and ghrelin (168, 169). However, most available evidence comes from studies conducted in elite athletes (166, 170), highlighting the need for research in the general population to clarify the magnitude of the effect of physical activity during childhood on pubertal timing.

Recent findings indicate that sedentary behaviour is associated with an increased risk of early puberty, particularly in girls, with obesity likely playing a mediating role (171). Nonetheless, the role of sedentary behaviours in pubertal timing remains largely unexplored, and further in-depth prospective studies are needed to elucidate this relationship.

2.5. Sleep duration

Evidence from previous studies suggest that longer sleep duration may influence the timing of pubertal onset, but research on this topic has only emerged relatively recently. Longer sleep duration has been associated with a lower risk of early pubertal development (172), whereas insufficient sleep appears to increase the risk of earlier puberty (173). Also, a higher prevalence of early puberty has been described among patients with sleep disorders (173). However, other studies report only weak or inconsistent associations between sleep duration and sexual maturation, indicating that further research is needed to clarify the role of sleep duration on pubertal timing (174).

The pathways through which sleep may influence pubertal timing remain unclear, although some indirect mechanisms have been proposed. One possible explanation is through its association with obesity, as insufficient sleep duration and late bedtime have been identified as risk factors for overweight and obesity (173). In addition, sleep deprivation may increase food intake by altering appetite-regulating hormones, such as leptin and ghrelin (173, 175).

2.6. Environmental determinants

2.6.1. Exposure to chemical pollutants

Chemical pollutants, particularly EDCs may also influence the timing of puberty onset (127, 176-178). Individuals are exposed to a multitude of EDCs daily through food and everyday consumer products (179-185). These chemicals can interfere with hormonal homeostasis through various signalling pathways, directly or indirectly affecting hormonal receptors (186).

Several studies suggest that exposure to EDCs during critical developmental periods - such as in utero, breastfeeding, early childhood, and the pre-pubertal period - is associated with pubertal timing. However, the direction of this association is not always clear and consistent. Exposure to EDC such as Bisphenol A (BPA), benzophenone-3, phthalates, parabens, and pesticides has been linked to earlier puberty onset (187-193), while some studies also report delayed puberty (189, 192, 193) or no association at all (188-190, 193, 194).

This disparity in findings may relate to the pleiotropism of EDCs' mechanisms of action (176, 195). Additionally, individual characteristics such as overweight and obesity may play a role, as some EDCs have obesogenic properties, mediating the relationship between obesity and early puberty onset (196). Therefore, further studies are needed to better understand the effects of EDCs on pubertal timing.

2.6.2. Pre- and post-natal urban environmental exposures

The impact of urban environmental exposures on puberty onset has been scarcely studied, and results have been contradictory. Overall, an earlier puberty onset has been linked with urban areas (197, 198). This could be due to improved health, socioeconomic and living conditions (199, 200), better nutritional status and exposure to environmental hazards during childhood (200), compared to the rural contra parts (201-203).

Most of the studies have focused on studying the impact of air pollution (204-207). A study described that girls living within 150 meters of a major road reached one pubertal stage 2 to 9 months earlier (204). In contrast, some studies reported that in-utero and childhood exposure to pollutants such as Particulate Matter (PM) 10 (205), sulfur dioxide and nitrogen dioxide was associated with delayed puberty (206). A posterior study with a different study population could not find associations between ambient air pollutants and pubertal development determined by estradiol and testosterone levels in children (198).

Some authors speculate that green space proximity can affect the age at menarche (207). The proximity to an urban greening area could bring numerous beneficial health effects, such as lowering stress, lowering adiposity risk and promoting physical activity. These factors are known to be related to the timing of reproductive events (166, 200, 208). However, the availability of studies on green spaces' influence on puberty is almost null, with the only existing study on adolescent girls not supporting this association (209).

As outlined, current epidemiological evidence on the relationship between urban environmental exposures and pubertal timing is limited and largely inconsistent. Furthermore, most studies have focused on the effects of individual environmental exposures, without accounting for the complex interactions among multiple exposures. To date, the influence of the totality of environmental factors experienced throughout the life course, and the interactions among them on pubertal timing, remains largely

unexplored. Understanding these relationships is critical for identifying key environmental determinants of pubertal development.

2.7. The exposome and pubertal development

Previous research has typically examined these influences in isolation rather than within a comprehensive framework. Given that individuals are continuously exposed to a wide range of factors that may simultaneously affect the timing of puberty, adopting a broader perspective is essential. Many of these determinants fall under the concept of the exposome, which encompasses the totality of all non-genetic exposures an individual encounters from conception through life, along with their interactions. Recognizing that genetic predisposition alone does not fully account for disease risk, the exposome provides a more holistic approach to understanding health outcomes. It is composed of three main domains:

1. The personal exposome, which includes factors such as physical activity, diet, and exposure to toxic chemicals.

2. The external exposome, which encompasses climate conditions and the characteristics of urban and rural environments.

3. The internal exposome, which involves biological processes such as epigenetics, the metabolome, and the microbiome.

These exposures can induce lasting changes in an individual's physiology, metabolism, and overall health, potentially increasing the risk of chronic diseases later in life (210, 211).

3. Knowlegde gaps

The field of early life non-genetic exposures and their impact on pubertal timing is still in its early stages, and despite of the growing interest in this topics, significant gaps remain. For one, only the effects of breastfeeding and selected aspects of dietary intake on pubertal timing have been widely studied, with evidence suggesting protective effects against early puberty, although findings are not entirely consistent. Moreover, the majority of the studies have primarily examined individual foods or nutrients rather than

overall diet quality, which may better capture the complexity of dietary behaviours and their long-term effects.

For the remaining factors, such as physical activity during the prepubertal period, sleep patterns, exposure to EDCs and urban environmental exposures, the existing data is scarce and sometimes contradictory. In addition, the majority of studies have focused predominantly in females, with very few including male participants.

Methodological limitations remain an important challenge in this field, particularly regarding the assessment of pubertal development in epidemiological studies. Tanner staging is considered the gold standard method for evaluating pubertal maturation. However, it requires a physical examination by a trained professional and is often perceived as invasive by children and their parents. Consequently, there is still a need to improve methodological approaches for assessing pubertal development in epidemiological studies.

Above all, there are no studies exploring the impact of the totality of environmental exposures an individual experiences throughout life, nor the interactions between these exposures, known as the exposome, on pubertal timing. Addressing these gaps is essential, as it could provide a more comprehensive understanding of the determinants of pubertal development and inform strategies to support public health measures.

OBJECTIVES

The main aim of this thesis was to explore how early-life eating habits, lifestyle, and environmental exposures, from childhood to adolescence, are associated with pubertal timing using longitudinal data from two population-based cohorts, studying the effect beyond obesity.

The specific aims are as follows:

1. To evaluate the tracking of diet quality from pre-school age (4 years) to adolescence (13 years) and its associated factors (Generation XXI) (Paper I);

2. To determine whether the predicted Maturity Offset is a good proxy of puberty timing, in comparison with the Tanner stage evaluation (GENOBOX and Generation XXI) (Paper II);

3. To specifically examine whether breastfeeding and its duration are associated with pubertal timing (Generation XXI) (Paper III);

4. To understand the role of diet quality and other lifestyle behaviours across childhood on pubertal timing (Generation XXI) (Paper IV);

5. To evaluate the effects of early-life urban environmental exposures on pubertal timing, within an exposome framework (Generation XXI and INMA) (Paper IV).

METHODS & PARTICIPANTS

1. Participants

This thesis uses data from three independent European children's population: the population-based birth cohort Generation XXI (G21) (212, 213), assembled in the metropolitan area of Porto, Portugal (2005-2006); the multicentre case-control GENOBOX study (214, 215), conducted in Zaragoza, Santiago de Compostela and Córdoba, Spain; and the INMA project (216), a population-based prospective birth cohort established in Ribera d'Ebre, Menorca, Granada, Valencia, Sabadell, Asturias and Gipuzkoa, Spain. The papers included in this thesis use prospective data from G21 collected at birth and at 6, 15 and 24 months and at 4, 7, 10, 13 and 18 years of age, as well as prospective data from INMA collected during pregnancy, at birth, at 14-18 months, at 4-5 years and at 9 years. Cross-sectional data from the GENOBOX study, including children aged between 6 and 18 years, were also considered. A general description of the participants and data collection procedures of each study is provided below. The selection of eligible participants for each analysis depended on the specific objectives of each paper and is described in detail in the Methods section of the respective manuscript.

1.1. The population-based birth cohort Generation XXI (G21)

Objective 1 to 5 were answered partially using data from G21, which is a Portuguese population-based birth cohort. The G21 project aimed to estimate indicators of maternal and child health and to characterize the prenatal and postnatal growth and development, identifying their determinants to better understand health status in childhood and later in life (212, 213). Recruitment was conducted between April 2005 and August 2006 in the five public maternity hospitals of the metropolitan area of Porto, according to the following eligibility criteria: mothers residing in one of the six municipalities of the Porto metropolitan area (Figure 2); delivery in one of the public maternity hospitals located in these municipalities; and live-born infants with a gestational age ≥24 weeks. Mothers were invited to participate between 24 and 72 hours after delivery, and 91.4% of those invited accepted to participate, with a total of 8647 children enrolled at baseline. Participant subsamples were assessed at 6 (n=1558), 15 (n=1042) and 24 months (n=855). The entire cohort was invited to attend a follow-up when children were aged 4 (86% of participation), 7 (80% of participation), 10 (76% of participation), 13 (54% of

participation – follow-up was interrupted due to COVID-19 pandemic), and 18 years old (41% of participation). For families who were unable to attend in person, a shorter version of the questionnaire was administered by telephone. The same assessment procedures were used across all follow-ups. Data were collected by trained interviewers using structured questionnaires, including information on sociodemographic characteristics, clinical and lifestyle factors, and the child's health status, together with objective anthropometric measurements.

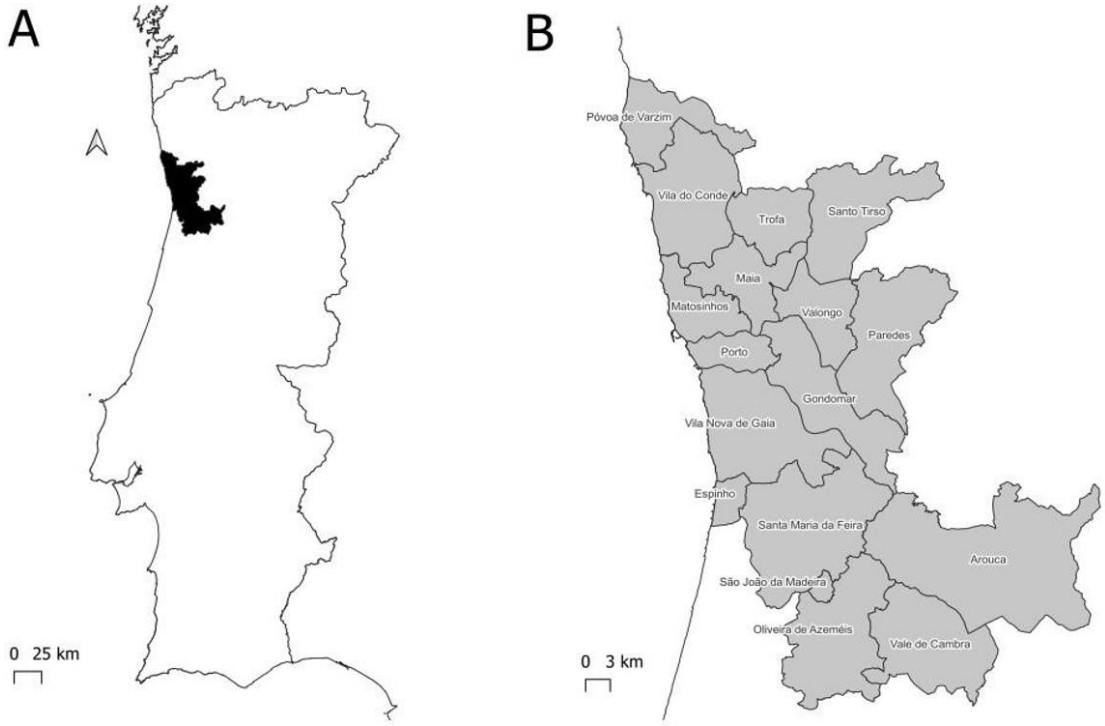


Figure 2. Porto municipalities included in the G21 project.

1.2. The GENOBOX study

Objective 2 was partially addressed using data from the GENOBOX study, a multicentre case-control cross-sectional design study that recruited 1699 Spanish children and adolescents (821 boys and 878 girls), aged 2 to 18 years (Figure 3). For our analysis, only data from children aged 8 to 18 years were included. The project aimed to identify genetic variants and environmental influences (e.g. physical activity and diet) associated with the development of obesity in children and adolescents (214, 215). Participants were

recruited at the primary care centre, and children fulfilling the inclusion criteria were invited to the Encodrine Departments of three Spanish public health institutions: Lozano Blesa University Clinical Hospital in Zaragoza, Santiago de Compostela University Clinical Hospital in Santiago de Compostela and Reina Sofia University Clinical Hospital in Córdoba. The study was conducted between 2012 and 2015 according to the following eligibility criteria: European-Caucasian heritage and absence of congenital metabolic diseases (e.g. diabetes or hyperlipidaemia). Exclusion criteria were non-European-Caucasian heritage; presence of congenital metabolic diseases or psychomotor disability; presence of chronic or inflammatory diseases; undernutrition; and the use of any medication that affects blood pressure, hormonal regulation, or glucose or lipid metabolism. The case group (children with overweight or obesity) was recruited randomly among children attending hospital for diagnosis of minor gastrointestinal disorders that were not confirmed after clinical and laboratory examinations or suspecting overweight or obesity. The control group (normal-weight children) was randomly recruited among children attending the emergency department for acute banal infectious conditions or for diagnosis of minor gastrointestinal disorders that were not confirmed after clinical and laboratory examinations.

During the assessment, clinical data were collected, information on diet and physical activity was obtained, anthropometric measurements were performed, and blood samples were collected for general biochemical analyses.

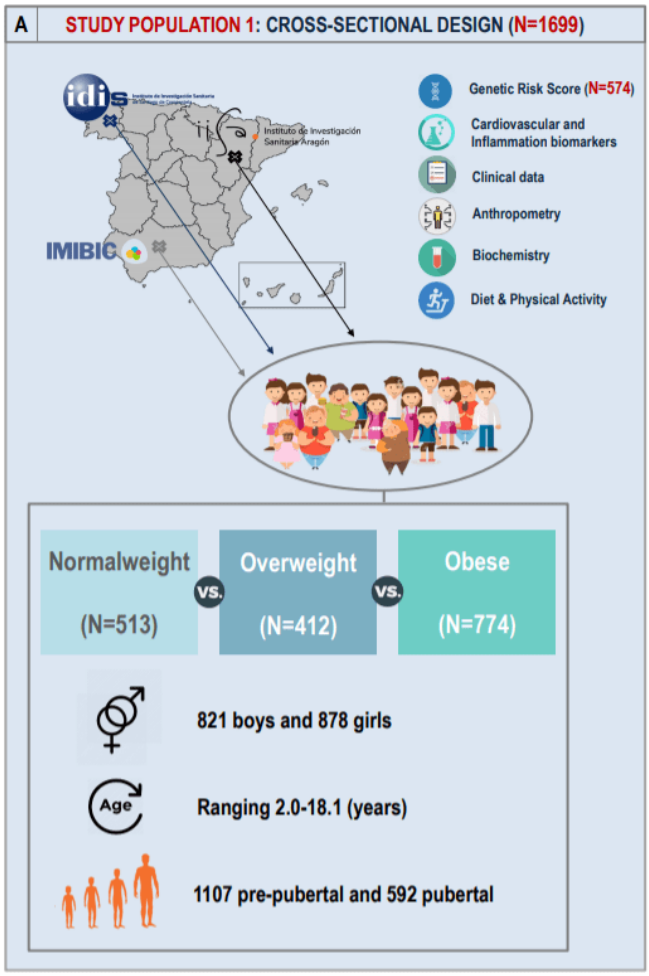


Figure 3. Overview of the GENOBOX study population and design

1.3. INMA Project

Objective 5 was partially answered using data from the INMA (*Infancia y Medio Ambiente*) Project, a Spanish prospective network of population-based birth cohorts that recruited 3768 mother-child pairs during pregnancy between 1997 and 2008 in the Spanish regions of Ribera d'Ebre, Menorca, Granada, Valencia, Sabadell, Asturias and Gipuzkoa (Figure 4) (216). The project was design to investigate the role of environmental pollutants in air, water and diet during pregnancy and early childhood in relation to child growth and development. Eligibility criteria for mothers included residence in one of the study areas, age ≥ 16 years, a singleton pregnancy, no use of assisted reproduction techniques, intention to deliver at the reference hospital, and the ability to communicate in Spanish or the corresponding regional language.

Figure 4. Geographical location of INMA cohorts in Spain.

Each cohort had a different recruitment period and follow-up waves, as presented in Figure 5, although follow-ups were performed in all cohorts at the main time points (during pregnancy, at birth and at 4 years of age). Of the invited mothers, 45% to 98% agreed to participate (96% in Ribera d'Ebre, 98% in Menorca, 54% in Valencia, 60% in Sabadell, 45% in Asturias and 68% in Gipuzkoa; information was not available for Granada). After baseline assessment during pregnancy, follow-up visits were conducted at birth, at 6 months, at 1-1.5 years, at 2-2.5 years, at 3 years, at 4-5 years, at 6-7 years, at 9-10 years and at 12-13 years. Data were collected through face-to-face interviews by INMA trained personnel and included questionnaire-based information, clinical records, physical examinations, ultrasound scans, biological samples, biomarkers, dietary assessments, and environmental measurements. Anthropometric measures were also performed.

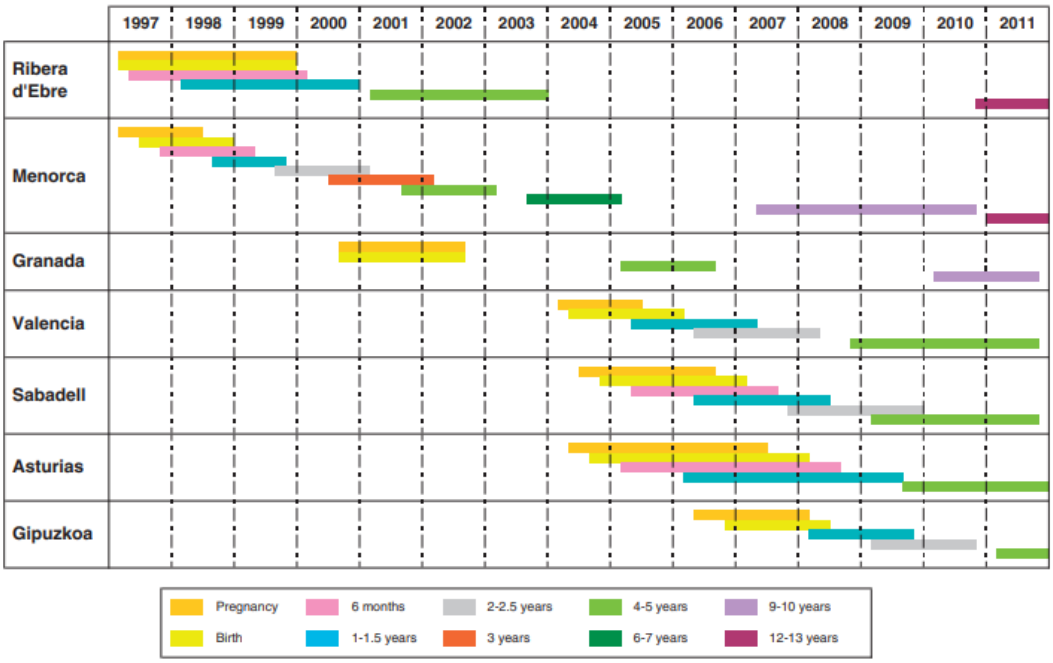


Figure 5. Follow-up periods of INMA cohorts.

2. Ethical considerations

All projects included in this thesis were conducted in accordance with the principles of the Declaration of Helsinki. In all three projects, the legal representatives of each participant received detailed information about the study objectives, procedures, potential benefits and possible discomforts, and provided written informed consent prior to participation, covering all examinations performed during the assessments. Additionally, for the follow-ups at ages 13 and 18, the participants also provided written informed consent.

The G21 baseline and follow-up evaluations were approved by the University of Porto Medical School/São João Hospital Centre Ethics Committee, except the 13- and 18-year follow-ups which were approved by the ISPUP Ethics Committee.

The GENOBOX study was conducted in accordance with the principles of Good Clinical Practice of the European Economic Community (Document 111/3976/88, July 1990) and the Spanish regulations governing biomedical research in humans, and was approved by the corresponding Ethics Committees on Human Research (registration code: 2011/198).

For the INMA project, the research protocol was approved by the Ethics Committees of each participating region.

3. Data collection

3.1. Generation XXI

At baseline, trained interviewers visited the five public maternity hospitals to present the Generation XXI project and invite eligible mothers to participate, 24–72 hours after delivery. At that time, information on both parents was collected using structured questionnaires, which included sociodemographic characteristics (e.g., parental age and educational level), lifestyle behaviours (e.g. diet, physical activity, alcohol consumption and smoking during pregnancy), and medical history. Anthropometric measurements of the parents and the newborns were also obtained. In addition, information on pregnancy complications, delivery characteristics (e.g., mode of delivery) and neonatal characteristics (e.g., birth weight) was abstracted from medical records.

3.1.1. Sociodemographic characteristics (G21)

The socioeconomic characteristics used in this thesis were maternal age and maternal completed years of education, both of which were collected at baseline and treated as continuous variables. Household monthly income was assessed at baseline and at the child's ages of 4, 7, 10 and 13 years of age and was recorded as a categorical variable with the following brackets: <€500, €501–1000, €1001–1500, €1501–2000, €2001–2500, €2501–3000 and >€3000. For the analyses conducted in the present thesis, household income was grouped into four categories: <€1000, €1001–1500, €1501–2000 and >€2000. Additionally, whether the child livied with siblings (yes/no) was considered, based on information collected at ages 4, 7, 10 and 13.

3.1.2. Maternal characteristics (G21)

Maternal age at menarche (used as a continuous variable) was collected at baseline and was considered in this thesis as a proxy for genetic heritability (217).

Gestational age was also treated as a continuous variable, estimated by integrating information from different sources, with preference given to the date of the last menstrual period (amenorrhoea).

The mode of delivery was extracted from medical records and categorized into five types: spontaneous vaginal delivery, vacuum-assisted delivery, forceps delivery, caesarean section, and caesarean section with vacuum assistance.

Maternal pre-pregnancy BMI was calculated as pre-pregnancy weight (in kilograms) divided by height (in metres) squared, and it was used as a continuous variable.

3.1.3. Children's dietary intake (G21)

3.1.3.1. Breastfeeding

According to the World Health Organization (218), exclusive breastfeeding is defined as the period during which the infant receives only breast milk and no other liquids or foods, including infant formula or water, with the exception of vitamins, minerals or medicines. In G21, exclusive breastfeeding was defined as exclusive even if the child had received water.

Information on breastfeeding was collected through questionnaires administered by trained interviewers to the main caregiver at the 6-, 15- and 24-month subsample follow-ups, and at the 4-year follow-up in the complete sample. At all follow-ups, caregivers were asked the question: "Do you feed or have you ever fed your baby with breast milk?". If the answer was positive, the interviewer asked whether the child was still being breastfed. If breastfeeding had already stopped, the caregiver was asked to report the child's age (in months, weeks or days) at cessation of both exclusive and non-exclusive breastfeeding. Breastfeeding duration was converted into weeks for this research. To minimise potential misreporting of exclusive breastfeeding duration, children with reported exclusive breastfeeding durations longer than 7 months were considered as missing information.

To increase power and accuracy, final exclusive and non-exclusive breastfeeding duration variables were derived based on the first report available across follow-ups. For example, if at the 6-month follow-up the child was no longer being breastfed and a duration was

reported, this value was considered final. If the child was still being breastfed or no information was available at that follow-up, information from the 15-month follow-up was used. The same procedure was applied sequentially to the 24-month and 4-year follow-ups. To account for potential recall bias, agreement between breastfeeding duration reports across different follow-ups was assessed. Intraclass correlation coefficients ranged from low agreement (0.26 for 6 versus 24 months) to excellent agreement (0.91 for 24 months versus 4 years).

Exclusive and non-exclusive breastfeeding information was subsequently combined into a single categorical variable with four categories ('never breastfed'; 'any breastfeeding but never exclusive'; 'exclusive breastfeeding for less than 4 months'; and 'exclusive breastfeeding for 4 months or more'). This categorisation allowed a clear distinction between children who were never breastfed (fed only infant formula), those who were exclusively breastfed (with or without water), and those who were non-exclusively breastfed (complemented with infant formula and/or other foods or beverages). The 4-month cut-off was selected because most children in the study sample were exclusively breastfed for less than 4 months and because, in Portugal, fully paid maternity leave typically extends up to four months (219).

3.1.3.2. Child's diet

Data on children's dietary intake at all follow-ups were collected through a Food Frequency Questionnaire (FFQs). The questionnaire were applied by trained interviewers to the child's main caregiver at the 4-, 7- and 10-year follow-ups, assessing the overall frequency of consumption during the previous six months, and directly applied to the adolescent at the 13-year follow-up, considering usual food consumption over the previous 12 months.

The FFQ included thirty-five food items at age 4 (including questions on the consumption of dairy products, meat, fish, eggs, bread, cookies, vegetables, fruit, pasta, rice, potatoes and added fats); thirty-eight food items at age 7 (added items: chocolate milk, breakfast cereals and natural fruit juice, and with all meat items (red and white meat) grouped together); forty-one food items at age 10 (added items: soy products, legumes and the butter/margarine group were divided in two separate items); and thirty-nine food items at

age 13 (only one group for the milk in total, the group rice, pasta and potatoes was split in two, and the items about tea were removed). Response options were collected using nine categories: ≥4 times/day, 2-3 times/day, once a day, 5-6 times/week, 2-4 times/week, once a week, 1-3 times/month, <once a month, and never. These frequency options were then converted into daily consumption frequencies.

For the papers included in this thesis, similar food groups were created accross all ages based on the dietary recommendations of the World Health Organization (WHO) (220), namely 'Dairy' (milk, yoghurt and cheese), 'Fish and eggs', 'Cereal products and potatoes' (rice, pasta, potatoes, bread and other grains), 'Fruit and vegetables' (fresh fruits, natural fruit juice and vegetables), 'Meat and meat products' (white meat, red meat and processed meat), 'Sweet foods' (cakes, candies, sweet pastry, chocolate, biscuits, added sugar and ice cream), 'Salty snacks' (salty snacks, pizzas and chips) and 'Soft drinks' (nectars and soft drinks).

Intakes of total energy and nutrients were estimated for each participant using data from the FFQ. Except animal and vegetable protein, values for all macro- and micronutrients were obtained from the Portuguese Food Composition Table (TCAP) (221), updated with data from the EUROFIR AISBL for the National Food, Nutrition and Physical Activity Survey 2015–2016 (222). To estimate animal and vegetable protein, a standardized procedure was applied. First, for unprocessed or non-commercial foods, protein was classified according to its source (e.g. beef was considered 100% animal protein, and potato 100% vegetable protein). Second, for processed or commercial foods, product labels were consulted and the proportion of each type of protein was estimated based on the list of ingredients; when labels were unavailable or when it was not possible to estimate the proportion of each protein type, the protein content of the main ingredient was assumed (e.g. jarred meat baby food was considered 100% animal protein). Third, for selected recipes available in PortFIR, the Portuguese food information platform maintained by the *Instituto Nacional de Saúde Doutor Ricardo Jorge*, the percentage of each ingredient in the recipe was used to estimate the proportion of each protein type. Finally, for non-specific food items, the mean proportion of protein type from the foods composing that group (e.g. apple, pear, banana and strawberry) was used to estimate the contribution of animal and vegetable protein.

Total energy intake for each participant was calculated in kilocalories per day (kcal/day). The total intakes of protein, carbohydrates, fat, and fibre were calculated in grams per day

(g/day), while the total intakes of iron, zinc, magnesium and calcium were recorded in miligrams per day (mg/day). Vitamin D intake was expressed in micrograms per day (μg/day).

The FFQ was validated and calibrated at ages 4 and 7 years in a subsample of the present cohort, using 3-day food diaries and biomarkers for comparison. This validation study demonstrated that the FFQ is a effective tool for assessing a wide range of food groups and key nutrient intakes in children at these ages (223).

3.1.3.3. Healthy Eating Index (HEI)

A Healthy Eating Index (HEI), previously developed to assess children's diet quality (224, 225), was adapted to be included in this thesis. For each of the eight food groups previously described, quartiles of frequency of consumption were calculated using the mean of quartiles across all ages, to define the same cut-off points for all follow-ups. A score ranging from 1 to 4 points was assigned to each food group as follows. For food groups considered healthier ("Dairy", "Fish and eggs", "Cereal products and potatoes", and "Fruit and vegetables"), the lowest quartile of consumption was assigned 1 point, the intermediate quartiles were assigned 2 and 3 points, and the highest quartile of consumption was assigned 4 points. For food groups considered less healthy ("Meat and meat products", "Sweet foods", "Salty snacks", and "Soft drinks"), the scoring was reversed, with the highest quartile of consumption receiving 1 point, the intermediate quartiles receiving 2 and 3 points, and the lowest quartile of consumption receiving 4 points. The points attributed to each food group were summed to obtain the total HEI score, which ranged possibly from 8 to 32 points at all ages, with higher scores reflecting a better diet quality.

The HEI was previously validated in a subsample of children evaluated at 4 and 7 years of age (225). For validation purposes, a subsample of children with dietary information gathered from 3-day food diaries was used to compare the HEI score with nutrient intake from those food diaries, including total energy intake, carbohydrates, protein, fat and fiber. The comparison revealed that the HEI was able to discriminate diet quality at both ages. Specifically, the HEI score showed a positive and significant correlation with

protein and fiber intake, while it was inversely correlated with total energy intake, total fat intake, and saturated fat intake.

3.1.3.4. Ultra processed foods (UPF)

All foods reported in the FFQ at all G21 follow-ups were classified (226) according to their processing degree and their purpose using the NOVA food classification system (227, 228). This classification system, developed by researchers at the University of São Paulo (Brazil), is based on the extent and purpose of food processing and categorises all foods into four groups: (1) unprocessed or minimally processed foods (e.g. fresh fruit and vegetables, fresh meat and fish); (2) processed culinary ingredients (e.g. olive oil, butter and sugar); (3) processed foods (e.g. bread and cheese); and (4) ultra-processed foods (UPF; e.g. soft drinks, fast food and chocolates). As each FFQ item may include foods belonging to different NOVA groups, classification was assigned according to the most commonly consumed subtype in Portugal, based on national consumption data (e.g. yoghurt was classified as group 4, although the FFQ item also included natural yoghurt without added sugar (group 1) and yoghurt with natural sweeteners (group 3)). For composite foods prepared at home, classification was based on the main ingredient (e.g. vegetable soup was classified as NOVA group 1, because its main components are fresh vegetables, despite the use of salt and olive oil during preparation) (226).

3.1.4. Children's physical activity, sleep duration and sedentary behaviour (G21)

Data on regular physical activity were collected at all follow-ups through reports from the child's main caregiver, who was asked whether the child engaged in any scheduled and regular sports activity outside school (yes/no). Regular practice of structured physical activity was considered as a categorical variable.

Night-time sleep duration (hours per night) was calculated based on the difference between parent-reported bedtime and wake-up time. At 4 and 13 years of age, bedtime and wake-up times were reported for the entire week, so no additional calculations were necessary to determine sleep duration at these ages. At 7 years of age, bedtime and wake-

up times were collected separately for weekdays and weekends, and weekly average sleep duration was computed as follows:

$$Sleep\ duration_{7y} = \frac{(weekday\ sleep\ duration\ \times\ 5) + (weekend\ sleep\ duration \times\ 2)}{7}$$

At the 10-year follow-up, sleep duration data were not collected. To estimate sleep duration at 10 years, an estimative was calculated considering the number of hours the child slept per night collected at the 7- and 13-year follow-ups, applying the following formula:

$$Sleep\ duration_{10y} = sleep\ duration_{7\ y} + \frac{(sleep\ duration_{13y} - sleep\ duration_{7y})}{156 - 84} \times 36$$

where 36 represents the difference in months between 13 years (156 months) and 10 years (120 months), and 10 years (120 months) and 7 years (84 months).

Sleep duration was then categorized into ≤7, 8, 9, 10, and ≥11 hours per night for Paper I. For Paper III, sleep duration was dichotomised as <10h and ≥10h per night, according to the WHO recommendations (229).

Time spent in sedentary behaviours such as reading, watching TV, and playing video games, were collected at all ages separately for weekdays and weekends. Weekly average sedentary time was calculated as:

$$Sedentary\ activity\ time = \frac{(sedentary\ activity\ week\ \times 5) + (sedentary\ activity\ weekend\ \times 2)}{7}$$

Sedentary activity time was categorized into ≤1, 2, and ≥3 hours per day for paper I. In paper IV, sedentary time was dichotomised as <1h and ≥1h per day at 4 years of age, and as <2h and ≥2h per day at 7 years of age. This classification alligns with the recommendations of the Australian Physical Activity and Sedentary Behaviour Guidelines (230, 231) and the WHO (229), which advises that children under 5 years of age should have no more than one daily hour of sedentary recreational screen time, while children aged 5-17 years should be limited to no more than 2 hours per day.

3.1.5. Pubertal timing assessment (G21)

Pubertal timing was assessed by trained nurses at the 10- and 13-year follow-ups through palpation and visual inspection, according to the Tanner staging criteria developed by Tanner and Marshall (22, 23). Pubic hair development (at 10 and 13 years of age) and axillary hair development (only at 13 years of age) were evaluated in both sexes. Breast development was assessed in girls, whereas testicular development was assessed in boys using a Prader orchidometer.

Each characteristic was classified into one of the five Tanner stages: stage 1 (prepubertal; puberty has not yet started), stages 2 to 4 (pubertal), and stage 5 (postpubertal; full sexual maturation). Girls were classified as having initiated puberty (Tanner stage ≥2) if they presented slightly pigmented and downy pubic hair and breast budding, defined as elevation of the breast and papilla with enlargement of the areola. Boys were classified as having initiated puberty (Tanner stage ≥2) if they presented a testicular volume greater than 4 mL and slightly pigmented and downy pubic hair.

In this thesis, some degree of misclassification of Tanner stages cannot be excluded, particularly for testicular volume assessment in boys, due to its invasive nature involving testicular palpation (26). In addition, defining pubertal timing based on a single pubertal characteristic is limited, since different pubertal markers may progress at different rates. Therefore, the agreement between the Tanner stage for genital development and the Tanner stage for pubic hair was evaluated. Agreement was relatively low, particularly among boys ($\kappa = 0.26$ for males and $\kappa = 0.56$ for females). Considering these limitations, a score, designated by the Puberty score, was developed. This score corresponds to the sum of the Tanner stage for genital (or breast, in girls) development and the Tanner stage for pubic hair, resulting in a total score ranging from 2 to 10. The Puberty score was used to reduce measurement error associated with the assessment of each pubertal characteristic separately, particularly the Tanner stage based on testicular volume. A Puberty score of 3 or higher was considered indicative of pubertal onset.

At the 13- and 18-year follow-ups, when pubertal development was assessed by trained nurses, girls were asked to report their age at menarche, defined as the age at which their first menstrual cycle occurred. The final age at menarche variable used in the analyses was derived as follows: when age at menarche was reported at only one follow-up, that

value was used; when it was reported at both follow-ups and the difference between the two reports did not exceed two years, the mean of the two values was used.

3.1.6. Anthropometrics (G21)

A team of trained staff performed objective measurements of children's weight and height at all follow-up visits, according to standardized procedures. Before the start of each follow-up assessment, all health professionals were instructed to read a detailed procedures manual describing how each measurement should be performed, and training sessions, including role-playing for anthropometric assessments, were conducted.

At all follow-ups, children's height was measured using a wall stadiometer, from the top of the head to the bottom of the feet, without shoes. Weight was measured to the nearest 0.1 kg using a calibrated digital scale (Tanita®, Arlington Heights, Illinois, USA), with children wearing light clothing and no shoes.

BMI was calculated as weight in kilograms divided by height in metres squared (kg/m^2). BMI was categorised according to age- and sex-specific cut-offs defined by the WHO as underweight (<-2 standard deviations [SD]), normal weight (≥-2 SD to ≤1 SD), overweight (>1 SD to ≤ 2 SD) and obesity (>2 SD) (232).

3.1.7. Urban environmental exposures (G21)

Residential address information was collected at birth and updated annually throughout the child's life. Urban environmental exposures considered in this thesis were obtained at each evaluation using Geographic Information System (GIS)–based modelling, based on the mother's self-reported residential address updated at each follow-up.

Urban environment variables were selected according to the existing literature and data availability in the current and ongoing cohort and included indicators of air pollution (e.g., NO_2 and $PM_{2.5}$), built environment (e.g., building and population density, facility density and richness, and a walkability index), natural environment (e.g., Normalized Difference Vegetation Index [NDVI] and distance to the nearest major green space), and traffic-related indicators (e.g., inverse distance to the nearest road and traffic load on all roads

within a 100-m buffer). These exposures were calculated only for participants living within the Porto Metropolitan Area. Built environment and natural environment variables were initially computed using multiple buffer sizes (100 m, 300 m and 500 m); however, in the present thesis, only variables derived from the 300-m buffer were considered.

3.2. GENOBOX

Participants in the GENOBOX study were evaluated at baseline using a standardized assessment protocol. A total of 1699 children aged between 2 and 18 years were included at baseline. This assessment included anthropometric measurements (weight and height), pubertal stage assessment, blood pressure measurements, collection of biological samples and genetic material, as well as sociodemographic, clinical and lifestyle-related information obtained through questionnaires and medical history.

3.2.1. Pubertal timing assessment (GENOBOX)

Similar to G21, the evaluation of sexual maturity was assessed in all participants by trained clinicians using standardized procedures, based on palpation and visual inspection, according to the Tanner's five-stage scale developed by Marshall and Tanner (22, 23). Pubertal development was classified into five stages (Tanner I-V), where stage I corresponds to the prepubertal stage and stages II to V represent progressive pubertal maturation.

3.2.2. Anthropometrics (GENOBOX)

Weight and height were assessed by a single examiner following standardized procedures within each hospital. Weight was measured using an electronic scale, with children wearing only underwear and without shoes. Standing height was measured barefoot using a precision stadiometer. BMI was calculated as weight (kg) divided by height squared (m^2). For this thesis, BMI was classified according to age- and sex-specific cut-off points

proposed by the WHO, into underweight (<-2 SD), normal weight (≥-2 SD to ≤ 1 SD), overweight (> 1 SD to ≤ 2 SD), and obesity (> 2 SD).

3.3. INMA Project

In the INMA Project, extensive assessments were conducted during pregnancy and throughout childhood. At baseline and at each follow-up, structured questionnaires were administered in face-to-face interviews by trained staff to collect parental sociodemographic information (e.g., maternal age and occupational characteristics), clinical data, and information on maternal and child dietary determinants. In addition, physical examinations (including pubertal assessment) and anthropometric measurements were performed. Additionally, biological samples were collected for biomarker assessment, and environmental exposures (e.g., air pollution and traffic-related pollution) were estimated.

3.3.1. Maternal characteristics (INMA)

Maternal sociodemographic characteristics were collected through a face-to-face questionnaire administered during the first trimester of pregnancy. Maternal age at delivery was used as a continuous variable. As a proxy for household socioeconomic position, the logarithm of equivalised total disposable household income at baseline was used, estimated through a prediction model based on data from the European Union Statistics on Income and Living Conditions (EU-SILC). This indicator corresponds to the household's total disposable income, adjusted for household size and composition (233).

Maternal pre-pregnancy weight was also collected at baseline, and maternal pre-pregnancy body mass index (BMI) was calculated as pre-pregnancy weight (kg) divided by height squared (m^2), and analysed as a continuous variable.

3.3.2. Maternal and child dietary intake (INMA)

3.3.2.1. Breastfeeding

Information on breastfeeding was obtained through a questionnaire administered by a trained interviewer to the mother when the child was 6 months old (Asturias and Sabadell sub-cohorts), 14 months, and 4 years old, in all sub-cohorts. In the present thesis, information on exclusive and non-exclusive breastfeeding of the INMA cohort participants was considered. Exclusive breastfeeding was defined as the child receiving breast milk only, without consuming any other type of food or drink, not even water, but allowing the child to receive oral rehydration salts solution, drops, and syrups. Information on exclusive breastfeeding was not collected in the Valencia sub-cohort. The duration of non-exclusive breastfeeding was considered as a continuous variable, and the duration of exclusive breastfeeding was considered both as a continuous and a categorical variable. Children were grouped into two categories: a) exclusive breastfeeding for <24 weeks (6 months); b) exclusive breastfeeding for ≥24 weeks.

3.3.2.2. Diet quality

Information on the mother's diet during pregnancy was collected during the first trimester through a validated FFQ (234). For the mother's diet quality, only the food groups 'Fruit and vegetables' and 'Energy-dense foods' were considered.

An FFQ derived from the validated version for pregnant women (234) was adapted to include foods and portions appropriate for children aged 4–5 years. This 105-item questionnaire was validated in a subsample of the same population (235). Validity was examined by comparing FFQ estimates with the average of three 24-hour dietary recalls and with blood biomarkers. The questionnaire was completed by the main caregiver, considering usual food consumption over the previous 12 months. The FFQ includes 9 possible frequencies of consumption, ranging from 'never or less than once a month' to 'six or more times a day'. These response categories were subsequently converted into daily intake frequencies. For the present thesis, only two food groups were considered as possible indicators of diet quality, the 'Fruit and vegetables' group and the 'Energy-dense foods' group.

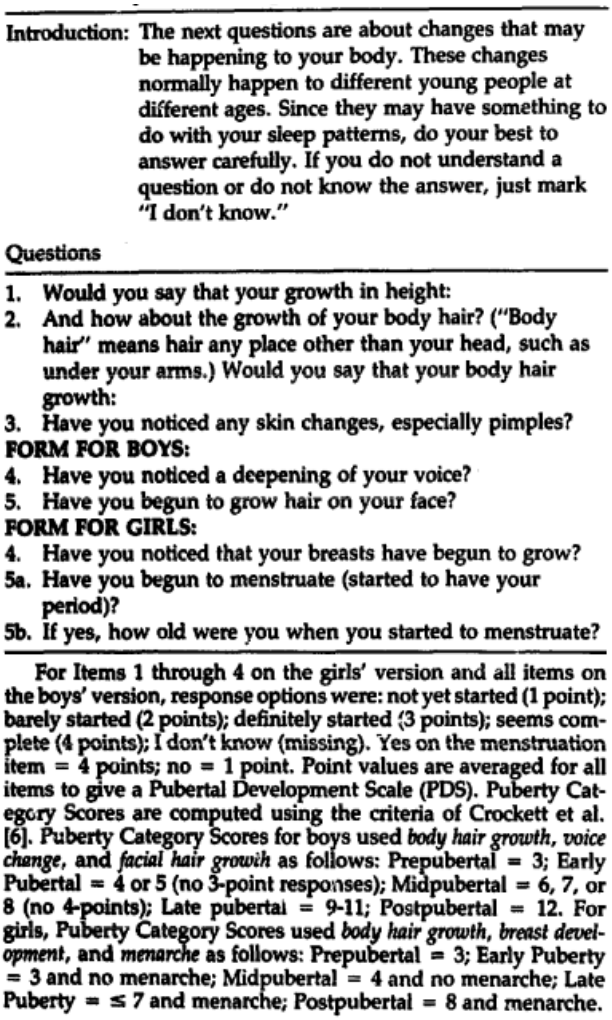
3.3.3. Pubertal timing assessment (INMA)

In INMA subcohorts, pubertal timing was assessed using the Tanner staging (22, 23), parent-reported Peterson's Pubertal Development Scale (PDS) (24), and/or age at menarche (girls only). A pediatrician or a trained health professional assessed genital development by visual inspection and palpation, pubic hair development by visual inspection, and breast development by palpation. The development stage was classified into one of the five Tanner stages, ranging from 1 (prepubertal) to 5 (complete development). A Tanner stage of 2 or higher was considered indicative of pubertal onset. Only children from the Asturias and Valencia sub-cohorts were evaluated for the Tanner stages, and the Valencia sub-cohort also has PDS data available.

The PDS is an interview-based continuous measure of pubertal development (Figure 6), obtained through parent report, and is a reliable and valid instrument for assessing pubertal development in children, based on physical characteristics. It includes items on breast/genital development, body hair, and skin changes for all children, with sex-specific items on facial hair and deepening voice for males, and on menarche for females. The four response options are 'not yet started', 'barely started', 'definitely started' and 'seems complete'. If the girl has already had her first period, a score of 4 is assigned; if not, a score of 1 is given. Point values were averaged across all items to provide a PDS score. The continuous score was then transformed into a five-point ordinal scale (1 - pre-puberty, 2 - early puberty, 3 - mid-puberty, 4 - late puberty, and 5 - post-puberty), using the criteria of Carskadon (37) and Acebo and Shirtcliff (43).

The PDS has been validated in previous studies, including within the INMA-Valencia sub-cohort, and the results indicate moderate to good agreement with the Tanner stages evaluated by physical examination (38). Although PDS categories cannot be directly mapped onto the five-point Tanner scale, they are effective in distinguishing between pre-, mid-, and post-pubertal stages (38).

During the assessment at ages 9 and 10, parents were asked whether the child had already begun menarche, and if so, at what age she had her first menstruation. Age at menarche was used as a continuous variable.



Introduction: The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. Since they may have something to do with your sleep patterns, do your best to answer carefully. If you do not understand a question or do not know the answer, just mark "I don't know."

Questions

1. Would you say that your growth in height:
2. And how about the growth of your body hair? ("Body hair" means hair any place other than your head, such as under your arms.) Would you say that your body hair growth:
3. Have you noticed any skin changes, especially pimples?

FORM FOR BOYS:

4. Have you noticed a deepening of your voice?
5. Have you begun to grow hair on your face?

FORM FOR GIRLS:

4. Have you noticed that your breasts have begun to grow?
5a. Have you begun to menstruate (started to have your period)?
5b. If yes, how old were you when you started to menstruate?

For Items 1 through 4 on the girls' version and all items on the boys' version, response options were: not yet started (1 point); barely started (2 points); definitely started (3 points); seems complete (4 points); I don't know (missing). Yes on the menstruation item = 4 points; no = 1 point. Point values are averaged for all items to give a Pubertal Development Scale (PDS). Puberty Category Scores are computed using the criteria of Crockett et al. [6]. Puberty Category Scores for boys used *body hair growth, voice change,* and *facial hair growth* as follows: Prepubertal = 3; Early Pubertal = 4 or 5 (no 3-point responses); Midpubertal = 6, 7, or 8 (no 4-points); Late pubertal = 9-11; Postpubertal = 12. For girls, Puberty Category Scores used *body hair growth, breast development,* and *menarche* as follows: Prepubertal = 3; Early Puberty = 3 and no menarche; Midpubertal = 4 and no menarche; Late Puberty = ≤ 7 and menarche; Postpubertal = 8 and menarche.

Figure 6. Pubertal Development Scale (PDS).

3.3.4. Anthropometrics (INMA)

BMI was calculated at all ages as weight (kg) divided by height squared (m^2). BMI was then converted into age- and sex-specific z-scores using the cut-off points for children and adolescents aged 5–19 years established by the WHO (232). Participants were subsequently classified as underweight (< −2 SD), normal weight (≥ −2 SD to ≤ 1 SD), overweight (> 1 SD to ≤ 2 SD), or obese (> 2 SD).

3.3.5. Urban environmental exposures (INMA)

Outdoor air pollution exposure to nitrogen dioxide (NO_2) and particulate matter (PM) was estimated using environmental monitoring data combined with land-use regression models, incorporating geographical information on land use, traffic indicators, and altitude (236, 237). These models were used to predict individual residential exposure.

Residential surrounding greenness was characterized using the Normalized Difference Vegetation Index (NDVI) within a 300m buffer around each participant's home. NDVI is a widely used satellite-based indicator of vegetation cover, with values ranging from -1 to 1, where higher values indicate greater photosynthetically active vegetation. Satellite images were selected for each study area during the greenest months and under cloud-free conditions, and greenness was estimated at the time of delivery (238). Access to green and blue spaces was calculated as the straight-line distance from the participant's residence to the nearest green space and to the nearest blue space, respectively, using an urban atlas database (238).

Built environment indicators were derived from different spatial data sources. Traffic data and public transport accessibility were obtained from municipal records, while population density was derived from the Spanish Statistical Office (INE). Street connectivity and the location of facilities were obtained from digital street maps provided by Navteq, and building density data were retrieved from the regional cartographic institutes (238). Connectivity was calculated as the number of intersections per square kilometre within 300m buffers. Facilities were characterized using two indicators: a facility richness index, defined as the number of different types of facilities divided by the maximum possible number of facility types within the 300-m buffer, and a facility density index, calculated as the number of facilities divided by the buffer area (300m). Land-use characteristics were summarized using a land-use mix indicator based on Shannon's Evenness Index, which reflects the degree of diversity of land-use categories within the area. Traffic exposure was described using three indicators: total traffic load within a 100m buffer, traffic density on the nearest road, and the inverse distance to the nearest road. Accessibility to public transport was assessed using two measures within 300m buffers: the total length of public bus lines and the number of bus stops. The food environment was defined as the density of unhealthy food, and was calculated as the number of facilities related to unhealthy food divided by the area of the 300m buffer, assessed for

the first trimester of pregnancy. Lastly, a walkability index was constructed by combining population density, street connectivity, facility richness, and land-use evenness. The index was computed as the mean and the sum of the deciles of these components within 300m buffers, resulting in a walkability score ranging from 0 to 1 (238).

RESULTS

Paper I

Longitudinal tracking of diet quality from childhood to adolescene: The Interplay of individual and sociodemographic factors

Marta Pinto da Costa, Milton Severo, Joana Araújo, Sofia Vilela

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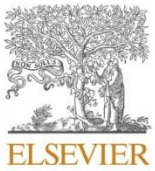

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Longitudinal tracking of diet quality from childhood to adolescence: The Interplay of individual and sociodemographic factors

Marta Pinto da Costa [a,b,c], Milton Severo [a,b,d], Joana Araújo [a,b,c], Sofia Vilela [a,b,*]

[a] EPIUnit – Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600, Porto, Portugal
[b] Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, n° 135, 4050-600, Porto, Portugal
[c] Departamento de Ciências da Saúde Pública e Forenses, e Educação Médica, Faculdade de Medicina, Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal
[d] Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Rua de Jorge Viterbo Ferreira, n°288, 4050-321, Porto, Portugal

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ABSTRACT

This study aimed to examine diet tracking from childhood to adolescence, using 4 time-points, and the influence of socioeconomic and individual characteristics in this transition. The sample included 6893 children from the Portuguese birth cohort Generation XXI with complete information on Food Frequency Questionnaire in at least one of the considered follow-ups. A Healthy Eating Index (HEI), previously developed to assess adherence to WHO's dietary recommendations, was applied at all ages (4, 7, 10 and 13y). The intraclass correlation coefficient (ICC) was used to analyse the tracking of diet quality. Linear mixed-effect models were performed to estimate the association of the child's socioeconomic and individual characteristics with the HEI score and respective trajectories over time. The overall diet quality decreased from childhood (22.2 ± 3.6 at 4y) to adolescence (18.2 ± 3.9 at 13y), with moderate tracking (ICC = 0.53), showing that children who start a healthy diet earlier might have a better diet quality as time goes by. Children of older mothers (β = 0.079, 95%CI = 0.061–0.097) and with higher education (β = 0.203, 95%CI = 0.178–0.229) and a higher household monthly income (β = 0.024,95%CI = 0.007–0.041) had a higher diet quality over time. Besides family characteristics, the child's sedentary activities (β = −0.009, 95%CI = −0.014–-0.003) negatively influence diet quality throughout life. In contrast, being a girl (β = −0.094, 95%CI = −0.132–-0.056) and having higher sleep duration (β = 0.039, 95%CI = 0.015–0.064) are associated with a higher diet quality over time. The presence of dietary tracking from childhood to adolescence implies that promoting healthy eating habits during the first years of life is crucial for a healthier diet quality during late childhood and early adolescence, focusing on maternal and individual child characteristics.

1. Introduction

Children are often the targets for primary prevention through the promotion of healthy eating habits due to the evidence that the physiological risk of chronic diseases can develop early in childhood (CDC, 2011). Diet quality, both in childhood and adolescence, could have a lifelong effect on the incidence of several diseases, namely obesity, diabetes, heart diseases and some types of cancer (Herman et al., 2009; Ng et al., 2014; Park et al., 2012).

In Portugal, more than half the children do not meet the World Health Organization (WHO) recommendation of the consumption of five or more servings of fruit and vegetables per day (Lopes et al., 2018), a classical indicator of diet quality. Although some changes in food intake mark the transition from preschool to school, the eating habits developed early in preschool tend to track to some degree into school age (da Costa et al., 2019). In the same way, the existence of dietary tracking from 7 to 10 years old was previously demonstrated (da Costa et al., 2022). Later in life, adolescents' eating behaviours are also frequently considered unhealthy (Seddon, 2003). According to the most recent Portuguese National Food, Nutrition and Physical Activity Survey (2015–2016), adolescents are the group with the greatest dietary inadequacy of fruit and vegetables, processed meat, and soft drinks (Lopes et al., 2018). Given that, it is important to identify what changes in diet quality occur from childhood to adolescence and at what age, to understand better the optimal stage to intervene and change the prevalence of unhealthy eating habits. However, information on changes in

* Corresponding author. EPIUnit – Instituto de Saúde Pública, Universidade do Porto Rua das Taipas, no 135, 4050-600, Porto, Portugal.
E-mail address: sofia.vilela@ispup.up.pt (S. Vilela).

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diet quality from childhood to adolescence is usually scarce and not so consistent. While some studies show that eating habits developed in childhood tend to track into adolescence and adulthood (Mikkilä et al., 2005; Madruga et al., 2012; Movassagh et al., 2017; Ambrosini et al., 2014), others show that eating habits acquired during childhood may not be maintained during adolescence (Madruga et al., 2012; Patterson et al., 2009) with studies showing evidence for slight tracking for some food groups and nutrients or not supporting that nutrient intakes are stable between earlier and later ages (Zive et al., 2002; Patterson et al., 2009), since behaviours are prone to change in this life stage (Meredith & Dwyer, 1991).

Considerable changes in diet quality between childhood and adolescence have been proposed to occur because of physiologic changes and the maturation process (Trahms & Peggy, 1997). Dietary tracking can be affected by family socioeconomics but also due to adolescents' growing independence in this transition and influence from peers, social environment, and new challenges, such as the influence of social media (Arruda et al., 2014; Sawyer et al., 2012; Totland et al., 2013). In this stage of life, the impact of the parents on diet quality diminishes (De Bourdeaudhuij, 1997). The adolescent begins a period where his healthy eating habits will be shaped (Sawyer et al., 2012), with evidence suggesting that eating habits developed in this phase usually 'track' into adulthood (Mikkilä et al., 2004; Cruz et al., 2018). Nonetheless, one of the hypotheses raised in the present study is that family socioeconomic characteristics continue to influence dietary tracking over time, namely from childhood to adolescence. Maternal age and educational level have usually been described as socioeconomic factors with a major impact on diet quality during childhood and adolescence. Children and adolescents with older and more educated mothers are more likely to have better dietary quality (MacFarlane et al., 2007; da Costa et al., 2019). Also, higher dietary scores were observed in children and adolescents with higher household incomes (Hinning et al., 2018; Kurotani et al., 2021)

Individual behaviours could, still, have an important role in determining changes in food intake and choices throughout childhood and adolescence. The practice of physical activity and less sedentary behaviours were previously associated with higher diet quality during childhood in cross-sectional studies (Fisk et al., 2011). In addition, evidence suggests that sedentary activities are associated with increased consumption of energy-dense foods, namely soft drinks, and sweets, between 11 and 13 years old (Gebremariam et al., 2013). Better diet quality has been associated with adequate sleep duration (González-Treviño et al., 2022). The opposite has also been described, with sleep deficiency in childhood and adolescence linked to poor diet quality (Córdova et al., 2018). Most studies that describe the association between sedentary behaviours, sleep duration, and diet quality have a cross-sectional design. This study fills the gap of longitudinal studies on these associations, which makes it easier to detect the development or changes in behaviour patterns that may arise over time and influence diet quality trajectories. Individual characteristics also seem to impact diet quality during these two stages of life, such as sex. Several studies report that girls frequently have healthier food choices than boys, especially a higher consumption of fruit and vegetables and fibre-rich foods in childhood and adolescence (Cooke & Wardle, 2005; Lopes et al., 2018). Understanding when and how sociodemographic and individual characteristics and behaviours can affect dietary tracking over time is essential to support strategies to improve diet quality at these stages of life.

To the best of our knowledge, this is the first study on the tracking of diet quality between two important stages of the life cycle that consider 4 different ages, with a focus on transitions from preschool to adolescence. Therefore, the present study aimed to evaluate diet tracking from childhood to adolescence, spanning over a period of 10 years, and the influence of socioeconomic, individual and behaviours characteristics in the diet quality trajectories. The specific hypotheses tested were that: a) there is stability in the diet quality over time, with diet quality at 4 years of age being a consistent predictor of healthier eating habits later in life; b) socioeconomic characteristics of the family predict stability of diet quality over time; c) healthy habits throughout life, namely practice of physical activity and adherence to the recommended number of sleep hours per night, predict better diet quality.

2. Methods

2.1. Study population

This study included participants of the previously described population-based cohort Generation XXI (Alves et al., 2012; Larsen et al., 2013). Briefly, a total of 8647 new-borns in the five public maternity hospitals in the metropolitan area of Porto were recruited between 2005 and 2006. Of the invited mothers, 91.4% accepted to participate. All families were invited to attend a follow-up when children were aged 4 (86% of participation), 7 (80% of participation), 10 (76% of participation) and 13 years old (54% of participation – follow-up was interrupted due to COVID-19 pandemic). At each evaluation wave, participants were invited to participate in a face-to-face interview and trained interviewers collected the data through structured questionnaires with information about sociodemographic conditions, health status, lifestyle, and objective anthropometric measures. There were included 3456 participants with 4 complete dietary follow-up data; 1543 participants with 3 complete dietary follow-up data; 955 participants with 2 complete dietary follow-up data; 939 participants with 1 complete dietary follow-up data. The final sample included all children who attended at least one evaluation with complete dietary data (n = 6893), corresponding to a total of 21302 observations over the four time-points.

2.2. Healthy Eating Index (HEI)

Data on children's food consumption were collected through a Food Frequency Questionnaire (FFQ) by trained interviewers applied to the main caregiver of the child at 4-, 7- and 10-year follow-up, assessing usual food consumption in the previous 6 months, and directly applied to the adolescent at the 13-year follow-up, considering frequent food consumption in the last 12 months. The FFQ included thirty-five food items at 4 years of age, thirty-eight food items at 7 (added items: chocolate milk, breakfast cereals and natural fruit juice), forty-one food items at 10 (added items: soy products, legumes and butter/margarine group were divided into two), and thirty-nine food items at 13 years of age (only one group for the milk in total, the group rice, pasta and potatoes was split in two, and the items about tea were removed). At 4 and 7 years old, the FFQ was validated and calibrated in a subsample of the present cohort against 3-day food diaries, showing that the FFQ is a useful instrument for evaluating a wide range of food groups and key nutrient intake in children at this age (Vilela et al., 2019). The nine possible frequency options, ranging from 'never' to '≥4 times per day', were converted into daily consumption frequencies. Similar food groups were created for all ages, based on the dietary recommendations of the World Health Organization, 2006): 'Dairy' (milk, yoghurt and cheese), 'Fish and eggs', 'Cereal products and potatoes' (rice, pasta, potatoes, bread and other grains), 'Fruit and vegetables' (fresh fruits, natural fruit juice and vegetables), 'Meat and meat products' (white meat, red meat and processed meat), 'Sweet foods' (cakes, candies, sweet pastry, chocolate, biscuits, added sugar and ice cream), 'Salty snacks' (salty snacks, pizzas and chips) and 'Soft drinks' (nectars and soft drinks).

An HEI previously developed to evaluate children's diet quality (Vilela et al., 2014; da Costa et al., 2019) was adapted for this study to assess diet quality from childhood to adolescence. For each of the eight food groups described above, quartiles of frequency of consumption were obtained considering the mean of quartiles at all ages to define the same cut-off points for all follow-ups (Supplementary Table 1). The score was assigned, ranging from 1 to 4 points, as follows: for food

groups considered healthier ('Dairy', 'Fish and eggs', 'Cereal products and potatoes' and 'Fruit and vegetables'), the lowest quartile of consumption was scored with 1 point, intermediate quartiles with 2–3 points and the highest consumption quartile were attributed 4 points; for food groups considered less healthy ('Meat and meat products', 'Sweet foods', 'Salty snacks' and 'Soft drinks'), the score was assigned reversely, that is, the highest quartile of consumption was scored with 1 point. The scores assigned were summed up, resulting in a total score index possibly ranging from 8 to 32 points for all ages, with a higher score reflecting a better diet quality. The HEI was previously validated in a subsample of children evaluated at 4 and 7 years of age (da Costa et al., 2019). To validate the HEI, children with dietary information based on 3-day food diaries were considered to compare the HEI score with nutrient intake evaluated by food diaries (total energy intake, carbohydrates, proteins, fat, and fiber). The comparison showed that the HEI was assessing a better diet quality at both ages since the HEI was positively and significantly correlated with protein and fiber intake but inversely correlated with energy intake, total fat intake and saturated fat.

2.3. Child's characteristics

The child's characteristic variables were analysed similarly at all ages. Night-time sleep duration was calculated according to the difference between parent reports of bedtime and awake time. At 4 and 13 years old, the bedtime and awake time were collected considering the entire week, not being needed to apply any formula to obtain sleep duration at these ages. At 7 years of age, the bedtime and awake time were assessed separately for week and weekend days and computed as follows: [(week sleep duration x 5) + (weekend sleep duration x 2)]/7 days, and then categorized into $\leq$7, 8, 9, 10 and $\geq$ 11 h of sleep per night. At the 10-year follow-up, sleep time or duration information was not collected. To overcome the sleep duration missing data, an estimative was calculated considering the number of hours the child slept per night collected at the 7- and 13-year follow-ups. The following formula was applied: sleep $\text{duration}_{7 \text{ years of age (y)}}$ + (($\text{sleep duration}_{13y}$ - $\text{sleep duration}_{7y}$)/($156_m$–$84_m$) * 36)), where 36 is the difference between 156 and 120 months, and 120 and 84 months. For example, if the child slept 9 h per night at 7 years old and 8 h per night at 13 years old, the estimated sleep duration at 10 years old was 8.6 h [9 + ((8–9)/(156 - 84) * 36)).

Daytime activities, including the time spent on sedentary activities (e.g., reading, watching TV, and playing video games), were collected separately by weekdays and weekend days and computed as: [(time spent on sedentary activities week x 5) + (time spent on sedentary activities weekend x 2)]/7 days. Then, it was categorized into $\leq$1, 2 and $\geq$ 3 h of sedentary activities per day, considering the recommendations of no more than 2 h of sedentary recreational screen time per day between 5 and 17 years of the Australian Physical Activity and Sedentary Behaviour Guidelines (Department of Health, 2014). Regular practice of structured physical activity was used as a qualitative variable. The main caregiver/adolescent was asked if the child practised any scheduled and regular sports activity outside of school (yes or no), and then children were classified as non-practitioners or practitioners in each evaluation.

2.4. Maternal and family characteristics

The following maternal variables were collected at baseline and considered non-modifiable variables over time: maternal age and complete years of education (considered as continuous variables). Household monthly income was measured at each evaluation wave as a categorical variable (<500, 500–1000, 1001–1500, 1501–2000, 2001–2500, 2501–3000, >3000 euros) and then grouped into four categories (<1000, 1001–1500, 1501–2000, >2001 euros), according to the distribution of the variable. Living with siblings was also considered a modifiable variable over time, and the study included information collected in all follow-ups.

2.5. Ethical considerations

The Generation XXI project was conducted in accordance with the guidelines defined in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Hospital de São João/University of Porto Medical School. The project was approved by the Portuguese Authority of Data Protection. The legal representatives of each participant were informed about benefits and potential discomforts, through written informed consent, with information on all the examinations to be carried out during the evaluation, at baseline and in the subsequent follow-up evaluations. After a brief explanation of the evaluation, the child's oral assent was requested. At the 13-year follow-up, the adolescent also signed a written informed consent.

2.6. Statistical analysis

Normality was evaluated through visual inspection (histograms and Normal Q-Q plots). Descriptive statistics, such as mean and standard deviation (SD) for continuous variables and numbers and percentages for categorical variables, were calculated to describe the participant's characteristics. Comparisons between the characteristics of eligible participants and the remaining cohort were performed using the *t-test* for continuous variables and the χ^2 test for categorical variables. Comparisons between the descriptive statistics of each follow-up were performed using the Friedman test for categorical variables and the ANOVA for Repeated Measures for continuous variables.

The tracking of diet quality from childhood to adolescence was analysed using the intraclass correlation coefficient (ICC) with a 95% confidence interval (95% CI), considering values < 0.30 as low, between 0.30 and 0.60 as moderate and >0.60 as high (Malina, 2001). Linear mixed-effect models were performed to estimate the association of the child's socioeconomic and individual characteristics with the HEI score and respective trajectories over time. Three models were calculated. Model 1 was the crude model; Model 2 was adjusted for the child's sex, maternal age, and educational level; Model 3 was adjusted for model 2 plus monthly household income, child practice of physical activity, living with siblings, sleep hours per night, and sedentary activities over the time. The variables included in the models were selected based on previous literature (Vereecken & Maes, 2010; Fisk et al., 2011; Vilela et al., 2015; Córdova et al., 2018; Hinning et al., 2018;da Costa et al., 2019; Ragelienė & Grønhøj, 2020; Souza Neto et al., 2021). Interactions between time and all the previously described variables were tested, and only the statistically significant ones are presented. The variables that might change over time, including household income, practice of physical activity, living with siblings, sleep hours per night and sedentary activities, have been included in the models as continuous. All variables that showed a statistically significant interaction with time have been categorized for better visualization of the dispersion in the graphical representation (Fig. 2). All models included random intercept and slope for time and fixed linear and quadratic terms for time. Quadratic terms have been included in the models because as it is that the variability of the diet quality would be greater at older ages than at younger ages. Considerable differences in diet quality from 4 to 7 years old are expected, as it is a transition period with a significant change in food intake, as observed in a previous study using a sample from the Generation XXI cohort (da Costa et al., 2019). We also anticipate a great difference in diet quality from age 7 to 10 because of the children's growing independence in this transition and the influence of peers, social environment, and social media (Sawyer et al., 2012; Totland et al., 2013). Nonetheless, a significant difference in the HEI score between the ages of 10 and 13 is not expected since there were no major changes in the influence of peers and the social environment on adolescent eating at these ages (Sawyer et al., 2012). The mixed-effects model assumes

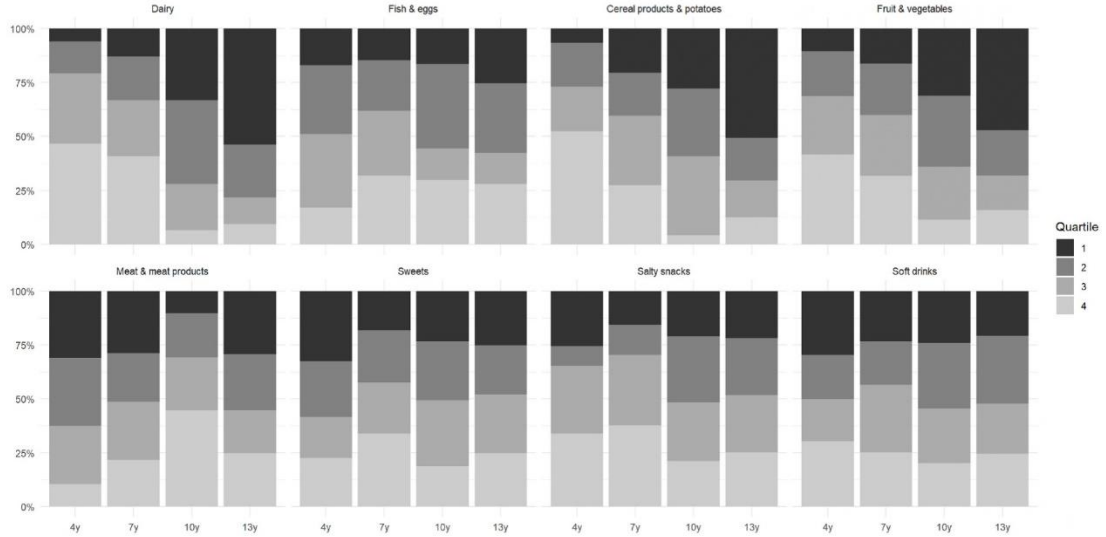

Fig. 1. Proportion of participants in each quartile of consumption of each food group of the Healthy Eating Index at 4, 7, 10 and 13 years old[1]
[1]Q4 is considered better for intakes of the 8 food groups.

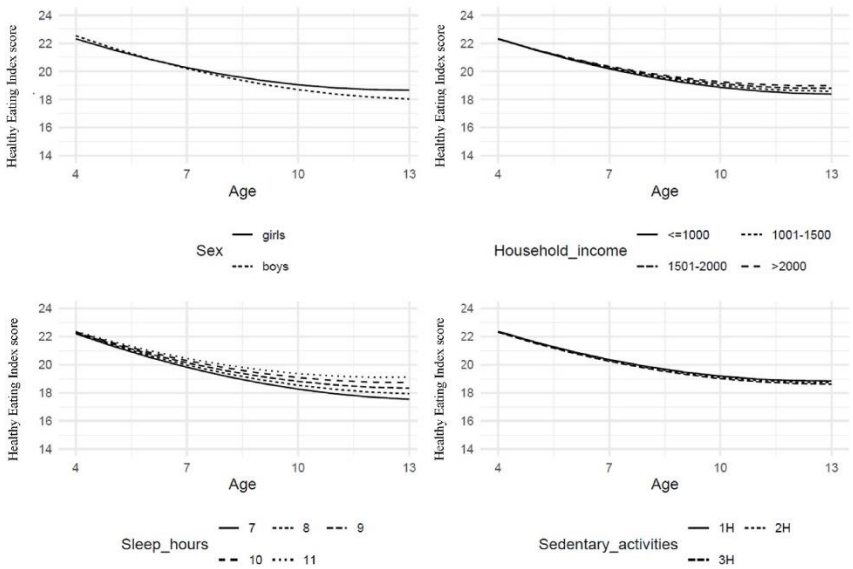

Fig. 2. Trajectories of the interactions between the Healthy Eating Index (HEI) score over time and the child's sex, sleeping hours per night, time spent on sedentary activities, and family monthly household income.[1]
[1]The prediction was based on the results of model 3 from the mixed effect model, adjusted for child's sex, maternal age and educational level, monthly household income, child practice of physical activity, siblings, sleep hours per night, and sedentary activities over time. The prediction was conditioned on mother's education level equal to 10 years, mother's age equal to 29 years, practice of physical activity equal to practice, living with siblings equal to yes, and household income (2.4), sedentary activities (152 min) and sleep hours per night (9:09 a.m.) equal to the average of the 4 follow-ups. The variables have been categorized for a better visualization of the dispersion in the graphical representation. Given that the HEI score has a minimum value of 8 and a maximum of 32, with extreme scores falling as outliers in the distribution curve, the y-axis limits were set at mean±1SD. Legend: Household income, in euros; Sleep hours/night: ≤7h; 8h; 9h; 10h; ≥11h; Sedentary activities, in hours: ≤1h; 2h; ≥3h.

missing at random and extends the curve according to the available points without losing the sample size. Linear mixed-effect models were repeated as a sensitivity analysis, considering only participants with complete data at all follow-ups (n = 3456).

Another sensitivity analysis for misreporting was done to account for possible over- and under-reporting of dietary intake. The misreporting was estimated by calculating the ratio between the reported total energy intake (TEI) through the FFQ and the estimated energy requirement (EER) (Huang et al., 2005; Leech et al., 2018). EER was calculated using validated sex- and age-specific equations (National Academies of Sciences, Engineering and Medicine, 2023), considering the physical activity category (low active), exact age and objectively measured weight and height. Participants were categorized as plausible reporters, under-reporters, or over-reporters of TEI using the ±1SD cut-off for the ratio TEI/EER (Huang et al., 2005; Leech et al., 2018).

An additional sensitivity analysis was conducted in a subsample (n =

4550), considering parental feeding practices as a potential confounder. A questionnaire combining a version of the Child Feeding Practices (Birch et al., 2001) and the scales of overt and covert control (Ogden et al., 2006), previously adapted and validated for Portuguese school-age children (Real et al., 2014), have been self-completed by parents or main caregivers at the 4-year follow-up. Answers were given on a 5-point Likert scale, with higher scores indicating higher levels in each subscale. The food parenting practices have been evaluated through three main domains (Perceived monitoring, Restriction and Pressure to eat) (Costa et al., 2021) (Supplementary Table 4). No different associations and tendencies have been found in the additional analysis. Since the main objective of the study was to assess the influence of individual and sociodemographic factors on diet quality and the parental feeding practices were only available for a subsample at 4-year-evaluation we decided to consider the final models without the parental feeding practices variables.

The software used was the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 27.0.), and the R® 3.0.1 R language and software environment for statistical computation (version 4.2.1) package 'nlme' (Pinheiro et al., 2017). Since interactions with four variables were observed, the statistical analysis considered a significance level of 0.01 as an appropriate alpha value to indicate statistical significance, considering the Bonferroni correction.

3. Results

The differences between the study participants and the remaining cohort are presented in Table 1. There were no statistically significant differences regarding the child's sex. Mothers had a mean age of 29 years and 11 years of schooling at baseline. The mothers in the study sample were slightly older and more educated than the mothers of the remaining cohort. However, the effects don't seem to affect the analysis since the effect size is considered modest (d = 0.361 and d = 0.390. respectively) (Valladares-Neto, 2018).

Table 2 presents the child and family characteristics at the four follow-ups. The number of children living with at least one sibling decreased over time (3084 at 4 years and 2984 at 13 years, $p < 0.001$). The percentage of children who practised physical activity increased from 4 years (69.6%) to 7 years (85.1%) but decreased in the subsequent follow-ups, with only 58.4% of the adolescents practising physical activity at 13 years. The percentage of households in the lowest income class decreased from 4 to 13 years, potentially associated with the increase in the minimum wage in Portugal. The number of night-time sleep hours slightly decreased over time (10 ± 0.9 h at 4 years vs 9 ± 0.8 at 13 years, $p < 0.001$), while the time that the child spent in sedentary activities per day slightly increased (104.8 min, SD = 69.0 at 4 years vs 175.0 min, SD = 90.0 at 13 years, $p < 0.001$). The HEI, a marker of diet quality, decreased from 4 to 13 years old (22.2, SD = 3.6 at 4 years vs 18.2, SD = 3.9 at 13 years). The largest decrease occurred between 7 (20.5, SD = 3.7) and 10 years old (18.6, SD = 3.7). Then, the score HEI seems to stabilize between ages 10 and 13 (18.2, SD = 3.69). The scores ranged from 10 to 32 points at 4 and 7 years, 8 to 30 points at 10 years, and 8 to 31 points at 13 years. The decrease in the HEI score is explained by the reduction in the consumption of 'Dairy', 'Cereal products' and 'Fruit and vegetables' from 4 to 13 years, which showed the same decreasing trend as the HEI over time (Fig. 1).

Table 3 presents the association between family and child characteristics and the HEI score from childhood to adolescence, as well as the ICC of the tracking of the HEI score over time. The ICC indicated moderate tracking for the HEI values between 4 and 7 years (ICC = 0.53, 95% CI = 0.51–0.55), 7 and 10 years (ICC = 0.57, 95%CI: 0.55–0.59), 10 and 13 years (ICC = 0.45, 95% CI = 0.42–0.47), and between school age (7y) and adolescence (13y) (ICC = 0.38, 95% CI = 0.36–0.41). The ICC between preschool age (4y) and adolescence (13y) indicates low tracking (ICC = 0.28, 95% CI = 0.25–0.31). As the participant got older, for each year that passed, the HEI score decreased (β = −1.619, 95%CI = −1.934; −1.305) but decreased less as time went by (β = 0.046, 95% CI = 0.039; 0.054). Boys (β = 0.590, 95% CI = 0.238; 0.943, model 3), children of mothers with higher age (β = 0.079, 95% CI = 0.061; 0.097) and higher educational level (β = 0.203, 95% CI = 0.178; 0.229, model 3) at baseline presented higher HEI scores at 4 years. Furthermore, children who practised physical exercise (β = 0.473, 95% CI = 0.308; 0.637) and lived with siblings (β = 0.128, 95% CI = 0.007; 0.248) also presented higher HEI scores at 4 years than counterparts. Likewise,

Table 1
Comparison between characteristics of the 6893 eligible participants and the remaining Generation XXI cohort evaluated at baseline.

	Study sample (n=6893)	Remaining cohort (n=1754)	Effect size
Maternal age (years), mean (SD)	29.4 (5.4)	27.4 (6.0)	0.361[a]
Maternal education (years), mean (SD)	10.8 (4.3)	9.1 (3.9)	0.390[a]
Maternal prepregnancy BMI (kg/cm^2), mean (SD)	23.9 (4.2)	23.8 (4.4)	0.020[a]
Child's sex (girl), n (%)	3371 (48.9)	865 (49.3)	0.003[b]

SD, standard deviation.
[a] Cohen's d.
[b] Cramer's V.

Table 2
Characterization of the sample of 6893 Portuguese children from the Generation XXI cohort according to the 4 different time-points data collections.

	Age at follow-up			
	4 years (n = 5699)	7 years (n = 5713)	10 years (n = 5341)	13 years (n = 4549)
Household income, **n (%)**				
≤1000€	1585 (28.4)	1619 (29.0)	1394 (27.0)	773 (17.4)
1001–1500€	1579 (28.3)	1567 (28.1)	1498 (29.0)	1183 (26.6)
1501–2000€	1055 (18.9)	1050 (18.8)	963 (18.6)	992 (22.3)
>2000€	1366 (24.5)	1336 (24.0)	1309 (25.3)	1494 (33.6)
Living with sibling, yes, **n (%)**	3084 (54.1)	3522 (61.6)	3441 (64.4)	2984 (64.8)
Child's practice of physical exercise, yes, **n (%)**	3882 (68.4)	4951 (85.1)	3365 (63.0)	2668 (58.4)
Child's sleeping hours per night, **mean (SD)**	10.1 (0.86)	10.2 (0.60)	9.7 (0.53)	9.1 (0.80)
Child's sleeping hours per night, **n (%)**				
≤7h00	11 (0.2)	0 (0.0)	0 (0.0)	42 (0.9)
7h01 – 8h00	85 (1.5)	7 (0.2)	2 (0.1)	407 (9.0)
8h01 – 9h00	611 (10.7)	92 (2.3)	231 (10.4)	1943 (43.0)
9h01–10h00	2195 (38.6)	1409 (34.9)	1488 (67.0)	1750 (38.7)
10h01–11h00	2957 (39.8)	2227 (55.2)	479 (21.6)	319 (7.1)
>11h00	499 (8.8)	300 (7.4)	21 (0.9)	57 (1.3)
Child's sedentary activity/day **(minutes), mean (SD)**	104.1 (67.62)	171.3 (68.22)	163.4 (66.86)	175.0 (89.97)
Child's sedentary activity/day **(hours), n (%)**				
≤1	1757 (31.6)	124 (2.2)	209 (3.3)	381 (8.4)
1h01-2h	2221 (40.0)	1261 (22.0)	1677 (26.5)	976 (21.5)
2h01-3h	1004 (18.1)	2186 (38.1)	2355 (37.2)	1245 (27.4)
>3h	574 (10.1)	2170 (37.8)	2083 (32.9)	1944 (42.8)
Healthy eating index score, mean (SD)	22.2 (3.6)	20.5 (3.7)	18.6 (3.7)	18.2 (3.9)

SD, standard deviation; The significance of the results is discussed in the text.

Table 3
Results from the linear mixed-effect models assessing the association between family and child characteristics from the Generation XXI cohort and Healthy Eating Index score from childhood to adolescence.

Predictors	Model 1		Model 2		Model 3	
	β (95%CI)	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**
Time	**−1.205 (-1.297; -1.113)**	**<0.001**	**−1.170 (-1.264; -1.007)**	**<0.001**	**−1.619 (-1.934; -1.305)**	**<0.001**
Time^2	**0.041 (0.036; 0.046)**	**<0.001**	**0.041 (0.035; 0.046)**	**<0.001**	**0.046 (0.039; 0.054)**	**<0.001**
Sex			**0.500 (0.166; 0.834)**	**<0.001**	**0.590 (0.238; 0.943)**	**<0.001**
Maternal age			**0.079 (0.063; 0.096)**	**<0.001**	**0.079 (0.061; 0.097)**	**<0.001**
Maternal educational level			**0.229 (0.208; 0.249)**	**<0.001**	**0.203 (0.178; 0.229)**	**<0.001**
Household income					−0.110 (−0.274; 0.053)	0.082
Practice of physical activity (yes)					**0.473 (0.308; 0.637)**	**<0.001**
Siblings (yes)					**0.128 (0.007; 0.248)**	**0.006**
Sleep hours per night					−0.116 (−0.328; 0.095)	0.156
Sedentary activities/day					**−0.006 (-0.007; -0.005)**	**<0.001**
Time*Sex			**−0.069 (-0.104; -0.035)**	**<0.001**	**−0.094 (-0.132; -0.056)**	**<0.001**
Time*Household income					**0.024 (0.007; 0.041)**	**<0.001**
Time*Sleep					**0.039 (0.015–0.064)**	**<0.001**
Time*Sedentary activities					**−0.009 (-0.014; -0.003)**	**<0.001**
σ^2	6.54		6.56		6.42	
τ_{00}	11.75		10.48		9.81	
τ_{11}	0.08		0.08		0.08	
ρ_{01}	−0.67		−0.71		−0.72	
ICC	0.53		0.48		0.48	
n	6893		6855		6451	
Observations	21302		21193		14264	

Statistically significant associations are highlighted in bold; Model 1: unadjusted; Model 2: adjusted for the child's sex, maternal age, and educational level; Model 3: adjusted for Model 2 plus monthly household income, child practice of physical activity, siblings, sleep hours per night, and sedentary activities over the time. Interactions between time and all the considered variables were tested; only the statistically significant are represented. Variables stables over time: Child's sex; Maternal age and education. Variables that might change over time: Household income; Practice of physical activity; Living with siblings; Sleep hours per night; Sedentary activities. σ^2 – Residual variance; τ_{00} – Random intercept variance; τ_{11} – Random slope variance; ρ_{01} – Random slope-interception correlation; ICC – Intraclass correlation.

children who spent more time in sedentary activities showed a lower diet quality at 4 years (β = −0.006, 95% CI = −0.007; −0.005) compared to those who spent 150 min per day in this kind of activity [Table 3]. Two sensitivity analysis were performed, one considering participants with all the measurements completed (Supplementary Table 2) and the other not accounting for participants with over- or under-reporting of dietary intake (Supplementary Table 3). It was possible to observe that the interaction values follow the same tendency as in the original analysis and that the results are similar.

The HEI score followed different trajectories depending on the child's sex, monthly household income, the number of hours that the children slept per night, and the number of hours that children spent on sedentary activities per day (Fig. 2). At 4 years old boys started with a higher HEI score. However, as time went by, the score declined faster in boys than in girls, with boys presenting a lower mean HEI score at age 13 (β = −0.094, 95% CI = −0.132; −0.056, interaction term, Table 3). A higher household income was associated with a slighter decrease in the HEI score throughout time (β = 0.024, 95% CI = 0.007; 0.041, interaction term, Table 3). At 13 years, adolescents with a lower household income were the ones who presented lower diet quality, and this difference was noticeable only from 7 years old onwards. Compared to children who slept 7 h a night, children who slept 8 or more hours per night followed a different trajectory, showing a smaller decrease in the HEI score (β = 0.039, 95% CI = 0.015; 0.064). The number of slept hours also started showing influence after 4 years, not affecting the HEI score at this age. Regarding the time spent on sedentary activities, the more time a child spent a day on these types of activities, the worse they performed on the index score (β = −0.009, 95% CI = −0.014; −0.003). The time spent on sedentary activities presented more influence when children were younger but lost its effect at the beginning of adolescence.

4. Discussion

The present study provided evidence that diet quality changes with the transition from childhood to adolescence. Previous studies showed the existence of diet quality tracking from 4 to 7 years old and from 7 to 10 years (da Costa et al., 2019; da Costa et al., 2022). In this study, we corroborate those findings since the dietary quality from childhood to adolescence presents moderate tracking. Nonetheless, the tracking seems to stabilize between 10 and 13 years, but a future follow-up is needed to confirm this hypothesis. Our results show that children with a healthier diet will have a healthier diet later. However, the same happens to not-so-healthy eating habits since the ICC of the model was moderately high. Besides that, the overall diet quality score decreases from childhood to adolescence. These modifications may be due to the decrease in children's intake of healthier food groups (dairy, cereal products and fruit and vegetables) while maintaining unhealthy dietary consumption. In this sense, it is important to understand when the changes in diet quality occur to know how and at each time we should intervene.

Maternal age and education were independently associated with better diet quality in all follow-ups. These results are in concordance with some previous studies that showed that a higher maternal age (Vereecken & Maes, 2010) and education (Aranceta et al., 2003; Robinson et al., 2007; Bjelland et al., 2013; da Costa et al., 2019) had been associated with overall healthier eating habits and particularly better diet quality in childhood. Moreover, in early adolescence, the offspring of mothers with a low educational level seems more prone to report that unhealthy foods are often available at home (MacFarlane et al., 2007). In contrast, those adolescents of mothers with a higher educational level are more prone to report a higher availability of fruit and vegetables at home and frequently served during the main meals (MacFarlane et al., 2007). As shown in previous studies, this could be due to the less supportive home food environment experienced by children with mothers with a low educational level (Neumark-Sztainer et al., 2003; Sandvik et al., 2010), which highlights the importance of parents as a role model for children's and adolescent's dietary intake. Family structure and household income also seem to influence diet quality (Reicks et al., 2015). As in our study, household income has been linked to diet quality, with offspring from higher-income families presenting a lower

adherence to an unhealthy dietary pattern (Hinning et al., 2018). This could be because families with a lower income tend to experience more restrained food environments, leading to poorer access to healthy food (Kant & Graubard, 2013).

The influence of parental factors on children's diet might be partly explained through the adopted parental feeding practices, i.e. eating-specific behaviours or strategies used by parents to regulate or modify their children's food intake, which influence what, when, and how much their children eat (Carnell et al., 2014; Russel et al., 2018; Ventura & Birch, 2008). These practices are hypothesized to play an important role in the development of children's eating behaviours and appetite and, subsequently, in the control of children's weight status (Faith et al., 2004; Peters et al., 2012). Given its importance, we tested the parental feeding practices as a potential confounder of the association of socio-economic and individual characteristics and the tracking of diet quality in a subsample at 4 years of age. No different associations or tendencies have been found in this additional analysis, leading us to conclude that individual and sociodemographic factors could weigh more in diet quality trajectories than parental feeding practices. Also, feeding practices are potentially modifiable over time (Russel et al., 2018). Nevertheless, a longitudinal analysis of parental feeding practices was not possible in the present study, given that we only have available data at the 4- and 7-year follow-ups, which, as they present differences between the two questionnaires at both ages, are not comparable, so future research on this topic is warranted.

As shown by our results, the sex of the child plays a role in the development of diet quality. In the first years of life, boys present a higher HEI score than girls, but this tendency is reversed later, with girls presenting a smaller decrease in diet quality score over time during childhood and early adolescence. Similar findings have been reported in other studies, where boys were found to consume less fruit and vegetables and dairy than girls, reporting sex differences in food preferences (Cooke & Wardle, 2005; Evers et al., 2022; Le Bigot Macaux, 2001). Women usually report higher intakes of fruit and vegetable and lower intakes of fat than men. Some previous studies have reported that mothers and daughters were more strongly correlated in their dietary quality than mothers and sons, which partly can explain why girls tend to have a better diet quality during childhood and early adolescence than boys (Westenhoefer, 2005; Vågstrand, 2010). Another possible explanation is that parents can be more worried about managing their daughters' weight and eating habits than their sons because of the idea of social standards of physical attraction, imposing healthier food choices and consequently a better diet quality (Katz-Wise et al., 2013).

In recent years, there has been a growing interest in studying the influence of sedentary activities, mainly screen time, on children's eating habits (Ciccone et al., 2013; Souza Neto et al., 2021; Tambalis et al., 2020). A drastic increase in the viewing time of various types of screens has been observed between children and adolescents, increasing their daily use of computers, video games, and smartphones/tablets (Shqair et al., 2019). In addition, a substantial decrease in the time spent on physical activities has been described (Cooper et al., 2015). Inevitably, this pattern increases the prevalence of sedentary behaviours among these age groups (Thivel et al., 2013). Previous studies have shown that greater screen time is linked to a higher intake of soft drinks, fast food, sugar, energy from fat and total energy, and a decrease in the daily servings of fruits and vegetables (Stiglic & Viner, 2019; Wärnberg et al., 2021). One possible explanation for these results is that children and adolescents who spend more time in front of screens pay less attention to what they eat during this period (Börnhorst et al., 2015). Another contributing factor could be the influence of advertisements promoting food and beverages with high energy, fat, salt and sugar content (Kelly et al., 2016). In both the current investigation and previous research, it was consistently observed that the longer the screen time, the unhealthier the dietary habits (Liang et al., 2009; Miller et al., 2008; Tambalis et al., 2020).

According to the American National Sleep Foundation, the recommended sleep duration per night is between 10 and 13 h in pre-schoolers, 9–11 h in school-aged children and 8–9 h in adolescents (Hirshkowitz et al., 2015). The mean sleep hours duration in the present analysis accomplishes these recommendations and is in accordance with the previously reported European children's (6–12 years) mean sleep duration (9.1 h) (Canet, 2010). However, in our sample, 32.5% of pre-schoolers sleep less than 10 h per night, and 22.9% of adolescents sleep more than 9 h per night (data not shown). Previous studies have linked short sleep duration with lower diet quality (Mikkilä et al., 2004; González-Treviño et al., 2022), especially due to the increased consumption of energy-dense foods and decreased consumption of fruit and vegetables (Westerlund et al., 2009; Moreira et al., 2010). Our findings align with the literature since we found that shorter sleep duration relates to worse dietary quality within this sample of Portuguese children and adolescents. This relationship is not so evident during preschool age, but over time, both in school-aged children and especially adolescents, the shorter the sleep duration, the faster the diet quality declines. There are several explanations for this association, but the most discussed in the literature is a hormonal dysfunction due to sleep disruption that alters the perception of hunger and appetite, namely a dysfunction in leptin and ghrelin that presents inhibitory effects on food intake and appetite stimulus, respectively (Chaput et al., 2007; Hart et al., 2013). It is also hypothesized that children with a short sleep duration extend their waking hours and have more eating opportunities, especially for food usually consumed during screen time (Córdova et al., 2018). Another explanation is that sleep deficiency will lead to daytime fatigue, reducing physical activity levels and leading to an unhealthy food intake (Córdova et al., 2018).

The strengths of this study include the use of a large representative population-based sample with high participation rates and repeated detailed and standardized measures, as well as its longitudinal design, allowing a time sequence of various exposures and greater control for a wide range of potential confounders. It is a study with dietary intake measures (HEI) at four different time points, from early childhood to early adolescence, with a longitudinal design that enables us to explore the potential causal direction of the described associations. Regarding the limitations, the FFQ was answered by parents until the child's 10-year follow-up and answered by the adolescent at 13 years in a face-to-face interview. The FFQ is limited by subjective reporting, which might lead to misreporting and social desirability bias. Parents and adolescents may have underestimated the consumption of less healthy foods or overestimated the consumption of more healthy foods. Also, parents might not be aware of all the foods eaten out-of-home by the child. However, in a subsample of children belonging to the Generation XXI cohort, dietary intake information collected from the FFQ was compared with the dietary intake information from 3-day food diaries, supporting the validity of these results with the previous validation and calibration of the FFQ, particularly for foods more frequently consumed (Vilela et al., 2019). To account for the impact of the misreporting on the analysis, we performed a sensitivity analysis not accounting for participants with potential over- and under-reporting of energy intake. We concluded that over- and under-reporting does not appear to have introduced bias in the analysis. The 13-year follow-up was prematurely ended due to the COVID-19 pandemic, not being possible to evaluate all the sample in this wave. This interruption could be a limitation since the assessed children may differ from the rest of the sample. Usually, the children of parents with a higher level of education attend the follow-ups earlier, right after the first contact, to schedule the visit. Another possible limitation is the inclusion of all the children with dietary information in at least one of the follow-ups for this analysis since dietary information of only one follow-up may not provide a representative picture of a child's overall diet. However, its exclusion could also be considered a limitation because it may introduce selection bias. In this sense, we have compared the HEI scores considering all the children with at least one follow-up completed versus children with complete information at all follow-ups, observing that the interaction values

continue to follow the same trend and that the results are practically the same. Besides, the linear mixed-effect models analysis used for this study corrects the analysis for missing at random. With the individual data available, we can complete the trajectory of diet quality with what would be expected for each individual over time.

5. Conclusions

This study suggests that the quality of dietary intake from childhood to adolescence decreases over time. However, the healthy eating habits established during childhood track to some degree into adolescence, with those with a better diet quality at 4 years maintaining a better diet quality at 13 years. This implies that promoting healthy eating habits during the first years of life is crucial for a healthier diet quality during late childhood and early adolescence. The influence of the mother, the structure of the family, individual behaviours such as the practice of sedentary activities during the first years of life and the duration of sleep, as well as individual characteristics such as the sex of the child, present a significant influence on the development of trajectories of diet quality throughout life. This study elucidates the importance of an adequate sleep duration per night for better diet quality from childhood to adolescence. It helps to define a target population to intervene with a higher chance of developing poor eating habits, more specifically, young boys with a lower socioeconomic environment and who engage in more sedentary activities.

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Data and code availability

The data from Generation XXI are not publicly available due to privacy or ethical restrictions. The data can be made available for research proposals on request to the Generation XXI Executive Committee (generationxxi@ispup.up.pt). Further information about Generation XXI can be obtained via the Generation XXI website [www.geracao21.com] or by emailing generationxxi@ispup.up.pt. The lead author has full access to the data reported in the manuscript.

Ethical considerations

The Generation XXI project was conducted in accordance with the guidelines defined in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Hospital de São João/University of Porto Medical School. The project was approved by the Portuguese Authority of Data Protection. The legal representatives of each participant received an explanation about benefits and potential discomforts, through written informed consent, with the information of all the examinations to be carried out during the evaluation, at baseline and in the subsequent follow-up evaluations. After a brief explanation of the evaluation, the child's oral consent was requested. At the 13-year follow-up, the adolescent also signed a written informed consent.

CRediT authorship contribution statement

Marta Pinto da Costa: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Milton Severo:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Joana Araújo:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization. **Sofia Vilela:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Formal analysis, Conceptualization.

Declaration of Competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.appet.2024.107279.

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Paper II

Predicted Maturity Offset and Tanner Stages show distinct aspects of biological maturation: Evidence from Two European Child Populations

Marta Pinto da Costa, Sofia Vilela, Joana Araújo, Alvaro Torres-Martos, Abel Plaza-Florido, Mercedes Gil-Campos, Rosaura Leis, Concepción M. Aguilera, Gloria Bueno, Augusto Anguita-Ruiz

[Submitted]

Abstract

Background: Biological maturation in epidemiological studies is commonly assessed using Tanner stages (sexual maturation), which may be perceived as invasive and reduce participation. Non-invasive methods, such as Maturity Offset (MO), reflecting somatic maturation, have been proposed and used for adjustment in epidemiological and pediatric physical activity studies as proxies for pubertal timing.

Objective: To evaluate whether pMO can approximate pubertal timing assessed by Tanner Stages in epidemiological cohorts, across different maturation strata.

Methods: This study included 2459 children from the Spanish GENOBOX study (n=485) and the Portuguese Generation XXI [G21] cohort (n=1974). Tanner Stage was determined by trained professionals. Early normal puberty was defined as Tanner Stage≥2 at >8-<9 years in girls and >9-<10 years in boys. G21 girls were excluded from pMO analysis due to normal development. pMO was estimated using sex-specific modified Moore equations.

Results: In G21, mean ages were 11.7±1.6 years (girls) and 12.5±1.4 years (boys), mostly in Tanner Stage 2-3. In GENOBOX, mean ages were 12.9±2.0 years (girls) and 13.4±1.7 years (boys); most boys were in Tanner Stage 2 and girls in Stage 5. Girls reached peak height velocity at Tanner Stage 3, whereas boys reached it later. Within the same Tanner stage, early normal developers had lower pMO values.

Conclusion: pMO may distinguish prepubertal from more advanced maturation among normal developers but is unsuitable for Tanner Stage stratification or predicting pubertal timing in early normal developers. Tanner stages and pMO should not be used interchangeably, as they reflect distinct components of biological maturation.

Keywords: Adolescence; Children; Maturity offset; Pubertal timing; Tanner Stages

1. Introduction

In humans, there is considerable variation in the age of pubertal onset. Puberty normally occurs between the ages of 8 and 13 years in females, and between 9 and 14 years in males, but variations in normal pubertal development can be expected to occur, by definition, at a frequency of roughly 3% of the population (1). Breast development in girls and an increase in testicular volume to 4 mL in boys are the initial physical findings of pubertal onset (2). Established indicators of pubertal onset and development, such as the Tanner stages assessment by physical examination – the most commonly used method for evaluating puberty development (sexual maturation) in epidemiological studies (3-5) - are often perceived as invasive (4) and, in some cases, have low acceptability among children/adolescents (6). Consequently, this may hinder participation in research studies, or lead to missing data for puberty outcomes (4). Given concerns related to the invasiveness of some methods and the challenges posed by others, simple and non-invasive methods have been suggested to assess biological maturity development in children/adolescents (7-10).

Among these is the Maturity Offset (MO), which is defined as the time (years) before or after peak height velocity (PHV) – an event that occurs around Tanner Stage 3 in girls and Tanner Stage 4 in boys (11) - indicating how far a child is from their period of greatest height increase, reflecting somatic maturation (10). However, a key limitation of MO is that it ideally requires the use of repeated height measurements over time. To address this limitation in settings where longitudinal data are unavailable, predicted MO (pMO) can be used (10). The pMO consists of an estimate of MO, calculated based on sex-specific predictive equations developed by Mirwald et al. (10) and Moore et al. (12). These equations allow for the estimation of biological maturity based on a single set of anthropometric measurements, including leg length, height, sitting height, weight and chronological age (10,12). Despite not being an indicator of puberty onset and development, pMO is an indicator of maturity timing and was designed for use in pediatric populations aged 8-16 years in girls and 9-18 years in boys (13). A previous study reported that pMO is quite accurate for assessing normal biological maturity development, especially in boys (7). However, the prediction of maturity offset using these equations might have limitations in youth with early or late normal biological maturation in both sexes (7). Nonetheless, pMO has been widely used to assess biological

maturational status in studies of physical activity and sports among youth athletes (8,9,14,15).

Exercise plays a key role in healthy growth and development (16). Physiological and molecular responses to a single aerobic exercise session vary across Tanner stages in boys and girls1(7-19) and prepubertal adolescents tend to be more physically active than their pubertal peers (20). On the other hand, biological maturation, as reflected by MO (an indicator of somatic maturation), was positively associated with physical performance, body size, and testosterone concentration in youth athletes(14). Moreover, different stages of maturity (represented by MO) might modulate physical performance and hormonal responses to acute and regular exercise interventions in youth (21-23). Otherwise, the literature commonly identifies observational studies exploring the association between physical activity and fitness levels and health-related outcomes in pediatric populations, with biological maturation, using maturity offset (24,25) or Tanner stage (26,27) as a covariate/confounder in the statistical models. Although MO and pubertal status (Tanner stages) are indicators of biological maturation, they should not be used interchangeably, as the former reflects somatic maturation and the latter sexual maturation. Accordingly, this cross-sectional study aimed to quantitatively evaluate whether pMO can approximate pubertal timing assessed by Tanner Stages in epidemiological cohorts, across different maturation strata, including normal and early normal developers.

2. Materials and Methods

2.1. Setting and Participants

The present study utilised data from two independent European children's populations: Generation XXI (G21) in Portugal and GENOBOX in Spain. G21 is a population-based birth cohort, as previously described (28,29). Briefly, all the newborns in the five public maternity hospitals in the metropolitan area of Porto were invited to participate between 2005 and 2006. Of the invited mothers, 91.4% accepted to participate, with a total of 8647 children enrolled at baseline. All families were invited to attend a face-to-face follow-up when children were aged 4 (86% of participation), 7 (80% of participation), 10 (76% of participation), and 13 years old (54% of participation – follow-up was interrupted due to

the COVID-19 pandemic). The 18-year evaluation is currently ongoing. Children who attended the evaluation at 10 and 13 years old were considered for this study.

GENOBOX is a case-control multicentre cross-sectional design study that recruited 915 Spanish children (438 boys and 477 girls), aged to 6 to 18 years, from three national health institutions (30,31). In this study, only one assessment was conducted, and it was performed at the time of recruitment.

The final sample included all children with complete data on Tanner Stage, sex, height and exact age at the evaluation moment in each study, counting a sample of 1974 children from G21 and 485 children from the GENOBOX study.

2.2. Anthropometric and Pubertal Timing Assessment

Objective anthropometric measurements were performed by a team of trained examiners according to standard procedures in both studies.

Sexual maturity was assessed by trained professionals through visual inspection and palpation, using Tanner's five-stage scale (32, 33). In both studies, pubertal development in girls was assessed through pubic hair and breast development. In boys, pubertal staging was based on Tanner´s scale for pubic hair and genital development, as well as testicular volume assessment using a Prader orchidometer. Girls were classified as a Tanner Stage 2 or higher, the mark of pubertal onset, if they present a breast bud stage characterized by bilateral elevation of the breast and papilla, along with enlargement of the areola32. Boys were classified as Tanner Stage 2 or higher if they presented a testicular volume higher than 4 mL (G21 cohort and GENOBOX study), and if scrotal had begun to enlarge, develop increased rugosity, and show darker pigmentation (GENOBOX study) (33).

Children were classified as normal pubertal developers if they presented a Tanner Stage ≥2 and were between 9 years and 12.9 years of age in girls, and between 10 and 13.9 years of age in boys. Conversely, physiological early normal pubertal developers – that is, children who initiate puberty within the normal age range but at its lower age limits – were defined as those with a Tanner Stage 2 or higher occurring between 8 and 9 years in girls, and between 9 and 10 years in boys, according to previously established cut-offs in the literature (34). All girls with Tanner Stage 2 before age 8 (precocious puberty) (n=4) or after age 13 (late puberty) (n=74), and boys with Tanner Stage 2 before age 9

(precocious puberty) (n=3) or after age 14 (late puberty) (n=21), were excluded from the present study. Moreover, as all G21 cohort girls of 10 years or older were classified as having normal pubertal development, the early normal developers' group was not represented in this cohort and therefore not included in the pMO analysis.

2.3. Predicted Maturity Offset (pMO)

Two equations are available in the literature to predict the Maturity Offset value. The original sex-specific equations require data on the child's chronological age, estimated leg length, sitting height, mass, and mass by stature ratio10. The modified sex-specific equations offer similar accuracy but are simpler, requiring only the child's chronological age and sitting height (12). In the present study, Maturity Offset was predicted using the modified sex-specific equations for girls (12). For boys, since sitting height was unavailable in both studies, the alternative modified equation previously described specifically for boys was used. The equations applied were:

Girls: Maturity offset (years) = -7.709133 + (0.0042232 x (age x stature) (1)

Boys (alternative equation): Maturity offset (years) = -7.999994 + (0.0036124 x (age x stature)) (2)

If the predicted MO value is negative, the child has not yet reached peak height velocity (PHV). If it is positive, the PHV has already occurred. If it is equal to zero, the PHV is occurring.

2.4. Ethical considerations

All described projects were conducted in accordance with the guidelines defined in the Declaration of Helsinki. The Generation XXI procedures involving human subjects were approved by the Ethics Committee of the Hospital de São João/University of Porto Medical School. The project was approved by the Portuguese Authority of Data Protection. The GENOBOX study followed the recommendations of both the Good Clinical Practice of the CEE (Document 111/3976/88 July 1990) and the legally enforced

Spanish regulation for clinical investigation of human beings. The Ethics Committees on Human Research of all participant institutions approved all experiments and analyses with registration Code: "2011/198". In both projects, the legal representatives of each participant received an explanation about benefits and potential discomforts, through written informed consent, with the information of all the examinations to be carried out during the evaluation, at baseline and in the subsequent follow-up evaluations. After a brief explanation of the evaluation, the child's oral assent was requested. At the Generation XXI 13-year follow-up, the adolescent also signed a written informed consent.

2.5. Statistical analysis

Descriptive statistics (means, standard deviations, ranges and frequencies) were calculated for normal and early normal pubertal developers, stratified by sex, study and Tanner Stage. The frequency and percentage of children with a pMO greater than 0 - indicating that PHV has already occurred - was also computed. Independent samples t-tests were used to compare mean pMO values between the two studies, stratified by sex and Tanner Stage.

All statistical analysis were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, version 28.0). A significance level of 0.05 was considered as an appropriate alpha value.

A post hoc power analysis was conducted using G*Power (version 3.1.9.7) to assess the statistical power of comparisons between early and normal pubertal developers at Tanner Stage 2 within each study and between studies. In both sexes, when comparing early and normal pubertal developers within each study, the achieved statistical power was high (~1.00), indicating sufficient power to detect differences despite the small sample size of the early pubertal developers' group. For the comparisons between studies, in normal pubertal developers of both sexes, the achieved statistical power was also high (~1.00), indicating adequate power to detect differences between the two studies. However, for the comparison between early pubertal boys from the G21 and GENOBOX studies, the achieved power was only 0.32, indicating limited ability to detect true differences between early developers across studies. This low power is primarily attributed to the small sample size of early normal developers in the GENOBOX study (n = 5).

3. Results

The majority of the sample consisted of girls (55.0% in G21 and 58.9% in GENOBOX). Among normal pubertal developers, the mean age was higher in GENOBOX for both sexes. In G21, most participants were in Tanner Stage 3 among girls (37.4%) and in Tanner Stage 2 among boys (33.4%). In GENOBOX, most girls were in Tanner Stage 5, whereas most boys were in Tanner Stage 2 (Table 1). Mean pMO values differed significantly between studies for both girls and boys (mean difference for girls = -1.040, $p<0.001$; mean difference for boys = -0.690, $p<0.001$). Early normal pubertal developers were mostly at Tanner Stage 2, with lower mean ages and significantly lower pMO values compared to normal pubertal developers, indicating a greater time to PHV (Table 1). Statistically significant differences in pMO between studies were also observed in early pubertal boys (mean difference for boys = 0.310, $p=0.003$).

Table 2 presents the percentage of children with a pMO greater than zero across all pubertal stages. Among normal pubertal developers, most girls had reached a pMO greater than zero by Tanner Stage 3, while most boys did so by Tanner Stage 4 in GENOBOX and Tanner Stage 5 in G21.

In the early pubertal developers' group, none of the children presented a pMO greater than zero. Statistically significant differences in pMO between studies were observed for girls at Tanner Stage 2 ($p<0.001$) and 5 ($p<0.001$) and for boys at Tanner Stage 2 ($p<0.001$), 4 ($p<0.001$) and 5 ($p<0.001$), with higher pMO values in GENOBOX (Table 3). Although the mean pMO in early-developing boys was slightly lower in GENOBOX, this difference was not statistically significant ($p=0.140$) (Table 3).

The pMO mean values at each Tanner Stage differed between early and normal pubertal developers. However, since the majority of early pubertal developers in both studies were in Tanner Stage 2 (89.7% in G21, and 100% in GENOBOX), comparisons were specifically focused on individuals at this stage to ensure consistency. In both sexes and in each study, early pubertal developers had significantly lower pMO values compared to normal pubertal developers ($p<0.001$) despite being in the same Tanner Stage (Supplementary Table S1). For example, in the GENOBOX study, at Tanner Stage 2, early puberty girls had a mean pMO of -2.97 ± 0.29, while girls at the same stage but in the normal puberty group had a mean pMO of -0.92 ± 1.0 (Supplementary Table S1).

4. Discussion

The present study provides evidence that the pMO can be used to distinguish prepubertal from more advanced pubertal stages (i.e., after PHV) only among normal pubertal developers, within the context of epidemiological studies, although this applicability should be interpreted with certain limitations. Similarly, a previous study has shown that pMO is quite accurate for average maturing boys (7). Additionally, pMO allows the detection of sex-related differences in the timing of PHV, as demonstrated in this study, where the majority of girls had a pMO greater than zero (indicating PHV had been reached) at earlier Tanner stages compared to boys (Tanner Stage 3 vs. Tanner Stage 4/5). However, pMO does not appear to be a reliable indicator of pubertal stages stratified by Tanner Stages, as expected, given that pMO and Tanner stages reflect somatic and sexual maturation, respectively. For instance, among girls in Tanner Stage 3, who had comparable mean ages across studies, no statistically significant differences in pMO were observed between the GENOBOX and G21 studies. A similar pattern was observed among boys at the same Tanner Stage. In contrast, statistically significant differences in pMO values were found between studies within Tanner Stage 2 and 5 among girls, and within Tanner Stage 2, 4, and 5 among boys. Moreover, if we inspect individual levels, we might find children with similar pMO values in very different Tanner stages - for example, some in Tanner Stage 2 and others in Tanner Stage 4 - as indicated by the overlapping pMO ranges across stages. This overlap suggests that, while pMO may offer a less invasive measure, it should not be interpreted as directly equivalent to Tanner stages in epidemiological studies, given that they reflect somatic and sexual maturation, respectively. Furthermore, pMO does not perform as well in early normal pubertal developers, as the mean pMO values differed between early and normal pubertal developers at the same Tanner Stage (stratified by sex). This limitation has been previously described in other studies, which highlighted the reduced accuracy in predicting age at PHV, particularly in earlier or later maturing individuals (7,13,35). The reduced accuracy of pMO equations in early and late developers may result from the fact that these equations were likely derived from samples predominantly composed of youths maturing around the expected average ages and did not include a significant proportion of early and late developers. In fact, new equations should be specifically developed for early maturing children, rather than incorporating them into a general equation designed for the selected reference population.

Future studies should explore whether the physiological and molecular adaptations to acute and exercise interventions differ across subgroups defined by biological maturation status (e.g., MO [pre-PHV vs post-PHV] or early vs. late pubertal stages [Tanner 1–2 vs. 4–5]). Also, when possible, both MO and Tanner stages should be considered as independent confounders in statistical models, as they reflect distinct aspects of biological maturation: somatic and sexual, respectively.

In the present study, it was also possible to observe that chronological age has a substantial impact on pMO values. When comparing pMO values by Tanner Stage between the G21 and GENOBOX studies, statistically significant differences were observed when the participants' ages differed between studies (e.g., 10.2 years in G21 vs. 10.8 years in GENOBOX, Tanner Stage 2). Conversely, when the ages were similar (e.g., 12.9 years in G21 vs. 13.2 years in GENOBOX, Tanner Stage 4), no statistically significant differences in pMO were found. Thus, these findings suggest that one of the reasons pMO may not be a reliable predictor for early normal pubertal developers is the fact that chronological age is part of the equation.

Further research is needed to determine whether an alternative equation can be derived from the predefined ones to improve the pMO accuracy, allowing its application as a non-invasive technique for assessing pubertal development in early normal, normal and late developers. One possible suggestion would be to introduce other indicators of body composition into the modified equation - such as fat mass and lean mass percentages - since children with excess adiposity may have higher body weight without necessarily being more advanced in terms of pubertal development (36). However, in practice, assessing body composition is not always straightforward and may not be feasible in large-scale population settings. Additionally, it is important to explore other non- or minimally invasive techniques that could be considered as alternatives to Tanner Stage clinical assessment by pediatricians and in epidemiological studies. In the meantime, Tanner Stages remains the gold standard method to be used as puberty estimator in epidemiological studies, particularly when the aim of the study is to assess pubertal timing, despite limitations such as the inter-observer variability, reliance on self-reporting and potential discomfort for participants.

This study should be interpreted in light of some limitations. Although Tanner Stage assessments were conducted by trained professionals through palpation and visual inspection, this method may be affected by inter- and intra-observer variability,

potentially leading to misclassification and compromising the consistency of data in population-based studies. Another limitation is the considerable interindividual variability in the chronological sequence of pubertal events, which may hinder the accurate assessment of pubertal development at the population level. Children of the same age and sex may initiate puberty and progress through its stages at different times and with different tempos of development. A longitudinal study considering pMO progression could help to better understand how pMO behaves throughout pubertal development. Furthermore, we did not account for potential factors that may influence growth and pubertal timing, such as genetic predispositions, environmental and behavioural exposures, and other individual variations. Lastly, the small sample size of the early normal developers group in the GENOBOX study (5 boys and 5 girls) should also be acknowledged as a limitation, as it may not provide sufficient power to detect true differences between early normal and normal developers across the two studies considered.

This study suggests that the pMO may help distinguish prepubertal from more advanced pubertal stages in normal pubertal developers, in population-based studies with sufficiently large sample size. However, pMO does not appear suitable for stratifying individuals by Tanner Stage, at either individual or population level. Although it provides a less invasive measure of somatic maturation, it should not be interpreted as directly equivalent to Tanner stages, which reflect sexual maturation, given the considerable overlap in values. Furthermore, the pMO does not appear to be a highly accurate predictor for pubertal onset and development in early normal pubertal developers, given the difference in mean values between early normal and normal pubertal developers within each Tanner Stage.

Given these limitations, pMO should be used with caution and cannot replace detailed clinical assessments of pubertal development. Despite being a more invasive method, the Tanner Stage is still the best approach to define puberty onset and development in epidemiological studies. Further research is needed to develop robust, non-invasive tools for puberty assessment and to establish standardized measurement protocols.

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Data Availability Statement

Data will be made available on request from the corresponding author due to privacy and ethical restrictions.

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Declaration of competing interests

The authors declare no conflicts of interest.

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Table 1. Descriptive statistics by sex, study and developmental timing.

	G21		GENOBOX	
	Girls	**Boys**	**Girls**	**Boys**
Normal pubertal developers	n=1059	n=866	n=279	n=195
Age (y), mean (SD)	11.7 (1.6)	12.5 (1.4)	12.9 (2.0)	13.4 (1.7)
Age (y), range	9.3; 14.7	10.0; 14.8	9.0; 17.3	10.0; 17.2
pMO, mean (SD)	-0.25 (1.46)	-0.87 (1.17)	0.79 (1.64)	-0.18 (1.46)
pMO, range	-2.53; 2.54	-3.29; 1.22	-2.66; 4.23	-3.47; 3.51
Tanner stages, n (%)				
2	348 (32.9)	289 (33.4)	61 (21.9)	63 (32.3)
3	396 (37.4)	149 (17.2)	54 (19.4)	57 (29.2)
4	273 (25.7)	259 (29.9)	65 (23.3)	40 (20.6)
5	42 (4.0)	169 (19.5)	99 (35.5)	35 (17.9)
Early pubertal developers	n=0	n=49	n=5	n=5
Age (y), mean (SD)	-	9.7 (0.2)	8.2 (0.2)	9.3 (0.3)
Age (y), range	-	9.3;10.0	8.0; 8.5	9.1; 9.9
pMO, mean (SD)	-	-2.99 (0.21)	-2.97 (0.29)	-3.15 (0.17)
pMO, range	-	-3.52; -2.58	-3.41; -2.61	-3.32; -2.92
Tanner stages, n (%)				
2	-	44 (89.8)	5 (100.0)	5 (100.0)
3	-	5 (10.2)	0 (0.0)	0 (0.0)
4	-	0 (0.0)	0 (0.0)	0 (0.0)
5	-	0 (0.0)	0 (0.0)	0 (0.0)

SD, standard deviation; pMO, predicted Maturity Offset

Table 2. Description of the pMO mean values by Tanner Stage (breast for girls, genitalia for boys) for normal and early developers, stratified by sex, study and Tanner Stage.

		Normal pubertal developers				Early pubertal developers			
		TS 2	**TS 3**	**TS 4**	**TS 5**	**TS 2**	**TS 3**	**TS 4**	**TS 5**
G21	**Girls**	n=348	n=396	n=273	n=42				
	Age (y), mean (SD)	10.2 (0.4)	11.9 (1.6)	12.9 (1.1)	13.3 (0.2)				
	Age (y), range	9.4; 11.5	9.3; 14.7	9.4; 14.2	13.1; 14.1				
	pMO, mean (SD)	-1.65 (0.31)	0.001 (1.41)	0.93 (0.95)	1.36 (0.31)	Not performed			
	pMO, range	-2.53; -0.78	-2.32; 2.54	-2.02; 2.14	0.61; 1.99				
	pMO>0, n (%)	0 (0.0)	219 (55.3)	234 (85.7)	42 (100)				
	Boys	n=289	n=149	n=259	n=169	n=44	n=5	n=0	n=0
	Age (y), mean (SD)	11.1 (1.4)	13.1 (1.0)	13.3 (0.4)	13.4 (0.3)	9.7 (0.2)	9.6 (0.1)	-	-
	Age (y), range	10.0; 14.0	10.0; 14.4	10.1; 14.8	13.1; 14.4	9.3; 9.9	9.5; 9.7	-	-
	pMO, mean (SD)	-2.13 (1.01)	-0.59 (0.78)	-0.19 (0.44)	0.02 (0.41)	-3.01 (0.21)	-2.88 (0.18)	-	-
	pMO, range	-3.29; 0.33	-2.99; 1.18	-2.39; 1.09	-1.03; 1.22	-3.52; -2.58	-3.14; -2.74	-	-
	pMO>0, n (%)	9 (3.1)	25 (16.8)	83 (32.0)	84 (49.4)	0 (0.0)	0 (0.0)	-	-
GENOBOX	**Girls**	n=61	n=54	n=65	n=99	n=5	n=0	n=0	n=0
	Age (y), mean (SD)	10.8 (1.2)	11.9 (1.4)	13.2 (1.5)	14.4 (1.6)	8.2 (0.2)	-	-	-

Age (y), range	9.1; 13.0	9.3; 15.1	9.0; 17.0	10.0; 17.3	8.0; 8.5	-	-	-
pMO, mean (SD)	-0.92 (1.00)	-0.04 (1.15)	1.16 (1.21)	2.06 (1.19)	-2.97 (0.29)	-	-	-
pMO, range	-2.66; 0.94	-2.11; 2.86	-1.85; 4.14	-1.64; 4.23	-3.41; -2.61	-	-	-
pMO>0, n (%)	14 (23.0)	27 (50.0)	54 (83.1)	95 (96.0)	0 (0.0)	-	-	-
Boys	n=63	n=57	n=40	n=35	n=5	n=0	n=0	n=0
Age (y), mean (SD)	12.0 (1.1)	13.1 (1.4)	14.2 (1.2)	15.4 (1.3)	9.3 (0.3)	-	-	-
Age (y), range	10.0; 14.0	10.0; 15.7	12.1; 17.0	12.0; 17.2	9.1; 9.9	-	-	-
pMO, mean (SD)	-1.36 (0.88)	-0.44 (1.08)	0.51 (0.93)	1.60 (1.16)	-3.15 (0.17)	-	-	-
pMO, range	-3.47; 0.66	-2.99; 1.43	-1.31; 2.62	-1.24; 3.51	-3.32; -2.92	-	-	-
pMO>0, n (%)	2 (3.3)	18 (32.1)	30 (75.0)	30 (85.7)	0 (0.0)	-	-	-

SD, standard deviation; pMO, predicted Maturity Offset; TS, Tanner stage

Table 3. Comparison of pMO mean values between G21 and GENOBOX studies, by sex and Tanner Stage.

	G21		GENOBOX			
	Age, mean (SD)	**pMO**, mean (SD)	**Age**, mean (SD)	**pMO**, mean (SD)	**Mean difference**	**p-value**
Girls – Normal pubertal developers						
Tanner stage 2	10.2 (0.4)	-1.65 (0.31)	10.8 (1.2)	-0.92 (1.00)	-0.732	<0.001
Tanner stage 3	11.9 (1.6)	0.001 (1.41)	11.9 (1.4)	-0.04 (1.15)	0.040	0.817
Tanner stage 4	12.9 (1.1)	0.93 (0.95)	13.2 (1.5)	1.16 (1.21)	-0.232	0.153
Tanner stage 5	13.3 (0.2)	1.36 (0.31)	14.4 (1.6)	2.06 (1.19)	-0.698	<0.001
Boys – Normal pubertal developers						
Tanner stage 2	11.1 (1.4)	-2.13 (1.01)	12.0 (1.1)	-1.36 (0.88)	-0.776	<0.001
Tanner stage 3	13.1 (1.0)	-0.59 (0.78)	13.1 (1.4)	-0.44 (1.08)	-0.154	0.332
Tanner stage 4	13.3 (0.4)	-0.19 (0.44)	14.2 (1.2)	0.51 (0.93)	-0.692	<0.001
Tanner stage 5	13.4 (0.3)	0.02 (0.41)	15.4 (1.3)	1.60 (1.16)	-1.584	<0.001
Boys – Early pubertal developers						
Tanner stage 2	9.7 (0.2)	-3.01 (0.21)	9.3 (0.3)	-3.15 (0.17)	0.145	0.140

SD, standard deviation; pMO, predicted Maturity Offset.

Supplementary Material

Table S1

Descriptive statistics for participants in Tanner stage 2.

Supplementary Table S1. Descriptive statistics by sex, study and developmental timing for participants in Tanner stage 2.

	G21		GENOBOX	
	Girls	**Boys**	**Girls**	**Boys**
Normal pubertal developers	n=348	n=289	n=61	n=63
Age (y), mean (SD)	10.2 (0.4)	11.1 (1.4)	10.8 (1.2)	12.0 (1.1)
Age (y), range	9.4; 11.5	10.0; 14.0	9.1; 13.0	10.0; 14.0
pMO, mean (SD)	-1.65 (0.31)	-2.13 (1.01)	-0.92 (1.00)	-1.36 (0.88)
pMO, range	-2.53; -0.78	-3.29; 0.33	-2.66; 0.94	-3.47; 0.66
pMO>0, n (%)	0 (0.0)	9 (3.1)	14 (23.0)	2 (3.3)
Early pubertal developers	n=0	n=44	n=5	n=5
Age (y), mean (SD)	-	9.7 (0.2)	8.2 (0.2)	9.3 (0.3)
Age (y), range	-	9.3-10.0	8.0; 8.5	9.1; 9.9
pMO, mean (SD)	-	-3.00 (0.21)	-2.97 (0.29)	-3.15 (0.17)
pMO, range	-	-3.52; -2.58	-3.41; -2.61	-3.32; -2.92
pMO>0, n (%)		0 (0.0)	0 (0.0)	0 (0.0)
p-value for pMO differences between early and normal developers	-	<0.001	<0.001	<0.001

SD, standard deviation; pMO, predicted Maturity Offset

Paper III

Human Milk Feeding Practices and Pubertal Timing: Insights from the Generation XXI Population-Based Birth Cohort

Marta Pinto da Costa, Milton Severo, Carla Lopes, Augusto Anguita-Ruiz, Joana Araújo, Sofia Vilela

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Human Milk Feeding Practices and Pubertal Timing: Insights from the Generation XXI Population-Based Birth Cohort

Marta Pinto da Costa [1,2,*], Milton Severo [1,3], Carla Lopes [1,2], Augusto Anguita-Ruiz [4,5], Joana Araújo [1,2], Sofia Vilela [1]

[1] EPIUnit ITR, Instituto de Saúde Pública da Universidade do Porto, Universidade do Porto, Porto, Portugal; [2] Departamento de Ciências da Saúde Pública e Forenses, e Educação Médica, Faculdade de Medicina, Universidade do Porto, Porto, Portugal; [3] Departamento de Ensino Pré-Graduado, Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Porto, Portugal; [4] CIBEROBN, Institute of Health Carlos III, Madrid, Spain; [5] ISGlobal, Barcelona Institute for Global Health,, Barcelona, Spain

ABSTRACT

Background: Evidence regarding the potential influence of human milk feeding on pubertal timing remains inconsistent.
Objectives: This study explored the association between human milk feeding (never, nonexclusive, or exclusive) and its duration with pubertal timing, using longitudinal data from a population-based birth cohort.
Methods: The sample included 4745 children from the population-based Portuguese birth cohort Generation XXI, with data on human milk feeding, feeding duration, and Tanner stages at 10 years of age. A Puberty score was derived from Tanner stages (range: 2–10). Human milk feeding was categorized according to exclusivity and duration (never; nonexclusive; exclusive < 4 mo; and exclusive ≥ 4 mo). Multinomial logistic regression and Cox proportional hazard models were used to estimate the association between human milk feeding categories and both puberty score and age at menarche, adjusting for maternal and child characteristics.
Results: After adjustment for confounders, females who received human milk had lower odds ratio (OR) of a higher puberty score at 10 y of age (exclusive ≥4 mo—OR: 0.33; 95% CI: 0.24, 0.47) than those never fed human milk. Exclusive human milk feeding was associated with later age at menarche. No significant associations were found in males, except that those receiving "any but never exclusive" human milk feeding, who had lower OR of being in the highest puberty score (≥5; OR: 0.48; 95% CI: 0.25, 0.94).
Conclusions: These findings suggest that human milk feeding, particularly exclusive human milk feeding for ≥4 mo, may be associated with later pubertal timing among females.

Keywords: human milk, puberty, Tanner stages, children, cohort study, longitudinal study

Introduction

Puberty is a critical transitional phase from childhood to adulthood, characterized by a series of physiological events. In females, the main characteristics of pubertal development are breast growth, the development of pubic hair, and, at a later stage, the appearance of menarche. In males, the characteristics defined as the major milestones of puberty are genital growth, voice change, and the development of pubic hair [1].

A growing body of evidence suggests a declining tendency of the age of puberty onset worldwide, especially among females, including Portugal, configuring itself as a public health concern [2–7]. Early pubertal development has important public health implications, as it has been associated with an increased risk of both short- and long-term adverse health outcomes. Previous studies have shown a significant association between early puberty and several adult diseases, such as endocrine-related disorders, type 2 diabetes [8], metabolic syndrome [9], cardiovascular diseases, and reproductive cancers [10–12], such as breast [13] and prostate cancers [10].

Puberty onset and development is a complex process that can be influenced by multiple factors, including prenatal and postnatal exposures [14]. Although genetic factors are considered the main determinants of the age of puberty onset [15–17], nutrition [18], BMI [19–23], endocrine disrupting chemicals

Abbreviations: ICC, intraclass correlation coefficient; OR, odds ratio.

* Corresponding author. *E-mail address:* marta.costa@ispup.up.pt (M.P. da Costa).

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[24], environmental factors [15–17], and socioeconomic factors [25] have also been shown to affect the onset of puberty. Prepubertal obesity, as stated earlier, has been associated with an earlier puberty onset [6,23,26,27]. However, as observed in previous studies, prepubertal weight status does not entirely explain the decline in the age of puberty onset in recent years, since this tendency has been observed especially in females of varying BMI categories [23,28,29]. Interestingly, some studies suggested early feeding practices, specifically human milk feeding, may significantly affect the timing of pubertal development [14,18,30].

Human milk is the first food a newborn should ideally consume, with a well-known positive impact on both mother and infant health. The WHO recommends exclusive human milk feeding for the first 6 mo of life and continued human milk feeding with complementary food ≤2 y of age or beyond [31, 32]. Human milk feeding has been associated with a reduced risk of several childhood health outcomes, including lower incidence of respiratory, gastroenterological, and endocrinological pathologies [33], as well as reduced risk of faster weight gain and obesity [34–36]. However, evidence regarding its potential influence on pubertal development remains inconsistent. One study described that human milk feeding for ≥6 mo reveals a protective association against early pubertal development in both males and females [37]. However, the authors did not distinguish between exclusive and nonexclusive human milk feeding, and the findings may not be generalized to populations outside of Korea due to cultural and genetic differences. Another study found that compared with females who received human milk feeding for ≥6 mo, females who did not receive human milk feeding are at increased risk of earlier breast and pubic hair onset, although the authors were unable to precisely estimate human milk feeding duration and exclusivity [38]. Moreover, a more recent study indicated that the absence of human milk feeding appears to accelerate pubertal timing but only in males [39]. On the contrary, 2 European cohort studies that included males and females did not find an association between human milk feeding duration and pubertal onset [40,41].

Therefore, the present study aimed to examine whether human milk feeding (never, nonexclusive, or exclusive) and its duration are associated with pubertal timing, using longitudinal data from a population-based birth cohort.

Methods

Study design and participants

The present study included data from the previously described Generation XXI cohort [42,43]. Between April 2005 and August 2006, all newborns in the 5 public maternity hospitals in the metropolitan area of Porto were invited to participate. Of the invited mothers, 91.4% agreed to participate, with a total of 8647 children enrolled at baseline. Starting from an initial assessment at birth, participant subsamples were assessed at 6 (n = 1558), 15 (n = 1042), and 24 mo (n = 855). The entire cohort was invited to attend a follow-up when children were aged 4 (86% of participation), 7 (80% of participation), 10 (76% of participation) and 13 y (54% of participation—follow-up was interrupted due to the COVID-19 pandemic). The 18-y-old follow-up has recently been completed. During these evaluation waves, participants were invited to participate in a face-to-face interview where data on social and demographic conditions, children's health status, and lifestyle were collected by trained interviewers through structured questionnaires. Objective anthropometric measures were collected by trained health professionals at all follow-up waves following a standardized protocol based on WHO recommendations, while puberty development was assessed at 10 and 13 y of age. Anthropometric measurements were conducted under supervision, with ongoing quality control to ensure consistency across waves. For the final sample, inclusion criteria comprised children with data on exclusive and nonexclusive human milk feeding duration collected at 6, 15, and/or 24 mo, and/or at 4 y of age, complete information on Tanner stages assessed at 10 y and self-reported status and age at menarche recorded at 13 and 18 y of age. After excluding twins (n = 144) and children with congenital anomalies or diseases that could affect the analysis of food intake, such as celiac disease, allergy, or food intolerance (n = 50), the final sample included 4515 children (2239 females and 2276 males) (Supplementary Figure 1).

Human milk feeding duration

Data on human milk feeding practices were collected through a questionnaire applied by trained interviewers to the main caregiver at 6-, 15- and 24-mo subsample follow-up evaluations and at 4-y complete sample follow-up. In all evaluations, the following question was asked: Do you feed, or have you ever fed your baby with human milk? If the answer was yes, the interviewer asked, "Are you still giving him/her human milk?" If the child was no longer being fed with human milk, the main caregiver was asked how old the child was when the mother completely stopped providing human milk, both exclusively and nonexclusively (in months, weeks, or days). The total duration of exclusive and/or nonexclusive human milk feeding were converted into weeks. Exclusive human milk feeding duration is defined by the WHO as the total time when the infant receives only human milk and not any other foods or liquids (including water or infant formula), with the exception of vitamins, minerals, or medicines [44]. However, in the Generation XXI cohort, human milk feeding was classified as exclusive even if the child had already been given water. To reduce the possible reporting error of the exclusive human milk feeding duration, given that the WHO only recommends exclusive human milk feeding for the first 6 m of life [45,46], we only considered in this study data reported ≤7 mo, excluding all children with a reported exclusive human milk feeding duration higher than this cutoff point (n = 77). The final variables of the exclusive and nonexclusive human milk feeding duration were obtained considering the first human milk feeding duration value reported in any of the follow-ups. To account for possible recall bias of the main caregiver, the agreement in the reported duration of human milk feeding between different follow-ups was analyzed. The results are presented in Supplementary Table 1. The agreement across different follow-up waves varied substantially. Intraclass correlation coefficients (ICC) ranged from 0.26 (6 mo compared with 24 mo), indicating low agreement, to 0.91 (24 mo compared with 4 y), indicating excellent agreement. Moderate agreement was observed for earlier time points (6 mo compared with 15 mo; ICC: 0.51), with higher agreement observed in later

TABLE 1
Maternal and child characteristics by sex

Characteristics	Total sample (N = 4515)	Female (n = 2239)	Male (n = 2276)	P
Maternal age (y)	29.9 ± 5.1	30.0 ± 5.3	29.9 ± 5.0	0.370[1]
Maternal education (y)	11.3 ± 4.3	11.2 ± 4.4	11.4 ± 4.2	0.155[1]
Maternal age at first menarche (y)	12.4 ± 1.5	12.4 ± 1.6	12.4 ± 1.5	0.995[1]
Child's age at first menarche (y)	—	11.6 ± 1.0	—	
Child's birth weight (g)	3192 ± 487	3143 ± 469	3240 ± 499	<0.001[1]
Child's exact age at 10-y follow-up (y)	10.2 ± 0.3	10.2 ± 0.3	10.1 ± 0.3	0.004[1]
Total human milk feeding duration (wk)	28.3 ± 33.7	27.6 ± 32.7	29.0 ± 34.7	0.183[1]
Total human milk feeding duration (wk)	20.0 (8.0–36.0)	18 (8.0–32.5)	20.0 (8.0–36.0)	
Exclusive human milk feeding duration (wk)	11.0 ± 8.3	11.1 ± 8.3	10.9 ± 8.3	0.328[1]
Exclusive human milk feeding duration (wk)	12 (3.0–16.0)	12 (3.0–16.0)	12.0 (3.0–16.0)	
Human milk feeding categories				
Never	272 (6.0)	138 (6.2)	134 (5.9)	
Any but never exclusive	441 (9.8)	207 (9.3)	234 (10.3)	
Exclusive, <4 mo	1835 (40.6)	903 (40.3)	932 (40.9)	0.567[2]
Exclusive, ≥4 mo	1967 (43.6)	991 (44.3)	976 (42.9)	
BMI z-score at 10 y follow-up				
Underweight	46 (1.0)	20 (0.9)	26 (1.1)	0.019[2]
Normal weight	2556 (56.6)	1282 (57.3)	1274 (56.0)	
Overweight	1163 (25.8)	601 (26.8)	562 (24.7)	
Obesity	750 (16.6)	336 (15.0)	414 (18.2)	
Genitalia Tanner stages at 10-y follow-up				
1	2257 (50.0)	578 (25.8)	1679 (73.8)	<0.001[2]
2	1568 (34.7)	1019 (45.5)	549 (24.1)	
3	585 (13.0)	546 (24.4)	39 (1.7)	
4	104 (2.3)	96 (4.3)	8 (0.4)	
5	1 (0.0)	0 (0.0)	1 (0.0)	
Pubic hair Tanner stages at 10-y follow-up				
1	2949 (65.3)	1003 (44.8)	1946 (85.5)	<0.001[2]
2	992 (22.0)	716 (32.0)	276 (12.1)	
3	477 (10.6)	432 (19.3)	45 (2.0)	
4	96 (2.1)	87 (3.9)	9 (0.4)	
5	1 (0.0)	1 (0.0)	0 (0.0)	
Puberty score				
2	1987 (44.0)	466 (20.8)	1521 (66.8)	<0.001[2]
3	1075 (23.8)	532 (23.8)	543 (23.9)	
4	726 (16.1)	564 (25.2)	162 (7.1)	
5	334 (7.4)	301 (13.4)	33 (1.5)	
6	277 (6.1)	269 (12.0)	8 (0.4)	
7	43 (1.0)	38 (1.7)	5 (0.2)	
8	71 (1.6)	68 (3.1)	3 (0.1)	
9	2 (0.0)	1 (0.0)	1 (0.0)	

Values are n (%), mean ± SD, or median (p25–p75).
[1] Student t test.
[2] χ^2 test.

follow-ups (15 mo compared with 24 mo; ICC: 0.89) (Table 1). The duration of exclusive and nonexclusive human milk feeding was considered as a categorical variable. Children were grouped into 4 categories: *1*) never received human milk (n = 327); *2*) received human milk but never exclusively (n = 521); *3*) exclusively received human milk for <4 mo (<16 wk, n = 1902); and *4*) exclusively received human milk for ≥4 mo (≥16 wk, n = 1995). These categories allow for a clear distinction between children who never received human milk (and were fed with infant formula) and those who were exclusively (with water or without) and nonexclusively fed with human milk (complementary with infant formula and/or other food or beverage, beyond water). The 4-mo (16 wk) cutoff point for the exclusive human milk feeding duration was selected due to the majority of the sample reporting having exclusively fed human milk for <4 mo (57.9%) and because, in Portugal, maternity leave consists of only 4 mo of fully paid leave (120 d) [47].

Pubertal timing

Pubertal development was assessed by trained nurses at the 10-y follow-up by physical examination, using palpation and visual inspection, following the 5-level Tanner stages criteria developed by Marshall and Tanner [48,49]. Pubic hair and breast development were evaluated in females, and pubic hair and testicular development were assessed in males. Each parameter was classified into 1–5 stages—1, prepubertal; 2–4, pubertal; and 5, postpubertal. Tanner stage 1 suggests that puberty has not yet started, while Tanner stage 5 for pubic hair and both genital and breast development defines full sexual maturation. Females were classified as a Tanner stage 2 or higher, the mark of the beginning of puberty, if they present slightly pigmented and downy pubic hair and a breast bud stage with an elevation of breast and papilla and enlargement of the areola [48,50]. Males were classified as Tanner stage 2 or higher if they presented a testicular volume >4 mL and a slightly pigmented

and downy pubic hair [49,50]. In the present sample, some misclassification of Tanner stages cannot be discarded, particularly for testicular volume in males. Moreover, relying on a single parameter to define pubertal timing is limited, as pubic hair and breast or genital development progress at different rates [48,49]. Therefore, the concordance between the genitalia and pubic hair stages has been tested, and it was possible to conclude that the agreement was relatively low, especially in males ($k = 0.26$ for males and $k = 0.56$ for females). Given that, a puberty score has been developed by summing the Tanner stage for genital development and the Tanner stage for pubic hair, resulting in a total score ranging from 2 to 10. This combined score helps reduce the measurement error associated with evaluating each characteristic individually, especially the Tanner stage based on testicular volume, which can be less reliably assessed by the nurses due to its invasive nature involving testicular palpation [51]. A puberty score of ≥3 indicates that the child has already started puberty. At 10 y of age, given the small number of children in puberty score categories 5 through 9, especially males ($n = 58$), we combined these categories for the main analysis.

At ages 13 and 18 y, when nurses assessed pubertal development, females self-reported their age at menarche, defined as the age of the occurrence of their first menstrual cycle. The final variable for age at menarche used in analyses was derived as follows: if reported at only 1 follow-up, that value was used; if reported at both follow-ups and the difference between values did not exceed 2 years, the mean of the 2 was calculated. Among females with information available at both follow-ups ($n = 700$), the mean difference between the 2 reported ages was 0.36 y, and the ICC was 0.60, indicating moderate agreement between reports.

Other child and maternal characteristics

From the baseline evaluation, maternal age and completed years of education and maternal age at menarche were collected by trained interviewers using structured questionnaires. Maternal age and maternal educational level were collected as indicators of socioeconomic status, while maternal age at menarche was considered as a proxy for genetic heritability [52].

The child's birth weight, previously described elsewhere [53], was abstracted from medical records by trained interviewers. A team of trained interviewers performed objective measurements of children's heights and weights at 10 y, following standard procedures. Children's BMI was defined by weight in kilograms divided by height in meters squared. This variable was categorized using specific cutoffs for sex and age as defined by the WHO: underweight, less than −2 SD; normal weight, −2 SD or more to ≤1 SD; overweight, >1 to ≤2 SD; and obesity, >2 SD [54]. Given the small number of children classified as underweight ($n = 55$), this category was combined with the normal weight category for analysis.

Ethical considerations

All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. The baseline and follow-up evaluations were approved by the University of Porto Medical School/S. João Hospital Centre Ethics Committee, except the 13-y follow-up that was approved by the ISPUP Ethics Committee. At baseline and follow-up evaluations, all procedures were explained to participants, and an informed consent was signed by one of the parents or legal guardians (at 13 y, it was also signed by the participants). At 18 y, informed consent was signed by the participants themselves. The baseline evaluation was approved by the Data Protection National Commission and the study follows the EU General Data Protection Regulation under close supervision of the Data Protection Office of ISPUP.

Statistical analysis

Descriptive statistics of participants were calculated as mean and SD for continuous variables and numbers and percentages for categorical variables. Comparison between child's sex was achieved using Student t test or the $\chi 2$ test. Statistical analyses were conducted separately by sex because males and females exhibit different pubertal trajectories and timing—females typically begin puberty earlier than males [48,49]—and use different markers to define pubertal timing. A descriptive analysis stratified by human milk feeding categories and sex was also performed (Supplementary Table 2).

To study the agreement between the pubic hair stages and testicular volume and breast stages, Cohen κ statistic was applied. The agreement was considered as follows: none to slight, $0 < \kappa < 0.20$, fair, $0.21 \leq \kappa < 0.40$; moderate, $0.41 \leq \kappa < 0.60$; to be substantial, $0.61 \leq \kappa \leq 0.80$; and to be almost perfect, $\kappa > 0.80$ [55,56].

The association between the child's human milk feeding categories (reference group: never received human milk) and the pubertal timing was examined with 2 outcomes: the puberty score at 10 y of age and age at menarche. For the puberty score, multinomial logistic regression models were fitted, stratified by sex, as the proportional odds assumption required for ordinal logistic regression models was violated (Brant test). For age at menarche, Cox proportional hazards models for interval-censored data were used, restricted to females. Females were considered at risk of menarche from age 8 y and remained at risk until the age at which menarche was reported across the follow-up assessments at 10, 13, and 18 y. This cutoff was established because menarche occurring at or before this age is considered abnormally early and generally indicative of precocious pubertal development. Participants who had not experienced menarche or had missing information were treated as right-censored at the last available follow-up. For both outcomes, 4 models were considered. Model 1 was the crude model; model 2 was adjusted for maternal age and years of education and for the physical examiner code; model 3 was adjusted for Model 2 in addition to maternal age at menarche, child's birth weight, and gestational age at birth; model 4 was adjusted for model 3 in addition to child's BMI z-score. All models were adjusted for children's exact age. The analyses were adjusted for identification number of the nurse who performed the physical examination to account for interrater variability in Tanner stage assessment, thereby reducing the likelihood that observed associations were driven by systematic measurement error. Household income was also tested as a covariate, but its inclusion did not change the observed associations. Ordinal logistic regression models were repeated as 2 sensitivity analyses, considering only breast (for females) or testicular (for males) Tanner stages, and pubic hair (for both) Tanner stages as outcomes (Supplementary Tables 3 and 4), as the proportional odds

assumption was met for these outcomes. Multinomial logistic regression and Cox proportional hazards regression analyses were repeated for puberty score and age at menarche as outcomes, respectively, stratified by BMI category (normal weight and overweight/obese) (Supplementary Tables 5 and 6). An additional sensitivity analysis was conducted considering the categories of exclusively human milk fed for <6 mo (<24 wk, n = 3260) and exclusively human milk fed for ≥6 mo (≥24 wk, n = 542) (Supplementary Table 7).

The software used was R language and software environment for statistical computation (version 4.4.3; R Foundation for Statistical Computing), package MASS for ordinal logistic regression [57], package iceReng for Cox proportional hazards regression, and package nnet for multinomial logistic regression. A significance level of 0.05 was considered.

Results

Table 1 shows the maternal and children characteristics according to sex. Females included in the analysis (49.6% of the sample) were slightly older than males at the time of Tanner stage assessment and showed more advanced pubertal development, with a higher proportion having initiated puberty, whereas most males remained in Tanner stage 1. The mean duration of human milk feeding, including total and exclusive duration, were similar between females and males. Among the children included in the analysis, the majority were normal weight in both sexes. The prevalence of obesity at age 10 y was higher in males than in females.

Table 2 presents the distribution of human milk feeding categories across puberty score groups at 10 y of age. Overall, children exclusively fed with human milk for ≥4 mo were more frequently represented in the lower puberty score categories, while those never fed human milk were more represented in the higher categories. Although a similar pattern is observed in males, it is not pronounced as in females (Table 2).

After adjustment for all the considered covariates (Table 3, model 4), all human milk feeding categories were significantly associated with lower odds ratio (OR) of a higher puberty score (≥5) among females, than that among those who were never fed with human milk. Specifically, females who were exclusively fed with human milk for ≥4 mo had significantly lower OR of being in puberty score category 4 and ≥5. For males, most human milk feeding categories were not significantly associated with puberty score. An exception was observed for males who received human milk, but never exclusively, showing lower OR of being in puberty score of ≥5, compared with those who were never fed with human milk. However, this estimate should be interpreted cautiously given the small numbers of males in puberty score of ≥5 (n = 50).

Compared with those who were never fed with human milk, exclusive human milk feeding was significantly associated with later age at menarche (Table 4). However, this association was no longer observer after adjusted in model 4 (Table 4).

When the analysis was conducted using pubic hair Tanner stage (Supplementary Table 3) or genital Tanner stage (Supplementary Table 4) as outcomes, and after adjusting for all covariates (Model 4), human milk feeding was significantly associated with lower OR of being in a higher Tanner stage, among females. Specifically, all human milk feeding categories were associated with lower OR of being in a higher pubic hair Tanner stage, while only exclusive human milk feeding categories were associated with lower OR of a higher genital Tanner stage, compared with females who were never fed with human milk. No statistically significant associations were observed among males.

Interactions between child BMI categories at age 10 y and human milk feeding in the associations with puberty score and age at menarche were tested. No statistically significant interactions were observed in either analysis (data not shown). Supplementary Table 5 shows that human milk feeding was associated with lower puberty scores among females, in both normal weight and overweight/obese categories. No significant associations were observed among males, except for males in the overweight/obese category who received human milk, but never exclusive.

Supplementary Table 6 shows the association between human milk feeding categories and menarche, stratified by BMI category. No statistically significant associations were observed in any BMI category.

TABLE 2
Distribution of human milk feeding type and duration by puberty score categories at 10 y of age

Human milk feeding	Puberty score at 10 y			
	2	3	4	5-9
Total	n = 1987	n = 1075	n = 726	n = 727
Never	102 (5.1)	48 (4.5)	52 (7.2)	70 (9.6)
Any but never exclusive	195 (9.8)	109 (10.1)	74 (10.2)	63 (8.7)
Exclusive, <4 mo	814 (41.0)	397 (36.9)	307 (42.3)	317 (43.6)
Exclusive, ≥4 mo	876 (44.1)	521 (48.5)	293 (40.3)	277 (38.1)
Females	n = 466	n = 532	n = 564	n = 677
Never	15 (3.2)	21 (3.9)	36 (6.4)	66 (9.7)
Any but never exclusive	37 (7.9)	54 (10.2)	56 (9.9)	60 (8.9)
Exclusive, <4 mo	181 (38.9)	189 (35.5)	240 (42.6)	293 (43.3)
Exclusive, ≥4 mo	233 (50.0)	268 (50.4)	232 (41.1)	258 (38.1)
Males	n = 1521	n = 543	n = 162	n = 50
Never	87 (5.7)	27 (5.0)	16 (9.9)	4 (8.0)
Any but never exclusive	158 (10.4)	55 (10.1)	18 (11.1)	3 (6.0)
Exclusive, <4 mo	633 (41.6)	208 (38.3)	67 (41.4)	24 (48.0)
Exclusive, ≥4 mo	643 (42.3)	253 (46.6)	61 (37.6)	19 (38.0)

Discussion

The current study aimed to explore the association between human milk feeding type and duration and earlier pubertal timing. After the adjustment for potential confounders, the present study showed that, in females, human milk feeding was associated with less advanced pubertal development at age 10 years, as well as later pubertal timing. In males, no consistent statistically significant associations were observed across models. This sex difference may be explained by the fact that females typically begin puberty earlier than males, making it more likely to detect associations when the pubertal timing is assessed at 10 y of age [58]. Furthermore, genital development in males progresses more slowly, making it more difficult to detect visual changes in genitalia from Tanner stage 1 to stage 2 than breast changes in females [59]. Body composition may also influence this sex difference, as greater adiposity in females

TABLE 3
Results from the multinomial logistic regression models assessing the association between human milk feeding type and duration and puberty score at 10 y old

Puberty score	Human milk feeding	Model 1		Model 2		Model 3		Model 4	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Females		$n = 2238$		$n = 2231$		$n = 2196$		$n = 2196$	
3	Never	Reference		Reference		Reference		Reference	
	Any but never exclusive	1.06	0.48, 2.32	1.14	0.51, 2.53	1.20	0.77, 1.87	1.17	0.74, 1.87
	Exclusive, <4 mo	0.76	0.38, 1.52	0.77	0.38, 1.57	0.74	0.51, 1.09	0.70	0.47, 1.04
	Exclusive, ≥4 mo	0.86	0.43, 1.71	0.92	0.46, 1.85	0.88	0.61, 1.28	0.89	0.60, 1.31
4	Never	Reference		Reference		Reference		Reference	
	Any but never exclusive	0.69	0.31, 1.35	0.70	0.33, 1.46	0.75	0.49, 1.17	0.73	0.47, 1.14
	Exclusive, <4 mo	0.57	0.30, 1.07	0.57	0.30, 1.09	0.59[1]	0.42, 0.84	0.55[1]	0.38, 0.78
	Exclusive, ≥4 mo	0.44[1]	0.23, 0.83	0.46[1]	0.25, 0.88	0.47[1]	0.33, 0.67	0.48[1]	0.33, 0.68
≥5	Never	Reference		Reference		Reference		Reference	
	Any but never exclusive	0.40[1]	0.20, 0.81	0.44[1]	0.21, 0.90	0.46[1]	0.30, 0.71	0.46[1]	0.29, 0.72
	Exclusive, <4 mo	0.39[1]	0.21, 0.71	0.41[1]	0.22, 0.75	0.42[1]	0.30, 0.58	0.39[1]	0.27, 0.55
	Exclusive, ≥4 mo	0.27[1]	0.16, 0.52	0.32[1]	0.17, 0.59	0.32[1]	0.23, 0.45	0.33[1]	0.24, 0.47
Males		$n = 2276$		$n = 2259$		$n = 2229$		$n = 2229$	
3	Never	Reference		Reference		Reference		Reference	
	Any but never exclusive	1.15	0.68, 1.96	1.20	0.70, 2.07	1.21	0.70, 2.08	1.27	0.74, 2.18
	Exclusive, <4 mo	1.08	0.68, 1.72	1.12	0.70, 1.79	1.14	0.71, 1.82	1.19	0.74, 1.92
	Exclusive, ≥4 mo	1.36	0.86, 2.16	1.45	0.91, 2.32	1.51	0.94, 2.42	1.59	0.99, 2.54
4	Never	Reference		Reference		Reference		Reference	
	Any but never exclusive	0.66	0.32, 1.36	0.69	0.33, 1.45	0.67	0.32, 1.40	0.73	0.35, 1.53
	Exclusive, <4 mo	0.61	0.34, 1.11	0.64	0.35, 1.17	0.61	0.33, 1.11	0.65	0.36, 1.20
	Exclusive, ≥4 mo	0.57	0.32, 1.05	0.63	0.34, 1.15	0.62	0.33, 1.14	0.66	0.36, 1.23
≥5	Never	Reference		Reference		Reference			
	Any but never exclusive	0.47	0.10, 2.19	0.47	0.10, 2.20	0.43[1]	0.22, 0.83	0.48[1]	0.25, 0.94
	Exclusive, <4 mo	0.94	0.31, 2.82	0.95	0.31, 2.84	0.90	0.51, 1.57	1.02	0.58, 1.80
	Exclusive, ≥4 mo	0.83	0.27, 2.52	0.83	0.27, 2.55	0.86	0.48, 1.53	0.95	0.53, 1.70

Model 1, crude; model 2, adjusted for the ID number of the physical examiner in addition to maternal age and educational level; model 3, adjusted for model 2 in addition to maternal age at menarche, child's birth weight, and gestational age at birth; model 4, adjusted for model 3 in addition to child's BMI *z*-score. All models were adjusted for children's exact age. The stratified analysis by BMI category is presented in Supplementary Table 5.
[1] Significant difference ($P < 0.05$).

TABLE 4
Results from the Cox proportional hazards regression for time to event assessing the association between human milk feeding type and duration and age of menarche

Human milk feeding	Model 1		Model 2		Model 3		Model 4	
	β	*P*	β	*P*	β	*P*	β	*P*
Females	$n = 1715$		$n = 1704$		$n = 1679$		$n = 1679$	
Never	Reference		Reference		Reference		Reference	
Any but never exclusive	0.63	0.35, 1.11	0.64	0.35, 1.14	0.72	0.40, 1.32	0.75	0.40, 1.41
Exclusive, <4 mo	0.58[1]	0.36, 0.98	0.60[1]	0.37, 0.96	0.59[1]	0.37, 0.96	0.63	0.38, 1.05
Exclusive, ≥4 mo	0.49[1]	0.31, 0.79	0.52[1]	0.32, 0.84	0.54[1]	0.33, 0.87	0.62	0.37, 1.03

Model 1, crude; model 2, adjusted for the ID number of the physical examiner in addition to maternal age and educational level; model 3, adjusted for model 2 in addition to maternal age at menarche, child's birth weight, and gestational age at birth; model 4, adjusted for model 3 in addition to child's BMI *z*-score. All models were adjusted for children's exact age. The stratified analysis by BMI category is presented in Supplementary Table 6.
[1] Significant difference ($P < 0.05$).

appears to affect pubertal timing, whereas this relationship is less clearly established in males [60].

The results of the present study corroborate previous findings, which mostly indicate that human milk feeding and its duration play a role in the timing of puberty in females, with a later age at the onset of menarche and onset and development of breast and pubic hair development [38,61–63]. In males, the results are less consistent, likely due to a relative scarcity of studies focusing on this group compared with females. Some of the few existent studies have suggested that human milk feeding and its duration may have a protective role against early puberty onset [37,38], whereas others, as our study, have found no association [40,41]. These discrepancies between studies may be explained by differences in human milk feeding practices across countries and by differences in the age at assessment on pubertal outcomes. For example, in a longitudinal study conducted in a Hong Kong population, where maternity leave is very short, ~57% of the sample were never fed with human milk, and only 6.4% were exclusively fed with human milk for >3 mo [41]. These conflicting findings highlight the need for further studies

including both females and males, to better understand the effect of human milk feeding on pubertal timing.

Human milk contains bioactive components, including regulatory hormones, such as leptin [64–66]. Beyond its role in promoting early satiety in infants and regulating energy balance and expenditure during childhood [67], leptin has been implicated in the onset and development of puberty [67–69]. Leptin acts as a signal of the body's nutritional status [70], influencing the hypothalamic–pituitary–gonadal axis [71]. In females, low prepubertal leptin levels have been shown to predict subsequent changes in body composition and greater gains in fat percentage [72], which may, in turn, indirectly affect the timing of pubertal onset [73,74]. However, leptin levels were not measured in the present study, and there is no evidence that leptin in human milk contributes to leptin levels during childhood. Therefore, any connection between early-life leptin exposure via human milk and pubertal timing is purely speculative.

It is possible that childhood body weight status could mediate the pathway between human milk feeding and pubertal timing. Previously, a longer duration of human milk feeding was shown to be protective against childhood obesity [75]. Childhood obesity, in turn, seems to be linked to earlier pubertal timing [23,76]. However, some studies have reported no association between obesity and earlier puberty onset [77,78], and findings among males have been inconsistent [21,79]. In our study, the association between a longer duration of exclusive human milk feeding and a lower puberty score remained significant among females, although slightly attenuated, after adjustment for BMI at age 10 y. Similarly, in the Cox proportional hazards regression for age at menarche, adjustment for child BMI was the covariate that most noticeably attenuated the crude association, suggesting that childhood adiposity may partially mediate this relationship, although other factors are also likely to contribute.

An alternative pathway may be that human milk feeding is linked with other maternal behavior patterns that support healthier lifestyles. Consistent with literature, maternal education may serve as a social determinant of child health, as more educated mothers tend to initiate and maintain human milk feeding for longer periods [80], establish more consistent sleep routines that improve children's sleep quality [81], and foster healthier dietary habits [82]. Recent studies have highlighted a strong association between sleep and puberty development [83], including a higher prevalence of early puberty among patients with sleep disorders, as well as an association between insufficient sleep duration and late bedtime with the increased risk of early puberty development [84]. Similarly, children with lower diet quality appear to enter puberty at an earlier age [85], whereas a later onset of puberty has been prospectively associated with higher diet quality [86].

The main strengths of this study include its prospective design, based on a relatively large population-based sample with a high participation rate and the inclusion of both males and females. The pubertal development was assessed by trained nurses, thereby minimizing errors commonly associated with self-reports or parental reports. Clinical examination with visualization and palpation [87] is considered more reliable than subjective reporting. Another strength of the study was the control for multiple potential confounding factors, including potential maternal genetic influences, by adjusting for maternal age at menarche. Nevertheless, some limitations should be acknowledged. Some errors can arise from measuring Tanner stages, especially when measuring testicular volume in males, even following a standard guideline for staging and using an orchidometer, as this is a highly invasive measurement and can affect measurement accuracy. However, it was possible to overcome this limitation through the construction of the puberty score, which, in addition to considering the genitalia Tanner staging, considered the pubic hair Tanner stage. Additionally, the fact that the human milk feeding duration information was collected only at specific time points and by the parental report can introduce a possible recall bias. To minimize this reporting error, we integrated information from the subsample follow-ups and the complete sample follow-up at 4 y of age to construct the final variable for exclusive and nonexclusive human milk feeding duration. As confirmed by the agreement analysis, the agreement on the parental report across the different follow-ups was considered satisfactory. The COVID-19 pandemic may have affected participation in the 13-y follow-up, potentially introducing selection bias [88]. However, this bias is expected to be limited, as it is unlikely to be directly associated with pubertal timing, thereby reducing the likelihood of differential bias.

Being fed with human milk, whether exclusively or not, was associated with later pubertal timing in females. In our study, the associations were similar across human milk feeding categories, suggesting that any exposure to human milk, rather than the exact duration or exclusivity, was related to later pubertal timing. These findings are consistent with public health recommendations promoting human milk feeding. No significant association was observed in males, except for males who received human milk but never exclusive, who had lower OR of being in the highest puberty score ($\geq$5). These findings highlight the public health importance of policies that support sustained human milk feeding, including extended maternity leave, flexible feeding breaks, dedicated workplace lactation facilities, and even onsite childcare centers to enable mothers to continue provision of human milk.

Author contributions

The authors' responsibilities were as follows—MPdC, JA, AA-R, CL, SV: designed research; MPdC, MS, SV: analyzed data; MPdC, MS, SV, JA, AA-R, CL: wrote the paper; MPdC, SV: had primary responsibility for final content; and all authors: have read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending (e.g., application and approval, payment, and other). The data can be made available for research proposals on request to the Generation XXI Executive Committee (generationxxi@ispup.up.pt).

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI or AI-assisted technologies were used in the writing of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2026.101535.

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Supplementary Tables

Table S1

Agreement of maternal reports on human milk feeding duration across follow-ups.

Table S2

Maternal and child's characteristics by human milk feeding category and sex.

Table S3

Association between human milk feeding type and duration and pubic hair Tanner stage at 10 years (ordinal logistic regression).

Table S4

Association between human milk feeding type and duration and genital Tanner stage at 10 years (ordinal logistic regression).

Table S5

Association between human milk feeding type and duration and Puberty score at 10 years by BMI categories (multinomial logistic regression).

Table S6

Association between human milk feeding type and duration and age at menarche by BMI categories (Cox proportional hazards regression).

Table S7

Association between human milk feeding type and duration and Puberty score at 10 years using the 6-month breastfeeding cut-off (multinomial logistic regression).

Supplementary Table 1. Agreement of human milk feeding duration maternal report between different follow-ups.

	Intraclass correlation coefficient (ICC)		
	15 months	24 months	4 years
6 months	0.51	0.26	0.48
15 months	-	0.89	0.83
24 months	-	-	0.91

Supplementary Table 2. Maternal and child's characteristics by human milk feeding category and sex.

	Girls				Boys			
	Never (n=138)	Any, but never exclusive (n=207)	Exclusive, <4 months (n=903)	Exclusive, ≥4 months (n=990)	Never (n=134)	Any, but never exclusive (n=234)	Exclusive, <4 months (n=932)	Exclusive, ≥4 months (n=977)
Maternal age (years), mean (SD)	30.7 (5.9)	29.9 (5.6)	29.4 (5.4)	30.5 (4.9)	29.4 (6.1)	30.1 (5.1)	29.5 (5.1)	30.2 (4.7)
Maternal education (years), mean (SD)	8.9 (4.2)	11.5 (4.5)	11.0 (4.2)	11.6 (4.4)	10.0 (4.5)	11.7 (4.1)	11.1 (4.2)	11.7 (4.2)
Maternal age at first menarche (years), mean (SD)	12.5 (1.5)	12.3 (1.5)	12.4 (1.6)	12.5 (1.6)	12.4 (1.8)	12.2 (1.4)	12.3 (1.5)	12.5 (1.5)
Child's age at first menarche (years), mean (SD)	11.3 (1.1)	11.4 (0.8)	11.6 (1.0)	11.6 (0.9)	-	-	-	-
Child's birth weight (grams), mean (SD)	2993 (598.0)	3077 (585.0)	3144 (455.0)	3176 (428.0)	3100 (605.0)	3109 (602.0)	3233 (481.0)	3297 (463.0)
Child's exact age at 10y follow-up (years), mean (SD)	10.2 (0.4)	10.2 (0.3)	10.2 (0.3)	10.1 (0.3)	10.2 (0.4)	10.1 (0.3)	10.1 (0.3)	10.1 (0.3)
Total human milk feeding duration (weeks), mean (SD)	0	9.2 (8.7)	14.3 (18.8)	47.6 (36.5)	0	10.9 (15.0)	15.0 (19.6)	50.8 (39.2)
Exclusive human milk feeding duration (weeks), mean (SD)	0	0	6.7 (4.0)	19.2 (3.6)	0	0	6.5 (4.0)	19.2 (3.6)
BMI z-score at 10y follow-up, n (%)								
Underweight	1 (0.7)	3 (1.4)	5 (0.6)	11 (1.1)	1 (0.7)	4 (1.7)	16 (1.7)	5 (0.5)

Normal weight	68 (49.3)	114 (55.1)	504 (55.8)	596 (60.2)	73 (54.5)	133 (56.8)	511 (54.8)	557 (57.0)
Overweight	41 (29.7)	56 (27.1)	254 (28.1)	249 (25.2)	25 (18.7)	57 (24.4)	233 (25.0)	248 (25.4)
Obesity	28 (20.3)	34 (16.4)	140 (15.5)	134 (13.5)	35 (26.1)	40 (17.1)	172 (18.5)	167 (17.1)
Genitalia tanner stages at 10y follow-up, n (%)								
1	21 (15.2)	48 (23.2)	213 (23.6)	296 (29.9)	99 (73.9)	177 (75.6)	692 (74.3)	711 (72.8)
2	53 (38.4)	100 (48.3)	414 (45.8)	451 (45.6)	32 (23.9)	54 (23.1)	220 (23.6)	244 (25.0)
3	56 (40.6)	52 (25.1)	227 (25.1)	211 (21.3)	1 (0.7)	3 (1.3)	16 (1.7)	19 (1.9)
4	8 (5.8)	7 (3.4)	49 (5.4)	32 (3.2)	1 (0.7)	0 (0.0)	4 (0.4)	3 (0.3)
5	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
Pubic hair tanner stages at 10y follow-up, n (%)								
1	40 (29.0)	94 (45.4)	394 (43.6)	475 (48.0)	105 (78.4)	200 (85.5)	800 (85.8)	841 (86.1)
2	46 (33.3)	62 (30.0)	288 (31.9)	319 (32.2)	22 (16.4)	29 (12.4)	105 (11.3)	121 (12.4)
3	41 (29.7)	42 (20.3)	179 (19.8)	170 (17.2)	5 (3.7)	4 (1.7)	22 (2.4)	14 (1.4)
4	11 (8.0)	9 (4.3)	41 (4.6)	26 (2.6)	2 (1.5)	1 (0.4)	5 (0.5)	1 (0.1)
	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Puberty score, n (%)								

2	15 (10.9)	37 (17.8)	181 (20.1)	233 (23.5)	87 (64.9)	158 (67.5)	633 (67.9)	643 (65.8)
3	21 (15.2)	54 (26.1)	189 (20.9)	268 (27.1)	27 (20.1)	55 (23.5)	208 (22.3)	253 (25.9)
4	36 (26.1)	56 (27.0)	240 (26.6)	231 (23.4)	16 (11.9)	18 (7.7)	67 (7.2)	62 (6.4)
5	26 (18.8)	24 (11.6)	141 (15.6)	110 (11.1)	1 (0.8)	3 (1.3)	16 (1.7)	13 (1.3)
6	28 (20.3)	27 (13.0)	98 (10.9)	116 (11.7)	1 (0.8)	0 (0.0)	4 (0.5)	3 (0.3)
7	5 (3.6)	2 (1.0)	21 (2.3)	10 (1.0)	0 (0.0)	0 (0.0)	2 (0.2)	3 (0.3)
8	7 (5.1)	7 (3.4)	32 (3.5)	22 (2.2)	1 (0.8)	0 (0.0)	2 (0.2)	0 (0.0)
9	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)

SD, standard deviation; BMI, body mass index

Supplementary Table 3. Results from the ordinal logistic regression models assessing the association between human milk feeding type and duration and pubic hair tanner stage at 10 years old

	Model 1		Model 2		Model 3		Model 4	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Females	n=2239		n=2232		n=2197		n=2197	
Human milk feeding								
Never	Ref.		Ref.		Ref.		Ref.	
Any, but never exclusive	0.540	0.360; 0.808	0.558	0.371; 0.838	0.578	0.383; 0.871	0.594	0.392; 0.899
Exclusive, <4mo	0.558	0.400; 0.779	0.570	0.407; 0.799	0.597	0.425; 0.839	0.607	0.430; 0.856
Exclusive, ≥4mo	0.483	0.346; 0.674	0.504	0.360; 0.706	0.522	0.371; 0.733	0.555	0.394; 0.784
Males	n=2276		n=2259		n=2229		n=2229	
Human milk feeding								
Never	Ref.		Ref.		Ref.		Ref.	
Any, but never exclusive	0.643	0.370; 1.124	0.673	0.385; 1.182	0.649	0.371; 1.143	0.695	0.394; 1.234
Exclusive, <4mo	0.633	0.406; 1.013	0.650	0.415; 1.046	0.628	0.399; 1.014	0.670	0.422; 1.090

Exclusive, ≥4mo	0.632	0.405; 1.012	0.670	0.427; 1.079	0.685	0.435; 1.107	0.725	0.457; 1.181

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age and educational level; Model 3: adjusted for Model 2 plus maternal age at menarche, child's birth weight and gestational age at birth; Model 4: adjusted for Model 3 plus child's BMI z-score. All the models were adjusted for children's exact age.

Supplementary Table 4. Results from the ordinal logistic regression models assessing the association between human milk feeding type and duration and genitalia tanner stage at 10 years old

	Model 1		**Model 2**		**Model 3**		**Model 4**	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Females	n=2245		n=2238		n=2203		n=2203	
Human milk feeding								
Never	Ref.		Ref.		Ref.		Ref.	
Any, but never exclusive	0.571	0.382; 0.853	0.612	0.407; 0.919	0.622	0.527; 0.735	0.673	0.566; 0.800
Exclusive, <4mo	0.600	0.429; 0.838	0.629	0.448; 0.883	0.623	0.546; 0.711	0.660	0.576; 0.757
Exclusive, ≥4mo	0.463	0.332; 0.647	0.507	0.361; 0.711	0.494	0.433; 0.563	0.565	0.493; 0.647
Males	n=2280		n=2263		n=2232		n=2232	
Human milk feeding								
Never	Ref.		Ref.		Ref.		Ref.	
Any, but never exclusive	0.952	0.585; 1.563	0.984	0.600; 1.632	0.961	0.784; 1.178	1.000	0.816; 1.226
Exclusive, <4mo	1.033	0.688; 1.583	1.076	0.711; 1.663	1.056	0.901; 1.238	1.100	0.938; 1.289

Exclusive, ≥4mo	1.171	0.782; 1.793	1.241	0.820; 1.918	1.240	1.061; 1.450	1.288	1.101; 1.507

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age and educational level; Model 3: adjusted for Model 2 plus maternal age at menarche, child's birth weight and gestational age at birth; Model 4: adjusted for Model 3 plus child's BMI z-score. All the models were adjusted for children's exact age.

Supplementary Table 5. Results from the multinomial logistic regression models assessing the association between human milk feeding type and duration and Puberty score at 10 years old, by BMI categories

		Model 1		Model 2		Model 3	
Puberty score	**Human milk feeding**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Normal weight							
Females		n=1282		n=1277		n=1254	
3	Never	Ref.		Ref.		Ref.	
	Any, but never exclusive	1.542	0.593; 4.016	1.758	0.654; 4.726	1.703	0.948; 3.057
	Exclusive, <4m	0.860	0.370; 2.003	0.917	0.383; 2.197	0.925	0.551; 1.552
	Exclusive, ≥4m	1.033	0.448; 2.379	1.144	0.481; 2.717	1.137	0.686; 1.884
4	Never	Ref.		Ref.		Ref.	
	Any, but never exclusive	0.873	0.362; 2.110	0.927	0.378; 2.273	0.915	0.512; 1.636
	Exclusive, <4m	0.596	0.281; 1.264	0.591	0.276; 1.263	0.635	0.392; 1.028
	Exclusive, ≥4m	0.476	0.225; 1.007	0.490	0.229; 1.046	**0.499**	**0.309; 0.807**
≥5	Never	Ref.		Ref.		Ref.	
	Any, but never exclusive	0.435	0.173; 1.094	0.465	0.182; 1.188	**0.442**	**0.234; 0.835**
	Exclusive, <4m	**0.454**	**0.217; 0.955**	**0.468**	**0.220; 0.995**	**0.506**	**0.311; 0.822**
	Exclusive, ≥4m	**0.334**	**0.159; 0.701**	**0.358**	**0.168; 0.761**	**0.366**	**0.225; 0.597**
Males		n=1274		n=1264		n=1243	
3	Never	Ref.		Ref.		Ref.	

	Any, but never exclusive	1.631	0.794; 3.352	1.812	0.857; 3.830	1.796	0.836; 3.858
	Exclusive, <4m	1.005	0.529; 1.910	1.159	0.593; 2.262	1.192	0.601; 2.365
	Exclusive, ≥4m	1.469	0.778; 2.775	1.740	0.895; 3.380	1.808	0.915; 3.571
4	Never		Ref.		Ref.		Ref.
	Any, but never exclusive	0.412	0.112; 1.508	0.461	0.120; 1.769	0.419	0.109; 1.604
	Exclusive, <4m	0.549	0.221; 1.363	0.667	0.252; 1.767	0.598	0.225; 1.588
	Exclusive, ≥4m	0.660	0.266; 1.635	0.860	0.325; 2.280	0.831	0.314; 2.197
≥5	Never		Ref.		Ref.		Ref.
	Any, but never exclusive	0.807	0.106; 6.158	0.671	0.087; 5.163	0.549	0.215; 1.400
	Exclusive, <4m	0.828	0.171; 4.017	0.709	0.145; 3.471	0.632	0.289; 1.380
	Exclusive, ≥4m	0.754	0.150; 3.802	0.632	0.125; 3.203	0.745	0.337; 1.646
Overweight + obese							
Females			n=936		n=934		n=923
3	Never		Ref.		Ref.		Ref.
	Any, but never exclusive	0.442	0.079; 2.463	0.432	0.079; 2.352	0.475	0.215; 1.051
	Exclusive, <4m	0.579	0.119; 2.818	0.576	0.121; 2.730	**0.481**	**0.254; 0.911**
	Exclusive, ≥4m	0.809	0.166; 3.941	0.775	0.164; 3.653	0.687	0.363; 1.301
4	Never		Ref.		Ref.		Ref.
	Any, but never exclusive	0.345	0.065; 1.832	0.364	0.070; 1.890	**0.431**	**0.203; 0.915**
	Exclusive, <4m	0.509	0.110; 2.361	0.547	0.121; 2.474	**0.499**	**0.276; 0.903**
	Exclusive, ≥4m	0.514	0.110; 2.396	0.516	0.114; 2.334	**0.509**	**0.279; 0.931**

≥5	Never		Ref.		Ref.		Ref.
	Any, but never exclusive	0.251	0.050; 1.264	0.269	0.054; 1.325	**0.314**	**0.155; 0.636**
	Exclusive, <4m	0.311	0.070; 1.386	0.340	0.078; 1.475	**0.314**	**0.181; 0.546**
	Exclusive, ≥4m	0.319	0.071; 1.427	0.330	0.076; 1.431	**0.321**	**0.183; 0.565**
Males			n=976		n=969		n=960
3	Never		Ref.		Ref.		Ref.
	Any, but never exclusive	0.823	0.363; 1.868	0.819	0.359; 1.865	0.870	0.387; 1.955
	Exclusive, <4m	1.259	0.640; 2.475	1.204	0.611; 2.373	1.246	0.639; 2.430
	Exclusive, ≥4m	1.320	0.673; 2.590	1.297	0.658; 2.556	1.384	0.708; 2.703
4	Never		Ref.		Ref.		Ref.
	Any, but never exclusive	0.856	0.335; 2.183	0.864	0.337; 2.213	0.896	0.353; 2.271
	Exclusive, <4m	0.701	0.314; 1.565	0.687	0.307; 1.538	0.680	0.307; 1.508
	Exclusive, ≥4m	0.546	0.242; 1.236	0.552	0.243; 1.255	0.553	0.244; 1.252
≥5	Never		Ref.		Ref.		Ref.
	Any, but never exclusive	0.241	0.021; 2.815	0.257	0.022; 2.991	**0.244**	**0.096; 0.623**
	Exclusive, <4m	1.042	0.222; 4.885	1.102	0.239; 5.087	1.085	0.430; 2.738
	Exclusive, ≥4m	0.832	0.173; 3.994	0.868	0.182; 4.131	0.803	0.306; 2.105

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age and educational level; Model 3: adjusted for Model 2 plus maternal age at menarche, child's birth weight and gestational age at birth; Model 4: adjusted for Model 3 plus child's BMI z-score. All the models were adjusted for children's exact age.

Supplementary Table 6. Results from the Cox proportional hazards regression for time to event assessing the association between human milk feeding type and duration and age of menarche, by BMI categories.

	Model 1		Model 2		Model 3	
	β	95% CI	β	95% CI	β	95% CI
Normal weight	n=1023		n=1015		n=1000	
Human milk feeding						
Never	Ref.		Ref.		Ref.	
Any, but never exclusive	0.699	0.307; 1.594	0.714	0.309; 1.647	0.810	0.339; 1.932
Exclusive, <4m	0.569	0.288; 1.126	0.605	0.301; 1.216	0.611	0.296; 1.261
Exclusive, ≥4m	0.518	0.262; 1.023	0.536	0.267; 1.077	0.563	0.273; 1.160
Overweight + obese	n=677		n=674		n=665	
Human milk feeding						
Never	Ref.		Ref.		Ref.	
Any, but never exclusive	0.604	0.262; 1.394	0.622	0.264; 1.469	0.638	0.260; 1.562

Exclusive, <4m	0.648	0.329; 1.278	0.621	0.313; 1.234	0.643	0.319; 1.294
Exclusive, ≥4m	0.562	0.285; 1.109	0.604	0.303; 1.201	0.653	0.320; 1.331

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age and educational level; Model 3: adjusted for Model 2 plus maternal age at menarche, child's birth weight and gestational age at birth; Model 4: adjusted for Model 3 plus child's BMI z-score. All the models were adjusted for children's exact age.

Supplementary Table 7. Results from the multinomial logistic regression models assessing the association between human milk feeding type and duration and Puberty score at 10 years old, considering the 6-month breastfeeding cut-off.

		Model 1		**Model 2**		**Model 3**		**Model 4**	
Puberty score	**Human milk feeding**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Females		n=2238		n=2231		n=2196		n=2196	
3	Never	Ref.		Ref.		Ref.		Ref.	
	Any, but never exclusive	1.058	0.482; 2.321	1.138	0.512; 2.531	1.193	0.757; 1.882	1.168	0.725; 1.882
	Exclusive, <6m	0.835	0.423; 1.649	0.870	0.435; 1.737	0.836	0.576; 1.212	0.818	0.554; 1.206
	Exclusive, ≥6m	0.701	0.332; 1.480	0.750	0.352; 1.599	0.718	0.464; 1.110	0.670	0.425; 1.055
4	Never	Ref.		Ref.		Ref.		Ref.	
	Any, but never exclusive	0.648	0.310; 1.353	0.697	0.331; 1.465	0.756	0.485; 1.176	0.732	0.465; 1.152
	Exclusive, <6m	**0.520**	**0.279; 0.969**	0.540	0.288; 1.011	**0.553**	**0.392; 0.779**	**0.540**	**0.381; 0.766**
	Exclusive, ≥6m	**0.374**	**0.186; 0.754**	**0.388**	**0.192; 0.786**	**0.396**	**0.258; 0.608**	**0.365**	**0.236; 0.565**
≥5	Never	Ref.		Ref.		Ref.		Ref.	
	Any, but never exclusive	**0.397**	**0.195; 0.809**	**0.439**	**0.213; 0.903**	**0.464**	**0.300; 0.717**	**0.456**	**0.288; 0.722**
	Exclusive, <6m	**0.338**	**0.189; 0.611**	**0.368**	**0.203; 0.669**	**0.371**	**0.268; 0.513**	**0.371**	**0.264; 0.522**
	Exclusive, ≥6m	**0.299**	**0.154; 0.582**	**0.321**	**0.164; 0.629**	**0.328**	**0.219; 0.491**	**0.308**	**0.201; 0.472**
Males		n=2276		n=2259		n=2229		n=2229	
3	Never	Ref.		Ref.		Ref.		Ref.	
	Any, but never exclusive	1.151	0.675; 1.961	1.197	0.697; 2.058	1.205	0.701; 2.072	1.264	0.734; 2.176

	Exclusive, <6m	1.171	0.747; 1.837	1.224	0.774; 1.935	1.252	0.790; 1.983	1.317	0.830; 2.090
	Exclusive, ≥6m	1.545	0.923; 2.584	1.658	0.982; 2.801	**1.731**	**1.022; 2.933**	**1.790**	**1.055; 3.038**
4	Never		Ref.		Ref.		Ref.		Ref.
	Any, but never exclusive	0.658	0.318; 1.362	0.694	0.331; 1.453	0.671	0.322; 1.398	0.730	0.349; 1.525
	Exclusive, <6m	0.575	0.324; 1.022	0.607	0.338; 1.092	0.587	0.327; 1.053	0.634	0.352; 1.142
	Exclusive, ≥6m	0.717	0.351; 1.464	0.802	0.389; 1.656	0.789	0.382; 1.630	0.829	0.400; 1.718
≥5	Never		Ref.		Ref.		Ref.		Ref.
	Any, but never exclusive	0.473	0.102; 2.193	0.473	0.102; 2.204	**0.426**	**0.204; 0.889**	0.477	0.227; 1.001
	Exclusive, <6m	0.869	0.298; 2.534	0.873	0.298; 2.560	0.845	0.487; 1.467	0.956	0.550; 1.662
	Exclusive, ≥6m	1.013	0.273; 3.751	1.012	0.272; 3.765	1.117	0.535; 2.333	1.201	0.574; 2.514

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age and educational level; Model 3: adjusted for Model 2 plus maternal age at menarche, child's birth weight and gestational age at birth; Model 4: adjusted for Model 3 plus child's BMI z-score. All the models were adjusted for children's exact age.

Paper IV

Pubertal Timing: The Role of Nutrition and other Lifestyle Behaviours

Marta Pinto da Costa, Milton Severo, Vânia Magalhães, Carla Lopes, Augusto Anguita-Ruiz, Joana Araújo, Sofia Vilela

Abstract

Background: Although nutrition is recognised as a non-genetic determinant of pubertal timing, most studies have focused on single foods or nutrients and primarily on girls. Evidence addressing overall diet quality and other lifestyle behaviours remains limited.

Objective: To explore the associations of specific food group m intake, nutrients, diet quality, ultra-processed foods (UPF) consumption, sleep duration, physical activity, and sedentary behaviour during childhood with pubertal timing.

Methods: The sample included 4174 children evaluated at 4 years and 4522 children evaluated at 7 years from the population-based Portuguese birth cohort Generation XXI. Dietary intake was assessed using a food frequency questionnaire, and information on sleep duration, physical activity, and sedentary behaviour was collected at 4 and/or 7 years. Pubertal development was assessed at 10 years using Tanner stages. A Puberty score was developed based on genital and pubic hair Tanner stages (range: 2-10). Ordinal logistic regression and Cox proportional hazards models were used to estimate associations with Puberty score at 10 years of age and age at menarche.

Results: Higher diet quality at 4 years was associated with a less advanced Puberty score (OR=0.976; 95%CI:0.955;0.997) in girls and later age at menarche (β=0.967; 95%CI: 0.935;0.999), although the latter association was attenuated after further adjustment for maternal characteristics. Higher consumption of EDF at 4 years of age and meat and meat products at 7 years of age were associated with an earlier age at menarche. Among boys, higher consumption of the 'Fish and eggs' food group at 4 years of age and higher consumption of saturated fat and trans fatty acids, vitamin D, zinc and calcium were associated with a more advanced Puberty score. No consistent associations were found for sleep duration or sedentary behaviour, whereas physical activity was associated with age at menarche only in BMI-stratified analyses.

Conclusion: Early childhood dietary exposures, particularly overall diet quality, EDF consumption and some specific food groups and nutrients, are associated with pubertal timing in both boys and girls. These findings highlight early childhood as a critical and potentially modifiable window for promoting healthy dietary patterns that may contribute to healthier pubertal timing.

Keywords: Dietary quality, Sleep quality, Sedentary activity, Puberty, Tanner stages, Children

Introduction

Over the past decades, accumulating evidence has suggested a global declining tendency in the age at menarche and an earlier onset of puberty, particularly among girls (1-5). This trend represents a public health concern, as earlier pubertal development has been associated with several adverse health outcomes in childhood and later in life (6-11). Genetic predisposition has long been considered the main determinant of pubertal timing (12, 13). However, accumulating evidence also highlights the important role of non-genetic factors. Nutrition (14, 15), childhood adiposity (16), lifestyle behaviours (17, 18), environmental exposures (12, 19), and socioeconomic factors (16) also appear to influence pubertal development.

Nutritional status during childhood plays a crucial role in pubertal development and has been estimated to account for up to 25% of the variation in pubertal timing (15). Although current literature includes observational studies suggesting that specific foods (e.g., dairy (15, 20), red meat (21), energy dense foods (22-24)), macro- (energy (25), fat (26), protein (27), carbohydrates (25), fiber (28, 29)) and micronutrient (iron (30, 31), zinc(32)) may influence the onset and progression of puberty, the results are inconsistent (33-35). Moreover, most evidence comes from girls, with a primary focus on age at menarche, and examines foods in isolation rather than considering the influence of overall diet quality (36, 37). Another limitation of the existing literature is that only a few studies have specifically evaluated the potential influence of higher consumption of ultra-processed foods on pubertal timing (24). Further research is therefore needed to address the current lack of robust evidence in boys, to evaluate diet as a whole, and to clarify the potential influence of ultra-processed food consumption on pubertal development.

Beyond nutrition, other lifestyle factors have also been suggested to influence pubertal timing potentially. Previous studies have shown that longer sleep duration is associated with a lower risk of early puberty (38), whereas insufficient sleep has been linked to an increased risk of early pubertal onset (39). However, other evidence suggests only a limited association between sleep duration and sexual maturation (40). Regarding physical activity and sedentary behaviours, children who are physically active and spend less time in sedentary activities tend to experience a relatively delayed pubertal onset

(18). Nevertheless, the relationship between physical activity and pubertal timing remains limited, as most available evidence comes from studies conducted in elite athletes (41), and the role of sedentary behaviour in pubertal timing is still largely unexplored.

Therefore, the present study aimed to explore the influence of dietary intake and diet quality, ultra-processed foods consumption, sleep duration, physical activity and sedentary behaviour during childhood on pubertal timing.

Methods

Study Design and Participants

This study included participants from the ongoing prospective population-based birth cohort Generation XXI (G21), previously described (42, 43). Briefly, between April 2005 and August 2006, all newborns delivered at the five public maternity hospitals in the metropolitan area of Porto were invited to participate. Of those invited, 91.4% of mothers accepted to participate in the study, resulting in a baseline sample of 8647 children. All families were invited to follow-up assessments when the children were aged 4 years (86% of participation), 7 years (80% of participation), 10 years (76% of participation), 13 years (54% of participation – follow-up was interrupted due to the COVID-19 pandemic), and 18 years (41% of participation). At each evaluation wave, participants were invited to participate in a face-to-face interview conducted by trained interviewers using structured questionnaires to collect data on sociodemographic factors, child health status, lifestyle, and objective anthropometric measures. The final sample for the present study included all children who attended a face-to-face follow-up at 4 and/or 7 years of age and at 10 years of age, with complete data on dietary intake, practice of physical activity, sleep duration and sedentary activity at 4 or 7 years, as well as information on pubertal development assessed using the Tanner stage scale at 10-year follow-up and self-reported status and age at menarche recorded at 13 or 18 years of age. After excluding twins (n=144) and children with congenital anomalies or conditions that could affect the analysis of food intake (e.g., celiac disease, allergies, or food intolerances) (n=50), the final sample comprised 4174 children evaluated at 4 years of age (2061 girls and 2113 boys) and 4522 children evaluated at 7 years of age (2209 girls and 2313 boys).

Dietary Assessment

The present study assessed children's food consumption using a validated Food Frequency Questionnaire (FFQ) (44), administered by trained interviewers to the child's main caregiver at the 4- and 7-year follow-ups, referring to usual intake in the previous six months. The FFQ included thirty-five food items at 4 years of age and thirty-eight food items at 7 years of age (added items: chocolate milk, breakfast cereals and natural fruit juice). The nine frequency options, ranging from 'never' to '≥4 times per day', were converted into daily consumption frequencies. At both ages, food items were grouped into similar food groups based on the World Health Organization (WHO) dietary recommendations (45): 'Dairy' (milk, yogurt, and cheese), 'Fish and eggs', 'Cereal products and potatoes' (rice, pasta, potatoes, bread, and other grains), 'Fruit and vegetables' (fresh fruit, natural fruit juice, and vegetables), 'Meat and meat products' (white meat, red meat, and processed meat), 'Sweet foods' (cakes, candies, sweet pastry, chocolate, biscuits, added sugar, and ice cream), 'Salty snacks' (salty snacks, pizzas, and chips), and 'Soft drinks' (nectars and soft drinks).´

Dietary data from all waves were processed by instructed nutritionists using the software eAT24 (Electronic Assessment Tool for 24-hour recall) developed for the National Food, Nutrition and Physical Activity Survey of the Portuguese Population (IAN-AF 2015–2016) (46). Food items were classified according to the FoodEx2 system (47); complex food or recipes were disaggregated into individual ingredients following the EU Menu methodology (48). Total energy, macronutrients and micronutrients were estimated based on the Portuguese Food Composition Table at PortFir (http://portfir.insa.pt), supplemented with the European Food Information Resource (EuroFIR) data (49). Total energy intake was calculated in kcal/day for each individual. Total intake of protein, carbohydrates, fat, and fiber was calculated in g/day, while total intake of iron, zinc, magnesium and calcium was calculated in mg/day, and vitamin D in μg/day.

For the micronutrient analyses, cut-off points were defined based on the Population Reference Intake (PRI) from the European Food Safety Authority (EFSA), which represents the intake of a nutrient that is likely to meet the needs of almost all healthy individuals in a population (50-53). When the PRI value was not available (i.e. magnesium and vitamin D), the Adequate Intake (AI) was considered. The AI corresponds to the average nutrient intake level assumed to be adequate for the needs of the population, based on observations or experiments (52, 54). For vitamin D, the cut-off

was defined using the median intake of the study sample, since all participants presented intake levels well below the AI (15µg/day). For nutrients for which an Upper Intake Level (UL) was available - the maximum level of chronic daily intake unlikely to pose a risk of adverse health effects - this value was also used as a cut-off point (i.e. zinc and magnesium) (55).

Healthy Eating Index

A Healthy Eating Index (HEI), previously developed and validated to evaluate children's diet quality (56-58) was adapted for this study to assess the influence of overall diet quality during childhood on pubertal timing. For each of the eight food groups described above, quartiles of frequency of consumption were calculated using the mean of quartiles across all ages to define consistent cut-off points for all follow-ups, as previously described (56-58). The assigned scores ranged from 1 to 4 points, as follows: for food groups considered healthier ('Dairy', 'Fish and eggs', 'Cereal products and potatoes' and 'Fruit and vegetables'), the lowest quartile of consumption received 1 point, intermediate quartiles 2–3 points, and the highest consumption quartile 4 points; for food groups considered less healthy ('Meat and meat products', 'Sweet foods', 'Salty snacks' and 'Soft drinks'), the score was assigned reversely, with the highest quartile of consumption receiving 1 point. The scores assigned were summed up to obtain a total HEI score, possibly ranging from 8 to 32 points, with a higher score indicating a better diet quality.

Ultraprocessed Foods (UPF)

Food items from the FFQ were previously classified according to the processing degree and its purpose in this cohort (59) using the NOVA classification (60, 61), which categorizes all foods into four groups: 1) Unprocessed and minimally processed foods such (e.g., fresh fruit and vegetables, fresh meat and fish); 2) Processed culinary ingredients (e.g., olive oil, butter, sugar); 3) Processed foods (e.g., bread and cheese); and 4) UPF (e.g., soft drinks, fast food and chocolates).

Other Lifestyle Behaviours

Night-time sleep duration was calculated as the difference between bedtime and wake-up times reported by parents at 4 and 7 years of age. At 4 years old, usually bedtime and awake time were collected for the entire week, without needing to apply any formula to derive sleep duration at this age. At 7 years old, the bedtime and wake-up time were reported separately for weekdays and weekend days, and night-time sleep duration was calculated using the following formula: [(weekday sleep duration x 5) + (weekend sleep duration x 2)]/7 days. Sleep duration was then categorised as <10h and ≥10h of sleep per night, according to the WHO recommendation (62).

Sedentary activities (e.g., reading, watching TV, playing video games) were assessed through parental report and collected separately by weekdays and weekend days, and computed as: [(time spent on sedentary activities week x 5) + (time spent on sedentary activities weekend x 2)]/7 days. Then, it was categorized as <1h and ≥1h per day at 4 years of age and as <2h and ≥2h per day at 7 years of age, considering the recommendations of no more than 1h per day of sedentary recreational screen time for children under 5 years of age (62) and no more than 2h per day for children aged 5-17 years (63).

Regular practice of structured physical activity was assessed as a qualitative variable. At both follow-ups, the main caregiver was asked whether the child practised any scheduled and regular sports activity outside school (yes/no). Children were then classified as practitioners or non-practitioners in each follow-up.

Pubertal Timing

At the 10-year follow-up, pubertal timing was assessed by trained nurses through physical exam, using palpation and visual inspection according to the 5-level Tanner stages criteria developed by Marshall and Tanner (64, 65). In girls, pubic hair and breast development were evaluated, while in boys, pubic hair and testicular development were assessed. Stages were classified as: 1 - prepubertal; 2-4 - pubertal; and 5 - postpubertal. Tanner stage 2 or higher was considered the onset of puberty, defined by slightly pigmented, downy pubic hair and a breast bud stage with an elevation of breast and papilla and enlargement of the areola in girls (64, 66), and by testicular volume higher than 4 ml with slightly pigmented and downy pubic hair (65, 66). Previously, a Puberty score has been developed, derived by summing Tanner stage for pubic hair and genital/breast

development, to address potential misclassification of Tanner stages, particularly in boys, where testicular volume assessment can be less reliably assessed by the nurses due to its invasive nature involving testicular palpation (67). A Puberty score of 3 or higher indicates that the child has already started puberty. Due to the small number of children in higher Puberty score categories (5-9), especially boys (n=48), these categories were combined for the main analysis.

At the 13- and 18-year follow-ups, girls self-reported their age at menarche, defined as the age at their first menstrual cycle. A final age at menarche variable was derived using information from both follow-ups as follows: if age at menarche was reported at only one follow-up, that value was used; if it was reported at both follow-ups and the difference between the two reports did not exceed two years, the mean of the two values was calculated. Otherwise, the value was considered as missing (n=2044).

Other Child and Maternal Characteristics

Maternal age and education were considered markers of socioeconomic position, whereas maternal age at menarche was used as a proxy for genetic predisposition (68).

Children's early-life factors, including type of delivery and birth weight, were also collected at baseline, both abstracted from medical records by trained interviewers (69).

Information on breastfeeding type and duration was obtained from structured questionnaires applied to the main caregiver at 6-, 15-, and 24-month subsample follow-ups, as well as at the 4-year complete sample follow-up. Breastfeeding was previously categorised into four groups: (a) never breastfed; (b) any breastfeeding but never exclusive; (c) exclusively breastfed for less than 4 months (<16 weeks); (d) exclusively breastfed for 4 months or more (≥16 weeks). The 4-month cut-off was chosen based on the distribution of the data and the duration of fully paid maternity leave in Portugal (70).

At the 10-year follow-up, trained interviewers measured children's height and weight following standard procedures. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²) and categorised according to WHO age- and sex-specific cut-offs: underweight (<-2 Standard Deviation (SD)), normal weight (≥-2 SD to ≤1 SD), pre-obesity (>1 SD to ≤2 SD), and obesity (>2 SD) (71).

Ethical Considerations

All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. The baseline and follow-up evaluations were approved by the University of Porto Medical School/ S. João Hospital Centre Ethics Committee, except the 13-year follow-up that was approved by the ISPUP Ethics Committee. At baseline and follow-up evaluations, all procedures were explained to participants, and an informed consent was signed by one of the parents or legal guardians (at 13 years, it was also signed by the participants). The baseline evaluation was approved by the Data Protection National Commission, and the study follows the EU General Data Protection Regulation under close supervision of the Data Protection Office of ISPUP.

Statistical Analysis

Descriptive statistics, such as mean and SD for continuous variables and frequencies and percentages for categorical variables, were calculated to describe participants' characteristics. All analyses were performed separately by sex, given that boys and girls follow distinct pubertal trajectories and timing, with girls typically experiencing pubertal onset earlier than boys (64, 65), and require different indicators to define pubertal timing.

Associations between the HEI, specific food groups, macro- and micronutrient intake, sleep duration, sedentary activity time, and physical activity (yes/no), assessed at both 4 and 7 years of age, and pubertal timing were examined using two outcomes: the Puberty score at age 10 and age at menarche. For the Puberty score sex-stratified ordinal logistic regression models were fitted. For age at menarche, interval-censored Cox proportional hazards models were fitted, restricted to females. Females were considered at risk of menarche from 8 years of age and remained at risk until the interval in which menarche was reported during the follow-up assessments at 10, 13, or 18 years. The lower age limit of 8 years was selected because menarche occurring at or before this age is generally considered abnormally early and indicative of precocious pubertal development. Four models were considered in all analyses. Model 1 was the crude model; Model 2 was adjusted for child's birth weight, type of delivery, breastfeeding categories, maternal age at menarche, and the ID number of the physical examiner; Model 3 was adjusted for Model 2 plus practice of physical activity, HEI score, sleep duration and sedentary

activity, assessed at ages 4 and/or 7, depending on the timing of the exposures. For exposures assessed at the 4-year follow-up, the adjustment included only covariates measured at 4 years of age; for exposures assessed at the 7-year follow-up, the adjustment included covariates measured at both 4 and 7 years. When any of these variables corresponded to the exposure of interest, the model was adjusted for the remaining variables only; Model 4 was adjusted for Model 3 plus maternal age and years of education. All models were adjusted for the child's exact age at the 10-year follow-up. The selection of covariates was guided by the directed acyclic graph and supported by evidence from the literature. Analyses were adjusted for the ID number of the physical examiner to account for potential inter-rater variability in Tanner stage assessment and to minimize the risk of systematic measurement error. Ordinal logistic regression and Cox proportional hazards regression analyses were repeated for Puberty score and age at menarche as outcomes, stratified by BMI category (normal weight and overweight/obese) (Supplementary Tables 1 and 2).

All statistical analyses were performed using the R software environment for statistical computing (version 4.4.3), package 'MASS' for ordinal logistic regression (72). A significance level of 0.05 was considered for all analyses.

Results

Table 1 presents the characteristics of the mothers and their children according to sex. At baseline, mothers had a mean age of 29.9 years (SD = 5.2) and an average of 11.2 years of education (SD = 4.3). Slightly more than half of the sample were boys (51.1%). At 10 years of age, most children were classified as normal weight (56.3%) 1.0% were underweight, 26.0% were overweight and 16.7% were obese. There were statistically significant differences between sexes in weight status. As expected, most girls had already initiated puberty at 10 years of age, with a Puberty score ≥3 (79%), whereas most boys had not (67.0%), p-value <0.001.

Table 2 presents the results of the ordinal logistic regression analyses exploring the associations between pre-pubertal lifestyle factors and pubertal development, assessed using the Puberty score, in girls. Among girls, a higher HEI score at 4 years of age was significantly associated with lower odds of a higher Puberty score at 10 years of age (Model 4, OR=0.976; 95%CI: 0.955; 0.997). Higher consumption of energy-dense foods

(EDF) and UPF at 4 years of age was associated with higher odds of a higher Puberty score, with statistical significance observed in Models 1 and 2 (EDF, OR=1.041, 95%CI: 1.003; 1.081; 'Ultra-processed food', OR=1.053, 95%CI: 1.000; 1.108), but not in Models 3 and 4.

Among boys, higher HEI scores at both ages were associated a higher Puberty score in the crude model, however, these associations were attenuated and became non-significant after adjustment for the considered covariates (Table 3). A higher consumption of 'Fish and eggs' at 4 years of age and a higher intake of saturated and trans fatty acids at 7 years of age, were consistently associated with higher odds of higher Puberty score after adjustment for all covariates Furthermore, higher intakes of vitamin D at 4 years of age (OR=1.248, 95%CI: 1.027; 1.515), zinc at 4 and 7 years of age (5.5-10mg/day: OR=1.910, 95%CI: 1.140; 3.365 vs. OR=1.913, 95%CI: 1.094; 3.492, respectively), and higher calcium intake at 7 years of age (OR=1.428, 95%CI: 1.030; 1.996), were consistently associated with a higher Puberty score at 10 years of age across all models (Table 3).

When menarche was considered as the outcome (Table 4), a higher HEI score at 4 years of age was associated with a later age at menarche up to Model 3 (β =0.967, 95%CI: 0.935; 0.999). After further adjustment for maternal education and age, the direction of the association remained, although it was no longer statistically significant. The HEI score at 7 years of age also showed a significant association with age at menarche in Models 1 and 2. Higher consumption of EDF at 4 years of age (β=1.077, 95%CI: 1.020; 1.138) and meat and meat products at 7 years of age (β=1.295, 95%CI: 1.044; 1.607) was associated with an earlier age at menarche. These association remained statistically significant after adjustment for all covariates. EDF consumption at 7 years of age was also associated with an increased risk of earlier menarche in Models 1 and 2. Higher consumption of unprocessed and minimally processed foods plus processed culinary ingredients at 4 years of age was consistently associated with an earlier age at menarche (Model 4: β=1.060, 95%CI: 1.019; 1.103). Similarly, higher intakes of energy, protein, animal protein, carbohydrates, fiber, total fat, saturated fat, monounsaturated fatty acids (MUFA), and trans fatty acids at 4 years of age were also associated with an earlier age at menarche, and these associations remained statistically significant after adjustment for all covariates. Regarding micronutrients, higher intake iron, calcium and magnesium at 4 years of age were signficantly associated with an earlier age at menarche.

Supplementary Table 1 shows the associations between diet quality and lifestyle behaviours and Puberty score at 10 years, stratified by BMI categories. The association between the HEI at 4 years and Puberty score in girls observed in the main analysis was not statistically significant among either children with a normal-weight or children with overweight or obesity, and no interaction with BMI was observed (p-value for interaction = 0.424). Among girls with overweight or obesity, in contrast to the main analysis, a higher consumption of the 'Fish and eggs' food group at 4 years was positively associated with Puberty score (Model 4, OR = 1.921, 95% CI: 1.194–3.103), although no interaction with BMI was detected (p for interaction = 0.590). Among boys, the positive association between 'Fish and eggs' consumption at 4 years and Puberty score observed in the main analysis was only observed among boys with overweight or obesity, with no statistically significant interaction between 'Fish and eggs' consumption and BMI category was detected (p for interaction = 0.777). Among boys with normal-weight, a higher energy and carbohydrate intake at 7 years were associated with higher odds of a more advanced Puberty score at 10 years of age. In contrast to the main analysis, vitamin D intake at 4 years and calcium intake at 7 years were not significantly associated with Puberty score among normal-weight boys. Adequate zinc intake at 4 years (5.5–10 mg/day) was no longer significantly associated with Puberty score in either BMI group, and no statistically significant interaction with BMI was observed (p for interaction = 0.567).

Supplementary Table 2 presents the associations between diet quality, dietary intake and lifestyle behaviours, and age at menarche, stratified by BMI categories. Among girls with a normal weight, a higher HEI score at 4 years of age was associated with a later age at menarche (Model 4, β=0.955, 95% CI: 0.912-0.999), whereas higher consumption of EDF at 4 years (Model 4, β =1.081, 95% CI: 1.004-1.163) and meat and meat products at 7 years (Model 4, β =1.344, 95% CI: 1.007-1.794) was associated with an earlier age at menarche. Among girls with overweight or obesity, a higher HEI score and higher calcium and zinc intakes at 7 years were associated with a later age at menarche. In contrast, higher consumption of fruit and vegetables, dairy products, fish and eggs, unprocessed and minimally processed foods plus processed culinary ingredients, EDF, and UPF at 4 years, as well as meat and meat products at 7 years, was associated with an earlier age at menarche. Similarly, higher intakes of energy, protein (total, animal, and vegetable), carbohydrates, fiber, total fat, saturated fat, MUFA, PUFA, trans fatty acids, iron, calcium, magnesium, and zinc at 4 years of age were associated with an earlier age

at menarche. Additionally, not engaging in physical activity at 7 years of age was associated with an earlier age at menarche. A statistically significant interaction between UPF consumption and BMI category was observed (p for interaction = 0.038), whereas no interaction was observed for EDF (p for interaction = 0.172).

Discussion

The present study provides evidence that early-life patterns, particularly diet quality and the intake of specific food groups and nutrients, may influence pubertal timing in both sexes. In contrast, lifestyle behaviours such as sleep duration and sedentary behaviour did not show consistent associations with pubertal outcomes. These findings suggest that dietary factors during early childhood may play a more important role in pubertal timing than other lifestyle behaviours. Furthermore, the observed differences according to BMI category indicate that body weight status may modify the relationship between childhood diet and pubertal development.

A higher diet quality at 4 years of age was linked to a lower Puberty score at 10 years among girls and with a later age at menarche. Although the association with age at menarche was attenuated after adjustment for maternal age and educational level, its direction remained unchanged, suggesting that socioeconomic factors may partly explain the relationship between childhood diet quality and pubertal timing. Most previous epidemiological studies have focused on single foods or individual nutrients (20-22, 26, 27, 30). However, the available evidence on overall diet aligns with our findings, showing that a higher dietary quality is associated with a later onset of menarche (73) and a slower progression of pubertal stages (36, 74). Therefore, the present study adds to the growing evidence that overall diet quality may play an important role in shaping pubertal timing, particularly among girls.

In contrast to the HEI, higher consumption of EDF at pre-school age and meat and meat products at 7 years of age were associated with an earlier age at menarche. These findings are consistent with current evidence, as previous studies have linked precocious puberty and earlier age at menarche to dietary patterns rich in desserts, snacks, soft drinks, and fried foods, as well as to higher consumption of animal source foods (21, 24, 75, 76). These associations may be explained by increased energy availability and higher animal protein intake, which have been associated with greater circulating concentrations of

insulin, insulin-like growth factor-1 (IGF-1), and leptin, all of which may promote earlier activation of the hypothalamic–pituitary–gonadal axis (77, 78).

The BMI-stratified analyses revealed markedly different patterns according to weight status. Among girls with normal weight, only a limited number of dietary exposures were associated with age at menarche, namely higher HEI scores, higher consumption of EDF at 4 years of age, and meat and meat products at 7 years of age. In contrast, among girls with overweight or obesity, a broader range of dietary exposures, together with physical activity, was associated with pubertal timing. This overall pattern suggests that these associations may reflect greater overall food intake or a state of positive energy balance rather than independent effects of specific foods or nutrients. Because dietary exposures were analysed as absolute rather than energy-adjusted intakes, the independent contribution of individual dietary components cannot be completely disentangled from that of total energy intake. Moreover, it is possible that, among girls with overweight or obesity, the influence of diet on pubertal timing is partly masked by the strong influence of excess adiposity, a well-established determinant of pubertal timing (79). Interestingly, in contrast to our findings, a higher intake of carbohydrates has previously been associated with a later age at menarche in several studies (34, 80, 81). Similarly, higher consumption of foods that are major sources of vegetable protein, such as grains, nuts, beans, legumes, and fruits, has been associated with a delayed age at menarche (29, 34). Taken together, these findings suggest that overall diet quality may provide a more robust indicator of the relationship between childhood diet and pubertal timing than individual foods or nutrients, as it captures the combined effects of multiple dietary components.

In the main analysis, higher consumption of the ‘Fish and eggs’ group at age 4 was associated with a higher Puberty score among boys, whereas no such association was observed among girls. However, after stratification by BMI category, a similar positive association was observed among both boys and girls with overweight/obesity, while no statistically significant interaction with BMI category was detected. Although the available literature does not allow a clear interpretation of this finding, one previous study reported a protective association between higher fish consumption and an earlier onset of menarche (82). A possible explanation for the observed association is that this food group represents an important source of animal protein, which may influence the hypothalamic-pituitary-gonadal axis and potentially contribute to an earlier pubertal timing (83). Nevertheless, this result should be interpreted with caution, as it may reflect differential

parental reporting according to the child's BMI category, potentially influenced by social desirability. In this context, parents of children with higher BMI may be more likely to overreport foods perceived as healthy and underreport foods perceived as less healthy.

Among boys, a higher intake of foods rich in saturated fatty acids (SFA) and trans fats at age 7 was also associated with a higher Puberty score. The same associations were observed in the BMI-stratified analyses, in both BMI categories. Most previous studies have reported associations with total fat intake or polyunsaturated fatty acids (PUFA), whereas the literature does not provide evidence supporting an association between trans-fat intake and pubertal timing (87). In addition, the available evidence on SFA intake does not show a significant association with pubertal timing in either sex (84).

Higher intakes of vitamin D and zinc at age 4, as well as calcium at age 7, were associated with a higher Puberty score at age 10 among boys. Notably, all children in our sample reported intake level that were below the recommended amounts. Since these nutrients intakes were estimated using a FFQ and circulating concentrations were not assessed, the observed associations may partly reflect reporting patterns of foods rich in these nutrients, such as fish, eggs, dairy products and meat, rather than true biological effects of the micronutrients themselves.

One of the main strengths of the present study is its prospective design, which includes a large, population-based sample with a high participation rate and repeated, detailed and standardized assessments. This design allows for better control of a wide range of potential confounders. Unlike most previous studies that primarily focused on girls, this study included data from both girls and boys. Additionally, pubertal timing was assessed through clinical examination with visual inspection and palpation performed by a trained nurse, a method considered more reliable than subjective reporting and therefore less prone to the measurement error commonly associated with self- or parent reports (85). Although pubertal development was assessed by a trained nurse, some measurement error may still have occurred in Tanner staging, particularly in evaluating testicular volume in boys. Even when following standardized staging guidelines and using an orchidometer, the accuracy of testicular volume assessment may be affected, partly because this is an invasive procedure. This limitation was partly addressed using a composite Puberty score that includes both genital development, and pubic hair development. Moreover, all analyses were adjusted for the ID number of the physical examiner in order to account for potential inter-rater variability in Tanner stage assessment and to reduce the likelihood

that the observed associations were driven by systematic measurement errors. In addition, data from children assessed by a specific examiner who was not a nurse were excluded from the analyses. Another limitation of this study is that dietary intake was assessed using a FFQ, which relies on subjective reporting and may be affected by misreporting and social desirability bias. For instance, parents might report what they perceive their child should consume rather than what is actually consumed, or overreport foods perceived as healthy and underreport less healthy foods. However, it is worth noting that the FFQ used in this study was previously calibrated and validated (44), particularly for the foods most frequently consumed, supporting the validity of the dietary data.

Conclusion

A worldwide declining trend in the age at menarche and pubertal onset raises public health concerns, as earlier pubertal timing has been associated with several adverse health outcomes across the life course. Our findings suggest that overall diet quality as well as the intake of specific food groups (EDF, 'Meat and meat products' and 'Fish and eggs') and nutrients (vitamin D, zinc and calcium), may influence age at menarche and pubertal timing in both sexes. Our findings also indicate that these associations are not uniform across children, with BMI acting as an effect modifier in some associations. Although other lifestyle behaviours have been proposed as potential contributors to pubertal timing, these results indicate that overall diet quality and dietary intake may play a more prominent role.

These findings highlight early childhood as a crucial and potentially modifiable window during which dietary exposures may influence subsequent pubertal timing. From a public health perspective, strategies aimed at promoting healthy dietary patterns should be implemented from early childhood. Family-based interventions, including parental nutrition education, together with improvements in food environments both at home and in school settings, may contribute to healthier dietary habits and, subsequently, to more favourable pubertal development.

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Table 1. Characteristics of the study population by sex.

	Total sample (n=4522)	Female (n=2209)	Male (n=2313)	p-value
Maternal age (years), mean (SD)	29.9 (5.2)	30.0 (5.3)	29.9 (5.1)	0.595[a]
Maternal education (years), mean (SD)	11.2 (4.3)	11.2 (4.4)	11.3 (4.3)	0.331[a]
Maternal age at first menarche (years), mean (SD)	12.4 (1.5)	12.4 (1.6)	12.4 (1.5)	0.782[a]
Type of delivery, n (%)				
Eutocia	2126 (47.0)	1072 (48.5)	1054 (45.6)	
Vento use	607 (13.4)	273 (12.4)	334 (14.4)	
Forceps	59 (1.3)	28 (1.3)	31 (1.3)	0.189
Caesarean	1713 (37.9)	827 (37.4)	886 (38.3)	
Caesarean with vendeuse	17 (0.4)	9 (0.4)	8 (0.4)	
Child's age at first menarche (years), mean (SD)	-	12.0 (1.4)	-	
Child's birth weight (grams), mean (SD)	3199 (483.4)	3146 (470.0)	3250 (490.0)	<0.001[a]
Child's exact age at 10y follow-up (years), mean (SD)	10.1 (0.3)	10.1 (0.3)	10.1 (0.3)	0.006[a]
Breastfeeding categories, n (%)				
Never	241 (5.7)	123 (5.9)	118 (5.5)	
Any, but never exclusive	406 (9.7)	189 (9.2)	217 (10.2)	0.621[b]
Exclusive, <4 months	1703 (40.6)	833 (40.4)	870 (40.8)	
Exclusive, ≥4 months	1844 (44.0)	918 (44.5)	926 (43.5)	
Child's practice of physical exercise at 4y, yes, n (%)	3112 (70.3)	1579 (72.9)	1533 (68.0)	<0.001
Child's practice of physical exercise at 7y, yes, n (%)	3883 (86.2)	1915 (87.0)	1968 (85.4)	0.114
Healthy eating index score at 4y, mean (SD)	22.4 (3.6)	22.3 (3.6)	22.5 (3.5)	0.150[a]
Healthy eating index score at 7y, mean (SD)	20.6 (3.7)	20.5 (3.7)	20.7 (3.7)	0.108[a]
Child's sleeping hours per night at 4y, n (%)				
<10h	1457 (32.8)	700 (32.1)	757 (33.4)	0.351[b]
≥10h	2991 (67.2)	1483 (67.9)	1508 (66.6)	
Child's sleeping hours per night at 7y, n (%)				
<10h	754 (32.6)	341 (30.8)	413 (34.3)	0.078[b]
≥10h	1556 (67.4)	766 (69.2)	790 (65.7)	
Child's sedentary activity/day at 4y (hours), n (%)				
<1h	923 (23.1)	493 (25.1)	430 (21.1)	0.003[b]
≥1h	3079 (76.9)	1468 (71.9)	1611 (78.9)	
Child's sedentary activity/day at 7y (hours), n (%)				
<2h	971 (21.8)	464 (21.3)	507 (22.2)	0.468[b]

≥2h	3488 (78.2)	1715 (78.7)	1773 (77.8)	
Fish and eggs at 4y (frequency/d), mean (SD)	0.77 (0.30)	0.77 (0.29)	0.76 (0.30)	0.105[a]
Fish and eggs at 7y (frequency/d), mean (SD)	0.87 (0.35)	0.87 (0.34)	0.88 (0.35)	0.435[a]
Bmi z-score at 10y follow-up, n (%)				
Underweight/normal weight	2591 (57.3)	1275 (57.8)	1316 (56.9)	
Overweight	1173 (26.0)	603 (27.3)	570 (24.7)	0.003[b]
Obesity	754 (16.7)	328 (14.9)	426 (18.4)	
Puberty score, n (%)				
2	2012 (44.5)	463 (21.0)	1549 (67.0)	
3	1088 (24.1)	532 (24.1)	556 (24.0)	
4	719 (15.9)	559 (25.3)	160 (6.9)	
5	319 (7.0)	288 (13.0)	31 (1.3)	<0.001[b]
6	271 (6.0)	262 (11.9)	9 (0.4)	
7	45 (1.0)	40 (1.8)	5 (0.2)	
8	65 (1.4)	62 (2.8)	3 (0.1)	
9	3 (0.1)	3 (0.1)	0 (0.0)	

SD, standard deviation; BMI, body mass index. [a] Student T-test. [b] χ^2 test

Table 2. Results from the ordinal logistic regression models assessing the associations between diet quality and lifestyle behaviours and Puberty score at 10 years of age in females.

	Model 1			Model 2			Model 3			Model 4		
	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI
Diet quality												
Healthy eating index, 4y	2061	0.976	0.955; 0.997	2061	0.976	0.955; 0.997	2061	0.976	0.955; 0.997	2061	0.976	0.955; 0.997
Healthy eating index, 7y	2209	0.981	0.962; 1.001	2031	0.980	0.960; 1.001	898	0.979	0.943; 1.017	898	0.982	0.945; 1.020
Food groups												
Fruit and vegetables, 4y	2062	0.988	0.945; 1.033	1973	0.987	0.942; 1.033	1921	0.990	0.945; 1.037	1917	1.001	0.954; 1.049
Fruit and vegetables, 7y	2209	0.992	0.949; 1.037	2031	0.989	0.944; 1.036	898	1.045	0.968; 1.127	898	1.052	0.975; 1.136
Energy dense food, 4y	2062	1.044	1.007; 1.083	1973	1.041	1.003; 1.081	1921	1.037	0.998; 1.078	1917	1.026	0.986; 1.067
Energy dense food, 7y	2209	1.012	0.974; 1.051	2031	1.018	0.978; 1.060	898	0.998	0.932; 1.069	898	0.992	0.926; 1.063
Dairy, 4y	2062	1.025	0.971; 1.082	1973	1.022	0.967; 1.081	1921	1.021	0.965; 1.081	1917	1.013	0.956; 1.073
Dairy, 7y	2209	1.026	0.981; 1.074	2031	1.025	0.977; 1.075	898	1.000	0.930; 1.076	898	0.994	0.924; 1.070
Cereal products and potatoes, 4y	2062	0.996	0.941; 1.054	1973	0.997	0.940; 1.057	1921	1.000	0.942; 1.062	1917	0.997	0.939; 1.059
Cereal products and potatoes, 5y	2209	0.984	0.927; 1.045	2031	0.984	0.924; 1.047	898	0.989	0.900; 1.086	898	0.987	0.899; 1.084
Fish and eggs, 4y	2062	1.058	0.812; 1.379	1973	1.110	0.847; 1.455	1921	1.121	0.851; 1.478	1917	1.196	0.903; 1.587
Fish and eggs, 7y	2209	1.016	0.814; 1.267	2031	1.005	0.795; 1.271	898	1.133	0.785; 1.634	898	1.178	0.812; 1.711
Meat and meat products, 4y	2062	1.041	0.912; 1.189	1973	1.057	0.924; 1.211	1921	1.050	0.916; 1.205	1917	1.029	0.897; 1.181
Meat and meat products, 7y	2209	1.006	0.907; 1.117	2031	1.007	0.903; 1.124	898	0.987	0.841; 1.158	898	0.982	0.837; 1.153
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	2062	0.972	0.947; 0.998	1973	0.976	0.950; 1.002	1921	0.977	0.950; 1.004	1917	0.978	0.952; 1.006
Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	2209	0.984	0.959; 1.010	2031	0.982	0.955; 1.009	898	1.004	0.960; 1.049	898	1.006	0.962; 1.052
Processed foods, 4y	2062	1.068	0.892; 1.278	1973	1.072	0.890; 1.292	1921	1.087	0.898; 1.316	1917	1.068	0.882; 1.294
Processed foods, 7y	2209	1.034	0.923; 1.160	2031	1.038	0.920; 1.172	898	1.071	0.876; 1.309	898	1.069	0.874; 1.306
Ultra processed foods, 4y	2062	1.059	1.007; 1.114	1973	1.053	1.000; 1.108	1921	1.052	0.997; 1.109	1917	1.035	0.979; 1.094
Ultra processed foods, 7y	2209	1.018	0.981; 1.057	2031	1.019	0.980; 1.060	898	1.009	0.946; 1.076	898	1.001	0.938; 1.069
Macronutrients												

Energy, 4y		2061	1.008	0.981; 1.035	1973	1.010	0.982; 1.038	1921	1.009	0.981; 1.038	1917	1.005	0.977; 1.034
Energy, 7y		2209	0.996	0.977; 1.017	2031	0.996	0.975; 1.017	898	1.003	0.970; 1.036	898	1.002	0.970; 1.035
Protein, 4y		2061	1.000	0.995; 1.006	1973	1.001	0.995; 1.006	1921	1.001	0.995; 1.007	1917	1.001	0.995; 1.006
Protein, 7y		2209	0.999	0.994; 1.004	2031	0.998	0.994; 1.003	898	1.000	0.992; 1.007	898	1.000	0.992; 1.007
Vegetable protein, 4y		2061	1.000	0.979; 1.021	1973	1.000	0.979; 1.022	1921	1.002	0.980; 1.024	1917	1.001	0.980; 1.023
Vegetable protein, 7y		2209	0.994	0.980; 1.009	2031	0.994	0.979; 1.009	898	1.002	0.979; 1.026	898	1.003	0.980; 1.026
Animal protein, 4y		2061	1.000	0.994; 1.006	1973	1.001	0.995; 1.007	1921	1.001	0.995; 1.007	1917	1.001	0.995; 1.007
Animal protein, 7y		2209	0.999	0.994; 1.005	2031	0.999	0.993; 1.004	898	0.999	0.990; 1.008	898	1.000	0.991; 1.009
Carbohydrate, 4y		2061	1.001	0.999; 1.002	1973	1.001	0.999; 1.003	1921	1.001	0.998; 1.003	1917	1.000	0.998; 1.002
Carbohydrate, 7y		2209	1.000	0.999; 1.001	2031	1.000	0.998; 1.001	898	1.000	0.998; 1.002	898	1.000	0.998; 1.002
Fiber, 4y		2061	0.991	0.967; 1.016	1973	0.991	0.967; 1.016	1921	0.993	0.968; 1.018	1917	0.996	0.971; 1.022
Fiber, 7y		2209	0.993	0.975; 1.011	2031	0.993	0.974; 1.012	898	1.010	0.981; 1.041	898	1.012	0.982; 1.043
Total fat, 4y		2061	1.002	0.995; 1.010	1973	1.004	0.996; 1.012	1921	1.004	0.996; 1.011	1917	1.003	0.995; 1.011
Total fat, 7y		2209	0.999	0.992; 1.005	2031	0.999	0.992; 1.005	898	1.001	0.991; 1.011	898	1.001	0.991; 1.011
Saturated fat, 4y		2061	1.010	0.992; 1.028	1973	1.013	0.994; 1.031	1921	1.011	0.993; 1.030	1917	1.008	0.990; 1.028
Saturated fat, 7y		2209	1.002	0.987; 1.018	2031	1.003	0.987; 1.019	898	1.003	0.979; 1.028	898	1.002	0.978; 1.027
Mufa, 4y		2061	0.996	0.976; 1.016	1973	1.000	0.980; 1.021	1921	1.001	0.980; 1.022	1917	1.000	0.979; 1.021
Mufa, 7y		2209	0.988	0.972; 1.005	2031	0.989	0.971; 1.006	898	0.998	0.971; 1.026	898	0.999	0.972; 1.027
Pufa, 4y		2061	1.028	0.976; 1.083	1973	1.034	0.981; 1.090	1921	1.036	0.982; 1.093	1917	1.035	0.982; 1.092
Pufa, 7y		2209	0.991	0.953; 1.029	2031	0.990	0.951; 1.031	898	1.011	0.951; 1.075	898	1.013	0.953; 1.078
Trans, 4y		2061	1.229	0.943; 1.604	1973	1.301	0.992; 1.709	1921	1.259	0.956; 1.659	1917	1.218	0.922; 1.609
Trans, 7y		2209	1.151	0.917; 1.447	2031	1.172	0.925; 1.486	898	1.191	0.835; 1.700	898	1.173	0.820; 1.687
	Micronutrients												
Iron, 4y													
	<7mg/day			Ref.			Ref.			Ref.			Ref.
	≥7mg/day	2061	0.969	0.829; 1.134	1973	0.989	0.841; 1.162	1921	0.997	0.846; 1.174	1917	0.987	0.838; 1.164
Iron, 7y													
	<11mg/day			Ref.			Ref.			Ref.			Ref.
	≥11mg/day	2209	0.924	0.756; 1.130	2031	0.946	0.764; 1.171	898	0.925	0.673; 1.273	898	0.921	0.669; 1.267
Vitamin D, 4y													
	<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
	≥2.4347mg/day	2061	1.017	0.867; 1.194	1973	1.030	0.874; 1.214	1921	1.055	0.893; 1.247	1917	1.081	0.913; 1.278
Vitamin D, 7y													

<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	2209	1.039	0.893; 1.208	2031	1.042	0.889; 1.222	898	1.128	0.883; 1.439	898	1.150	0.899; 1.472
Calcium, 4y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	2061	1.050	0.870; 1.267	1973	1.059	0.874; 1.283	1921	1.020	0.839; 1.239	1917	1.000	0.822; 1.216
Calcium, 7y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	2209	1.064	0.909; 1.245	2031	1.026	0.869; 1.210	898	0.992	0.775; 1.271	898	0.986	0.770; 1.264
Magnesium, 4y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	2061	1.035	0.818; 1.310	1973	1.059	0.829; 1.353	1921	1.098	0.857; 1.407	1917	1.105	0.863; 1.417
≥250mg/day	2061	1.178	0.936; 1.483	1973	1.207	0.954; 1.528	1921	1.225	0.965; 1.557	1917	1.214	0.956; 1.544
Magnesium, 7y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	2209	0.803	0.642; 1.004	2031	0.855	0.677; 1.079	898	0.889	0.624; 1.268	898	0.903	0.633; 1.288
≥250mg/day	2209	1.014	0.860; 1.196	2031	1.029	0.866; 1.224	898	1.009	0.777; 1.310	898	1.017	0.782; 1.322
Zinc, 4y												
<5.5mg/day			Ref.			Ref.			Ref.			Ref.
≥5.5-<10mg/day	2061	0.843	0.590; 1.202	1973	0.860	0.597; 1.236	1921	0.825	0.570; 1.190	1917	0.825	0.569; 1.192
≥10mg/day	2061	0.893	0.599; 1.331	1973	0.929	0.617; 1.394	1921	0.907	0.600; 1.367	1917	0.889	0.587; 1.344
Zinc, 7y												
<7.4mg/day			Ref.			Ref.			Ref.			Ref.
≥7.4-<13mg/day	2209	1.000	0.836; 1.196	2031	0.985	0.815; 1.190	898	0.915	0.694; 1.207	898	0.904	0.685; 1.193
≥13mg/day	2209	0.872	0.561; 1.360	2031	0.887	0.553; 1.422	898	1.036	0.445; 2.411	898	1.016	0.433; 2.383
Other lifestyle behaviours												
Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	2398	0.901	0.771; 1.053	2204	0.934	0.793; 1.099	1921	0.958	0.805; 1.141	1917	0.969	0.813; 1.154
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	1267	0.963	0.775; 1.197	1137	0.999	0.792; 1.258	898	1.139	0.868; 1.494	898	1.133	0.864; 1.487
Sedentary activity, 4y												
<1h			Ref.			Ref.			Ref.			Ref.

	≥1h	2101	1.128	0.944; 1.348	2012	1.143	0.951; 1.372	1921	1.062	0.879; 1.284	1917	1.054	0.872; 1.275
Sedentary activity, 7y													
	<2h			Ref.			Ref.			Ref.			Ref.
	≥2h	2216	1.047	0.871; 1.258	2037	1.061	0.875; 1.286	898	1.049	0.777; 1.417	898	1.024	0.755; 1.388
Physical exercise, 4y													
	Yes			Ref.			Ref.			Ref.			Ref.
	No	2380	1.104	0.935; 1.301	2189	1.125	0.948; 1.336	1921	1.099	0.913; 1.322	1917	1.067	0.885; 1.287
Physical activity, 7y													
	Yes			Ref.			Ref.			Ref.			Ref.
	No	2240	1.166	0.932; 1.458	2058	1.189	0.939; 1.507	898	0.684	0.419; 1.118	898	0.669	0.408; 1.094

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age at menarche, type of delivery, breastfeeding category and child's birth weight; Model 3: For exposures at 4 years of age, Model 2 plus physical activity/HEI score/sleep time/sedentary activity at 4 years; For exposures at 7 years of age, Model 2 plus physical activity, HEI score, sleep time and sedentary activity at 4 years, and physical activity/HEI score/sleep time/sedentary activity at 7 years; Model 4: Model 3 plus maternal age and educational level. All the models were adjusted for children's exact age.

Table 3. Results from the ordinal logistic regression models assessing the associations between diet quality and lifestyle behaviours and Puberty score at 10 years of age in males.

	Model 1			Model 2			Model 3			Model 4		
	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI
Diet quality												
Healthy eating index, 4y	2113	1.030	1.003; 1.057	1999	1.023	0.996; 1.051	1949	1.022	0.994; 1.050	1938	1.025	0.997; 1.055
Healthy eating index, 7y	2313	1.030	1.007; 1.054	2098	1.016	0.991; 1.041	944	0.989	0.941; 1.039	944	0.993	0.944; 1.044
Food groups												
Fruit and vegetables, 4y	2114	0.990	0.940; 1.042	2000	0.984	0.933; 1.039	1950	0.976	0.924; 1.031	1938	0.983	0.929; 1.039
Fruit and vegetables, 7y	2313	0.997	0.948; 1.049	2098	0.973	0.921; 1.027	944	0.942	0.858; 1.033	944	0.948	0.864; 1.040
Energy dense food, 4y	2114	0.965	0.925; 1.006	2000	0.970	0.928; 1.013	1950	0.976	0.933; 1.021	1939	0.972	0.928; 1.018
Energy dense food, 7y	2313	0.990	0.949; 1.031	2098	0.983	0.940; 1.027	944	1.037	0.959; 1.121	938	1.029	0.949; 1.114
Dairy, 4y	2114	1.028	0.967; 1.092	2000	1.012	0.950; 1.078	1950	1.022	0.959; 1.090	1939	1.019	0.955; 1.088
Dairy, 7y	2313	1.045	0.994; 1.100	2098	1.026	0.972; 1.082	944	1.065	0.976; 1.163	938	1.060	0.969; 1.158
Cereal products and potatoes, 4y	2114	1.014	0.949; 1.083	2000	0.998	0.932; 1.068	1950	1.001	0.934; 1.073	1939	1.003	0.935; 1.075
Cereal products and potatoes, 5y	2313	1.045	0.974; 1.122	2098	1.014	0.940; 1.093	944	1.064	0.939; 1.207	938	1.061	0.936; 1.204
Fish and eggs, 4y	2114	1.466	1.093; 1.965	2000	1.385	1.021; 1.887	1950	1.428	1.049; 1.943	1939	1.456	1.063; 1.993
Fish and eggs, 7y	2313	1.114	0.872; 1.422	2098	1.042	0.802; 1.353	944	0.864	0.547; 1.359	938	0.879	0.555; 1.389
Meat and meat products, 4y	2114	0.933	0.795; 1.091	2000	0.910	0.769; 1.074	1950	0.914	0.770; 1.082	1939	0.919	0.772; 1.088
Meat and meat products, 7y	2313	0.973	0.872; 1.087	2098	0.943	0.836; 1.061	944	0.952	0.771; 1.166	938	0.938	0.758; 1.151
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	2114	1.019	0.987; 1.052	2000	1.012	0.980; 1.046	1950	1.013	0.980; 1.047	1939	1.015	0.982; 1.050
Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	2313	1.007	0.977; 1.037	2098	0.997	0.966; 1.029	944	0.992	0.942; 1.046	938	0.993	0.943; 1.047
Processed foods, 4y	2114	0.927	0.753; 1.136	2000	0.876	0.703; 1.087	1950	0.897	0.718; 1.116	1939	0.882	0.703; 1.101
Processed foods, 7y	2313	1.074	0.954; 1.208	2313	1.074	0.954; 1.208	944	1.187	0.964; 1.453	938	1.184	0.960; 1.450
Ultra processed foods, 4y	2114	0.965	0.912; 1.019	2000	0.971	0.915; 1.028	1950	0.979	0.923; 1.038	1950	0.979	0.923; 1.038
Ultra processed foods, 7y	2313	0.987	0.949; 1.027	2098	0.980	0.939; 1.022	944	1.027	0.952; 1.105	938	1.017	0.940; 1.098
Macronutrients												

Energy, 4y		2113	1.008	0.977; 1.040	1999	1.000	0.967; 1.034	1949	1.005	0.972; 1.040	1939	1.006	0.972; 1.041
Energy, 7y		2312	1.012	0.990; 1.035	2098	1.001	0.978; 1.026	944	1.028	0.988; 1.070	938	1.026	0.986; 1.068
Protein, 4y		2113	1.005	0.999; 1.011	1999	1.002	0.996; 1.009	1949	1.004	0.997; 1.010	1939	1.004	0.997; 1.011
Protein, 7y		2312	1.004	0.999; 1.010	2098	1.002	0.997; 1.008	944	1.005	0.996; 1.015	938	1.005	0.995; 1.014
Vegetable protein, 4y		2113	0.993	0.970; 1.018	1999	0.990	0.965; 1.015	1949	0.991	0.966; 1.016	1939	0.991	0.966; 1.017
Vegetable protein, 7y		2312	1.006	0.989; 1.022	2098	0.999	0.981; 1.017	944	1.007	0.977; 1.038	938	1.007	0.977; 1.038
Animal protein, 4y		2113	1.007	1.000; 1.014	1999	1.004	0.997; 1.011	1999	1.004	0.997; 1.011	1939	1.005	0.998; 1.013
Animal protein, 7y		2312	1.005	0.999; 1.012	2098	1.003	0.997; 1.010	944	1.006	0.995; 1.017	938	1.006	0.995; 1.017
Carbohydrate, 4y		2113	1.000	0.998; 1.002	1999	1.000	0.997; 1.002	1949	1.000	0.998; 1.002	1939	1.000	0.997; 1.002
Carbohydrate, 7y		2312	1.000	0.999; 1.002	2098	1.000	0.998; 1.001	944	1.001	0.999; 1.004	938	1.001	0.999; 1.004
Fiber, 4y		2113	0.999	0.971; 1.027	1999	0.997	0.969; 1.027	1949	0.995	0.966; 1.025	1939	0.997	0.968; 1.027
Fiber, 7y		2312	1.003	0.982; 1.023	2098	0.993	0.972; 1.015	944	1.000	0.964; 1.037	938	1.002	0.966; 1.039
Total fat, 4y		2113	1.003	0.994; 1.012	1999	1.000	0.990; 1.009	1949	1.002	0.992; 1.011	1939	1.002	0.993; 1.012
Total fat, 7y		2312	1.005	0.998; 1.012	2031	1.003	0.995; 1.010	944	1.012	0.999; 1.025	938	1.011	0.999; 1.024
Saturated fat, 4y		2113	1.009	0.988; 1.029	1999	1.002	0.980; 1.023	1949	1.006	0.984; 1.028	1939	1.006	0.984; 1.029
Saturated fat, 7y		2312	1.016	0.999; 1.033	2098	1.009	0.991; 1.027	944	1.040	1.010; 1.071	938	1.038	1.008; 1.070
Mufa, 4y		2113	1.002	0.979; 1.026	1999	0.995	0.971; 1.020	1949	0.999	0.975; 1.024	1939	1.001	0.976; 1.026
Mufa, 7y		2312	1.011	0.992; 1.031	2098	1.006	0.986; 1.027	944	1.019	0.985; 1.054	938	1.018	0.984; 1.053
Pufa, 4y		2113	1.040	0.979; 1.104	1999	1.023	0.960; 1.089	1949	1.033	0.969; 1.101	1939	1.037	0.973; 1.105
Pufa, 7y		2312	1.018	0.974; 1.063	2098	1.006	0.959; 1.054	944	1.024	0.946; 1.107	938	1.022	0.945; 1.105
Trans, 4y		2113	1.304	0.954; 1.778	2113	1.304	0.954; 1.778	1949	1.225	0.875; 1.708	1939	1.232	0.877; 1.726
Trans, 7y		2312	1.456	1.125; 1.885	2312	1.456	1.125; 1.885	944	1.944	1.278; 2.945	938	1.906	1.247; 2.900
	Micronutrients												
Iron, 4y													
	<7mg/day			Ref.			Ref.			Ref.			Ref.
	≥7mg/day	2113	0.951	0.793; 1.138	1999	0.943	0.782; 1.137	1949	0.971	0.803; 1.174	1939	0.974	0.804; 1.178
Iron, 7y													
	<11mg/day			Ref.			Ref.			Ref.			Ref.
	≥11mg/day	2312	0.924	0.748; 1.138	2098	0.881	0.701; 1.103	944	0.806	0.544; 1.175	938	0.798	0.537; 1.166
Vitamin D, 4y													
	<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
	≥2.4347mg/day	2113	1.251	1.041; 1.504	1999	1.189	0.982; 1.437	1949	1.232	1.015; 1.494	1939	1.248	1.027; 1.515
Vitamin D, 7y													

<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	2312	1.174	0.988; 1.395	2098	1.113	0.928; 1.336	944	1.049	0.774; 1.422	938	1.046	0.770; 1.419
Calcium, 4y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	2113	1.017	0.814; 1.276	1999	0.977	0.775; 1.235	1949	1.005	0.794; 1.277	1939	1.008	0.796; 1.283
Calcium, 7y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	2312	1.235	1.025; 1.491	2098	1.188	0.976; 1.450	944	1.434	1.037; 2.001	938	1.428	1.030; 1.996
Magnesium, 4y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	2113	1.148	0.886; 1.479	1999	1.180	0.903; 1.533	1950	1.181	0.899; 1.541	1950	1.181	0.899; 1.541
≥250mg/day	2113	1.197	0.930; 1.535	1999	1.106	0.847; 1.436	1950	1.124	0.859; 1.462	1950	1.124	0.859; 1.462
Magnesium, 7y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	2312	1.140	0.886; 1.461	2098	1.098	0.844; 1.426	944	1.395	0.911; 2.120	938	1.419	0.925; 2.159
≥250mg/day	2312	1.041	0.861; 1.259	2098	0.958	0.784; 1.172	944	0.948	0.679; 1.324	938	0.954	0.683; 1.333
Zinc, 4y												
<5.5mg/day			Ref.			Ref.			Ref.			Ref.
≥5.5-<10mg/day	2113	1.709	1.069; 2.844	1999	1.656	1.018; 2.806	1950	1.883	1.125; 3.315	1939	1.910	1.140; 3.365
≥10mg/day	2113	1.857	1.117; 3.194	1999	1.609	0.948; 2.825	1950	1.870	1.072; 3.406	1939	1.913	1.094; 3.492
Zinc, 7y												
<7.4mg/day			Ref.			Ref.			Ref.			Ref.
≥7.4-<13mg/day	2312	1.295	1.032; 1.635	2098	1.252	0.984; 1.601	944	1.382	0.937; 2.079	938	1.393	0.944; 2.095
≥13mg/day	2312	1.276	0.787; 2.028	2098	1.091	0.645; 1.806	944	1.477	0.619; 3.314	938	1.437	0.600; 3.236
Other lifestyle behaviours												
Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	2447	1.010	0.846; 1.209	2237	1.030	0.854; 1.244	1949	1.012	0.828; 1.238	1949	1.012	0.828; 1.238
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	1949	1.012	0.828; 1.238	1949	1.012	0.828; 1.238	944	0.904	0.655; 1.252	938	0.889	0.642; 1.235
Sedentary activity, 4y												
<1h			Ref.			Ref.			Ref.			Ref.

	≥1h	2160	0.998	0.805; 1.245	2160	0.998	0.805; 1.245	1949	0.974	0.772; 1.233	1949	0.974	0.772; 1.233
Sedentary activity, 7y													
	<2h			Ref.			Ref.			Ref.			Ref.
	≥2h	2320	1.138	0.923; 1.407	2109	1.166	0.935; 1.460	944	1.193	0.821; 1.759	944	1.193	0.821; 1.759
Physical exercise, 4y													
	Yes			Ref.			Ref.			Ref.			Ref.
	No	2436	0.964	0.805; 1.153	2228	0.989	0.818; 1.194	1949	1.029	0.838; 1.261	1938	0.997	0.809; 1.227
Physical activity, 7y													
	Yes			Ref.			Ref.			Ref.			Ref.
	No	2347	1.272	1.007; 1.600	2130	1.240	0.967; 1.584	2130	1.240	0.967; 1.584	2130	1.240	0.967; 1.584

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age at menarche, type of delivery, breastfeeding category and child's birth weight; Model 3: For exposures at 4 years of age, Model 2 plus physical activity/HEI score/sleep time/sedentary activity at 4 years; For exposures at 7 years of age, Model 2 plus physical activity, HEI score, sleep time and sedentary activity at 4 years, and physical activity/HEI score/sleep time/sedentary activity at 7 years; Model 4: Model 3 plus maternal age and educational level. All the models were adjusted for children's exact age.

Table 4. Results from the Cox proportional hazards regression for time to event assessing the associations between diet quality and lifestyle behaviours and age at menarche.

	Model 1			Model 2			Model 3			Model 4		
	n	β	95% CI	n	β	95% CI	n	β	95% CI	n	β	95% CI
Diet quality												
Healthy eating index, 4y	1597	**0.958**	**0.930; 0.988**	1528	**0.967**	**0.937; 0.998**	1491	**0.967**	**0.935; 0.999**	1488	0.982	0.949; 1.015
Healthy eating index, 7y	1712	**0.956**	**0.927; 0.986**	1578	**0.957**	**0.926; 0.989**	742	0.973	0.923; 1.025	742	0.981	0.932; 1.033
Food groups												
Fruit and vegetables, 4y	1598	1.028	0.966; 1.095	1528	1.024	0.958; 1.094	1491	1.026	0.956; 1.101	1488	1.063	0.989; 1.142
Fruit and vegetables, 7y	1712	1.008	0.943; 1.076	1578	1.016	0.948; 1.088	742	1.006	0.908; 1.115	742	1.043	0.940; 1.157
Energy dense food, 4y	1598	**1.104**	**1.055; 1.155**	1528	**1.102**	**1.050; 1.157**	1491	**1.103**	**1.048; 1.160**	1488	**1.077**	**1.020; 1.138**
Energy dense food, 7y	1712	**1.069**	**1.015; 1.126**	1578	**1.084**	**1.024; 1.147**	742	0.978	0.896; 1.069	742	0.953	0.867; 1.047
Dairy, 4y	1598	1.060	0.980; 1.146	1528	**1.087**	**1.002; 1.180**	1491	**1.091**	**1.003; 1.187**	1488	1.068	0.979; 1.165
Dairy, 7y	1712	0.987	0.924; 1.053	1578	1.019	0.949; 1.094	742	0.980	0.890; 1.080	742	0.951	0.853; 1.060
Cereal products and potatoes, 4y	1598	0.990	0.915; 1.070	1528	1.017	0.935; 1.105	1491	1.012	0.929; 1.101	1488	0.987	0.905; 1.076
Cereal products and potatoes, 5y	1712	0.922	0.847; 1.003	1578	0.928	0.848; 1.016	742	0.936	0.831; 1.055	742	0.909	0.801; 1.031
Fish and eggs, 4y	1598	0.880	0.606; 1.279	1528	0.875	0.596; 1.285	1491	0.860	0.581; 1.274	1488	1.017	0.683; 1.514
Fish and eggs, 7y	1712	1.200	0.885; 1.628	1578	1.194	0.858; 1.662	742	0.997	0.614; 1.618	742	1.219	0.741; 2.006
Meat and meat products, 4y	1598	1.112	0.942; 1.314	1528	1.080	0.901; 1.295	1491	1.084	0.900; 1.306	1488	1.046	0.867; 1.263
Meat and meat products, 7y	1712	**1.204**	**1.047; 1.385**	1578	**1.262**	**1.082; 1.473**	742	**1.304**	**1.054; 1.613**	742	**1.295**	**1.044; 1.607**
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	1598	**1.040**	**1.003; 1.079**	1528	**1.058**	**1.018; 1.100**	1491	**1.055**	**1.014; 1.097**	1488	**1.060**	**1.019; 1.103**
Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	1712	0.967	0.961; 1.033	1578	1.014	0.975; 1.054	742	0.993	0.931; 1.059	742	0.993	0.930; 1.061
Processed foods, 4y	1598	1.179	0.896; 1.552	1528	1.196	0.903; 1.584	1491	1.231	0.922; 1.642	1488	1.166	0.870; 1.564
Processed foods, 7y	1712	1.188	1.001; 1.410	1578	1.201	0.989; 1.457	742	1.244	0.937; 1.651	742	1.229	0.919; 1.644
Ultra processed foods, 4y	1598	**1.128**	**1.053; 1.207**	1528	**1.111**	**1.035; 1.192**	1491	**1.112**	**1.032; 1.199**	1488	1.073	0.990; 1.164
Ultra processed foods, 7y	1712	**1.055**	**1.001; 1.112**	1578	**1.061**	**1.002; 1.124**	742	1.005	0.927; 1.090	742	0.991	0.907; 1.082
Macronutrients												

Energy, 4y		1597	**1.058**	**1.020; 1.098**	1528	**1.072**	**1.031; 1.115**	1491	**1.068**	**1.026; 1.112**	1488	**1.058**	**1.016; 1.101**
Energy, 7y		1712	1.018	0.988; 1.048	1578	1.031	0.999; 1.064	742	1.004	0.960; 1.051	742	1.003	0.956; 1.052
Protein, 4y		1597	**1.010**	**1.002; 1.018**	1528	**1.012**	**1.003; 1.020**	1491	**1.011**	**1.003; 1.019**	1488	**1.011**	**1.003; 1.019**
Protein, 7y		1712	1.003	0.996; 1.009	1578	1.005	0.998; 1.012	742	1.003	0.992; 1.014	703	1.003	0.992; 1.015
Vegetable protein, 4y		1597	1.018	0.987; 1.050	1528	1.031	0.998; 1.066	1491	1.026	0.993; 1.061	1488	1.022	0.988; 1.057
Vegetable protein, 7y		1712	0.996	0.975; 1.018	1578	1.002	0.979; 1.027	742	0.993	0.960; 1.026	742	0.993	0.960; 1.028
Animal protein, 4y		1597	**1.011**	**1.002; 1.019**	1528	**1.012**	**1.004; 1.021**	1491	**1.012**	**1.003; 1.021**	1488	**1.012**	**1.003; 1.021**
Animal protein, 7y		1712	1.004	0.997; 1.012	1578	1.007	0.999; 1.015	742	1.005	0.992; 1.019	742	1.005	0.992; 1.019
Carbohydrate, 4y		1597	**1.004**	**1.002; 1.007**	1528	**1.005**	**1.002; 1.008**	1491	**1.005**	**1.002; 1.008**	1488	**1.004**	**1.001; 1.007**
Carbohydrate, 7y		1712	1.001	0.999; 1.003	1578	**1.002**	**1.000; 1.004**	742	1.001	0.998; 1.004	742	1.000	0.997; 1.004
Fiber, 4y		1597	1.028	0.992; 1.066	1528	1.035	0.996; 1.076	1491	1.031	0.990; 1.073	1488	**1.042**	**1.001; 1.084**
Fiber, 7y		1712	1.010	0.983; 1.038	1578	1.020	0.991; 1.050	742	1.014	0.972; 1.059	742	1.023	0.979; 1.068
Total fat, 4y		1597	**1.012**	**1.002; 1.022**	1528	**1.015**	**1.004; 1.026**	1491	**1.014**	**1.003; 1.025**	1488	**1.012**	**1.001; 1.023**
Total fat, 7y		1712	1.003	0.994; 1.013	1578	1.006	0.996; 1.016	742	0.995	0.980; 1.010	742	0.995	0.980; 1.010
Saturated fat, 4y		1597	**1.026**	**1.002; 1.051**	1528	**1.034**	**1.009; 1.060**	1491	**1.034**	**1.008; 1.061**	1488	**1.027**	**1.001; 1.054**
Saturated fat, 7y		1712	1.004	0.981; 1.027	1578	1.010	0.985; 1.035	742	0.978	0.942; 1.015	742	0.976	0.939; 1.014
Mufa, 4y		1597	**1.034**	**1.006; 1.063**	1528	**1.042**	**1.012; 1.072**	1491	**1.038**	**1.008; 1.069**	1488	**1.036**	**1.006; 1.066**
Mufa, 7y		1712	1.005	0.981; 1.030	1578	1.011	0.985; 1.038	742	0.983	0.944; 1.025	742	0.985	0.944; 1.027
Pufa, 4y		1597	1.072	0.999; 1.150	1528	**1.083**	**1.006; 1.167**	1491	1.073	0.995; 1.157	1488	1.074	0.998; 1.156
Pufa, 7y		1712	1.055	0.998; 1.116	1578	**1.334**	**1.003; 1.130**	742	1.028	0.944; 1.118	742	1.045	0.958; 1.140
Trans, 4y		1597	**1.637**	**1.128; 2.375**	1528	**1.812**	**1.237; 2.653**	1491	**1.848**	**1.250; 2.731**	1488	**1.746**	**1.167; 2.611**
Trans, 7y		1712	1.118	0.786; 1.590	1578	1.223	0.841; 1.779	742	0.782	0.441; 1.385	742	0.752	0.417; 1.356
	Micronutrients												
Iron, 4y													
	<7mg/day			Ref.			Ref.			Ref.			Ref.
	≥7mg/day	1597	**1.320**	**1.048; 1.663**	1528	**1.453**	**1.141; 1.850**	1491	**1.452**	**1.136; 1.855**	1488	**1.424**	**1.111; 1.826**
Iron, 7y													
	<11mg/day			Ref.			Ref.			Ref.			Ref.
	≥11mg/day	1712	1.237	0.933; 1.640	1578	**1.427**	**1.051; 1.936**	742	1.420	0.930; 2.167	742	1.489	0.968; 2.291
Vitamin D, 4y													
	<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
	≥2.4347mg/day	1597	0.903	0.716; 1.139	1528	0.946	0.740; 1.209	1491	0.921	0.716; 1.184	1488	0.991	0.768; 1.280
Vitamin D, 7y													

<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	1712	1.187	0.944; 1.493	1578	1.263	0.991; 1.612	742	1.100	0.783; 1.546	742	0.930	0.635; 1.362
Calcium, 4y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	1597	**1.718**	**1.280; 2.304**	1528	**1.909**	**1.405; 2.594**	1491	**1.912**	**1.401; 2.609**	1488	**1.807**	**1.318; 2.478**
Calcium, 7y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	1712	0.999	0.792; 1.261	1578	1.002	0.782; 1.285	742	0.949	0.674; 1.335	742	0.860	0.601; 1.232
Magnesium, 4y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	1597	1.072	0.777; 1.479	1528	1.134	0.805; 1.596	1491	1.146	0.809; 1.624	1488	1.180	0.831; 1.675
≥250mg/day	1597	**1.430**	**1.054; 1.940**	1528	**1.658**	**1.203; 2.285**	1491	**1.656**	**1.194; 2.297**	1488	**1.672**	**1.676; 2.269**
Magnesium, 7y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	1712	0.729	0.511; 1.040	1578	0.818	0.567; 1.182	742	0.717	0.420; 1.225	742	0.704	0.408; 1.216
≥250mg/day	1712	0.941	0.737; 1.201	1578	1.082	0.832; 1.407	742	1.053	0.723; 1.533	742	1.058	0.723; 1.549
Zinc, 4y												
<5.5mg/day			Ref.			Ref.			Ref.			Ref.
≥5.5-<10mg/day	1597	1.048	0.643; 1.710	1528	1.111	0.672; 1.836	1491	1.086	0.656; 1.798	1488	1.166	0.704; 1.932
≥10mg/day	1597	1.415	0.830; 2.413	1528	1.736	0.998; 3.018	1491	1.657	0.949; 2.893	1488	1.725	0.982; 3.029
Zinc, 7y												
<7.4mg/day			Ref.			Ref.			Ref.			Ref.
≥7.4-<13mg/day	1712	0.942	0.722; 1.229	1578	0.977	0.735; 1.299	742	0.872	0.586; 1.297	742	0.821	0.545; 1.237
≥13mg/day	1712	1.114	0.600; 2.071	1578	1.262	0.637; 2.498	742	1.020	0.339; 3.072	742	1.411	0.434; 4.583
Other lifestyle behaviours												
Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	1822	1.094	0.872; 1.372	1685	1.028	0.805; 1.312	1491	1.094	0.841; 1.423	1488	1.164	0.891; 1.521
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	986	0.834	0.625; 1.111	906	0.887	0.647; 1.216	742	0.919	0.624; 1.353	742	0.987	0.669; 1.460
Sedentary activity, 4y												
<1h			Ref.			Ref.			Ref.			Ref.

	≥1h	1631	**1.318**	**1.002; 1.732**	1560	**1.396**	**1.048; 1.860**	1491	1.306	0.969; 1.759	1488	1.264	0.936; 1.707
Sedentary activity, 7y													
	<2h			Ref.			Ref.			Ref.			Ref.
	≥2h	1716	0.914	0.696; 1.200	1583	0.826	0.618; 1.106	742	0.630	0.424; 1.093	742	0.541	0.360; 1.012
Physical exercise, 4y													
	Yes			Ref.			Ref.			Ref.			Ref.
	No	1810	0.959	0.757; 1.215	1674	0.888	0.687; 1.147	1491	0.889	0.667; 1.184	1488	0.798	0.594; 1.072
Physical activity, 7y													
	Yes			Ref.			Ref.			Ref.			Ref.
	No	1735	1.236	0.897; 1.704	1600	1.047	0.730; 1.500	742	0.938	0.493; 1.787	742	0.802	0.412; 1.561

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age at menarche, type of delivery, breastfeeding category and child's birth weight; Model 3: For exposures at 4 years of age, Model 2 plus physical activity/HEI score/sleep time/sedentary activity at 4 years; For exposures at 7 years of age, Model 2 plus physical activity, HEI score, sleep time and sedentary activity at 4 years, and physical activity/HEI score/sleep time/sedentary activity at 7 years; Model 4: Model 3 plus maternal age and educational level. All the models were adjusted for children's exact age.

Supplementary Tables

Table S1

Ordinal logistic regression of diet and lifestyle associations with Puberty score at 10 years, by BMI in females.

Table S2

Ordinal logistic regression of diet and lifestyle associations with Puberty score at 10 years, by BMI in males.

Table S3

Ordinal logistic regression of diet and lifestyle associations with age at menarche by BMI.

Supplementary Table 1. Results from the ordinal logistic regression models assessing the associations between diet quality and lifestyle behaviours and Puberty score at 10 years old, by BMI categories, in females.

	Model 1			Model 2			Model 3			Model 4		
	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI
NORMAL WEIGHT												
Diet quality												
Healthy eating index, 4y	1202	0.988	0.960; 1.017	1149	0.984	0.955; 1.014	1119	0.987	0.958; 1.018	1116	0.989	0.958; 1.021
Healthy eating index, 7y	1275	0.993	0.968; 1.019	1169	0.991	0.965; 1.018	535	0.986	0.939; 1.035	535	0.985	0.937; 1.035
Food groups												
Fruit and vegetables, 4y	1203	1.023	0.965; 1.084	1149	1.014	0.955; 1.076	1119	1.015	0.954; 1.079	1116	1.016	0.955; 1.082
Fruit and vegetables, 7y	1275	1.017	0.960; 1.079	1169	1.015	0.955; 1.079	535	1.080	0.979; 1.192	535	1.080	0.978; 1.192
Energy dense food, 4y	1203	1.042	0.994; 1.092	1149	1.038	0.989; 1.090	1119	1.033	0.983; 1.085	1116	1.032	0.980; 1.087
Energy dense food, 7y	1275	1.006	0.957; 1.058	1169	1.018	0.966; 1.073	535	0.989	0.904; 1.081	535	0.986	0.900; 1.079
Dairy, 4y	1203	1.046	0.972; 1.125	1149	1.039	0.963; 1.121	1119	1.039	0.962; 1.123	1116	1.039	0.960; 1.124
Dairy, 7y	1275	1.054	0.993; 1.119	1169	1.051	0.988; 1.119	535	0.988	0.896; 1.088	535	0.984	0.892; 1.085
Cereal products and potatoes, 4y	1203	1.008	0.935; 1.087	1149	1.017	0.941; 1.100	1119	1.023	0.944; 1.108	1116	1.024	0.945; 1.110
Cereal products and potatoes, 7y	1275	0.990	0.915; 1.072	1169	1.009	0.928; 1.097	535	1.016	0.896; 1.151	535	1.011	0.892; 1.147
Fish and eggs, 4y	1203	1.056	0.750; 1.486	1149	1.086	0.767; 1.536	1119	1.097	0.772; 1.559	1116	1.116	0.780; 1.596
Fish and eggs, 7y	1275	1.062	0.787; 1.434	1169	1.138	0.831; 1.558	535	1.046	0.643; 1.704	535	1.022	0.619; 1.688
Meat and meat products, 4y	1203	0.890	0.740; 1.070	1149	0.904	0.748; 1.090	1119	0.887	0.733; 1.073	1116	0.878	0.723; 1.063
Meat and meat products, 7y	1275	1.008	0.878; 1.158	1169	1.042	0.901; 1.205	535	1.053	0.852; 1.301	535	1.051	0.849; 1.301
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	1203	0.999	0.965; 1.035	1149	0.999	0.963; 1.036	1119	1.000	0.964; 1.038	1116	1.000	0.963; 1.038
Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	1275	1.003	0.969; 1.039	1169	1.004	0.968; 1.042	535	1.016	0.957; 1.078	535	1.014	0.956; 1.076
Processed foods, 4y	1203	1.142	0.905; 1.440	1149	1.152	0.908; 1.463	1119	1.169	0.918; 1.490	1116	1.170	0.917; 1.493
Processed foods, 7y	1275	1.014	0.863; 1.192	1169	1.026	0.867; 1.213	535	0.987	0.755; 1.290	535	0.979	0.747; 1.281
Ultra processed foods, 4y	1203	1.047	0.980; 1.118	1149	1.043	0.974; 1.117	1119	1.037	0.967; 1.113	1116	1.037	0.963; 1.118
Ultra processed foods, 7y	1275	1.023	0.974; 1.075	1169	1.031	0.979; 1.086	535	1.009	0.924; 1.101	535	1.006	0.918; 1.102

Macronutrients													
Energy, 4y	1202	1.022	0.986; 1.060	1149	1.024	0.986; 1.062	1119	1.022	0.984; 1.061	1116	1.022	0.983 1.061
Energy, 7y	1275	1.008	0.982; 1.035	1169	1.013	0.986; 1.042	535	1.008	0.965; 1.053	535	1.006	0.963; 1.051
Protein, 4y	1202	1.001	0.994; 1.008	1149	1.001	0.994; 1.009	1119	1.002	0.994; 1.009	1116	1.001	0.994; 1.009
Protein, 7y	1275	1.002	0.996; 1.008	1169	1.003	0.997; 1.010	535	1.003	0.992; 1.013	535	1.002	0.992; 1.013
Vegetable protein, 4y	1202	1.014	0.986; 1.042	1149	1.016	0.988; 1.046	1119	1.018	0.989; 1.047	1116	1.018	0.989; 1.047
Vegetable protein, 7y	1275	1.004	0.985; 1.023	1169	1.008	0.988; 1.028	535	1.012	0.982; 1.043	535	1.011	0.981; 1.042
Animal protein, 4y	1202	1.000	0.992; 1.008	1149	1.000	0.992; 1.009	1119	1.001	0.992; 1.009	1116	1.000	0.992; 1.009
Animal protein, 7y	1275	1.003	0.996; 1.010	1169	1.004	0.996; 1.011	535	1.002	0.990; 1.014	535	1.003	0.991; 1.015
Carbohydrate, 4y	1202	1.002	0.999; 1.004	1149	1.002	0.999; 1.004	1119	1.001	0.999; 1.004	1116	1.001	0.999; 1.004
Carbohydrate, 7y	1275	1.000	0.999; 1.002	1169	1.001	0.999; 1.002	535	1.000	0.998; 1.003	535	1.000	0.998; 1.003
Fiber, 4y	1202	1.011	0.979; 1.044	1149	1.009	0.976; 1.043	1119	1.010	0.976; 1.044	1116	1.010	0.976; 1.045
Fiber, 7y	1275	1.006	0.982; 1.029	1169	1.009	0.984; 1.034	535	1.027	0.989; 1.066	535	1.025	0.987; 1.065
Total fat, 4y	1202	1.007	0.996; 1.017	1149	1.008	0.997; 1.018	1119	1.007	0.997; 1.018	1116	1.007	0.996; 1.018
Total fat, 7y	1275	1.003	0.995; 1.012	1169	1.005	0.996; 1.014	535	1.001	0.987; 1.015	535	1.001	0.987; 1.015
Saturated fat, 4y	1202	1.018	0.994; 1.042	1149	1.020	0.995; 1.044	1119	1.018	0.993; 1.044	1116	1.018	0.993; 1.044
Saturated fat, 7y	1275	1.011	0.991; 1.032	1169	1.014	0.992; 1.035	535	0.998	0.965; 1.033	535	0.996	0.963; 1.031
Mufa, 4y	1202	1.010	0.983; 1.038	1149	1.014	0.986; 1.043	1119	1.015	0.987; 1.044	1116	1.014	0.986; 1.044
Mufa, 7y	1275	1.006	0.983; 1.028	1169	1.011	0.987; 1.035	535	1.010	0.972; 1.049	535	1.009	0.972; 1.048
Pufa, 4y	1202	1.037	0.968; 1.112	1149	1.049	0.977; 1.126	1119	1.047	0.974; 1.125	1116	1.047	0.974; 1.125
Pufa, 7y	1275	1.013	0.962; 1.067	1169	1.033	0.978; 1.091	535	1.045	0.959; 1.138	535	1.042	0.956; 1.135
Trans, 4y	1202	1.292	0.905; 1.844	1149	1.329	0.921; 1.916	1119	1.286	0.884; 1.866	1116	1.277	0.874; 1.863
Trans, 7y	1275	1.246	0.905; 1.718	1169	1.266	0.908; 1.769	535	0.938	0.555; 1.588	535	0.912	0.538; 1.549
Micronutrients												
Iron, 4y												
<7mg/day			Ref.			Ref.			Ref.			Ref.
≥7mg/day	1202	1.024	0.834; 1.257	1149	1.058	0.856; 1.308	1119	1.066	0.860; 1.320	1116	1.060	0.855; 1.314
Iron, 7y												
<11mg/day			Ref.			Ref.			Ref.			Ref.
≥11mg/day	1275	1.000	0.771; 1.296	1169	1.063	0.807; 1.399	535	0.928	0.611; 1.409	535	0.920	0.603; 1.396
Vitamin D, 4y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	1202	0.974	0.791; 1.200	1149	1.013	0.818; 1.255	1119	1.028	0.827; 1.279	1116	1.037	0.833; 1.291

Vitamin D, 7y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	1275	1.114	0.912; 1.360	1169	1.158	0.939; 1.429	535	1.168	0.846; 1.611	535	1.153	0.831; 1.600
Calcium, 4y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	1202	1.179	0.919; 1.512	1149	1.172	0.908; 1.514	1119	1.137	0.877; 1.474	1116	1.127	0.867; 1.465
Calcium, 7y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	1275	1.181	0.958; 1.456	1169	1.110	0.891; 1.384	535	0.931	0.671; 1.291	535	0.932	0.671; 1.293
Magnesium, 4y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	1202	1.083	0.796; 1.473	1149	1.135	0.823; 1.562	1119	1.191	0.861; 1.648	1116	1.194	0.862; 1.651
≥250mg/day	1202	1.131	0.842; 1.519	1149	1.173	0.864; 1.591	1119	1.193	0.875; 1.626	1116	1.193	0.874; 1.627
Magnesium, 7y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	1275	0.759	0.567; 1.015	1169	0.789	0.582; 1.069	535	0.723	0.448; 1.169	535	0.733	0.452; 1.182
≥250mg/day	1275	1.181	0.949; 1.469	1169	1.221	0.971; 1.537	535	1.130	0.802; 1.592	535	1.116	0.791; 1.573
Zinc, 4y												
<5.5mg/day			Ref.			Ref.			Ref.			Ref.
≥5.5-<10mg/day	1202	1.214	0.732; 2.011	1149	1.212	0.719; 2.049	1119	1.192	0.702; 2.032	1116	1.181	0.689; 2.033
≥10mg/day	1202	1.260	0.724; 2.193	1149	1.264	0.713; 2.245	1119	1.269	0.711; 2.274	1116	1.257	0.698; 2.272
Zinc, 7y												
<7.4mg/day			Ref.			Ref.			Ref.			Ref.
≥7.4-<13mg/day	1275	1.182	0.930; 1.503	1169	1.194	0.926; 1.542	535	1.067	0.731; 1.560	535	1.073	0.735; 1.570
≥13mg/day	1275	1.106	0.637; 1.921	1169	1.189	0.657; 2.150	535	1.061	0.366; 3.036	535	0.963	0.328; 2.779
Other lifestyle behaviours												
Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	1391	1.009	0.820; 1.241	1274	1.053	0.847; 1.309	1119	1.064	0.843; 1.342	1116	1.065	0.843; 1.345
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	742	1.136	0.847; 1.527	669	1.213	0.887; 1.664	535	1.158	0.804; 1.672	535	1.154	0.799; 1.670
Sedentary activity, 4y												

<1h			Ref.			Ref.			Ref.			Ref.
≥1h	1222	1.046	0.829; 1.322	1166	1.089	0.856; 1.385	1119	1.048	0.818; 1.344	1116	1.044	0.814; 1.340
Sedentary activity, 7y												
<2h			Ref.			Ref.			Ref.			Ref.
≥2h	1281	0.888	0.700; 1.128	1174	0.888	0.692; 1.138	535	0.937	0.638; 1.380	535	0.933	0.631; 1.379
Physical exercise, 4y												
Yes			Ref.			Ref.			Ref.			Ref.
No	1384	1.187	0.956; 1.475	1268	1.218	0.971; 1.527	1119	1.206	0.946; 1.538	1116	1.206	0.941; 1.545
Physical activity, 7y												
Yes			Ref.			Ref.			Ref.			Ref.
No	1295	1.192	0.880; 1.616	1185	1.227	0.889; 1.693	535	0.762	0.403; 1.424	535	0.764	0.401; 1.436
OVERWEIGHT + OBESE												
Diet quality												
Healthy eating index, 4y	857	0.994	0.960; 1.030	823	0.996	0.960; 1.033	801	0.996	0.959; 1.034	800	1.000	0.961; 1.040
Healthy eating index, 7y	931	0.989	0.956; 1.023	861	0.984	0.949; 1.020	363	0.947	0.886; 1.011	363	0.940	0.878; 1.005
Food groups												
Fruit and vegetables, 4y	857	0.981	0.913; 1.053	823	0.988	0.919; 1.063	801	0.991	0.921; 1.068	800	0.993	0.921; 1.072
Fruit and vegetables, 7y	931	0.993	0.926; 1.066	861	0.985	0.914; 1.061	363	1.010	0.892; 1.145	363	0.998	0.878; 1.133
Energy dense food, 4y	857	1.030	0.971; 1.093	823	1.031	0.970; 1.097	801	1.031	0.968; 1.098	800	1.029	0.964; 1.100
Energy dense food, 7y	931	1.006	0.945; 1.070	861	1.018	0.953; 1.088	363	1.088	0.966; 1.227	363	1.101	0.975; 1.246
Dairy, 4y	857	1.009	0.927; 1.098	823	1.012	0.928; 1.104	801	1.008	0.923; 1.100	800	1.011	0.926; 1.104
Dairy, 7y	931	0.996	0.927; 1.071	861	1.003	0.929; 1.083	363	0.991	0.878; 1.119	363	1.001	0.886; 1.132
Cereal products and potatoes, 4y	857	0.966	0.883; 1.056	823	0.953	0.869; 1.045	801	0.953	0.868; 1.046	800	0.956	0.871; 1.049
Cereal products and potatoes, 5y	931	0.979	0.890; 1.076	861	0.934	0.845; 1.032	363	0.929	0.799; 1.081	363	0.923	0.794; 1.073
Fish and eggs, 4y	857	1.740	1.124; 2.694	823	1.754	1.120; 2.757	801	1.871	1.171; 3.000	800	1.921	1.194; 3.103
Fish and eggs, 7y	931	1.115	0.795; 1.566	861	1.026	0.710; 1.483	363	1.310	0.715; 2.416	363	1.247	0.677; 2.309
Meat and meat products, 4y	857	1.142	0.934; 1.395	823	1.156	0.945; 1.419	801	1.160	0.944; 1.431	800	1.145	0.931; 1.414
Meat and meat products, 7y	931	0.961	0.816; 1.134	861	0.942	0.794; 1.119	363	0.940	0.722; 1.228	363	0.935	0.718; 1.221
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	857	0.981	0.941; 1.021	823	0.986	0.946; 1.028	801	0.987	0.946; 1.030	800	0.988	0.947; 1.031

Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	931	1.003	0.963; 1.044	861	0.996	0.954; 1.040	363	1.014	0.944; 1.089	363	1.007	0.937; 1.082
Processed foods, 4y	857	1.041	0.775; 1.397	823	1.072	0.787; 1.469	801	1.090	0.790; 1.510	800	1.099	0.795; 1.527
Processed foods, 7y	931	0.989	0.838; 1.172	861	0.982	0.822; 1.180	363	1.084	0.790; 1.488	363	1.073	0.781; 1.472
Ultra processed foods, 4y	857	1.030	0.952; 1.117	823	1.021	0.941; 1.110	801	1.027	0.944; 1.118	800	1.025	0.939; 1.120
Ultra processed foods, 7y	931	0.984	0.927; 1.045	861	0.985	0.924; 1.050	363	1.021	0.921; 1.132	363	1.025	0.923; 1.140
Macronutrients												
Energy, 4y	857	1.003	0.961; 1.047	823	1.003	0.961; 1.048	801	1.004	0.961; 1.050	800	1.006	0.962; 1.051
Energy, 7y	931	0.996	0.965; 1.028	861	0.988	0.954; 1.022	363	1.009	0.956; 1.065	363	1.006	0.953; 1.062
Protein, 4y	857	1.003	0.995; 1.012	823	1.003	0.995; 1.012	801	1.003	0.995; 1.012	800	1.003	0.995; 1.012
Protein, 7y	931	0.998	0.990; 1.005	861	0.995	0.987; 1.003	363	0.998	0.985; 1.010	363	0.997	0.985; 1.009
Vegetable protein, 4y	857	0.989	0.957; 1.022	823	0.985	0.952; 1.018	801	0.987	0.954; 1.021	800	0.988	0.955; 1.022
Vegetable protein, 7y	931	0.996	0.972; 1.019	861	0.986	0.961; 1.011	363	0.996	0.958; 1.036	363	0.992	0.954; 1.031
Animal protein, 4y	857	1.005	0.996; 1.015	823	1.005	0.996; 1.015	801	1.005	0.995; 1.015	800	1.005	0.996; 1.015
Animal protein, 7y	931	0.997	0.989; 1.006	861	0.995	0.986; 1.005	363	0.997	0.983; 1.012	363	0.997	0.983; 1.012
Carbohydrate, 4y	857	1.000	0.996; 1.003	823	0.999	0.996; 1.003	801	1.000	0.996; 1.003	800	1.000	0.996; 1.003
Carbohydrate, 7y	931	1.000	0.998; 1.002	861	1.000	0.997; 1.002	363	1.001	0.997; 1.005	363	1.001	0.997; 1.005
Fiber, 4y	857	0.987	0.950; 1.026	823	0.988	0.950; 1.027	801	0.990	0.952; 1.030	800	0.991	0.952; 1.031
Fiber, 7y	931	0.997	0.967; 1.027	861	0.988	0.957; 1.021	363	1.000	0.950; 1.053	363	0.992	0.941; 1.046
Total fat, 4y	857	1.002	0.990; 1.014	823	1.003	0.991; 1.016	801	1.003	0.991; 1.016	800	1.004	0.991; 1.016
Total fat, 7y	931	0.997	0.988; 1.007	861	0.995	0.985; 1.006	363	1.002	0.986; 1.018	363	1.001	0.985; 1.017
Saturated fat, 4y	857	1.006	0.978; 1.035	823	1.009	0.980; 1.039	801	1.008	0.979; 1.038	800	1.009	0.980; 1.039
Saturated fat, 7y	931	0.996	0.972; 1.020	861	0.994	0.969; 1.019	363	1.006	0.969; 1.046	363	1.006	0.969; 1.046
Mufa, 4y	857	0.994	0.963; 1.025	823	0.997	0.966; 1.029	801	0.998	0.967; 1.030	800	0.999	0.967; 1.031
Mufa, 7y	931	0.985	0.959; 1.011	861	0.978	0.951; 1.006	363	0.995	0.953; 1.038	363	0.990	0.949; 1.034
Pufa, 4y	857	1.039	0.959; 1.127	823	1.034	0.953; 1.122	801	1.041	0.958; 1.132	800	1.042	0.959; 1.133
Pufa, 7y	931	0.985	0.927; 1.046	861	0.961	0.901; 1.025	363	0.998	0.907; 1.099	363	0.990	0.900; 1.091
Trans, 4y	857	1.203	0.793; 1.825	823	1.284	0.840; 1.977	801	1.252	0.815; 1.937	800	1.277	0.830; 1.981
Trans, 7y	931	0.990	0.704; 1.388	861	1.017	0.713; 1.448	363	1.268	0.757; 2.258	363	1.286	0.763; 2.325
Micronutrients												
Iron, 4y												
<7mg/day			Ref.			Ref.			Ref.			Ref.

≥7mg/day	857	1.014	0.787; 1.306	823	1.025	0.789; 1.333	801	1.055	0.808; 1.377	800	1.055	0.808; 1.378
Iron, 7y												
<11mg/day			Ref.			Ref.			Ref.			Ref.
≥11mg/day	931	0.908	0.652; 1.267	861	0.872	0.613; 1.244	363	0.953	0.556; 1.649	363	0.950	0.553; 1.645
Vitamin D, 4y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	857	1.197	0.923; 1.552	823	1.151	0.881; 1.504	801	1.204	0.918; 1.581	800	1.223	0.930; 1.612
Vitamin D, 7y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	931	1.099	0.863; 1.401	861	1.070	0.829; 1.382	363	1.161	0.778; 1.738	363	1.131	0.755; 1.696
Calcium, 4y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	857	0.952	0.707; 1.281	823	1.008	0.743; 1.364	801	0.959	0.705; 1.303	800	0.976	0.716; 1.327
Calcium, 7y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	931	1.004	0.783; 1.286	861	0.989	0.763; 1.282	363	0.864	0.573; 1.299	363	0.859	0.569; 1.294
Magnesium, 4y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	857	1.008	0.688; 1.483	823	0.985	0.659; 1.478	801	1.039	0.690; 1.573	800	1.036	0.687; 1.569
≥250mg/day	857	1.391	0.955; 2.039	823	1.356	0.925; 1.999	801	1.402	0.950; 2.084	800	1.406	0.952; 2.090
Magnesium, 7y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	931	1.051	0.734; 1.511	861	1.107	0.762; 1.613	363	1.117	0.640; 1.960	363	1.086	0.620; 1.913
≥250mg/day	931	0.902	0.695; 1.173	861	0.888	0.675; 1.171	363	0.900	0.583; 1.391	363	0.873	0.564; 1.353
Zinc, 4y												
<5.5mg/day			Ref.			Ref.			Ref.			Ref.
≥5.5-<10mg/day	857	0.743	0.439; 1.241	823	0.783	0.459; 1.322	801	0.727	0.423; 1.231	800	0.741	0.431; 1.255
≥10mg/day	857	0.894	0.488; 1.625	823	0.920	0.499; 1.685	801	0.889	0.478; 1.639	800	0.904	0.486; 1.667
Zinc, 7y												
<7.4mg/day			Ref.			Ref.			Ref.			Ref.
≥7.4-<13mg/day	931	0.860	0.651; 1.135	861	0.836	0.623; 1.118	363	0.714	0.457; 1.110	363	0.712	0.455; 1.107
≥13mg/day	931	0.818	0.378; 1.815	861	0.810	0.355; 1.897	363	1.358	0.265; 10.094	363	1.426	0.274; 10.751
Other lifestyle behaviours												

Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	1004	0.865	0.676; 1.105	929	0.888	0.686; 1.148	801	0.921	0.698; 1.214	800	0.953	0.720; 1.259
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	524	1.000	0.713; 1.401	468	0.978	0.680; 1.405	363	1.551	1.002; 2.407	363	1.549	1.001; 2.404
Sedentary activity, 4y												
<1h			Ref.			Ref.			Ref.			Ref.
≥1h	877	1.232	0.925; 1.642	845	1.229	0.916; 1.648	801	1.136	0.837; 1.540	800	1.159	0.853; 1.574
Sedentary activity, 7y												
<2h			Ref.			Ref.			Ref.			Ref.
≥2h	932	1.233	0.915; 1.662	862	1.256	0.914; 1.724	363	1.092	0.659; 1.804	363	1.107	0.664; 1.840
Physical exercise, 4y												
Yes			Ref.			Ref.			Ref.			Ref.
No	994	1.004	0.769; 1.310	920	1.016	0.771; 1.341	801	0.998	0.740; 1.348	800	0.976	0.722; 1.321
Physical activity, 7y												
Yes			Ref.			Ref.			Ref.			Ref.
No	942	0.995	0.706; 1.410	872	0.983	0.683; 1.414	363	0.643	0.275; 1.528	363	0.647	0.279; 1.528

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age at menarche, type of delivery, breastfeeding category and child's birth weight; Model 3: For exposures at 4 years of age, Model 2 plus physical activity/HEI score/sleep time/sedentary activity at 4 years; For exposures at 7 years of age, Model 2 plus physical activity, HEI score, sleep time and sedentary activity at 4 years, and physical activity/HEI score/sleep time/sedentary activity at 7 years; Model 4: Model 3 plus maternal age and educational level. All the models were adjusted for children's exact age.

Supplementary Table 2. Results from the ordinal logistic regression models assessing the associations between diet quality and lifestyle behaviours and Puberty score at 10 years old, by BMI categories, in males.

	Model 1			Model 2			Model 3			Model 4		
	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI
NORMAL WEIGHT												
Diet quality												
Healthy eating index, 4y	1214	1.022	0.986; 1.060	1151	1.020	0.983; 1.059	1123	1.018	0.980; 1.058	1117	1.017	0.977; 1.058
Healthy eating index, 7y	1316	1.050	1.016; 1.085	1197	1.029	0.994; 1.066	543	1.002	0.931; 1.079	538	1.002	0.929; 1.080
Food groups												
Fruit and vegetables, 4y	1215	1.004	0.934; 1.079	1152	1.007	0.934; 1.085	1124	0.998	0.924; 1.077	1118	0.996	0.922; 1.076
Fruit and vegetables, 7y	1316	1.037	0.964; 1.115	1197	0.995	0.920; 1.075	543	1.015	0.888; 1.160	538	1.022	0.893; 1.169
Energy dense food, 4y	1215	0.987	0.932; 1.044	1152	0.986	0.927; 1.047	1124	0.993	0.932; 1.055	1118	0.998	0.934; 1.065
Energy dense food, 7y	1316	0.986	0.929; 1.047	1197	0.999	0.938; 1.064	543	1.080	0.959; 1.210	538	1.084	0.962; 1.217
Dairy, 4y	1215	1.051	0.965; 1.144	1152	1.049	0.960; 1.146	1124	1.057	0.966; 1.156	1118	1.059	0.967; 1.160
Dairy, 7y	1316	1.033	0.962; 1.110	1197	1.034	0.958; 1.116	543	1.084	0.953; 1.231	538	1.087	0.955; 1.237
Cereal products and potatoes, 4y	1215	1.021	0.931; 1.119	1152	1.008	0.916; 1.108	1124	1.007	0.914; 1.109	1118	1.016	0.921; 1.120
Cereal products and potatoes, 7y	1316	1.097	0.994; 1.210	1197	1.058	0.952; 1.175	543	1.142	0.948; 1.377	538	1.153	0.956; 1.391
Fish and eggs, 4y	1215	1.269	0.824; 1.954	1152	1.193	0.760; 1.886	1124	1.234	0.781; 1.940	1118	1.192	0.747; 1.893
Fish and eggs, 7y	1316	1.014	0.719; 1.428	1197	0.992	0.683; 1.435	543	0.603	0.295; 1.218	538	0.564	0.273; 1.151
Meat and meat products, 4y	1215	0.865	0.686; 1.091	1152	0.854	0.664; 1.089	1124	0.873	0.676; 1.119	1118	0.882	0.684; 1.138
Meat and meat products, 7y	1316	0.916	0.778; 1.079	1197	0.922	0.772; 1.095	543	1.074	0.790; 1.441	538	1.065	0.780; 1.435
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	1215	1.021	0.976; 1.068	1152	1.017	0.971; 1.065	1124	1.014	0.969; 1.063	1118	1.014	0.968; 1.063
Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	1316	1.023	0.981; 1.067	1197	1.018	0.974; 1.065	543	1.035	0.959; 1.116	538	1.039	0.964; 1.121
Processed foods, 4y	1215	0.994	0.748; 1.320	1152	0.948	0.697; 1.279	1124	0.958	0.703; 1.294	1118	0.970	0.710; 1.326
Processed foods, 7y	1316	1.047	0.878; 1.241	1197	0.966	0.795; 1.168	543	1.109	0.823; 1.475	538	1.115	0.827; 1.482
Ultra processed foods, 4y	1215	0.984	0.912; 1.062	1152	0.991	0.914; 1.074	1124	1.002	0.923; 1.087	1118	1.013	0.930; 1.103
Ultra processed foods, 7y	1316	0.977	0.923; 1.035	1197	0.982	0.924; 1.043	543	1.048	0.939; 1.164	538	1.055	0.942; 1.175

Macronutrients												
Energy, 4y	1214	1.017	0.974; 1.062	1151	1.012	0.966; 1.059	1123	1.015	0.969; 1.064	1117	1.020	0.972; 1.069
Energy, 7y	1316	1.017	0.985; 1.050	1197	1.013	0.979; 1.049	543	1.059	1.000; 1.121	538	1.063	1.003; 1.125
Protein, 4y	1214	1.005	0.996; 1.014	1151	1.003	0.994; 1.012	1123	1.004	0.995; 1.013	1117	1.005	0.995; 1.014
Protein, 7y	1316	1.004	0.996; 1.011	1197	1.003	0.995; 1.011	543	1.012	0.998; 1.026	538	1.012	0.998; 1.026
Vegetable protein, 4y	1214	1.002	0.969; 1.035	1151	0.999	0.965; 1.034	1123	0.998	0.964; 1.034	1117	1.001	0.966; 1.037
Vegetable protein, 7y	1316	1.019	0.996; 1.043	1197	1.012	0.987; 1.039	543	1.033	0.989; 1.078	538	1.036	0.992; 1.082
Animal protein, 4y	1214	1.006	0.996; 1.015	1151	1.004	0.994; 1.014	1123	1.005	0.995; 1.015	1117	1.005	0.995; 1.015
Animal protein, 7y	1316	1.002	0.993; 1.011	1197	1.003	0.993; 1.012	543	1.012	0.996; 1.029	538	1.012	0.995; 1.028
Carbohydrate, 4y	1214	1.001	0.998; 1.004	1151	1.001	0.998; 1.004	1123	1.001	0.998; 1.004	1117	1.001	0.998; 1.005
Carbohydrate, 7y	1316	1.001	0.999; 1.003	1197	1.001	0.999; 1.003	543	1.004	1.000; 1.007	538	1.004	1.000; 1.008
Fiber, 4y	1214	1.011	0.972; 1.052	1151	1.013	0.972; 1.056	1123	1.008	0.967; 1.051	1117	1.010	0.968; 1.053
Fiber, 7y	1316	1.022	0.993; 1.052	1197	1.010	0.979; 1.042	543	1.036	0.984; 1.092	538	1.040	0.988; 1.096
Total fat, 4y	1214	1.002	0.990; 1.015	1151	1.000	0.987; 1.014	1123	1.002	0.989; 1.016	1117	1.003	0.990; 1.017
Total fat, 7y	1316	1.004	0.994; 1.014	1197	1.003	0.992; 1.014	543	1.015	0.997; 1.033	538	1.016	0.997; 1.034
Saturated fat, 4y	1214	1.010	0.981; 1.040	1151	1.006	0.976; 1.037	1123	1.010	0.979; 1.042	1117	1.012	0.981; 1.045
Saturated fat, 7y	1316	1.012	0.988; 1.037	1197	1.011	0.985; 1.037	543	1.045	1.002; 1.089	538	1.047	1.003; 1.091
Mufa, 4y	1214	0.998	0.965; 1.031	1151	0.993	0.959; 1.027	1123	0.998	0.963; 1.033	1117	0.999	0.965; 1.035
Mufa, 7y	1316	1.010	0.983; 1.038	1197	1.009	0.979; 1.038	543	1.031	0.981; 1.082	538	1.032	0.983; 1.084
Pufa, 4y	1214	1.021	0.940; 1.109	1151	1.010	0.925; 1.101	1123	1.020	0.934; 1.112	1117	1.025	0.938; 1.119
Pufa, 7y	1316	1.020	0.958; 1.086	1197	1.016	0.948; 1.088	543	1.055	0.939; 1.184	538	1.053	0.938; 1.182
Trans, 4y	1214	1.438	0.920; 2.246	1151	1.360	0.842; 2.185	1123	1.437	0.885; 2.320	1117	1.494	0.914; 2.442
Trans, 7y	1316	1.419	0.983; 2.048	1197	1.342	0.901; 1.973	543	1.876	1.065; 3.194	538	1.924	1.086; 3.299
Micronutrients												
Iron, 4y												
<7mg/day			Ref.			Ref.			Ref.			Ref.
≥7mg/day	1214	0.976	0.759; 1.256	1151	0.987	0.760; 1.282	1123	0.982	0.753; 1.280	1117	0.994	0.761; 1.299
Iron, 7y												
<11mg/day			Ref.			Ref.			Ref.			Ref.
≥11mg/day	1316	0.969	0.724; 1.286	1197	0.994	0.727; 1.348	543	1.180	0.704; 1.936	538	1.219	0.726; 2.009
Vitamin D, 4y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	1214	1.152	0.889; 1.490	1151	1.089	0.833; 1.422	1123	1.119	0.852; 1.467	1117	1.111	0.846; 1.460

Vitamin D, 7y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	1316	1.131	0.889; 1.440	1197	1.115	0.863; 1.442	543	1.039	0.666; 1.618	538	0.998	0.636; 1.562
Calcium, 4y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	1214	1.032	0.755; 1.411	1151	1.029	0.746; 1.433	1123	1.051	0.758; 1.471	1117	1.049	0.752; 1.464
Calcium, 7y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	1316	1.193	0.918; 1.551	1197	1.205	0.914; 1.595	543	1.305	0.822; 2.107	538	1.343	0.844; 2.176
Magnesium, 4y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	1214	1.115	0.785; 1.583	1151	1.160	0.802; 1.659	1123	1.153	0.790; 1.665	1117	1.162	0.795; 1.678
≥250mg/day	1214	1.225	0.864; 1.737	1151	1.209	0.835; 1.733	1123	1.213	0.835; 1.745	1117	1.227	0.841; 1.773
Magnesium, 7y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	1316	1.116	0.780; 1.587	1197	1.027	0.701; 1.492	543	1.146	0.596; 2.151	538	1.164	0.602; 2.197
≥250mg/day	1316	1.145	0.877; 1.497	1197	1.140	0.859; 1.517	543	1.231	0.762; 2.003	538	1.269	0.784; 2.069
Zinc, 4y												
<5.5mg/day			Ref.			Ref.			Ref.			Ref.
≥5.5-<10mg/day	1214	1.872	0.953; 3.678	1151	1.818	0.948; 3.795	1123	1.945	0.991; 4.200	1117	1.931	0.983; 4.173
≥10mg/day	1214	2.216	1.072; 4.580	1151	1.968	0.963; 4.317	1123	2.168	1.038; 4.909	1117	2.191	1.045; 4.980
Zinc, 7y												
<7.4mg/day			Ref.			Ref.			Ref.			Ref.
≥7.4-<13mg/day	1316	1.310	0.945; 1.815	1197	1.408	0.998; 2.014	543	1.688	0.943; 3.201	538	1.722	0.962; 3.265
≥13mg/day	1316	1.209	0.619; 2.359	1197	1.087	0.502; 2.223	543	2.362	0.728; 7.045	538	2.406	0.740; 7.219
Other lifestyle behaviours												
Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	1391	0.873	0.680; 1.120	1271	0.906	0.695; 1.186	1123	0.905	0.682; 1.204	1117	0.898	0.675; 1.196
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	764	0.857	0.595; 1.234	666	1.052	0.705; 1.570	543	1.077	0.679; 1.727	538	1.073	0.673; 1.731
Sedentary activity, 4y												

<1h			Ref.			Ref.			Ref.			Ref.
≥1h	1236	0.996	0.739; 1.342	1180	0.948	0.695; 1.291	1123	0.961	0.698; 1.332	1117	0.965	0.698; 1.334
Sedentary activity, 7y												
<2h			Ref.			Ref.			Ref.			Ref.
≥2h	1317	1.157	0.865; 1.548	1198	1.209	0.890; 1.658	543	1.261	0.751; 2.182	538	1.285	0.762; 2.232
Physical exercise, 4y												
Yes			Ref.			Ref.			Ref.			Ref.
No	1387	0.940	0.729; 1.214	1268	0.903	0.686; 1.182	1123	0.968	0.722; 1.292	1117	0.986	0.732; 1.327
Physical activity, 7y												
Yes			Ref.			Ref.			Ref.			Ref.
No	1333	1.039	0.736; 1.451	1211	1.028	0.709; 1.471	543	0.655	0.257; 1.454	538	0.650	0.255; 1.447
OVERWEIGHT + OBESE												
Diet quality												
Healthy eating index, 4y	899	1.046	1.007; 1.087	848	1.036	0.995; 1.078	826	1.034	0.993; 1.078	821	1.041	0.997; 1.086
Healthy eating index, 7y	996	1.015	0.982; 1.049	901	1.009	0.973; 1.046	401	0.982	0.915; 1.053	400	0.987	0.919; 1.060
Food groups												
Fruit and vegetables, 4y	899	0.991	0.920; 1.068	848	0.979	0.905; 1.060	826	0.968	0.892; 1.050	821	0.980	0.902; 1.065
Fruit and vegetables, 7y	996	0.979	0.911; 1.051	901	0.973	0.901; 1.051	401	0.904	0.788; 1.035	400	0.905	0.788; 1.038
Energy dense food, 4y	899	0.939	0.881; 0.998	848	0.951	0.891; 1.012	826	0.957	0.896; 1.020	821	0.950	0.888; 1.014
Energy dense food, 7y	996	0.993	0.937; 1.052	901	0.967	0.908; 1.029	401	1.011	0.906; 1.125	400	0.992	0.885; 1.107
Dairy, 4y	899	0.991	0.906; 1.083	848	0.967	0.880; 1.061	826	0.972	0.883; 1.068	821	0.966	0.876; 1.064
Dairy, 7y	996	1.055	0.981; 1.134	901	1.015	0.940; 1.096	401	1.052	0.925; 1.194	400	1.036	0.910; 1.178
Cereal products and potatoes, 4y	899	1.027	0.933; 1.131	848	1.006	0.911; 1.111	826	1.013	0.915; 1.120	821	1.013	0.915; 1.121
Cereal products and potatoes, 5y	996	0.991	0.893; 1.099	901	0.962	0.861; 1.074	401	1.012	0.848; 1.207	400	0.996	0.835; 1.189
Fish and eggs, 4y	899	1.637	1.095; 2.458	848	1.579	1.038; 2.409	826	1.645	1.075; 2.529	821	1.685	1.091; 2.617
Fish and eggs, 7y	996	1.193	0.839; 1.697	901	1.048	0.721; 1.520	401	0.994	0.536; 1.845	400	1.054	0.565; 1.968
Meat and meat products, 4y	899	0.976	0.785; 1.212	848	0.947	0.754; 1.190	826	0.936	0.739; 1.178	821	0.951	0.749; 1.198
Meat and meat products, 7y	996	1.006	0.865; 1.170	901	0.959	0.811; 1.128	401	0.869	0.635; 1.166	400	0.850	0.620; 1.143
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	899	1.025	0.980; 1.073	848	1.016	0.969; 1.066	826	1.019	0.971; 1.070	821	1.022	0.973; 1.073

Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	996	1.007	0.964; 1.052	901	0.994	0.948; 1.041	401	0.969	0.895; 1.047	400	0.962	0.889; 1.041
Processed foods, 4y	899	0.835	0.613; 1.126	848	0.792	0.576; 1.089	826	0.820	0.590; 1.128	821	0.791	0.566; 1.095
Processed foods, 7y	996	1.068	0.907; 1.257	901	1.012	0.849; 1.201	401	1.209	0.893; 1.624	400	1.190	0.877; 1.603
Ultra processed foods, 4y	899	0.932	0.857; 1.012	848	0.940	0.862; 1.023	826	0.941	0.862; 1.026	821	0.930	0.849; 1.017
Ultra processed foods, 7y	996	0.991	0.937; 1.046	901	0.970	0.913; 1.031	401	1.016	0.911; 1.129	400	0.995	0.889; 1.110
Macronutrients												
Energy, 4y	899	1.001	0.956; 1.048	848	0.991	0.943; 1.040	826	0.996	0.948; 1.046	821	0.996	0.948; 1.047
Energy, 7y	995	1.011	0.980; 1.043	901	0.995	0.962; 1.029	401	1.018	0.960; 1.080	400	1.011	0.952; 1.073
Protein, 4y	899	1.006	0.997; 1.015	848	1.003	0.993; 1.012	826	1.004	0.994; 1.014	821	1.004	0.994; 1.014
Protein, 7y	995	1.005	0.998; 1.013	901	1.002	0.994; 1.010	401	1.001	0.988; 1.015	400	1.000	0.987; 1.013
Vegetable protein, 4y	899	0.991	0.957; 1.027	848	0.986	0.950; 1.023	826	0.988	0.952; 1.026	821	0.989	0.952; 1.027
Vegetable protein, 7y	995	0.996	0.973; 1.021	901	0.991	0.965; 1.017	401	0.996	0.953; 1.041	400	0.992	0.949; 1.037
Animal protein, 4y	899	1.008	0.998; 1.017	848	1.004	0.994; 1.015	826	1.005	0.995; 1.016	821	1.006	0.995; 1.016
Animal protein, 7y	995	1.007	0.999; 1.016	901	1.003	0.994; 1.013	401	1.002	0.987; 1.018	400	1.001	0.985; 1.017
Carbohydrate, 4y	899	0.999	0.996; 1.002	848	0.999	0.995; 1.002	826	0.999	0.996; 1.002	821	0.999	0.995; 1.002
Carbohydrate, 7y	995	1.000	0.998; 1.002	901	0.999	0.997; 1.001	401	1.001	0.997; 1.004	400	1.000	0.996; 1.004
Fiber, 4y	899	0.995	0.956; 1.036	848	0.991	0.949; 1.034	826	0.989	0.947; 1.033	821	0.994	0.951; 1.039
Fiber, 7y	995	0.992	0.964; 1.022	901	0.986	0.956; 1.018	401	0.982	0.930; 1.038	400	0.980	0.927; 1.035
Total fat, 4y	899	1.003	0.990; 1.016	848	0.999	0.986; 1.013	826	1.001	0.987; 1.015	821	1.001	0.987; 1.015
Total fat, 7y	995	1.007	0.997; 1.017	901	1.003	0.992; 1.013	401	1.014	0.996; 1.033	400	1.012	0.994; 1.031
Saturated fat, 4y	899	1.004	0.975; 1.034	848	0.995	0.964; 1.027	826	0.998	0.967; 1.031	821	0.999	0.966; 1.031
Saturated fat, 7y	995	1.019	0.995; 1.043	901	1.007	0.982; 1.033	401	1.052	1.005; 1.100	400	1.047	1.000; 1.096
Mufa, 4y	899	1.009	0.976; 1.043	848	1.000	0.965; 1.036	826	1.003	0.968; 1.040	821	1.004	0.968; 1.041
Mufa, 7y	995	1.016	0.989; 1.044	901	1.008	0.979; 1.038	401	1.019	0.970; 1.070	400	1.014	0.966; 1.066
Pufa, 4y	899	1.067	0.976; 1.166	848	1.047	0.953; 1.150	826	1.060	0.963; 1.167	821	1.065	0.968; 1.173
Pufa, 7y	995	1.017	0.956; 1.080	901	0.998	0.933; 1.065	401	1.015	0.908; 1.136	400	1.009	0.902; 1.129
Trans, 4y	899	1.122	0.722; 1.744	848	0.948	0.590; 1.509	826	0.982	0.606; 1.573	821	0.976	0.601; 1.571
Trans, 7y	995	1.440	0.992; 2.092	901	1.208	0.804; 1.804	401	2.811	1.386; 5.743	400	2.700	1.323; 5.543
Micronutrients												
Iron, 4y												
<7mg/day			Ref.			Ref.			Ref.			Ref.

≥7mg/day	899	0.946	0.727; 1.231	848	0.919	0.699; 1.208	826	0.979	0.741; 1.293	821	0.985	0.744; 1.302
Iron, 7y												
<11mg/day			Ref.			Ref.			Ref.			Ref.
≥11mg/day	995	0.944	0.689; 1.293	901	0.825	0.582; 1.159	401	0.625	0.322; 1.150	400	0.578	0.296; 1.072
Vitamin D, 4y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	899	1.389	1.064; 1.813	848	1.347	1.021; 1.777	826	1.431	1.079; 1.897	821	1.472	1.105; 1.960
Vitamin D, 7y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	995	1.211	0.943; 1.555	901	1.104	0.848; 1.437	401	1.019	0.660; 1.574	400	1.034	0.668; 1.596
Calcium, 4y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	899	1.005	0.726; 1.400	848	0.914	0.649 1.287	826	0.947	0.669; 1.351	821	0.952	0.671; 1.360
Calcium, 7y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	995	1.304	0.996; 1.714	901	1.196	0.901; 1.595	401	1.674	1.045; 2.728	400	1.612	1.002; 2.639
Magnesium, 4y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	899	1.255	0.853; 1.830	848	1.264	0.849; 1.866	826	1.255	0.838; 1.864	821	1.260	0.840; 1.874
≥250mg/day	899	1.173	0.811; 1.683	848	1.035	0.699; 1.519	826	1.073	0.722; 1.579	821	1.083	0.728; 1.597
Magnesium, 7y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	995	1.196	0.836; 1.712	901	1.209	0.829; 1.755	401	1.770	0.977; 3.179	400	1.751	0.965; 3.148
≥250mg/day	995	1.004	0.763; 1.322	901	0.870	0.650; 1.162	401	0.819	0.502; 1.329	400	0.790	0.483; 1.285
Zinc, 4y												
<5.5mg/day			Ref.			Ref.			Ref.			Ref.
≥5.5-<10mg/day	899	1.478	0.720; 3.036	848	1.385	0.667; 3.109	826	1.634	0.736; 4.010	821	1.699	0.764; 4.173
≥10mg/day	899	1.442	0.667; 3.118	848	1.202	0.545; 2.835	826	1.457	0.619; 3.742	821	1.530	0.648; 3.939
Zinc, 7y												
<7.4mg/day			Ref.			Ref.			Ref.			Ref.
≥7.4-<13mg/day	995	1.272	0.916; 1.765	901	1.110	0.789; 1.575	401	1.119	0.654; 1.965	400	1.105	0.644; 1.943
≥13mg/day	995	1.385	0.700; 2.741	901	1.153	0.545; 2.369	401	1.197	0.286; 4.299	400	1.063	0.251; 3.869
Other lifestyle behaviours												

Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	1055	1.211	0.937; 1.569	966	1.191	0.911; 1.561	826	1.173	0.879; 1.570	821	1.188	0.889; 1.593
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	566	0.688	0.480; 0.988	498	0.679	0.461; 1.005	401	0.708	0.441; 1.140	400	0.687	0.425; 1.111
Sedentary activity, 4y												
<1h			Ref.			Ref.			Ref.			Ref.
≥1h	924	0.951	0.687; 1.318	872	0.906	0.650; 1.271	826	0.978	0.692; 1.390	821	0.988	0.699; 1.405
Sedentary activity, 7y												
<2h			Ref.			Ref.			Ref.			Ref.
≥2h	1002	1.059	0.778; 1.450	911	1.088	0.787; 1.513	401	1.007	0.577; 1.804	400	0.970	0.553; 1.745
Physical exercise, 4y												
Yes			Ref.			Ref.			Ref.			Ref.
No	1048	0.963	0.744; 1.245	960	1.030	0.786; 1.345	826	1.054	0.785; 1.410	821	1.001	0.743; 1.347
Physical activity, 7y												
Yes			Ref.			Ref.			Ref.			Ref.
No	1013	1.420	1.028; 1.961	919	1.377	0.974; 1.938	401	1.547	0.785; 2.963	400	1.447	0.729; 2.793

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age at menarche, type of delivery, breastfeeding category and child's birth weight; Model 3: For exposures at 4 years of age, Model 2 plus physical activity/HEI score/sleep time/sedentary activity at 4 years; For exposures at 7 years of age, Model 2 plus physical activity, HEI score, sleep time and sedentary activity at 4 years, and physical activity/HEI score/sleep time/sedentary activity at 7 years; Model 4: Model 3 plus maternal age and educational level. All the models were adjusted for children's exact age.

Supplementary Table 3. Results from the Cox proportional hazards regression for time to event assessing the associations between diet quality and lifestyle behaviours and age at menarche, by BMI categories.

	Model 1			Model 2			Model 3			Model 4		
	n	β	95% CI	n	β	95% CI	n	β	95% CI	n	β	95% CI
NORMAL WEIGHT												
Diet quality												
Healthy eating index, 4y	962	**0.940**	**0.902; 0.981**	920	**0.948**	**0.907; 0.991**	888	**0.947**	**0.906; 0.992**	886	**0.955**	**0.912; 0.999**
Healthy eating index, 7y	1032	**0.954**	**0.916; 0.993**	947	0.963	0.922; 1.005	442	0.995	0.927; 1.069	442	1.024	0.955; 1.099
Food groups												
Fruit and vegetables, 4y	963	0.990	0.905; 1.084	920	1.004	0.910; 1.109	888	1.009	0.912; 1.117	886	1.032	0.932; 1.142
Fruit and vegetables, 7y	1032	0.980	0.895; 1.073	947	1.016	0.920; 1.122	442	1.045	0.898; 1.215	442	1.133	0.964; 1.331
Energy dense food, 4y	963	**1.101**	**1.036; 1.170**	920	**1.095**	**1.024; 1.172**	888	**1.091**	**1.018; 1.169**	886	**1.081**	**1.004; 1.163**
Energy dense food, 7y	1032	**1.084**	**1.011; 1.163**	947	**1.089**	**1.008; 1.176**	442	0.940	0.825; 1.069	442	0.879	0.763; 1.013
Dairy, 4y	963	1.013	0.908; 1.132	920	1.004	0.890; 1.133	888	1.006	0.888; 1.138	886	1.004	0.881; 1.144
Dairy, 7y	1032	0.978	0.896; 1.067	947	1.016	0.922; 1.120	442	0.981	0.861; 1.118	442	0.960	0.828; 1.113
Cereal products and potatoes, 4y	963	0.934	0.839; 1.041	920	0.974	0.869; 1.093	888	0.959	0.851; 1.080	886	0.943	0.833; 1.068
Cereal products and potatoes, 7y	1032	**0.890**	**0.796; 0.996**	947	0.919	0.814; 1.036	442	0.879	0.744; 1.038	442	0.863	0.723; 1.031
Fish and eggs, 4y	963	0.661	0.398; 1.099	920	0.679	0.400; 1.153	888	0.667	0.388; 1.145	886	0.730	0.422; 1.262
Fish and eggs, 7y	1032	1.181	0.770; 1.814	947	1.136	0.708; 1.824	442	0.769	0.380; 1.555	442	1.072	0.515; 2.234
Meat and meat products, 4y	963	1.046	0.822; 1.330	920	1.053	0.814; 1.362	888	1.121	0.850; 1.479	886	1.086	0.825; 1.429
Meat and meat products, 7y	1032	1.159	0.963; 1.395	947	**1.268**	**1.029; 1.562**	442	**1.383**	**1.043; 1.834**	442	**1.344**	**1.007; 1.794**
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	963	1.000	0.999; 1.001	920	1.000	0.999; 1.001	888	1.000	0.999; 1.001	886	1.000	0.999; 1.001
Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	1032	0.999	0.999; 1.000	947	1.000	0.999; 1.001	442	0.999	0.999; 1.001	442	1.000	0.999; 1.001
Processed foods, 4y	963	1.001	0.997; 1.005	920	1.002	0.998; 1.007	888	1.003	0.998; 1.007	886	1.002	0.998; 1.007
Processed foods, 7y	1032	1.002	0.999; 1.005	947	1.002	0.999; 1.005	442	1.002	0.998; 1.006	442	1.001	0.997; 1.005
Ultra processed foods, 4y	963	**1.001**	**1.000; 1.002**	920	1.001	0.999; 1.002	888	1.001	0.999; 1.00	888	1.001	0.999; 1.002
Ultra processed foods, 7y	1032	**1.001**	**1.000; 1.002**	947	**1.001**	**1.000; 1.00**	442	0.999	0.865; 1.001	442	0.999	0.998; 1.001

Macronutrients												
Energy, 4y	962	1.021	0.968; 1.077	920	1.036	0.978; 1.097	888	1.034	0.975; 1.097	886	1.034	0.973; 1.098
Energy, 7y	1032	1.013	0.974; 1.054	947	1.031	0.987; 1.077	442	0.981	0.921; 1.046	442	0.976	0.911; 1.045
Protein, 4y	962	1.000	0.990; 1.011	920	1.002	0.991; 1.014	888	1.003	0.991; 1.015	886	1.004	0.992; 1.016
Protein, 7y	1032	1.000	0.992; 1.009	947	1.004	0.994; 1.013	442	0.998	0.983; 1.014	442	0.999	0.982; 1.015
Vegetable protein, 4y	962	0.986	0.944; 1.031	920	1.010	0.964; 1.059	888	1.004	0.957; 1.054	886	1.004	0.956; 1.055
Vegetable protein, 7y	1032	0.993	0.964; 1.022	947	1.004	0.972; 1.036	442	0.981	0.937; 1.026	442	0.984	0.938; 1.032
Animal protein, 4y	962	1.001	0.989; 1.013	920	1.002	0.989; 1.015	888	1.003	0.990; 1.017	886	1.004	0.991; 1.018
Animal protein, 7y	1032	1.001	0.991; 1.011	947	1.005	0.994; 1.016	442	1.001	0.982; 1.020	442	1.001	0.981; 1.021
Carbohydrate, 4y	962	1.002	0.998; 1.006	920	1.004	0.999; 1.008	888	1.003	0.999; 1.008	886	1.003	0.999; 1.008
Carbohydrate, 7y	1032	1.001	0.999; 1.004	947	1.002	0.999; 1.005	442	0.999	0.995; 1.003	442	0.990	0.995; 1.003
Fiber, 4y	962	0.996	0.945; 1.050	920	1.019	0.961; 1.081	888	1.015	0.956; 1.078	886	1.023	0.964; 1.086
Fiber, 7y	1032	1.002	0.966; 1.039	947	1.021	0.981; 1.063	442	1.005	0.948; 1.066	442	1.023	0.961; 1.090
Total fat, 4y	962	1.002	0.987; 1.017	920	1.004	0.989; 1.020	888	1.005	0.988; 1.021	886	1.004	0.988; 1.021
Total fat, 7y	1032	1.002	0.990; 1.014	947	1.005	0.992; 1.019	442	0.986	0.965; 1.007	442	0.984	0.962; 1.005
Saturated fat, 4y	962	1.010	0.977; 1.045	920	1.014	0.978; 1.051	888	1.014	0.978; 1.053	886	1.013	0.975; 1.053
Saturated fat, 7y	1032	1.000	0.971; 1.031	947	1.009	0.976; 1.043	442	0.959	0.911; 1.009	442	0.947	0.896; 1.000
Mufa, 4y	962	1.002	0.963; 1.044	920	1.010	0.968; 1.055	888	1.013	0.969; 1.059	886	1.013	0.969; 1.059
Mufa, 7y	1032	1.005	0.972; 1.038	947	1.013	0.976; 1.050	442	0.966	0.910; 1.024	442	0.963	0.907; 1.023
Pufa, 4y	962	0.989	0.894; 1.094	920	1.015	0.913; 1.129	888	1.015	0.909; 1.133	886	1.020	0.915; 1.136
Pufa, 7y	1032	1.042	0.966; 1.124	947	1.060	0.975; 1.152	442	0.969	0.857; 1.096	442	0.981	0.862; 1.116
Trans, 4y	962	1.398	0.817; 2.390	920	1.452	0.822; 2.562	888	1.487	0.823; 2.687	886	1.540	0.834; 2.843
Trans, 7y	1032	1.011	0.625; 1.634	947	1.124	0.662; 1.909	442	0.547	0.243; 1.230	442	0.430	0.179; 1.032
Micronutrients												
Iron, 4y												
<7mg/day			Ref.			Ref.			Ref.			Ref.
≥7mg/day	962	1.082	0.784; 1.494	920	1.200	0.849; 1.697	888	1.201	0.846; 1.704	886	1.213	0.845; 1.742
Iron, 7y												
<11mg/day			Ref.			Ref.			Ref.			Ref.
≥11mg/day	1032	1.209	0.825; 1.772	947	1.465	0.962; 2.233	442	1.343	0.729; 2.476	442	1.398	0.762; 2.566
Vitamin D, 4y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	962	0.744	0.536; 1.032	920	0.817	0.577; 1.157	888	0.772	0.540; 1.103	886	0.800	0.557; 1.147

Vitamin D, 7y												
<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
≥2.4347mg/day	1032	1.125	0.821; 1.541	947	1.169	0.832; 1.643	442	0.935	0.578; 1.511	442	1.227	0.744; 2.022
Calcium, 4y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	962	1.402	0.950; 2.071	920	1.382	0.915; 2.088	888	1.418	0.923; 2.177	886	1.416	0.906; 2.213
Calcium, 7y												
<800mg/day			Ref.			Ref.			Ref.			Ref.
≥800mg/day	1032	0.987	0.718; 1.358	947	1.000	0.708; 1.413	442	1.239	0.775; 1.982	442	1.215	0.732; 2.017
Magnesium, 4y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	962	0.876	0.552; 1.388	920	0.921	0.555; 1.530	888	0.917	0.546; 1.539	886	0.966	0.574; 1.627
≥250mg/day	962	1.184	0.767; 1.827	920	1.436	0.896; 2.301	888	1.470	0.906; 2.384	886	1.483	0.903; 2.437
Magnesium, 7y												
<230mg/day			Ref.			Ref.			Ref.			Ref.
≥230-<250mg/day	1032	0.736	0.456; 1.187	947	0.817	0.495; 1.351	442	0.616	0.273; 1.387	442	0.601	0.257; 1.408
≥250mg/day	1032	0.874	0.625; 1.223	947	1.025	0.705; 1.490	442	0.994	0.588; 1.680	442	1.038	0.600; 1.795
Zinc, 4y												
<5.5mg/day			Ref.			Ref.			Ref.			Ref.
≥5.5-<10mg/day	962	1.010	0.517; 1.973	920	1.008	0.489; 2.078	861	0.983	0.474; 2.036	886	1.086	0.520; 2.268
≥10mg/day	962	1.073	0.510; 2.258	920	1.153	0.515; 2.581	861	1.138	0.504; 2.567	886	1.279	0.555; 2.949
Zinc, 7y												
<7.4mg/day			Ref.			Ref.			Ref.			Ref.
≥7.4-<13mg/day	1032	0.922	0.640; 1.328	901	0.948	0.634; 1.417	442	0.922	0.514; 1.652	442	0.943	0.514; 1.728
≥13mg/day	1032	1.154	0.546; 2.439	901	1.256	0.528; 2.986	442	1.063	0.271; 4.161	442	2.081	0.450; 9.632
Other lifestyle behaviours												
Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	1101	1.006	0.738; 1.371	1014	0.911	0.647; 1.282	888	0.931	0.636; 1.361	886	0.975	0.661; 1.473
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	597	0.847	0.576; 1.244	547	0.885	0.577; 1.356	442	0.837	0.480; 1.460	442	0.925	0.524; 1.634
Sedentary activity, 4y												

<1h			Ref.			Ref.			Ref.			Ref.
≥1h	986	1.381	0.944; 2.020	941	1.562	1.040; 2.345	888	1.323	0.860; 2.036	886	1.289	0.835; 1.990
Sedentary activity, 7y												
<2h			Ref.			Ref.			Ref.			
≥2h	1036	0.946	0.654; 1.368	952	0.861	0.579; 1.282	442	0.709	0.398; 1.261	442	0.596	0.320; 1.111
Physical exercise, 4y												
Yes			Ref.			Ref.			Ref.			Ref.
No	1096	0.977	0.705; 1.355	1009	0.804	0.556; 1.163	888	0.916	0.614; 1.367	886	0.876	0.581; 1.321
Physical activity, 7y												
Yes			Ref.			Ref.			Ref.			Ref.
No	1047	1.076	0.693; 1.671	960	0.885	0.536; 1.460	442	1.208	0.221; 1.301	442	0.377	0.145; 1.001
OVERWEIGHT + OBESE												
Diet quality												
Healthy eating index, 4y	634	0.995	0.952; 1.041	607	1.007	0.961; 1.055	590	1.009	0.958; 1.062	589	1.037	0.981; 1.096
Healthy eating index, 7y	679	0.961	0.912; 1.012	630	0.937	0.887; 0.991	292	**0.863**	**0.778; 0.957**	292	**0.853**	**0.767; 0.950**
Food groups												
Fruit and vegetables, 4y	634	**1.102**	**1.003; 1.210**	607	1.089	0.987; 1.201	590	1.094	0.983; 1.217	589	**1.167**	**1.040; 1.309**
Fruit and vegetables, 7y	679	1.072	0.970; 1.185	630	1.042	0.941; 1.153	292	0.976	0.821; 1.160	292	0.975	0.811; 1.172
Energy dense food, 4y	634	**1.124**	**1.041; 1.214**	607	**1.129**	**1.040; 1.226**	590	**1.159**	**1.060; 1.266**	589	**1.134**	**1.032; 1.246**
Energy dense food, 7y	679	1.045	0.965; 1.131	630	1.090	0.998; 1.190	292	0.992	0.851; 1.156	292	0.993	0.848; 1.163
Dairy, 4y	634	**1.145**	**1.019; 1.287**	607	**1.207**	**1.073; 1.359**	590	**1.204**	**1.067; 1.359**	589	**1.181**	**1.045; 1.335**
Dairy, 7y	679	0.994	0.892; 1.109	630	0.996	0.886; 1.119	292	0.840	0.682; 1.034	292	0.839	0.679; 1.035
Cereal products and potatoes, 4y	634	1.069	0.944; 1.211	607	1.042	0.914; 1.189	590	1.021	0.893; 1.168	589	1.014	0.887; 1.160
Cereal products and potatoes, 7y	679	1.003	0.872; 1.153	630	0.942	0.810; 1.096	292	0.959	0.765; 1.203	292	0.956	0.761; 1.201
Fish and eggs, 4y	634	1.486	0.823; 2.682	607	1.466	0.803; 2.677	590	1.626	0.858; 3.082	589	**2.062**	**1.065; 3.990**
Fish and eggs, 7y	679	1.137	0.720; 1.795	630	1.240	0.759; 2.025	292	0.944	0.435; 2.046	292	0.967	0.439; 2.133
Meat and meat products, 4y	634	1.132	0.871; 1.469	607	1.052	0.796; 1.391	590	1.039	0.779; 1.385	589	1.004	0.753; 1.338
Meat and meat products, 7y	679	**1.312**	**1.030; 1.671**	630	**1.357**	**1.042; 1.767**	292	**1.743**	**1.093; 2.778**	292	**1.727**	**1.081; 2.761**
Ultraprocessed foods												
Unprocessed and minimally processed foods + Processed culinary ingredients, 4y	634	**1.001**	**1.000; 1.002**	607	**1.001**	**1.001; 1.002**	590	**1.001**	**1.000; 1.002**	589	**1.001**	**1.001; 1.002**

Unprocessed and minimally processed foods + Processed culinary ingredients, 7y	679	1.000	0.999; 1.001	630	1.000	0.999; 1.001	292	0.999	0.998; 1.001	292	0.999	0.998; 1.001
Processed foods, 4y	634	1.003	0.999; 1.006	607	1.002	0.998; 1.006	590	1.002	0.998; 1.006	589	1.002	0.997; 1.006
Processed foods, 7y	679	1.000	0.998; 1.003	630	1.000	0.997; 1.003	292	1.001	0.996; 1.007	292	1.001	0.996; 1.006
Ultra processed foods, 4y	634	**1.002**	**1.000; 1.003**	607	**1.002**	**1.000; 1.003**	590	**1.002**	**1.001; 1.003**	589	**1.001**	**1.000; 1.003**
Ultra processed foods, 7y	679	1.000	0.999; 1.001	630	1.000	0.999; 1.001	292	1.000	0.998; 1.002	292	1.000	0.998; 1.002
Macronutrients												
Energy, 4y	634	**1.141**	**1.073; 1.213**	607	**1.142**	**1.073; 1.216**	590	**1.139**	**1.068; 1.215**	589	**1.130**	**1.060; 1.204**
Energy, 7y	679	1.039	0.993; 1.088	630	**1.052**	**1.000; 1.106**	292	1.030	0.944; 1.124	292	1.032	0.946; 1.126
Protein, 4y	634	**1.023**	**1.011; 1.035**	607	**1.025**	**1.013; 1.038**	590	**1.025**	**1.013; 1.038**	589	**1.025**	**1.012; 1.038**
Protein, 7y	679	1.009	0.998; 1.020	630	1.009	0.998; 1.021	292	1.007	0.986; 1.028	292	1.007	0.987; 1.029
Vegetable protein, 4y	634	**1.071**	**1.022; 1.234**	607	**1.061**	**1.010; 1.114**	590	**1.053**	**1.002; 1.106**	589	**1.051**	**1.001; 1.104**
Vegetable protein, 7y	679	1.015	0.979; 1.051	630	1.014	0.975; 1.054	292	1.011	0.952; 1.074	292	1.011	0.952; 1.074
Animal protein, 4y	634	**1.022**	**1.010; 1.036**	607	**1.026**	**1.013; 1.040**	590	**1.027**	**1.013; 1.041**	589	**1.027**	**1.013; 1.041**
Animal protein, 7y	679	1.011	0.998; 1.024	630	1.011	0.998; 1.025	292	1.007	0.983; 1.031	292	1.008	0.984; 1.032
Carbohydrate, 4y	634	**1.009**	**1.005; 1.014**	607	**1.009**	**1.004; 1.013**	590	**1.009**	**1.004; 1.013**	589	**1.008**	**1.003; 1.013**
Carbohydrate, 7y	679	1.002	0.999; 1.005	630	1.003	0.999; 1.007	292	1.002	0.996; 1.007	292	1.002	0.996; 1.007
Fiber, 4y	634	**1.091**	**1.033; 1.152**	607	**1.078**	**1.020; 1.140**	590	**1.074**	**1.014; 1.137**	589	**1.094**	**1.032; 1.159**
Fiber, 7y	679	1.035	0.991; 1.082	630	1.036	0.989; 1.087	292	1.022	0.942; 1.108	292	1.024	0.941; 1.114
Total fat, 4y	634	**1.031**	**1.014; 1.049**	607	**1.035**	**1.017; 1.053**	590	**1.034**	**1.015; 1.053**	589	**1.033**	**1.014; 1.051**
Total fat, 7y	679	1.013	0.998; 1.028	630	1.014	0.999; 1.030	292	1.007	0.979; 1.035	292	1.008	0.980; 1.036
Saturated fat, 4y	634	**1.060**	**1.019; 1.104**	607	**1.073**	**1.029; 1.118**	590	**1.074**	**1.029; 1.121**	589	**1.068**	**1.024; 1.114**
Saturated fat, 7y	679	1.025	0.989; 1.062	630	1.027	0.989; 1.067	292	1.004	0.936; 1.077	292	1.006	0.939; 1.079
Mufa, 4y	634	**1.088**	**1.041; 1.137**	607	**1.095**	**1.046; 1.146**	590	**1.090**	**1.040; 1.142**	589	**1.090**	**1.041; 1.142**
Mufa, 7y	679	1.027	0.988; 1.069	630	1.035	0.993; 1.078	292	1.002	0.928; 1.083	292	1.003	0.928; 1.085
Pufa, 4y	634	**1.213**	**1.086; 1.356**	607	**1.195**	**1.066; 1.340**	590	**1.189**	**1.057; 1.337**	589	**1.193**	**1.062; 1.340**
Pufa, 7y	679	**1.100**	**1.007; 1.202**	630	**1.109**	**1.013; 1.214**	292	1.102	0.954; 1.274	292	1.109	0.959; 1.282
Trans, 4y	634	**1.997**	**1.145; 3.483**	607	**2.368**	**1.366; 4.108**	590	**2.362**	**1.377; 4.050**	589	**2.259**	**1.309; 3.899**
Trans, 7y	679	1.419	0.876; 2.298	630	1.543	0.951; 2.503	292	1.341	0.554; 3.244	292	1.380	0.590; 3.231
Micronutrients												
Iron, 4y												
<7mg/day		Ref.			Ref.			Ref.			Ref.	

	≥7mg/day	634	**1.744**	**1.229; 2.474**	607	**2.015**	**1.385; 2.932**	590	**2.074**	**1.409; 3.053**	589	**2.076**	**1.411; 3.056**
Iron, 7y													
	<11mg/day			Ref.			Ref.			Ref.			Ref.
	≥11mg/day	679	1.278	0.826; 1.977	630	1.425	0.884; 2.297	292	1.782	0.863; 3.680	292	1.826	0.881; 3.783
Vitamin D, 4y													
	<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
	≥2.4347mg/day	634	1.156	0.822; 1.626	607	1.138	0.795; 1.630	590	1.206	0.822; 1.767	589	1.332	0.904; 1.965
Vitamin D, 7y													
	<2.4347mg/day			Ref.			Ref.			Ref.			Ref.
	≥2.4347mg/day	679	1.234	0.871; 1.747	630	**1.464**	**1.009; 2.214**	292	1.381	0.798; 2.389	292	1.405	0.811; 2.434
Calcium, 4y													
	<800mg/day			Ref.			Ref.			Ref.			Ref.
	≥800mg/day	634	**2.342**	**1.452; 3.776**	607	**3.153**	**1.884; 5.279**	590	**3.211**	**1.900; 5.425**	589	**3.063**	**1.817; 5.164**
Calcium, 7y													
	<800mg/day			Ref.			Ref.			Ref.			Ref.
	≥800mg/day	679	0.999	0.697; 1.431	630	0.925	0.630; 1.359	292	**0.428**	**0.218; 0.838**	292	**0.430**	**0.219; 0.845**
Magnesium, 4y													
	<230mg/day			Ref.			Ref.			Ref.			Ref.
	≥230-<250mg/day	634	1.334	0.833; 2.137	607	1.444	0.864; 2.411	590	1.480	0.871; 2.516	589	1.505	0.886; 2.556
	≥250mg/day	634	**1.971**	**1.246; 3.119**	607	**2.139**	**1.315; 3.480**	590	**2.199**	**1.331; 3.633**	589	**2.151**	**1.308; 3.538**
Magnesium, 7y													
	<230mg/day			Ref.			Ref.			Ref.			Ref.
	≥230-<250mg/day	679	0.724	0.413; 1.268	630	0.692	0.380; 1.258	292	0.518	0.223; 1.205	292	0.512	0.217; 1.209
	≥250mg/day	679	1.027	0.706; 1.495	630	1.113	0.749; 1.653	292	0.872	0.456; 1.666	292	0.872	0.451; 1.685
Zinc, 4y													
	<5.5mg/day			Ref.			Ref.			Ref.			Ref.
	≥5.5-<10mg/day	634	1.253	0.597; 2.631	607	1.545	0.731; 3.269	590	1.545	0.729; 3.378	589	1.604	0.762; 3.376
	≥10mg/day	634	**2.374**	**1.060; 5.315**	607	**3.317**	**1.456; 7.555**	590	**3.309**	**1.432; 7.650**	589	**3.228**	**1.411; 7.383**
Zinc, 7y													
	<7.4mg/day			Ref.			Ref.			Ref.			Ref.
	≥7.4-<13mg/day	679	0.910	0.605; 1.369	630	0.959	0.630; 1.462	292	**0.411**	**0.190; 0.984**	292	**0.415**	**0.190; 0.903**
	≥13mg/day	679	1.768	0.538; 5.812	630	2.129	0.638; 7.106	292	2.585	0.304; 21.970	292	2.700	0.315; 23.170
Other lifestyle behaviours													

Sleep time, 4y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	720	1.306	0.924; 1.884	670	1.312	0.903; 1.910	590	1.374	0.913; 2.069	589	1.405	0.926; 2.131
Sleep time, 7y												
<10h			Ref.			Ref.			Ref.			Ref.
≥10h	389	1.070	0.680; 1.686	359	1.143	0.684; 1.911	292	1.594	0.832; 3.057	292	1.598	0.826; 3.093
Sedentary activity, 4y												
<1h			Ref.			Ref.			Ref.			Ref.
≥1h	644	1.333	0.887; 2.001	618	1.355	0.886; 2.073	590	1.517	0.959; 2.399	589	1.501	0.947; 2.380
Sedentary activity, 7y												
<2h			Ref.			Ref.			Ref.			Ref.
≥2h	679	0.871	0.571; 1.328	630	0.775	0.491; 1.223	292	0.544	0.288; 1.029	292	0.533	0.280; 1.015
Physical exercise, 4y												
Yes			Ref.			Ref.			Ref.			Ref.
No	713	0.903	0.629; 1.297	664	1.003	0.682; 1.475	590	0.879	0.565; 1.369	589	0.778	0.492; 1.230
Physical activity, 7y												
Yes			Ref.			Ref.			Ref.			Ref.
No	687	**1.945**	**1.172; 3.228**	639	1.609	0.922; 2.807	292	**3.319**	**1.079; 10.211**	292	**3.223**	**1.042; 9.967**

Statistically significant association are highlighted in bold. CI, confidence interval. Model 1: crude; Model 2: adjusted for the ID number of the physical examiner plus maternal age at menarche, type of delivery, breastfeeding category and child's birth weight; Model 3: For exposures at 4 years of age, Model 2 plus physical activity/HEI score/sleep time/sedentary activity at 4 years; For exposures at 7 years of age, Model 2 plus physical activity, HEI score, sleep time and sedentary activity at 4 years, and physical activity/HEI score/sleep time/sedentary activity at 7 years; Model 4: Model 3 plus maternal age and educational level. All the models were adjusted for children's exact age.

Paper V

Early-life Urban Environment, Nutrition, and Pubertal Timing in Southern Europe: An Exposome Analysis

Marta Pinto da Costa, María Arteaga Jover, Adrià Setó Llorens, Aurélie Portefaix, Ana Isabel Ribeiro, Susana Santos, Maria-Jose Lopez-Espinosa, Carmen Iñiguez, Mikel Subiza-Pérez, Ane Arregi, Rosaura Leis, Gloria Bueno, Xavier Basagaña, Marta Cirach, Mark Nieuwenhuijsen, Mònica Guxens, Martine Vrijheid, Carmen Freire, Joana Araújo, Sofia Vilela, Augusto Anguita-Ruiz

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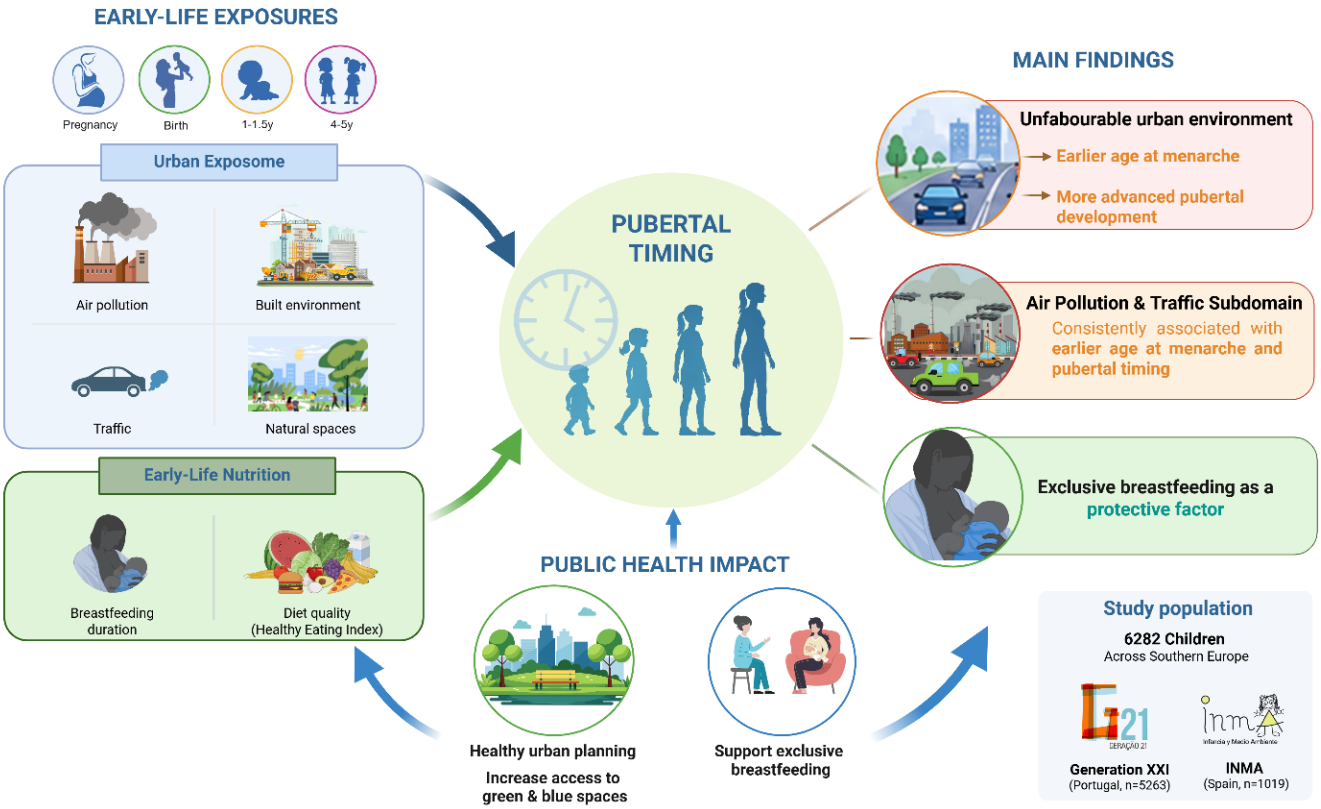

Abstract

Background: Urban environmental and lifestyle factors during early life may influence pubertal timing, but the combined effects of multiple environmental exposures within an exposome analytical framework remain poorly understood.

Objective: To examine the association between early-life urban environmental exposures and pubertal timing, and to explore whether these exposures interact with early-life nutritional factors, namely breastfeeding duration and childhood diet quality.

Methods: Data from two European population-based birth cohorts were analysed: Generation XXI (G21, Portugal; n=5263; 51.5% girls) and INfancia y Medio Ambiente (INMA, Spain; n=1019; 50.1% girls). Urban environmental exposures including indicators of air pollution, traffic, built environment, and natural spaces were estimated at 4 early-life stages at both cohorts: pregnancy (INMA only), birth, 1 year, and 4–5 years of age. Pubertal development timing was assessed using Tanner staging and/or the Pubertal Development Scale (PDS), and age at menarche was self-reported. Exposome-Wide Association Study (ExWAS) models and unsupervised clustering followed by ordinal logistic regression models were used to examine single- and multi-exposure associations, respectively. Regression models were fitted adjusting

for relevant child characteristics, maternal factors, and household socioeconomic conditions, and corrected for multiple testing.

Results: Individuals living in more unfavourable urban environments characterised by higher building density, air pollution, and lower access to natural spaces showed earlier pubertal timing according to multiple outcomes, across multiple early-life exposure periods, and in both cohorts. In the G21 cohort, these environmental profiles were associated with earlier age at menarche, particularly for exposures at 1-1.5 and 4-5 years (e.g., 1-1.5y: β=-0.172, FDR-adjusted p-value=0.041), while in the INMA cohort, boys exposed to more unfavourable environmental profiles showed more advanced pubertal development, also particularly for exposures at 1-1.5 and 4-5 years of age (e.g., 1-1.5y; β=0.572, FDR-adjusted p-value=0.008). Among environmental domains, air pollution and traffic were the factors most consistently associated with pubertal timing. Regarding early-life nutritional factors, longer duration of exclusive breastfeeding was associated with a lower Tanner stage among girls in G21. No significant interactions between breastfeeding duration and environmental exposure clusters were observed.

Conclusion: Early-life urban environmental exposures, particularly air pollution and traffic, may influence pubertal timing. Exclusive breastfeeding may have a protective role against earlier pubertal development. These findings highlight the importance of improving urban environmental conditions and promoting breastfeeding to support healthy developmental trajectories.

Keywords: Urban environment; Breastfeeding; Puberty; Children; Cohort study

1. Introduction

A growing body of evidence indicates a worldwide declining trend in the age at menarche and pubertal onset (1-6). This trend raises public health concerns, as earlier pubertal timing carries significant health implications, including an increased risk of metabolic disorders (7), cardiovascular disease (8, 9), psychological and behavioural problems (10-12), substance use (13), fertility impairment (14), and a higher risk of breast and other reproductive cancers (15-17).

Pubertal timing is a complex and multifactorial trait shaped by both genetic and non-genetic influences (18). Although genetic predisposition plays a major role in determining pubertal timing, existing scientific evidence points to specific external and personal environmental factors as additional relevant contributors to this relationship: breastfeeding, nutrition, socioeconomic status, and environmental exposures (18-21). The study of urban environmental factors as determinants of health and disease has gained a lot of attention with the advent of exposome research. The exposome framework provides a comprehensive strategy to explore environmental risk factors by capturing the totality of exposures across the life course (22, 23). Within the Developmental Origins of Health and Disease (DOHaD) paradigm, the prenatal and early-life period is increasingly recognized as a sensitive window in which environmental exposures can program long-term health. (22, 24).

Concerning urban environmental exposures, several studies have evaluated their association with pubertal timing. Most studies examining the impact of the urban environment on pubertal development have focused on air pollution, with some reporting associations with earlier pubertal onset, while others have not confirmed these findings (25-28). For instance, living within 150 meters of a major road has been associated with earlier pubertal onset in girls (25), and residence in urban area has been linked with earlier age at menarche (29,30), potentially reflecting differences in health, nutrition, socioeconomic conditions (31, 32), and exposure to environmental pollutants during childhood compared with rural settings (32). Conversely, other studies have found that in utero and childhood exposure to Particulate Matter (PM_{10}) was associated with a decreased age at menarche (27), while exposure to sulfur dioxide and nitrogen dioxide was associated with delayed pubertal onset in both sexes (26). In contrast, a study conducted in two European birth cohorts did not find any significant associations between ambient air pollutants and pubertal development (28).

Some authors have further hypothesized that proximity to green spaces may influence the age at menarche (33), by reducing stress, lowering adiposity risk, and promoting opportunities for

physical activity - factors known to be associated with the timing of reproductive development (32, 34, 35). Nevertheless, the only available study, conducted among adolescents in Germany and Australia, did not support the hypothesis (36).

As described, current epidemiological evidence on the association between urban environmental exposures and pubertal timing is limited. Moreover, previous studies have mainly assessed single environmental exposures, without accounting for the complex correlations and interactions between them, highlighting the need for exposome-based approaches in big multi-cohort studies. To date, the impact of the totality of urban environmental exposures (external exposome landscape) that an individual experiences across early life on pubertal timing remains largely unexplored.

Regarding personal exposome factors, breastfeeding and childhood nutrition are also modifiable factors that appear to play a significant role in pubertal timing. (37-39) While some studies have found no association between breastfeeding and puberty onset (40, 41), an increasing body of evidence suggests that breastfeeding, and longer breastfeeding duration, may act as protective factors against early pubertal onset, particularly in girls (42-44). Regarding childhood nutrition, although most studies have examined specific nutrients (21, 37, 45-49) or food groups (37, 46, 47) rather than overall dietary quality, higher diet quality has been associated with a later age at puberty onset (50, 51).

To address these gaps, the present study aimed to explore the influence of early-life urban environmental exposures, measured prenatally (pregnancy) and postnatally (at birth, 1 year, and 4-5 years), on pubertal timing within an exposome framework in two well-known birth exposome cohorts representative of South Europe (Spain and Portugal). In addition, this study explored how these environmental exposures interact with early-life nutritional factors, such as breastfeeding duration and diet quality, in order to determine which of these modifiable risk factors plays a more decisive role in pubertal timing (Figure 1).

2. Methods

2.1. Study population

The present study included data from two independent European birth cohorts: Generation XXI (G21) in Portugal and INfancia y Medio Ambiente (INMA) in Spain (Supplementary Figure 1). G21 is a population-based cohort previously described (52, 53). For this cohort, 8647 newborns delivered in the five maternity hospitals in the metropolitan area of Porto were invited and enrolled at baseline. All families were invited to participate in follow-up assessments when children were aged 4 (86% of participation), 7 (80% of participation), 10 (76% of participation), 13 (54% of participation – follow-up was interrupted due to the COVID-19 pandemic), and 18 years (41% of participation). At each evaluation wave, data were collected by trained interviewers through structured questionnaires, including information on sociodemographic conditions, health status, lifestyle, biological samples, as well as objective anthropometric measures. For the present study, we included individuals with complete data on pubertal development (Tanner Stage and/or age at menarche) assessed at 10, 13, and/or 18 years, as well as breastfeeding history and dietary intake collected at 4 years of age., and data on urban environmental exposures available at least at birth, 1 year and 4-5 years of age. After excluding twins (n=144) and children with congenital anomalies or diseases that might influence the analysis of foo5263d intake or pubertal development (n=50), a final sample of 5263 children from the G21 cohort was considered.

The INMA Project is a Spanish network of population-based birth cohorts that recruited 3768 mother-child pairs during pregnancy between 1997 and 2008 in the Spanish regions of Ribera d'Ebre, Menorca, Granada, Valencia, Sabadell, Asturias and Gipuzkoa (54). The recruitment and general characteristics of the INMA cohorts are described elsewhere (54). For the present study, data from Valencia, Sabadell and Gipuzkoa cohorts were included. Eligibility criteria comprised: residence in one of the study areas, maternal age ≥16 years of age, singleton pregnancy, no assisted conception, intention to deliver at the reference hospital, and ability to communicate in Spanish or the regional language. Although follow-up time points varied slightly across cohorts, mother-child pairs were generally assessed during the third trimester of pregnancy, at birth, at 1-1.5 years, 2-2.5 years, 4-5 years and at approximately 9.5 years of age. At each evaluation wave, data were collected through face-to-face interviews by trained INMA personnel and included questionnaire-based information, clinical records, physical examinations, ultrasound scans, biological samples, biomarkers, dietary assessments, and environmental measurements. For this study, we included all children with complete data on pubertal development (assessed using the

Pubertal Development Scale and/or Tanner Stage) and age at menarche at 9.5 years, as well as breastfeeding history and dietary intake assessed at 1-1.5, 4-5 and 9.5 years, and environmental exposure data available during at least one of the following periods: pregnancy, birth, 1-1.5 years, and/or 4-5 years. A final sample of 1019 children from the INMA cohort was considered.

2.2. Pubertal timing assessment

In the INMA cohorts, pubertal development was assessed using clinical Tanner staging (55, 56) and/or parental assessment of the child's pubertal status using the Pubertal Development Scale (PDS) at the 9-year follow-up (57). The PDS is a reliable and valid instrument for assessing pubertal development in children (58, 59), and is based on five physical characteristics, including pubic hair development and skin changes in both sexes, voice deepening and facial hair growth in boys, and breast development in girls. Response options range from 1 ('not yet started') to 4 ('seems complete'). Following the criteria proposed by Carskadon and Acebo (58) and Shirtcliff et al. (60), PDS scores were converted into a 5-point ordinal scale (1 - Prepubertal, 2 - Early pubertal, 3 - Midpubertal, 4 - Late pubertal, 5 - Postpubertal). The Tanner staging of genital development and pubic hair growth was performed by a pediatrician or trained health care professional, according to the five Tanner stages (ranging from 1- prepubertal to 5 – postpubertal). The PDS was used to assess pubertal development in children from the Gipuzkoa and Sabadell cohorts, while both Tanner staging and the PDS were used in children from the Valencia cohort. Information on age at menarche was collected only in the Sabadell cohort.

In the G21 cohort, pubertal development was assessed following the Tanner stage criteria, performed by trained nurses at the 10-year follow-up. In females, pubic hair and breast development were evaluated, while in males, pubic hair development was assessed according to Tanner staging, and testicular volume was additionally measured. Each parameter was classified from Tanner stage 1 (prepubertal) to Tanner stage 5 (postpubertal). Tanner 1 indicated that puberty had not started yet, whereas Tanner stage 2 was defined as the mark of the beginning of puberty in both cohorts. Females were classified as Tanner stage 2 or higher if they presented slightly pigmented and downy pubic hair, and breast bud stage with an elevation of breast and papilla enlargement of the aureola. Males were classified as Tanner stage 2 if they presented a testicular volume higher than 4ml, and also a slightly pigmented and downy pubic hair. At the 13- and 18-year follow-ups, girls self-reported their age at menarche. The final age at menarche variable was derived as follows: if age at menarche was reported at only one follow-up, that value

was considered as the final one; if reported at both follow-ups, and the difference between reports did not exceed two years, the mean of the two values was calculated and considered as the final age at menarche.

2.3. Assessment of the urban environment during early-life

Urban environmental exposures considered in this study were obtained using Geographic Information Systems based on the mothers' residential addresses during pregnancy, at birth, and annually throughout the child's life in both cohorts. In the present study, information collected during pregnancy (available only in the INMA cohorts), at birth, at 1-1.5 years, and at 4-5 years of age was included. Urban environment variables were selected based on literature and data availability in the current and ongoing G21 and INMA studies, and included indicators of air pollution (e.g., NO2, PM2.5), built environment (e.g., building density, connectivity density, walkability index, Land use Shannon's Evenness Index), natural spaces (e.g., NDVI, distance to nearest major blue/green space), and traffic (e.g., inverse distance to nearest road) (Supplementary Table 1). The built environment and natural spaces were measured within different buffer sizes (100m, 300m, and 500m), but in this study, only variables corresponding to the 300m buffer were included. Further details on exposure assessment methods in the INMA and Generation XXI cohorts are available in Supplementary Section I, and have been described previously (61-64).

2.4. Breastfeeding and Healthy Eating Index

Information on the child's diet and breastfeeding was obtained using interviewer-administered questionnaires with the mothers when the child was 6 months old (G21 and INMA), 14–15 months (G21 and INMA), 24 months (G21 only), and 4–5 years of age (G21 and INMA).

In both cohorts, mothers were asked about the duration of any and exclusive breastfeeding. Any breastfeeding was defined as the child receiving any type of breast milk feeding, regardless of whether it was combined with other foods. Exclusive breastfeeding was defined according to the World Health Organization (WHO) definition as the period during which the child received only breast milk and no other foods or liquids (including water or infant formula), while allowing the intake of oral rehydration salt solutions, vitamins, minerals, and medicines (65). However, in the G21 cohort, breastfeeding was classified as exclusive even if the child had already been given

water. Information on exclusive breastfeeding was not collected in the Valencia cohort. The duration of non-exclusive breastfeeding was considered as a continuous variable, and the duration of exclusive breastfeeding was considered as both continuous and categorical variables. Children were grouped into two categories: (a) exclusive breastfeeding for < 24 weeks (6 months); and (b) exclusive breastfeeding for ≥ 24 weeks.

Dietary information was collected using a Food Frequency Questionnaire administered at 4 years of age, validated in both cohorts (66, 67). Two food groups were considered for the analysis, 'Fruit and Vegetables (FV) (fresh fruit and vegetables) and 'Energy-dense Foods' (EDF) (cakes, candies, chocolate, sweet pastries, biscuits, added sugar, ice cream, salty snacks, pizza, and chips), as these can be considered indicators of dietary quality. Based on these two food groups, a Healthy Eating Index (HEI) was also developed, adapted from a previously established index in the G21 cohort (68, 69). For each food group, quartiles of consumption were calculated, and the mean values of each quartile were used to define cut-off points. Each quartile was assigned a score ranging from 1 to 4. For the FV group, considered a healthier food group, the lowest quartile of consumption was assigned 1 point, the intermediate quartiles were assigned 2–3 points, and the highest quartile of consumption was assigned 4 points. For the EDF group, which includes less healthy foods, the score was assigned reversely. The scores were summed to obtain a total HEI score, ranging from 2 to 8, with higher scores indicating better overall diet quality.

2.5. Other maternal and child characteristics

A comprehensive set of covariates was selected based on previous studies examining associations between single urban environmental exposure, breastfeeding, diet, and pubertal timing. The following variables were considered: child exact age at pubertal assessment (years), child birthweight (grams), maternal pre-pregnancy body mass index (BMI) (kg/m2), maternal age at delivery (years), and household income.

Child BMI z-score was not included as a covariate because it could potentially act as a mediator rather than a confounder in the pathway between early-life exposures and pubertal timing. Therefore, instead of including BMI as a covariate, a sensitivity analysis restricted to children with normal weight was performed to explore the robustness of the associations, acknowledging the potential reduction in statistical power due to the smaller sample size.

2.6. Statistical analyses

The whole statistical pipeline is illustrated in Supplementary Figure 2.

Data pre-processing

The correlations between exposure variables within each cohort and at each time point were explored by computing Pearson correlation matrices. At this stage, variables with correlations > 0.9 were excluded. This procedure led to the exclusion of NOx due to its high correlation with NO2. To minimize the influence of extreme values, winsorization was applied to all numeric variables. Values below the 2.5th percentile and above the 97.5th percentile were replaced with their respective cut-off values.

To handle missing data, an imputation procedure was performed using the MissForest algorithm (70). Variables with more than 20% missing data were excluded from the imputation process, as was the case for child BMI in the INMA cohort (~38% missingness).

Association analysis between pre- and postnatal environmental exposures and pubertal timing

To explore the influence of prenatal and postnatal environmental factors on pubertal timing, we included 23 exposures during pregnancy (INMA only); at birth, 17 exposures in G21 and 9 in INMA; at 1-1.5 years, 19 exposures in G21 and 20 in INMA; and at 4-5 years of age, 20 exposures in G21 and 21 in INMA (Supplementary Table 1). Associations were evaluated considering both single-exposure and multiple-exposure effects.

Single-exposure analysis

In a first step, single-exposure associations were examined through an Exposome-Wide Association Study (ExWAS) stratified by sex, using ordinal logistic regression models to assess associations between individual environmental exposures and pubertal timing. All ExWAS models were stratified by sex and adjusted for key covariates (child exact age, birthweight, maternal pre-pregnancy BMI, maternal age, and household income). Statistical significance was assessed using p-values adjusted for multiple comparisons using the Benjamini–Hochberg procedure to control the false discovery rate (FDR). The correction was applied separately within each set of analyses, defined by cohort, outcome, sex stratum, and exposure time point. Thus, for each outcome, all exposure-specific associations tested within the same exposure period and sex stratum were jointly included in a single FDR adjustment, while associations from different

exposure periods, sex strata, outcomes, or cohorts were adjusted independently. Although FDR correction was applied in all analyses, FDR-corrected p-values were identical to nominal p-values in some analyses, as indicated in the tables.

Multi-exposure analysis

Given that individuals are simultaneously exposed to different urban environmental factors that may be correlated, and that these exposures may interact and co-occur within the same context, a multi-exposure approach was considered in the present study, focusing on the urban environmental exposome. This approach aimed to assess the combined effect of urban environmental exposures, rather than analysing each exposure separately.

In a second step, to account for the effect of simultaneous exposures. an unsupervised clustering analysis was performed to identify groups of children with similar patterns of environmental exposure using Agglomerative Hierarchical Clustering (AHC), followed by association analysis between identified clusters and puberty outcomes. To reduce dimensionality, Principal Component Analysis (PCA) was applied prior to clustering, retaining components explaining 80-90% of the total variance. Clustering was conducted separately by sex, for each time point at which exposure were available and for each cohort. In INMA, exposures were standardized within each subcohort prior to PCA and clustering to reduce the influence of between-city differences on cluster assignment. Subcohort and degree of urbanization were not included as clustering variables, as the aim was to identify clusters based on environmental exposure profiles rather than predefined geographic or urbanization categories. Their distribution was subsequently examined descriptively across clusters, and sensitivity analyses excluding rural areas were performed. For consistency and interpretability, a two-cluster solution was predefined and applied across all analyses, while the Calinski–Harabasz (CH) index was used as complementary metric. As the main analysis, ordinal logistic regression models adjusting for key covariates, as described above, were fitted to evaluate the association between identified urban environmental exposure clusters and pubertal development. Following characterization of the clusters, the group with the lowest burden of environmental hazards was designated as Cluster 1 and was used as the reference category for this regression.

In a third step, the clustering procedure was repeated using AHC, focusing on urban environmental exposures grouped into subdomains (built environment, natural spaces, and traffic and air pollution) (Supplementary Table 1), to explore which subdomains might be driving the associations observed in the exposome analysis. For each subdomain, a two-cluster solution was

predefined. Associations between these subdomain-specific clusters and pubertal timing were then assessed using ordinal logistic regression models, adjusted for the same covariates.

In a fourth and final step, the association between combined exclusive breastfeeding duration-environmental exposure groups and pubertal development was examined using ordinal logistic regression models, adjusted for the same set of covariates. In this analysis, exclusive breastfeeding duration was considered as a categorical variable, and environmental exposure was represented by the AHC-derived clusters. The reference group (Group 1) included participants classified in the more favourable environmental exposure cluster who had been exclusively breastfed for 24 weeks or more.

All multi-exposure analyses were adjusted for multiple testing using Benjamini-Hochberg FDR approach. As a global FDR correction strategy, for k=2 clustering analyses, all exposure-outcome associations tested across outcomes and exposure periods within the same sex stratum were jointly included in a single FDR adjustment, while associations from different sex strata were adjusted independently. Additionally, alternative block-specific FDR corrections were examined depending on the analytical approach and were defined by outcome, sex stratum, exposure period, and clustering solution. However, because only one exposure variable was tested (cluster 2, using cluster 1 as reference), FDR-corrected p-values were identical to the nominal p-values. In subdomain analyses, associations across outcomes, exposure periods, and subdomains within the same sex stratum were jointly included in a single FDR adjustment. For interaction analyses, FDR correction was applied separately within each interaction type (e.g., exposome x diet, exposome x breastfeeding) and sex stratum, jointly considering all interaction tests across exposure periods and outcomes. All correction strategies are transparently described in the corresponding tables and supplementary material.

Statistical analyses were performed using R software (version 4.4.3).

Sensitivity analysis

As sensitivity analyses, ordinal logistic regression models based on the AHC-derived clusters were performed excluding participants living in rural areas in both cohorts. In addition, analyses restricted to participants with normal weight were conducted in the G21 cohort only. Ordinal logistic regression models were also fitted including only individuals who remained in the same environmental exposure cluster across all available time points. Subdomain-specific analyses (built environment, natural spaces, and traffic and air pollution) were also repeated after

excluding participants living in rural areas in both cohorts, as well as in analyses restricted to participants with normal weight in the G21 cohort.

2.7. Ethical considerations

All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. The baseline and follow-up evaluations were approved by the University of Porto Medical School/ S. João Hospital Centre Ethics Committee, except the 13-year follow-up that was approved by the ISPUP Ethics Committee. At baseline and follow-up evaluations, all procedures were explained to participants, and an informed consent was signed by one of the parents or legal guardians (at 13 years, it was also signed by the participants). The baseline evaluation was approved by the Data Protection National Commission, and the study follows the EU General Data Protection Regulation under close supervision of the Data Protection Office of ISPUP. In the INMA cohort, written informed consent was obtained from all mothers during pregnancy and from the child's legal guardians at the clinical examination, and the research protocol was approved by the Ethics Committees of each region.

3. Results

3.1. Study population

Figures 1 and Supplementary Figures 1 and 2 summarise the study design and analytical approach. The majority of children in both cohorts were girls (51.5% in G21 and 50.1% in INMA), with a mean age at outcome assessment of 8.7 years in INMA and 10.2 years in G21 ($p<0.001$) (Table 1). The prevalence of exclusive breastfeeding for ≥24 weeks was similar between sexes and did not differ significantly between cohorts.

In both cohorts, at the time of outcome assessment, most girls had already reached Tanner stage 2 or higher (74.3% in G21 and 58.5% in INMA), whereas the majority of boys had not yet initiated puberty, being classified as Tanner stage 1 (73.4% in G21 and 58.5% in INMA). Similarly, PDS distributions in INMA indicated more advanced development among girls compared to boys ($p<0.001$). The mean age at menarche was slightly lower in G21 compared to INMA, although this difference was not statistically significant.

Mothers in INMA were older than those in G21 ($p<0.001$), and there were small differences in pre-pregnancy BMI distributions ($p=0.005$).

Similar clustering patterns were observed across both cohorts (Supplementary Figure 4). In general, Cluster 1 was characterised by greater availability of blue and green spaces and lower levels of urbanicity and air pollution, whereas Cluster 2 reflected more urbanised environments with higher building density, connectivity, walkability, population density, and air pollution levels. Compared with INMA, the G21 cohort generally presented higher levels of urbanicity and air pollution indicators, but also greater availability of green and blue spaces.

3.2. Single-exposure models for pubertal timing

ExWAS analysis has been proposed as an initial approach to study the urban environmental and lifestyle determinants associated with pubertal timing. FDR-adjusted associations between urban environmental and lifestyle exposures at birth and at 1 and 4 years of age and pubertal timing are presented in Supplementary Figure 3. Overall, significant results were only observed in the G21 cohort. In G21, a longer duration of exclusive breastfeeding was associated with a lower Tanner stage among females, indicating less advanced pubertal development, after FDR correction. Regarding age at menarche, environmental exposures at birth and at 1 year of age related to built environment factors, including higher walkability index, greater density of public bus stops, higher building and population density, and higher concentrations of air pollutants such as NO_2 and $PM_{2.5}$ (at 1 year only), were negatively associated with earlier age at menarche, suggesting accelerated pubertal timing after FDR correction. Conversely, higher levels of NDVI, an indicator of access to natural spaces, were positively associated with a later age at menarche after FDR correction, indicating a protective effect against accelerated pubertal timing. At 4 years of age, exposure to built environment factors followed a similar pattern to that observed at birth and 1 year, with higher population density and walkability index being negatively associated with age at menarche after FDR correction, suggesting that greater exposure at 4 years was related to an earlier onset of menarche (Supplementary Figure 3). In both cohorts, no statistically significant associations after FDR correction were observed for diet quality variables.

3.3. Multi-exposure models for pubertal timing (urban environmental exposures)

To account for the combined effects of multiple correlated urban environmental exposures, we applied a clustering approach to derive environmental profiles, allowing the identification of real-life exposure patterns rather than isolated factors (Figure 2). For each exposure data point, our clustering approach revealed two urban environmental profiles. A cluster representing a more favourable or healthy environment, characterised by greater availability of green spaces, vegetation, and land-use diversity, together with lower building density, traffic intensity, and air pollution levels. On the other hand, the second cluster reflected a less favourable or unhealthy profile, involving higher building density, traffic intensity, and pollutant concentrations, as well as lower access to natural spaces. Those profiles were identified ubiquitously for each exposure follow-up and cohort (Figure 2 and Supplementary Figure 4).

Subsequently, the association between clusters at each exposure time point and pubertal outcomes, including Tanner stage, PDS score, and age at menarche was assessed. In the G21 cohort, no statistically significant associations were observed between urban environmental clusters and Tanner stage after FDR correction. When age at menarche was considered as the outcome, females in the unhealthier cluster exhibited an earlier age at menarche compared with those in the healthier cluster at exposure ages 1 (β=0-0.172, nominal p-value=0.041; FDR-adjusted p-value=0.041; global FDR-adjusted p-value=0.084) and 4 years (β=-0.181, nominal p-value=0.024; FDR-adjusted p-value=0.024; global FDR-adjusted p-value=0.084) (Supplementary Table 2 and Figure 3).

In the INMA cohort, no statistically significant associations were identified between urban environmental clusters and either Tanner stage or age at menarche. Regarding PDS score, males in the unhealthier cluster showed higher PDS scores than those in the healthier cluster at exposure ages 14–18 months (β=0.572, nominal p-value=0.008; FDR-adjusted p-value=0.008; global FDR-adjusted p-value=0.065) and 4–5 years (β=0.437, nominal p-value=0.027; FDR-adjusted p-value=0.027; global FDR-adjusted p-value=0.327) (Supplementary Table 2 and Figure 3).

3.4. Multi-exposure models by exposome subdomain for pubertal timing (urban environmental exposures)

Clustering analyses were also conducted within exposome subdomains to identify which external exposure domains are the most relevant for the pubertal timing outcome. The exposome was organized into three main subdomains according to the nature of the variables assessed: (i) Air pollution & Traffic, (ii) Natural spaces, and (iii) Built environment.

In G21, when Tanner stage was considered as the outcome, only one statistically significant association was observed at 4 years of age in the 'Air Pollution & Traffic' subdomain (β=0.187, nominal p-value=0.045; FDR-adjusted p-value=0.045; global FDR-adjusted p-value=0.153), with females in the unhealthier cluster presenting a higher Tanner stage compared with those in the healthier cluster, suggesting a more advanced pubertal development in the unhealthier cluster. No statistically significant associations were observed for the remaining exposure time-points or subdomains. When age at menarche was considered as the outcome, statistically significant associations were observed in the 'Air Pollution & Traffic' subdomain at all exposure time-points (Birth: β=-0.253, nominal p-value=0.028; FDR-adjusted p-value=0.028; global FDR-adjusted p-value=0.111; 1 year: β=-0.330, nominal p-value=0.004; FDR-adjusted p-value=0.004; global FDR-adjusted p-value=0.028; 4 years: β=-0.219, nominal p-value=0.024; FDR-adjusted p-value=0.024; global FDR-adjusted p-value=0.111), and in the 'Built environment' subdomain at 1 year (β=-0.224, nominal p-value=0.005; FDR-adjusted p-value=0.005; global FDR-adjusted p-value=0.032) and 4 years of age (β=-0.263, nominal p-value=0.001; FDR-adjusted p-value=0.001; global FDR-adjusted p-value=0.026). In all statistically significant associations, females in the unhealthier cluster presented a lower age at menarche compared with those in the healthier cluster, consistent with accelerated pubertal timing (Supplementary Table 3).

In INMA, considering Tanner stage as the outcome, a statistically significant association was observed only at the 1-1.5-year time-point. In the 'Natural spaces' subdomain, males in the unhealthier cluster, characterised by lower access to natural spaces compared with the healthier cluster, showed a higher Tanner stage (β=1.100, nominal p-value=0.012; FDR-adjusted p-value=0.012; global FDR-adjusted p-value=0.213), indicating more advanced pubertal development in this group. No statistically significant associations were observed for age at menarche at any exposure time-point or subdomain. When PDS was considered as the outcome, for exposure during pregnancy, females in the unhealthier cluster of the 'Built environment' (β=0.479, nominal p-value=0.031; FDR-adjusted p-value=0.031; global FDR-adjusted p-value=0.294) and 'Natural spaces' (β=0.486, nominal p-value=0.024; FDR-adjusted p-value=0.024; global FDR-adjusted p-value=0.294) subdomains presented higher PDS scores compared with females in the healthier cluster, also suggesting more advanced pubertal development. At 1-1.5 years and at 4-5 years of age, females in the unhealthier cluster of the 'Air Pollution & Traffic' subdomain also presented higher PDS scores than females in the healthier cluster (1-1.5 years: β=0.380, nominal p-value=0.033; FDR-adjusted p-value=0.033; global FDR-adjusted p-value=0.294; 4-5 years: β=0.381, nominal p-value=0.044; FDR-adjusted p-

value=0.044; global FDR-adjusted p-value=0.294). In addition, at 4-5 years of age, males in unhealthier cluster of the 'Built environment' subdomain also presented higher PDS scores compared with males in healthier cluster (β=0.599, nominal p-value=0.024; FDR-adjusted p-value=0.024; global FDR-adjusted p-value=0.213), consistent with more advanced pubertal development in males in the unhealthier cluster (Supplementary Table 3).

3.5. Multi-exposure models for pubertal timing and interaction with exclusive breastfeeding duration

To explore the potential combined effects of early-life nutrition and urban environmental exposures, a joint analysis was conducted integrating exclusive breastfeeding duration (<24 weeks vs. ≥24 weeks) with the previously defined urban environmental exposure clusters (healthier vs. unhealthier clusters). This approach aimed to assess how environmental exposures interact with early-life nutritional factors and to determine which one of these factors plays a more important role in pubertal timing.

In the combined analysis of exclusive breastfeeding duration and urban environmental exposure clusters, no statistically significant associations were observed on pubertal development after FDR correction for any outcome, exposure time point, or cohort (Supplementary Table 4).

3.6. Sensitivity analysis

In the sensitivity analysis restricted to the urban population (Supplementary Table 5), no FDR-statistically significant associations were observed in the G21 cohort. In the INMA cohort, a significant association was observed only among boys for exposures measured at 1-1.5 years, with boys in the unhealthier cluster presenting higher PDS scores than those in the healthier cluster (β=0.550, nominal p-value=0.014; FDR-adjusted p-value=0.014; global FDR-adjusted p-value=0.111), indicating more advanced pubertal development in the unhealthier cluster. In the sensitivity analysis restricted to participants with normal weight in G21 (Supplementary Table 6), no significant associations were observed for any outcome or exposure time point. In the sensitivity analysis restricted to individuals who remained in the same cluster over time (Supplementary Table 7), no significant associations were observed in G21, while in INMA, boys showed higher PDS scores in the unhealthier cluster, at all exposure time points (Pregnancy: β=1.013, nominal p-value=0.024; FDR-adjusted p-value=0.024; global FDR-adjusted p-

value=0.048; Birth: β=1.014, nominal p-value=0.024; FDR-adjusted p-value=0.024; global FDR-adjusted p-value=0.048; 1-1.5 years: β=1.015, nominal p-value=0.024; FDR-adjusted p-value=0.024; global FDR-adjusted p-value=0.048; 4-5 years: β=1.016, nominal p-value=0.024; FDR-adjusted p-value=0.024; global FDR-adjusted p-value=0.048).

In the subdomain-specific sensitivity analysis restricted to the urban population (Supplementary Table 8), G21 females in unhealthier cluster of the 'Built environment' subdomain had a lower age at menarche at exposure time-points 1 (β=-0.190, nominal p-value=0.022; FDR-adjusted p-value=0.046; global FDR-adjusted p-value=0.142) and 4 years of age (β=-0.239, nominal p-value=0.004; FDR-adjusted p-value=0.012; global FDR-adjusted p-value=0.071), indicating earlier menarche compared with females in the healthier cluster. In both time points, females in unhealthier cluster of the 'Built environment' subdomain had a lower age at menarche compared with females in the healthier cluster. At the 1-year exposure time point, the unhealthier cluster of the 'Air Pollution & Traffic' subdomain was also associated with a lower age at menarche (β= -0.282, nominal p-value=0.031; FDR-adjusted p-value=0.046; global FDR-adjusted p-value=0.142), consistent with earlier pubertal timing.

In the sensitivity analysis restricted to participants with normal weight in G21 (Supplementary Table 9), subdomain analyses showed statistically significant associations at 4 years of age in the 'Natural spaces' (β=0.222, nominal p-value=0.032; FDR-adjusted p-value=0.047; global FDR-adjusted p-value=0.189) and 'Air Pollution & Traffic' (β=0.278, nominal p-value=0.028; FDR-adjusted p-value=0.047; global FDR-adjusted p-value=0.189) subdomains, with girls in unhealthier cluster presenting a higher Tanner stage compared with girls in the healthier cluster, indicating more advanced pubertal development. At the 1-year exposure time point, girls in the unhealthier of the Built environment subdomain had a lower age at menarche (β=-0.266, nominal p-value=0.016; FDR-adjusted p-value=0.047; global FDR-adjusted p-value=0.189), suggesting earlier pubertal timing.

4. Discussion

This study provides evidence that living in an unfavourable urban environment, characterized by higher building and population density, traffic and air pollution, and lower access to natural spaces, is associated with an earlier age at menarche in Portuguese girls and with a more advanced pubertal stage among Spanish boys. To our knowledge, this is the first study to examine the effect of multiple early-life urban environmental exposures measured at repeated time points

across early-life and under an exposome approach on pubertal timing. All urban environmental exposome subdomains evaluated in our study, namely 'Built environment', 'Natural spaces', and 'Air Pollution & Traffic', were independently associated with pubertal development or age at menarche in the two cohorts. In particular, the 'Air Pollution & Traffic' subdomain appeared to be the most consistently associated with pubertal development across almost all exposure time points, especially among girls. Additionally, the ExWAS analysis, reported how a longer duration of exclusive breastfeeding was also associated with a lower Tanner stage at 10 years of age among females in the G21 Portuguese cohort. However, interaction analyses did not provide evidence of synergistic or combined effect between exclusive breastfeeding duration and environmental exposures on pubertal timing. Similarly, we found no evidence that exclusive breastfeeding duration attenuated the potential adverse effects of unhealthier urban environmental exposures on pubertal timing. Importantly, under the more conservative global FDR correction approach, statistically significant associations were substantially reduced and remained only in the subdomain analyses within the G21 cohort, specifically for age at menarche in relation to the Air pollution subdomain at 1–1.5 years and the Built environment subdomain at 1–1.5 and 4–5 years of age.

The present study corroborates previous evidence indicating that a longer duration of breastfeeding, in this case exclusive breastfeeding, is associated with a later age at menarche (42, 44, 71, 72). It is possible that childhood body weight status mediates the relationship between breastfeeding and pubertal timing, as longer breastfeeding duration appears to be protective against childhood obesity (73), and obesity, in turn, has been associated with earlier pubertal timing (74). However, some studies have also reported no association between weight status and earlier pubertal onset (75, 76). An alternative pathway may be that breastfeeding is associated with other maternal and family behaviours considered healthier, including higher diet quality during childhood (77) and better sleep habits (78), factors that have also been linked to pubertal development (50, 79). This study therefore supports the potential value of investing in breastfeeding policies as a public health intervention with potential long-term benefits for child pubertal development.

Regarding urban environmental exposures, air pollution and traffic showed the most consistently associations with pubertal timing across cohorts, with associations observed for age at menarche in the G21 cohort and PDS score in girls in the INMA cohort, suggesting a potential role of these exposures in pubertal development. Previous studies have also reported associations between exposure to air pollution and traffic-related factors and pubertal development (25, 80-84). For

example, a prospective cohort study conducted in the United States reported that girls with higher in utero and childhood exposure to air pollution were more likely to experience an earlier age at menarche (85). Several biological mechanisms may underlie these associations. Air pollution derived from industrial and vehicle emissions contains multiple compounds, including PM and NO, as well as persistent organic pollutants and polycyclic aromatic hydrocarbons (PAHs), which may act as endocrine-disrupting chemicals or have endocrine-disrupting properties (86). These pollutants can interfere with hormonal pathways that are key regulators of reproductive development, and may activate the aryl hydrocarbon, androgen, and estrogen receptors (86, 87). PM has been suggested to influence estrogen signalling, which may stimulate the release of kisspeptin and subsequently trigger gonadotropin-releasing hormone secretion, a central regulator of pubertal onset (88). In addition, exposure to PM may exert obesogenic effects. Previous studies have shown that higher prenatal and postnatal exposure to PM2.5 is associated with higher BMI z-scores and an increased risk of childhood obesity (89, 90). Increased adiposity, in turn, has long been recognised as an important trigger of pubertal onset in girls (74). In general, all these findings highlight that air pollution and traffic exposure may have broader implications beyond respiratory health, influencing key stages of child development. Notably, in our sensitivity analysis restricted to participants with normal weight, the previously observed subdomain associations were no longer statistically significant in the majority of cases, suggesting that child BMI may play an important role in the relationship between urban environmental exposures and pubertal timing, potentially acting as a mediator. However, the reduced sample size in these restricted analyses may also have limited statistical power.

Access to natural spaces also appears to play an important role in pubertal development in both boys and girls in the INMA cohort. Only few studies have investigated the influence of access to natural spaces on pubertal timing (91, 92). One study found no association between living in areas with more green spaces and age at menarche (92), whereas another study showed that African American girls who lived in neighbourhoods with greater availability of parks, walking or hiking trails, playing fields, and basketball or tennis courts experienced lower rates of onset of breast and pubic hair development over four years of follow-up (91). To our knowledge, our study is the first to suggest a potential protective association between natural spaces and pubertal timing in both sexes. Natural spaces, including green and blue spaces, may promote higher levels of physical activity and reduce sedentary behaviours (33, 93). Moreover, increased exposure to nature also seems to be associated with reduced stress levels, as nature induces a response that

reduce cortisol levels (94). In turn, early-life stress appears to act as a potential trigger for menarche (32).

Finally, we also observed an association between the 'Built Environment' subdomain and pubertal timing, including age at menarche. This subdomain may influence pubertal timing more indirectly, as neighbourhoods with higher population and built density are often associated with fewer opportunities for physical activity (95). In addition, less favourable built environments may also be linked to higher exposure to air pollution (96).

In the sensitivity analysis restricted to the urban population, most associations observed in the main analysis were no longer statistically significant, particularly in the G21 cohort. This suggests that the observed associations may be driven by differences between rural and urban environments, rather than contrasts within urban settings. Nonetheless, the association that remained in the INMA cohort among boys at 1-1.5 years, with higher PDS scores in the unhealthier cluster, suggests that this effect may be less dependent on the urban-rural context for evaluated Spanish cities.

Based on data from these two Iberian cohorts, pubertal timing in Southern European populations appears to follow similar patterns to those reported in other European settings and worldwide (97). In our study, exposure profiles characterised by higher air pollution and lower access to green spaces were consistently associated with more advanced pubertal development and a younger age at menarche, suggesting that the quality of the urban environment may play a relevant role in shaping pubertal development. Compared with other European regions, Portugal and Spain are generally characterised by intermediate levels of air pollution, higher than those observed in Northern Europe but lower than those reported in parts of Eastern Europe (98). However, access to green spaces shows marked intra-urban heterogeneity, particularly in densely built-up areas. In major cities such as Barcelona, large urban parks exist but are unevenly distributed across neighbourhoods (99). These findings highlight the importance of incorporating child health considerations into urban environmental policies, with a particular focus on reducing traffic-related emissions and promoting equitable access to green infrastructure.

One of the main strengths of this study is the use of data from two longitudinal cohorts, based on relatively large population-based samples with high participation rates, in which some of the findings were replicated across cohorts. However, several limitations should be considered. First, some measurement error may arise from the assessment of Tanner stages, particularly from the measurement of testicular volume in males, even when following standardized guidelines and

using an orchidometer, as this is a sensitive and highly invasive procedure. Another limitation is that most children in the INMA cohort were assessed using the PDS rather than Tanner stages, which is considered the gold standard. Although the PDS does not provide a complete estimation of Tanner stages, it has been previously validated, including in the INMA-Valencia subcohort, showing moderate to good agreement with physician-assessed Tanner stages (100, 101). In addition, a substantial proportion of missing data on child BMI in the INMA cohort (~37% missingness) could not be imputed and, therefore, BMI could not be considered as an adjustment variable in the present study. Nevertheless, a sensitivity analysis restricted to normal-weight children was conducted to address this limitation. Furthermore, recall bias may affect breastfeeding information, as it was collected only at specific time points. Finally, maternal and child dietary information was based on self-reported data, which may be subject to misreporting and social desirability bias.

5. Conclusion

This study highlights that exposures to the urban environment, from pregnancy through childhood, may influence pubertal timing in both boys and girls. Air pollution and traffic appear to be the determinants most consistently associated with pubertal timing. Duration of exclusive breastfeeding also seems to act as a protective factor against early onset of menarche. No statistically significant associations were observed in the interaction analysis between exclusive breastfeeding duration and urban environment clusters, thereby limiting the determination of which factor plays a more prominent role in pubertal timing, and suggesting that these factors may operate through independent pathways rather than synergistic effects. These results reinforce the importance of the first years of life as a sensitive window for environmental influences on development. Improving the urban environment through policies that explicitly consider child health, and implementing strategies that support longer breastfeeding duration, may represent complementary public health strategies to reduce the risk of earlier puberal development in both sexes.

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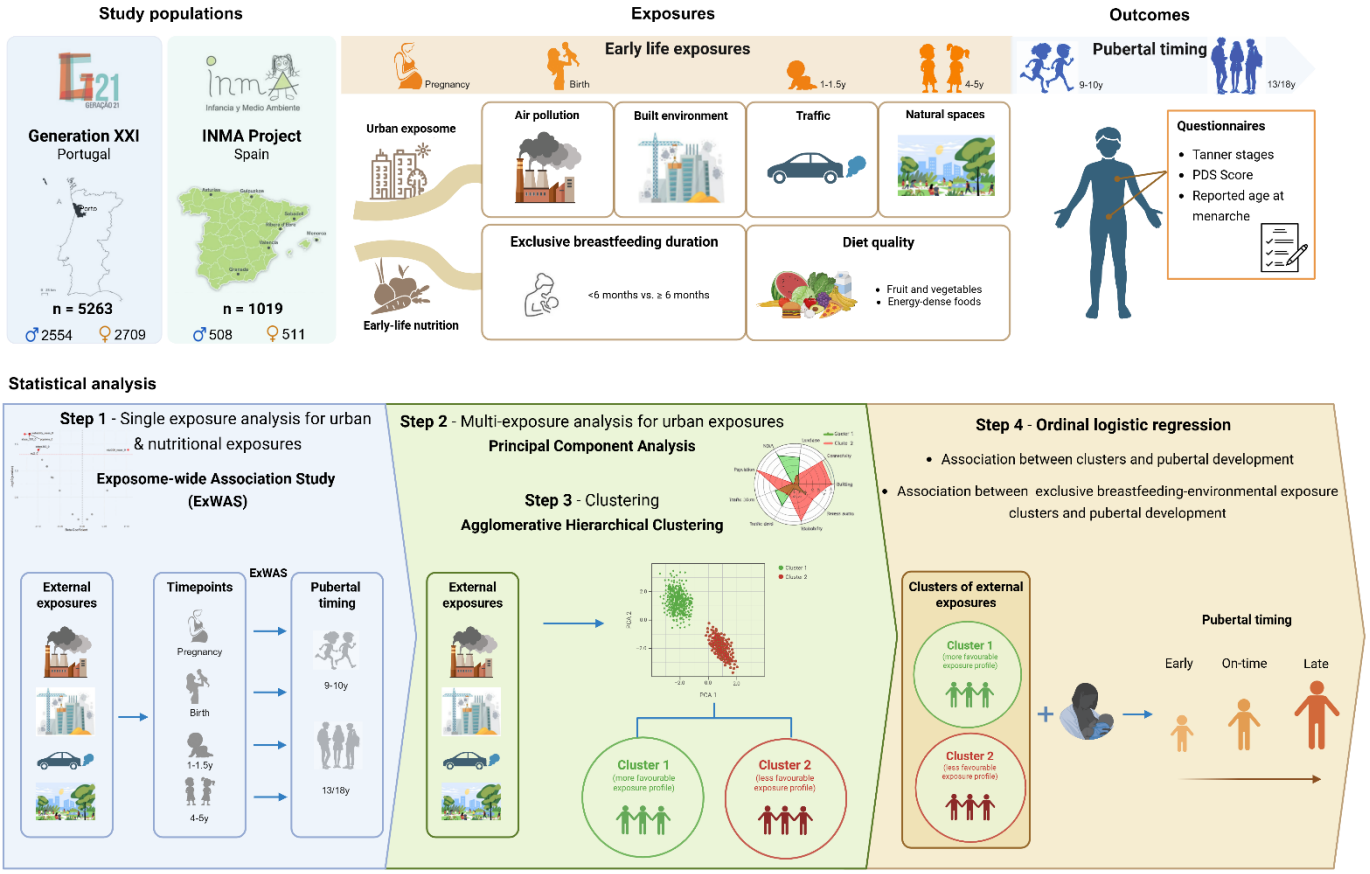


Figure 1. Overview of the study design, exposure and outcome assessment, and analytical framework. Figure 1 presents an overview of the study design, including the participating cohorts, the early-life urban environmental and nutritional exposures, pubertal outcomes, and the analytical strategy applied in the INMA and G21 cohorts. Early-life urban environmental exposures, including built environment, natural spaces, air pollution and traffic, were assessed across different life stages. Statistical analysis included Exposome-wide Association Study (ExWAS), Principal Component Analysis, Agglomerative Hierarchical Clustering, and Ordinal Logistic Regression models to evaluate associations with pubertal timing.

Table 1. Maternal and child characteristics by sex and cohort.

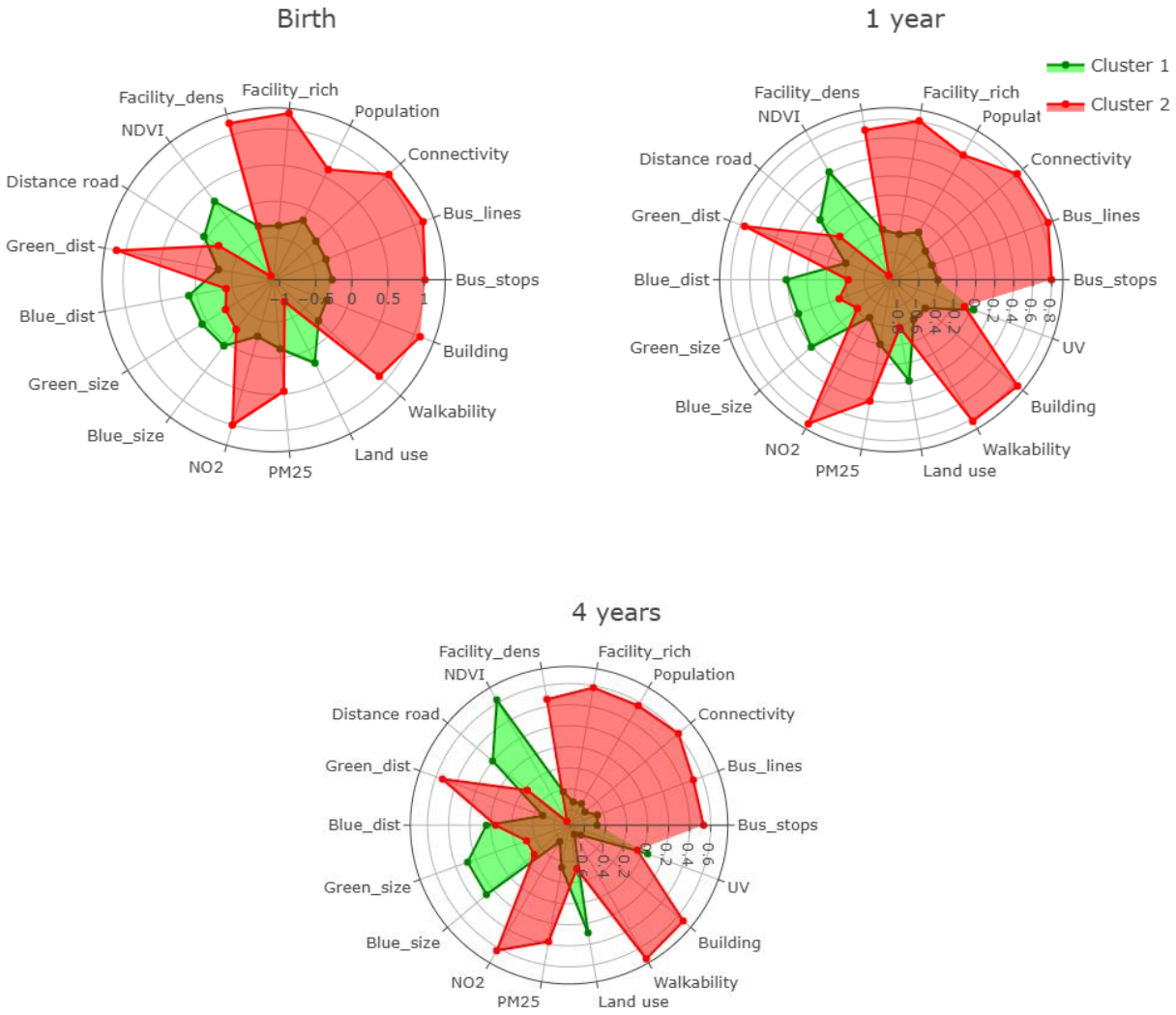
	G21			*INMA*			
	Female n=2709	Male n=2554	p-value	Female n=511	Male n=508	p-value	p-value for differences between cohorts
Child's characteristics							
Birthweight (g), mean (SD)	3150 (430.0)	3240 (456.0)	<0.001[a]	3210 (403.0)	3340 (421.0)	<0.001[a]	<0.001[a]
Exclusive breastfeeding duration (weeks), n (%)							
<24 weeks	2178 (88.3)	2040 (88.0)	0.767[b]	444 (92.1)	444 (92.9)	0.609[b]	0.358[b]
≥24 weeks	288 (11.7)	277 (12.0)		38 (7.9)	34 (7.1)		
Child's exact age at outcome assessment follow-up (years), mean (SD)	10.2 (0.3)	10.1 (0.3)	0.010[a]	8.7 (0.7)	8.7 (0.7)	0.713[a]	<0.001[a]
INMA cohort, n (%)							
Valencia				148 (29.0)	142 (28.0)		
Sabadell	-	-		190 (37.2)	187 (36.8)	0.886[b]	
Gipuzkoa				173 (33.9)	179 (35.2)		
Age at menarche (years), mean (SD)	12.0 (1.4)	-		12.3 (1.5)	-		0.054[a]
Tanner stage, n (%)							
1	636 (25.7)	1875 (73.4)		61 (41.5)	83 (58.5)		
2	1113 (45.1)	622 (24.4)		75 (51.0)	55 (38.7)		
3	597 (24.2)	46 (1.8)	<0.001[b]	11 (7.5)	4 (2.8)	0.008[b]	<0.001[b]
4	121 (4.9)	10 (0.4)		0 (0.0)	0 (0.0)		
5	2 (0.1)	1 (0.0)		0 (0.0)	0 (0.0)		
Pubertal Development Score, n (%)							
1				212 (42.0)	348 (68.8)		
1.5				160 (31.7)	112 (22.1)		
2				82 (16.2)	37 (7.3)		
2.5				32 (6.3)	6 (1.2)		
3	-	-		13 (2.6)	3 (0.6)	<0.001[b]	
3.5				3 (0.6)	0 (0.0)		
4				0 (0.0)	0 (0.0)		
4.5				1 (0.2)	0 (0.0)		

5				2 (0.4)	0 (0.0)		
Maternal characteristics							
Maternal age (years), mean (SD)	29.8 (5.2)	29.8 (4.9)	0.887[a]	32.0 (3.7)	32.3 (3.5)	0.100[a]	<0.001[a]
Maternal pre-pregnancy BMI (kg/m^2), n (%)							
>18.5kg/m^2	93 (3.9)	95 (4.0)		23 (4.5)	26 (5.1)		
18.5 – 24.9kg/m^2	1606 (67.4)	1586 (67.3)	0.605[b]	365 (71.4)	347 (68.3)	0.885[b]	0.005[b]
25.0-29.9 kg/m^2	489 (20.7)	487 (20.7)		91 (17.8)	91 (17.9)		
≥30 kg/m^2	194 (8.1)	190 (8.0)		32 (6.3)	44 (8.7)		
Household income (€), n (%)							
<500€	155 (5.7)	99 (3.9)					
500-1000€	901 (33.3)	788 (30.9)					
1001-1500€	801 (29.6)	830 (32.5)					
1501-2000€	424 (15.7)	439 (17.2)	0.006[b]	-	-		
2001-2500€	201 (7.4)	198 (7.8)					
2501-3000€	115 (4.2)	106 (4.2)					
>3000€	112 (4.1)	94 (3.7)					
Equivalised disposable income quintile (EU-SILC)							
1st quintile				58 (11.4)	71 (14.0)		
2nd quintile				92 (18.0)	88 (17.3)		
3rd quintile	-	-		106 (20.7)	104 (20.5)	0.751[b]	
4th quintile				125 (24.5)	114 (22.4)		
5th quintile				130 (25.4)	131 (25.8)		
Urbanicity (Pregnancy)							
Urban Centre				209 (59.6)	202 (56.4)		
Dense Urban Cluster				84 (23.9)	84 (23.5)		
Semi-Dense Urban Cluster				0 (0.0)	0 (0.0)		
Suburban/Peri-Urban	-	-		45 (12.8)	46 (12.8)	0.218[b]	
Rural Cluster				2 (0.6)	6 (1.7)		
Low-Density Rural				11 (3.1)	16 (4.5)		
Very Low-Density Rural				0 (0.0)	4 (1.1)		

	Wilderness				0 (0.0)	0 (0.0)		
Urbanicity (Birth)								
	Urban Centre	3291 (80.9)	3403 (80.1)		309 (61.2)	305 (60.8)		
	Dense Urban Cluster	88 (2.2)	122 (2.9)		114 (22.5)	101 (20.1)		
	Semi-Dense Urban Cluster	31 (0.8)	46 (1.1)		6 (1.2)	3 (0.6)		
	Suburban/Peri-Urban	472 (11.6)	490 (11.5)	0.323[b]	56 (11.1)	57 (11.3)	0.104[b]	<0.001[b]
	Rural Cluster	58 (1.4)	55 (1.3)		3 (0.6)	5 (1.0)		
	Low-Density Rural	116 (2.8)	120 (2.8)		16 (3.2)	21 (4.2)		
	Very Low-Density Rural	11 (0.3)	10 (0.2)		1 (0.2)	10 (2.0)		
	Wilderness	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		
Urbanicity (1-1.5y)								
	Urban Centre	3291 (80.9)	3403 (80.1)		299 (62.6)	294 (61.7)		
	Dense Urban Cluster	88 (2.2)	122 (2.9)		106 (22.2)	93 (19.5)		
	Semi-Dense Urban Cluster	31 (0.8)	46 (1.1)		4 (0.8)	4 (0.8)		
	Suburban/Peri-Urban	472 (11.6)	490 (11.5)	0.323[b]	51 (10.7)	51 (10.7)	0.105[b]	<0.001[b]
	Rural Cluster	58 (1.4)	55 (1.3)		4 (0.8)	3 (0.6)		
	Low-Density Rural	116 (2.8)	120 (2.8)		13 (2.7)	21 (4.4)		
	Very Low-Density Rural	11 (0.3)	10 (0.2)		1 (0.2)	10 (2.1)		
	Wilderness	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		
Urbanicity (4-5y)								
	Urban Centre	3232 (79.4)	3325 (78.3)		266 (61.0)	261 (59.7)		
	Dense Urban Cluster	100 (2.5)	139 (3.3)		103 (23.6)	91 (20.8)		
	Semi-Dense Urban Cluster	38 (0.9)	51 (1.2)		3 (0.7)	3 (0.7)		
	Suburban/Peri-Urban	487 (12.0)	528 (12.4)	0.135[b]	49 (11.2)	50 (11.4)	0.146[b]	<0.001[b]
	Rural Cluster	55 (1.4)	66 (1.6)		2 (0.5)	4 (0.9)		
	Low-Density Rural	143 (3.5)	127 (3.0)		12 (2.7)	19 (4.3)		
	Very Low-Density Rural	13 (0.3)	10 (0.2)		1 (0.2)	9 (2.1)		
	Wilderness	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		

BMI, body mass index; SD, standard deviation. [a] Wilcoxon test; [b] χ^2 test/Fisher test.

a)


Birth
1 year
4 years
Cluster 1
Cluster 2
Facility_dens
Facility_rich
Population
Connectivity
Bus_lines
Bus_stops
UV
Building
Walkability
Land use
PM25
NO2
Blue_size
Green_size
Blue_dist
Green_dist
Distance road
NDVI

b)

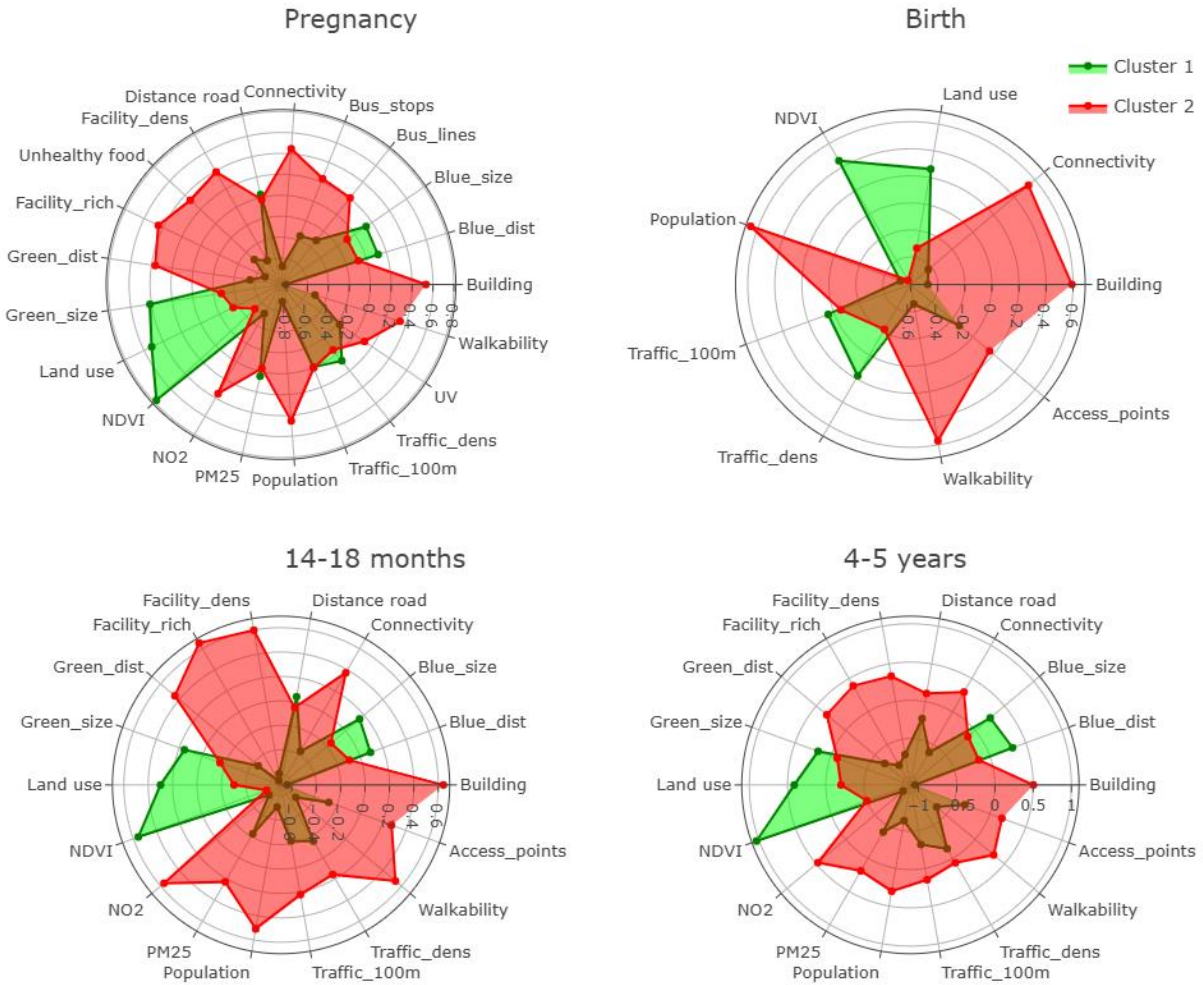
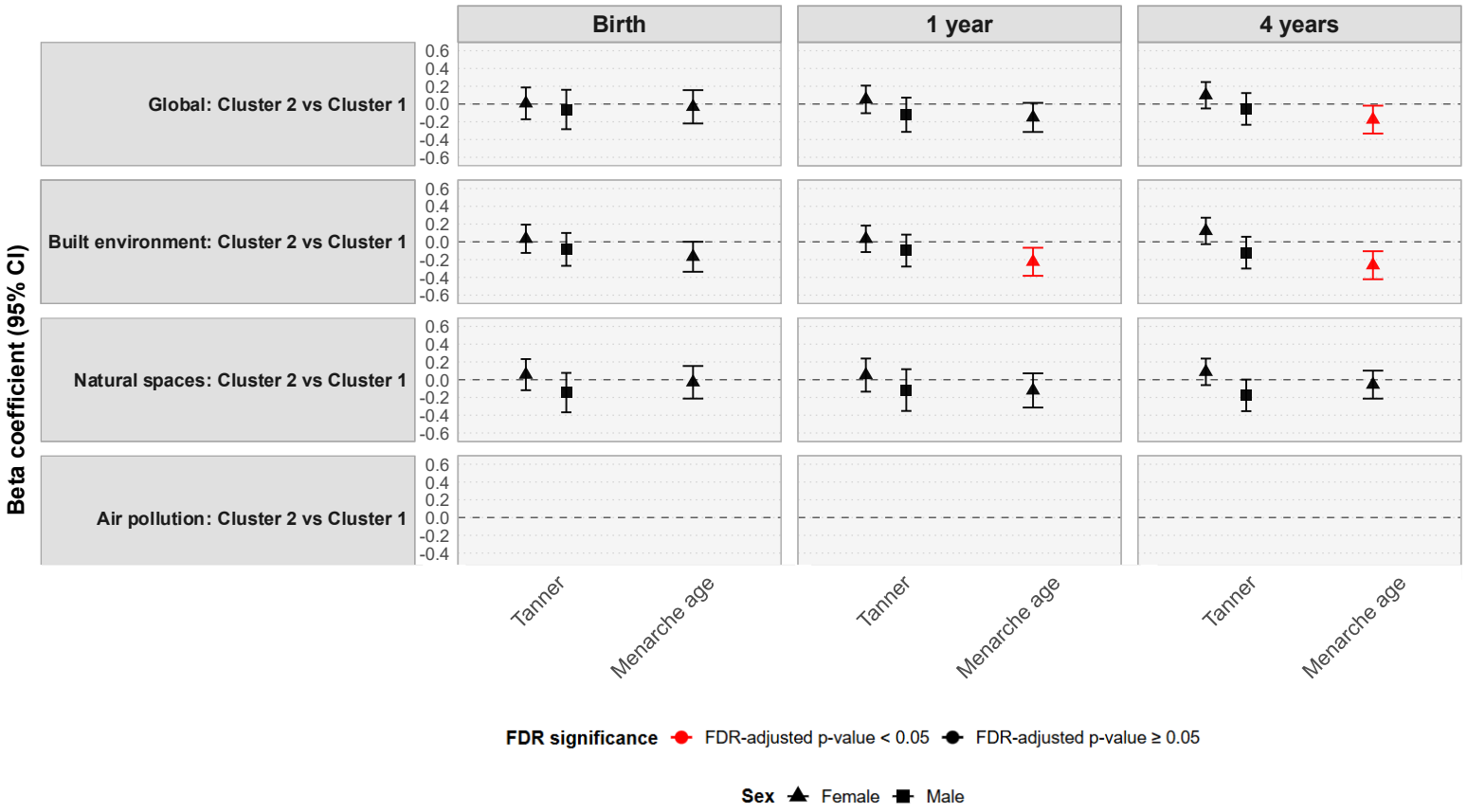


Figure 2. Radar plots of environmental exposure clusters across early-life time windows in the G21 and INMA cohorts. a) G21 cohort; b) INMA cohort. Green areas represent Cluster 1 and red areas represent Cluster 2. Abbreviations: Access_points,; Building, building density; Blue_dist, distance to nearest major blue space; Blue_size, size of the nearest major blue space; Green_dist, distance to nearest major green space;

Green_size, size of the nearest major green space; Bus_lines, length of public transport lines; Bus_stops, density of public bus stops; Connectivity, connectivity density; Distance road, inverse distance to nearest road; Facility_dens, facility density; Facility_rich, facility richness; Unhealthy food, unhealthy food facility density; Land use, land use Shannon's Evenness Index; NDVI, Normalized Difference Vegetation Index ; NO2, nitrogen dioxide; PM25, particulate matter with an aerodynamic diameter of less than 2.5 μm; Population, population density; Traffic_100, traffic load on all roads in 100m buffer; Traffic_dens, traffic density in nearest road; UV, average of vitamin D UV dose; Walkability, walkability index; F&V, fruit and vegetables; EDF, energy-dense foods; HEI, healthy eating index; BF_any, non-exclusive breastfeeding duration; BF_exc, exclusive breastfeeding duration.

a)


Birth
1 year
4 years
Global: Cluster 2 vs Cluster 1
Built environment: Cluster 2 vs Cluster 1
Natural spaces: Cluster 2 vs Cluster 1
Air pollution: Cluster 2 vs Cluster 1
Beta coefficient (95% CI)
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
Tanner
Menarche age
FDR significance
FDR-adjusted p-value < 0.05
FDR-adjusted p-value ≥ 0.05
Sex
Female
Male

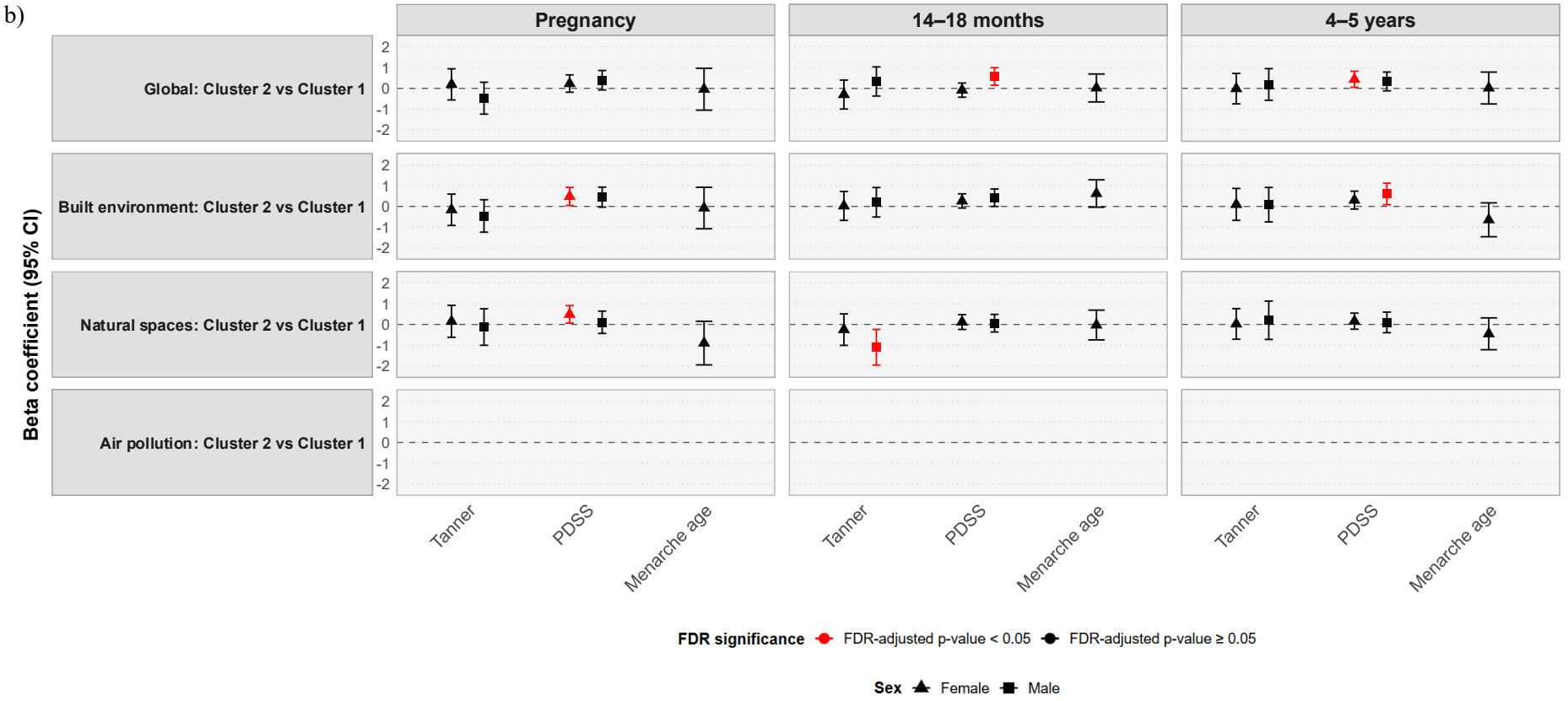


Figure 3. Standardized beta estimates from cluster-based ordinal regression models for pubertal outcomes across different exposure time points in the G21 and INMA cohorts. a) G21 cohort; b) INMA cohort. Estimates represent standardized beta coefficients comparing Cluster 2 (less favourable environmental profile) versus Cluster 1 (favourable environmental profile). Results with FDR-adjusted p-values <0.05 are highlighted in red. Results are shown for global environmental exposure and each environmental subdomain (built environment, natural spaces, and air pollution & traffic). Models are stratified by sex.

In the G21 cohort (a), statistically significant associations after FDR correction were observed for the global analysis at 4 years and for the built environment subdomain at 1 and 4 years with age at menarche as the outcome. At 4 years, belonging to Cluster 2 (less favourable urban environment) was associated with a lower age at menarche. Similarly, at 1 and 4 years, belonging to Cluster 2 of the built environment subdomain was also associated with a lower age at menarche, compared with girls belonging to Cluster 1. Associations with Tanner stage as the outcome were not statistically significant after FDR correction.

In the INMA cohort (b), statistically significant associations after FDR correction were observed for the global analysis and for the built environment and natural spaces subdomains. During pregnancy, belonging to Cluster 2 in the built environment and natural spaces subdomains was positively associated with PDSS scores in females. At 14–18 months, significant associations were observed for the global analysis and the natural spaces subdomain, where Cluster 2 was associated with higher PDSS scores in males and lower Tanner stages, also in males, respectively. At 4–5 years, significant positive associations were identified between Cluster 2 from the global analysis and PDSS scores in females, and between Cluster 2 from the built environment subdomain and PDSS scores in males. Associations with age at menarche were not statistically significant after FDR correction.

Supplementary Methods, Tables & Figures

Table S1

Environmental exposures and outcomes considered at each time point and cohort.

Table S2

Early-life environmental exposure clusters and pubertal timing.

Table S3

Early-life environmental exposure subdomains and pubertal timing.

Table S4

Exclusive breastfeeding duration, environmental exposure clusters, and pubertal timing.

Table S5

Environmental exposure clusters and pubertal timing restricted to urban populations.

Table S6

Environmental exposure clusters and pubertal timing in normal-weight children (G21).

Table S7

Environmental exposure clusters and pubertal timing in participants remaining in the same cluster.

Table S8

Environmental exposure subdomains and pubertal timing in the urban populations.

Table S9

Environmental exposure subdomains and pubertal timing in normal-weight children (G21).

Figure S1

Timeline of exposures and pubertal outcomes in the G21 and INMA cohorts.

Figure S2

Statistical analysis pipeline.

Figure S3

Standardized ExWAS estimates for urban environment, breastfeeding and diet in relation to pubertal timing.

Figure S4

Urban environmental cluster characteristics by exposure time point.

Figure S5

Volcano plots of ExWAS analyses (uncorrected p-values).

Section I. Exposure assessment methods

In this section, we describe the methods used to estimate all exposures included in the exposome for Generation XXI and INMA cohorts. A detailed list of all exposure variables included in the analyses is provided in **Table 1**.

1.1.Air pollution and traffic

Exposure to outdoor air pollution, including nitrogen dioxide (NO_2) and particulate matter (PM), was estimated using environmental monitoring data combined with land-use regression models. These models incorporated geographical information on land use, traffic indicators, and altitude (61,62), and were used to predict individual-level residential exposure.

1.2.Natural spaces

Residential greenness was assessed using the Normalized Difference Vegetation Index (NDVI) within a 300m buffer around each participant's residence. NDVI is a satellite-based indicator of vegetation cover, with values ranging from -1 to 1, where higher values indicate greater photosynthetically active vegetation. Satellite images were selected for each study area during peak greenness periods under cloud-free conditions, and NDVI was estimated at the time of delivery (63). Access to green and blue spaces was measured as the straight-line distance from the participant's residence to the nearest green space and blue space, respectively, using data from an urban atlas database (63).

1.3.Built environment

Built environment indicators were derived from multiple spatial data sources. Traffic data and public transport accessibility were obtained from municipal records, while population density was derived from the Spanish Statistical Office (INE). Street connectivity and facility locations were obtained from digital street maps provided by Navteq, and building density data were retrieved from regional cartographic institutes (63). Street connectivity was defined as the number of intersections per square kilometre within 300m buffers. Facilities were characterized using two complementary indicators: (i) a facility richness index, defined as the number of different facility types divided by the maximum possible number of facility types within the 300-m buffer; and (ii) a facility density index, calculated as the number of facilities per unit area within the 300m buffer.

Land-use characteristics were summarized using a land-use mix indicator based on Shannon's Evenness Index, reflecting the diversity of land-use categories within the area.

Traffic exposure was characterised using three indicators: total traffic load within a 100m buffer, traffic density on the nearest road, and the inverse distance to the nearest road. Accessibility to public transport was assessed using two measures within 300m buffers using two measures: the total length of public bus lines and the number of bus stops. The food environment was defined as the density of unhealthy food outlets, calculated as the number of such facilities divided by the area of the 300m buffer, and assessed for the first trimester of pregnancy. Finally, a walkability index was constructed by combining population density, street connectivity, facility richness, and land-use evenness. The index was calculated using both the mean and the sum of the deciles of these components within 300m buffers, resulting in a walkability score ranging from 0 to 1 (63).

References

61. Beelen R, Hoek G, Vienneau D, Eeftens M, Dimakopoulou K, Pedeli X, et al. Development of NO2 and NOx land use regression models for estimating air pollution exposure in 36 study areas in Europe – The ESCAPE project. Atmospheric Environment. 2013;72:10-23.

62. Eeftens M, Beelen R, de Hoogh K, Bellander T, Cesaroni G, Cirach M, et al. Development of Land Use Regression Models for PM2.5, PM2.5 Absorbance, PM10 and PMcoarse in 20 European Study Areas; Results of the ESCAPE Project. Environmental Science & Technology. 2012;46(20):11195-205.

63. Tamayo-Uria I, Maitre L, Thomsen C, Nieuwenhuijsen MJ, Chatzi L, Siroux V, et al. The early-life exposome: Description and patterns in six European countries. Environment International. 2019;123:189-200.

Section II. Supplementary tables

Supplementary Table 1. Environmental exposures considered at each time point, by cohort.

	Pregnancy	Birth	1-1.5y	4-5y	9-10y	13/18y
INMA						
Air pollution	NO_2 $PM_{2.5}$	-	NO_2 $PM_{2.5}$	NO_2 $PM_{2.5}$	-	-
Built environment	Building density Population density Lenght of public transport lines Density of public bus stops Connectivity density Facility density Facility richness Unhealthy food facility density Land use Shannon's Evenness Index Walkability index	Building density Population density Number of accesspoints Connectivity density Land use Shannon's Evenness Index Walkability index	Building density Population density Number of accesspoints Connectivity density Land use Shannon's Evenness Index Facility density Facility richness Walkability index	Building density Population density Number of accesspoints Connectivity density Land use Shannon's Evenness Index Facility density Facility richness Walkability index	-	-
Natural spaces	Distance to nearest major blue space Size of nearest major blue space Distance to nearest major green space Size of nearest major green space Normalized Difference Vegetation Index (NDVI)	NDVI	Distance to nearest major blue space Size of nearest major blue space Distance to nearest major green space Size of nearest major green space NDVI	Distance to nearest major blue space Size of nearest major blue space Distance to nearest major green space Size of nearest major green space NDVI	-	-
Traffic	Inverse distance to nearest road	Traffic load on all roads in 100m buffer	Inverse distance to nearest road	Inverse distance to nearest road	-	-

	Traffic load on all roads in 100m buffer Traffic density in nearest road	Traffic density in nearest road	Traffic load on all roads in 100m buffer Traffic density in nearest road	Traffic load on all roads in 100m buffer Traffic density in nearest road		
Lifestyle	Energy-dense foods (EDF) Fruit and vegetables (FV) Healthy Eating Index (HEI)	-	Total breastfeeding duration Exclusive breastfeeding duration	EDF FV HEI	-	-
Outcome assessment	-	-	-	-	Pubertal Development Scale Tanner stages (Valencia) Age at menarche (Sabadell)	-
G21						
Air pollution	-	NO_2 $PM_{2.5}$	NO_2 $PM_{2.5}$	NO_2 $PM_{2.5}$	-	-
Built environment	-	Building density Population density Lenght of public transport lines Density of public bus stops Connectivity density Facility density Facility richness Land use Shannon's Evenness Index Walkability index	Building density Population density Lenght of public transport lines Density of public bus stops Connectivity density Facility density Facility richness Land use Shannon's Evenness Index Walkability index	Building density Population density Lenght of public transport lines Density of public bus stops Connectivity density Facility density Facility richness Land use Shannon's Evenness Index Walkability index	-	-
Natural spaces	-	Distance to nearest major blue space Size of nearest major blue space Distance to nearest major green space Size of nearest major green space	Distance to nearest major blue space Size of nearest major blue space Distance to nearest major green space Size of nearest major green space	Distance to nearest major blue space Size of nearest major blue space Distance to nearest major green space Size of nearest major green space	-	-

		NDVI	NDVI	NDVI		
Traffic	-	Inverse distance to nearest road	Inverse distance to nearest road	Inverse distance to nearest road	-	-
Lifestyle	-	-	Total breastfeeding duration Exclusive breastfeeding duration	EDF FV HEI	-	-
Outcome assessment					Tanner stages	Age at menarche

NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with an aerodynamic diameter of less than 2.5 μm; NDVI, Normalized Difference Vegetation Index; EDF, Energy-dense foods; FV, Fruit and vegetables; HEI, Healthy Eating Index.

Supplementary Table 2. Associations between early-life environmental exposure clusters and pubertal timing in the G21 and INMA cohorts.

	G21				INMA					
	Tanner stage		Menarche age		Tanner stage		Menarche age		PDS	
	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Pregnancy										
Female										
Cluster 1		-		-		Ref.		Ref.		Ref.
Cluster 2					0.192	0.616	-0.041	0.937	0.231	0.279
Male										
Cluster 1		-		-		Ref.		-		Ref.
Cluster 2					-0.473	0.228			0.391	0.102
Birth										
Female										
Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Cluster 2	0.019	0.835	-0.053	0.579	-0.137	0.699	0.082	0.806	0.129	0.449
Male										
Cluster 1		Ref.		-		Ref.		-		Ref.
Cluster 2	-0.071	0.535			0.112	0.752			0.282	0.168
1-1.5 years										
Female										
Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Cluster 2	0.060	0.454	-0.172	0.041	-0.299	0.402	0.019	0.957	-0.086	0.623
Male										
Cluster 1		Ref.		-		Ref.		-		Ref.
Cluster 2	-0.122	0.216			0.332	0.354			0.572	0.008
4-5 years										
Female										
Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.

	Cluster 2	0.099	0.191	-0.181	0.024	-0.014	0.970	0.016	0.968	0.333	0.150
Male											
	Cluster 1	Ref.				Ref.				Ref.	
	Cluster 2	-0.059	0.517	-		0.188	0.630	-		0.437	0.027

Cluster 1 represents a more favourable or healthier environmental profile, characterised by greater availability of green spaces, vegetation and land-use diversity, and lower building density, traffic intensity, and air pollution levels. Cluster 2 represents a less favourable or unhealthier environmental profile, characterised by higher building density, traffic intensity, and pollutant concentrations, and lower access to natural spaces. Cluster 1 was used as the reference category in the present analysis. P-values are FDR adjusted using the Benjamini-Hochberg procedure. FDR correction was applied separately by cohort, sex, outcome, and exposure time point. In all analysis, FDR-corrected p-values were identical to the nominal p-values.

Supplementary Table 3. Associations between clusters of environmental subdomains (natural spaces, built environment, and air pollution and traffic) and pubertal timing indicators.

		G21				INMA					
		Tanner stage		Menarche age		Tanner stage		Menarche age		PDS	
		β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Pregnancy											
Built environment	Female										
	Cluster 1		-		-		Ref.		Ref.		Ref.
	Cluster 2					-0.164	0.671	-0.079	0.877	0.479	0.031
	Male										
	Cluster 1		-		-		Ref.		-		Ref.
	Cluster 2					-0.461	0.248			0.446	0.070
Natural spaces	Female										
	Cluster 1		-		-		Ref.		Ref.		Ref.
	Cluster 2					0.150	0.703	-0.900	0.093	0.486	0.024
	Male										
	Cluster 1		-		-		Ref.		-		Ref.
	Cluster 2					-0.126	0.779			0.104	0.704
Air pollution & traffic	Female										
	Cluster 1		-		-		Ref.		Ref.		Ref.
	Cluster 2					-0.923	0.133	-0.451	0.435	0.161	0.525
	Male										
	Cluster 1		-		-		Ref.		-		Ref.
	Cluster 2					0.305	0.596			0.086	0.773
Birth											
Built environment	Female										
	Cluster 1		Ref.		Ref.						
	Cluster 2	0.035	0.671	-0.168	0.051		-		-		-

	Male										
	Cluster 1		Ref.		-						
	Cluster 2	-0.085	0.369				-		-		-
	Female										
	Cluster 1		Ref.		Ref.						
Natural spaces	Cluster 2	0.057	0.524	-0.029	0.755		-		-		-
	Male										
	Cluster 1		Ref.		-						
	Cluster 2	-0.145	0.200				-		-		-
	Female										
	Cluster 1		Ref.		Ref.						
Air pollution & traffic	Cluster 2	0.014	0.895	-0.253	0.028		-		-		-
	Male										
	Cluster 1		Ref.		-						
	Cluster 2	-0.031	0.813				-		-		-
1-1.5 years											
	Female										
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Built environment	Cluster 2	0.034	0.657	-0.224	0.005	0.023	0.949	0.621	0.065	0.262	0.133
	Male										
	Cluster 1		Ref.		-		Ref.		-		Ref.
	Cluster 2	-0.098	0.284			0.198	0.585			0.418	0.051
	Female										
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Natural spaces	Cluster 2	0.052	0.585	-0.121	0.219	0.251	0.516	0.028	0.939	-0.115	0.528
	Male										
	Cluster 1		Ref.		-		Ref.		-		Ref.
	Cluster 2	-0.117	0.330			1.100	0.012			-0.059	0.784

Air pollution & traffic	Female											
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.	
	Cluster 2	0.041	0.702	-0.330	0.004	-0.549	0.148	-0.303	0.393	0.380	0.033	
	Male											
	Cluster 1		Ref.		-		Ref.		-		Ref.	
	Cluster 2	-0.040	0.750			0.101	0.792			0.197	0.354	
4-5 years												
Built environment	Female											
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.	
	Cluster 2	0.123	0.106	-0.263	0.001	0.096	0.807	-0.646	0.121	0.303	0.170	
	Male											
	Cluster 1		Ref.		-		Ref.		-		Ref.	
	Cluster 2	-0.122	0.183			0.083	0.846			0.599	0.024	
Natural spaces	Female											
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.	
	Cluster 2	0.088	0.246	-0.055	0.490	0.023	0.951	-0.452	0.248	0.158	0.426	
	Male											
	Cluster 1		Ref.		-		Ref.		-		Ref.	
	Cluster 2	-0.176	0.052			0.201	0.670			0.098	0.699	
Air pollution & traffic	Female											
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.	
	Cluster 2	0.187	0.045	-0.219	0.024	-0.186	0.601	-0.351	0.368	0.381	0.044	
	Male											
	Cluster 1		Ref.		-		Ref.		-		Ref.	
	Cluster 2	-0.058	0.597			0.479	0.203			0.334	0.131	

Cluster 1 represents a more favourable or healthier environmental profile, characterised by greater availability of green spaces, vegetation and land-use diversity, and lower building density, traffic intensity, and air pollution levels. Cluster 2 represents a less favourable or unhealthier environmental profile, characterised by

higher building density, traffic intensity, and pollutant concentrations, and lower access to natural spaces. Cluster 1 was used as the reference category in the present analysis. P-values are FDR adjusted using the Benjamini-Hochberg procedure. FDR correction was applied separately by cohort, sex, outcome, exposure time point, and urban environmental subdomain. In all analysis, FDR-corrected p-values were identical to the nominal p-values.

Supplementary Table 4. Ordinal logistic regression models of pubertal development by combined exclusive breastfeeding duration and environmental exposure clusters.

		G21				INMA			
		Tanner stage		Menarche age		Menarche age		PDS	
		β	p-value	β	p-value	β	p-value	β	p-value
Pregnancy									
Female									
	Group 1		Ref.		Ref.		Ref.		Ref.
	Group 2					0.970	0.611	0.114	0.923
	Group 3					-1.528	0.611	0.536	0.923
	Group 4					0.919	0.611	0.364	0.923
Male									
	Group 1		Ref.						Ref.
	Group 2							-0.935	0.379
	Group 3				-		-	-0.750	0.630
	Group 4							-0.486	0.630
Birth									
Female									
	Group 1		Ref.		Ref.		Ref.		Ref.
	Group 2	0.173	0.506	-0.184	0.447	0.253	0.742	-0.174	0.923
	Group 3	-0.042	0.884[#]	-0.494	0.447	-1.462	0.611	-0.442	0.923
	Group 4	0.217	0.506	-0.163	0.573	0.510	0.611	-0.076	0.923
Male									
	Group 1		Ref.						Ref.
	Group 2	-0.326	0.056					-0.809	0.379
	Group 3	-0.720	0.056		-		-	-1.384	0.379
	Group 4	-0.395	0.056					-0.824	0.379
1-1.5 years									

Female									
	Group 1		Ref.		Ref.		Ref.		Ref.
	Group 2	0.106	0.762	-0.044	0.856	-0.584	0.611	0.224	0.923
	Group 3	-0.154	0.762	-0.063	0.856	-2.638	0.127	0.237	0.923
	Group 4	0.193	0.506	-0.226	0.447	-0.194	0.781[#]	0.042	0.923[#]
Male									
	Group 1		Ref.						Ref.
	Group 2	-0.269	0.134		-		-	-0.445	0.630
	Group 3	-0.369	0.204[#]					-0.352	0.751
	Group 4	-0.413	0.056					-0.149	0.817
4-5 years									
Female									
	Group 1		Ref.		Ref.		Ref.		Ref.
	Group 2	-0.071	0.762	-0.033	0.856[#]	-0.565	0.646	-0.188	0.923
	Group 3	-0.376	0.506	-0.097	0.856	-1.376	0.611	0.122	0.923
	Group 4	0.085	0.762	-0.246	0.447	-0.338	0.742	0.248	0.923
Male									
	Group 1		Ref.						Ref.
	Group 2	-0.409	0.056		-		-	-0.574	0.630
	Group 3	-0.590	0.056					-0.186	0.817[#]
	Group 4	-0.460	0.056					-0.338	0.751

Four groups were defined according to combinations of urban environmental exposure clusters and exclusive breastfeeding duration (<24 weeks vs. ≥24 weeks): Group 1 (favourable environmental profile and ≥24 weeks of exclusive breastfeeding; reference category), Group 2 (favourable environmental profile and <24 weeks), Group 3 (unfavourable environmental profile and ≥24 weeks), and Group 4 (unfavourable environmental profile and <24 weeks). P-values are FDR adjusted using the Benjamini-Hochberg procedure. FDR correction was applied separately within each interaction type (e.g., exposome x diet, exposome x breastfeeding) and sex stratum. [#]FDR-corrected p-values were identical to the nominal p-values.

Supplementary Table 5. Associations between early-life environmental exposure clusters and pubertal timing in the G21 and INMA cohorts, restricted to the urban population.

	G21				INMA					
	Tanner stage		Menarche age		Tanner stage		Menarche age		PDS	
	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Pregnancy										
Female										
Cluster 1		-		-		Ref.		Ref.		Ref.
Cluster 2					0.192	0.616	-0.041	0.937	0.142	0.521
Male										
Cluster 1		-		-		Ref.		-		Ref.
Cluster 2					-0.492	0.215			0.299	0.234
Birth										
Female										
Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Cluster	0.013	0.891	-0.018	0.856	-0.185	0.604	0.082	0.806	0.062	0.719
Male										
Cluster 1		Ref.		-		Ref.		-		Ref.
Cluster 2	-0.054	0.641			0.055	0.878			0.213	0.314
1-1.5 years										
Female										
Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Cluster 2	0.055	0.494	-0.126	0.139	-0.316	0.378	0.072	0.835	-0.176	0.325
Male										
Cluster 1		Ref.		-		Ref.		-		Ref.
Cluster 2	-0.099	0.323			0.260	0.471			0.550	0.014
4-5 years										

Female											
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
	Cluster 2	0.107	0.170	-0.143	0.083	-0.087	0.820	0.016	0.968	0.335	0.111
Male											
	Cluster 1		Ref.				Ref.				Ref.
	Cluster 2	-0.031	0.738		-	0.121	0.764		-	0.308	0.243

Cluster 1 represents a more favourable or healthier environmental profile, characterised by greater availability of green spaces, vegetation and land-use diversity, and lower building density, traffic intensity, and air pollution levels. Cluster 2 represents a less favourable or unhealthier environmental profile, characterised by higher building density, traffic intensity, and pollutant concentrations, and lower access to natural spaces. Cluster 1 was used as the reference category in the present analysis. P-values are FDR adjusted using the Benjamini-Hochberg procedure. FDR correction was applied separately by cohort, sex, outcome, and exposure time point. In all analysis, FDR-corrected p-values were identical to the nominal p-values.

Supplementary Table 6. Associations between early-life environmental exposure clusters and pubertal timing in the G21 cohorts, restricted to normal weight.

	G21			
	Tanner stage		**Menarche age**	
	β	**p-value**	**β**	**p-value**
Birth				
Female				
Cluster 1		Ref.		Ref.
Cluster 2	0.078	0.527	-0.090	0.487
Male				
Cluster 1		Ref.		
Cluster 2	0.077	0.618		-
1-1.5 years				
Female				
Cluster 1		Ref.		Ref.
Cluster 2	0.166	0.122	-0.174	0.128
Male				
Cluster 1		Ref.		
Cluster 2	-0.094	0.491		-
4-5 years				
Female				
Cluster 1		Ref.		Ref.
Cluster 2	0.103	0.315	-0.117	0.285
Male				
Cluster 1		Ref.		
Cluster 2	0.090	0.478		-

Cluster 1 represents a more favourable or healthier environmental profile, characterised by greater availability of green spaces, vegetation and land-use diversity, and lower building density, traffic intensity, and air pollution levels. Cluster 2 represents a less favourable or unhealthier environmental profile, characterised by

higher building density, traffic intensity, and pollutant concentrations, and lower access to natural spaces. Cluster 1 was used as the reference category in the present analysis. P-values are FDR adjusted using the Benjamini-Hochberg procedure. FDR correction was applied separately by cohort, sex, outcome, and exposure time point. In all analysis, FDR-corrected p-values were identical to the nominal p-values.

Supplementary Table 7. Associations between early-life environmental exposure clusters and pubertal timing in the G21 and INMA cohorts of individuals always in the same cluster.

	G21				INMA					
	Tanner stage		Menarche age		Tanner stage		Menarche age		PDS	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Pregnancy										
Female										
Cluster 1		-		-		Ref.		Ref.		Ref.
Cluster 2					-0.470	0.394	0.607	0.476	0.580	0.100
Male										
Cluster 1		-		-		Ref.		-		Ref.
Cluster 2					0.232	0.662			1.013	0.024
Birth										
Female										
Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Cluster	0.104	0.454	-0.159	0.286	-0.470	0.394	0.608	0.476	0.578	0.101
Male										
Cluster 1		Ref.		-		Ref.		-		Ref.
Cluster 2	0.137	0.442			0.232	0.662			1.014	0.024
1-1.5 years										
Female										
Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Cluster 2	0.104	0.454	-0.159	0.286	-0.470	0.394	0.607	0.476	0.579	0.101
Male										
Cluster 1		Ref.		-		Ref.		-		Ref.
Cluster 2	0.137	0.442			0.232	0.662			1.015	0.024
4-5 years										
Female										

	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
	Cluster 2	0.104	0.454	-0.159	0.286	-0.470	0.394	0.608	0.476	0.577	0.102
Male											
	Cluster 1		Ref.				Ref.				Ref.
	Cluster 2	0.137	0.442		-	0.232	0.662		-	1.016	0.024

Cluster 1 represents a more favourable or healthier environmental profile, characterised by greater availability of green spaces, vegetation and land-use diversity, and lower building density, traffic intensity, and air pollution levels. Cluster 2 represents a less favourable or unhealthier environmental profile, characterised by higher building density, traffic intensity, and pollutant concentrations, and lower access to natural spaces. Cluster 1 was used as the reference category in the present analysis. P-values are FDR adjusted using the Benjamini-Hochberg procedure. FDR correction was applied separately by cohort, sex, outcome, and exposure time point. In all analysis, FDR-corrected p-values were identical to the nominal p-values.

Supplementary Table 8. Associations between clusters of environmental subdomains (natural spaces, built environment, and air pollution and traffic) and pubertal timing indicators, restricted to the urban population.

		G21				INMA					
		Tanner stage		Menarche age		Tanner stage		Menarche age		PDS	
		β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Pregnancy											
Built environment	Female										
	Cluster 1		-		-		Ref.		Ref.		Ref.
	Cluster 2					-0.164	0.703	-0.079	0.877[#]	0.407	0.118
	Male										
	Cluster 1		-		-		Ref.		-		Ref.
	Cluster 2					-0.477	0.707			0.354	0.536
Natural spaces	Female										
	Cluster 1		-		-		Ref.		Ref.		Ref.
	Cluster 2					0.150	0.703[#]	-0.900	0.280	0.444	0.118
	Male										
	Cluster 1		-		-		Ref.		-		Ref.
	Cluster 2					-0.144	0.748[#]			0.053	0.953
Air pollution & traffic	Female										
	Cluster 1		-		-		Ref.		Ref.		Ref.
	Cluster 2					-0.923	0.398	-0.451	0.652	0.081	0.753[#]
	Male										
	Cluster 1		-		-		Ref.		-		Ref.
	Cluster 2					0.299	0.748			0.018	0.953[#]
Birth											
Built environment	Female										
	Cluster 1		Ref.		Ref.		-		-		-
	Cluster 2	0.028	0.741[#]	-0.108	0.353						

	Male										
	Cluster 1		Ref.		-						
	Cluster 2	-0.040	0.915				-		-		-
	Female										
	Cluster 1		Ref.		Ref.						
Natural spaces	Cluster 2	0.051	0.741	0.005	0.960#		-		-		-
	Male										
	Cluster 1		Ref.		-						
	Cluster 2	-0.129	0.779				-		-		-
	Female										
	Cluster 1		Ref.		Ref.						
Air pollution & traffic	Cluster 2	0.080	0.741	-0.207	0.346		-		-		-
	Male										
	Cluster 1		Ref.		-						
	Cluster 2	0.016	0.915#				-		-		-
1-1.5 years											
	Female										
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Built environment	Cluster 2	0.029	0.711#	-0.190	0.046	0.013	0.971#	0.700	0.118	0.179	0.319#
	Male										
	Cluster 1		Ref.		-		Ref.		-		Ref.
	Cluster 2	-0.059	0.799			0.108	0.849			0.392	0.262
	Female										
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Natural spaces	Cluster 2	0.047	0.711	-0.088	0.372#	0.304	0.659	0.070	0.849#	-0.258	0.310
	Male										
	Cluster 1		Ref.		-		Ref.		-		Ref.
	Cluster 2	-0.101	0.799			1.047	0.052			-0.035	0.881#

	Female										
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Air pollution & traffic	Cluster 2	0.057	0.711	-0.282	0.046	-0.563	0.424	-0.370	0.450	0.233	0.310
	Male										
	Cluster 1		Ref.		-		Ref.		-		Ref.
	Cluster 2	0.011	0.937[#]			0.073	0.849[#]			0.161	0.707
4-5 years											
	Female										
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Built environment	Cluster 2	0.141	0.109	-0.239	0.012	0.098	0.979	-0.646	0.362	0.217	0.536
	Male										
	Cluster 1		Ref.		-		Ref.		-		Ref.
	Cluster 2	-0.093	0.486			0.000	1.000[#]			0.521	0.278
	Female										
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Natural spaces	Cluster 2	0.115	0.143[#]	-0.040	0.627[#]	-0.010	0.979[#]	-0.452	0.368	0.100	0.618[#]
	Male										
	Cluster 1		Ref.		-		Ref.		-		Ref.
	Cluster 2	-0.160	0.262			0.124	1.000			0.117	0.649[#]
	Female										
	Cluster 1		Ref.		Ref.		Ref.		Ref.		Ref.
Air pollution & traffic	Cluster 2	0.219	0.095	-0.182	0.135	-0.295	0.979	-0.351	0.368[#]	0.280	0.466
	Male										
	Cluster 1		Ref.		-		Ref.		-		Ref.
	Cluster 2	-0.012	0.919[#]			0.384	0.946			0.295	0.331

Cluster 1 represents a more favourable or healthier environmental profile, characterised by greater availability of green spaces, vegetation and land-use diversity, and lower building density, traffic intensity, and air pollution levels. Cluster 2 represents a less favourable or unhealthier environmental profile, characterised by

higher building density, traffic intensity, and pollutant concentrations, and lower access to natural spaces. Cluster 1 was used as the reference category in the present analysis. P-values are FDR adjusted using the Benjamini-Hochberg procedure. FDR correction was applied separately by cohort, sex, outcome, exposure time point, and urban environmental subdomain. [#]FDR-corrected p-values were identical to the nominal p-values.

Supplementary Table 9. Associations between clusters of environmental subdomains (natural spaces, built environment, and air pollution and traffic) and pubertal timing indicators, restricted to normal weight (G21 only).

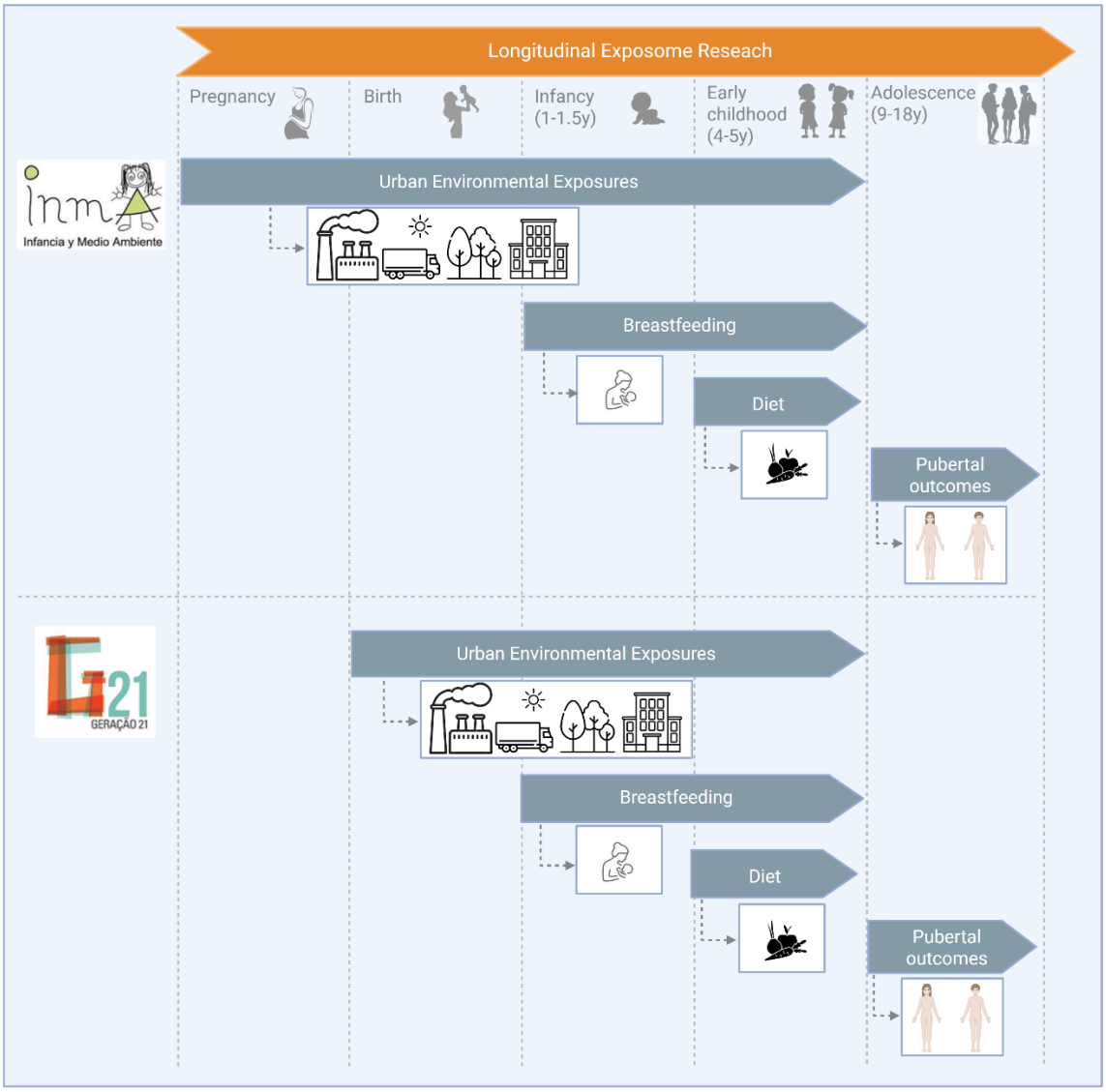
		G21			
		Tanner stage		Menarche age	
		β	95% CI	β	95% CI
Birth					
Built environment	Female				
	Cluster 1		Ref.		Ref.
	Cluster 2	0.118	0.280[#]	-0.200	0.260
	Male				
	Cluster 1		Ref.		-
	Cluster 2	-0.054	0.887		
Natural spaces	Female				
	Cluster 1		Ref.		Ref.
	Cluster 2	0.208	0.243	-0.095	0.455[#]
	Male				
	Cluster 1		Ref.		-
	Cluster 2	0.022	0.887[#]		
Air pollution & traffic	Female				
	Cluster 1		Ref.		Ref.
	Cluster 2	0.166	0.280	-0.195	0.344
	Male				
	Cluster 1		Ref.		-
	Cluster 2	0.079	0.887		
1-1.5 years					
Built environment	Female				
	Cluster 1		Ref.		Ref.

	Cluster 2	0.089	0.387[#]	-0.266	0.047
	Male				
	Cluster 1		Ref.		-
	Cluster 2	-0.039	0.906		
Natural spaces	Female				
	Cluster 1		Ref.		Ref.
	Cluster 2	0.147	0.361	-0.151	0.295
	Male				
	Cluster 1		Ref.		-
	Cluster 2	-0.019	0.906[#]		
Air pollution & traffic	Female				
	Cluster 1		Ref.		Ref.
	Cluster 2	0.188	0.361	-0.165	0.295
	Male				
	Cluster 1		Ref.		-
	Cluster 2	0.084	0.906		
4-5 years					
Built environment	Female				
	Cluster 1		Ref.		Ref.
	Cluster 2	0.199	0.053[#]	-0.209	0.170
	Male				
	Cluster 1		Ref.		-
	Cluster 2	-0.013	0.920		
Natural spaces	Female				
	Cluster 1		Ref.		Ref.
	Cluster 2	0.222	0.047	-0.137	0.320
	Male				
	Cluster 1		Ref.		-

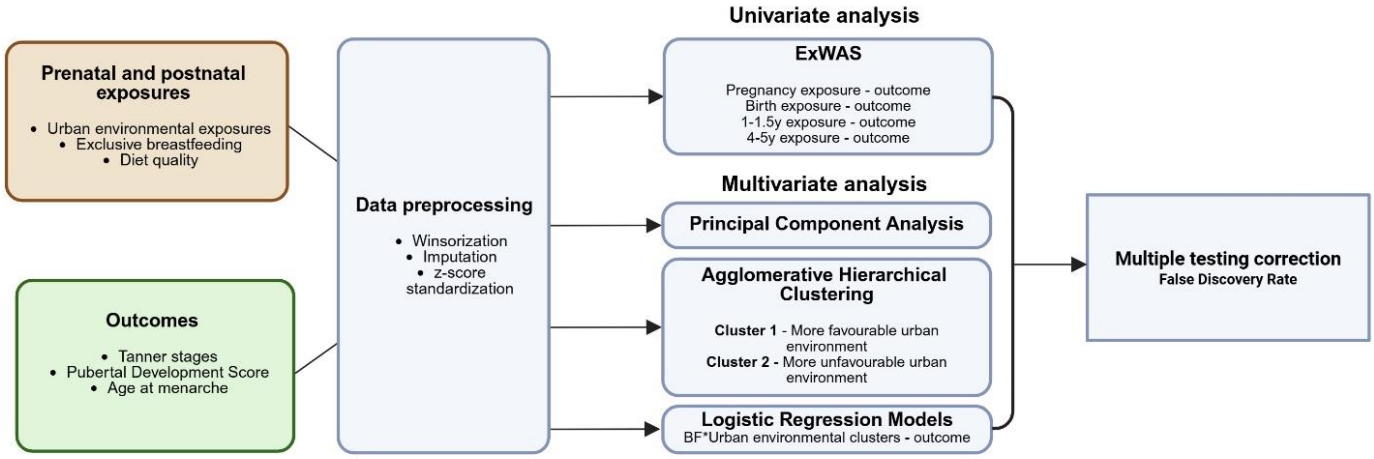
	Cluster 2	0.041	0.920		
Air pollution & traffic	Female				
	Cluster 1	Ref.		Ref.	
	Cluster 2	0.278	0.047	-0.081	0.537[#]
	Male				
	Cluster 1	Ref.		-	
	Cluster 2	0.160	0.917		

Cluster 1 represents a more favourable or healthier environmental profile, characterised by greater availability of green spaces, vegetation and land-use diversity, and lower building density, traffic intensity, and air pollution levels. Cluster 2 represents a less favourable or unhealthier environmental profile, characterised by higher building density, traffic intensity, and pollutant concentrations, and lower access to natural spaces. Cluster 1 was used as the reference category in the present analysis. P-values are FDR adjusted using the Benjamini-Hochberg procedure. FDR correction was applied separately by cohort, sex, outcome, exposure time point, and urban environmental subdomain. [#]FDR-corrected p-values were identical to the nominal p-value.

Section III. Supplementary figures

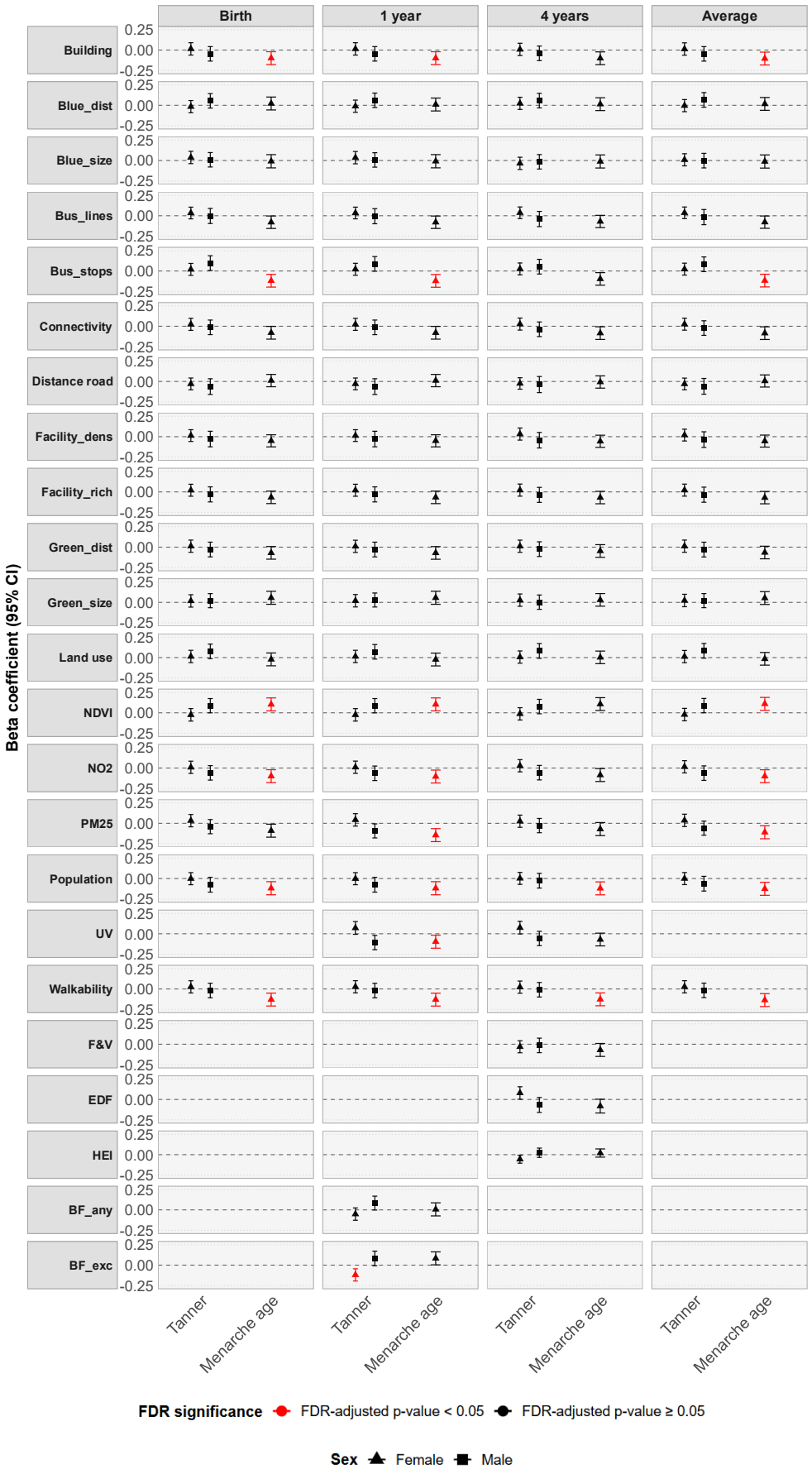


Supplementary Figure 1. **Timeline of exposures and pubertal outcomes assessed in the INMA and G21 cohorts.** The figure illustrates the timing of assessment of urban environmental exposures (built environment, natural spaces, and air pollution & traffic), exclusive breastfeeding duration, diet quality, and pubertal outcomes across different time points in the INMA and G21 birth cohorts.

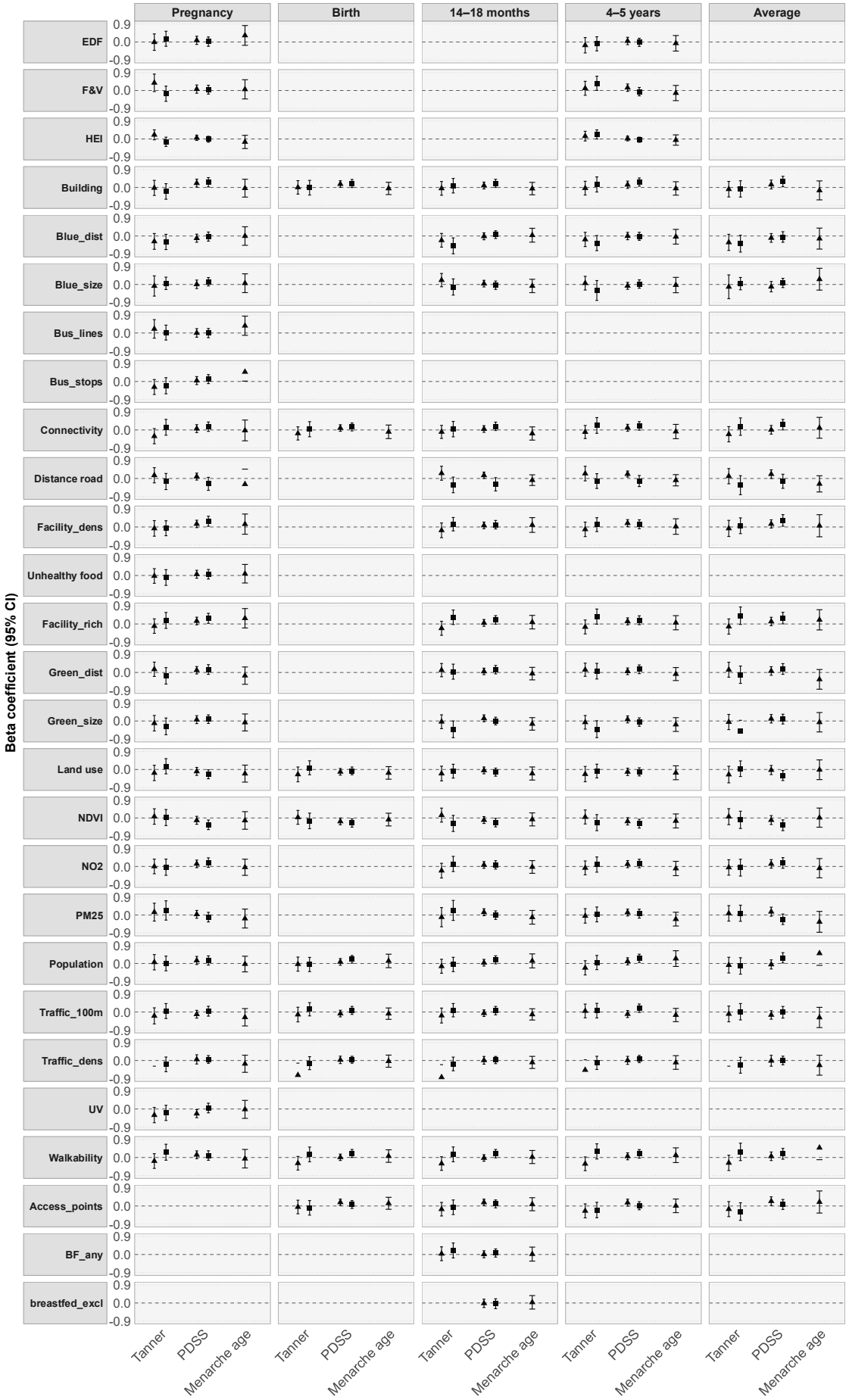


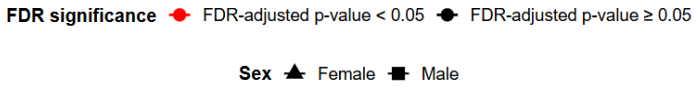
Supplementary Figure 2. Statistical analysis pipeline. Abbreviations: IQR, Interquartile range; ExWAS, Exposome-wide association study; BF, breastfeeding.

a)


Birth
1 year
4 years
Average
Building
Blue_dist
Blue_size
Bus_lines
Bus_stops
Connectivity
Distance road
Facility_dens
Facility_rich
Green_dist
Green_size
Land use
NDVI
NO2
PM25
Population
UV
Walkability
F&V
EDF
HEI
BF_any
BF_exc
0.25
0.00
-0.25
Beta coefficient (95% CI)
Tanner
Menarche age
FDR significance
FDR-adjusted p-value < 0.05
FDR-adjusted p-value ≥ 0.05
Sex
Female
Male

b)


Pregnancy
Birth
14–18 months
4–5 years
Average
EDF
F&V
HEI
Building
Blue_dist
Blue_size
Bus_lines
Bus_stops
Connectivity
Distance road
Facility_dens
Unhealthy food
Facility_rich
Green_dist
Green_size
Land use
NDVI
NO2
PM25
Population
Traffic_100m
Traffic_dens
UV
Walkability
Access_points
BF_any
breastfed_excl
Beta coefficient (95% CI)
0.9
0.0
-0.9
Tanner
PDSS
Menarche age



Supplementary Figure 3 - Standardized beta estimates from ExWAS analyses of urban environment, breastfeeding duration, and diet in relation to pubertal development at 10 years and age at menarche in the G21 and INMA cohorts. a) G21 cohort; b) INMA cohort. Each ExWAS was conducted separately for each outcome and exposure time point, with false discovery rate (FDR) correction applied based on the number of exposures tested in each ExWAS. Results with FDR-adjusted p-values <0.05 are highlighted in red. Points show beta coefficients and vertical bars show standard 95% confidence intervals. Models are stratified by sex. Abbreviations: Access_points, number of bus public transport mode stops; Building, building density; Blue_dist, distance to nearest major blue space; Blue_size, size of the nearest major blue space; Green_dist, distance to nearest major green space; Green_size, size of the nearest major green space; Bus_lines, length of public transport lines; Bus_stops, density of public bus stops; Connectivity, connectivity density; Distance road, inverse distance to nearest road; Facility_dens, facility density; Facility_rich, facility richness; Unhealthy food, unhealthy food facility density; Land use, land use Shannon's Evenness Index; NDVI, Normalized Difference Vegetation Index ; NO2, nitrogen dioxide; PM25, particulate matter with an aerodynamic diameter of less than 2.5 μm; Population, population density; Traffic_100, traffic load on all roads in 100m buffer; Traffic_dens, traffic density in nearest road; UV, average of vitamin D UV dose; Walkability, walkability index; F&V, fruit and vegetables; EDF, energy-dense foods; HEI, healthy eating index; BF_any, non-exclusive breastfeeding duration; BF_exc, exclusive breastfeeding duration.


Main barplots: Birth
Main barplots: 1-1.5 years
Main barplots: 4-5 years
Mean value (95% CI)
Building
Connectivity
Land_use
NDVI
Population
Walkability
BF_any
Blue_dist
Blue_size
Distance_road
Facility_dens
Facility_rich
Green_dist
Green_size
NO2
PM25
EDF
F_V
Cluster comparison
Not significant
FDR p < 0.05
Cluster
Cluster 1
Cluster 2
Cohort border
INMA
G21

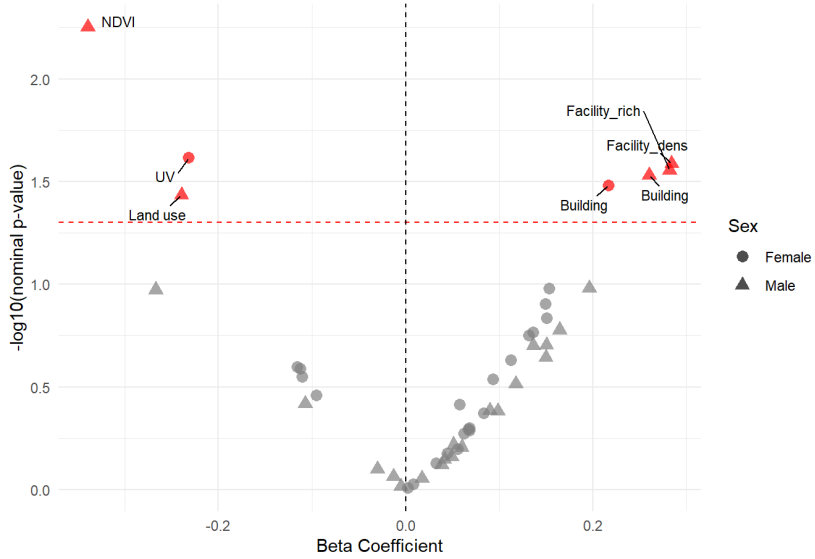
Supplementary Figure 4. Urban environmental cluster characteristics across exposure time points in the G21 and INMA cohorts. Cluster 1 represents a more favourable or healthier environmental profile, characterised by greater availability of green spaces, vegetation and land-use diversity, and lower building density, traffic intensity, and air pollution levels. Cluster 2 represents a less favourable or unhealthier environmental profile, characterised by higher building density, traffic intensity, and pollutant concentrations, and lower access to natural spaces. Stars compare INMA vs G21 cohorts within the same cluster based on FDR-adjusted p-values (* FDR-adjusted $p < 0.05$; ** FDR-adjusted $p < 0.01$; *** FDR-adjusted $p < 0.001$). Lighter bars indicate non-significant INMA vs G21 differences within that cluster. Abbreviations: Building, building density; Blue_dist, distance to nearest major blue space; Blue_size, size of the nearest major blue space; Green_dist, distance to nearest major green space; Green_size, size of the nearest major green space; Connectivity, connectivity density; Distance road, inverse distance to nearest road; Facility_dens, facility density; Facility_rich, facility richness; Land use, land use Shannon's Evenness Index; NDVI, Normalized Difference Vegetation Index ; NO2, nitrogen dioxide; PM25, particulate matter with an aerodynamic diameter of less than 2.5 μm; Population, population density; Walkability, walkability index; F_V, fruit and vegetables; EDF, energy-dense foods; HEI, healthy eating index; BF_any, non-exclusive breastfeeding duration; BF_exc, exclusive breastfeeding duration.

Across all exposure windows in both cohorts, Cluster 1 was characterised by greater availability of green and blue spaces, higher vegetation levels, and lower building density, connectivity, population density, and air pollution levels. In contrast, Cluster 2 was characterised by higher building density, connectivity, population density, walkability, and air pollution levels, together with lower availability of natural spaces. Similar clustering patterns were observed across the G21 and INMA cohorts, although some differences were identified between cohorts. Compared with INMA, the G21 cohort presented higher values for building density, connectivity, walkability, and air pollution indicators, but also greater availability of blue and green spaces.

Section IV. Results with no corrected p-values

INMA – PDS score

a)



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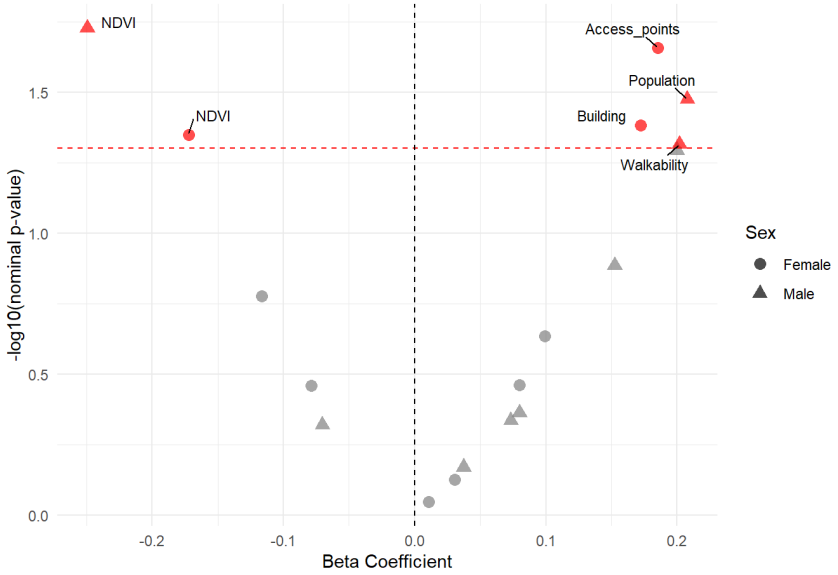

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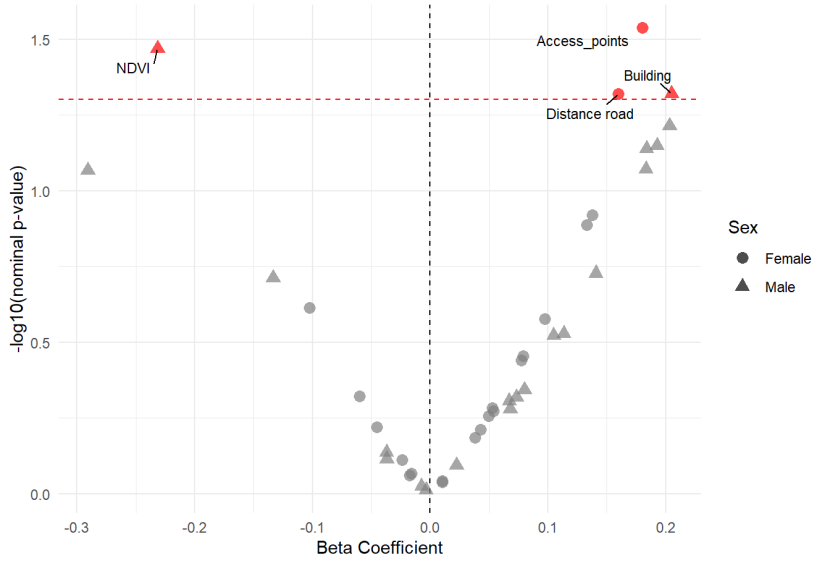

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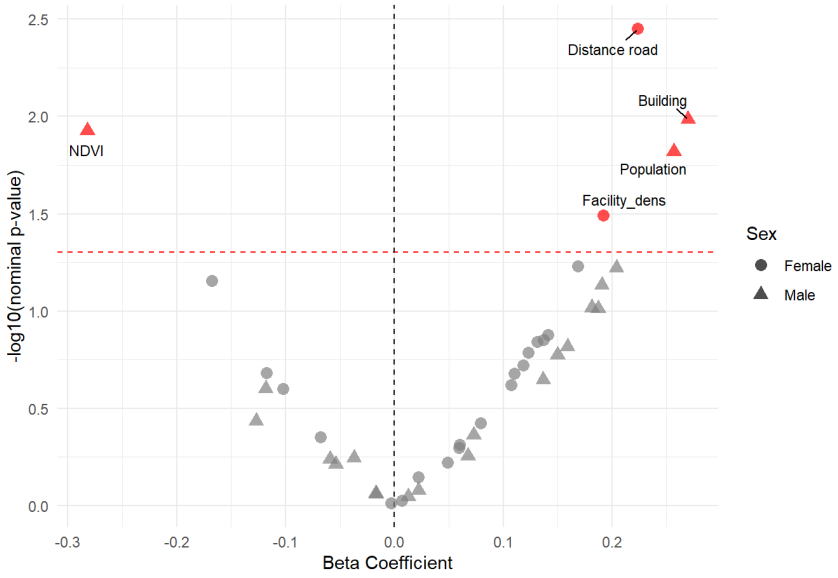
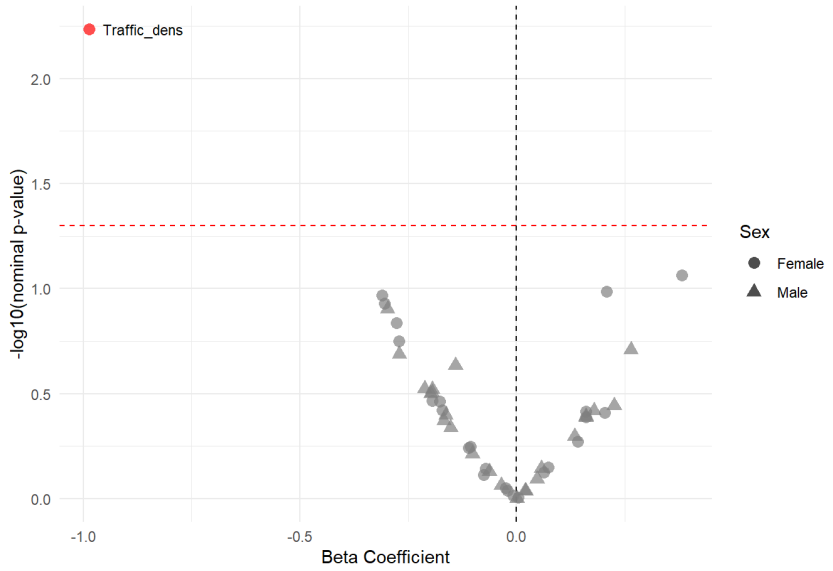
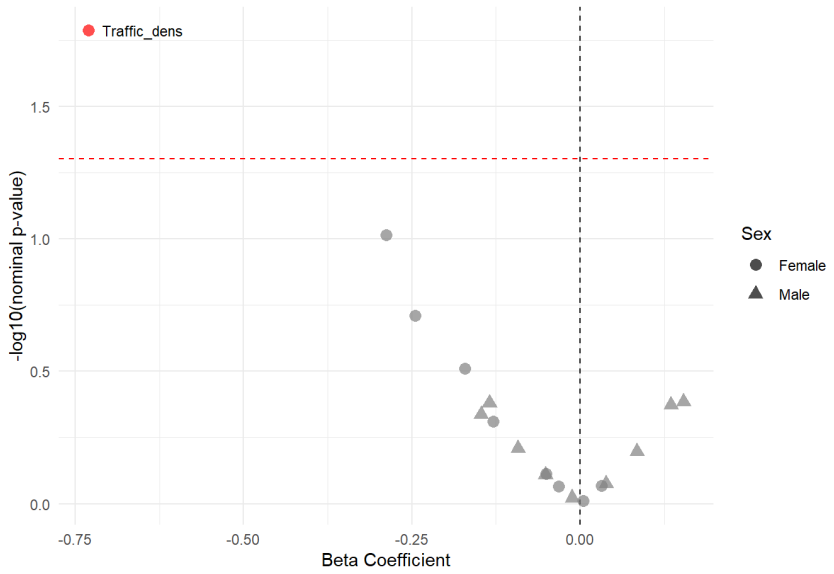


INMA - Tanner stages

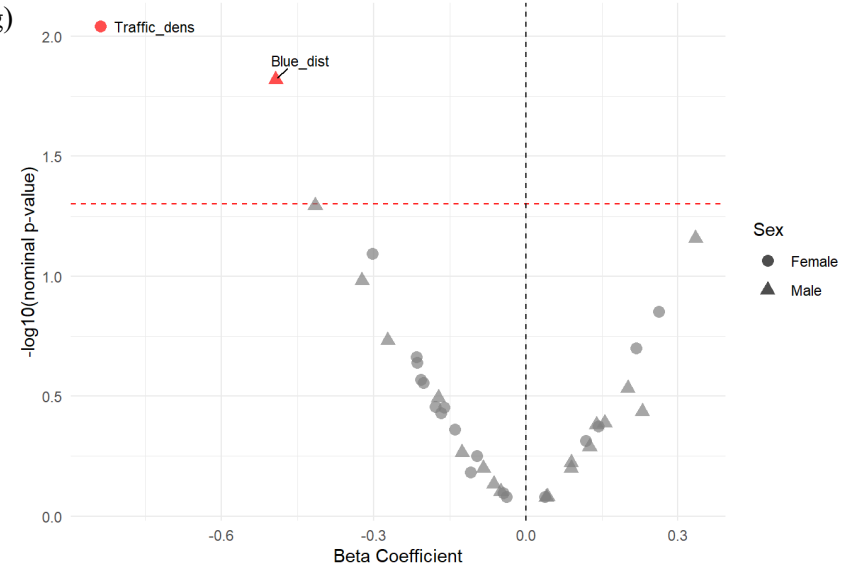
e)



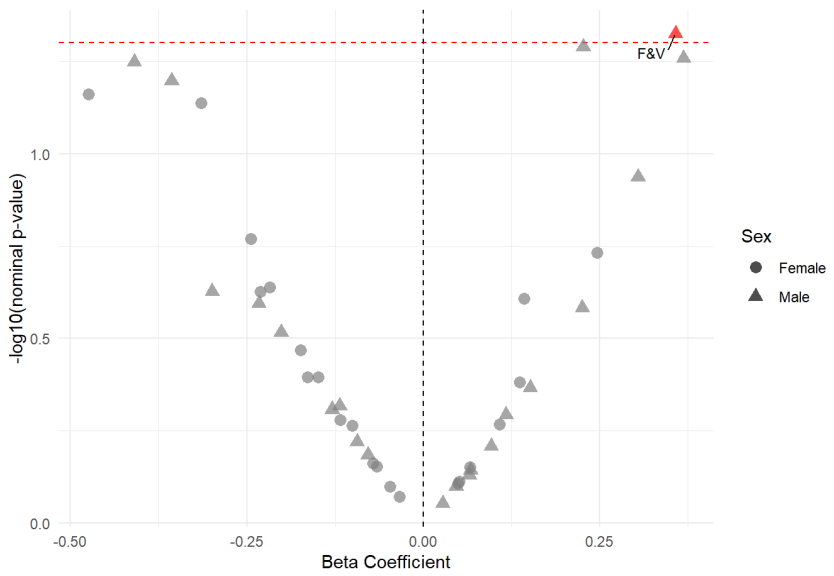
f)



g)

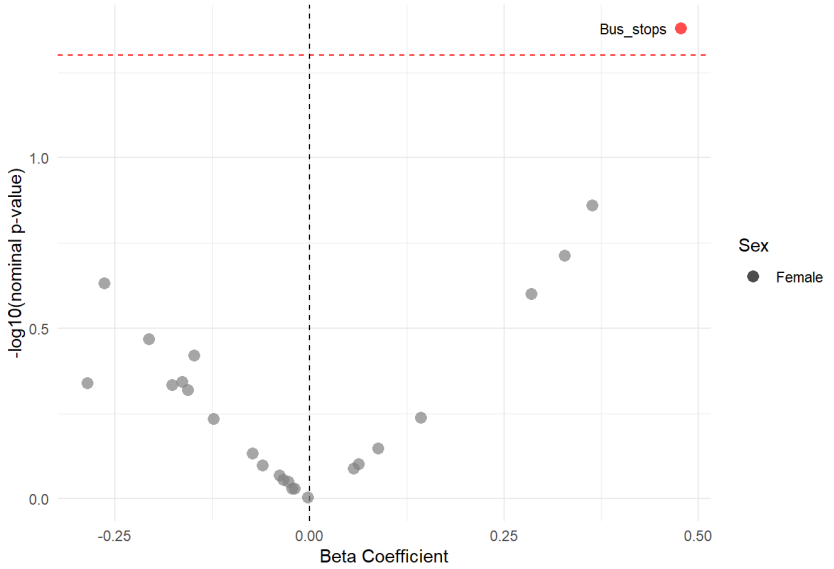


h)



INMA – Age at menarche

i)



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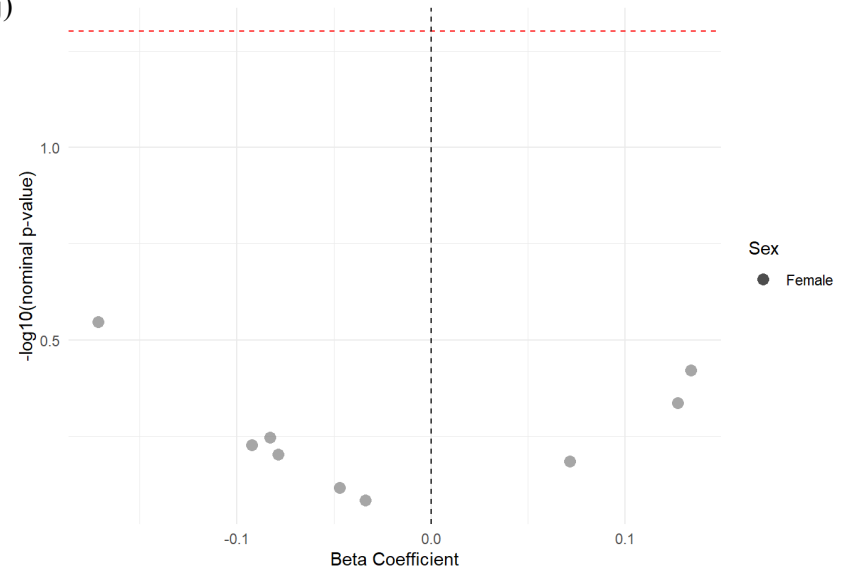
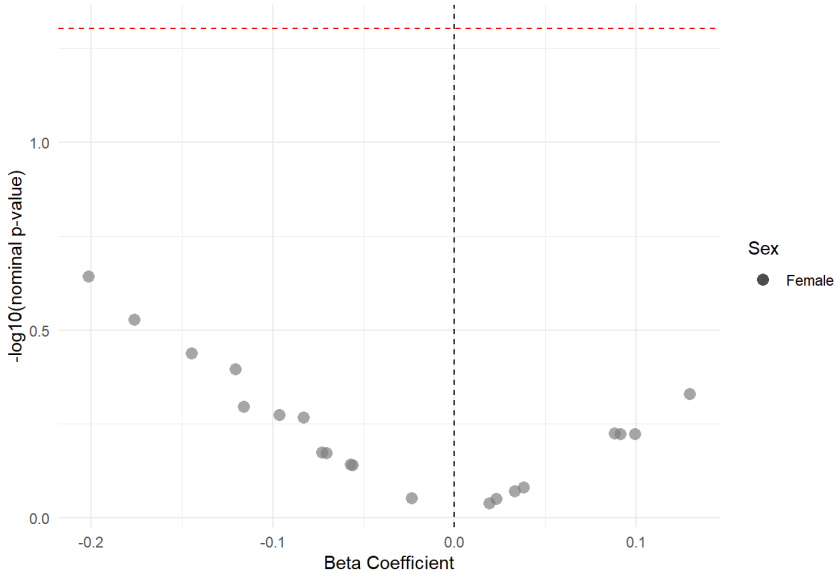
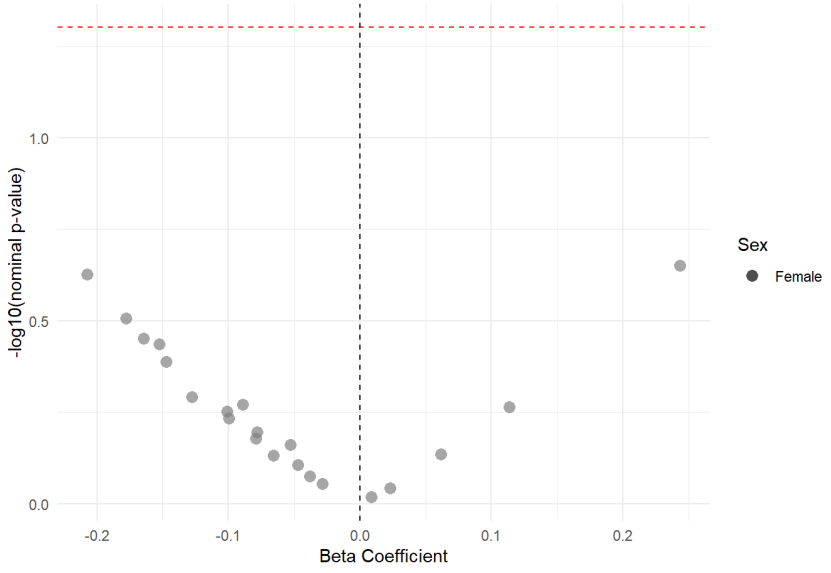


k)

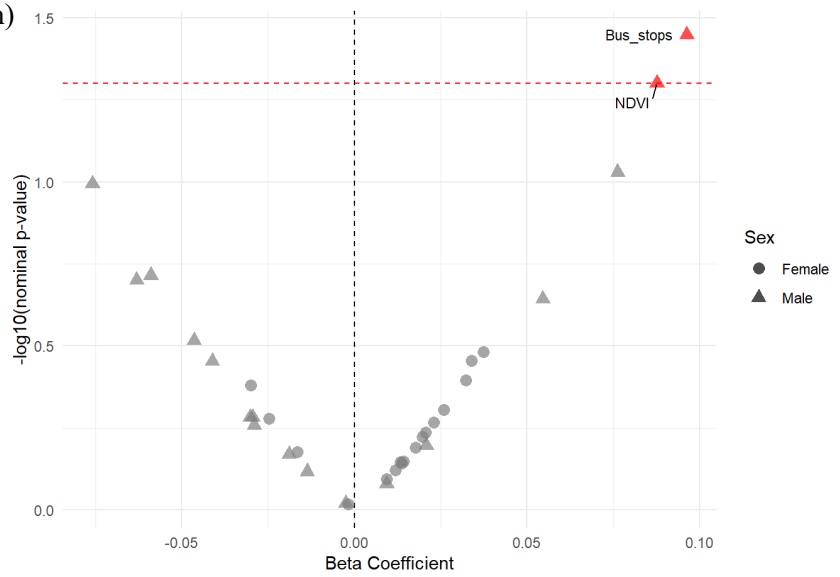


l)

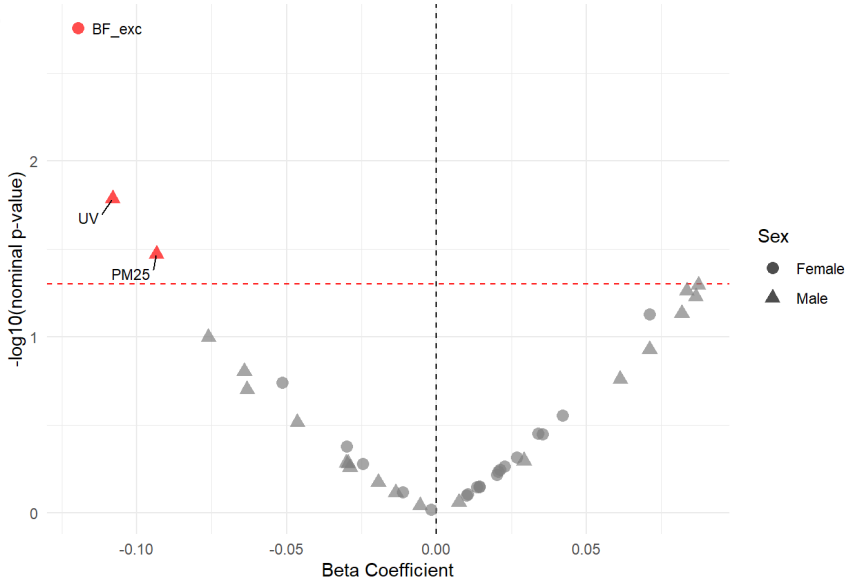


G21 – Tanner stages

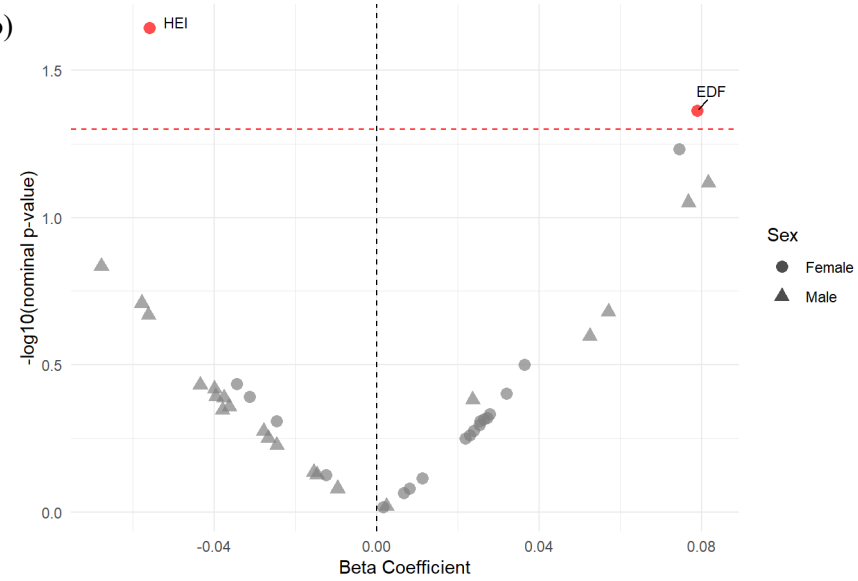
m)



n)

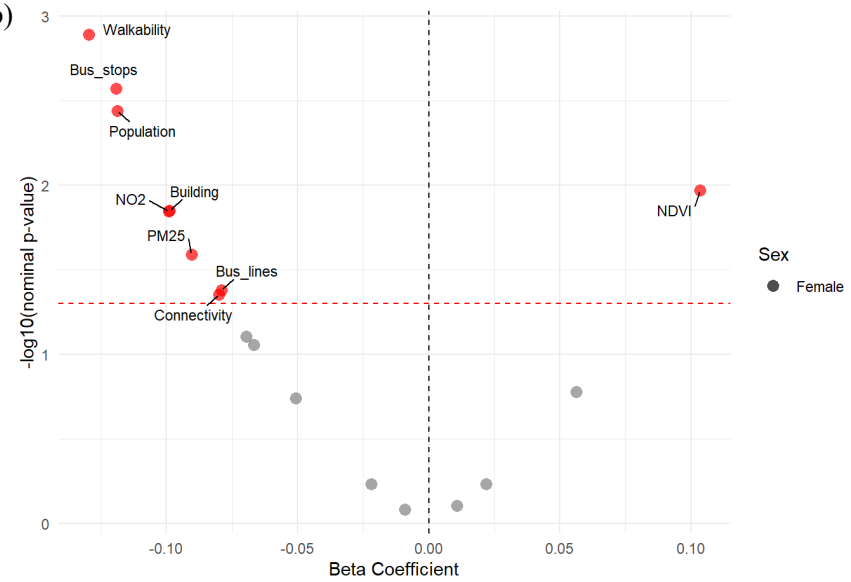


o)

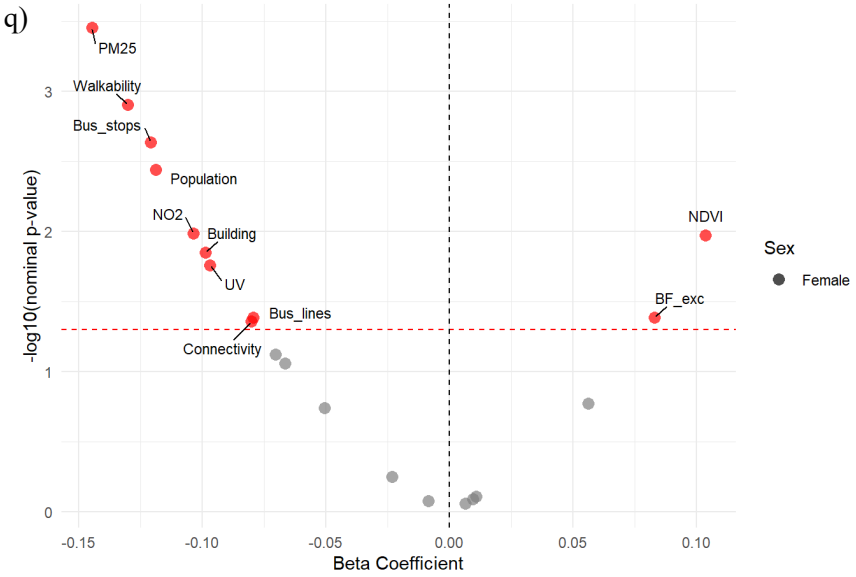


G21 – Age at menarche

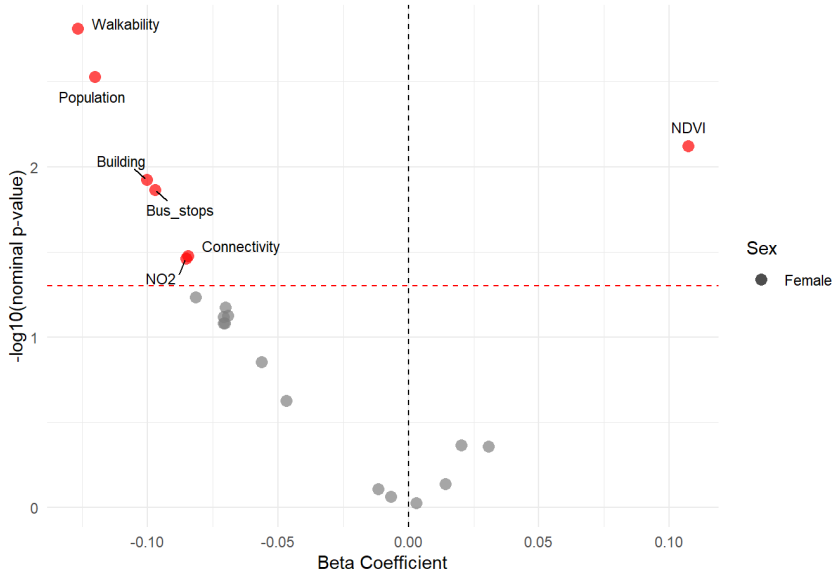
p)



q)



r)



Supplementary Figure 5. Volcano plots of the Exposure Wild Association Study (ExWAS, single exposure models) analyses between the urban environment, breastfeeding duration and diet, and pubertal development at 9-10 years old, using non-FDR-corrected p-values. INMA: a) Pregnancy exposure and pubertal timing defined by PDS score; b) Birth exposure and pubertal timing defined by PDS score; c) 14-18 months exposure and pubertal timing defined by PDS score; d) 4-5 years exposure and pubertal timing defined by PDS score; e) Pregnancy exposure and pubertal timing defined by Tanner stages; f) Birth exposure and pubertal timing defined by Tanner stages; g) 14-18 months exposure and pubertal timing defined by Tanner stages; h) 4-5 years exposure and pubertal timing defined by Tanner stages; i) Pregnancy exposure and age at menarche; j) Birth exposure and age at menarche; k) 14-18 months exposure and age at menarche; l) 4-5 years exposure and age at menarche; G21: m) Birth exposure and pubertal timing defined by Tanner stages; n) 1 year exposure and pubertal timing defined by Tanner stages; o) 4 years exposure and pubertal timing defined by Tanner stages; p) Birth exposure and age at menarche; q) 1 year exposure and age at menarche; r) 4 years exposure and age at menarche. Abbreviations: Access_points, number of bus public transport mode stops; Building, building density; Blue_dist, distance to nearest major blue space; Blue_size, size of the nearest major blue space; Green_dist, distance to nearest major green space; Green_size, size of the nearest major green space; Bus_lines, length of public transport lines; Bus_stops, density of public bus stops; Connectivity, connectivity density; Distance road, inverse distance to nearest road; Facility_dens, facility density; Facility_rich, facility richness; Unhealthy food, unhealthy food facility density; Land use, land use Shannon's Evenness Index; NDVI, Normalized Difference Vegetation Index ; NO2, nitrogen dioxide; PM25, particulate matter with an aerodynamic diameter of less than 2.5 μm; Population, population density; Traffic_100, traffic load on all roads in 100m buffer; Traffic_dens, traffic density in nearest road; UV, average of vitamin D UV dose; Walkability, walkability index; F&V, fruit and vegetables; EDF, energy-dense foods; HEI, healthy eating index; BF_any, non-exclusive breastfeeding duration; BF_exc, exclusive breastfeeding duration.

GENERAL DISCUSSION AND CONCLUSIONS

In this thesis, we investigated the role of early-life exposures, such as breastfeeding, diet quality, physical activity, sleep and urban environment, in shaping pubertal timing. We also examined the tracking of diet quality from childhood to adolescence and explored the influence of socioeconomic, individual and behavioural characteristics on diet quality trajectories. To address these aims, analyses were conducted using data from two large, well-designed population-bases European birth cohorts: Generation XXI and INMA. Overall, the findings of this thesis suggest that diet quality established during childhood tends to track into adolescence. Additionaly, early-life exposure, namely breastfeeding, childhood diet quality and urban environmental exposures, may contribute to variability in pubertal timing. These results support the hypothesis that pubertal timing is influenced not only by genetic predisposition but also by modifiable environmental and behavioural factors.

To assess the development of eating habits from childhood to adolescence, we evaluated the tracking of overall diet quality from preschool to adolescence [paper I]. The development of a healthy eating index to summarise overall diet quality represents a complementary approach to the analysis of single foods or nutrients, allowing for a more comprehensive assessment by capturing the cumulative and interactive effects of foods and nutrients. Our findings indicate a moderate tracking of diet quality between ages 4 and 13, suggesting that children with better diet quality early in life tend to maintain healthier diet quality over time. However, overall diet quality decreased from early childhood to adolescence, suggesting that this transition between life-course stages may represent a sensitive period for the deterioration of eating habits.

Our findings are consistent with previous evidence from the Avon Longitudinal Study of Parents and Children (ALSPAC), which reported moderate tracking of an energy-dense, high-fat and low-fibre dietary pattern between 7 and 13 years of age, supporting the hypothesis that dietary behaviours established in childhood tend to persist into adolescence (144). However, while the ALSPAC study focused on the tracking of a specific dietary pattern, our study extends this evidence by examining the tracking of overall diet quality across early childhood and adolescence. To the best of our knowledge, this is the first study to specifically assess the tracking of overall diet quality, rather than the tracking of individual dietary patterns, across this critical development window.

These findings further emphasise the importance of implementing dietary interventions early in life, as dietary habits established during childhood tend to persist over time. The

transition from childhood to adolescence is characterised by increased autonomy in food choices and a gradual reduction in parental influence and control over dietary intake, accompanied by a greater influence of peers and the social environment (239, 240). Our results suggest that despite this progressive reduction in parental influence, children who had better diet quality at 4 years of age were more likely to maintain relatively better diet quality at 13 years of age. Thus, even though an overall deterioration in diet quality was observed over time, parental influences during early childhood appear to have a lasting impact on dietary behaviours into adolescence.

In addition, socioeconomic and individual factors appear to play an important role in shaping diet quality trajectories. Children from older and more educated mothers, as well as those from households with higher income, consistently presented higher diet quality over time, which is consistent with previous studies (225, 241-245). These associations may be explained by differences in the home food environment, as families with lower socioeconomic status may experience a less supportive food environment and more limited access to healthy foods (246-248). Furthermore, children with higher sedentary time and shorter sleep duration presented poorer diet quality, confirming patterns previously described in the literature (249-252). One of the most frequently proposed explanations for the association between sleep and diet quality is that sleep disruption may lead to hormonal dysregulation involving leptin and ghrelin, hormones that play a key role in appetite regulation and satiety (253, 254). Another possible explanation, which may also help explain the association with higher sedentary time, is that longer waking hours provide more opportunities for food consumption, particularly of energy-dense foods commonly consumed during screen time (255-258).

Early-life dietary exposures represent an important pathway through which environmental factors may influence pubertal development. An important finding of this thesis relates to the potential effect of early feeding practices on pubertal development. To evaluate the association between breastfeeding practices and pubertal timing at 10 years of age, data from 4515 children from the Generation XXI cohort were analysed [paper III]. The results showed that females who had been breastfed (either exclusively or non-exclusively) were less likely to present higher Puberty scores, indicating a lower likelihood of more advanced pubertal development. Similarly, girls who were exclusively breastfed tended to experience a later age at menarche compared with those who were never breastfed. No significant associations were observed among boys.

In our study, the association between exclusive breastfeeding and age at menarche was notably attenuated and was no longer statistically significant after adjustment for BMI at 10 years of age, whereas the association with the Puberty score remained. This finding may suggest that childhood adiposity could partially mediate this relationship, particularly regarding age at menarche, although other mechanisms may also contribute. Childhood obesity has previously been associated with earlier pubertal timing (128, 259), especially among girls, and may represent an important pathway linking early-life nutritional exposures to pubertal development, given that previous studies have shown that a longer duration of breastfeeding may be protective against childhood obesity (260). However, the persistence of the association with the Puberty score after adjustment for BMI suggests that the influence of human milk feeding on pubertal timing may not be entirely explained by differences in body composition.

Human milk contains several bioactive components, including regulatory hormones such as leptin, ghrelin and adipoinectin (120, 261-263). Beyond its role in appetite regulation and energy balance, leptin has also been implicated in the activation of the HPG axis, potentially influencing pubertal maturation (120-122). In addition, breastfeeding practices may also reflect maternal and family characteristics that support healthier early-life environments, such as higher maternal education, better sleep quality (264) and healthier dietary patterns (129), factors that have previously been associated with a later onset of puberty. Differences between the indicators of pubertal development used in this study may also help explain the observed results. Age at menarche represents a single pubertal event that typically occurs approximately two to three years after breast budding, around Tanner stage IV (265). In contrast, the Puberty score reflects pubertal progression at 10 years of age and may be considered a more sensitive indicator at this stage, as it captures earlier stages of sexual maturation based on the Tanner staging. Taken together, these findings support the hypothesis that early-life nutritional exposures may contribute to shaping pubertal timing and reinforce the importance of early-life interventions.

While breastfeeding represents one of the earliest nutritional exposures in life, other dietary behaviours established during childhood may also play an important role in pubertal development. Therefore, in the present thesis we explored whether dietary intake, diet quality, energy-dense food and UPF consumption, and other lifestyle behaviours were associated with pubertal timing, using data from the Generation XXI cohort [paper IV]. The results showed that a higher diet quality at 4 years of age was

associated with a lower Puberty score at 10 years among girls, as well as with a later age at menarche, which is consistent with previous findings (129, 138, 142). In addition, higher consumption of energy-dense foods at 4 years of age and meat and meat products at 7 years of age was associated with an earlier age at menarche. Among boys, higher consumption of the 'Fish and eggs' food group at age 4 was associated with a higher Puberty score in early adolescence. Furthermore, higher intake of saturated fatty acids and trans fats at age 7 was also associated with a higher Puberty score. Regarding micronutrients, higher intakes of vitamin D, zinc, and calcium were also associated with a higher Puberty score at age 10 among boys. Lifestyle behaviours such as sleep duration and sedentary behaviour were not associated with pubertal timing in the present study, whereas physical activity was associated with age at menarche only among girls with overweight or obesity. However, in the previous study examining trajectories of diet quality [paper I], these lifestyle behaviours were shown to influence children's diet quality. Therefore, even though they were not directly associated with pubertal timing in the present analysis, these lifestyle factors may influence pubertal development indirectly through their effect on diet quality.

Several biological mechanisms may explain the observed associations. Overall diet quality, together with dietary patterns characterised by higher consumption of EDFs, has been associated with increased adiposity, as well as metabolic and hormonal dysregulation (139, 140). Increased adiposity may raise circulating leptin levels and alter insulin signalling, which may accelerate activation of the HPG axis and consequently influence the timing of puberty (266). Nevertheless, a significant interaction with BMI was observed only for the association between UPF consumption and age at menarche, with the association being evident among girls with overweight or obesity. This finding may suggest that body weight status may modify the relationship between UPF consumption and pubertal timing. One possible explanation is that excess adiposity itself, which is known to play an important role in pubertal development (267), may interact with dietary exposures to influence pubertal timing. However, no interaction with BMI was observed for the reamining dietary exposures, suggesting that the influence of childhood diet on pubertal timing may not be entirely mediated by adiposity, highlighting the potential role of diet composition and metabolic pathways beyond obesity. Moreover, the observation that overall diet quality remained consistently associated with pubertal timing, whereas numerous individual food groups and nutrients were associated with

earlier menarche only among girls with overweight or obesity, suggests that different aspects of dietary exposure may underlie these associations. While the HEI captures the overall quality of the diet, the associations observed for multiple foods and nutrients may primarily reflect greater overall food intake. Since dietary variables were analysed as absolute rather than energy-adjusted intakes, the independent contribution of individual dietary components cannot be completely disentangled from that of total energy intake. Taken together, these findings support the use of dietary pattern-based approaches, rather than focusing exclusively on individual foods or nutrients, when investigating the role of childhood nutrition in pubertal development.

Another possible explanation relates to the fact that UPF are frequently packaged in materials containing EDCs, which may migrate from packaging into food, leading to dietary exposure (268). EDCs can interfere with pubertal development through several pathways, including effects on the HPG axis and neuroendocrine signalling (269). Phthalates, for example, are among the chemicals known to interfere with hormonal signalling and have been associated with an increase in the risk of earlier pubertal onset (270). The gut microbiome may also play a role. Healthier dietary patterns and breastfeeding may promote a more favourable gut microbiota composition, which has increasingly been recognised as a metabolic and hormonal modulator involved in pubertal development (271-273). One potential mechanism involves the microbial conversion of dietary polyphenols into metabolites with estrogen-like activity, which may contribute to the development of secondary sexual characteristics (274). In addition, poor diet quality has been associated with low-grade systemic inflammation (275), which may interfere with endocrine signalling and metabolic regulation, potentially influencing pubertal timing. For example, high consumption of sugar-sweetened beverages may contribute to increased insulin secretion and higher circulating levels of IGF-1, which may promote earlier pubertal onset (135, 276). Finally, children's diet quality during early life may also reflect broader family and lifestyle characteristics, including healthier behaviours and socioeconomic factors, which have previously been associated with pubertal timing (264, 277-279).

In addition to early-life and childhood feeding practices being proposed as modifiable factors that may influence pubertal timing, children are also exposed to a wide range of environmental factors that may affect the process of pubertal development. Among these, urban environmental exposures have increasingly been recognised as potentially relevant

determinants of child health (280). In this context, the present thesis aimed to explore the influence of early-life urban environmental exposures, breastfeeding and diet quality (from pregnancy until 4 years of age) on pubertal timing, using an exposome-based approach [paper V]. This approach allows for a more comprehensive assessment of the complex environmental context in which children live. Furthermore, the study sought to examine how these environmental exposures may interact with early-life nutritional factors, specifically breastfeeding and diet quality. To address this objective, data from the Generation XXI (Portugal) and INMA (Spain) cohorts were used. The use of two independent cohorts allows confirmation and validation of the results, strengthening the evidence for the observed associations between environmental exposures and pubertal timing, and increasing the validity and generalizability of the results. This study provides evidence that early-life exposure to unfavourable urban environments, characterised by higher building and population density, greater traffic and air pollution levels, and lower access to natural space, may contribute to earlier age at menarche in the Generation XXI cohort and to more advanced pubertal stages in the INMA cohort. To our knowledge, this is the first study to explore the combined effects of multiple early-life urban environmental exposures on pubertal timing within an exposome framework.

Among the environmental exposome subdomains considered, exposure to air pollution and greater traffic at birth and at ages 1 and 4 years showed the most consistent associations with pubertal development. These findings align with previous studies that have examined individual exposures within this subdomain, such as exposure to PM, NO_2, and residential proximity to traffic (204, 206, 281). In contrast, relatively few studies have investigated the influence of proximity to green spaces on pubertal timing. In the limited evidence available, associations have mainly been reported among females (209, 282). In our study, however, greater access to green spaces appeared to have a protective effect against earlier pubertal onset in both sexes.

Several biological mechanisms may underlie the observed associations. Air pollutants contain several compounds with endocrine-disrupting properties, including PM, NO_2, persistent organic pollutants, and polycyclic aromatic hydrocarbons, which may interfere with hormonal pathways involved in pubertal development (283). These pollutants may disrupt hormones that regulate reproductive development by interacting with molecular targets such as the aryl hydrocarbon receptor, androgen and estrogen receptors, and kisspeptin signalling pathways (87, 283, 284). In addition, air pollution may exert

obesogenic effects, which could indirectly influence pubertal maturation (87). The absence of significant associations in the sensitivity analysis restricted to participants with normal weight in the Generation XXI cohort suggests that BMI may act as a mediator in the relationship between urban environmental exposures and pubertal timing. Another potential pathway relates to access to green spaces, which may promote higher levels of physical activity and reduce sedentary behaviours, while also contributing to lower stress levels and reduced cortisol concentrations, factors that may influence hormonal regulation and pubertal development (207, 285, 286).

Regarding early-life nutritional factors, diet quality was not associated with pubertal timing in any of the time points or cohorts analysed. In contrast, longer duration of exclusive breastfeeding was associated with lower Tanner stages at 10 years of age among girls in the Generation XXI cohort. The absence of associations with diet quality in the Generation XXI cohort may be partly explained by the dietary score used in this study, which only considered the consumption of fruits and vegetables and EDFs, potentially limiting its ability to capture overall diet quality.

The interaction analysis between breastfeeding and urban environmental exposures did not reveal statistically significant results, and therefore, it was not possible to determine whether one of these factors plays a more decisive role in influencing pubertal timing. Additionally, the sex-specific patterns observed in our results, namely the associations with Tanner stage and the PDS among boys, and age at menarche among girls, suggest that pubertal development may involve different biological pathways according to sex. Future studies are needed to clarify these potential sex-specific mechanisms better.

Childhood adiposity has been widely recognised as an important determinant of pubertal timing (99, 287, 288). However, the findings of this thesis suggest that the relationship between early-life exposures and pubertal development may extend beyond mechanisms solely related to obesity. While some associations, such as those observed for breastfeeding and UPF consumption in relation to age at menarche, were attenuated after adjustment for BMI, others remained largely unchanged. These findings indicate that adiposity may partially mediate some of the observed relationships, but does not fully explain them. Several mechanisms that may underlie these associations beyond obesity have been discussed throughout this thesis. These include dietary and environmental exposure to ECDs, low-grade systemic inflammation, alterations in insulin signalling and metabolic regulation, as well as broader family socioeconomic characteristics and

lifestyle behaviours that may shape both early-life exposures and developmental trajectories.

An additional contribution of this thesis relates to methodological considerations in the assessment of pubertal development in epidemiological studies. Accurate measurement of pubertal timing is essential when investigating both the factors and consequences of pubertal maturation. The tanner staging, assessed by clinical examination, which was used in several studies included in this thesis, is the method most commonly applied in epidemiological studies to assess sexual maturation in pediatric populations (25, 27, 289). However, its application in large population-based studies is not always feasible and may present several challenges. In particular, clinical examination may be perceived as invasive (289), which can lead to lower acceptability among children and adolescents (30) and may consequently hinder participation (289). For this reason, some non-invasive proxy methods of biological maturation have been proposed (290-293), especially when other data are not available (e.g., PDS score). Among these, Maturity Offset (MO) has been widely used. MO is defined as the time before or after peak height velocity (PHV), indicating how far a child is from the period of greatest height increase and reflecting somatic maturation (293). Predicted maturity offset (pMO), an estimation of MO calculated using sex-specific predictive equations (293), has been increasingly used in studies of physical activity and sports among youth atlhetes as a marker of maturational status (290, 291, 294, 295). In this thesis, we evaluated whether pMO could approximate pubertal timing assessed by Tanner stages across different maturation strata in two European studies: Generation XXI and GENOBOX [paper II]. Our findings showed that although pMO was able to distinguish prepubertal children from those in more advanced pubertal stages, it did not accurately reflect sexual maturation stages. Consequently, pMO is unsuitable for stratifying Tanner stages or predicting pubertal timing, either at the individual or population level. These findings reinforce that pMO and Tanner staging capture distinct biological processes, somatic maturation and sexual maturation, respectively, and should not be used interchangeably in epidemiological research. Despite being a more invasive approach, Tanner staging remains the most appropriate approach for assessing pubertal timing in epidemiological studies.

So, throughout this thesis, Tanner Staging was used to evaluate pubertal timing whenever possible, for example, in all studies using data from Generation XXI. When this was not feasible, due to the absence of Tanner assessments, the PDS score was considered, as in

the case of the INMA cohort. The main disadvantage of the PDS is that it is a self-reported tool, making it less invasive than Tanner Staging. However, although the PDS does not provide a complete estimate of Tanner stages, it has been previously and demonstrated moderate to good agreement with physician-assessed Tanner stages. (41, 296)

Strengths and limitations

The main strengths of this thesis include the use of data from two European longitudinal cohorts and one case control study, with a relatively large sample size and high participation rates. Children from Generation XXI and INMA are part of population-based birth cohorts with regular follow-ups and detailed, standardized assessments, enabling us to address the proposed objectives using a longitudinal approach. In addition, a wide range of relevant variables was collected, including pregnancy characteristics, early-life and childhood dietary habits, socioeconomic circumstances, maternal characteristics, child lifestyle, behaviours and anthropometric measurements, including pubertal assessment, allowing adjustment for multiple potential confounding factors. Furthermore, the longitudinal design also allowed the assessment of exposures during critical developmental periods prior to the onset of puberty. The GENOBOX study, despite its cross-sectional design, reinforced the importance of using Tanner stages in epidemiological research across different settings and populations. Another important strength is that pubertal timing was overall assessed through clinical examination in Generation XXI, which is considered more reliable than subjective reporting and therefore less prone to measurement error commonly associated with self- or parent-reported data. Finally, all research objectives were addressed using data from both males and females, which represents an additional strength, given that much of the existing literature on pubertal development focuses primarily on females.

Nonetheless, potential limitations of the present thesis should be acknowledged. First, the FFQ relies on subjective reporting, and therefore social desirability bias and misreporting may have occurred. Dietary intake was assessed through face-to-face interviews using the FFQ, completed by parents during the 4-, 7-, and 10-year follow-ups, and by the participant at age 13. However, parents may not be aware of all foods their children consumed when out-of-home, despite the fact that other caregivers were also asked. Additionally, parents and adolescents may have overreported healthy foods and/or

underreported unhealthy foods. However, in a subsample of the Generation XXI cohort, dietary intake data collected through the FFQ were compared with data from 3-day food diaries and biomarkers, supporting the validity of the FFQ estimates (223). Misreporting of food consumption may also represent a limitation. Underreporting of dietary intake among individuals with overweight and obesity is widely described in the literature (297, 298) and may affect the association between eating habits and children's weight status. To address for the potential impact of misreporting, a sensitivity analysis excluding participants with potential over- and under-reporting of energy intake was performed [Paper I]. The results suggested that over- and under-reporting did not introduce bias into the analyses.

In Generation XXI and INMA, information on the type and duration of breastfeeding was collected at specific time points (6, 15, and 24 months, and 4 years). As this information was based on parental reporting, it may have been affected by recall bias. To minimize this limitation and increase power, information collected from the follow-ups of the subsamples and from the full cohort at the 4-year follow-up was combined to construct a final categorical variable. This variable was used as the exposure in Paper II and as a confounder in Paper III. Agreement analyses of parental reports across different follow-ups showed satisfactory consistency.

The Tanner stage assessment, performed by trained nurses, may also be subject to measurement error, particularly in the evaluation of testicular volume in boys. This procedure involves palpation following standardized guidelines, and may cause some discomfort to the child, potentially affecting measurement accuracy. To account for this potential measurement error, in G21, analyses using Tanner stage as the outcome were adjusted for the ID code of the physical examiner to control for possible inter- and intra-observer variability. In addition, data from children assessed by a specific examiner who was not a nurse were excluded from the analyses (n=30). To further reduce measurement error associated with Tanner stage assessment, a Pubertal score was constructed [Papers II and III], defined as the sum of genital and pubic hair Tanner stages for males, and breast and pubic hair Tanner stages for females. This combined score may reduce measurement error compared with evaluating each characteristic separately.

Finally, another limitation relates to the assessment of pubertal development in the INMA cohort [paper V], where most children did not have Tanner stage information available, but instead completed the PDS. Although the PDS does not provide a complete estimation

of Tanner stages, this scale has been previously validated, including in the INMA-Valencia subcohort, showing good agreement with physician-assessed Tanner stages (296).

Public health implications and strategies

Understanding the determinants of pubertal timing has important public health implications. As described previously, earlier puberty has been associated with an increased risk of several chronic conditions in adulthood, including cardiometabolic diseases, hormone-related cancers, adverse mental health outcomes, and higher all-cause mortality. In this thesis, we identified several modifiable early-life exposures that may influence pubertal timing, highlighting the importance of discussing potential preventive interventions. Our findings underscore the potential role of early-life and childhood nutrition, as well as urban environmental exposures, in shaping pubertal timing. From a public health perspective, promoting optimal early-life nutrition may be a key strategy. Policies supporting sustained breastfeeding should be implemented, including extended maternity leave, at least until six months, to ensure that mothers have the necessary conditions to maintain exclusive breastfeeding, as recommended by the WHO. Promoting breastfeeding-friendly workplaces and breastfeeding education interventions during the prenatal and postnatal periods may further support mothers in initiating and maintaining breastfeeding.

Similarly, interventions aimed at improving childhood dietary are crucial, as childhood represents a period during which exposures can be modified to influence long-term health outcomes. Strategies may include promoting healthy school food environments, increasing balanced diets rich in fruits and vegetables, and reducing consumption of ultra-processed foods. Family-based interventions, including parental nutrition education and workshops that engage the whole family in healthy eating practices, may also contribute to reducing the likelihood of earlier pubertal timing. Implementing strategies to promote adequate sleep duration is also important, since results observed in one of our studies [Paper I], suggested that adequate sleep may support healthier dietary habits and, consequently, more favourable pubertal development.

Finally, interventions targeting the urban environment are likely to be important. Policies that improve urban planning to reduce exposure to air pollution and increase access to green spaces, which themselves promote opportunities for physical activity, may have lasting effects on pubertal timing and long-term health outcomes.

Conclusions

This thesis aimed to explore how early-life eating habits, lifestyle behaviours, and environmental exposures, from pregnancy to adolescence, are associated with pubertal timing. Overall, the findings suggest that pubertal timing may be influenced by a range of modifiable early-life exposures, including early-life feeding practices such as breastfeeding, dietary habits during childhood, and environmental exposures related to the urban context. Together, this thesis contributes to the growing body of evidence showing that early-life environments play a crucial role in shaping developmental trajectories.

The findings also reinforce the importance of adopting a broader exposome perspective when studying the timing of puberty, acknowledging that children are simultaneously exposed to multiple environmental and behavioural factors throughout early life. Importantly, some of the observed associations were not fully explained by adiposity, suggesting that these factors may influence pubertal development through additional biological and environmental mechanisms.

Further research is needed to better understand these pathways and clarify the complex mechanisms linking early-life exposures to pubertal development. From a public health perspective, these findings reinforces the importance of developing and implementing interventions that promote breastfeeding, support healthier dietary patterns from early childhood, and improve the urban environments in which children grow and develop.

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