Aetiological role of early lifestyle exposures in puberty timing

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DECLARATION

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text.

It is not substantially similar to any work that I have submitted or is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution.

This thesis does not exceed 60,000 words excluding references, tables, figures and appendices, as prescribed by the Degree Committee for the Faculties of Clinical Medicine and Veterinary Medicine.

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ABSTRACT

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Amidst the global secular trends towards earlier timing of pubertal development, there are abundant evidence from various populations that early puberty timing is associated with higher risks for a wide range of subsequent adverse physical and mental health outcomes. Despite the increasing recognition that rapid growth and obesity during prepubertal childhood may promote earlier puberty, it is unclear whether lifestyle behaviours are determinants of puberty timing. This thesis aimed to explore whether diet and physical activity in childhood are associated with puberty timing, using a combination of robust analytical approaches.

First, to understand the disease relevance of puberty timing independent of adiposity, published findings on the associations between puberty timing and risks for type 2 diabetes and/or impaired glucose tolerance (T2D/IGT) were systematically reviewed and pooled by inverse-variance-weighted random-effects models. Based on 28 studies in Western and Asian settings, earlier timing of puberty in females, indicated by age at menarche, was consistently associated with higher T2D/IGT risk in women (n=1,228,306). Similarly, in the only one identified study in males (n=197,714 British men), relatively younger (versus 'about average') voice breaking was associated with higher risk of T2D. These associations were only partially mediated by adiposity, thus warranting examination of the determinants of puberty timing that act through or are independent of changes in adiposity.

I then investigated the associations of total energy and macronutrient intakes with timings of several puberty traits in boys (n=3811) and girls (n=3919) in the Avon Longitudinal Study of Parents and Children in the United Kingdom (UK). Integrating comprehensive data on dietary intakes and puberty

development from early childhood until young adulthood, higher prepubertal childhood intakes of total energy in both sexes, and protein (including animal and plant-based proteins) in girls were associated with earlier timings of puberty onset and progression and peak height velocity, independent of adiposity during puberty. Further, in the same cohort, I examined the associations of dietary and plasma phospholipid fatty acids with puberty timing in boys (n=3654) and girls (n=3872). Based on repeatedly assessed dietary intakes and objective measures of fatty acids during prepuberty, higher dietary intake of polyunsaturated fatty acids, and higher plasma concentrations of dihomo-γ-linolenic acid (20:3n6) and palmitoleic acid (16:1n7) were associated with earlier puberty timing in girls, but not in boys. In Mendelian Randomization (MR) analyses, higher genetically predicted circulating dihomo-γ-linolenic acid but not palmitoleic acid was associated with earlier menarche in girls. Finally, I tested the associations between accelerometer-measured physical activity prior to puberty and subsequent puberty timing in boys (n=2531) and girls (n=3079) in the UK Millennium Cohort Study. Lower total daily movement, regardless of physical activity intensity, was consistently associated with higher risk for earlier puberty timing in both sexes, independent of body mass index. Using a MR model approach, higher genetically predicted sedentary activities were associated with earlier puberty timing.

This thesis uses a variety of data types and analytical triangulation approaches to demonstrate specific lifestyle behaviours, namely dietary intakes and physical activity, during prepubertal childhood, as potential determinants of the timing of pubertal development in both boys and girls. These findings may inform future interventions to avoid early puberty timing as part of a life course approach to prevention of non-communicable diseases.

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PUBLICATIONS AND PRESENTATIONS

Publications resulting from this thesis

Published work

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Cheng, TS., Sharp, SJ., Brage S., Emmett, PM., Forouhi, NG., Ong, KK. (2021). Longitudinal associations between prepubertal childhood total energy and macronutrient intakes and subsequent puberty timing in UK boys and girls. *European Journal of Nutrition*. https://doi.org/10.1007/s00394-021-02629-6

Cheng, TS., Day, FR., Perry, JRB., Luan J., Langenberg, C., Forouhi, NG., Wareham, NJ., Ong, KK. (2021). Prepubertal dietary and plasma phospholipid fatty acids related to puberty timing: Longitudinal cohort and Mendelian Randomization analyses. *Nutrients,* 13(6), 1868.

Under review

Cheng, TS., Brage, S., van Sluijs, E., Ong, KK. Prepubertal objectively measured physical activity and puberty timing in British boys and girls.

Other publications

Chadda, KR., **Cheng, TS.**, Ong, KK. (2020). GLP-1 agonists for obesity and type 2 diabetes in children: Systematic review and meta-analysis. *Obesity Reviews*. 22(6), e13177.

Ong, KK., **Cheng, TS.**, Olga, L., Prentice, PM., Petry, CJ., Hughes, IA., Dunger, DB. (2020). Which infancy growth parameters are associated with later adiposity? The Cambridge Baby Growth Study. *Annals of Human Biology*, 47(2), 142-149.

Olwi, DI., Day, FR., **Cheng, TS.**, Olga, L., Petry, CJ., Hughes, IA., Dunger, DB., Ong, KK. Associations of appetitive traits with growth velocities in infancy and childhood. (under review)

Conference presentations

Longitudinal cohort and Mendelian Randomization analyses of dietary fat quality and plasma phospholipid fatty acid concentrations in relation to puberty timing. (2021) Longitudinal Studies (virtual). *Poster presentation*

Associations between prepubertal dietary intakes and puberty timing in boys and girls from the Avon Longitudinal Study of Parents and Children (ALSPAC). (2020). Nutrition Society of Malaysia 35th Scientific Conference (virtual). *Oral presentation*

Differential associations between childhood macronutrient intakes and puberty timing in UK boys and girls. (2020). 7th International Conference on Nutrition and Growth (virtual). *Oral presentation*

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LIST OF ABBREVIATIONS

%BF: body fat percentage

ALSPAC: Avon Longitudinal Study of Parents and Children

B2: Tanner stage 2 breast development

BMI: body mass index

CI: confidence intervals

CPM: counts per minute

DOHaD: Developmental Origins of Health and Disease

DONALD: DOrtmund Nutritional and Anthropometric Longitudinally Designed

EAR: estimated average requirement

EPIC: European Prospective Investigation into Cancer and Nutrition Study

FA: fatty acid

FFQ: Food Frequency Questionnaire

G2: Tanner stage 2 genital development

GCSE: General Certificate of Secondary Education

GWAS: genome-wide association studies

HR: hazard ratio

IGT: impaired glucose tolerance

IVW: inverse-variance weighted

kcal: kilocalories

kJ: kilojoules

MET: Metabolic Equivalent Task

MR: Mendelian Randomization

MUFA: monounsaturated fatty acid

NCD: Non-communicable disease

OR: odds ratio

PH2: Tanner stage 2 pubic hair growth

PHV: peak height velocity

PUFA: polyunsaturated fatty acid

RR: relative risk

SD: standard deviation

SITAR: SuperImposition by Translation and Rotation

SFA: saturated fatty acid

SNP: single nucleotide polymorphism

T2D: type 2 diabetes

TEI: total energy intake

UK: United Kingdom

US: United States

WHO: World Health Organization

CHAPTER 1 INTRODUCTION

1.1 Global burden of non-communicable diseases

Non-communicable diseases (NCDs), also known as chronic diseases, represent the leading threats to global public health in the twenty-first century. The common types of NCDs include cardiovascular diseases, chronic respiratory diseases, diabetes mellitus and cancers. According to the Global Burden of Disease Study, 523 million people lived with cardiovascular diseases worldwide in 2019, which has increased dramatically from 271 million in 1990[1]. A similar increasing trend was seen for chronic respiratory diseases, which affected 544.9 million people in 2017, as compared to 389.7 million in 1990[2]. Strikingly, the global prevalence of diabetes mellitus has more than doubled from 211 million in 1990 to 476 million in 2017, and will continue to rise to 570.9 million in 2025 without effective interventions[3]. A total of 19.3 million cases with cancer across the world was also estimated in 2020, and this figure is expected to reach 28.4 million by 2040[4]. Besides physiological disorders, mental illness is another major contributor to NCD burden globally. The prevalence of common mental health problems including mood disorder and anxiety is 29.2% at any point during lifetime[5].

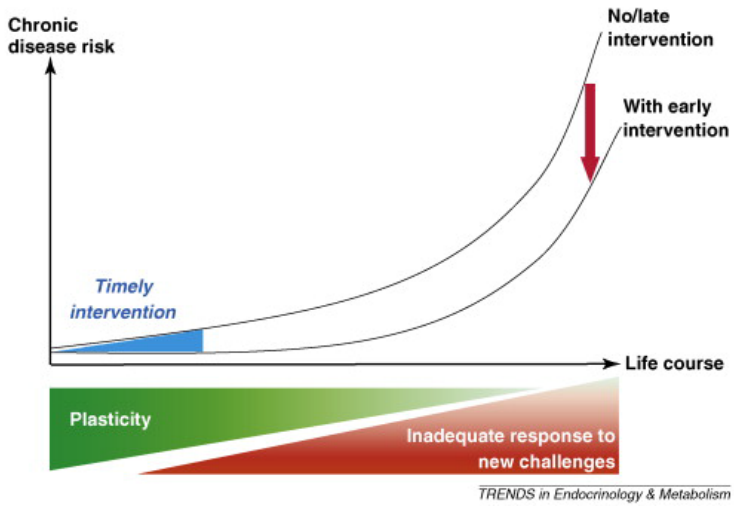
More alarmingly, NCDs are responsible for nearly three quarters of all deaths worldwide annually[6-9]. The number of deaths due to NCDs was 41.1 million in 2017[9] and is projected to increase to 52 million by 2030[7]. Of the NCD mortality, about 40% were premature deaths that occurred before age 70 years in 2012[7]. A higher proportion of premature deaths among NCD deaths (over 50%), which could be amenable to effective healthcare interventions, was estimated across all age groups from birth to 95 years and older in 2017[6]. The majority of global NCD deaths is attributable to cardiovascular diseases (mainly ischaemic heart diseases and stroke), followed by cancers and chronic respiratory diseases (predominantly chronic obstructive pulmonary disease)[9]. Other cause of NCD deaths includes diabetes mellitus, mainly type 2 diabetes

(T2D)[9]. Similarly in the United Kingdom (UK), in 2016, NCDs accounted for 89% of all-cause deaths, of which, more than half were contributed by cancers (28%) and cardiovascular diseases (25%)[8].

1.2 Early programming of adult diseases

Historically, prevention strategies for the NCDs focused on modifying unhealthy lifestyles in adulthood (including tobacco use, harmful use of alcohol, physical inactivity and excess salt or sodium intake) and risk factors (including high blood pressure, hyperlipidaemia, hyperglycaemia, and overweight and obesity)[8]. Attention has now shifted to include a life course approach to health within the basic principles of human biology[10], in view of the optimal benefits of early interventions in reducing future NCD risk[11] (**Figure 1.1**).

Figure 1.1 A life course approach to health



Adapted from Godfrey et al.[11] This figure shows a nonlinear increase in NCD risk throughout the life course as a result of declining developmental plasticity (green triangle) and accumulative effects of inadequate responses to new challenges (red triangle). Early life interventions (blue area) can have a large effect on later NCD risk (red arrow).

According to the Developmental Origins of Health and Disease (DOHaD) hypothesis, adverse environmental exposures across developmental stages from preconception to pregnancy, infancy, childhood, adolescence until adulthood may lead to NCDs in later life[12], through physiological processes of developmental plasticity rather than pathophysiological processes[13]. This concept originated from the thrifty phenotype hypothesis or foetal origins hypothesis based on the earlier works from David Barker and his colleagues, showing the associations of low birth weight with later susceptibility to cardiovascular disease, and T2D in adulthood[14-17]. More evidence that support DOHaD could be seen from epidemiological research in early life famines and historical cohorts in different countries[18]. In particular, in the Dutch Hunger Winter cohort, adults who had been exposed to famine in utero (especially at the third trimester) near the end of World War II in 1944 and 1945 had poorer glucose tolerance[19] and higher blood pressure[20] at age 50-59 years, compared to those never exposed to famine. Further epidemiological research across diverse populations has well-documented that greater or rapid growth in infancy and childhood are associated with higher later metabolic risk factors including obesity, glucose intolerance, insulin resistance and high blood pressure[21-25]. Therefore, research from a life course perspective provides potential opportunities to understand how different exposures throughout the entire life stages influence subsequent health and disease risks.

1.3 Puberty

Since the proposal of the DOHaD concept, there have been an abundance of studies examining early life risk factors of future diseases. In particular, puberty timing has increasingly received attention. In this section, the developmental process of puberty is first discussed, followed by an introduction to puberty timing.

1.3.1 Pubertal development

Puberty is a transitional period from childhood to adulthood, which involves a series of physiological and physical changes to attain reproductive capacity. It begins with the secretion of gonadotropin-releasing hormone from hypothalamus after a quiescent period during infancy and childhood, which triggers the release of luteinising hormone and follicle-stimulating hormone from the pituitary to promote gametogenesis and sex hormone synthesis[26]. The sex hormones including testosterone in boys and oestrogen in girls subsequently induce the development of secondary sexual characteristics[27].

As well-described by Marshall and Tanner[28, 29], processes of pubertal development can be divided into five Tanner stages in boys (**Table 1.1**) and girls (**Table 1.2**). While Tanner stage 1 represents prepuberty, Tanner stage 2 to 5 are indicative of the progression from onset to completion of puberty. The onset of puberty can be observed from the appearance of breast buds (i.e. thelarche) in girls and penis and testis enlargement in boys, followed by pubic hair growth (i.e. pubarche)[28, 29] and growth spurt in height (i.e. peak height velocity)[27] in both sexes, between Tanner stage 2 and 3 genital or breast development[30] (**Figure 1.2**). At the later stages of puberty (i.e. Tanner stage 3 or 4), boys and girls experience voice break[31] and first menstruation (i.e. menarche)[32], respectively. Finally, puberty completes with axillary hair growth in both sexes, and facial hair growth in boys[27]. Most of these physical changes during puberty in girls and boys are a gradual process, except menarche in girls and voice breaking in boys, which are distinctive events[31, 33]. The whole process of pubertal development from onset to completion usually takes about 2 to 3 years.

Several methods are available to capture pubertal development status in children[27]. Among these, a widely used instrument is a non-invasive questionnaire, namely Petersen Pubertal Development Scale[34], which is described in detail in **Section 3.1.2.3**.

Table 1.1 Tanner puberty stages for genital development in boys

Tanner stages	Description
Stage 1 Prepubertal	Testes, scrotum and penis are of about the same size and proportion as in early childhood.
Stage 2	The scrotum and testes have enlarged, and there is a change in the texture of the scrotal skin. There is also some reddening of the scrotal skin.
Stage 3	Growth of the penis has occurred, at first mainly in length but with some increase in breadth. There has been further growth of testes and scrotum.
Stage 4	Penis further enlarged in length and breadth with development of glans. Testes and scrotum further enlarged. There is also further darkening of the scrotal skin.
Stage 5 Adult	Genitalia adult in size and shape. No further enlargement takes place after stage 5 is reached.

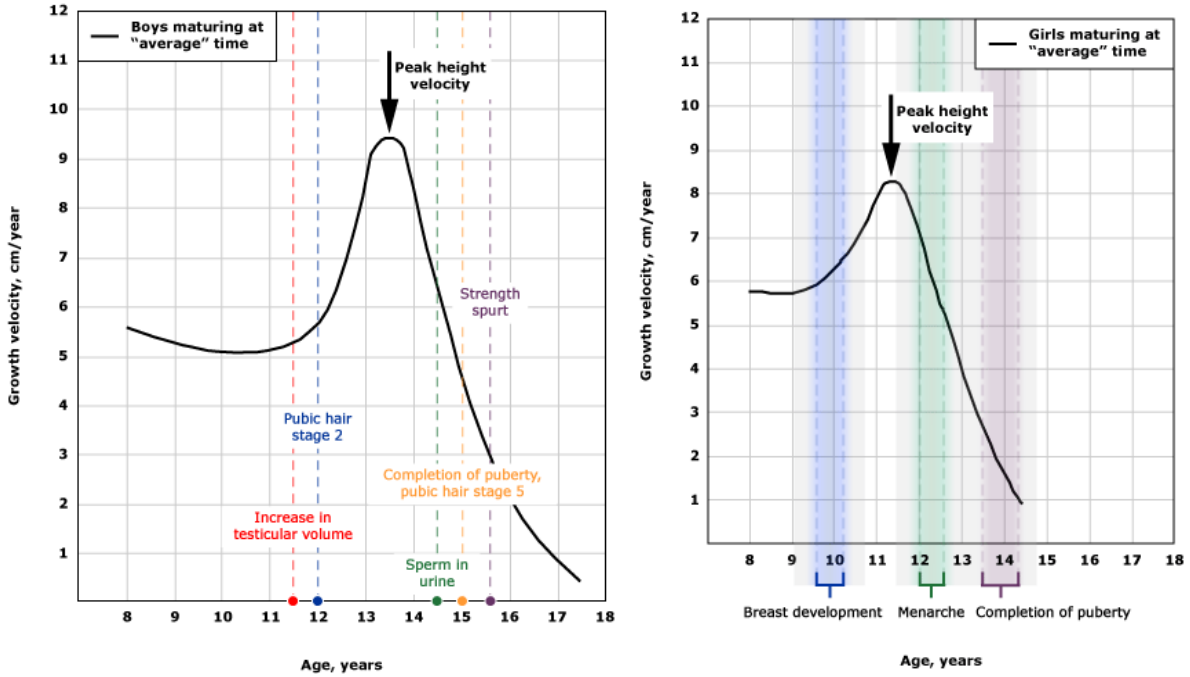
Based on Marshall et al.[28]

Table 1.2 Tanner puberty stages for breast development in girls

Tanner stages	Description
Stage 1 Prepubertal	Elevation of papilla only.
Stage 2	Breast bud stage; elevation of breast and papilla as a small mound, enlargement of areola diameter.
Stage 3	Further enlargement of breast and areola, with no separation of their contours.
Stage 4	Projection of areola and papilla to form a secondary mound above the level of the breast.
Stage 5 Adult	Mature stage; projection of papilla only, owing to recession of the areola to the general contour of the breast.

Based on Marshall et al.[29]

Figure 1.2 Physical changes at puberty



Adapted from Biro[35]. This figure shows the sequence of the occurrence of secondary sexual characteristics in boys (left) and girls (right). The timings presented here are for demonstration purposes as they vary by populations.

1.3.2 Puberty timing

The staging pattern of pubertal development is consistent across normal populations[27, 36]. However, ages at the occurrence of different secondary sexual characteristics vary widely between individuals, typically ranging from 8 to 17 years in girls and from 9 to 18 years in boys[37, 38], with girls generally starting and going through pubertal development at earlier ages than boys[27]. The average timing of puberty may also differ by ethnicity within the same populations in both sexes[39-42].

More notably, average age at puberty has been becoming earlier over the last decades worldwide. Such trends are particularly denoted by average age at menarche in girls in Western and Asian countries, which has declined from age 17 years in the early nineteenth century to 12 years in the recent century[43, 44]. A meta-analysis of 30 studies across all continents showed that

age at the onset of breast development in girls decreased by 0.24 years (almost 3 months) per decade between 1977 and 2013[45]. In both girls and boys in Western countries, similar magnitude of trends towards earlier puberty (declining by 0.3-0.5 years) over decades have been also observed based on age at peak height velocity[46-48]. These further support that secular trends towards earlier puberty exist not only in girls but also in boys. Conversely, findings on the secular trends towards earlier ages at genital and pubic hair development in boys are limited and inconsistent[43, 49], likely due to the lack of data availability especially in earlier decades.

The timings of most pubertal traits in girls and boys can only be captured cross-sectionally or longitudinally but not retrospectively, given the nature of pubertal development (**Section 1.3.1**). On the other hand, age at menarche can be expediently recalled in women even several decades later, which was shown to be moderately correlated with the prospectively recorded age at menarche[50, 51]. Age at voice breaking has been also recently demonstrated as a reasonably well-recalled indicator of puberty timing in men[52], and when prospectively assessed, it was shown to be correlated with childhood growth[53, 54]. Furthermore, unlike other timings of pubertal traits that rely on physical examination or self-report, age at peak height velocity represents a precise and objective measure of puberty timing in both sexes[55]. Nonetheless, recent cohorts tend to focus on age at the occurrence of secondary sexual characteristics rather than age at peak height velocity, because the latter requires frequent repeated measures of height during adolescence.

It has been postulated that the global secular trends towards earlier puberty timing indicate the decreases in childhood undernutrition and increases in childhood adiposity over years[32]. Moreover, physiologically, timing of puberty reflects various underlying hormone levels[27, 56]. Women with early menarche were found to have higher oestrogen[57, 58] and lower sex-hormone binding globulin concentrations[59], compared to their peers with later menarche. Earlier puberty timing may also indicate a longer lifetime duration of exposure to sex hormones[60].

1.4 Puberty timing and risk of NCDs

Amid the worldwide secular trends towards earlier puberty (**Section 1.3.2**), timing of puberty has been widely examined in relation to subsequent health outcomes in different populations. In the following section, findings on puberty timing and future health among women and men are appraised.

1.4.1 Women

A wealth of epidemiological evidence has demonstrated the associations between early puberty timing, typically indicated by recalled age at menarche (≤11-13 years), and a wide range of adverse physical and mental health outcomes in later life in women, mainly using a cross-sectional or case-control design.

Several meta-analyses of 8-17 studies generally in Western settings showed that early menarche (versus later menarche) was associated with higher cardiometabolic risk factors including higher body mass index (BMI)[61], metabolic syndrome[62], insulin resistance[63] and hypertension[64] in adulthood. Similarly, a systematic review found that the majority of studies (eight out of twelve) in Western populations reported an association between early menarche and higher risk for cardiovascular diseases including coronary heart disease and ischemic heart disease[65]. A meta-analysis of twelve cohort studies mainly in Asia (n=7) added that each 1-year earlier age at menarche was associated with 2-3% higher relative risks of death from all causes and ischemic heart disease[66]. Furthermore, early menarche was associated with higher risks for diabetes mellitus, specifically T2D[67] and gestational diabetes mellitus[68, 69], and asthma[70], as reported by meta-analyses of 5-10 studies largely in Western settings. Emerging studies also showed the associations of early menarche with more depressive symptoms and depression in adolescence to adulthood[71, 72]. The large UK Biobank study (n=250,037) estimated that early menarche (8-11 years versus 13 years) and/or

continuous earlier age at menarche (in linear models) was associated with higher risks of 41 (32 and 22, respectively) adverse health outcomes including cancers, cardiometabolic, gynaecological or obstetric, gastrointestinal, musculoskeletal, neurocognitive and respiratory disorders[33]. Additionally, late menarche (15-19 years versus 13 years) was associated with higher risks of 17 diseases (including three outcomes also for continuous later age at menarche) across the aforementioned categories, especially cervical cancer, malabsorption or coeliac disease, osteoporosis, low intelligence and poor lung function[33].

The associations between timings of other puberty traits and later health have been also reported, albeit in fewer and smaller studies. Earlier age at peak height velocity was associated with higher BMI, fasting insulin and diastolic blood pressure and lower high-density lipoprotein in adulthood among Finnish women[73] and higher risk for self-harm including suicidal intent at 16-21 years in British girls[74]. Earlier age at pubic hair development was associated with atopic conditions at 6-13 years among girls in the United States (US)[75]. Likewise, earlier onset of breast development was associated with depression at 13.6 years amongst Hong Kong girls[76].

Together these findings suggest that puberty timing, especially age at menarche, may potentially affect a variety of short-term and long-term health outcomes in women, as supported by a recent Mendelian Randomization (MR) analysis[77].

1.4.2 Men

Among men, few studies on puberty timing and health have been conducted due to the lack of accessible markers of puberty timing. A longitudinal British birth cohort study, the National Survey of Health and Development, found that earlier voice breaking when assessed at age 14-15 years was associated with higher weight at 36 years and adiposity at 60-64 years[53], and higher systolic and diastolic blood pressure at 53 years[78]. In the same cohort, early

timings of other pubertal traits (i.e. genital development, pubic hair and axillary hair growth) were also prospectively associated with higher weight at 36 years[53]. Further, using extensive data on a wide range of diseases, a large UK Biobank study showed that relatively younger (versus about average) recalled voice breaking was associated with 14 adverse cardiometabolic, psychiatric and gastrointestinal outcomes[33]. Relatively older voice breaking was also associated with lower risks of five cardiometabolic-related outcomes but higher risks of six psychiatric, allergic-related and gastrointestinal diseases[33]. Similarly, earlier peak height velocity was associated with higher risk of self-harm at 16 years[74], and higher cardiometabolic risk factors including higher BMI, fasting insulin and blood pressure, and adverse lipid profile at 31 years among Western populations[73]. However, age at onset of genital or pubic hair development was not associated with systolic blood pressure at 13 years in Hong Kong boys[79], possibly due to a short follow-up. Overall, the existing evidence suggests that puberty timing may influence later health in men, similar to that in women.

1.5 Potential determinants of puberty timing

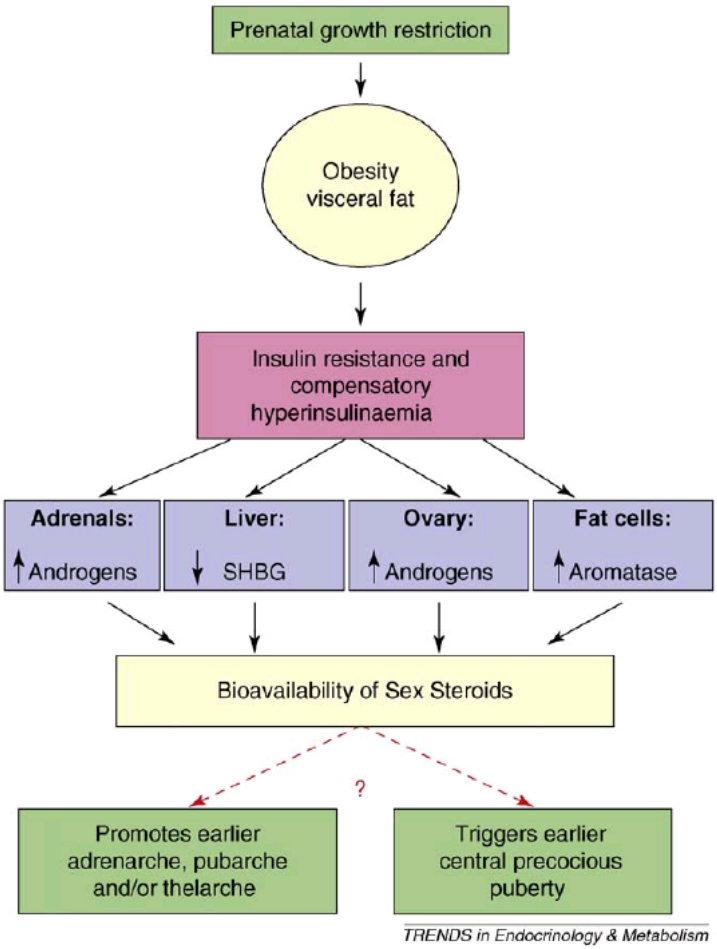
The increasing recognition of public health implications of puberty timing (**Section 1.4**) highlight the importance of identifying determinants of puberty timing as part of early intervention strategies. Here the biological factors and related modifiable exposures that potentially underlie the timing of pubertal development are elaborated.

1.5.1 Biological factors

It is well-recognized that rapid growth and overweight and obesity during prepubertal childhood have been associated with earlier puberty timing especially in girls[32, 80]. The causality of such associations is further supported by evidence from MR analyses[81, 82]. One of the possible mechanisms is insulin

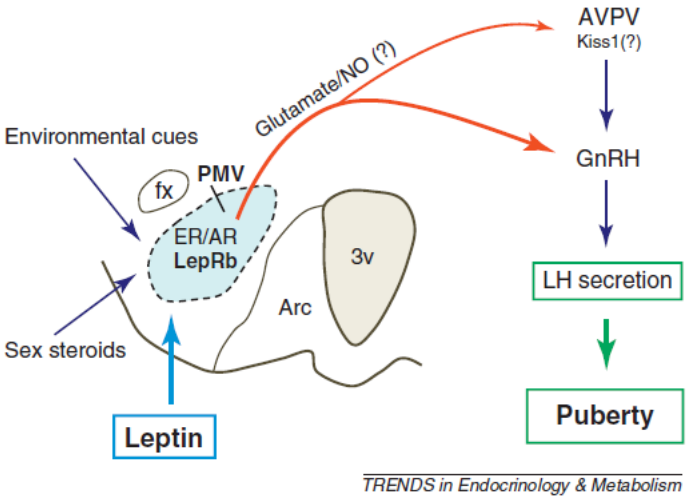
resistance and peripheral hyperinsulinemia, due to adiposity and specifically visceral fat, which increase sex steroid bioavailability at various organs including the adrenals, liver, ovary and fat cells (**Figure 1.3**)[32]. Consequently, the elevated circulating sex steroids would promote earlier hypothalamic-pituitary puberty and sexual maturation[32]. Secondly, it has been hypothesised that higher leptin secretion from adipose tissue may activate the ventral premammillary nucleus[83] and upregulate gonadotropin secretion that is directly responsible for pubertal development[84] (**Figure 1.4**). These biological pathways suggest that modifiable factors which influence childhood adiposity may affect puberty timing.

Figure 1.3 Potential endocrine pathways linking insulin to pubertal development



Adapted from Ahmed et al.[32]. SHBG, sex-hormone-binding globulin

Figure 1.4 Potential neural pathway linking leptin to pubertal development



Adapted from Elias[83]. PMV, ventral premammillary nucleus; LepRb, leptin long form; ER, oestrogen receptor; AR, androgen receptor; NO, nitric oxide; AVPV, anteroventral periventricular nucleus; GnRG, gonadotropin-releasing hormone; LH, luteinizing hormone

1.5.2 Dietary intake

1.5.2.1 Dietary compositions and assessments

Dietary intake refers to the foods and beverages consumed daily by an individual, which provides energy and various nutrients to maintain life and growth. Energy intake is the calorie content of foods measured in kilocalories (kcal) or kilojoules (kJ) (1 kcal = 4.18 kJ) and predominantly contributed by macronutrients, namely carbohydrate, protein and fat. While one gram of dietary carbohydrate or protein yields circa 4 kilocalories, one gram of dietary fat generates approximately 9 kilocalories[85]. Further, macronutrients can be divided into several types which have different physiological and metabolic effects. Based on food sources, dietary protein is categorised as animal- and plant-based protein[86]. Differently, based on molecular size, dietary fat is categorized as saturated, monounsaturated and polyunsaturated fatty acids[87], whilst dietary carbohydrate is classified into simple sugars, oligosaccharides and polysaccharides[88]. Other nutrients from foods are micronutrients, specifically vitamins and minerals, which contribute little energy but are

involved mainly in the regulation and maintenance of different metabolic and cellular functions[89].

Individual dietary intakes can be assessed in several ways. The most common method for dietary assessment in epidemiological studies is the Food Frequency Questionnaire (FFQ) which can be self- or interviewer-administered to record usual intakes over a long period but its low precision due to restrictions imposed by a pre-defined food list and recall bias is acknowledged[90]. Another method is the food diary which is self-administered using open-ended questionnaires and records actual intake in detail at the time when foods are consumed over certain days[90] with high validity for 9-day food diaries, but it is subjected to day-to-day variations within individuals and season-to-season variations[91]. The 24-hour dietary recall is another dietary assessment using open-ended questionnaires, which is administered by a trained interviewer, to record actual intake in detail over the previous 24 hours, but it may be limited by recall bias and not representative of usual intake[90]. It is noteworthy that no subjective dietary assessment is deemed superior, unless dietary nutrients are validated against their respective biomarkers which are more robust to measurement error[92]. However, reliable dietary biomarkers have been lacking[93] and do not reflect habitual intakes of the particular nutrients if only measured once[94]. It has been therefore suggested that a combination of different subjective dietary assessments[95], together with objective biomarkers[96], can more accurately estimate dietary intakes than any single dietary assessment method and substantially strengthen findings of the association between diet and disease. Such approach is termed as data triangulation.

In this thesis, the nutrients of particular interest are energy and macronutrients for their major role in child growth and obesity.

1.5.2.2 Current evidence gaps

Several prospective studies have examined the associations of childhood dietary intakes with puberty timing. However, existing findings have been broadly contradicting and largely constrained by study designs and analytical approaches[97]. Some studies showed inconsistent associations of higher total energy intake (TEI) with earlier[98, 99] or later age at menarche[100] in girls, whilst others reported no association of TEI with age at menarche[101, 102] or breast development[103]. Likewise, higher total protein intake was differentially associated with timings of several puberty traits including earlier[99] or later age at menarche and onset of breast development[104] in girls, and earlier peak height velocity in both sexes[105]. Conversely, no association between total protein intake and puberty timing (i.e. age at menarche in girls[100-103, 105, 106] or voice breaking in boys[105]) was found in many studies. A study demonstrated that higher total fat intake was associated with early menarche[107] but this finding was mainly not seen in other studies[99-101, 103, 106]. Similarly, although one study observed that higher carbohydrate intake was associated with later age at menarche[106], most studies found null findings[99-103]. Notably, the typical measure of puberty timing used in previous studies was age at menarche, thus limiting their findings predominantly to girls. A single dietary assessment method (either FFQ or food diaries) was also generally employed to evaluate dietary intakes. To the best of my knowledge, no study has considered dietary biomarkers for fat quality such as plasma phospholipid fatty acids in relation to puberty timing. Moreover, the associations between macronutrient intakes and puberty timing were not examined in isocaloric macronutrient substitution models, with more emphasis on macronutrient quantity than quality. Further, previous studies assessed dietary intakes at a single timepoint at later age after puberty had probably started and obtained a single self-reported age at menarche or dichotomous menarche status at a specific age before puberty had completed, thus raising the question of reverse causality in their findings (i.e. changing dietary intakes after the onset of puberty).

1.5.3 Physical activity

1.5.3.1 Definition and measurements

Physical activity is 'the behaviour that involves human movement, resulting in physiological attributes including increased energy expenditure and improved physical fitness'[108]. It is quantified as the daily total volume of physical activity which comprises three components: i) frequency (i.e. the number of times for performing physical activity within a certain period), ii) intensity (i.e. the physiological effort involved in a particular activity), and iii) duration (i.e. the amount of time in minutes or hours spent in an activity)[108]. Based on the energy expenditure expressed as Metabolic Equivalent Tasks (METs) which are the multiples of resting metabolic rate, physical activity can be classified into light (1.5-2.9 METs), moderate (3.0-5.9 METs) and vigorous (≥6.0 METs) intensities[109]. Examples of usual physical activities across intensities are i) standing and slow walking for light intensity, ii) playing with balls and strength exercises for moderate intensity and ii) running and swimming for vigorous intensity. On the other hand, sedentary behaviours represent any waking behaviours with energy expenditure ≤1.5 METs, while in a sitting, reclining or lying posture[110], which are typically exemplified by reading, writing and watching television.

The most commonly used method to capture multi-dimensional physical activity is the subjective report-based measures including questionnaires that estimate physical activity as the time spent across different physical activity intensities but are susceptible to misreporting bias[111]. With the increasing available wearable devices including accelerometers, objectively measuring physical activity in epidemiological research has now become possible. In particular, accelerometers measure the amplitude and frequency of acceleration of body resulted from muscular forces during movement in one (vertical), two (vertical and medo-lateral) or three (vertical, medo-lateral and anterior-posterior) planes[111]. The output data of accelerometers are recorded in counts (i.e. the product of the amplitude and frequency of the vertical

acceleration) and can be further converted to meaningful estimates including time spent in different physical activity intensity levels using valid intensity thresholds[111].

1.5.3.2 Current evidence gap

A paucity of studies have explored the association between physical activity and puberty timing especially using a prospective study design[112]. Previous findings were also conflicting and limited to self-reported menarche status in girls. Among historical cohort studies which assessed recalled premenarcheal physical activity in postmenarcheal girls, majority reported associations of longer duration of physical activity[113-115] and being more active with later age at menarche[116], but one study showed no difference in age at menarche between those with and without participation in sports[117]. In contrast, among prospective cohort studies, whereas one found that higher weight-adjusted energy expenditure and physical activity duration were associated with lower risk for earlier menarche[107], most showed null associations[118-121]. Nonetheless, physical activity was recorded among premenarcheal girls at ages when puberty had likely already started, whilst menarche status was measured after short follow-up durations of 1 to 3 years even though puberty may have not yet completed[107, 118-121]. Such data collection method draws the same concern of reverse causation (i.e. changing physical activity after onset of puberty) as those among previous studies on dietary intakes and puberty timing (**Section 1.5.2.2**). Moreover, previous studies relied on parent- or self-reported overall physical activity, and did not consider different intensities, although both total volume and intensities of physical activity are increasingly recognized to play a role in cardiometabolic risk factors among children[122]. In a meta-analysis of 13 case-control studies among girls, athletes were found to have 1.13 years later age at menarche than non-athletes but confounding by other differences between those groups, such as diet and weight regulation, still exist[112]. To my knowledge, similar studies have yet been conducted among boys.

1.5.4 Summary

Overall, there are vast discrepancies in existing findings on the associations of dietary intakes and physical activity with puberty timing from observational studies. On the contrary, in a randomised controlled trial, a school-based intervention that successfully prevented obesity through healthy dietary intakes (i.e. decreasing consumption of high-fat foods and increasing consumption of fruits and vegetables) and increased physical activity (i.e. decreasing television viewing and increasing moderate and vigorous physical activity) delayed onset of menarche in girls[123]. This finding supports that the typical lifestyle behaviours may affect puberty timing, whether or not acting through adiposity. Nevertheless, it is difficult to deduce the role of specific dietary factors and/or physical activity intensity in regulating pubertal development to unveil aetiological pathways to early puberty timing and inform future implementation of targeted interventions.

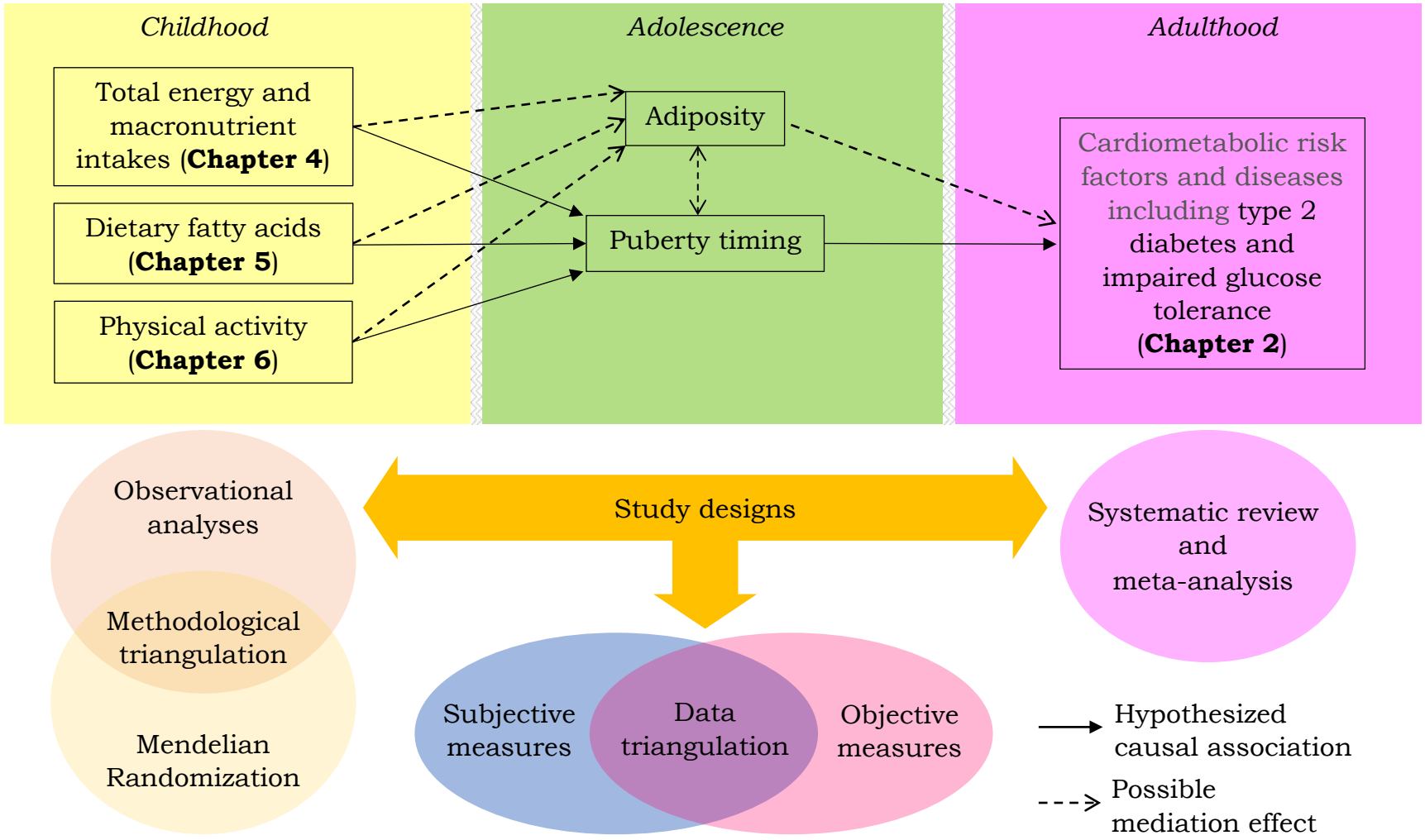
1.6 Thesis aims

The overall aim of this thesis is to identify specific aetiological lifestyle-related factors that potentially modify puberty timing, by analysing more comprehensive data with integrative analytical approaches and incorporating insights from multiple disciplines including life course, nutritional, molecular, physical activity and genetic epidemiology. This thesis is divided into two parts, as illustrated in **Figure 1.5**. The first part examines whether the associations between puberty timing and future diseases are independent of adiposity. This would warrant the subsequent investigations in the second part to discover individual dietary factors (i.e. macronutrient quantity and quality) and intensity of physical activity that may influence puberty timing in boys and girls, with and without adjustment for adiposity.

The specific research aims are outlined below:

1) to systematically review publications on the association between puberty timing and risk for T2D and/or impaired glucose tolerance, with and without adjustment for adiposity indicators (**Chapter 2**),
2) to investigate associations of total energy and macronutrient intakes throughout prepubertal childhood with subsequent puberty timing (**Chapter 4**),
3) to investigate causal associations between prepubertal dietary fatty acids (FAs) and puberty timing (**Chapter 5**), and
4) to test whether total volume and intensities of physical activity were associated with puberty timing (**Chapter 6**).

Figure 1.5 Flow chart of thesis aims, study designs and hypothesis testing



CHAPTER 2 META-ANALYSIS OF PUBERTY TIMING AND TYPE 2 DIABETES

Publication

Cheng, TS., Day, FR., Lakshman, R., Ong, KK. (2020). Association of puberty timing with type 2 diabetes: A systematic review and meta-analysis. *PLoS Medicine*, 17(1), e1003017.[124]

Contributions

I conceived this project together with my supervisors (Dr. Felix Day and Prof. Ken Ong). I also extracted the published data, conducted statistical analyses, jointly interpreted the findings and completed writing for this chapter and the resulting manuscript.

2.1 Summary

Background

The associations between puberty timing, particularly age at menarche, and type 2 diabetes have been increasingly examined in different populations. Whether this association is independent of adiposity is however unclear. This chapter aimed to systematically review published evidence on the associations of puberty timing with type 2 diabetes and/or impaired glucose tolerance (T2D/IGT), with and without adjustment for adiposity.

Methods

Relevant publications (up to 28 February 2019) were ascertained from online databases (i.e. PubMed, Medline and Embase) with the search terms related to the timing of any secondary sexual characteristic in boys or girls and T2D/IGT. Reported estimates were pooled using inverse-weighted random-

effects meta-analyses, while sources of heterogeneity between studies was tested using meta-regression.

Findings

A total of 28 observational studies were found; of which, all assessed age at menarche in women and only one additionally examined voice breaking in men. Meta-analyses demonstrated that earlier age at menarche and early (versus later) menarche were associated with higher T2D/IGT risk among women. These associations were still evident, despite weaker, after adjustment for adult adiposity indicators. Also, these associations were stronger in white than Asians, and in populations with earlier average age at menarche. The only one identified study in white men reported that relatively younger (versus about average) voice breaking was associated with T2D.

Conclusions

These findings suggest that puberty timing was associated with higher T2D/IGT risk, independent of adiposity, thus providing a strong foundation for further research to identify influential factors of puberty timing in **Chapters 4, 5** and **6**.

2.2 Background

Numerous observational studies including meta-analyses have reported findings on the associations between puberty timing and NCDs especially cardiometabolic diseases, as described in **Section 1.4**. Among previous meta-analyses, most were recently conducted between 2017 and 2019, whereas a meta-analysis of age at menarche and T2D was based on 10 publications (315,428 participants) dated until the end of 2013[67]. That meta-analysis also included studies mainly from Western populations, with only two studies in non-Western settings (both from China)[67], which did not allow to compare differences in findings between populations. Since that review, several large published studies among Asians have been subsequently available[125, 126].

Furthermore, previous meta-analysis considered only effect estimates that were adjusted for BMI and hence deliberately excluded those studies that did not include BMI as one of the potential confounders in adjusted analysis[67]. This may nevertheless induce biases in the findings since BMI was typically measured in adulthood (i.e. after the occurrence of menarche) rather than in childhood in previous studies which retrospectively collected age at menarche in women. Indeed, adult BMI is more likely to be considered as a mediator between puberty timing and T2D than simply as a confounder. However, it can be arguable that age at BMI measurement may have little effect on the findings since BMI, overweight and obesity may track from early childhood to adulthood[127, 128]. With respect to these concerns, comparing findings with and without adjustment for adiposity would better inform the role of adiposity in the association between puberty timing and T2D.

It has been reported that association between age at menarche and incident diabetes differed by year of birth among Chinese women, with a stronger association in women born between 1960s and 1970s than those born in 1950s and between 1920s and 1940s[125]. Such potential effect modification deserves further investigation, for which meta-analysis offers a great opportunity. Moreover, in the recent recognition of physical markers of puberty timing for men, association between puberty timing and T2D in men has been examined[33]. Whether similar studies have been performed in more populations is unclear.

2.3 Study aims

This study aimed to perform a systematic review and meta-analysis to i) examine the associations of puberty timing with T2D and IGT, with and without adjustment for adiposity, in both women and men, ii) evaluate study-design-related factors that may explain heterogeneity between study estimates, and iii) estimate potential contribution of early menarche to the population burden of T2D.

2.4 Methods

2.4.1 Data sources and searches

Online databases (i.e., PubMed, Medline, and Embase) were used to search relevant publications until 28 February 2019. The search terms were keywords and/or measures related to (i) puberty timing (e.g., puberty, menarche, voice break, Tanner) and (ii) diabetes (e.g., diabetes, glucose, insulin, glycated haemoglobin) and (iii) epidemiological studies (based on guidelines from the Scottish Intercollegiate Guidelines Network)[129], as fully presented in **Supplementary Table A-1**.

All papers that were identified from the searches were first screened based on title and abstract. If any paper was considered potentially relevant according to study inclusion criteria, full text was then read for inclusion decision. Whenever there was any uncertainty about the eligibility of a particular study, it was resolved through discussion between authors (Prof. Ken Ong and myself). Studies included in the previous systematic review[67] and the reference lists of our included papers were further checked through to ensure no relevant paper was missed out from searches. This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO registration number: CRD42019124353), and the protocol is available at: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42019124353.

2.4.2 Study inclusion and exclusion criteria

The present systematic review included published papers which reported (i) any measure of puberty timing collected in childhood or adulthood (i.e. pubertal onset: age at breast or genital development or pubic hair; pubertal progression: age at menarche or voice breaking) and (ii) T2D/IGT based on

fasting plasma glucose, oral glucose tolerance test, glycated haemoglobin, self-report by participants or medical records/physician diagnosis. Inclusion was not restricted to specific sex, geographical locations of studied populations nor the type of study design, whether observational or experimental. Publications without a full report available in English language were not excluded; however, no such paper was considered potentially relevant on screening of titles and abstracts in English.

Any study among populations with specific diseases such as breast cancer, polycystic ovary syndrome, Turner syndrome, premature adrenarche, and type 1 or 2 diabetes, and animal studies were excluded.

2.4.3 Data extraction

Data from eligible studies for systematic review were extracted by one author (myself), while 20% of the data was independently extracted by a second author (Dr. Rajalakshmi Lakshman) who was blinded to the original dataset. The extracted data were then compared and verified by a third author (Prof. Ken Ong), which showed 100% agreement.

Extracted information were first author, publication year, sample size, study population and ethnicity, year at enrolment, ages at puberty and outcome assessment, mean age at menarche, number of cases, definition of outcome, types of outcomes (prevalent or incident T2D/IGT cases), risk estimates with corresponding confidence intervals (CIs), definition of early puberty and its reference category, and variables controlled for in multivariable models.

The specific data selected for meta-analysis were (i) risk estimates for T2D/IGT per year later age at menarche as a continuous variable (i.e. dose–response relationship) and (ii) risk estimates for T2D/IGT in the earlier age at menarche category compared to the middle or later age at menarche category (i.e. categorical relationship). These risk estimates were from the models adjusted for potential confounders (but not adiposity) and/or the models

adjusted for an adiposity indicator (usually BMI or waist circumference; if available, estimates adjusted for both were prioritized). If several risk estimates for multiple outcomes (i.e. T2D and IGT, T2D only or IGT only) were reported in a study, only one risk estimate for an outcome per study was extracted, where the risk estimate for combined T2D/IGT was first chosen to maximize power of analyses, followed by T2D only and IGT only.

Some extracted risk estimates were further transformed accordingly if they were reported in the opposite direction of association of interest. If risk estimates for T2D/IGT per year earlier (rather than later) age at menarche were reported[130], the reciprocals were calculated to produce risk estimates per year later age at menarche. Similarly, if risk estimates for T2D/IGT in an older (rather than earlier) age at menarche category[125, 131-134] compared to an earlier age at menarche category as the reference were reported, the reciprocals were calculated to produce risk estimates in the earlier age at menarche category compared to the older age at menarche category as the reference. In the present study, odds ratios (ORs) and hazard ratios (HRs) were considered to be similar estimates of the relative risk (RR) since findings were similar by these measures of association.

2.4.4 Data synthesis and analysis

The association between age at menarche and T2D/IGT was summarized using inverse-variance-weighted random-effects models, which allow for heterogeneity among individual study effect estimates. Estimates from models with and without adjustment for adiposity indicators were analysed separately. Heterogeneity between studies was assessed using the inconsistency index (I^2) (<50%, 50%–75%, and >75% indicate mild, moderate, and high heterogeneity, respectively). Potential sources of heterogeneity were evaluated using meta-regression analyses. Asymmetry of individual study effect estimates was tested using visual inspection of funnel plots and Egger's regression test. Sensitivity analyses by the trim-and-fill and leave-one-out methods were further performed. All statistical analyses were computed using

the "metafor" package in R software[135]. P value<0.05 was considered as statistical significance.

The population attributable risk for T2D/IGT due to early menarche among British women was further calculated using the formula $\frac{p(\mathrm{RR}-1)}{p(\mathrm{RR}-1)+1}$, where p is the prevalence of early menarche (<12 years) in the large population-based UK Biobank study[33], and RR is the pooled risk estimate among white populations. Such calculation is conditional on the causal assumption that age at menarche affects T2D/IGT risk, which underlies the interpretation of population attributable risk as the proportion of preventable disease[136].

2.4.5 Study quality assessment

To assess the quality of each study included in the present systematic review, the Newcastle–Ottawa Quality Assessment Scale for cohort studies[137] was employed. Criteria for each item in the assessment scale were pre-determined according to the present research topic before study quality assessments were performed. All eight items (maximum score of 9) were used to evaluate longitudinal studies of incident T2D/IGT and longitudinal studies that assessed puberty timing in adolescence and early adulthood and subsequent prevalent T2D/IGT. On the other hand, only six items (maximum score of 7) were used for cross-sectional studies of prevalent T2D/IGT since two items on presence of T2D/IGT at baseline and follow-up duration were not relevant.

2.5 Results

2.5.1 Study characteristics

Figure 2.1 shows study inclusion flow chart. Initially, 6,155 records were identified from the search strategy; of which, 49 texts were selected for full-text reading after screening based on titles and abstracts, and removing

duplicates and non-relevant studies. Finally, 28 eligible studies were included in the current review. All studies included in the previous review[67] and studies in the reference lists of included studies were found from our search strategy.

Figure 2.1 Flow chart of study inclusion

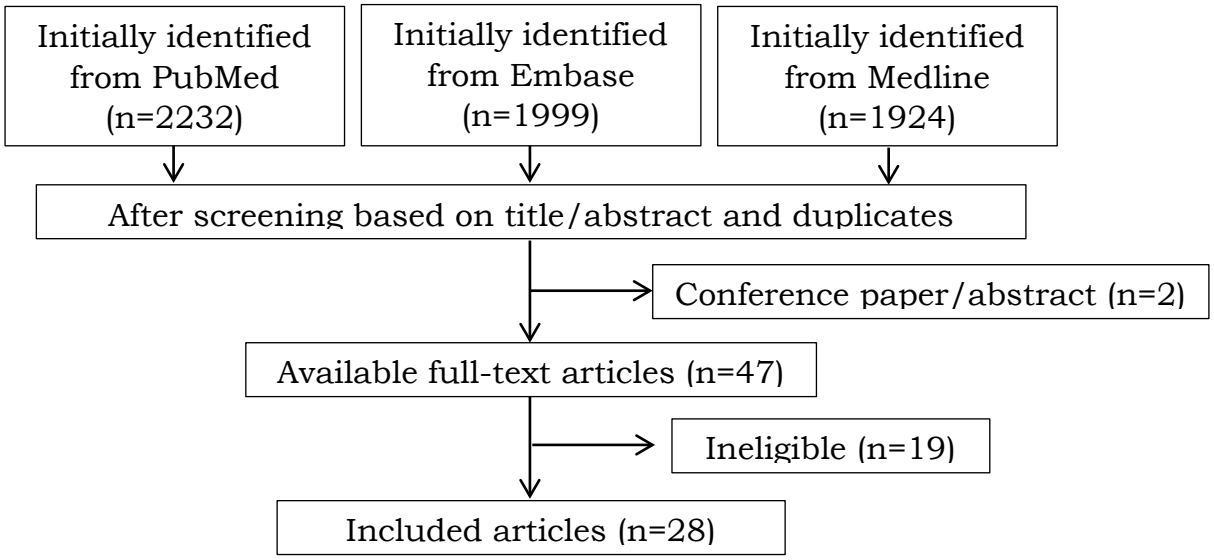


Table 2.1 and **Table 2.2** (more details in **Supplementary Table A-2** and **A-3**) present the characteristics of the included studies analysing prevalent and incident cases of T2D/IGT as the outcomes, respectively. Among the 28 included studies, all were observational studies, while one additionally included a MR analysis[126]. All assessed age at menarche in women (combined N=1,228,306), but only one additionally considered age at voice breaking in men[33]. Most studies collected puberty timing during middle to late adulthood (mean age ranging from 35 to 70 years) (n=25), except two in early adulthood (age <25 years)[130, 138] and one during adolescence[139]. Early menarche was determined as age at menarche <14 years in 13 studies[125, 131, 133, 134, 140-148] and <12 years in nine studies[33, 130, 149-155], whereas reference category was defined as age at menarche ≥14 years in 10 studies[125, 130, 131, 133, 134, 140, 142, 146, 147, 152] and ≥12 years in 12 studies[33, 141, 143-145, 148-151, 153-155]. The reference category of age at menarche was the middle category in 12 studies[33, 142-144, 146, 148-151, 153-155] and the oldest category in 10 studies[125, 130, 131, 133, 134, 140, 141, 145, 147, 152].

Half of the included studies (n=14) examined prevalent T2D, two prevalent IGT[134, 140], three prevalent T2D and IGT[141, 143, 156], eight incident T2D[125, 130, 133, 147-149, 151, 153], and one prevalent and incident T2D[154]. Various definitions of T2D and IGT were used across studies, and four studies intended to exclude participants with potential type 1 diabetes based on age at diagnosis[33, 139, 154, 155]. Adiposity indicators were adjusted for in 25 studies; of which, mostly were BMI alone (n=19)[130-134, 138, 139, 143, 145-151, 153, 155-157], followed by both BMI and waist circumference (n=4)[125, 142, 144, 154], waist circumference alone (n=1)[140], and body composition (n=1)[33].

Majority of the included studies were conducted among Asians (Chinese, Bangladeshi, Korean, and Japanese) (n=13)[125, 126, 133, 134, 140-147, 152], followed by studies among white populations (n=9)[33, 131, 132, 138, 139, 149-151, 156] and studies among multi-ethnic populations (white, Hispanic, Asian, African-American, and Latino) (n=6)[130, 148, 153-155, 157]. Also, most studies (n=18) tested the association of age at menarche with T2D/IGT risk using logistic regression models and reported ORs, while others used Cox proportional-hazards models and reported HRs (n=6)[125, 130, 133, 139, 148, 149], or performed Poisson regression[155, 156], log binomial regression[157] or generalized linear modelling[151] and reported RRs (n=4).

In models without adjustment for adiposity, almost all studies (n=20/24) found a statistically significant association between earlier menarche[33, 125, 126, 130, 132, 133, 139, 140, 143-146, 148-151, 153-156] or earlier voice breaking[33] and higher T2D/IGT risk, whereas the rest reported null (n=3)[142, 147, 152] or positive (n=1)[141] associations. In models with adjustment for adiposity, about half of the studies (n=11/24) reported a statistically significant association of earlier menarche[33, 125, 140, 143-146, 150, 151, 155, 156] or earlier voice breaking[33] with higher T2D/IGT risk, while other studies showed null findings (n=11)[130-134, 138, 139, 142, 147, 148, 154] or inconsistent findings (n=2) between dose–response and categorical age at menarche models[149] or between sub-cohorts[153].

Table 2.1 Summary of eligible studies of prevalent T2D/IGT

First author, year	N Total (N cases)	Study; Ethnicity	Year at enrolment	Age at menarche (mean±SD) (year)	Age at outcome assessment (year)	Outcome: definition	Adiposity covariate
Cooper, 2000[138]	668 (49)	Menstruation and Reproductive History: white	1934-39	12.4 (range: 8-18)	73 (range: 63-81)	Diabetes: Self-reported physician diagnosis	BMI
Saquib, 2005[131]	997 (125)	Rancho Bernardo: white	1984-87	<12: 14.5 % 12–15: 78.9 % ≥16: 6.6 %	69.5±9.3 (range: 50-92)	Diabetes: OGTT, physician diagnosis or anti-diabetic medication	BMI
Heys, 2007[140]	7108 (-)	Guangzhou Biobank; Chinese	2003-04	15.4±2.1 (range: 8-25)	64.0±6.0 (range: 50-94)	IGT: fasting glucose or anti-diabetic medication	WC
Lakshman, 2008[132]	13,308 (734)	EPIC-Norfolk; mainly white	1993-97	13.0±1.6	40-75	Diabetes: Self-reported physician diagnosis or anti-diabetic medication	BMI
Akter, 2012[141]	1423 (-)	Gabindagonj Upazilla; Bangladeshi	2009-10	Unknown	40.9 to 42.7 (by age at menarche group)	Diabetes: physician diagnosis or anti-diabetic medication AND IGT: fasting glucose)	-
Dreyfus, 2012[154]	8491 (990)	ARIC; white, African-American	1987-89	12.9±1.6	50.6±9.3	Diabetes: fasting/non-fasting glucose, self-reported physician-diagnosis or anti-diabetic medication	BMI, WC
Pierce, 2012[139]	1632 (26)	NSHD; white	1946	13.2 (range: 8.5-19.5)	31, 36, 43, 53	Diabetes: Ever treated	BMI
Stockl, 2012[156]	1503 (366)	KORA; white	2006-08	13.5±1.6	25-74	Diabetes: OGTT, physician diagnosis or anti-diabetic medication AND IGT: OGTT	BMI
Qiu, 2013[142]	3304 (738)	Chinese	2011-12	Median: 16 (IQR: 15-18)	59 (range: 37-92)	Diabetes: OGTT, physician diagnosis or anti-diabetic medication	BMI, WC
Mueller, 2014[155]	8075 (1335)	ELSA-Brasil; White and Black Brazilian	2008-10	12.7±1.7	52.0±8.8 (range: 35-74)	Diabetes: OGTT, HbA1c, physician diagnosis or anti-diabetic medication	BMI

OGTT, oral glucose tolerance test; IQR, interquartile range; WC, waist circumference

Table 2.1 Summary of eligible studies of prevalent T2D/IGT (continued)

First author, year	N Total (N cases)	Study; Ethnicity	Year at enrolment	Age at menarche (mean±SD) (year)	Age at outcome assessment (year)	Outcome: definition	Adiposity covariate
Baek, 2015[143]	2,039 (905)	Sungkyunkwan University; Korean	2012-13	14.6±1.6	48.9±3.5 (range: 44-56)	Diabetes: OGTT, HbA1c, physician diagnosis or anti-diabetic medication AND IGT: fasting glucose or HbA1c	BMI
Day, 2015[33]	250,037 (4836)	UK Biobank; white	2006-10	13.0±1.6 (range:8-19)	56.52±8.09 (range: 40-69)	Diabetes: Self-report physician diagnosis	Body Comp.
Hwang, 2015[144]	3,254 (-)	KNHANES IV; Korean	2007-09	15.67	64.1 (range: 50-85)	Diabetes: Self-report physician diagnosis (including Type 1 and 2)	BMI, WC
Lim, 2015[145]	4,326 (119)	KNHANES IV; Korean	2007-09	13.0 to 14.3 (by age group)	20-50	Diabetes: fasting glucose, self-report physician diagnosis or anti-diabetic medication	BMI
Cao, 2016[134]	1,625 (-)	Changsha Women's Health Screening Program; Chinese	2011-14	-	60.45±8.19 (range: 40-75)	IGT: fasting glucose	BMI
Won, 2016[152]	12,336 (-)	KNHANES; Korean	2010-13	14.6	45.7	Diabetes: Self-reported physician diagnosis	-
Yang, 2016[146]	16,114 (832)	Jinchang Cohort; Chinese	2011-13	14.8±2.0	45.8±11.8	Diabetes: fasting glucose or use of anti-diabetic medication	BMI
Au Yeung, 2017[126]	12,484 (-)	Guangzhou Biobank; Chinese	2003-08	14.3 to 15.9 (by age group)	≥50	Diabetes: fasting glucose or use of anti-diabetic medication	-
Farahmand, 2017[150]	4,952 (187)	Tehran Lipid and Glucose; white	1998	13.3±1.5	28.1 to 36.9 (by age at menarche group)	Diabetes: OGTT	BMI
Petersohn, 2019[157]	30,626 (2,328)	Mexican National Health survey; Mexican	1999-2000	13	37-45 (by age at menarche group)	Diabetes: self-report physician diagnosis or OGTT	BMI

OGTT, oral glucose tolerance test; WC, waist circumference

Table 2.2 Summary of eligible studies of incident T2D/IGT

First author, year	N Total (N cases)	Study; Ethnicity	Year at enrolment	Age at menarche (mean±SD) (year)	Age at outcome assessment (year)	Outcome: definition	Adiposity covariate
He, 2010[153]	101,415 (7,963)	Nurses' Health; Multi-ethnic	1980	-	63.5	Diabetes: OGTT; ≥1 diabetes symptom; anti-diabetic medication	BMI
	100,547 (2,739)	Nurses' Health II; Multi-ethnic	1991	-	47.4		BMI
Conway, 2012[133]	69,385 (1,831)	Shanghai Women's Health; Chinese	1997-2000	-	60.1±2.0	Diabetes: OGTT or anti-diabetic medication	BMI
Dreyfus, 2012[154]	7,501 (755)	ARIC; white, African-American	1987-89	12.9±1.6	56.8±8.0	Diabetes: fasting/non-fasting glucose, self-reported physician-diagnosis or anti-diabetic medication	
Elks, 2013[149]	10,903 (4,242)	EPIC-InterAct; white	1991	13.14±1.58	52	Diabetes: Health record confirmed self-reported physician diagnosis	BMI
Dreyfus, 2015[130]	1,970 (271)	CARDIA; Caucasian, African-American	1985	12.6±1.5 (range: 8-16)	50 (range: 42-59)	Diabetes: OGTT or anti-diabetic medication	BMI
LeBlanc, 2017[148]	124,379 (11,262)	Women's Health Initiative; Multi-ethnic	1993-98	-	(Follow-up: 12.2±4.2)	Diabetes: self-report diagnosis, use of anti-diabetes medication	BMI
Yang, 2018[125]	270,345 (5,391)	China Kadoorie Biobank; Chinese	2004-08	15.4±1.9	(Follow-up: 7)	Diabetes: Health record	BMI, WC
Pandeya, 2018[151]	126,721 (4,073)	InterLACE; mainly white	1985-09	13.1 (range:8-20)	56.1±11.4	Diabetes: self-reported physician diagnosis or Health records	BMI
Nanri, 2019[147]	37,511 (513)	Japan Public Health Center-based Study; Japanese	1990, 1993	14.7±1.9	(Follow-up: 10)	Diabetes: Health record confirmed self-reported physician diagnosis	BMI

OGTT, oral glucose tolerance test; WC, waist circumference

2.5.2 Study quality

Supplementary Table A-4 shows more than half of studies for prevalent T2D/IGT (n=11 studies) scored 6 out of max 7 scores, followed by 5/7 (n=4), 7/7 (n=3) and 5/9 (n=2). **Supplementary Table A-5** shows that longitudinal studies for incident T2D/IGT were rated 9 (n=5) or 8 (n=4) out of max 9 scores.

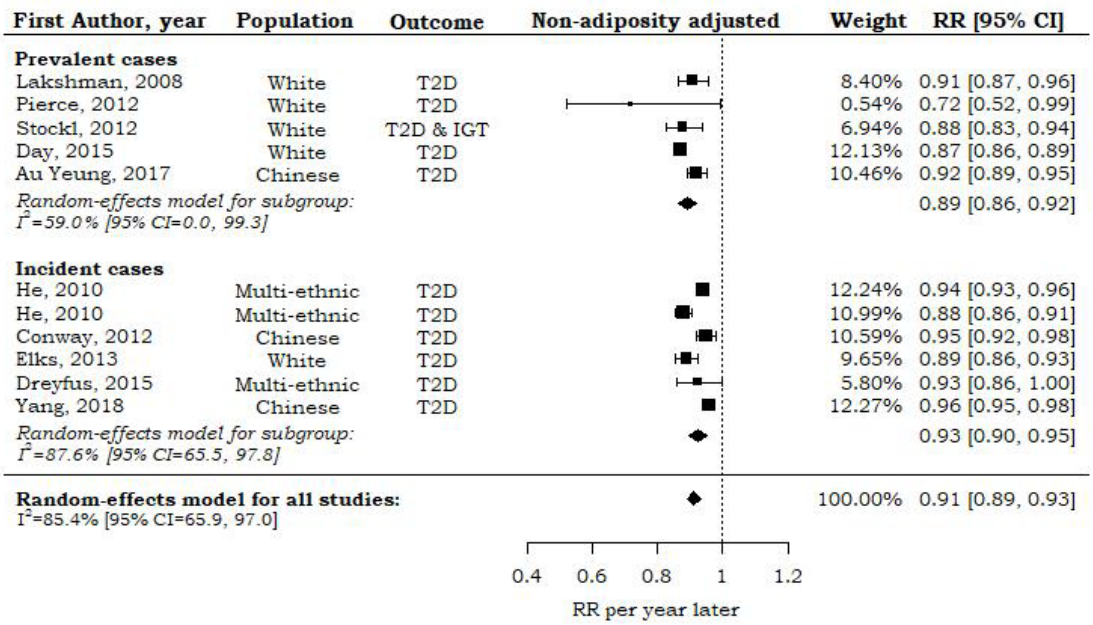
2.5.3 Age at menarche and T2D/IGT

All 28 observational studies on age at menarche and T2D/IGT in women were included in meta-analysis. Majority of the outcomes was T2D only (n=23), followed by T2D/IGT (n=3) and IGT only (n=2).

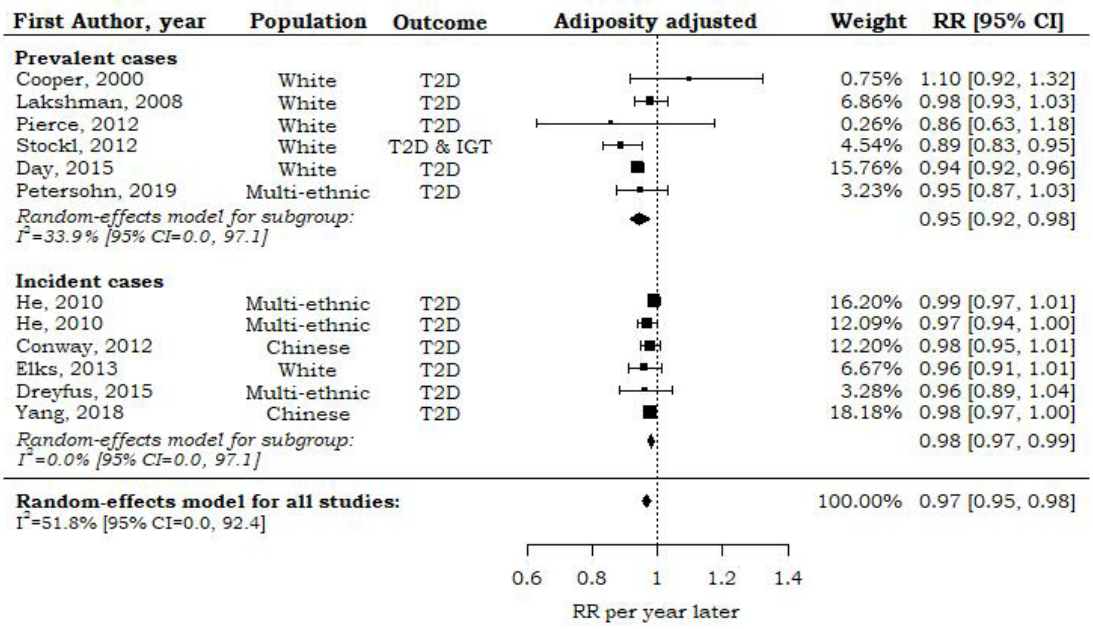
Figure 2.2 plots the associations between continuous age at menarche and T2D/IGT. Pooled analysis of 11 estimates from models without adjustment for adult adiposity in 10 studies showed that later age at menarche was associated with lower T2D/IGT risk (RR=0.91 per year, n=833,529; **Figure 2.2a**). This association was weaker but still evident in models with adjustment for adiposity (pooled analysis of 12 estimates from 11 studies: RR=0.97 per year, n=852,268; **Figure 2.2b**). Heterogeneity between studies was high in estimates without adjustment for adiposity (I^2=85.4%) and moderate in estimates with adjustment for adiposity (I^2=51.8%). Further, similar findings for pooled estimates were found in subgroup analyses by prevalent and incident T2D/IGT (**Figure 2.2**), and in separate analyses by T2D only and IGT only (**Supplementary Figure B-1**).

Figure 2.2 Forest plots of the associations between age at menarche and T2D/IGT

(a)



(b)

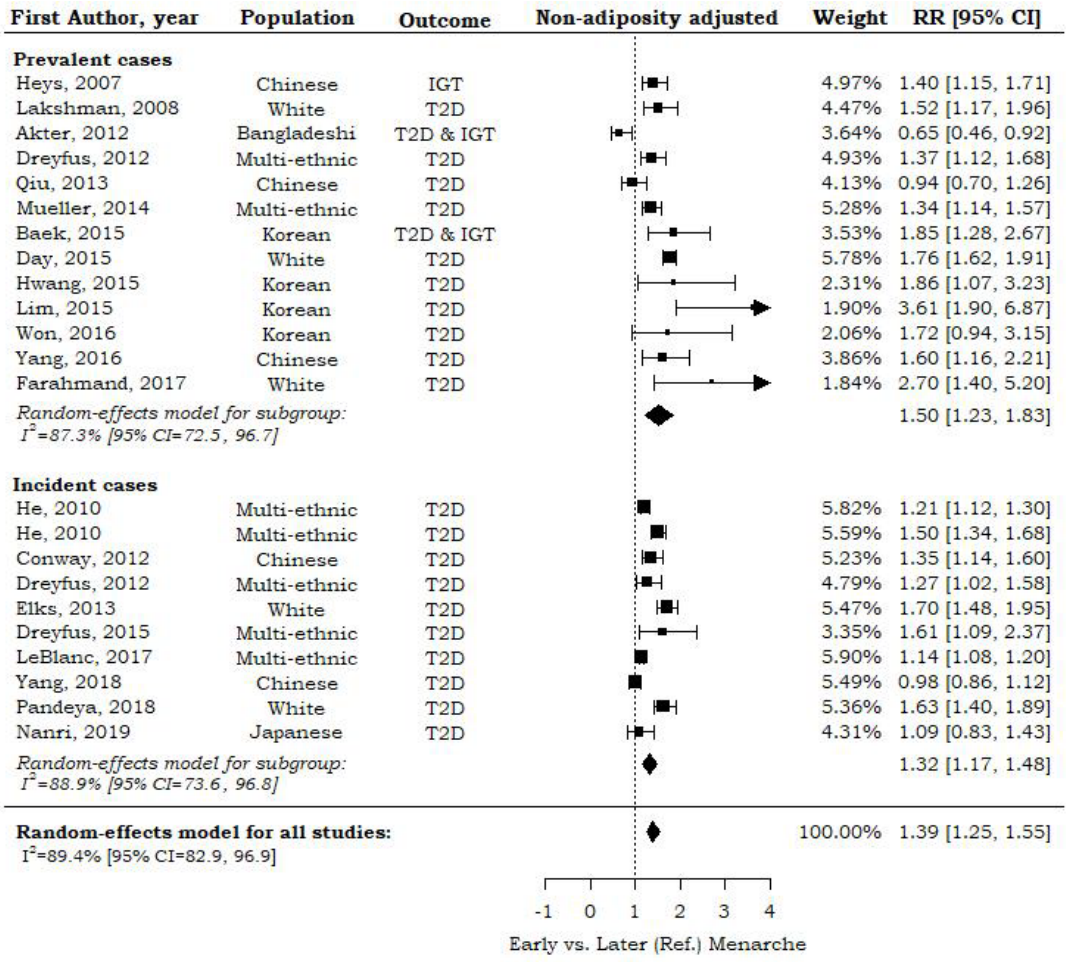


The plots illustrate the relative risks for combined T2D/IGT per each year later age at menarche, (a) without and (b) with adjustment for adiposity indicators. He et al.[153] consisted two cohort studies.

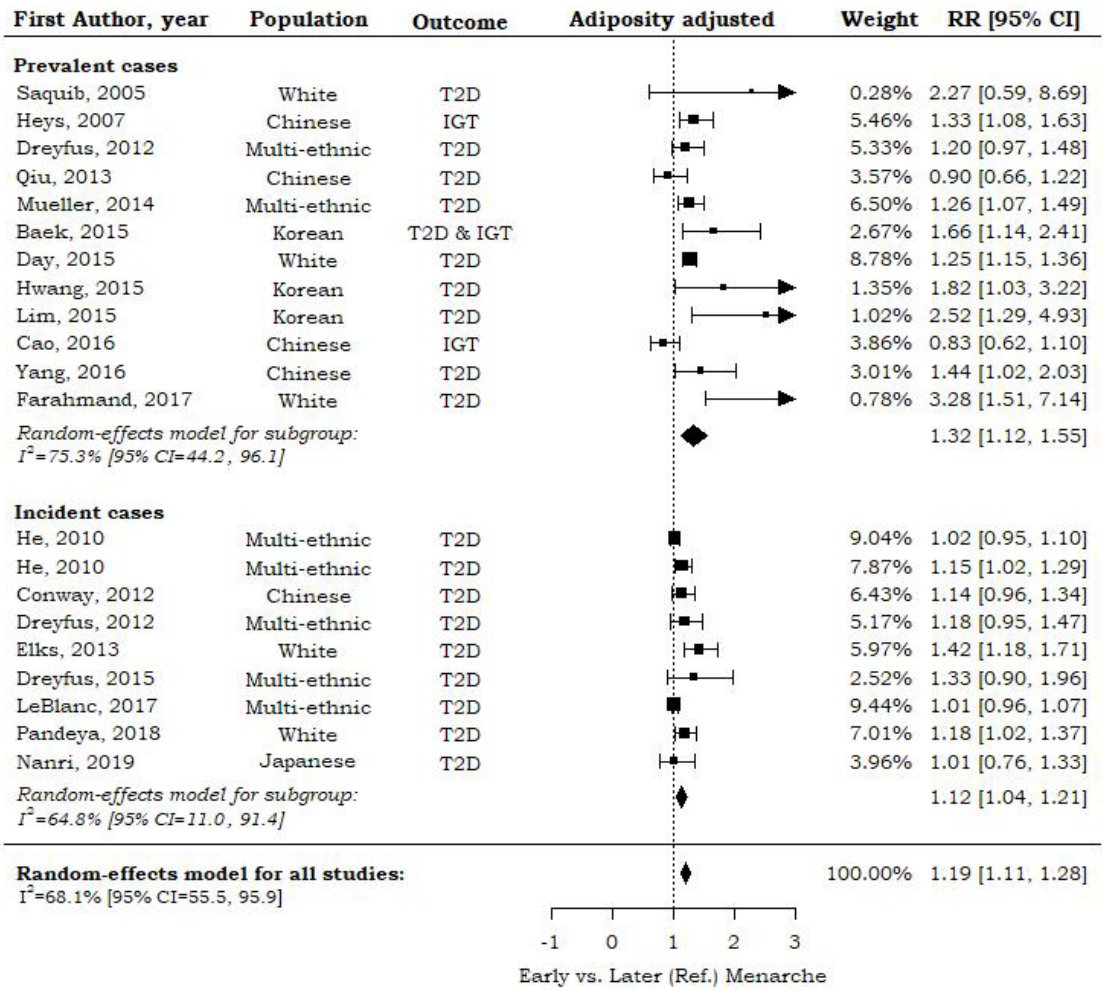
Figure 2.3 plots the associations between categorical early versus later menarche and T2D/IGT. Pooled analysis of 23 estimates from models without adjustment for adult adiposity in 21 studies showed that early menarche was associated with higher T2D/IGT risk (RR=1.39, n=1,185,444; **Figure 2.3a**). This association was weaker but still evident in models with adjustment for adiposity (pooled analysis of 21 estimates from 19 studies: RR=1.19, n=890,583; **Figure 2.3b**). Heterogeneity between studies was high in estimates without adjustment for adiposity (I^2=87.8%) and moderate in estimates with adjustment for adiposity (I^2=68.1%). Further, similar findings for pooled estimates were obtained in subgroup analyses by prevalent or incident T2D/IGT (**Figure 2.3**), and in separate analyses by T2D only and IGT only (**Supplementary Figure B-2**).

Figure 2.3 Forest plots of the associations between early versus later menarche and T2D/IGT

(a)



(b)



The plots illustrate the relative risks for combined T2D/IGT associated with early (versus later) menarche, (a) without and (b) with adjustment for adiposity indicators. He et al.[153] consisted two cohort studies. Ref., Reference

2.5.4 Meta-regression results

Table 2.3 shows results of univariable meta-regression and pooled RRs by subgroups of studies. Heterogeneity between studies was partially explained by study-level differences in ethnicity and average age at menarche. Higher T2D/IGT risk associated with earlier menarche (both continuous and categorical) was observed among white than Asian individuals, as well as among populations with younger than older average age at menarche. Other study-design-related factors including year of enrolment, age at outcome assessment, number of variables adjusted for, age cutoff used to define early menarche and the reference category, and measures of association (OR, HR, or RR) did not explain the heterogeneity between study estimates (**Supplementary Table A-6**).

Table 2.3 Univariable meta-regression results and pooled RRs for T2D/IGT by study subgroups

Study-design-related factors	Non-adiposity adjusted				Adiposity adjusted			
	N	RR (95% CI)	P value[a]	R^2 (%)	N	RR (95% CI)	P value[a]	R^2 (%)
				Continuous age at menarche				
Ethnicity				53.7				99.63
Asian	3	0.95 (0.92, 0.97)			2	0.98 (0.97, 0.99)		
White	5	0.88 (0.86, 0.90)	0.002		6	0.95 (0.92, 0.98)	0.001	
Multi-ethnic	3	0.91 (0.87, 0.96)	0.154		4	0.98 (0.96, 1.00)	0.871	
Study average age at menarche, years[b]				33.3				0
<13.5	5	0.89 (0.86, 0.91)			6	0.96 (0.93, 0.98)		
≥13.5	2	0.92 (0.85, 1.01)	0.154		2	0.94 (0.86, 1.03)	0.719	
P value for linear trend[c]			<0.001				0.400	
				Early versus later (Ref.) menarche				
Ethnicity				37.9				16.3
Asian	11	1.33 (1.06, 1.69)			9	1.23 (1.02, 1.49)		
White	5	1.72 (1.61, 1.83)	0.013		5	1.27 (1.18, 1.36)	0.290	
Multi-ethnic	7	1.30 (1.18, 1.42)	0.743		7	1.11 (1.02, 1.20)	0.472	
Study average age at menarche, years[b]				38.0				18.9
<13.5	8	1.59 (1.45, 1.75)			7	1.26 (1.19, 1.34)		
≥13.5	8	1.36 (1.17, 1.58)	0.030		6	1.27 (1.03, 1.55)	0.820	
P value for linear trend[c]			0.014				0.677	

[a]The reference category in meta-regression models was the first subgroup in each factor
[b]Studies that did not report the information were excluded
[c]Using study average age at menarche as a continuous variable
R^2 (%), % heterogeneity explained

2.5.5 Sensitivity analyses

Supplementary Figure B-3 indicates some asymmetry in funnel plots especially for studies on the association between categorical early menarche and T2D/IGT without (Egger's test, P value=0.052) and with adjustment for adiposity (Egger's test, P value=0.001). The source of asymmetry was predominantly the small studies, whereas findings of the larger studies appeared to be consistent with the overall estimates.

Supplementary Figure B-4 shows that when the predicted missing studies using trim-and-fill method were added to the meta-analyses, the associations of earlier continuous age at menarche (non-adiposity adjusted RR=0.91 per year; adiposity-adjusted RR=0.97 per year) and early menarche (non-adiposity adjusted RR=1.32; adiposity-adjusted RR=1.15) with higher T2D/IGT risk remained similar.

Supplementary Figure B-5 illustrates that when one of the study estimates was iteratively removed from the meta-analysis using leave-one-out analyses, pooled estimates remained nearly unchanged for the associations between earlier (both continuous and categorical) age at menarche and higher T2D/IGT risk, with or without adjustment for adiposity.

2.5.6 Contribution of early menarche to the burden T2D

Given that higher T2D/IGT risk associated with early menarche was observed in white than Asian individuals, the pooled RR in white populations was used to estimate current maximum contribution of early menarche to the burden of T2D, together with the prevalence of early menarche (20.15%) in white women in UK Biobank which is a very large population study of predominantly white adults. The estimated population attributable risk for T2D/IGT due to early menarche (<12 years) among white British women, unadjusted for adult adiposity was 12.6% (95% CI 11.0% to 14.3%) and adjusted for adult adiposity was 5.1% (95% CI 3.6% to 6.7%).

2.6 Discussions

2.6.1 Summary of findings

The present meta-analyses of 28 observational studies demonstrated that both earlier age at menarche and early (versus later) menarche were associated with higher T2D/IGT risk in women. These associations were still evident, though weaker, after adjustment for adult adiposity indicators. Similar findings were obtained in sensitivity analyses that included predicted missing studies, despite asymmetry in study estimates owing to small study effects which was more evidently in one of the four models. While high study quality was generally obtained, high heterogeneity between studies was present and may be explained by study differences in ethnicity and average age at menarche, with stronger associations among white women and populations with lower average age at menarche. A significant proportion of T2D/IGT among white British women possibly attributable to early menarche (before age 12 years) was estimated, assuming a causal relationship. Also, this review observed scarce studies on puberty timing and T2D/IGT in men.

2.6.2 Comparisons with previous evidence

Our meta-analysis is consistent with a previous review[67] which reported an association of combined earlier age at menarche and early menarche with higher T2D risk, adjusted for adiposity based on 10 studies (n=315,428 women). We further (i) included a larger number of studies (n=28) and women (n=890,583), (ii) compared differences between findings using continuous and categorical age at menarche and between findings unadjusted and adjusted for adiposity, and (iii) explored a variety of reasons for heterogeneity between studies. Moreover, while the previous meta-analysis[67] found an association of early menarche with higher T2D risk in Europe and the US, we included more Asian studies and added that such association was also apparent among Asians, although weaker than in white individuals, possibly due to their later

average age at menarche. One study in China demonstrated that HR for incident diabetes associated with earlier age at menarche was higher in women born in more recent decades, along with the decreasing mean age at menarche over time, from 16.2 years in the 1920s–1940s to 14.7 years in the 1960s–1970s[125]. These findings suggest that in the midst of worldwide secular trends towards earlier average age at menarche[40,41], not only more women are moving into the high-risk category (early menarche), but also the magnitude of risk for T2D/IGT in this category would be increasing.

2.6.3 Potential mechanisms

Mechanisms that elucidate the association between earlier age at menarche and higher T2D/IGT risk are unclear. It has been hypothesized that rapid postnatal weight gain[158] and childhood obesity[80, 81] may lead to early menarche which may then promote adulthood obesity[159], consequently raising T2D risk[32, 160, 161]. Therefore, adiposity may act as both a partial confounder and a partial mediator. Nonetheless, the present meta-analysis showed that earlier menarche remained associated with higher T2D/IGT risk, though attenuated, after considering the potential confounding and mediating effects of adiposity. These observations may imply that other non-adiposity underlying mechanisms may be involved. Early menarche may alternatively reflect particular sex hormone exposures, such as higher levels of oestradiol[57, 58] and lower sex-hormone-binding globulin concentrations (owing to increased insulin level)[59], in women, which in turn possibly alter glycaemic control and increase T2D risk[162-164]. In contrast, hormone replacement therapy mainly using oestrogen was shown to lower diabetes risk in women[165]. Such various effects of oestrogen may be mediated by different oestrogen receptors at different parts of body including brain, adipose tissue, breast, endometrium and endothelium[166].

2.6.4 Strengths and Limitations

Several limitations exist in the present meta-analysis. First, the attenuation in the associations adjusted for adiposity could not be directly tested or quantified since the studies that contributed non-adiposity- and adiposity-adjusted estimates were not completely, though largely, overlapping. All risk estimates analysed were from observational studies, and thus findings may be subject to residual confounding. Some asymmetry was detected especially for the adiposity-adjusted associations between categorical early menarche and T2D/IGT, which indicates a possible bias towards reporting positive findings; however, such bias may be only applicable to small studies and thus possibly have little effect on the overall findings as supported by sensitivity analyses.

Age at menarche was self-reported and recalled during adulthood in most studies, which may affect its accuracy, but prospectively and retrospectively collected age at menarche have been reported to be moderately correlated[50, 51]. Study average age at menarche and cutoffs for early menarche and reference category also varied across studies, and the former factor was considered as the source of heterogeneity between study estimates, but the subgroup analyses by study average age at menarche were limited to studies that reported this value. Moreover, while current meta-analysis investigated associations of both continuous and categorical age at menarche with T2D/IGT risk, whether there was a particular threshold of age at menarche for elevated risk of T2D/IGT could not be determined, as was revealed by a large study[149].

The present systematic review may be affected by selection bias due to the inclusion of only papers with full reports in English. Nevertheless, no potentially relevant papers in other languages during screening of titles and abstracts in English was found, and many studies in non-English-speaking populations were included in this review. We still however could not exclude

the possibility that other non-English studies could be identifiable only in other search databases.

Finally, only one study of puberty timing and T2D/IGT in men was found, likely because measures of puberty timing in men were not obtained in most studies. The one identified study reported a robust association between relatively younger (versus about average) voice breaking and T2D in white men (non-adiposity-adjusted RR=1.44, 95% CI 1.30 to 1.59, P value<0.001; adiposity-adjusted RR=1.24, 95% CI 1.11 to 1.37, P value<0.001), using a very large sample size (n=197,714). However, more such studies are needed especially among non-white men to explore whether the association could vary by populations as observed among women.

2.6.5 Conclusions

This systematic review and meta-analysis of 28 observational studies mainly among Asian and white populations revealed that earlier age at menarche is consistently associated with higher T2D/IGT risk, independent of adult adiposity. This association is stronger among white women and populations with earlier average age at menarche. A substantial proportion of T2D cases related to early menarche was also found amongst the UK women, which would be likely to increase given the current global secular trends towards earlier puberty timing. Further studies are required to unveil potential underlying mechanisms linking early menarche to future T2D/IGT risk, including the potential modifiable factors of puberty timing.

CHAPTER 3 DATA SOURCES AND COMMON METHODS

This chapter describes data sources and common methods in relation to **Chapters 4, 5** and **6**. Relevant information on two British birth cohort studies, namely the Avon Longitudinal Study of Parents and Children (**Section 3.1**) and the Millennium Cohort Study (**Section 3.2**), including study population and data collection, and commonly used statistical analyses in this thesis are detailed in this chapter. More information on other cohort studies used in specific studies are discussed within the related chapters.

Contributions

I performed the data integration and data transformation to derive variables of interest in this thesis, including dietary intakes, physical activity and puberty timing, as described in **Sections 3.1.4** and **3.2.4**.

3.1 The Avon Longitudinal Study of Parents and Children

3.1.1 Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC), also known as "Children of the 90s", is an ongoing population-based birth cohort study which aims to gain insights into how genetic, epigenetic, biological, psychological, social and environmental exposures influence health and development across life course[167, 168]. The initial recruitment enrolled 14,541 pregnant women from the former Avon county with three Health Districts in the South West of England (**Figure 3.1**), who were expected to deliver between 1 April 1991 and 31 December 1992. Of these recruited pregnancies, 13,988 children were alive at age 1 year. This cohort was then extended to recruit

additional children from age 7 to 18 years (n=913) who met the original enrolment criteria to maximize the number of children in the cohort, giving 14,901 children in the final sample to date.

Figure 3.1 The ALSPAC eligible study area

Adapted from Boyd et al.[167]. The figure shows the study area in the UK, consisting of three eligible National Health Service District Health Authorities (DHAs).

3.1.2 Data collection

A large volume of data has been collected from mothers and their child at regular visits using several assessment methods. In brief, during pregnancy, up to three questionnaires were self-administered to mothers to record their information including socio-demographic characteristics and lifestyle behaviours. After delivery, children were frequently followed-up, with 68 data collection time points from birth to 18 years old. These included child-based questionnaires completed by caregivers (mainly mothers) and children themselves, and clinical assessments to record child's phenotype and environmental measures such as growth and development, and lifestyle behaviours. Biological samples including blood were also obtained from children. More details on protocols and data catalogue are available at:

http://www.bristol.ac.uk/alspac/researchers/our-data/. For this thesis, specific data that are used for main analyses are described below.

3.1.2.1 Dietary assessments

Children's dietary intakes were reported by parents using FFQ at ages ~3 (mean ± standard deviation (SD): 3.22±0.11 years) (n=9797, 70.0%), ~4 (4.54±0.10 years) (n=9484, 67.8%) and ~7 years (6.80±0.11 years) (n=8234, 58.9%), and using 3-day food diaries at age ~7.5 years (7.56±0.31 years) (n=7277, 52.0%)[169, 170].

The FFQ for children was adapted from the FFQ for pregnant mothers and further adapted at each age to cover age-appropriate child's diets. It has been also validated against 3-day food diaries collected from a 10% subcohort at each age. The FFQ comprised a list of ~60 foods and beverages, and asked parents to indicate how often their child currently consumed each food/drink item, with five response options: '*never or rarely*', '*once in 2 weeks*', '*1-3 times a week*', '*4-7 times a week*' and '*more than once a day*'. Also, more details for other five food/drink groups normally consumed daily were captured, including numbers of cups of tea or coffee and slices of bread, as well as usual types of milk (full fat, semi-skimmed, skimmed, or others), breads (white, brown/granary, wholemeal or others) and spread (butter, types of margarine, or others). To quantify individual dietary intakes, the consumption frequency categories were first converted to 0, 0.5, 2, 5.5 and 10 times per week. Missing response in any FFQ item was assumed to be no consumption and thus given a value of 0 only for those with 10 or fewer unanswered items, whereas those with more than 10 unanswered items were excluded from the estimation analysis. Finally, daily nutrient intakes at each age were estimated by summing the products between the reported weekly frequency and the nutrient content for each food consumed at a standard portion tailored to the particular age of the child[169, 170], and then dividing the sum by seven days. Plausibility of reporters for energy intakes at 3 to 7 years could not be assessed since body weight during the periods were not measured in the

whole cohort. Highly implausible estimated dietary data were however removed (n=223-329) for those with extreme low and high values for total energy intakes based on visual inspection of histograms at each age (age 3: ≤349kcal and ≥2617kcal; age 4: ≤514kcal and ≥3263kcal; age 7: ≤545kcal and ≥3970kcal).

The 3-day food diaries were posted to families prior to the child's visit to a research clinic, and parents recorded all foods and drinks consumed by their child in household measures over any three (consecutive or non-consecutive) days including one weekend day and two weekdays. At 7.5 years, majority completed all three days of food records (n=6020, 82.7%), followed by two days (n=994, 13.7%) and one day (n=263, 3.6%). The dietary records were then transformed to daily energy and nutrient intakes using DIDO (Diet In, Data Out) software (developed by the Human Nutrition Research Unit in Cambridge, UK)[171], based on the 5th edition of McCance & Widdowson's food tables and the supplements to the tables, the National Diet and Nutrition Survey database and information from manufacturers[169, 170]. When dietary records were not fully described, weights were based on the age-specific standard portion sizes for children[169, 170] , or information from packets or manufacturers. If there was no equivalence in the food tables for any particular food recorded, composite foods and recipes were analyzed by component parts. At 7.5 years, the previously calculated 95% CI for the accuracy of the ratio of reported energy intake to estimated energy requirement[172], that accounts for the amount of variation inherent in the methods used to estimate energy intake and energy requirement[173], was 0.79-1.21[170]. Based on this range, we found 74% plausible reporters of energy intake values in the whole cohort, which was similar to the range of the previously reported rate (76%) for 3-day food diaries in the 10% subcohort (n=814)[170]. We also found 79% plausible reporters based on the individual cut-off points[174] of basal metabolic rate calculated using sex-, age- and body weight-specific Schofield equations[175].

3.1.2.2 Profiling of plasma phospholipid fatty acids

Non-fasting venous blood samples were taken at 7.5 years (7.58±0.33 years) (n=5425, 38.8%) and stored at -70°C, which have been suggested as a more reliable indicator of average lipid concentration levels than fasting blood samples[176]. Using these samples, plasma phospholipid FA concentrations were profiled at the National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland, United States of America in 2009–2010[177]. The serial assay methods were: i) transmethylation of lipids with acetyl chloride and methanol using a simplified method based on the Lepage and Roy procedure[178] by a high throughput automated method[179], ii) adding internal standards to each assay for internal calibration and a second standard to quantify the exact amount of internal standard in each batch for ongoing assay of experimental variability, iii) transmethylation and extraction of FAs using Freedom Evo Instrument 200 (TECAN Trading AG, Switzerland), which was automated by the customized control and automation software (EVOware V.2.0, SP1, Patch3) and iv) separation of FAs using gas chromatography 6890 Plus LAN system (Agilent Technologies, Inc, Santa Clara, California, United States of America). The assay was linear in the range from 1 to 600 μg/ml plasma, while the within- and between-day imprecision were 3.26±1.2% and 2.95±1.6%, respectively. In total, 23 FAs were identified, consisting of 12 polyunsaturated FAs (PUFAs), five monounsaturated FAs (MUFAs) and six saturated FAs (SFAs).

3.1.2.3 Assessment of pubertal development

Children's pubertal status was reported annually by caregivers (mainly parent) from ages 8 to 13 years and children themselves from ages 14 to 17 years (29.3%-47.1%) (**Table 3.1**), using an adapted version of the Petersen Pubertal Development Scale[34]. The measures were status of genital development in boys and breast development in girls, and pubic hair growth in both sexes, resembled by the modified version of validated sex-specific line drawings (i.e. uncircumcised penises in boys were presented instead)[180], which were

indicated from a choice of five Tanner stages ranging from prepuberty (stage 1) to postpuberty (stage 5) (**Supplementary Figure B-6**), over all nine visits. Further questions were included in the scale to measure other traits of puberty, specifically: i) voice breaking status in boys over eight visits (except the first puberty assessment), with options of '*no, it is the same*', '*yes, occasionally it is a lot lower*' and '*yes, it has now changed totally*'; ii) menarche in girls over all nine visits, recorded as the date (in month and year) and/or age (in years) at first occurrence of menstruation; and iii) axillary hair growth status in both sexes over seven visits (except the first two puberty assessment), reported as '*no*' or '*yes*'. At each assessment, response date was also noted to calculate child's age.

Table 3.1 The average ages at puberty assessment

Puberty assessment visits	Age (years)	Number
Visit 1	8.2±0.24	6255
Visit 2	9.7±0.13	7017
Visit 3	10.7±0.13	6629
Visit 4	11.7±0.12	6293
Visit 5	13.2±0.16	6075
Visit 6	14.7±0.15	5163
Visit 7	15.4±0.26	4867
Visit 8	16.1±0.15	4760
Visit 9	17.0±0.09	4370

3.1.2.4 Measurements of standing height

Standing height of children was measured by trained fieldworkers at research clinics, using Leicester height measure at 5-6 years and Harpenden Stadiometer at 7-20 years.

3.1.3 Ethical approval and funding

Informed consent was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time, and biological samples were collected in accordance with the Human Tissue Act (2004). Ethical approval for this cohort study was obtained from the ALSPAC

Ethics and Law Committee and the Local Research Ethics Committees (http://www.bristol.ac.uk/alspac/researchers/research-ethics/).

The UK Medical Research Council and Wellcome (Grant ref:102215/2/13/2) and the University of Bristol provide core support for ALSPAC (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). Specifically, the intramural research programme of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) with funding from the National Oceanic and Atmospheric Administration (NOAA) supported the assays of child's blood, and the Center for Disease Control (AY5350) supported pubertal assessments.

3.1.4 Data processing

3.1.4.1 Prepubertal childhood dietary intakes

To ascertain habitual dietary intakes throughout prepubertal childhood, repeated measures of intakes from age 3 to 7.5 years were synthesised as the intakes at age 6 years (i.e. the middle age across the ages at dietary measures), using random intercepts linear regression models[181], separately in boys and girls. For these estimations, all reported dietary data at age 3 to 7.5 years, except those with extreme values (**Section 3.1.2.1**), were included, since restricting analyses to plausible reporters can potentially induce selection bias in the later findings[182]. First, age was centred to 6 years old, and the longitudinally time-varying intakes of a particular nutrient were modelled as a function of time using random intercepts. Next, from the model, the individual Best Unbiased Linear Predictor estimates were obtained, which represent the difference between the person-specific intercept and the overall intercept. Finally, the Best Unbiased Linear Predictor estimates were added to a constant value (i.e. the population mean intake of the nutrient of interest at age 6 years) to calculate individual intakes of a particular nutrient at 6 years old. Random intercepts linear regression model was used rather than a simpler alternative calculation of mean values across visits because the latter

method could be limited by data which are unbalanced and missing not at random[181].

3.1.4.2 Plasma phospholipid fatty acid concentrations

The assayed absolute concentrations of plasma phospholipid FAs in μg/ml were converted to relative concentrations expressed as percentage of total plasma phospholipid FAs (μg%). The relative concentrations of plasma phospholipid FAs were further adjusted for age using linear regression models to obtain their residuals.

3.1.4.3 Timings of puberty traits

Repeated measures of pubertal status from 8 to 17 years were combined to determine individual estimates of: i) age at pubertal onset, based on Tanner stage 2 genital (G2) and breast (B2) development in boys and girls, respectively, ii) age at pubertal progression, based on partial/total voice breaking in boys and menarche in girls, and iii) other puberty timing traits, namely age at Tanner stage 2 pubic hair growth (PH2), age at axillary hair growth and pubertal tempo (i.e. time from puberty onset to progression).

Ideally, age at the first occurrence of each puberty trait was estimated at individual level as the midpoint age between the last reported prepubertal status and the first reported pubertal appearance, as expressed as the equation below:

$$\text{age at occurrence} = \frac{\text{age at first reported puberty} - \text{age at previous annual report}}{2}$$

However, due to the nature of data collection, missing response on pubertal status and age at response at some visits, and inconsistent reporting of pubertal status across visits (i.e. non-sequential order with increasing age) are inevitable, hence making the abovementioned calculation difficult. Therefore, a series of strategy to estimate timings of puberty traits (i.e. G2, B2,

PH2, axillary hair growth and voice breaking) were considered as follows (**Figure 3.2**).

1) If puberty was first reported at age 8 years:

   age at occurrence = age at the first reported puberty – 6 months

2) If puberty was first reported after age 8 years and the one previous annual report was prepuberty:

   $$\text{age at occurrence} = \text{age at the first reported puberty} - \frac{x}{2},$$

   where x is the population average interval between the first report and the previous annual report

3) If puberty was first reported after age 8 years

   (a) but the one previous annual report was missing:

   age at occurrence = age at the first reported puberty – x

   (b) but the two previous annual reports were missing:

   $$\text{age at occurrence} = \text{age at the first reported puberty} - \left(x + \frac{y}{2}\right),$$

   where y is the interval between average ages at each annual report

   (c) but more than two previous annual reports were missing:

   $$\text{age at occurrence} = \text{age at the first reported puberty} - \left(x + \frac{y}{2} + \frac{y}{2} * + \ldots\right),$$

   $* \frac{y}{2}$ for each additional missing report (up to three missing annual reports for genital and breast development, and pubic hair growth; up to five missing annual reports for axillary hair growth, and voice breaking)

4) If puberty was never reported (only for voice breaking and axillary hair growth) and

   (a) the last prepuberty report was at age 17 years:

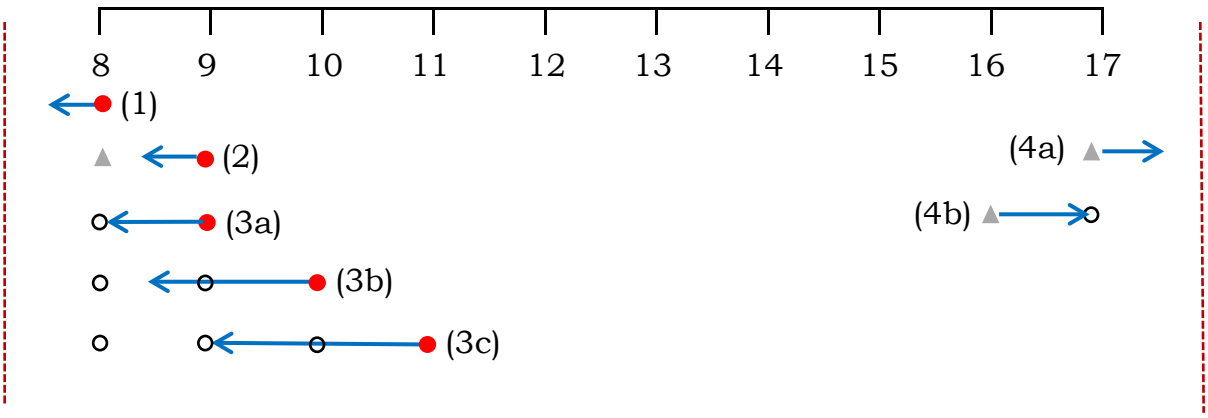
   age at occurrence = age at the last reported prepuberty + 6 months

   (b) the subsequent annual reports were missing:

   age at occurrence = age at the last reported prepuberty + z,

   where z is the aforementioned time intervals from (2) to (3)

Figure 3.2 Estimations of individual puberty timing traits



The figure illustrates the examples of strategies to estimate individual timings of puberty traits. ● First reported occurrence of puberty ▲ Last reported prepuberty ○ Missing report → backward/forward calculation ¦ typical minimum (7 years) and maximum (18 years) ages at puberty

For each puberty trait, only a proportion of children had the reported measures that followed a consistent sequential order with increasing age (i.e. from prepuberty to the first occurrence of puberty) (59.2%-98.7%). Therefore, to reduce the numbers of children excluded from these analyses, children with reported sequential measures of genital and breast developmental status, and pubic hair growth with only one inconsistency were additionally included (3.9%-9.1%). For example, while the first report was Tanner stage 3, this was followed by Tanner stages 1, 2 and 3 in subsequent reports in sequential order; in this case, the first report was deemed to be a likely error and thus treated missing. Also, boys whose reported measures of voice breaking status followed a consistent sequential order for at least three consecutive visits before or after an inconsistent report were included. For instance, 'no voice breaking' was reported at fourth visit after partial or total voice breaking had been reported in the previous three visits, and thus the reported 'no voice breaking' was considered an error and treated missing.

Overall, amongst children with reported puberty status, 68.3%-98.7% were effectively estimated for G2 (3152 out of 4617) and B2 (3874/4883), PH2 (boys: 3308/4627; girls: 3745/4887), axillary hair growth (boys: 4134/4251; girls: 4521/4580) and voice breaking (3577/4547). Timing of puberty in G2, B2,

PH2 and voice breaking could not be confidently calculated in 20.7%-31.7% of children, owing to inconsistent reports (i.e. non-sequential ordering of pubertal status that alternated between prepuberty and puberty over time, and/or missing data).

Among the repeated measures of age at menarche, the earliest reported age (from reported date or age) was considered on the assumption that it was closer to the event and thus more accurate. Values for age at voice breaking (n=27) and menarche (n=39) were further excluded if these occurred earlier than age at G2 and B2, respectively. Pubertal tempo was calculated as the difference between ages at G2 and voice breaking in boys, and between ages at B2 and menarche in girls.

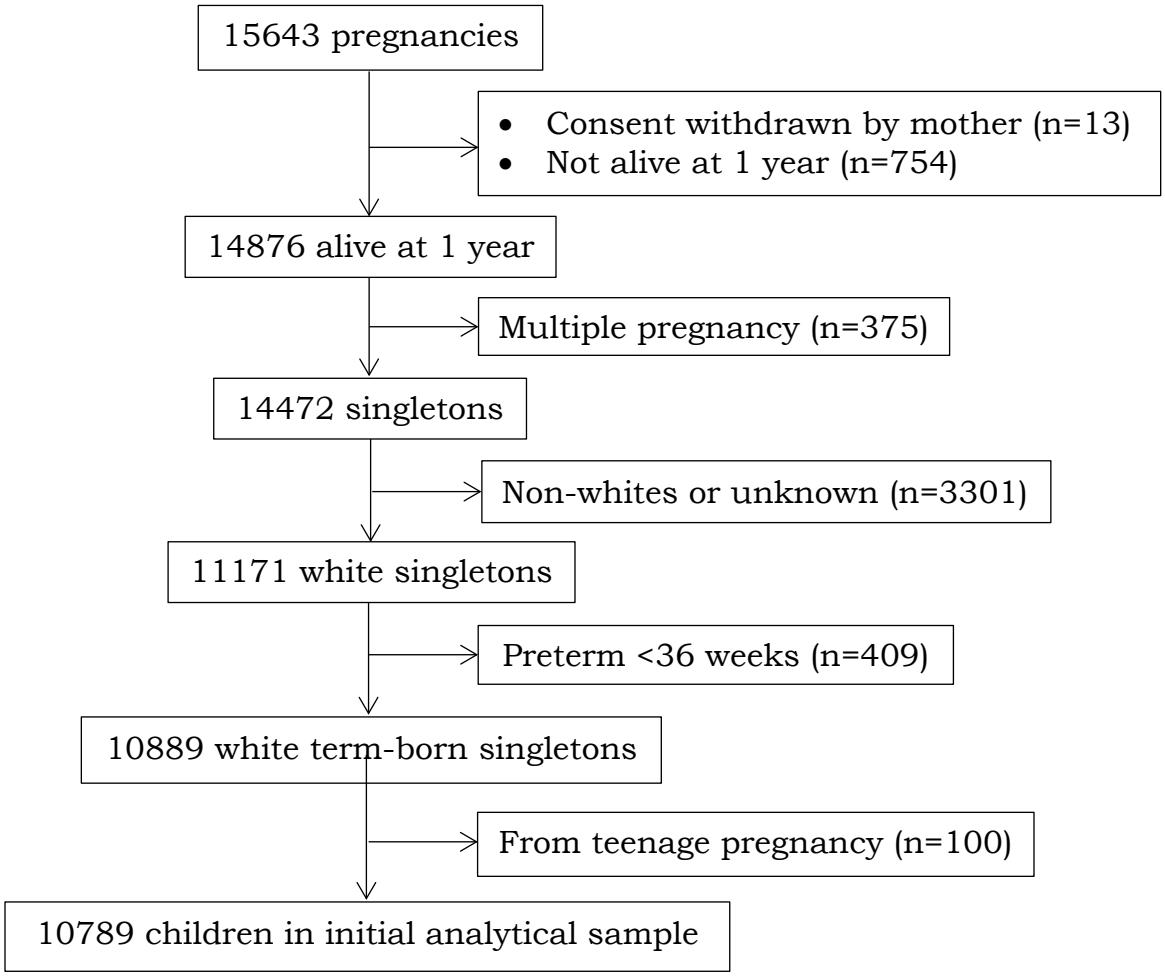
An objective measure of puberty timing, namely age at peak height velocity (PHV), was also considered. It was previously derived by Frysz et al.[55] using 46,246 measurements of standing height between age 5 and 20 years in 5,707 children with at least one height measurement in the time periods: 5 to <10 years, 10 to <15 years and 15 to 20 years (i.e. the pre-, peri- and post-pubertal periods for the majority of children)[55]. The computation of PHV was performed separately in boys (n=2688) and girls (n=3019), by transformation of the random age intercept that indicates the timing of the height growth spurt using SuperImposition by Translation and Rotation (SITAR) analysis which is the mixed effects shape-invariant growth curve model[55].

3.1.5 Common methods

3.1.5.1 Inclusion criteria

To reduce potential confounding, the general inclusion criteria for this thesis were: singleton pregnancy, white ethnicity (reported by mother and her partner), gestational age ≥36 weeks and mother's age at delivery ≥18 years, giving 10,789 children in the initial analytical sample (**Figure 3.3**). Further inclusion criteria related to data availability are discussed in specific chapters.

Figure 3.3 General thesis inclusion criteria using ALSPAC data



3.1.5.2 Potential confounders

Potential confounders were selected as those which have been associated with child's dietary intakes and puberty timing[183]. These included i) maternal and family characteristics (i.e. age at delivery, age at menarche, highest education level [none/Certificate of Secondary Education, vocational, O-level, A-level, Degree], pre-pregnancy BMI, parity [0, 1, 2, ≥3], passive smoking during pregnancy [none, <1 hour per day, ≥1 hour per day], active smoking during pregnancy [no, yes], highest maternal or mothers' partner's occupational socioeconomic group at 18 weeks of gestation [partly skilled and unskilled, skilled manual and non-manual, professional, managerial and technical]), and ii) infant characteristics (birth weight, gestational age, breastfeeding duration [never, <3 months, 3-<6 months, ≥6 months]). These characteristics

were mainly obtained from self-administered questionnaires during pregnancy and first year after delivery.

3.1.5.3 Energy adjustment models

The effects of macronutrients can be explored using three different energy adjustment models. First, the *energy partition model* functions to explore relative roles of macronutrients and includes the energy contributions from carbohydrate, fat and protein simultaneously in the same model[184]. Second, the *nutrient density model* represents a more robust approach and explores the substitution of a macronutrient for another macronutrient in an isocaloric diet by including the percentages of energy contributed by the two macronutrients and total energy intake in the same model[184]. For example, in a model that includes the percentages of energy from fat and protein and total energy intake, the resulting beta value for protein indicates the effect of each 10% increase in protein intake as carbohydrate intake (the only excluded macronutrient) concomitantly decreases by 10%, while fat and total energy intakes are held constant; this model refers to the substitution of protein for carbohydrate. Third, the *residual model* includes the residuals from two macronutrients (obtained from the regressions of each macronutrient intake on total energy intake)[184] and total energy intake in the same model, and can be used as a sensitivity analysis which accounts for the potential underreporting of dietary intakes[185].

3.1.5.4 Multiple imputation

Missing data are inevitable in longitudinal studies. To test the robustness of the results to missing values particularly on covariates, multiple imputation by chained equations were computed, assuming that data are missing at random (i.e. the probability of missingness of data is independent of the unobserved data but conditional on the observed data)[186]. The variables included in the multiple imputation model were all variables used for analysis, those highly correlated with explanatory variables and those related to data

missingness[187]. In this thesis, 50 imputed datasets were generated and results were summarised into single regression estimates with 95% CI based on Rubin's combination rules[188].

3.1.5.5 Statistical software

All statistical analyses were performed using Stata 15.1 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

3.2 The UK Millennium Cohort Study

3.2.1 Study population

The UK Millennium Cohort Study (MCS) is an on-going multidisciplinary, nationally representative, longitudinal observational study that aims to understand health inequalities and health outcomes throughout the lives among children born at the turn of the new century[153,154]. Its eligibility criteria were all children born between 1 September 2000 and 31 August 2001 (for England and Wales), and between 24 November 2000 and 11 January 2002 (for Scotland and Northern Ireland), alive, living in the UK, and entitled to child benefit at age 9 months, except a very small group of children such as asylum seekers. Eligible children were identified using government child benefit records, a benefit with almost universal coverage. At baseline, 18,827 children from 18,552 families were recruited, and a small number of eligible children who were missed at the initial recruitment were further included at subsequent visits, giving over 19,000 children in the cohort to date. Also, particular sub-groups of the population including children of ethnic minority backgrounds were intentionally oversampled in this cohort to ensure sufficient sample sizes for analysis.

3.2.2 Data collection

Information on diverse topics including behaviour and physical growth have been collected mainly using parent-reported questionnaires at various visits: age 9 months (MCS1) (n=18,818), and 3 (MCS2) (n=15,808), 5 (MCS3) (n=15,460), 7 (MCS4) (n=14,043), 11 (MCS5) (n=13,469), 14 (MCS6) (n=11,872) and 17 (MCS7) years (n=10,757). Further details about this cohort including available data can be found at https://cls.ucl.ac.uk/cls-studies/millennium-cohort-study/. For this thesis, specific data that are used for main analyses are described below.

3.2.2.1 Physical activity measures

Physical activity in children was measured at age 7 years (7.23±0.25) (MCS4) using uniaxial accelerometers (Actigraph GT1M, Pensacola, Florida). The accelerometer is a small (38 X 37 X 18 mm), lightweight (27g) and non-waterproof device, and has been extensively validated in children against observational techniques[189], heart rate monitoring[190], indirect and room calorimetry[191, 192] and doubly labelled water techniques[193]. All children surveyed at age 7 years were invited, and participating children with written consent from their parents (n=13,219, 94.1%) were posted an accelerometer preset with 15-seconds sampling epochs (i.e. the shortest possible epoch allowed to record data every 15 seconds, given the number of days of wear required) (n=12,625, 95.5%; others were not sent because details were not transmitted to fieldwork team). Children were then instructed to wear the accelerometer from the morning after they received it, on the right hip with an elastic belt around their waist during waking time (except during bathing and other aquatic activities) for up to seven consecutive days. After the monitoring period, families returned their accelerometer in a prepaid envelope (n=10,034, 79.5%).

Raw accelerometry data (n=8939, 89.1%) were downloaded using the ActiLife Lifestyle Monitoring System software version 3.2.1, and processed in multi-

stages to derive physical activity summary data[194, 195]. First, after basic data cleaning including the removal of low and high end days which likely indicated the posting periods, non-wear time was identified as any time period of consecutive zero-counts for at least 20 minutes and excluded from the summation of physical activity[196]. Next, extreme high values (≥11715 CPM) possibly owing to accelerometer malfunction or misuse of accelerometers such as vigorous shaking were removed[197]. Finally, the 'cleaned' accelerometry data were summarised as i) the overall movement volume expressed as daily total counts and ii) time spent in sedentary (<100 counts per minute (CPM)), light (100-2240 CPM), moderate (2241-3840 CPM) and vigorous (>3840 CPM) intensities, based on the previously developed thresholds[198]. The thresholds were calibrated against energy expenditure measures in kcal kg^{-1} $hour^{-1}$ obtained over a range of exercise intensities (lying down viewing television, sitting upright playing a computer game, slow walking, brisk walking, jogging, hopscotch and basketball) using a COSMED (Model $K4b_2$, Rome) portable metabolic unit in 53 children aged 7 years[198]. To determine children's typical daily physical activity, a minimum daily wear time was proposed as a monitor wear time period of at least two days, each day containing at least 10 hours of valid wear data[199] (n=6675, 74.7%).

3.2.2.2 Assessment of pubertal development

Pubertal status in growth spurt, body hair and skin changes for boys and girls, voice breaking and facial hair only for boys, and breast development only for girls were reported by parents at age 11 years (11.17±0.28) (MCS5) (n=12,771, 94.8%) and by children themselves at 14 years (14.26±0.27) (MCS6) (n=11,277, 95.0%). The response options were: "has not yet begun", "has barely started", "has definitely started" and "seems completed". Also, a binary response (no or yes) on menarcheal status and age at menarche in girls were reported: "How old was your child when she started to menstruate?" by parents at age 11 years (MCS5) (n=6206, 93.1%), and "How old were you when you had your first period?" by girls themselves at 14 (MCS6) (n=5630, 95.1%)

and 17 years (17.18±0.25) (MCS7) (n=5164, 96.3%). At each assessment, response date was also noted to calculate child's age.

3.2.3 Ethical approval and funding

Informed consent was obtained from parents, as well as from the children themselves as they grow up. The ethical approval for this cohort study was obtained from the South West Multi-Centre Research Ethics Committee (MCS1), the London Multi-Centre Research Ethics Committee (MCS2 and MCS3), the Northern and the Yorkshire Research Ethics Committee (MCS4), the Yorkshire and Humber Research Ethics Committee (MCS5), the National Research Ethics Service Research Ethics Committee London – Central (MCS6) and the National Research Ethics Service Research Ethics Committee North East – York (MCS7).

The MCS was funded by grants from the Economic and Social Research Council and a consortium of government funders. The accelerometer data collection was specifically funded by the Wellcome Trust (grant 084686/Z/08/A).

3.2.4 Data processing

3.2.4.1 Physical activity intensities

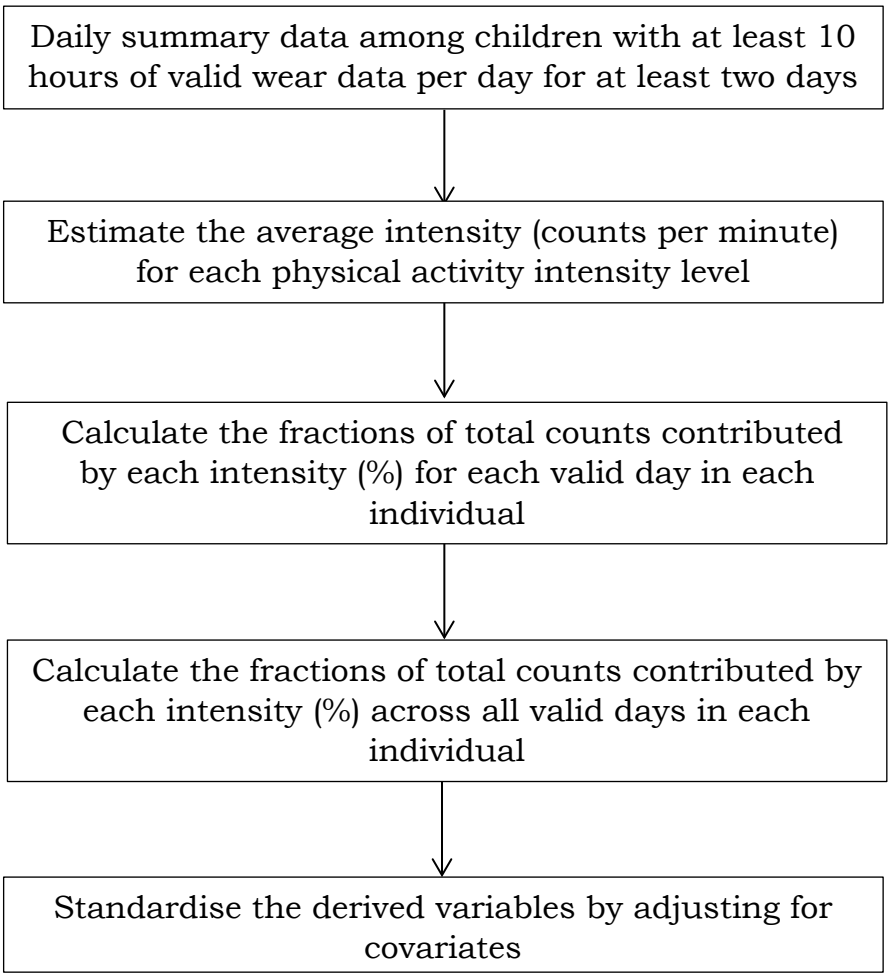
To evaluate physical activity intensity beyond the overall volume, daily summary data from **Section 3.2.2.1** (37,341 person-days of data in 6675 children) were used to estimate individual average daily fractions of total counts accumulated in each of the four intensity categories (**Figure 3.4**). First, average movement intensity (in CPM) across physical activity intensities were determined using a single linear regression with interval constraints. This regression model i) included the empirical total daily counts as the dependent variable, daily times spent across four physical activity intensities as the independent variables and no constant value, as expressed in formula below:

Empirical total counts = $Time_{sedentary}$ + $Time_{light}$ + $Time_{moderate}$ + $Time_{vigorous}$ and ii) predefined the resulting regression coefficients (i.e. average intensities) to be higher as each intensity level increases. From this analysis, the average intensities for sedentary, light, moderate, and vigorous activities were 0, 645, 3481 and 5754 CPMs, respectively, which were within the above designated ranges[198].

To validate these average intensity estimates, the estimated total counts, which was calculated by the summation of the products between time spent and derived average intensity, was compared with the empirical total counts at the individual daily level. The estimated total counts was 0.19% higher than the empirical total counts (854±18200, paired t-test: P value<0.001), but they were highly correlated (Pearson's correlation coefficient=0.99). Next, at each day in each individual, the fraction of total counts contributed by each physical activity intensity (in %) was calculated by dividing the estimated counts from each physical activity intensity by their estimated total counts. Finally, in each individual, averages across all included measure days were calculated for empirical total daily counts and fractions of total daily counts contributed by each physical activity intensity.

To allow comparison of physical activity across children, individual average empirical total daily counts, and fractions of total daily counts and daily time spent across all four intensities were internally standardised using linear regression models, by adjusting for age at accelerometry measurement, total wear time, number of valid measure days, proportion of weekdays measured and season during physical activity measurement (coded as two orthogonal sine functions[200]; "Winter" peaking at 1 on January 1st and reaching a minimum of − 1 on July 1st, and Spring peaking at 1 on April 1st and reaching a minimum of − 1 on October 1st).

Figure 3.4 Flow chart for derivation of fractions of total daily counts contributed by individual physical activity intensities



3.2.4.2 Timings of puberty traits

Repeated measures of pubertal status at age 11 to 17 years were summarised to determine individual timing of puberty traits. Given the widely ranging ages at each assessment (i.e. from 10.2 to 12.3 years at MCS5, and 13.1 to 15.3 years at MCS6), categorical instead of continuous ages at growth spurt, body hair, skin changes, voice breaking, facial hair and breast development for boys and/or girls were estimated using a series of approaches. First, reported puberty status were reclassified into binary categories at each timepoint: i) prepuberty - "has not yet begun" or "has barely started" and ii) puberty - "has definitely started" or "seems completed" to diminish inconsistencies between timepoints. Those with inconsistent reporting (i.e. puberty at one visit but prepuberty at the next) were then removed from estimation. Second, median

age for the report of each puberty trait across the whole sample was obtained using probit modelling of the combined data at 11 and 14 years, separately in boys and girls. In boys, the median ages at reporting growth spurt, body hair, skin changes, voice breaking and facial hair were 12.1, 13.0, 13.8, 13.8 and 14.9 years, whereas in girls, the median ages at reporting growth spurt, breast development, body hair and skin changes were 10.4, 11.3, 11.7 and 12.6, respectively. Finally, for each individual, timing of each puberty trait was classified into: i) earlier puberty - if puberty was first reported at the age earlier than the median or ii) later puberty - if the last prepuberty was reported at the age same as or later than the median. Many responses were unclassifiable because they were uninformative and thus excluded (n=3190-9039): if puberty was first reported at the age after the median age; or if the last prepuberty was reported at the age before the median age.

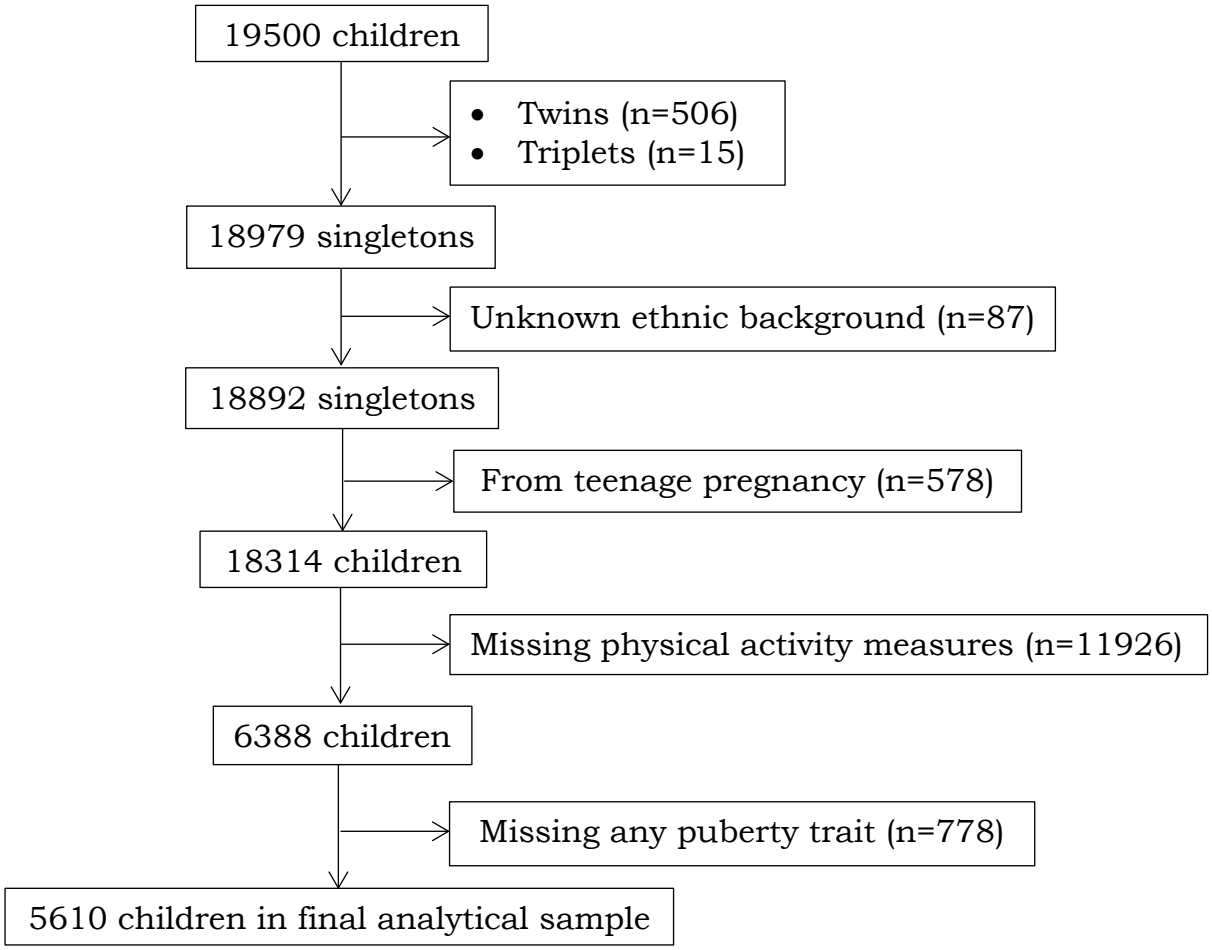
For age at menarche in girls, the first reported age at menarche among the three timepoints (age 11, 14, 17 years) was considered (n=6124), assuming it was closer to the event and thus more accurate, or if missing, calculated as the midpoint age between the last reported premenarcheal status and the next reported menarche status (n=56). The age at menarche was analysed as a continuous variable and also a categorical variable in tertiles: i) 8-12 years, ii) >12-13 years and iii) >13 years.

3.2.5 Common methods

3.2.5.1 Inclusion criteria

The initial inclusion criteria for this thesis were children who were a singleton pregnancy and had known ethnic background and mother's age at delivery ≥18 years (n=18314). Further, children with both data on accelerometry measures of physical activity and timing of any puberty trait were only included for analysis (**Figure 3.5**).

Figure 3.5 Study inclusion criteria using MCS data



3.2.5.2 Potential confounders

Potential confounders were selected as those factors that are likely to precede child's physical activity and puberty timing[183]. These included the most contemporary (i.e. closest to age 7 years or MCS4) self-reported maternal characteristics (i.e. age at delivery, active smoking during pregnancy [no, yes], alcohol consumption during pregnancy [never, <1 per month, 1-2 per month, ≥3 per month], highest academic qualification level [none/other, General Certificate of Secondary Education (GCSE) D-G, GCSE A-C, A-level/diploma, first/higher degree], and pre-pregnancy BMI), family income in quintiles and child characteristics (i.e. ethnicity [White, Asian, Black, mixed], birth weight, gestational age, breastfeeding duration [never, <3 months, 3-<6 months, ≥6 months], mental health [normal, borderline-abnormal], dietary behaviour [eat most things, eat a reasonable variety of foods, a fussy eater], regular sleep

time [no/yes sometimes, yes usually, yes always], long-term health status [no longstanding illness, longstanding illness, longstanding illness limits activity], and BMI-for-age z scores at 7 years). Specifically, pre-pregnancy BMI was either directly self-reported or calculated by dividing self-reported pre-pregnancy weight in kilogram by squared height in meter. Family income was derived by dividing self-reported total net weekly household income by the number of household members according to respective assigned weight (i.e. 1 for first adult, 0.5 for each remaining adult, and 0.3 for each child under 14 years) on the Organisation for Economic Co-operation and Development's equivalised income scale[201]. Child's mental health status was measured using the Strength and Difficulties Questionnaire[202] and classified into normal (0-13) or borderline to abnormal (14-40) based on total difficulties scores in four domains (peer problems, conduct disorders, hyperactivity and emotional problems)[203]. Child's weight and height were measured by trained staffs using electronic Tanita scales (Tanita UK Ltd., Middlesex, UK) and a Leicester Height Measure Stadiometers (Seca Ltd., Birmingham, UK), respectively, and used to calculate BMI-for-age z scores based on the WHO growth references[204].

3.2.5.3 Modelling of physical activity intensities

The relative role of physical activity intensities can be examined in three ways. First, *isomovement substitution analysis* aims to explore the effect of the reallocation of movement from an intensity to other intensities or vice versa, with consideration of total volume of physical activity. For example, in such model, when fractions of total counts contributed by moderate and vigorous intensities and empirical total daily counts are simultaneously included, while fraction of total counts from sedentary behaviour was excluded because its value is 0 CPM and thus does not contribute to the movement volume, the resulting estimates for moderate and vigorous intensities represent the independent effects of the reallocation of movement from light intensity to moderate and vigorous intensities. Second, *compositional analysis*[205, 206] allows to examine all codependent-data simultaneously and models the combined relative effects of physical activity intensities by including

isometrically log-ratio transformed fractions of counts from all three light, moderate and vigorous intensities (excluding sedentary behaviour since its value is 0 CPM) and empirical total daily counts in the same model. The isometric log-ratio was calculated by dividing fractions of total counts by geometric mean and then taking the logs, for each physical activity intensity. Third, *isotemporal substitution analysis*[207] enables testing the relative role of physical activity intensities and also sedentary behaviour. In this model, when times spent in light, moderate and vigorous intensities are simultaneously included, without adjusting for total movement volume, the resulting estimates for light, moderate and vigorous intensities are interpreted as the independent effects of the reallocation of time spent from sedentary behaviour to all these three intensities.

3.2.5.4 Multiple imputation

Missing values were predicted using multiple imputation by chained equations with 50 imputed datasets. Such approach is fully described in **Section 3.1.5.4**.

3.2.5.5 Statistical software

All statistical analyses were performed using Stata 15.1 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

CHAPTER 4 LONGITUDINAL ASSOCIATIONS OF PREPUBERTAL CHILDHOOD TOTAL ENERGY AND MACRONUTRIENT INTAKES WITH PUBERTY TIMING

Publication

Cheng, TS., Sharp, SJ., Brage S., Emmett, PM., Forouhi, NG., Ong, KK. Longitudinal associations between prepubertal childhood total energy and macronutrient intakes and subsequent puberty timing in UK boys and girls. *European Journal of Nutrition*. https://doi.org/10.1007/s00394-021-02629-6.[208]

Contributions

I conceived and designed this study together with my supervisor (Prof. Ken Ong). I generated new ALSPAC study dietary intake variables specifically on protein quality, with advice from Dr. Pauline M. Emmett (University of Bristol), which could be used in this study and also other projects. I also applied for, received and processed ALSPAC study data on dietary intakes and puberty development, conducted statistical analyses, jointly interpreted the findings and completed writing for this chapter and the resulting manuscript.

4.1 Summary

Background

Early timing of puberty is increasingly associated with adverse health outcomes including T2D in men and women, possibly independent of adiposity, as reported in **Chapter 2**. These findings suggest the importance to identify potential modifiable factors for puberty timing. This chapter aimed to

examine the associations of prepubertal childhood macronutrient (carbohydrate, fat, protein) intakes with puberty timing in boys and girls.

Methods

In the ALSPAC study, macronutrient intakes at 3, 4, 7 (assessed by food frequency questionnaires) and 7.5 years (by 3-day food diaries) were summarised as the intakes at age 6 years using random intercepts linear regression models. Individual timings of puberty onset (G2 and B2 in boys and girls, respectively) and puberty progression (voice breaking and menarche) were estimated from annual parental and child reports at 8-17 years. Age at PHV was determined from repeated measures of height at 5-20 years using SITAR. The associations of TEI and macronutrient intakes with puberty timing were tested using linear regression models, adjusting for maternal and infant characteristics.

Results

Among 3811 boys, only higher TEI was associated with earlier voice breaking. Among 3919 girls, higher TEI, as well as higher protein intake (in *energy partition models*) and substitution of dietary protein for carbohydrate (in *nutrient density* and *residual models*) were associated with earlier ages at B2, PHV and menarche. These associations remained on additional adjustment for body fat percentage during adolescence.

Conclusions

These findings suggest that habitual intakes of total energy and protein especially in girls may potentially modify subsequent puberty timing.

4.2 Background

Puberty is the critical transitional process relating to sexual maturation from childhood to adulthood. Earlier timing of puberty is crucially related to a wide range of psychological and physiological diseases, as described in **Chapter 1**.

These associations may be independent of adiposity, as suggested by a large UK biobank study[33] and findings in **Chapter 2**, underlining the significance of identifying specific modifiable determinants of puberty timing. Given the negative association between BMI and puberty timing remarkably in girls[32, 209], it is possible that dietary intakes especially energy and macronutrients (i.e. carbohydrate, fat and protein) which largely regulate child growth may be correspondingly relevant to puberty timing[97].

The associations between dietary intakes in childhood and subsequent puberty timing have been investigated in different populations. However, previous prospective studies largely obtained a single measure of dietary intakes and puberty timing (mainly age at menarche in girls), which may be subject to measurement error. Also, they analysed intakes of total energy and macronutrients individually and reported differential associations of dietary intakes with puberty timing. Furthermore, there may be the existence of reverse causality in previous findings despite prospective study design, due to the assessments of dietary intakes and puberty timing during the typical periods between puberty onset and completion. Findings for energy and each macronutrient are detailed as follows:

4.2.1 Total energy intake

In the previous ALSPAC study in the UK (n=3298), higher TEI at age 10 years (assessed by 3-day food diaries) but not at 3 and 7 years (by FFQ) was associated with earlier (versus later than 12 years 8 months) onset of menarche in girls[99]. Similarly, a Canadian study (n=666) identified that higher TEI at age 10.7 years (by 3-day food diaries) was associated with early menarche by 11.4 years[98]. Although a recent meta-analysis[210] of two studies (i.e. Rogers et al.[99] and Petridou et al.[211]) showed association between higher TEI and early menarche, despite high heterogeneity, Petridou et al. recorded dietary intakes in previous year using FFQ and age at menarche at the same visit, and thus it was rather a cross-sectional study[211]. In contrast, a study in the US (n=679) found a positive association between TEI at age 10.6 years (by

FFQ) and continuous age at menarche after 4-year follow-up[100]. No association between TEI and puberty timing (age at menarche and/or breast development) was also reported by another large Canadian study (n=2299; 3-day food diaries at age 11.1 years; 17-month follow-up)[101] and small studies in Canada (n=109; 3-day food diaries at age 9-15 years; 6-year follow-up)[102] and the Netherlands (n=63 pubertal girls; 7-day food diaries at age 9.6 years; 3-year follow-up)[103].

4.2.2 Protein

The previous ALSPAC analysis found that higher total protein intakes at 3 and 7 years (not at 10 years) were associated with early menarche among girls[99]. A German study, DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study (n=112), reported that higher total protein intake at 5-6 years (not at 3-4 years) (assessed by 3-day weighed dietary records) was associated with earlier PHV, but not with age at voice breaking and menarche (measured until 13 years), in boys and girls, without sex stratification[105]. On the contrary, in an Australian study (n=92), positive associations of total protein intake at age 8 years (by 3-day food diaries) with age at breast development and menarche (estimated from reports at 13 and15 years) in girls was observed, while total protein intake was not associated with age at genital development in boys[104]. Other studies in the US[100, 106], Canada[101, 102] and the Netherlands[103] mostly showed null associations between total protein intake and age at menarche in girls.

4.2.3 Carbohydrate

An earlier study in the US (n=99) demonstrated that premenarcheal girls who consumed carbohydrate (assessed by 24-hour recall) at the highest quartile (0.28-0.76 kg) at 9-15 years had 7 months later age at menarche than those at the lowest quartile (0-0.25 kg) after one-year follow up[106]. This association was however mostly not seen in subsequent studies in the UK[99], the US[100], Canada[101, 102] and the Netherlands[103].

4.2.4 Fat

A German study (n=167) showed that higher total fat intake at age 8-15 years (assessed by 7 days food diaries) was associated with earlier onset of menarche after 2-year follow-up[107]. Nevertheless, most studies reported null findings[99-101, 103, 106].

4.3 Study aims

This study aimed to examine the prospective associations of habitual energy and macronutrient intakes during prepubertal childhood with timing of several puberty traits in boys and girls. Given the broader inconsistency in the existing evidence on the associations between macronutrient intakes and puberty timing, all three main macronutrient intakes were considered as potential determinants of puberty timing and were explored in an isocaloric diet. Next, whether any observed association was independent of adiposity during puberty was tested.

4.4 Methods

4.4.1 Study population

The present study included 7730 children (71.6%) (3811 boys and 3919 girls) with data on at least one puberty timing trait from the initial analytical sample in the ALSPAC study. For a full description of the ALSPAC study, see **Section 3.1**.

4.4.2 Total energy and macronutrient intakes

Children's dietary intakes were repeatedly assessed between 3 and 7.5 years, as detailed in **Section 3.1.2.1** and summarised as the intakes at 6 years each for total energy and macronutrients (carbohydrate, fat and protein) using random intercepts linear regression models[181], separately in boys and girls, as described in **Section 3.1.4.1**.

4.4.3 Timings of puberty traits

Children's pubertal development at 8-17 years were annually reported (**Section 3.1.2.3**) and used to estimate individual timings of several puberty traits (**Section 3.1.4.3**). In this study, the primary outcomes included ages at puberty onset, puberty progression and PHV, while secondary outcomes were ages at pubic hair growth, axillary hair growth and pubertal tempo.

4.4.4 Potential confounders

The general potential confounders were those maternal and infant characteristics associated with intakes of total energy and macronutrients, and puberty timing, which are detailed in **Section 3.1.5.2**. Baseline BMI was not included since childhood anthropometric measures were not collected at age 3-7 years but were only available from age 7.5 years in the whole cohort, which may rather act as a mediator of dietary intakes at 3-7.5 years. Additional covariates included in some analyses were TEI and macronutrients in substitution models and also age at assessment in age-specific models.

4.4.5 Adiposity during adolescence

Children's body fat percentage (%BF) were measured biennially from age 9 to 15 years (Pearson's correlation coefficients=0.90 to 0.95) using dual-energy X-ray absorptiometry (Lunar prodigy). These repeated measures were

combined at age 11 years using random intercepts linear regression models[181] in a similar estimation method to **Section 3.1.4.1**. At age 11 years, majority (n=4378, 75.2%) had started puberty, but most (n=6025, 93.7%) had not completed puberty. In this study, the %BF at 11 years was used as an estimate of adiposity during adolescence.

4.4.6 Statistical analyses

Differences between excluded (owing to missing puberty timing) and included children were compared using chi-squared test for categorical variables and t-test for continuous variables.

The associations of TEI and macronutrient intakes with puberty timing were investigated using multivariable linear regression models, separately in boys and girls, with adjustment for the general potential confounders. Additional adjustment for %BF at age 11 years as a potential mediator was performed.

In this study, the *energy partition model* was first employed to simultaneously explore the associations of all three macronutrients with puberty timing. Next, the *nutrient density model* was performed as the primary analysis to examine the associations between substitution of the macronutrient of interest (identified from the *energy partition model*) for another macronutrient in an isocaloric diet and puberty timing. Finally, the *residual model* was used as a sensitivity analysis to test the associations of isocaloric macronutrient substitution with puberty timing, with the consideration of potential underreporting of TEI. Detailed methods for these energy adjustment models can be found at **Section 3.1.5.3**.

A series of post-hoc analyses for primary puberty timing traits was conducted to inform public health implications. First, to identify possible critical window of interventions, observed associations were re-tested in primary models by ages at dietary assessment, and the differences or heterogeneity between findings across ages were assessed using the inconsistency index (I^2) (<50%,

50%–75%, and >75% indicate mild, moderate, and high heterogeneity, respectively) in fixed effect models. Second, to explore the potential early puberty risks of dietary intakes, the associations of intakes above the UK recommendations (estimated average requirement (EAR) for TEI at age 6 years: 1577 kcal for boys and 1483 kcal for girls[212] and dietary recommended value for protein at age 6 years: 19.7g per day for boys and girls[213]) with dichotomous puberty timing status (earlier and equal to/later than the population mean by sex) were evaluated using multivariable logistic regressions. Third, to determine whether observed associations between dietary intakes and puberty timing were linear, potential associations of dietary intakes with nonlinear puberty timing (categorised into tertiles; 1st tertile versus 2nd tertile as reference, and 2nd tertile versus 3rd tertile as reference) were explored using multivariable logistic regressions, and the resulting ORs were compared using Wald tests. Fourth, to identify specific protein quality or type related to puberty timing, associations of substitutions of protein from animal sources (i.e. red meat (both processed and unprocessed), white meat (poultry and fish), dairy and eggs) and plant-based sources for carbohydrate with puberty timing were examined using multivariable linear regressions, by including intakes from protein types and fat, and TEI in the same model.

Missing data on dietary intakes (n=204, 2.7%) and more missing data on covariates (n=17 to 1238, 0.21 to 16.0%; n=3401, 44.0% with at least one missing) were predicted using multiple imputation by chained equations as described in **Section 3.1.5.4**.

4.5 Results

4.5.1 Children's characteristics

This study analysed 7730 children, comprising 3811 boys and 3919 girls, with average follow-up period of 11.5±2.9 years. As shown in **Supplementary Table A-7**, compared to mothers of the excluded children (n=3059), those of the included children were more likely to be older, nulliparous, from higher socioeconomic groups, have higher education level, and less likely to smoke and have second-hand smoke exposure during pregnancy. Included children were more likely to be girls, breastfed for longer, and tended to have lower intakes of total energy, carbohydrate and fat at age 3-7 years than excluded children.

Table 4.1 summarises TEI and macronutrient intakes at age 6 years, puberty timing traits and %BF at age 11 years in boys and girls. Compared to boys, girls consumed lower total energy, relatively less energy from carbohydrate, and relatively more energy from protein, particularly from red and white meats but less from plant sources. Total energy and macronutrient intakes predicted at age 6 years were moderately to highly correlated with the respective intakes reported at other ages (Pearson's correlation coefficients=0.55 to 0.81) (**Supplementary Table A-8**). As would be expected, most pubertal traits occurred earlier in girls than boys, except that unexpectedly G2 in boys was reported earlier than B2 in girls (**Table 4.1**). Correlations between pubertal traits were stronger in girls than in boys (**Supplementary Table A-9**). Also, girls had higher %BF at 11 years than boys (**Table 4.1**).

Table 4.1 Total energy and macronutrient intakes at age 6 years, puberty timing and body fat percentage at age 11 years in boys and girls in the ALSPAC study

	Boys (n=3811)	**Girls (n=3919)**	**P value**
	mean±SD	mean±SD	
Dietary intakes at age 6 years			
Total energy intake, kcal	1654±160	1579±148	<0.001
Total carbohydrate intake, g	207.2±20.8	196.9±19.4	<0.001
Total fat intake, g	67.4±7.4	64.4±6.8	<0.001
Total protein intake, g	56.6±5.8	54.7±5.3	<0.001
from red meat	7.2±1.8	6.9±1.7	<0.001
from white meat	9.6±1.9	9.8±1.9	<0.001
from dairy and eggs	17.0±3.2	16.3±2.9	<0.001
from plant sources	22.8±2.6	21.6±2.5	<0.001
Percentage of energy from carbohydrate, %	50.1±1.7	49.9±1.5	<0.001
Percentage of energy from fat, %	36.5±1.6	36.6±1.4	0.055
Percentage of energy from protein, %	13.8±1.0	14.0±0.9	<0.001
from red meat	1.7±0.5	1.8±0.4	0.035
from white meat	2.4±0.5	2.5±0.5	<0.001
from dairy and eggs	4.2±0.7	4.2±0.7	0.256
from plant sources	5.54±0.5	5.50±0.4	0.001
Puberty timing, years			
Age at genital/breast development	8.7±1.6	10.0±1.6	<0.001
Age at voice breaking/menarche	13.6±1.9	12.7±1.2	<0.001
Age at peak height velocity	13.6±0.9	11.7±0.8	<0.001
Age at pubic hair growth	11.0±1.7	10.8±1.5	<0.001
Age at axillary hair growth	13.7±1.7	12.2±1.6	<0.001
Pubertal tempo	4.8±2.3	2.7±1.3	<0.001
Body fat percentage at age 11 years, %	21.0±7.8	27.3±7.4	<0.001

4.5.2 Dietary intakes and puberty timing

Table 4.2 and **Figure 4.1** show the associations of prepubertal childhood total energy and macronutrient intakes with subsequent puberty timing in boys and girls, with adjustment for maternal and infant characteristics. In boys, higher TEI was associated with earlier voice breaking (β=-0.29 years per 500kcal). In girls, higher TEI was associated with earlier B2 (β=-0.28), PHV (β=-0.15) and menarche (β=-0.14). For secondary outcomes, higher TEI was associated with earlier PH2 in both sexes (boys: β=-0.26; girls: β=-0.19) (**Supplementary Table A-10**).

In *energy partition models*, higher protein intake was associated with earlier B2 (β=-2.52 years per 500kcal), PHV (β=-1.69) and menarche (β=-1.40) in girls (**Table 4.2**). Higher protein intake was also associated with earlier timing of secondary puberty traits in boys (PH2: β=-2.94) and girls (PH2: β=-2.49; axillary hair growth: β=-2.75) (**Supplementary Table A-10**). Total carbohydrate and fat intakes were not associated with most puberty timing traits, except for higher total fat intake and later axillary hair growth in girls (β=1.15) (**Table 4.2** and **Supplementary Table A-10**). Therefore, for subsequent analyses, we focused on the substitution of protein for carbohydrate intake.

In *nutrient density models*, the substitution of dietary protein for carbohydrate was associated with earlier B2 (β = -0.88 years per 10%) and PHV (β=-0.48), and a tendency to earlier menarche (β=-0.41) in girls, but not with any primary pubertal timing trait in boys (**Table 4.2** and **Figure 4.1**). Also, the substitution of dietary protein for carbohydrate was associated with earlier PH2 in both sexes (boys: β=-0.88; girls: β=-0.65), and earlier axillary hair growth in girls (β=-0.69) (**Supplementary Table A-10**). These findings were consistent in *residual models* (**Table 4.2** and **Supplementary Table A-10**).

In the analyses by age at dietary assessment (**Supplementary Table A-11**), moderate heterogeneity was found among the associations between TEI at

different ages and puberty timing especially in girls, which was more apparent at 7.5 years old (by 3-day food diaries) but not at 3-7 years (by FFQ). Associations between protein intake and puberty timing in girls were consistent across ages (3-7.5 years old), with low heterogeneity.

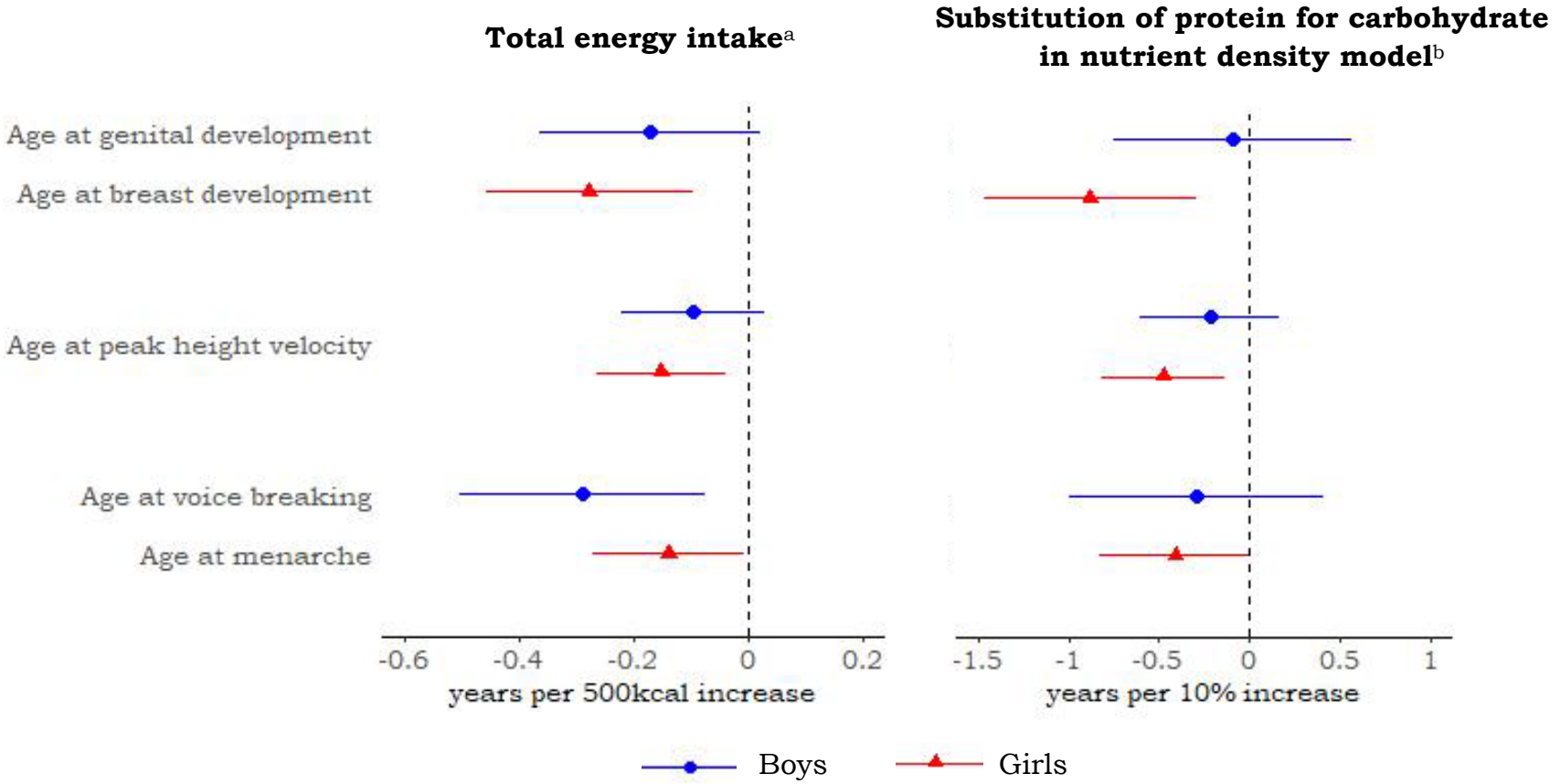
Also, with additional adjustment for body fat percentage at 9 to 15 years, the observed associations of childhood total energy and protein intakes with subsequent puberty timing traits were not attenuated (**Supplementary Table A-12**).

Table 4.2 Adjusted associations of total energy and macronutrient intakes at age 6 years with puberty timing

Dietary intakes	Age at genital/breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value
Boys	n=2619		n=2215		n=3017	
Total energy intake (per 500kcal increase)[a]	-0.17 (-0.36, 0.02)	0.085	-0.10 (-0.22, 0.03)	0.129	-0.29 (-0.50, -0.07)	0.009
Energy partition model (per 500kcal increase)[a]						
Carbohydrates	-0.42 (-1.02, 0.18)	0.173	-0.01 (-0.37, 0.36)	0.981	-0.40 (-1.05, 0.25)	0.231
Fat	0.32 (-0.47, 1.10)	0.428	-0.03 (-0.49, 0.44)	0.910	0.30 (-0.56, 1.16)	0.490
Protein	-0.74 (-2.80, 1.32)	0.479	-0.69 (-1.89, 0.52)	0.265	-1.73 (-3.96, 0.49)	0.127
Substitution of protein for carbohydrate						
Nutrient density model (per 10% increase)[b]	-0.09 (-0.75, 0.56)	0.781	-0.22 (-0.60, 0.16)	0.261	-0.29 (-0.99, 0.41)	0.418
Residual model (per 50g increase)[c]	-0.12 (-1.07, 0.83)	0.809	-0.27 (-0.82, 0.28)	0.342	-0.47 (-1.49, 0.54)	0.363
Girls	n=3204		n=2509		n=3414	
Total energy intake (per 500kcal increase)[a]	-0.28 (-0.46, -0.10)	0.003	-0.15 (-0.26, -0.04)	0.008	-0.14 (-0.27, -0.01)	0.040
Energy partition model (per 500kcal increase)[a]						
Carbohydrates	0.17 (-0.38, 0.73)	0.543	-0.21 (-0.53, 0.12)	0.208	-0.05 (-0.45, 0.36)	0.820
Fat	-0.19 (-0.94, 0.57)	0.627	0.39 (-0.05, 0.82)	0.082	0.12 (-0.42, 0.65)	0.673
Protein	-2.52 (-4.45, -0.59)	0.010	-1.69 (-2.81, -0.57)	0.003	-1.40 (-2.79, -0.02)	0.047
Substitution of protein for carbohydrate						
Nutrient density model (per 10% increase)[b]	-0.88 (-1.47, -0.29)	0.003	-0.48 (-0.82, -0.13)	0.007	-0.41 (-0.83, 0.01)	0.058
Residual model (per 50g increase)[c]	-1.12 (-2.01, -0.24)	0.013	-0.59 (-1.11, -0.08)	0.024	-0.56 (-1.19, 0.08)	0.084

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration)
[b]Additionally adjusted for energy from fat (%), total energy intake (kcal)
[c]Additionally adjusted for total fat intake (g), total energy intake (kcal)

Figure 4.1 Adjusted associations of total energy and protein intakes at age 6 years with primary puberty timing traits



[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration)

[b]Additionally adjusted for energy from fat (%), total energy intake (kcal)

4.5.3 Categorical associations

In this study, over two thirds of children (boys: 68.3%; girls: 73.9%) were estimated to have higher TEI than the UK recommendations at age 6 years. Among girls, those with TEI above EAR tended to have higher risks for earlier (versus later) B2 (OR=1.27, 95% CI 1.07 to 1.50) and menarche (OR=1.16, 95% CI 0.98 to 1.36) than those with TEI below EAR (**Supplementary Table A-13**). Also, all children had higher total protein intake than the UK recommendations at age 6 years. With each 5% or 10g increase in dietary protein substituting for carbohydrate intake, girls had ~30% higher risk for earlier (versus later) puberty timing (**Supplementary Table A-13**).

When timing of each puberty trait was analysed as tertiles (**Supplementary Table A-14**), similar risks for early (versus average) and average (versus late) puberty timing associated with dietary intakes were largely observed, despite wide confidence intervals. However, in boys, higher TEI was associated with lower risk of early (versus average) G2 (OR=0.41) but higher risk of early (versus late) G2 (OR=3.59) (Wald test, P value=0.009).

4.5.4 Protein quality

For the analyses of protein quality (**Supplementary Table A-15**), in *energy partition models*, higher plant-based protein was associated with earlier voice breaking in boys, and higher intakes of both animal and plant-based proteins were associated with earlier B2, PHV and menarche in girls. Similar associations with earlier puberty timing were found for the substitution of protein quality for carbohydrate in *nutrient density* and *residual models*.

4.6 Discussions

4.6.1 Summary of findings

The present long follow-up British study from 3 to 17 years old demonstrated that higher prepubertal childhood habitual intakes of total energy and protein at the expense of carbohydrate were associated with earlier timings of several puberty traits (i.e. puberty onset and progression, and an objective measure of puberty timing), particularly in girls. The findings for protein intake were consistently obtained using different energy adjustment methods (i.e. *energy partition, nutrient density* and *residual models*). Also, all observed associations remained robust after additional adjustment for adiposity during adolescence.

4.6.2 Comparisons with previous evidence

Our findings of higher TEI throughout prepuberty and subsequent earlier puberty timing corroborate previous ALSPAC analysis[99] and a Canadian study[98] which reported the associations between higher TEI at relatively late ages (when puberty have likely started in many, if not most, girls) and early onset of menarche. We further added that the inverse association between TEI and puberty timing was seen in both girls and boys, and these associations were not mediated by adiposity during puberty. Conversely, other prospective studies reported positive association between TEI and age at menarche[100] or null findings[101, 102] in girls.

The present observations of higher dietary protein intake and earlier puberty are broadly consistent with an earlier systematic review in 2013 which concluded that there was 'probable evidence' on the association between animal protein intakes and earlier puberty in boys and girls[214] based on three prospective studies in the US[215], Germany[105, 216] and UK[99]. Berkey et al. found that higher protein intakes specifically animal protein (assessed by dietary history interviews 6-monthly from birth to 11 years) were associated with

earlier menarche and PHV among 67 girls in the US[215]. The DONALD studies in Germany reported that higher animal protein intake (by 3-day food diaries) at age 5-6 years but not earlier 3 time points (12 months, 18-24 months, 3-4 years) (n=112), or 1-2 year prior to puberty onset (n=109), was associated with earlier menarche/voice breaking and PHV in both sexes, without sex stratification[105, 216]. The earlier ALSPAC study observed that higher intakes of total and animal protein at 3 and 7 years were associated with higher risk of early menarche[99]. Two of these studies also reported opposite associations between higher plant-based protein intakes and later puberty timing[105, 215]. Nonetheless, the current analysis found same direction of associations with earlier puberty for both animal and plant-based protein intakes in girls. Unlike previous studies, we considered TEI and all macronutrients together in the same analyses, so that findings for protein intakes are independent of compensatory changes in the other macronutrients in an isocaloric diet. Moreover, the present findings were adjusted for a wider range of covariates. It is however difficult to determine whether our analytical approach might be susceptible to over-adjustment but our adjusted findings for TEI and total protein intake remained similar to earlier ALSPAC analysis with adjustment for fewer covariates[99]. In fact, understanding the role of specific protein types on puberty timing require experimental studies. On the other hand, some earlier prospective studies which reported null association between any macronutrient intake and age at menarche were not evaluated in the systematic review[214], probably because they assessed diets at relatively late ages[100, 101] or even during puberty[103]. Surprisingly, a recent Australian study identified positive association between protein intake and puberty timing in girls, which was seen only using nutritional geometry analysis that considers multiple nutrient interactions but not in linear regression models; however their sample size was modest (50 boys; 92 girls)[104].

4.6.3 Potential mechanisms

Findings of the inverse associations of total energy and protein intakes with puberty timing in this study can be explained by several possible mechanisms. The robustness of findings to additional adjustment for adiposity during puberty possibly suggest adiposity-independent effects of the intakes on pubertal onset and/or progression. Higher TEI may enhance the secretion and bioactivities of sex hormones, probably through leptin and insulin, consequently inducing earlier pubertal development[217]. Higher consumption of protein may provide more readily available amino acids which promote the production of enzymes and hormones including the growth hormone/insulin-like growth factor-1 pathway that are involved in pubertal development[218]. Alternatively, high protein intake can lead to hyperinsulinemia and insulin resistance[219] which subsequently increases bioavailability of sex hormones[32] particularly in girls given the sexual dimorphism in protein metabolism[220]. Such pathway may not vary by protein quality since no difference in glucose homeostasis and fasting insulin was observed between plant and animal-based proteins in most intervention studies[221]. This explains our same findings for plant and animal-based proteins which were nearly equally consumed in this study and highlights the importance of both total protein as well as all protein types in regulating puberty development.

4.6.4 Strengths and limitations

The present study extended previous studies including earlier ALSPAC analysis[99], by synthesising repeated measures of prepubertal childhood macronutrient intakes from two dietary assessments (i.e. FFQ at age 3, 4 and 7 years and 3-day food diaries at 7.5 years) and by including more complete data on several pubertal traits into young adulthood. Acknowledging the strengths and weaknesses of FFQ and 3-day food diaries as discussed in **Section 1.5.2.1**, improvement of the accuracy of estimating individual usual intakes during child growth were attempted with the use of random intercepts linear regressions[181] that consider the expected age-related increase in

intakes as well as individual overestimation (by FFQ) and underestimation (by 3-day food diaries) of dietary assessments. Such approach concomitantly avoids differential findings by dietary assessments and ages owing to study design. Indeed, our secondary analyses showed findings for protein intakes were not heterogeneous by ages, suggesting a critical window of effect is unlikely. Although associations for TEI at 7.5 years was stronger than at 3-7 years, it is rather likely due to higher validity of TEI estimation by 3-day food diary than by FFQ.

Other strengths related to study design and analytical approach are present in this study. While previous studies mostly considered individual macronutrient intakes and dichotomous menarche status all within the typical timings of puberty development, we analysed isocaloric substitution of macronutrients throughout childhood prior to puberty onset and estimated continuous timings of several puberty traits including an objective measure (i.e. PHV) in both sexes. Although macronutrient intakes were predicted at age 6 years, our findings represent effects of intakes at ages 3 to 7.5 years which were the periods for dietary assessments. Similarly, predicted adiposity at 11 years is generalizable to the age range 9 to 15 years. Moreover, multiple imputation was used to predict missing data mainly on covariates to allow comparisons of findings between models, unlike previous findings which may be biased by the complete case analyses in adjustment models.

This study contains several limitations. First, there were some differences in maternal and infant characteristics between included and excluded children, but the differences were modest and these factors have been adjusted for in analyses. Next, individual implausible values for TEI were removed only by visual inspection of histograms because body weight was not measured in the whole cohort at 3, 4 and 7 years but only at 7.5 years. However, sensitivity analyses using *residual method* have been conducted to account for potential underreporting of dietary intakes. Also, there were relatively weak correlations between intakes by FFQ and 3-day food diaries in the present study, as observed elsewhere[91], possibly partly due to differences in ages of assessment.

Nevertheless, predicted intakes at age 6 years were moderately to highly correlated with all reported intakes, and may be considered as the balanced measures between the potential overreporting and underreporting of dietary intakes from FFQ and 3-day food diaries, respectively. While the present study focused mainly on TEI, total protein and protein quality, and findings for total fat and carbohydrate intakes were almost null, this does not exclude the possibility of the role of other macronutrient types (i.e. carbohydrate and fat) in puberty timing, which warrants future research. Further, Tanner stages of puberty were parent- or self-reported, which were generally shown to have moderate agreement with the gold standard – physical exam by health professionals[222]. Timings of several puberty traits in 20-30% of children could also not be estimated due to frequent inconsistencies in the repeated parent- or self-reports of pubertal development even after extensive data cleaning. In particular, estimated timing of G2 in boys was unusually early, which led to an unusually long duration of puberty, and thus these data should be cautiously interpreted. Nonetheless, findings using subjective and objective (i.e. PHV) measures of puberty timing were consistent. Finally, despite adjustment for several potential confounders, findings may be still confounded by unmeasured factors such as BMI at 3-7 years (which was available only from 7.5 years) and physical activity (which was measured only during adolescence).

4.6.5 Conclusions

With the integration of repeated measures over long period, this study showed associations of higher prepubertal childhood total energy and protein intakes, regardless of quality, with earlier pubertal development. These findings were independent of adiposity during adolescence and more evidently in girls than in boys. This suggests that dietary intakes, specifically current high levels of TEI and protein intake among children worldwide[223, 224], may be responsible for the global secular trends towards earlier puberty timing[43, 45, 46, 48].

CHAPTER 5 PREPUBERTAL DIETARY FAT QUALITY AND PLASMA PHOSPHOLIPID FATTY ACIDS IN RELATION TO PUBERTY TIMING

Publication

Cheng, TS., Day, FR., Perry, JRB., Luan J., Langenberg, C., Forouhi, NG., Wareham, NJ., Ong, KK. Prepubertal dietary and plasma phospholipid fatty acids related to puberty timing: Longitudinal cohort and Mendelian Randomization analyses. Nutrients, 13(6), 1868.[225]

Contributions

I conceived and designed this study together with my supervisors (Prof. Ken Ong, Prof Nita Forouhi, Prof Nicholas Wareham). I also processed data on dietary intakes and puberty development, conducted statistical analyses, jointly interpreted the findings and completed writing for this chapter and the resulting manuscript.

5.1 Summary

Background

Inconsistent associations have been reported between dietary fat quality (i.e. PUFAs, MUFAs, SFAs) and puberty timing. This chapter aimed to investigate prospective associations of prepubertal childhood dietary and plasma phospholipid FAs with several puberty timing traits in boys and girls.

Methods

In the ALSPAC study, prepubertal dietary fat intakes from FFQ or 3-day food diaries at 3-7.5 years were summarised using random intercepts linear regressions. Individual plasma phospholipid FA concentrations at 7.5 years were measured using gas chromatography. Timings of G2 or B2 and voice

breaking or menarche from annual parental and child reports at 8-17 years, and age at PHV from repeated height measures at 5-20 years were estimated. Associations between fat quality and puberty timing were tested using linear regressions with adjustment for maternal and infant characteristics, and MR analyses.

Results

Dietary substitution of PUFAs for SFAs was associated with earlier B2, PHV and menarche in girls (n=3919). Similarly, higher plasma concentrations of dihomo-γ-linolenic acid (20:3n6) and palmitoleic acid (16:1n7) were associated with earlier timings of puberty traits in girls. These associations were not seen in boys (n=3811). In MR models, higher genetically predicted circulating dihomo-γ-linolenic acid was associated with earlier menarche in girls.

Conclusions

Using data and methods triangulation approaches (i.e repeated measures of dietary fat quality, objectively measured FA concentrations, genetic causal modelling), these findings suggest that dietary and endogenous metabolic pathways that increase circulating dihomo-γ-linolenic acid, an intermediate metabolite of n-6 PUFAs, may promote earlier puberty development in girls.

5.2 Background

Early timing of puberty is of great relevance to extensive health issues including cardiometabolic diseases (**Chapter 1**). Emerging studies have therefore explored different modifiable factors that possibly influence puberty timing, including adiposity[32] and dietary intakes[97]. In particular, **Chapter 4** indicated that total energy and protein intakes were negatively associated with puberty timing remarkably in girls, independent of adiposity during puberty. In addition to this, dietary fat quality, namely PUFAs, MUFAs and SFAs, which is well-known as a key dietary determinant of cardiometabolic health[226], may

be related to puberty timing[210]. Further, individual FAs, either obtained from dietary intakes or endogenously synthesised especially at adipose tissue, have distinct biochemical properties and hence may have very different effects on physiological and metabolic processes[227], probably including the progression of pubertal maturation[210].

Studies that examined the prospective associations between dietary fat quality and puberty timing have been scarce and limited to girls[97]. Previous findings were also mixed[97] and some were seemingly in contradiction to the general recognition of PUFAs as a healthy type of dietary fat[227]. Furthermore, previous studies typically recorded a single parent- or self-reported measure of dietary intakes and puberty timing, and did not include objective measures of fat quality including individual FAs or puberty timing, which are more robust to measurement errors due to recall bias. Other limitations in previous studies include the lack of isocaloric macronutrient substitution analysis and the risk of reverse causality due to short follow-up periods, with dietary assessment at ages likely after puberty onset had occurred. Detailed findings for fat quality are elaborated as below:

5.2.1 Saturated fatty acids

Three prospective observational studies in the UK (ALSPAC; n=3298 premenarcheal girls; FFQ at age 3 and 7 years and 3-day food diaries at 10 years; follow-up at age 12.89±0.23 years)[99], the Netherlands (n=63 pubertal girls; 7-day food diaries at age 9.6 years; 3-year follow-up)[103] and Canada (n=589 premenarcheal girls; FFQ at age 9.7 (6.2-13.6) years; 2.4 years (13 days to 3.0 years) follow-up)[120] have consistently reported null associations between dietary SFAs intake and categorical age at onset of menarche.

5.2.2 Polyunsaturated fatty acids

A previous ALSPAC paper added that higher PUFA intake was associated with early age at menarche[99], but this was not seen in other studies in the Netherlands[103] and Canada[120]. More specifically, a study in the US (n=194) found higher dietary n-3 PUFAs intake but not linoleic acid (18:2n6) which is the shortest chain n-6 PUFAs (estimated from FFQ at 10±0.75 years) was associated with early menarche by age 12.5 years[228].

5.2.3 Monounsaturated fatty acids

A Canadian study showed that girls with higher quartiles of dietary MUFAs intake tended to have lower risk for early menarche, but the linear trend did not reach statistical significance[120]. A larger Canadian study (n=666) observed that MUFAs intake at age 10.7 years (by 3-day food diaries) was higher among girls who had not yet experienced menarche, compared to peers with early menarche by 11.4 years[98]. Further, a study in the US reported association between lower dietary oleic acid (18:1n9) and early menarche[228].

5.2.4 Replacement within fat quality

Two randomised controlled trials, i) Dietary Intervention Study in Children in the US (286 girls, 354 boys), that promoted lower intakes of SFA (<8% of total energy intake) and PUFA (<9%) and higher intake of MUFA (>11% within 28% energy contributed by total fat intake) at age 8-10 years[229, 230], and ii) the Special Turku Coronary Risk Factor Intervention Project in Finland (n=193-1062), that provided dietary counselling to maintain a ratio of 1:2 for SFA and MUFA or PUFA intakes since age 7 months[231, 232] observed similar timings of genital development in boys[229, 230], and similar timings of breast development and menarche in girls[229, 231, 232] between intervention and control groups, after 7- and 14-year follow-up, respectively.

5.3 Study aims

The aim of the present study was to examine the longitudinal associations between fat quality during prepubertal childhood and puberty timing traits in boys and girls. Firstly, more comprehensive data on prepubertal dietary intakes and puberty timing were analysed to confirm the previous ALSPAC finding of higher PUFA intake and early menarche in girls[99]. Next, we explored associations with puberty timing for objectively measured plasma phospholipid FA concentrations, specifically n-3 and n-6 PUFAs and their metabolites, and the two most abundant MUFAs in adipose tissue (i.e. palmitoleic acid (16:1n7) and oleic acid (18:1n9))[233, 234]. Finally, likely causation in any observed associations between specific FAs and puberty timing was evaluated using MR analyses, which take advantage of the naturally randomized allocation of allelic variation in genes affecting specific exposures[235, 236].

5.4 Methods

5.4.1 Study population

5.4.1.1 The ALSPAC cohort

The present study included 7526 children (69.8%) (3654 boys and 3872 girls) with data on at least one puberty timing trait from the initial analytical sample in the ALSPAC study. A full description of the ALSPAC study can be found at **Section 3.1**.

5.4.1.2 The EPIC–InterAct cohort

The European Prospective Investigation into Cancer and Nutrition Study (EPIC)-InterAct study[237] is a nested case-cohort study within eight of the

participating countries of the EPIC cohort study: France, Italy, Spain, UK, the Netherlands, Germany, Sweden and Denmark. The initial sample of EPIC-InterAct comprised 12,403 T2D cases and a randomly selected subcohort of 16,835 individuals with baseline plasma samples, who were part of the recruited 340,234 persons with 3.99 million person-years of follow-up (1991-2007) in the eight countries of the EPIC cohort. After further excluding those with prevalent diabetes (n=548) and uncertain diabetes status (n=133), the subcohort retained 16,154 individuals, of whom, 778 were the overlap group between the case-group and the subcohort as they had incident T2D during follow-up. Of the remaining 27,779 participants, another 483 were excluded due to missing data on fatty acid, giving 27,296 adults (12,132 T2D cases and 15,919 subcohort participants including 755 incident T2D cases) in the current analytical sample.

Written informed consent was obtained from all participants. The EPIC study was approved by the local ethics committees in the participating countries and the Internal Review Board of the International Agency for Research on Cancer. The EU FP6 programme provided support for the InterAct project (grant number LSHM_CT_2006_037197).

5.4.2 Dietary fat quality

In the ALSPAC cohort, repeated dietary intakes among children were ascertained from 3 to 7.5 years (**Section 3.1.2.1**) and were combined as the predicted intakes at 6 years for total energy and macronutrients (PUFAs, MUFAs, SFAs, carbohydrate, and protein), separately in boys and girls, using random intercepts linear regression models[181] (**Section 3.1.4.1**). The predicted dietary fat quality intake was moderately to highly correlated with reported intakes at each timepoint between 3 and 7.5 years (Pearson's correlation coefficients, r=0.64 to 0.78).

Given that seafood was the predominant source of n-3 PUFA in the UK, dietary n-3 PUFA, eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3)

were further calculated from the reported intakes of different fish groups (i.e. shellfish, white fish in breadcrumbs or batter, white fish without coating, tuna and other fish) listed in the FFQs. The fatty acid compositions and portion sizes were based on the profiles of typical species and typical consumption patterns in the UK[238], respectively. The estimated dietary intakes of n-3 PUFAs from fish sources at ages 3 to 7 years were similarly summarised using random intercepts linear regression models[181] (**Section 3.1.4.1**) and highly correlated with the predicted intakes at 6 years (r=0.71 to 0.85).

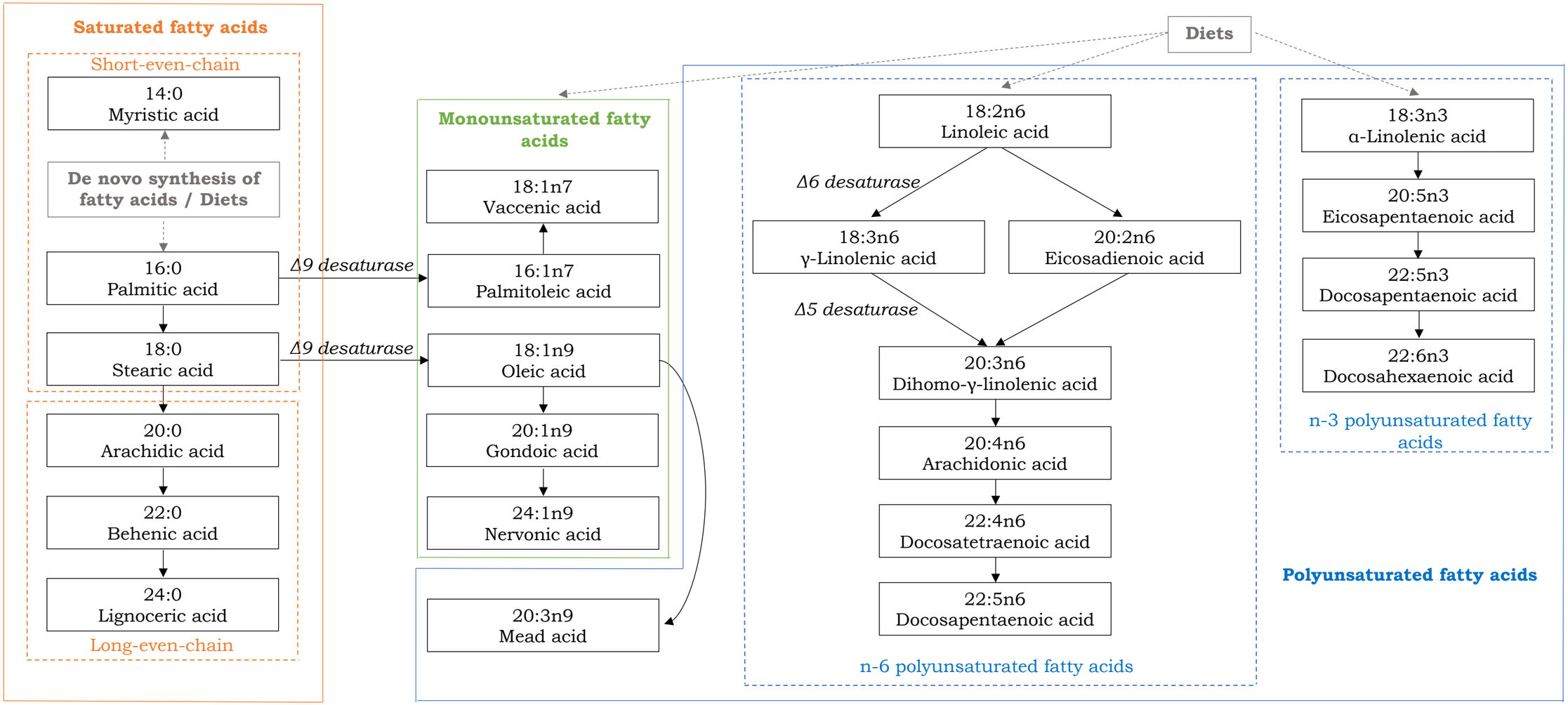
5.4.3 Individual plasma phospholipid fatty acids

In the ALSPAC cohort, individual plasma phospholipid FA concentrations were quantified from blood samples at age 7.5 years (**Section 3.1.2.2**) and standardised as age-specific percentage of total plasma phospholipid fatty acids (μg%) (**Section 3.1.4.2**). The identified 23 FAs comprised 12 PUFAs (including four long-chain n-3 PUFAs, seven n-6 PUFAs), five MUFAs (including palmitoleic acid (16:1n7) and oleic acid (18:1n9)) and six SFAs (three short-even-chain, three long-even-chain), as illustrated in **Figure 5.1**. Using the profiled plasma phospholipid FA concentrations, several product-to-precursor ratios of FAs were further calculated to estimate a) biological processes: i) activity of Δ6 (γ-linolenic acid (18:3n6) / linoleic acid (18:2n6)) and Δ5 (arachidonic acid (20:4n6) / dihomo-γ-linolenic acid (20:3n6)) desaturase enzymes, ii) the conversion of linoleic acid to dihomo-γ-linolenic acid (20:3n6 / 18:2n6) and iii) stearoyl-CoA desaturase-1 (palmitoleic acid (16:1n7) / palmitic acid (16:0) and oleic acid (18:1n9) / stearic acid (18:0)), and b) the balance between: i) two major PUFA subclasses (total n-6 PUFAs / total n-3 PUFAs) and ii) MUFAs and SFAs (total MUFAs / total SFAs).

In the EPIC-InterAct cohort, plasma samples at baseline were stored at -196°C (-150°C in Denmark). Plasma phospholipid FAs were profiled at the Medical Research Council Human Nutrition Research, Cambridge, UK, through solid phase extraction, hydrolysis and methylation to convert phospholipid FAs into more volatile fatty acid methyl esters which were subsequently separated by

gas chromatography (J&W HP-88, 30 meter length, 0.25 millimeter internal diameter [Agilent Technologies, CA, USA]) equipped with flame ionization detection (7890N GC [Agilent Technologies])[239]. Samples from T2D cases and subcohort participants were processed in a random manner by center and laboratory staff who were blinded to any participant characteristics with the use of anonymized aliquots. All FAs were identified by their retention times compared with those of commercial standards and their concentrations were expressed as percent of total phospholipid fatty acids (mol%).

Figure 5.1 Biosynthesis pathways of individual fatty acids profiled in the ALSPAC study



5.4.4 Timings of puberty traits

In the ALSPAC cohort, children's annual reports of pubertal development at 8-17 years (**Section 3.1.2.3**) were combined to determine individual timings of three puberty traits (i.e. ages at puberty onset, puberty progression and PHV) (**Section 3.1.4.3**).

5.4.5 Potential confounders

The basic potential confounders were those maternal and infant characteristics associated with dietary and plasma phospholipid FAs, and puberty timing (see **Section 3.1.5.2**). Additional covariates were considered in certain analyses, including TEI, macronutrients, food sources or BMI at age 7.5 years (since BMI was not available at 3-7 years).

5.4.6 Statistical analyses

5.4.6.1 Phenotypic analyses

In the ALSPAC cohort, differences between the excluded (due to missing data on all three puberty timing traits) and included children were compared using chi-squared test for categorical variables and t-test for continuous variables.

The associations between dietary fat quality and puberty timing were tested using multivariable linear regression models, with adjustment for the basic potential confounders, separately in boys and girls. The substitution of dietary PUFAs for SFAs in an isocaloric diet was modelled using the *nutrient density model* which simultaneously includes percentages of energy contributed by PUFAs, MUFAs, carbohydrate and protein, and TEI. The resulting estimate for dietary PUFAs is interpreted as the effect of each 5% increase intake of PUFAs as intake of SFAs (the only remaining macronutrient) concurrently decreases by 5%, while other macronutrients and total energy intakes are

kept constant. The substitution model was re-tested using the *residual method* as a sensitivity analysis that considers potential underreporting of TEI. The *nutrient density* and *residual models* were repeated to examine the substitution of dietary MUFAs for SFAs as supplementary analyses. For a full description of these energy adjustment models, refer to **Section 3.1.5.3**.

The associations of total and individual plasma phospholipid concentrations of n-6 PUFAs and MUFAs of interest (i.e. palmitoleic acid and oleic acid) with puberty timing were examined using multivariable linear regression models, adjusted for maternal and infant characteristics, and TEI at 6 years (synthesised from habitual energy intakes at 3-7.5 years). This basic model was further adjusted for i) total intakes in grams at 6 years (from intakes at 3-7.5 years) of foods that are: a) unlikely major sources of PUFAs (i.e. red meat, chicken, fruits and vegetables, dairy and eggs, and sugar confectionery), and then also b) possible dietary sources of PUFAs (fish, and cereals and nuts)[240]; or ii) BMI at 7.5 years to take into account potential confounding and/or mediating effects of adiposity. The basic model was also repeated as supplementary analyses for i) self-reported dietary and objectively measured n-3 PUFAs for comparisons of findings, ii) remaining MUFAs (vaccenic acid (18:1n7) which is a precursor of trans-palmitoleic acid to explore whether the effect of palmitoleic acid (16:1n7) was due to cis-palmitoleic acid or trans-palmitoleic acid, erucic acid (20:1n9), nervonic acid (24:1n9)), iii) two SFA categories, and iv) the FA ratios described above.

Missing data on dietary intakes (n=183 to 217, 2.4 to 4.2%), plasma phospholipid FAs (n=3425, 45.5%) and covariates (n=16 to 1205, 0.2 to 16.0%; n=2484, 33.0% with at least one missing) were predicted using multiple imputation by chained equations as described in **Section 3.1.5.4**.

5.4.6.2 Mendelian Randomization analyses

The likely causal nature of the observed associations between individual plasma phospholipid FAs and puberty timing was tested using MR analyses.

First, in the EPIC-InterAct cohort, genetic instruments for plasma phospholipid FAs of interest were separately identified from genome-wide association studies (GWAS) on the FAs (conducted by Dr. Jian'an Luan), using generalized linear mixed models adjusted for age, sex and population structure by BOLT-LMM[241], and independent signals were obtained using genome-wide complex trait analysis. Next, valid genetic instruments were selected as those variants with minor allele frequency ≥1%, P value <5.0 ×10-8 for association with the target FA, and weaker or null associations with concentrations of other FAs as well as of high- and low-density lipoproteins in the UK Biobank[242, 243]. For puberty timing outcome, the published GWAS summary statistics for age at menarche on up to 329,345 women of European ancestry from the ReproGen consortium (N=179,117), 23andMe (n=76,831) and UK Biobank (n=73,397)[60] was used.

All MR analyses were conducted using the "TwoSampleMR" package[244] in R software. These included the standard inverse variance-weighted (IVW) method which assumes all genetic variants are valid instrumental variables, and sensitivity analyses to address possible pleiotropy and heterogeneity between variants: i) MR-Egger method[245] that assumes a plurality of genetic variants are valid instrumental variables, ii) weighted median method and iii) penalized weighted median method[246], both assume a majority of genetic variants are valid instrumental variables[247].

5.5 Results

5.5.1 ALSPAC children's characteristics

This study analysed 7526 children (3654 boys and 3872 girls) in ALSPAC, with average follow-up period of 11.5±2.9 years. Compared to mothers of the excluded children (n=3263), those of the included children were more likely to be older, nulliparous, from higher socioeconomic groups, have higher

education level, and less likely to smoke or have second-hand smoke exposure during pregnancy (**Supplementary Table A-16**). Included children were also more likely to be girls, breastfed for longer duration, and tended to have modestly lower intakes of total energy and macronutrients at age 6 years than excluded children.

Table 5.1 summarises childhood macronutrient intakes, plasma phospholipid FAs and puberty timing traits in boys and girls. Compared to boys, girls had lower TEI, relatively less energy from carbohydrate, marginally more energy from protein and MUFAs. In plasma samples, girls than boys had modestly higher total FAs and concentrations of α-linolenic acid, docosahexaenoic acid, palmitoleic acid and oleic acid, but modestly lower concentrations of total and most individual n-6 PUFAs and docosapentaenoic acid. Also, PHV and puberty progression occurred earlier in girls than boys, whereas puberty onset was reported earlier in boys than girls.

Supplementary Table A-17 shows the correlations between dietary and plasma phospholipid PUFAs. In both boys and girls, total dietary PUFA intake was weakly correlated with concentrations of total n-6 PUFAs, linoleic acid and eicosadienoic acid (r=0.11 to 0.22). Dietary n-3 PUFA was modestly correlated with concentrations of total n-3 PUFAs and docosahexaenoic acid (r=0.22 to 0.32). Also, there were weak correlations between dietary intakes and plasma concentrations of the specific n-3 PUFAs: eicosapentaenoic acid (r=0.13 and 0.12, in boys and girls, respectively) and docosahexaenoic acid (r=0.28 and 0.32).

Supplementary Table A-18 shows the correlations between plasma phospholipid PUFAs and major MUFAs. Total n-6 PUFAs was strongly positively correlated with linoleic acid (r=0.92), and moderately negatively correlated with total and major MUFAs (r=-0.60 to -0.68). Total n-3 PUFAs was strongly positively correlated with eicosapentaenoic acid (r=0.76) and

docosahexaenoic acid (r=0.86). Total MUFAs was very strongly positively correlated with oleic acid (r=0.99).

Table 5.1 Macronutrient intakes, plasma phospholipid fatty acids and puberty timing in boys and girls in the ALSPAC study

	Boys (n=3811)	Girls (n=3919)	P value
	mean±SD	mean±SD	
Dietary intakes predicted at age 6 years			
Total energy intake, kcal	1654±159	1578±148	<0.001
Total carbohydrate intake, g	207.2±20.7	196.9±19.5	<0.001
Total protein intake, g	56.6±5.8	54.7±5.3	<0.001
Total fat intake, g	67.4±7.4	64.4±6.8	<0.001
Polyunsaturated fat intake, g	11.0±1.3	10.6±1.1	<0.001
Monounsaturated fat intake, g	21.8±2.4	20.9±2.2	<0.001
Saturated fat intake, g	27.3±4.0	26.1±3.6	<0.001
Percentage of energy from carbohydrate, %	50.1±1.7	49.9±1.6	<0.001
Percentage of energy from protein, %	13.8±1.0	13.9±0.9	<0.001
Percentage of energy from fat, %	36.5±1.6	36.6±1.4	0.049
Percentage of energy from polyunsaturated fat, %	6.0±0.6	6.0±0.5	0.419
Percentage of energy from monounsaturated fat, %	11.8±0.6	11.8±0.5	0.005
Percentage of energy from saturated fat, %	14.8±1.5	14.8±1.3	0.431
Plasma phospholipid fatty acids at 7.5 years			
Total fatty acids, µg/ml	2279.0±521.8	2378.5±547.4	<0.001
n-6 polyunsaturated fatty acids, µg%			
Total	39.9±4.0	39.7±3.9	0.034
Linoleic acid (18:2n6)	30.6±3.2	30.6±3.1	0.833
γ-Linolenic acid (18:3n6)	0.39±0.14	0.37±0.12	<0.001
Eicosadienoic acid (20:2n6)	0.21±0.04	0.20±0.04	0.029
Dihomo-γ-linolenic acid (20:3n6)	1.76±0.37	1.70±0.35	<0.001
Arachidonic acid (20:4n6)	6.5±1.3	6.3±1.3	0.001
Docosatetraenoic acid (22:4n6)	0.25±0.05	0.24±0.05	<0.001
Docosapentaenoic acid (22:5n6)	0.19±0.05	0.18±0.05	<0.001
n-3 polyunsaturated fatty acids, µg%			
Total	3.85±0.80	3.87±0.77	0.406
α-Linolenic acid (18:3n3)	0.71±0.27	0.73±0.29	0.026
Eicosapentaenoic acid (20:5n3)	0.64±0.22	0.63±0.20	0.086
Docosapentaenoic acid (22:5n3)	0.64±0.14	0.61±0.13	<0.001
Docosahexaenoic acid (22:6n3)	1.86±0.52	1.90±0.52	0.016
Monounsaturated fatty acids, µg%			
Palmitoleic acid (16:1n7)	1.27±0.40	1.32±0.41	<0.001
Oleic acid (18:1n9)	22.2±3.2	22.6±3.0	<0.001
Puberty timing, years			
Age at puberty onset (breast/genital development)	8.7±1.6	10.0±1.6	<0.001
Age at peak height velocity	13.6±0.9	11.7±0.8	<0.001
Age at puberty progression (voice breaking/menarche)	13.6±1.9	12.7±1.2	<0.001

5.5.2 Fat quality and puberty timing

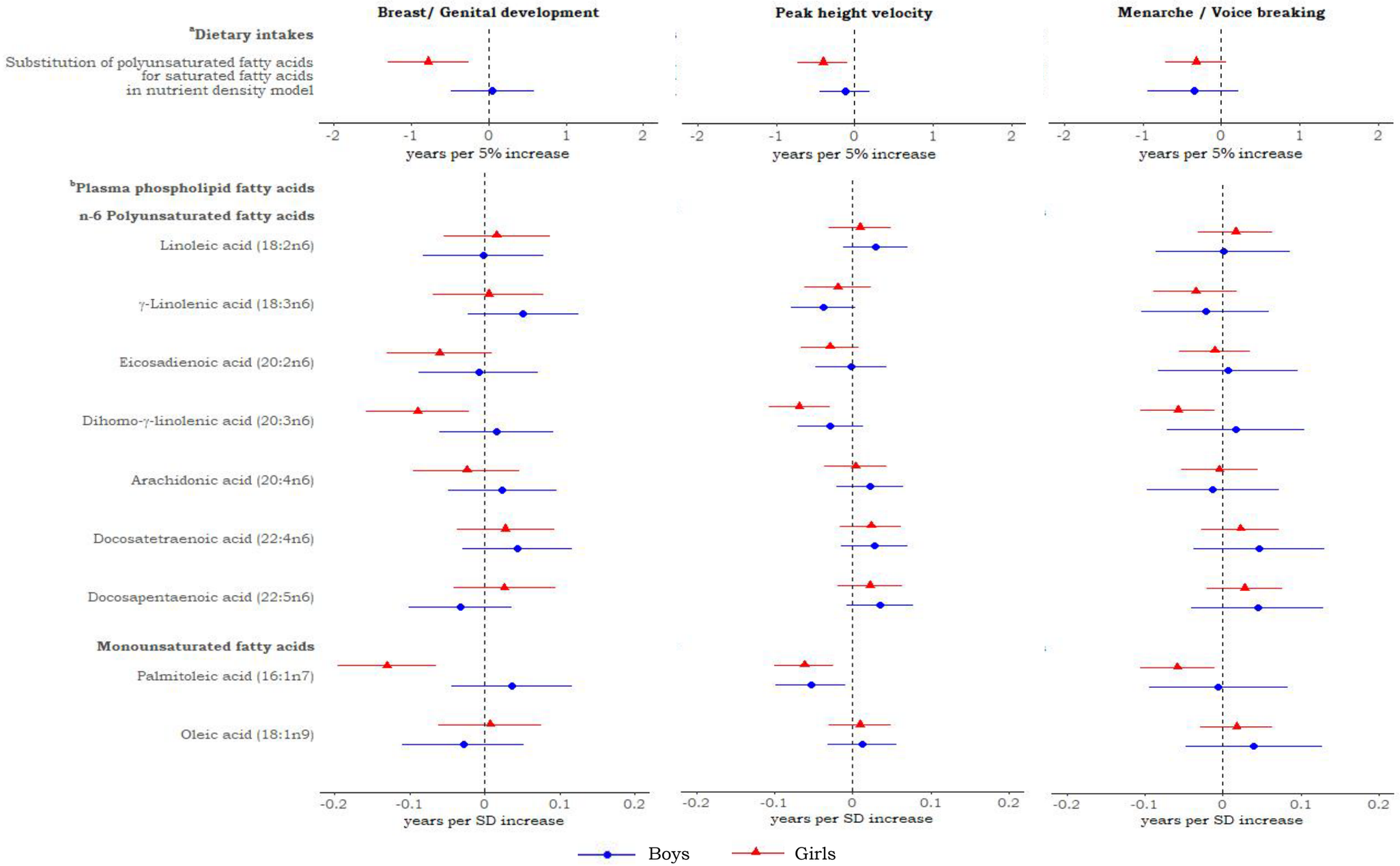
Table 5.2 and **Figure 5.2** show the associations between childhood dietary PUFAs intake and puberty timing in boys and girls. In *nutrient density models*, the substitution of dietary PUFAs for SFAs was associated with earlier B2 (β=-0.78 years per 5%) and PHV (β=-0.40), and a tendency to earlier menarche (β=-0.32) in girls. In contrast, these associations were not seen in boys. These findings remained robust in *residual models*. No association between dietary MUFAs intake and puberty timing in both sexes was found (**Supplementary Table A-19**).

Table 5.3 and **Figure 5.2** show the associations of plasma phospholipid n-6 PUFAs and major MUFAs with puberty timing in boys and girls. Higher concentrations of dihomo-γ-linolenic acid and palmitoleic acid were associated with earlier B2 (β=-0.09 years per SD and β=-0.13, respectively), PHV (β=-0.07 and β=-0.06) and menarche (β=-0.06 and β=-0.06) in girls, but not with any puberty timing trait in boys. These associations persisted after additional adjustment for non-major food sources and possible food sources of PUFAs at 6 years (**Supplementary Table A-20**). However, the associations between palmitoleic acid and puberty timing in girls were completely attenuated with further adjustment for BMI at 7.5 years (**Supplementary Table A-21**).

There was no strong evidence for the associations of dietary intakes and plasma phospholipid concentrations of total and individual n-3 PUFAs with puberty timing in boys and girls (**Supplementary Table A-22**).

Higher ratios of 20:3n6 to 18:2n6 (indicative of higher conversion of linoleic acid to dihomo-γ-linolenic acid activity) and 16:1n7 to 16:0 (indicative of higher Stearoyl-CoA desaturase-1 activity) were associated with earlier timing of puberty traits in girls but not boys (**Supplementary Table A-23**). No association of other MUFAs and ratios of FAs, and SFA categories with puberty timing was found.

Figure 5.2 Adjusted phenotypic associations of dietary and plasma phospholipid fatty acids with puberty timings in girls and boys in the ALSPAC study



Basic adjustment for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration)

[a]Additionally adjusted for energy from monounsaturated fatty acids, carbohydrate and protein (%), total energy intake (kcal)

[b]Additionally adjusted for total energy intake (kcal)

Table 5.2 Substitution of dietary polyunsaturated fatty acids for saturated fatty acids at age 6 years and puberty timing in the ALSPAC study

Dietary PUFAs intake	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value
Boys	n=2619		n=2215		n=3017	
Nutrient density model (per 5% increase)[a]	0.05 (-0.48, 0.59)	0.843	-0.12 (-0.44, 0.19)	0.446	-0.35 (-0.93, 0.22)	0.225
Residual model (per 10g increase)[b]	0.17 (-0.48, 0.81)	0.612	-0.13 (-0.51, 0.24)	0.485	-0.48 (-1.17, 0.20)	0.168
Girls	n=3204		n=2509		n=3414	
Nutrient density model (per 5% increase)[a]	-0.78 (-1.31, -0.25)	0.004	-0.40 (-0.72, -0.09)	0.011	-0.32 (-0.71, 0.07)	0.103
Residual model (per 10g increase)[b]	-0.86 (-1.50, -0.22)	0.008	-0.45 (-0.83, -0.08)	0.018	-0.35 (-0.81, 0.11)	0.139

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), carbohydrate intake (%), protein intake (%), monounsaturated fat intake (%), total energy intake (kcal)

[b]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), carbohydrate intake (g), protein intake (g), monounsaturated fat intake (g), total energy intake (kcal)

Table 5.3 Adjusted associations of plasma phospholipid fatty acids at age 7.5 years with puberty timing in the ALSPAC study

Fatty acids	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β per SD (95% CI)[a]	P value	Adjusted β per SD (95% CI)[a]	P value	Adjusted β per SD (95% CI)[a]	P value
Boys	n=2619		n=2215		n=3017	
n-6 PUFAs						
Total	0.01 (-0.07, 0.09)	0.813	0.03 (-0.01, 0.07)	0.186	-0.01 (-0.09, 0.09)	0.965
Linoleic acid (18:2n6)	-0.01 (-0.08, 0.08)	0.957	0.03 (-0.01, 0.07)	0.158	0.01 (-0.09, 0.09)	0.990
γ-Linolenic acid (18:3n6)	0.05 (-0.02, 0.12)	0.175	-0.04 (-0.08, 0.01)	0.078	-0.02 (-0.10, 0.06)	0.593
Eicosadienoic acid (20:2n6)	-0.01 (-0.09, 0.07)	0.839	-0.01 (-0.05, 0.04)	0.935	0.01 (-0.08, 0.10)	0.886
Dihomo-γ-linolenic acid (20:3n6)	0.02 (-0.06, 0.09)	0.688	-0.03 (-0.07, 0.01)	0.189	0.02 (-0.07, 0.11)	0.710
Arachidonic acid (20:4n6)	0.02 (-0.05, 0.10)	0.521	0.02 (-0.02, 0.07)	0.299	-0.01 (-0.10, 0.07)	0.765
Docosatetraenoic acid (22:4n6)	0.04 (-0.03, 0.11)	0.243	0.03 (-0.01, 0.07)	0.193	0.05 (-0.04, 0.13)	0.279
Docosapentaenoic acid (22:5n6)	-0.03 (-0.10, 0.04)	0.347	0.03 (-0.01, 0.08)	0.110	0.04 (-0.04, 0.13)	0.301
MUFAs						
Palmitoleic acid (16:1n7)	0.04 (-0.04, 0.12)	0.383	-0.05 (-0.10, -0.01)	0.020	-0.01 (-0.09, 0.08)	0.896
Oleic acid (18:1n9)	-0.03 (-0.11, 0.05)	0.492	0.01 (-0.03, 0.06)	0.584	0.04 (-0.05, 0.13)	0.374
Girls	n=3204		n=2509		n=3414	
n-6 PUFAs						
Total	-0.01 (-0.07, 0.07)	0.936	0.01 (-0.04, 0.04)	0.913	0.01 (-0.04, 0.05)	0.805
Linoleic acid (18:2n6)	0.02 (-0.05, 0.09)	0.656	0.01 (-0.03, 0.05)	0.640	0.02 (-0.03, 0.06)	0.498
γ-Linolenic acid (18:3n6)	0.01 (-0.07, 0.08)	0.893	-0.02 (-0.06, 0.02)	0.374	-0.03 (-0.09, 0.02)	0.199
Eicosadienoic acid (20:2n6)	-0.06 (-0.13, 0.01)	0.091	-0.03 (-0.07, 0.01)	0.125	-0.01 (-0.06, 0.04)	0.658
Dihomo-γ-linolenic acid (20:3n6)	-0.09 (-0.16, -0.02)	0.011	-0.07 (-0.11, -0.03)	6.3E-4	-0.06 (-0.11, -0.01)	0.018
Arachidonic acid (20:4n6)	-0.02 (-0.10, 0.05)	0.501	0.01 (-0.04, 0.04)	0.861	-0.01 (-0.05, 0.04)	0.863
Docosatetraenoic acid (22:4n6)	0.03 (-0.04, 0.09)	0.408	0.02 (-0.02, 0.06)	0.240	0.02 (-0.03, 0.07)	0.367
Docosapentaenoic acid (22:5n6)	0.03 (-0.04, 0.09)	0.448	0.02 (-0.02, 0.06)	0.294	0.03 (-0.02, 0.08)	0.252
MUFAs						
Palmitoleic acid (16:1n7)	-0.13 (-0.20, -0.06)	1.1E-4	-0.06 (-0.10, -0.02)	0.001	-0.06 (-0.11, -0.01)	0.017
Oleic acid (18:1n9)	0.01 (-0.06, 0.08)	0.833	0.01 (-0.03, 0.05)	0.645	0.02 (-0.03, 0.06)	0.462

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), total energy intake at 6 years (kcal)

5.5.3 Likely causal associations

Supplementary Table A-24 and **Supplementary Figure B-7** illustrate the identification and selection of single nucleotide polymorphisms (SNPs) as the instruments for genetic causal modelling for dihomo-γ-linolenic acid and palmitoleic acid that were observationally linked to puberty timing in girls (**Section 5.5.2**). Among identified SNPs, one SNP was very strongly associated with higher circulating dihomo-γ-linolenic acid concentrations (rs12928099 in *PDXCD1*, encoding pyridoxal dependent decarboxylase domain containing 1: P value=$2.3X10^{-196}$). The SNP was also less strongly associated with lower concentrations of its immediate upstream (γ-linolenic acid) and downstream (arachidonic acid) metabolites. This suggests a primary effect of the variant on higher dihomo-γ-linolenic acid concentrations. Similarly, one variant was very strongly and specifically associated with circulating palmitoleic acid concentrations (rs603424 near *SCD1*, encoding Stearoyl-CoA desaturase-1: P value=$6.2 X 10^{-38}$).

Table 5.4 shows the association of genetically predicted higher plasma dihomo-γ-linolenic acid with earlier menarche in girls (β=-0.05 years per SD); this genetic effect estimate was very similar to the observed phenotypic association (β=-0.06). Sensitivity analyses that included two additional variants less strongly associated with dihomo-γ-linolenic acid (rs721399 and rs499974) (**Supplementary Table A-24**) showed similar genetic associations between dihomo-γ-linolenic acid and earlier menarche in IVW (β=-0.04), weighted median (β=-0.05) and penalised weighted median models (β=-0.04). There was no evidence of horizontal pleiotropy (MR-Egger intercept=0.01, P value=0.6).

On the other hand, genetically predicted plasma palmitoleic acid was not associated with age at menarche (β=0.08) (**Table 5.4**), and this genetic effect estimate did not overlap the observed phenotypic association (β=-0.06). Sensitivity analyses that included an additional variant less strongly

associated with palmitoleic acid (rs4962238) also showed null association with age at menarche.

Table 5.4 Associations of genetically predicted fatty acids with age at menarche

Fatty acids	Number of SNPs	β (95% CI)	P value
Dihomo-γ-linolenic acid (20:3n6)			
rs12928099	1	-0.05 (-0.09, -0.01)	0.019
Inverse variance weighted	3	-0.04 (-0.09, 0.02)	0.166
Weighted median	3	-0.05 (-0.09, -0.01)	0.020
Penalised weighted median	3	-0.04 (-0.09, -0.01)	0.019
Palmitoleic acid (16:1n7)			
rs603424	1	0.08 (-0.01, 0.16)	0.082
Inverse variance weighted	2	0.05 (-0.09, 0.20)	0.472

5.6 Discussions

5.6.1 Summary of findings

In this longitudinal British birth cohort study, higher habitual intake of PUFAs substituting for SFAs, and higher plasma phospholipid concentrations of an intermediate metabolite of n-6 PUFAs (dihomo-γ-linolenic acid) during prepubertal childhood were associated with earlier timings of pubertal traits (indicated by puberty onset and progression, and an objective measure of puberty timing), in girls but not boys. Consistently, MR analyses supported a possible causal effect of higher dihomo-γ-linolenic acid concentrations on earlier menarche in girls. However, observed phenotypic association between higher concentrations of one of the most abundant MUFAs in adipose tissue (palmitoleic acid) and earlier puberty timing in girls was not seen in MR analyses.

5.6.2 Comparisons with previous evidence

The present study largely advances previous observational analyses of dietary fat quality and puberty timing[98, 99, 103, 120, 228] in many ways: i) by examining both boys and girls, ii) by combining the repeatedly assessed dietary intakes during prepubertal childhood, iii) by estimating several puberty traits based on longitudinal assessments from early adolescence to young adulthood, iv) by including objective measures of FAs (plasma phospholipid FA concentrations) and puberty timing (PHV), v) by analysing dietary intakes in isocaloric macronutrient substitution models and vi) by performing genetic causal modellings. The current findings are consistent with the previous ALSPAC study which reported associations between higher PUFA intakes during prepuberty and early menarche[99]. Moreover, our null findings of modelled substitution of dietary MUFAs for SFAs and puberty timing are similar to randomized controlled trials, namely the Dietary Intervention Study in Children[229, 230] and the Special Turku Coronary Risk Factor Intervention Project[231, 232] that found no difference in puberty timings between children who reduced SFAs intake (along with increase intake of MUFAs and/or PUFAs) and those who remained usual diet, in both sexes.

5.6.3 Interpretation and potential mechanisms

Using multiple analytical approaches with self-reported dietary fat intakes, plasma phospholipid FA concentrations and genetic causal modelling, findings in the present study showed high coherence. Higher dietary intake of PUFAs and higher plasma concentration of the intermediate metabolite of n-6 PUFAs (i.e. dihomo-γ-linolenic acid) were consistently associated with earlier puberty timing in girls; and these phenotypic associations were consistently null in boys. Also, no association of dietary and plasma phospholipid n-3 PUFAs (including eicosapentaenoic acid and docosahexaenoic acid) with puberty timing were consistently found. It is well-recognised that PUFAs, specifically n-6 and n-3 PUFAs, cannot be synthesised endogenously[227], and n-6 than n-3 PUFAs exist more typically in Western diets[223]. Further, among

n-6 PUFAs, linoleic acid is greatly ubiquitous in a wide variety of diets such as vegetable oils, nuts, seeds and meats[248], while dihomo-γ-linolenic acid is found in a small amount in animal sources such as meat and eggs[249]. After dietary PUFAs consumption, linoleic acid is catalyzed to γ-linolenic acid which is rapidly elongated to dihomo-γ-linolenic acid[250] (**Figure 5.1**). Subsequently, most of dihomo-γ-linolenic acid accumulates, while some is further converted to arachidonic acid but this conversion diminishes with increasing availability of dihomo-γ-linolenic acid[250]. Although oxidative metabolites of dihomo-γ-linolenic acid have anti-inflammatory effects[250], higher dihomo-γ-linolenic acid concentrations have been associated with increased risks for T2D and insulin resistance[249, 251, 252], which may in turn enhance bioavailability of sex steroids and induce earlier pubertal development[32] particularly in girls.

On the contrary, MUFAs can be sourced from diets or endogenously synthesised from SFAs (**Figure 5.1**). Hence, our observations of associations with puberty timings in girls for plasma palmitoleic acid but not for dietary MUFAs are not directly comparable. Moreover, given that palmitoleic acid is low in dietary concentration and rapidly oxidized, plasma concentration of palmitoleic acid is likely resulted from endogenous production from adipose tissue[234]. This might explain why the associations between plasma palmitoleic acid and puberty timings in girls were largely attenuated on additional adjustment for BMI, and also no overlap between phenotypic and MR effect estimates. We therefore speculate that the observed association of higher plasma palmitoleic acid with earlier puberty timing may be partly mediated by lower brown adipose tissue[253] or higher white adipose tissue predominantly composed of oleic acid (a precursor of palmitoleic acid) and palmitoleic acid[233, 234].

5.6.4 Strengths and limitations

Despite large sample size and use of data and methods triangulation approaches, limitations in this study are acknowledged. Modest differences in some maternal and infant characteristics were found between children with

and without data on puberty timing, but these factors were adjusted for in all analyses. Individuals with implausible reported energy intakes were not identifiable since body weight was not measured at 3-7 years. Such issue was however addressed using *residual method* which accounts for potential under-reporting of dietary intakes[182]. Dietary data from several timepoints were also synthesised to reduce random error and avoid multiple testing[181]. Although dietary intakes were parent-reported and single timepoint plasma samples were analysed after storage, expected modest positive correlations between habitual childhood dietary fat quality intakes and their respective biomarkers were still observed. Information on other plasma phospholipid FAs such as odd-chain SFAs (pentadecanoic acid (15:0) and heptadecanoic acid (17:0)) and trans FAs were not available. Moreover, report-based Tanner stages of puberty were recorded, but such method generally showed moderate agreement with physical exam by health professionals (the gold standard)[222]. Puberty timing traits in 20-30% of children could not be estimated due to unamendable inconsistencies in their repeated parent and/or self-reports. Nonetheless, findings with subjective (i.e. puberty onset and progression) and objective (i.e. PHV) measures of puberty timing were consistent. While our observed phenotypic associations may be affected by residual confounding due to unmeasured factors such as physical activity (which was not measured in ALSPAC before age 7 years), similar finding for dihomo-γ-linolenic acid was obtained using MR analysis which is considered to be less influenced by such confounding. Finally, causal inferences from MR analyses are subject to a number of assumptions. A single variant with a very strong and specific association with the target FA was selected to avoid pleiotropic effects. The genetic instrument for higher dihomo-γ-linolenic acid concentrations appeared to be specific for this FA, whilst the genetic instrument for palmitoleic acid is located near the gene encoding Stearoyl-CoA desaturase-1 which is the enzyme that catalyzes its biosynthesis.

5.6.5 Conclusions

The phenotypic analyses using subjective and objective measures of both dietary intakes and puberty timing indicated that higher prepubertal childhood habitual dietary intake of PUFAs, specifically higher concentrations of an intermediate metabolite of plasma phospholipid n-6 PUFAs (dihomo-γ-linolenic acid), were associated with earlier puberty timing in girls, but not in boys. More robust MR analyses further substantiated that increasing circulating dihomo-γ-linolenic acid concentrations, either from dietary or endogenous metabolic pathways, may cause earlier age at menarche in girls. Such sex disparity in the associations also suggests that there may be sex-specific individual fatty acid sensing pathway underlying pubertal development.

CHAPTER 6 ACCELEROMETERY MEASURED PHYSICAL ACTIVITY DURING PREPUBERTY AND SUBSEQUENT PUBERTY TIMING

Publication

The manuscript based on this chapter:

Cheng, TS., Brage, S., van Sluijs, EMF., Ong, KK. Prepubertal accelerometer-assessed physical activity and puberty timing in British boys and girls: The Millennium Cohort Study.

Contributions

I conceived and designed this study together with my supervisors (Prof. Ken Ong, Dr. Soren Brage and Dr. Felix Day). I also applied for and downloaded data, processed data on physical activity and puberty development in the UK MCS, searched for genetic instrumental variables for physical activity, conducted statistical analyses, jointly interpreted the findings and completed writing for this chapter and the resulting manuscript.

6.1 Summary

Background

Early puberty timing is associated with a variety of adverse health outcomes in men and women, possibly independent of adiposity, as discussed in **Chapter 2**. Although some studies have investigated childhood physical activity as a potential determinant of puberty timing, their findings are inconsistent and limited to self-reported physical activity in girls. The present chapter aimed to investigate the associations of objectively measured physical activity during prepuberty with puberty timing in boys and girls.

Methods

In the UK MCS, total volume and intensities of physical activity were assessed at age 7 years using accelerometers. Pubertal status of several traits reported at 11 and 14 years were classified into earlier or later than the median ages estimated from probit models, separately in boys and girls. Age at menarche in girls was also reported at 11, 14 and 17 years, and categorised into tertiles. The associations of total daily counts and fractions of counts across intensities (light, moderate or vigorous) with puberty timing were tested using multivariable regression models, adjusted for maternal and child characteristics including BMI at 7 years. The likely casual associations of physical activity and sedentary behaviours with puberty timing were also assessed using MR analyses in further large datasets.

Results

In MCS boys (n=2531) and girls (n=3079), higher total daily accelerometer counts, but not any specific intensity, was associated with lower risks for earlier puberty progression (voice breaking or menarche) and occurrence of other puberty traits (growth spurt, body hair growth and/or skin changes). These associations were not attenuated on additional adjustment for BMI at 11 years. In univariable and multivariable MR models adjusted for childhood size, genetically predicted longer time spent in television viewing in both sexes and computer use in girls were associated with earlier timing of puberty progression.

Conclusions

These findings suggest that more physical activity, regardless of intensity, concomitant with less sedentary behaviour, may be a potential strategy for the avoidance of earlier occurrence of puberty, independent of any changes in BMI, in both boys and girls.

6.2 Background

Chapters 1 and **2** revealed that earlier timing of puberty has been associated with higher risks for adverse health outcomes, which are likely independent of adiposity. These observations prompt the need to explore specific modifiable factors of puberty timing for targeted interventions. **Chapters 4** and **5** have demonstrated that prepubertal dietary intakes, namely higher intakes of total energy, protein and PUFAs (that increase circulating dihomo-γ-linolenic acid concentrations), may promote earlier pubertal development. These findings raise the question as to whether other behavioural factors, especially physical activity and sedentary behaviours may be potential determinants of puberty timing.

A recent systematic review found only five studies that have examined the prospective association between physical activity prior to menarche and risk of early menarche among girls, and reported conflicting findings[112]. Only one study in Germany (n=167) showed that higher physical activity duration and weight-adjusted energy expenditure (estimated from 7-day parent-reports of duration and type of sports activities) at age 8-15 years were associated with lower risk for early menarche after 2 years of follow-up[107]. These associations were however not seen in a larger study (n=2487) in Canada, which administered 7-day recalls to girls at age 11.1 (9.5-13.4) years to indicate the duration spent in each of 67 specified leisure activities over the last 7 days and followed-up menarche status 2 years later[118]. Also, no association between weight-unadjusted energy expenditure based on physical activity questionnaires and menarche status was found in studies in Taiwan (n=799, age 9-10 years, 1 year of follow-up)[119] and Canada (n=637, age 9.7 (6.2-13.6) years, 3 years of follow-up)[120]. A study in Iran reported no difference in subsequent age at menarche between active and nonactive premenarcheal girls at age 12-18 years[121]. It is noteworthy that previous studies assessed only overall physical activity based on self- or parent-reports which are susceptible to large errors. Moreover, the measures of physical activity were obtained during the typical ages of pubertal development, and so raises the

possibility of reverse causality. No study on physical activity and puberty timing in boys was noted.

6.3 Study aims

This study aimed to investigate the associations between prepubertal objectively measured physical activity and timings of several puberty traits in boys and girls, with simultaneous considerations of total volume and intensities of physical activity. Any observed association was further tested with additional adjustment for BMI during puberty. Also, the likely causality of the associations of physical activity and sedentary behaviours with puberty timing were examined in MR models using large-scale published GWAS summary statistics.

6.4 Methods

6.4.1 Study population

The phenotypic part of the present study included 5610 children (30.6%) (2531 boys and 3079 girls) with data on physical activity and at least one puberty timing trait from the initial analytical sample in the UK MCS. The details of the UK MCS are fully described in **Section 3.2**.

6.4.2 Total volume and intensities of physical activity

Child's physical activity was measured at age 7 years using an accelerometer and summarised as daily total counts and times spent in sedentary behaviour and light, moderate and vigorous intensities. For full description for the assessment and processing of accelerometry data, refer to **Section 3.2.2.1**. Individual daily times spent across physical activity intensities were further

converted to derive fractions of counts contributed by each intensity (**Section 3.2.4.1**).

6.4.3 Timings of puberty traits

Parent- and child-reported pubertal status at age 11 and 14 years were combined to classify earlier and later puberty for several puberty traits, while the first reported age at menarche at 11, 14 or 17 years was analysed as both continuous and categorical (tertiles) variables (**Section 3.2.2.2** and **3.2.4.2**).

6.4.4 Potential confounders

Potential confounders were maternal and infant characteristics (including BMI-for-age z score at 7 years) associated with prepubertal physical activity and puberty timing (**Section 3.2.5.2**). Total daily counts and fractions of counts from different intensities in *compositional* and *isomovement substitution models*, and times spent across intensities in *isotemporal substitution models*, were entered jointly.

6.4.5 Statistical analyses

6.4.5.1 Phenotypic analyses

The associations between total daily counts (as a single exposure) (per 100,000 counts) and puberty timing were explored using i) multivariable binary logistic regression - for binary puberty timing outcomes, with later puberty as the reference group, ii) multinomial logistic regression – for tertiles of age at menarche, with the middle tertile as the reference group, and iii) linear regression – for continuous age at menarche, with adjustment for potential confounders. These models were then additionally adjusted for BMI-for-age z score at age 11 years as a potential mediator.

Similar multivariable regression models were repeated to examine the associations of physical activity intensities (i.e. fractions of total daily counts contributed by each intensity) with puberty timing, adjusted for potential confounders. *Compositional models* were the primary method to simultaneously test the effects of (fractions of total daily counts contributed by) light, moderate and vigorous intensities, and total daily counts on puberty timing. Supplementary analyses for physical activity intensities included: i) *isomovement substitution models* to examine the effects of the reallocation of movement (per 10% of total daily counts) from light to moderate and vigorous intensities; these models also included total daily counts, and ii) *isotemporal substitution models* to test the effects of the reallocation of time spent (per 10 minutes) from sedentary behaviour to light, moderate and vigorous intensities. Details on the modelling of physical activity intensities can be found at **Section 3.2.5.3**.

The abovementioned models were repeated to assess the associations of total daily counts and physical activity intensities with BMI-for-age *z* score at 11 years, adjusted for potential confounders.

Multiple imputation by chained equations with 50 imputed datasets (**Section 3.2.5.4**) were performed to impute missing data on potential confounders (missing n=1-496, 0.02%-8.8%; at least one missing n=741, 13.2%).

6.4.5.2 Mendelian Randomization analyses

To evaluate the likely causal association between physical activity and puberty timing, two-sample MR analyses were conducted using IVW, MR-Egger[245], weighted median and penalized weighted median[246] methods. Assumptions of these methods were described in **Section 5.4.6.2**. The exposure data were the independent strongest-associated genetic variants identified from published GWASs for a) physical activity intensities: i) accelerometer-based average acceleration (in milli-gravities), and self-reported ii) strenuous sports or other exercises (≥2-3 versus 0 days per week), iii)

moderate-to-vigorous activity (in SD of MET-minutes per week) and iv) vigorous activity (≥3 versus 0 days per week) (adjusted for age, sex, genotyping chip, first ten genomic principal components, centre, season at centre visits or accelerometry monitor, Townsend Deprivation index, walk or standing at work, physical activity at work, and BMI; based on P value<5 X 10^{-9})[254], and b) sedentary behaviours: inverse rank normalized daily time spent in i) television watching and ii) leisure computer use (excluding computer use at work) (adjusted for age-squared, age, sex, age-sex interaction and the first 30 principal components to correct for population stratification and genotyping array; based on P value<1 X 10^{-8})[255] in the UK Biobank, which recruited nearly a half-million predominantly white adults aged 40-69 years[243]. The outcome data were obtained from unpublished GWAS summary statistics for continuous age at menarche from the ReproGen consortium[60], combined with expanded data from UK Biobank comprising in total ~566,000 women of European ancestry, and for categorical age at voice breaking ("younger", "about average" or "older" than peers) in men from the UK Biobank (n=191,235) (provided by Prof. Ken Ong and Dr. Felix Day). Where corresponding outcome data of a specific genetic variant for an exposure were not available, a highly correlated variant (within 1Mb and R^2>0.5) was identified as a proxy using the published GWAS summary statistics for the particular exposure (conducted by Stasa Stankovic, a PhD student in the same group).

To optimise the robustness of the findings, the exposure instrumental variable was filtered using the following steps. Firstly, to minimise the possibility of reverse causality (i.e. effects of puberty timing on physical activity), those genetic variants with stronger effects on puberty timing than each physical activity trait were filtered out using Steiger filtering by the "TwoSampleMR" package[244]. Secondly, to reduce heterogeneity in the modelled relationship between puberty timing and physical activity, heterogeneity was assessed using Rücker's *Q* statistic. Subsequent models were also performed removing those variants that were identified based on P value<0.05 from Radial regression models[256].

Supplementary Table A-25 describes the stages for selecting genetic variants associated with different physical activity traits for use in MR analyses, and lists those variants that were filtered out by Steiger filtering (to reduce reverse causality) or by Radial filtering (to reduce heterogeneity).

In addition to the primary univariable MR models, multivariable MR analyses were performed in order to explore whether any association between physical activity and puberty timing might be genetically mediated by childhood adiposity (using sex-specific data from the UK Biobank in response to the question; "When you were 10 years old, compared to average would you describe yourself as thinner, plumper, or about average?").

All MR analyses were conducted in R software, with the use of codes prepared by Dr. Felix Day.

6.5 Results

6.5.1 MCS children's characteristics

The MCS study sample included 5610 children, comprising 2531 boys and 3079 girls. Compared to mothers of the excluded children (n=12,704), mothers of the included children were more likely to be older, have higher family income and higher education level (**Supplementary Table A-26**). The included children were also more likely to be girls, white, and heavier at birth but lighter at 7 and 11 years (**Supplementary Table A-26**).

Table 6.1 summarises accelerometer-based physical activity, puberty timing and BMI of the included children. Compared to boys, girls had fewer days of valid physical activity data, more physical activity data on weekdays and shorter daily total monitor wear time, and were less active at 7 years and heavier at 11 years.

Supplementary Table A-27 shows the Pearson's correlations between physical activity measures. In both boys and girls, total daily counts was strongly negatively correlated with fractions of total counts from sedentary behaviour and light intensity (r=-0.74 to -0.85). Based on fractions of counts, vigorous intensity was strongly negatively correlated with light intensity (r=-0.88 and -0.87), whereas based on time spent, moderate and vigorous intensities were moderately positively correlated (r=0.67 and 0.70).

Supplementary Table A-28 shows the Spearman's correlations between puberty timing. In boys, timing of growth spurt was moderately correlated with timings of body hair growth, skin changes and voice breaking (r=0.39 to 0.64). In girls, timing of growth spurt was moderately to strongly correlated with timings of body hair growth, skin changes and breast development (r=0.47 to 0.60).

Table 6.1 Comparisons of physical activity, puberty timing and body mass index between boys and girls

	Boys (n=2531)	Girls (n=3079)	P value
Accelerometer at 7 years old (mean±SD)			
Age at measurement, years	7.23±0.25	7.22±0.25	0.661
Spring	-0.36±0.58	-0.38±0.57	0.214
Winter	-0.15±0.72	-0.14±0.71	0.610
Number of valid measure days	5.7±1.5	5.5±1.6	<0.001
Proportion of weekdays for measure, %	77.8±13.5	78.5±14.1	0.045
Daily total time for measure, minutes	740.5±61.6	732.8±60.9	<0.001
Sedentary behaviour	388.1±65.1	398.7±67.8	<0.001
Light activity	283.3±40.9	278.6±41.1	<0.001
Moderate activity	46.8±13.2	37.9±11.5	<0.001
Vigorous activity	22.3±11.4	17.6±9.4	<0.001
Total daily counts, 100,000 per day	4.70±1.13	4.15±1.03	<0.001
Fraction of total counts from, %			
Sedentary behaviour	$3.0E^{-20}$±$1.1E^{-21}$	$3.5E^{-20}$±$2.1E^{-20}$	<0.001
Light activity	41.6±8.4	46.4±8.6	<0.001
Moderate activity	33.7±4.1	31.2±4.1	<0.001
Vigorous activity	24.6±8.0	22.4±7.3	<0.001
Puberty timing			
Growth spurt (n, %)			-
Earlier (boys:<12.4; girls:<11.2)	847 (53.3)	563 (37.5)	
Later (boys:≥12.4; girls: ≥11.2)	741 (46.7)	940 (62.5)	
Body hair growth (n, %)			-
Earlier (boys:<12.4; girls:<11.7)	235 (29.8)	995 (79.9)	
Later (boys:≥12.4; girls: ≥11.7)	553 (70.2)	251 (20.1)	
Skin changes (n, %)			-
Earlier (boys:<12.4; girls:<12.6)	270 (21.8)	714 (51.9)	
Later (boys:≥12.4; girls: ≥12.6)	969 (78.2)	662 (48.1)	
Voice breaking (n, %)			-
Earlier (boys:<13.8 years)	138 (13.3)	-	
Later (boys:≥13.8 years)	900 (86.7)	-	
Facial hair growth (n, %)			-
Earlier (boys:<14.8 years)	863 (95.0)	-	
Later (boys:≥14.8 years)	45 (5.0)	-	
Breast development (n, %)			-
Earlier (girls:<11.5 years)	-	916 (59.1)	
Later (girls:≥11.5 years)	-	634 (40.9)	
Age at menarche (n, %)			-
Tertile 1 (8-12 years)	-	1515 (52.2)	
Tertile 2 (>12-13 years)	-	955 (32.9)	
Tertile 3 (>13-17 years)	-	434 (14.9)	
Age at menarche, years (mean±SD)	-	12.4±1.3	-
Body mass index at 11 years, kg/m^2 (mean±SD)	18.7±3.4	19.1±3.5	<0.001

6.5.2 Physical activity and puberty timing

Table 6.2 and **Supplementary Table A-29** show the associations of total daily counts and physical activity intensities with puberty timing, with adjustment for potential confounders. In single exposure models, higher total counts was associated with lower risks for earlier (versus later) skin changes (OR=0.86 per 100,000 counts/day) and voice breaking (OR=0.80) in boys, and earlier growth spurt (OR=0.85), body hair growth (OR=0.80), skin changes (OR=0.80) and menarche (OR=0.87) in girls.

No physical activity intensity was associated with risk for earlier puberty timing in *compositional* or *isomovement substitution analyses*. In *isotemporal substitution analyses*, reallocation of times spent in sedentary behaviour to light and vigorous intensities were associated with lower risks for earlier skin changes (OR=0.96 per 10 minutes/day) and breast development (OR=0.80) in girls, respectively.

Supplementary Table A-30 illustrates the associations of total daily counts and physical activity intensities with BMI at 11 years, with adjustment for potential confounders including BMI at 7 years. Total counts was not associated with BMI in the single exposure analysis. Higher fraction of counts from light intensity was associated with higher BMI in boys in *compositional analysis*. Similarly, reallocation of fraction of counts from light to vigorous intensity was associated with lower BMI in both sexes in the *isomovement substitution analysis*. Marginal positive and negative associations of reallocation of times spent in sedentary behaviour to light and vigorous intensities with BMI in both sexes were observed in *isotemporal analyses*, respectively.

Table 6.3 shows that all observed associations between physical activity, particularly total daily counts, and puberty timing were unattenuated on further adjustment for BMI at 11 years.

Table 6.2 Associations of physical activity with earlier (vs. later) timing of puberty traits

Physical activity	Adjusted OR (95% CI)[a]	P value	Adjusted OR (95% CI)[a]	P value	Adjusted OR (95% CI)[a]	P value	Adjusted OR (95% CI)[a]	P value
Boys	**Earlier growth spurt (n=1588)**		**Earlier body hair growth (n=788)**		**Earlier skin changes (n=1239)**		**Earlier facial hair (n=908)**	
Total counts (per 100,000)	0.92 (0.83, 1.01)	0.085	0.93 (0.79, 1.10)	0.397	0.86 (0.75, 0.98)	0.022	1.22 (0.87, 1.70)	0.249
Compositional analysis								
Total counts (per 100,000)	0.86 (0.73, 1.01)	0.065	0.76 (0.58, 1.00)	0.052	0.73 (0.58, 0.91)	0.006	1.14 (0.68, 1.91)	0.631
Log-fractions of counts								
Light intensity	0.27 (0.05, 1.36)	0.112	0.40 (0.03, 5.71)	0.501	0.30 (0.03, 2.55)	0.269	0.53 (0.01, 108.04)	0.813
Moderate intensity	0.50 (0.15, 1.70)	0.268	0.66 (0.09, 4.76)	0.679	1.18 (0.23, 5.99)	0.838	3.89 (0.12, 130.89)	0.449
Vigorous intensity	0.61 (0.28, 1.35)	0.221	1.41 (0.38, 5.20)	0.610	0.88 (0.32, 2.44)	0.803	0.77 (0.08, 7.58)	0.824
Girls	**Earlier growth spurt (n=1503)**		**Earlier body hair growth (n=1246)**		**Earlier skin changes (n=1376)**		**Earlier breast development (n=1550)**	
Total counts (per 100,000)	0.85 (0.76, 0.96)	0.008	0.80 (0.68, 0.94)	0.005	0.80 (0.71, 0.90)	2.6E-4	0.91 (0.81, 1.02)	0.106
Compositional analysis								
Total counts (per 100,000)	0.90 (0.75, 1.08)	0.261	0.73 (0.57, 0.94)	0.014	0.76 (0.62, 0.92)	0.005	0.88 (0.73, 1.06)	0.188
Log-fractions of counts								
Light intensity	1.45 (0.22, 9.55)	0.702	0.38 (0.02, 6.08)	0.495	1.24 (0.15, 10.46)	0.842	0.39 (0.05, 2.75)	0.343
Moderate intensity	0.66 (0.21, 2.08)	0.477	0.79 (0.14, 4.57)	0.796	1.50 (0.40, 5.57)	0.549	2.00 (0.63, 6.36)	0.238
Vigorous intensity	1.09 (0.50, 2.39)	0.828	0.85 (0.26, 2.79)	0.784	1.32 (0.55, 3.16)	0.533	0.52 (0.23, 1.20)	0.127

[a]Adjusted for maternal characteristics (age, active smoking during pregnancy, alcohol consumption during pregnancy, education, pre-pregnancy body mass index, OECD equivalized family income) and child characteristics (ethnicity, birth weight, gestational age, breastfeeding duration, mental health, dietary behaviour, regular sleep time, long-term health status and body-mass-index-for-age z score at 7 years

Table 6.2 Associations of physical activity with earlier (vs. later) timing of puberty traits (continued)

Physical activity	Adjusted OR (95% CI)[a]	P value	Adjusted OR (95% CI)[a]	P value	Adjusted β (95% CI)[a]	P value
Boys	**Earlier voice breaking (n=1038)**					
Total counts (per 100,000)	0.80 (0.66, 0.96)	0.018				
Compositional analysis						
Total counts (per 100,000)	0.66 (0.49, 0.90)	0.010				
Log-fractions of counts						
Light intensity	0.12 (0.01, 2.51)	0.173				
Moderate intensity	0.27 (0.03, 2.21)	0.224				
Vigorous intensity	0.66 (0.16, 2.79)	0.571				
Girls	**Earlier menarche (T1 vs. T2)**		**Later menarche (T3 vs. T2)**		**Age at menarche (n=2904)**	
Total counts (per 100,000)	0.87 (0.80, 0.95)	0.002	0.91 (0.81, 1.02)	0.111	0.03 (-0.01, 0.08)	0.156
Compositional analysis						
Total counts (per 100,000)	0.81 (0.70, 0.93)	0.004	0.84 (0.68, 1.02)	0.080	0.04 (-0.03, 0.12)	0.272
Log-fractions of counts						
Light intensity	0.36 (0.08, 1.59)	0.179	0.92 (0.11, 7.97)	0.939	0.26 (-0.53, 1.05)	0.518
Moderate intensity	1.13 (0.48, 2.64)	0.785	1.49 (0.42, 5.37)	0.538	-0.14 (-0.60, 0.32)	0.560
Vigorous intensity	0.71 (0.39, 1.30)	0.264	1.22 (0.49, 3.01)	0.673	0.16 (-0.16, 0.48)	0.330

[a]Adjusted for maternal characteristics (age, active smoking during pregnancy, alcohol consumption during pregnancy, education, pre-pregnancy body mass index, OECD equivalized family income) and child characteristics (ethnicity, birth weight, gestational age, breastfeeding duration, mental health, dietary behaviour, regular sleep time, long-term health status and body-mass-index-for-age z score at 7 years; T, tertile

Table 6.3 Associations of physical activity with earlier (vs. later) puberty timing, on further adjustment for body mass index during puberty

	Single exposure model (per 100,000)		Isotemporal substitution model (per 10 minutes spent)					
	Total counts		Light intensity		Moderate intensity		Vigorous intensity	
Physical activity	**Adjusted OR (95% CI)[a]**	**P value**	**Adjusted OR (95% CI)[a]**	**P value**	**Adjusted OR (95% CI)[a]**	**P value**	**Adjusted OR (95% CI)[a]**	**P value**
Boys								
Earlier skin changes (n=1239)	0.85 (0.75, 0.98)	0.021						
Earlier voice breaking (n=1038)	0.80 (0.66, 0.96)	0.018						
Girls								
Earlier growth spurt (n=1503)	0.85 (0.76, 0.96)	0.010						
Earlier body hair growth (n=1246)	0.79 (0.67, 0.93)	0.004						
Earlier skin changes (n=1376)	0.79 (0.70, 0.89)	1.7E-4	0.96 (0.92, 0.99)	0.013	0.95 (0.81, 1.13)	0.570	0.89 (0.74, 1.06)	0.187
Earlier breast development (n=1550)			0.97 (0.93, 1.00)	0.081	1.14 (0.96, 1.35)	0.142	0.81 (0.67, 0.98)	0.032
Menarche (n=2904)								
Earlier (T1 vs. T2)	0.87 (0.80, 0.95)	0.002						
Later (T3 vs. T2)	0.91 (0.81, 1.03)	0.122						

[a]Adjusted for maternal characteristics (age, active smoking during pregnancy, alcohol consumption during pregnancy, education, pre-pregnancy body mass index, OECD equivalized family income) and child characteristics (ethnicity, birth weight, gestational age, breastfeeding duration, mental health, dietary behaviour, regular sleep time, long-term health status, body-mass-index-for-age z scores at 7 years and 11 years

6.5.3 Probable causal associations

Table 6.4 illustrates the associations of genetically predicted physical activity traits with timing of puberty progression. Longer duration of television viewing was genetically associated with both earlier age at voice breaking in males (β=-0.02 unit per SD) and age at menarche in females (β=-0.07 years per SD). There was no evidence of horizontal pleiotropy (MR-Egger intercept P value>0.05). These associations persisted in sensitivity analyses (weighted median and penalised weighted median MR models) and in the multivariable MR model adjusting for childhood size (as a potential mediator).

Longer duration of leisure computer use was genetically associated only with earlier menarche in females (β=-0.18 years per SD) and there was no horizontal pleiotropy (MR-Egger intercept P value>0.05). The effect estimate was similar in sensitivity models and in the multivariable model adjusting for childhood size.

No genetic association between any physical activity intensity and puberty timing was obtained.

Table 6.4 Associations of genetically predicted physical activity traits with age at voice breaking in males and age at menarche in girls

Exposures	Number of SNPs	Inverse variance weighted		Weighted median		Penalised weighted median	
		β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
Males							
Physical activity intensities[a]							
Accelerometer-based average acceleration	2	0.005 (-0.001, 0.012)	0.361	0.004 (-0.002, 0.013)	0.386	0.004 (-0.002, 0.012)	0.385
Strenuous sports or other exercises	3	0.09 (-0.08, 0.26)	0.417	0.13 (-0.08, 0.33)	0.346	0.13 (-0.08, 0.34)	0.361
Moderate-to-vigorous intensity	7	-0.06 (-0.11, 0.01)	0.108	-0.06 (-0.14, 0.02)	0.200	-0.06 (-0.13, 0.02)	0.189
Vigorous intensity	4	-0.04 (-0.17, 0.08)	0.565	-0.05 (-0.20, 0.10)	0.559	-0.05 (-0.20, 0.10)	0.577
Sedentary behaviours							
Television viewing							
Univariable model	135	-0.02 (-0.03, -0.01)	0.002	-0.02 (-0.04, -0.01)	0.016	-0.02 (-0.04, -0.01)	0.017
Multivariable model[b]	135	-0.02 (-0.03, -0.01)	0.002	-		-	
Leisure computer use							
Univariable model	32	0.01 (-0.02, 0.03)	0.716	-0.01 (-0.05, 0.03)	0.560	-0.01 (-0.05, 0.03)	0.547
Multivariable model[b]	32	0.01 (-0.02, 0.04)	0.585	-		-	
Females							
Physical activity intensities[a]							
Accelerometer-based average acceleration		Not computable					
Strenuous sports or other exercises		Not computable					
Moderate-to-vigorous intensity	5	0.14 (-0.15, 0.43)	0.399	0.06 (-0.25, 0.37)	0.737	0.06 (-0.24, 0.36)	0.729
Vigorous intensity	4	0.83 (0.17, 1.49)	0.090	0.64 (0.14, 1.13)	0.086	0.52 (0.03, 1.02)	0.128
Sedentary behaviours							
Television viewing							
Univariable model	81	-0.07 (-0.12, -0.02)	0.009	-0.09 (-0.16, -0.02)	0.020	-0.02 (-0.04, -0.01)	0.017
Multivariable model[b]	81	-0.08 (-0.14, -0.03)	0.002	-		-	
Leisure computer use							
Univariable model	24	-0.18 (-0.28, -0.07)	0.002	-0.12 (-0.25, 0.02)	0.097	-0.12 (-0.25, 0.02)	0.100
Multivariable model[b]	24	-0.14 (-0.26, -0.02)	0.034	-		-	

[a]Univariable model using adult-BMI-adjusted estimates for exposures
[b]Adjusted for childhood size

6.6 Discussions

6.6.1 Summary of findings

The present study in the British MCS showed prospective associations of higher total daily counts based on accelerometers with lower risks for earlier timings of puberty indicated by puberty progression (i.e. voice breaking or menarche) and other puberty traits (growth spurt, body hair growth and/or skin changes) in boys and girls. Times spent in light and vigorous intensities reallocated from sedentary behaviour were associated with lower risks for earlier puberty timing in girls. These associations were not confounded by BMI at baseline and persisted on additional adjustment for BMI during puberty. Similarly, MR analyses demonstrated that more sedentary behaviour, denoted by television viewing in both sexes or leisure computer use in females, but not any physical activity intensity, may have a causal effect on earlier puberty progression, independent of childhood size.

6.6.2 Comparisons with previous evidence

Findings in this study greatly fill the current evidence gap on the role of physical activity in pubertal timing, with the use of several stronger approaches. Previous studies administered questionnaires to premenarcheal girls or their parent to record overall physical activity duration and energy expenditure at age 6-18 years, and subsequently obtained a single measure of menarche status 1 to 3 years later[107, 118-121]. On the other hand, the present study analysed accelerometry-based total volume and intensities of physical activity at age 7 years (i.e. before the typical physiological age range for puberty onset) to reduce measurement errors and followed-up pubertal development in several traits in boys and girls for at least two visits until 17 years, which minimises risk of reverse causality. Moreover, unlike most previous studies[107, 118-121], our findings had a high degree of internal validity resulting from the adjustment for a wide range of potential confounders

especially dietary behaviour and BMI at baseline. Consistent with only one study which reported an association between higher overall physical activity and lower risk for earlier menarche in girls[107], the present study added that higher overall physical activity but not any specific intensity was associated with lower risks for earlier timings of several puberty traits including puberty progression in girls and also boys, independent of BMI before and during puberty. To my knowledge, it is also the first study to use genetic MR approaches to test the direct adiposity-independent effect of sedentary behaviour on earlier puberty timing.

Additional findings included in the present study are the associations between physical activity and subsequent BMI, which has been previously examined in the same cohort[195]. In the single exposure regression models, previous analysis found that higher time spent in moderate-to-vigorous intensity but not sedentary behaviour and total counts was associated with lower percentage change in BMI at 11 years only in boys[195]. By contrast, the present study analysed times spent in light, moderate and vigorous intensities simultaneously in an *isomovement substitution model*, and observed that reallocations to light and vigorous intensities from sedentary behaviour were marginally associated with higher and lower BMI at 11 years, respectively, in both boys and girls. Together, these findings highlight the benefit of more vigorous physical activity, with less sedentary behaviour, regardless of total daily movement volume, in lowering subsequent BMI, in contrast to the role of total movement, regardless of intensity, in reducing the risk of early puberty timing.

6.6.3 Potential mechanisms

It has been speculated that adiposity may underlie the association between physical activity and puberty timing[257]. In particular, leptin from adipose tissue may regulate the reproductive hormone axis[114] or gonadotropin-releasing hormone pulsatility[258] that are involved in pubertal development. Nonetheless, in the present study, the associations of higher total counts with

lower risks for earlier timing of several puberty traits in boys and girls remained robust on adjustment for covariates including BMI before and during puberty. Furthermore, the associations of physical activity measures (i.e. total volume and intensities, respectively) with puberty timing and BMI were nearly mutually exclusive. These observations suggest that there may be other non-adiposity underlying mechanisms linking higher total movement volume to subsequent later puberty timing, as supported by genetic causal findings of sedentary behaviour and earlier puberty. Therefore, it is possible that a higher total volume of physical activity, achieved through increased intensity in any level or decreased sedentary time, may stimulate more gain in muscle mass, increase muscle absorption of glucose (by muscle GLUT4 isoforms)[259] and reduce insulin resistance, in turn lowering bioavailability of sex steroids and delaying pubertal development in boys and girls[32].

6.6.4 Strengths and limitations

Cautious interpretation of the present findings is required due to several limitations. A large proportion of MCS children had to be excluded due to missing accelerometery measures and even more due to unclassifiable timing of puberty traits as a result of the wide range of ages at each assessment of pubertal status. Such issue prompts future cohorts to assess pubertal development more frequently, with shorter intervals between visits (ideally one year), or record actual puberty timing. While modest differences in maternal and child characteristics were found between the excluded and included children, these factors were adjusted for in analyses to minimize the risk of selection bias.

Time spent in sedentary behaviour may be underestimated owing to the use of a short period (20 minutes) of consecutive zero-counts to define non-wear time. The average movement intensity across physical activity intensities were estimated rather than individually calculated, but in support of this approach, the estimated sum of the counts was nearly perfectly correlated with the empirical total counts. The potential bias due to estimation errors of counts

in each physical activity intensity was further minimised by adjusting analyses for the empirical total counts instead of the estimated total counts. The measure of physical activity was collected over two to seven consecutive days only at a single time period, which may not reflect the habitual activity throughout prepubertal childhood and this is likely to introduce random error.

Pubertal development and age at menarche were reported by parents and children using an unvalidated questionnaire, which introduce error. An objective measure of puberty timing such as age at PHV could not be examined since height was not regularly measured. Also, categorical timings of puberty traits were mainly analysed due to the nature of collected data, which does not allow to evaluate any continuous dose-effect relationship.

A modest surprisingly positive association between total counts and BMI was found in both sexes only in the models that also included physical activity intensities. Such appearance of associations only in multi-exposure models is suggestive of collider bias[260] – hence the primary models for total counts are those with the single exposure. Furthermore, the findings from current phenotypic analyses, despite the adjustment for many covariates, may be subject to residual confounding due to unmeasured factors such as specific dietary factors as revealed in **Chapters 4** and **5**. Nevertheless, the MR analyses that are more robust to confounding showed similar findings.

The current MR analyses may be still susceptible to other sources of biases. Physical activity measures analysed in previous GWASs were largely self-reported and thus errors may exist. The genetic instruments for the physical activity traits were identified using a statistical rather than biological approach and may not be specific, but Steiger filtering and Radial regressions were used to reduce the possibility of reverse causality and horizontal pleiotropy (heterogeneity). Nonetheless, given the weak instruments for the physical activity intensities (all comprising fewer than 10 SNPs), these MR findings should be considered as inconclusive rather than null. While television viewing and leisure computer use represent types of sedentary

behaviours, it is unclear why a sex difference was seen in the association of genetically predicted time spent in leisure computer use with puberty timing. Such differences may possibly be explained by sex-specific preference for the types of sedentary behaviour[261].

6.6.5 Conclusions

Using accelerometer-based measures during prepubertal childhood, higher total movement volume and lower sedentary behaviour but not any specific intensity of physical activity, were associated with lower risks for earlier timing of several puberty traits including puberty progression in both boys and girls. These associations were independent of BMI during prepuberty and puberty. Evidence from MR analyses supports a direct causal relationship between sedentary behaviour and early puberty timing in both sexes. These findings suggest that increasing overall physical activity by any intensity, and concomitantly reducing time in sedentary behaviour, may potentially contribute to interventions to avoid early timing of puberty in boys and girls.

CHAPTER 7 DISCUSSIONS AND IMPLICATIONS

7.1 Summary of research rationale and findings

This thesis aimed to gain insights into specific lifestyle-related factors that influence the timing of pubertal development in boys and girls, from an aetiological perspective. To provide robust research findings, integrative data analyses, study designs with high levels of evidence and triangulation approaches were adopted, along with the considerations of life course, nutritional, molecular, physical activity and genetic epidemiology. All main findings in this thesis are depicted in **Figure 7.1**.

7.1.1 The adiposity-independent association of early puberty with T2D

Puberty involves various physiological and physical development relating to sexual maturity between childhood and adulthood. Over the past decades, average age at the occurrence of puberty has been becoming earlier globally. Such secular trends are worrying since a large body of studies has reported the associations between early puberty timing and subsequent adverse health outcomes, particularly among women. However, whether its mechanism is dependent on higher adiposity has been uncertain. **Chapter 2** aimed to systematically review existing published findings of the associations of puberty timing with T2D/IGT risk in men and women, with and without adjustment for adiposity. A total of 28 studies in Western and Asian settings were identified. Meta-analyses of these studies using inverse-variance-weighted random-effects models showed that both earlier age at menarche (continuous) and early (versus later) menarche were consistently associated with higher T2D/IGT risk amongst 1,228,306 women. These associations were stronger among whites than Asians and among populations with earlier average age at menarche. A considerable percentage of T2D cases possibly due to early menarche among British women was further estimated. Similarly,

the only one identified male study reported that relatively younger (versus 'about average') voice breaking was associated with higher risk of T2D in 197,714 British men. The associations between puberty timing and T2D/IGT risk in both sexes were partially attenuated but persisted after adjustment for adiposity indicators mainly in adulthood.

7.1.2 The direct role of total energy and protein intakes in puberty timing

Given the associations of childhood rapid growth and overweight/obesity with earlier puberty timing and the partial mediation effect of adiposity in the association of early puberty with future diseases, thorough investigations into the role of lifestyle exposures in puberty timing are of importance to public health. Although there have been studies on dietary intakes and puberty timing, findings were conflicting and the strength of evidence was weakened by several methodologies, especially the collection of dietary data within the typical puberty periods (which risks bias by reverse causality). In **Chapter 4**, the prospective associations of intakes of total energy and macronutrients throughout prepubertal childhood (summarised from age 3 to 7.5 years using random intercepts linear regression models) with puberty timing were assessed. Within the ALSPAC study, higher TEI among boys (n=3811) and girls (n=3919), and higher total protein including both animal and plant-based proteins intakes (in *energy partition, nutrient density* and *residual models*) only among girls, were associated with earlier timings of puberty onset (G2 or B2) and progression (voice breaking or menarche) and peak height velocity (an objective measure). These associations did not change, with additional adjustment for adiposity during puberty (summarised from age 9 to 15 years).

7.1.3 Sexual disparity in the effect of n-6 PUFAs on puberty timing

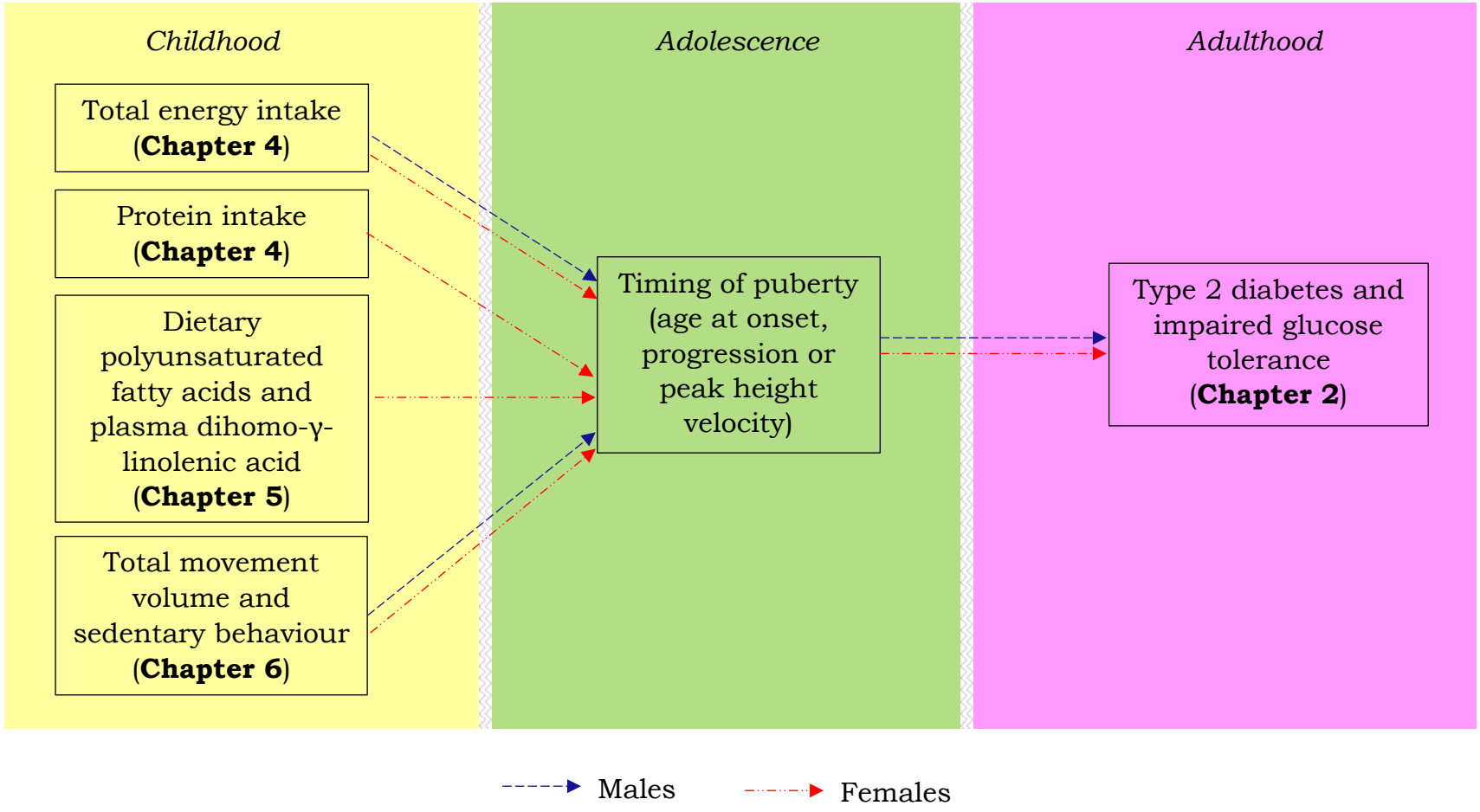
Since fat quality including individual FAs plays a vital role in determining cardiometabolic risk, **Chapter 5** continued to examine the associations of dietary fat quality and plasma phospholipid FAs with subsequent puberty timing. Using dietary data throughout prepubertal childhood in the ALSPAC study, higher dietary PUFA intake (in *nutrient density* and *residual models*) was associated with earlier timings of all primary puberty traits in girls (n=3872), but not boys (n=3654). Detailed analyses of individual plasma phospholipid FAs at age 7.5 years also revealed that higher plasma concentrations of an intermediate metabolite of plasma phospholipid n-6 PUFAs (dihomo-γ-linolenic acid, 20:3n6) and one of the most abundant MUFAs in adipose tissue (palmitoleic acid, 16:1n7) were associated with earlier puberty timing only in girls; however, the latter association was largely attenuated on additional adjustment for BMI at 7.5 years. Also, genetic causal modelling, namely two-sample MR analyses using GWAS summary statistics from large consortia, indicated that higher genetically predicted circulating dihomo-γ-linolenic acid but not palmitoleic acid was associated with earlier menarche in girls.

7.1.4 The differential associations of total volume and intensities of physical activity with puberty timing

Physical activity is another key lifestyle behaviour that affects health, besides dietary intakes. However, existing findings on physical activity and puberty timing were scarce and suffered from several limitations including reverse causality due to the assessment of physical activity after the typical ages at puberty onset and a short follow-up of puberty progression status. Therefore, **Chapter 6** aimed to investigate the longitudinal associations of physical activity measures with timings of several puberty traits. In the UK MCS which objectively measured physical activity at age 7 years using accelerometers,

higher daily total movement volume (i.e. total counts) but not intensities was associated with lower risks for earlier timing of puberty progression and other puberty traits (growth spurt, body hair growth and/or skin changes) in both boys (n=2531) and girls (n=3079). Longer time spent in sedentary behaviour was also associated with higher risks for earlier puberty timing in girls. These associations were independent of prepubertal and pubertal BMI. Consistently, in MR models, more time in sedentary behaviour but not any physical activity intensity was associated with earlier puberty timing in both sexes, and these associations persisted on adjustment for childhood size.

Figure 7.1 Summary of the main findings of the thesis



Arrows indicate possible causal associations, not or only partially mediated by adiposity

7.2 Interpretation of findings

Adiposity, either prior to or after puberty, has been postulated to link early puberty timing to subsequent adverse health outcomes. However, the only partial attenuation in the adult-adiposity-adjusted associations between early puberty and T2D/IGT risk in **Chapter 2** indicate that the associations may be not completely mediated by adiposity during adulthood and also not confounded by prepubertal adiposity tracking to adulthood[127, 128]. Therefore, together with the findings for other diseases from a large UK Biobank[33], it can be deduced that early puberty may lead to the development of many diseases through not only adiposity- but also other non-adiposity-related pathways. This highlights the importance of identifying potential modifiable factors to avoid early puberty and suggests that these could act only partly via adiposity.

The longitudinal cohort and MR analyses employed in **Chapters 4**, **5** and **6** attempt to draw causal inferences. In particular, in the longitudinal design, dietary intakes and physical activity during prepubertal childhood but not at later periods were intentionally analysed to represent the potential exposures. Additional evidence from MR analyses functions to consolidate the hypothesised causal directions since internal validity of phenotypic associations may be questioned due to residual confounding. Effectively, findings in **Chapters 4**, **5** and **6** could be confidently interpreted that TEI, dietary protein and PUFA intakes, plasma dihomo-γ-linolenic acid, total volume of physical activity and sedentary behaviour can affect the timings of pubertal onset and/or progression. These causal pathways are not mediated by other co-dietary or physical activity factors and adiposity, as implied by the robustness or the lack of attenuation in findings with considerations of those covariates. Moreover, the opposite associations of TEI and total movement volume (i.e. energy expenditure) with puberty timing in both sexes emphasise the possible contribution of energy balance in triggering the puberty processes. The effect of sedentary behaviour on puberty timing may be similarly explained by energy balance, but there may be other metabolic processes involved. The strong associations of dietary protein and PUFA

intakes with puberty timing in energy adjustment models may indicate possible alternative direct effects of macronutrient molecules rather than simply the energy contributed by macronutrients on pubertal development. The consistent associations of dietary protein and PUFA intakes, plasma dihomo-γ-linolenic acid with puberty timing only in girls further suggest sexual dimorphic response of pubertal initiation and development to macronutrients. Overall, the present findings demonstrate that there may be multiple independent pathways leading to early puberty especially among girls.

7.3 Public health implications

Results in this thesis, as summarised in **Section 7.1**, have several implications for public health policy. **Chapter 2** proposes early puberty (versus the population average) as the crucial early life risk factor for future development of glucose intolerance and diabetes, regardless of body weight status. Hence, the awareness of long-term adverse health consequences of early sexual maturation should be promoted among parents or caregivers. Also, assessment of pubertal status during early childhood should be implemented in any routine clinical screenings especially among the populations or subgroups with decreasing average age at puberty to identify the high-risk groups for early interventions.

Chapters 4, **5** and **6** recommend several specific lifestyle behaviours during young childhood, not merely focusing on child growth and adiposity, to avoid early puberty. Foremost, given that current intakes of dietary energy and protein are high among children worldwide[223, 224], **Chapter 4** gives prominence to following local recommended levels of their intakes, besides giving attention to the sources of protein. Also, while dietary PUFA is thought to be the alternative to SFA mainly for better cardiometabolic health, **Chapter 5** raises the concern on the possible detrimental effect of higher PUFA intake, especially n-6 PUFAs which are found in typical Western diets[223], in triggering

earlier puberty timing. Therefore, increase in intake of seafood as the source of n-3 PUFAs to substitute for n-6 PUFAs and SFA among British children may optimise overall health. In addition to controlling the daily amount of energy intake, **Chapter 6** suggests the possibility of increasing physical activity by any intensity, while reducing sedentary behaviour, to avoid the occurrence of early puberty. Although these prevention strategies may be independent of each other, they should be collectively considered to maximize their effectiveness.

7.4 Strengths and limitations

The present works contain strengths and limitations, which need to be considered when interpreting findings. In this section, overall strengths and limitations of the major part of this thesis (i.e. **Chapters 4**, **5** and **6**) are discussed. The strengths and limitations specific to each topic, especially for **Chapter 2**, have been described in the related chapters.

This thesis extends the previous evidence base in many ways including a prospective study design with a long follow-up duration, analyses of both subjective and objective measures that were repeatedly collected, and the use of multiple analytical approaches. While data on dietary intakes and physical activity likely before the usual age at puberty onset were analysed, pubertal status was recorded until young adulthood, which precludes the concern of reverse causality. More strategies were taken to reduce measurement errors, which were i) the synthesis of repeated parent-reports of dietary intakes from age 3 to 7.5 years, ii) the estimation of puberty timing in both boys and girls from the annual reports of pubertal status up to nine visits among those with high consistencies in the repeated reports of pubertal development (i.e. sequential events with increasing age) and iii) the inclusion of objective measures of dietary fat quality intake (i.e. plasma phospholipid FAs), puberty timing (i.e. age at PHV) and physical activity (i.e. accelerometry measures). Also, the robustness of findings from the same research questions were

examined, whenever possible, using two or more methods with different sources or directions of biases[262], namely subjective versus objective measures and/or phenotypic versus MR analyses. Other strengths of this thesis included larger sample sizes that enhance power of analysis, consideration of more specific dietary and physical activity factors, and adjustment for a number of covariates including potential confounders (i.e. maternal and child characteristics), mediators (i.e. adiposity indicators) and co-dietary or physical activity factors in phenotypic analyses.

Despite the abovementioned strengths, several common limitations in this thesis need to be mentioned. A considerable number of children was excluded due to missing data on exposures and/or outcomes and they were different in several maternal and child characteristics from those analysed children. These characteristics were however considered in the analyses to reduce possible selection bias. Also, there were some missing data on exposures and/or covariates, which were imputed using multiple imputation by chained equations, assuming data were missing at random, to avoid information bias. Although repeatedly reported measures of dietary intakes and pubertal development were combined, systematic error may arise when the measures were consistently under- or over-reported across visits. In the observational findings, the possibility of residual confounding due to unknown or unmeasured factors could not be excluded. On the other hand, in the MR analyses, identified genetic instrumental variables for exposures of interest may not be specific, which could lead to horizontal pleiotropic effects and bias the estimates of exposure-outcome associations towards the null. Finally, study populations in this thesis were predominantly White Europeans in the UK and hence findings may not be generalisable to other ethnic groups or populations.

7.5 Opportunities for future research

This thesis provides foundations for further studies related to puberty timing. The directions of future research are divided into different parts, as elaborated below.

7.5.1 The causal effect of puberty timing on later diseases

Given that current findings on the associations of puberty timing with adult T2D (and also other diseases) are largely based on observational studies (**Chapter 2**), future MR analyses using genetic instrumental variables for puberty timing are required to infer causality. Besides age at menarche in females, MR studies should include timing of other puberty traits in both sexes that have been analysed throughout this thesis. Also, multivariable MR models adjusting for potential confounders and/or mediators such as adiposity would be able to disentangle direct and indirect effects of puberty timing on diseases. Upon confirmation of the causal associations, pooling of phenotypic data in various ethnicities is warranted to define thresholds of age at puberty for increased disease risk and better inform intervention strategies in targeted populations.

7.5.2 Biological mechanisms underlying early puberty timing

Timings of several pubertal traits examined in this thesis (**Chapters 4**, **5** and **6**) were mainly based on the reported physical changes in secondary sexual characteristics. Future phenotypic or MR analyses may therefore consider investigating associations between the specific lifestyle factors identified in this thesis and biomarkers of puberty timing such as sex hormones, to confirm current findings. Moreover, the present studies could be advanced by analysing i) more dietary biomarkers detected from the emerging omics approaches including proteomic and metabolomic markers[263], ii) more levels of device-measured physical activity intensities to better exemplify different

real-life activities and iii) potential biomarkers of physical activity[264]. These objective measures of lifestyle factors should be repeatedly measured in future studies to better estimate habitual exposures prior to puberty. Furthermore, mediation analyses with additional consideration of other biological measures including fasting insulin and leptin are warranted to intensify the understanding of aetiological pathways to early sexual maturation.

7.5.3 Relevance of the observed associations between lifestyle factors and puberty timing to NCDs

Findings in **Chapters 4**, **5** and **6** suggest strategies to avoid early puberty timing, with the intention of lowering the risk for future development of NCDs including T2D (**Chapter 2**). Nevertheless, such extrapolation of findings does not completely explain the whole causal mechanisms. Whether the identified modifiable factors of puberty timing will affect disease risk in adulthood and whether such effects are mediated by physiological factors such as insulin sensitivity and IGF1 that regulate process of pubertal development deserve further studies. Outcomes of the research may enlighten the development or repurposing of drugs to prevent and treat early sexual maturation and/or related diseases.

7.6 Conclusions

This thesis undertakes an interdisciplinary approach and reveals specific dietary and physical activity factors during prepubertal childhood that potentially modify the timing of pubertal development in boys and girls. First, meta-analysis of Western and Asian studies showed that adiposity may not entirely mediate the causal pathway between early puberty and T2D, consistent with the findings of early puberty and other diseases from a large British study. Next, based on a variety of data types and analytical triangulation methodologies in analysing comprehensive phenotypic data

from young childhood to early adulthood in longitudinal British birth cohort studies and GWAS summary statistics from large consortia in the UK, higher dietary intakes of total energy in boys and girls, protein (including all protein types) and PUFA (that increases the circulating concentration of an intermediate metabolite n-6 PUFAs or dihomo-γ-linolenic acid, 20:3n6) only in girls, and lower total daily volume of physical activity and more time spent in sedentary behaviour in both sexes may be causally associated with earlier puberty timing, without acting through adiposity. These findings suggest that the timing of puberty is a behaviourally modifiable trait and inform future design of strategies to avoid early puberty timing as part of a life course approach to prevention of diseases.

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APPENDIX A SUPPLEMENTARY TABLES

Supplementary tables related to chapters in this thesis are as follows:

Chapter 2 - **Supplementary Table A-1** to **A-6**

Chapter 4 - Supplementary Table A-7 to **A-15**

Chapter 5 - Supplementary Table A-16 to **A-24**

Chapter 6 - **Supplementary Table A-25** to **A-30**

Supplementary Table A-1 Search strategy in different databases

PubMed	Medline	Embase
(puberty OR pubertal OR menarche OR Tanner[Text Word] OR (voice (break OR breaking)) OR sexual maturation) AND (diabetes OR diabetic OR insulin OR blood sugar OR glucose OR ((glycated OR glycosylated) AND (haemoglobin OR haemoglobin))) AND ("Epidemiologic studies"[Mesh] OR "case control studies"[Mesh] OR "cohort studies"[Mesh] OR Case control[Text Word] OR cohort stud*[Text Word] OR Cohort analy*[Text Word] OR Follow up stud*[Text Word] OR observational stud*[Text Word] OR (observ*[Text Word] association*[Text Word]) OR Longitudinal[Text Word] OR Retrospective[Text Word] OR Recall*[Text Word] OR Cross sectional[Text Word] OR "Cross-sectional studies"[Mesh] OR (nation*[Text Word] stud*[Text Word]) OR (nation*[Text Word] survey*[Text Word]) OR mendelian randomi*[Text Word])	1. puberty.mp. or exp puberty/ 2. pubertal.mp. 3. menarche.mp. or exp menarche/ 4. Tanner.tw. 5. (voice adj (break or breaking)).mp. 6. sexual maturation.mp. or exp sexual maturation/ 7. 1 or 2 or 3 or 4 or 6 8. diabetes.mp. or exp diabetes mellitus/ 9. diabetic.mp. 10. exp insulin sensitivity/ or insulin.mp. or exp insulin resistance/ 11. blood sugar.mp. or exp glucose blood level/ 12. glucose.mp. 13. exp glycosylated hemoglobin/ 14. or/8-13 15. 7 and 14 16. Epidemiologic studies/ 17. exp case control studies/ 18. exp cohort studies/ 19. Case control.tw. 20. (cohort adj (study or studies)).tw. 21. Cohort analy$.tw. 22. (Follow up adj (study or studies)).tw. 23. (observational adj (study or studies)).tw. 24. Longitudinal.tw. 25. Retrospective.tw. 26. Cross sectional.tw. 27. Cross-sectional studies/ 28. (observ* adj (association or associations)).tw. 29. (nation$ adj (study or studies or survey or surveys)).tw. 30. recall*.tw. 31. exp Mendelian Randomization Analysis/ or mendelian randomi*.mp. 32. or/16-31 33. 15 and 32	1. puberty.mp. or exp puberty/ 2. pubertal.mp. 3. menarche.mp. or exp menarche/ 4. Tanner.tw. 5. (voice adj (break or breaking)).mp. 6. sexual maturation.mp. or exp sexual maturation/ 7. 1 or 2 or 3 or 4 or 6 8. diabetes.mp. or exp diabetes mellitus/ 9. diabetic.mp. 10. exp insulin sensitivity/ or insulin.mp. or exp insulin resistance/ 11. blood sugar.mp. or exp glucose blood level/ 12. glucose.mp. 13. exp glycosylated hemoglobin/ 14. 8 or 9 or 10 or 11 or 12 or 13 15. 7 and 14 16. clinical study/ 17. Case control study/ 18. Family study/ 19. Longitudinal study/ 20. Retrospective study/ 21. Prospective study/ 22. Cohort analysis/ 23. (Cohort adj (study or studies)).mp. 24. (Case control adj (study or studies)).tw. 25. (follow up adj (study or studies)).tw. 26. (observational adj (study or studies)).tw. 27. (epidemiologic$ adj (study or studies)).tw. 28. (cross sectional adj (study or studies)).tw. 29. (observ* adj (association or associations)).tw. 30. (nation$ adj (study or studies or survey or surveys)).tw. 31. recall*.tw. 32. exp Mendelian randomization analysis/ or mendelian randomi*.mp. or exp Mendelian randomization/ 33. or/16-32 34. 15 and 33

Supplementary Table A-2 Summary of eligible studies for prevalent diabetes and/or impaired glucose tolerance

First author, year	Year at enrolment	Outcome, definition	Measures of association	Controlled variables without anthropometric adiposity measures		Controlled variables with anthropometric adiposity measures	
				Risk (95% CI)	Adjustment	Risk (95% CI)	Adjustment
Cooper, 2000[138]	1934-1939	Diabetes: Self-reported physician diagnosis	Odds Ratio	-	-	1.1 (0.9, 1.3) per year later	Age and BMI at age 30
Saquib, 2005[131]	1984-1987	Diabetes: fasting glucose≥7.0 mmol/L (≥126 mg/dL), 2-hour glucose≥11.1 mmol/L (≥200 mg/dl), a previous physician diagnosis or use of anti-diabetic medication		-	-	<12 vs. ≥16 (Ref): 2.27 (0.62, 9.09)[a]	Age, number of pregnancies, exercise≥3 times/week, cigarette smoking, oestrogen use, family history of diabetes and BMI
		Impaired glucose tolerance: fasting glucose=6.1 mmol/L (110 mg/dL) - 7.0 mmol/L or 2-hour glucose=7.8 mmol/L (140 mg/dL) - 11.1 mmol/L		-	-	<12 vs. ≥16 (Ref): 0.93 (0.47, 1.85)[a]	Age, number of pregnancies, exercise≥3 times/week, cigarette smoking, oestrogen use, family history of diabetes and BMI
Heys, 2007[140]	2003-2004	Impaired glucose tolerance: fasting glucose>5.6 mmol/L or use of anti-diabetic medication	Odds Ratio	<12.5 vs. ≥14.5 (Ref): 1.40 (1.15, 1.71)	Age, education and number of pregnancies	<12.5 vs. ≥14.5 (Ref): 1.33 (1.08, 1.63)	Previous model + waist circumference
Lakshman, 2008[132]	1993-1997	Diabetes: Self-reported physician diagnosis or use of diabetes-specific medication	Odds Ratio	0.91 (0.87, 0.96) per year later	Age at baseline, smoking, occupational social class, educational level, physical activity, family history of diabetes, reproductive factors parity, oral contraceptive and use hormone replacement therapy	0.98 (0.93, 1.03) per year later	Previous model + BMI
				8-11 vs. 15-18 (Ref): 1.52 (1.18, 1.96)[a]	Age at baseline, smoking, occupational social class, educational level, physical activity and family history of diabetes	-	-
Akter, 2012[141]	2009-2010	Diabetes: physician diagnosis or anti-diabetic medication and impaired glucose tolerance: fasting glucose≥6.1 mmol/L (≥110 mg/dL)	Odds Ratio	<12 vs. >13-16 (Ref): 0.65 (0.46, 0.93)	Age, education, marital status, use of tobacco products, ever use of contraceptives, and number of pregnancies	-	-

[a]Results were computed with the reciprocal of risk estimates at highest category

Supplementary Table A-2 Summary of eligible studies for prevalent diabetes and/or impaired glucose tolerance (continued)

First author, year	Year at enrolment	Outcome, definition	Measures of association	Controlled variables without anthropometric adiposity measures		Controlled variables with anthropometric adiposity measures	
				Risk (95% CI)	Adjustment	Risk (95% CI)	Adjustment
Dreyfus, 2012[154]	1987-1989	Diabetes: fasting glucose≥7.0 mmol/L (126 mg/dL), non-fasting glucose>11.1 mmol/L (200 mg/dl), self-reported physician-diagnosis or use of hypoglycaemic medication at 30 years or older	Odds Ratio	8-11 vs. 13 (Ref): 1.37 (1.12, 1.68)	Age at baseline, race, centre, family history of diabetes, smoking status, use of oral contraceptives and education	8-11 vs. 13 (Ref): 1.20 (0.97, 1.48)	Previous model + baseline BMI, height and waist circumference
Pierce, 2012[139]	1946	Diabetes: Ever treated with diet or oral hypoglycaemic agents, or who had insulin added more than 2 years after diagnosis at 30 years or older	Hazard Ratio	0.72 (0.52, 0.99) per year later	No	0.86 (0.63, 1.18) per year later	Adult BMI
Stockl, 2012[156]	2006-2008	Diabetes: use of glucose-lowering medication, self-reported physician diagnosis, fasting glucose≥7.0 mmol/L (≥126 mg/dL) or 2-hour glucose≥11.1 mmol/L (≥200 mg/dL)	Relative Risk	0.83 (0.73, 0.95) per year later	Year of birth, physical activity, education, marital status, smoking, alcohol consumption and menopausal status	0.84 (0.73, 0.98) per year later	Previous model + current BMI
		Prediabetes: fasting glucose=6.1 mmol/L - 6.9mmol/L or 2-hour glucose=7.8 mmol/L (140 mg/dL) - 11.1 mmol/L		0.91 (0.85, 0.98) per year later		0.92 (0.85, 0.99) per year later	
		Diabetes and prediabetes		0.88 (0.83, 0.94) per year later		0.89 (0.83, 0.95) per year later	
Qiu, 2013[142]	2011-2012	Diabetes: fasting glucose≥7.0 mmol/L (≥126 mg/dL) or 2-hour glucose≥11.1 mmol/L (≥200 mg/dL), a previous physician diagnosis or use of anti-diabetic medication	Odds Ratio	9-14 vs. 16 (Ref): 0.94 (0.70, 1.26)	Age at enrolment, physical activity, parity, smoking, alcohol consumption, family history of diabetes, age at menopause and type of menopause	9-14 vs. 16 (Ref): 0.90 (0.66, 1.21)	Previous model + BMI and waist circumference
Mueller, 2014[155]	2008-2010	Diabetes: self-report diagnosis, use of medication for diabetes, fasting glucose≥126 mg/dL, 2-hour glucose≥200 mg/dL or HbA1c≥6.5%	Relative Risk	<11 vs. 13-14 (Ref): 1.34 (1.14, 1.57)	Age at enrolment, study centre, race, maternal education, maternal diabetes, paternal diabetes and birth weight	<11 vs. 13-14 (Ref): 1.26 (1.07, 1.49)	Previous model + BMI at age 20 years

[a]Results were computed with the reciprocal of risk estimates at highest category

Supplementary Table A-2 Summary of eligible studies for prevalent diabetes and/or impaired glucose tolerance (continued)

First author, year	Year at enrolment	Outcome, definition	Measures of association	Controlled variables without anthropometric adiposity measures		Controlled variables with anthropometric adiposity measures	
				Risk (95% CI)	Adjustment	Risk (95% CI)	Adjustment
Baek, 2015[143]	2012-2013	Diabetes: self-report physician diagnosis, use of insulin or hypoglycaemic medication, fasting glucose≥126 mg/dL (7.0 mmol/L) or HbA1c≥6.5% (48 mmol/mol)	Odds Ratio	<13 vs. 13-16 (Ref): 2.43 (1.04, 5.69)	Age at enrolment, education, spouse, income, parity, menopause status, smoking, alcohol and physical activity	<13 vs. 13-16 (Ref): 2.10 (0.86, 5.14)	Previous model + BMI
		Prediabetes: fasting glucose=100-125 mg/dL (5.6mmol/L-6.9 mmol/L) or HbA1c=5.7%-6.4% (39-46 mmol/mol)		<13 vs. 13-16 (Ref): 1.80 (1.24, 2.61)		<13 vs. 13-16 (Ref): 1.63 (1.11, 2.39)	
		Diabetes + Prediabetes		13-16 (Ref), <13: 1.85 (1.28, 2.66)		13-16 (Ref), <13: 1.66 (1.14, 2.41)	
Day, 2015[33]	2006-2010	Diabetes: Self-report physician diagnosis, excluding possible type 1 diabetes (based on age at diagnosis≤35, use of insulin within 1 year of diagnosis or diagnosis less than 1 year before enrolment)	Odds Ratio	0.87 (0.85, 0.88) per year later; 8-11 vs. 13-14 (Ref): 1.76 (1.62, 1.91) relatively younger vs. about average (Ref): 1.44 (1.30, 1.59)	Birth year, age and age-squared	0.94 (0.92, 0.96) per year later; 8-11 vs. 13-14 (Ref): 1.25 (1.15, 1.36) relatively younger vs. about average (Ref): 1.24 (1.11, 1.37)	Previous model + socioeconomic position (11 principle components) and adiposity/body composition (5 principle components)
Hwang, 2015[144]	2007-2009	Diabetes: Self-report physician diagnosis	Odds Ratio	10-12 vs. 13-15 (Ref): 1.86 (1.07, 3.23)	Age, education, income, use of hormonal medication, smoking status, alcohol use, exercise status and diagnosis of hypertension, dyslipidaemia, and cardiac disease	10-12 vs. 13-15 (Ref): 1.82 (1.03, 3.23)	Previous model + BMI and waist circumference
Lim, 2015[145]	2007-2009	Diabetes: use of a glucose-lowering medication, self-report physician diagnosis, fasting glucose≥126 mg/dL	Odds Ratio	<12 vs. ≥12 (Ref): 3.61 (1.90, 6.88)	Age	<12 vs. ≥12 (Ref): 2.52 (1.29, 4.94)	Previous model + BMI
Cao, 2016[134]	2011-2014	Impaired glucose tolerance: fasting glucose≥5.6 mmol/L	Odds Ratio	-	-	11-13vs. 16-20 (Ref): 0.83 (0.62, 1.10)[a]	Age, education level, physical activity and BMI

[a]Results were computed with the reciprocal of risk estimates at highest category

Supplementary Table A-2 Summary of eligible studies for prevalent diabetes and/or impaired glucose tolerance (continued)

First author, year	Year at enrolment	Outcome, definition	Measures of association	Controlled variables without anthropometric adiposity measures		Controlled variables with anthropometric adiposity measures	
				Risk (95% CI)	Adjustment	Risk (95% CI)	Adjustment
Won, 2016[152]	2010-2013	Diabetes: Self-reported physician diagnosis	Odds Ratio	<11 vs. ≥17 (Ref): 1.72 (0.94, 3.15)	Age, current smoking, college graduation and menstruation	-	-
Yang, 2016[146]	2011-2013	Diabetes: use of anti-diabetic medication, fasting glucose ≥7.0 mmol/L (126 mg/dl.)	Odds Ratio	≤12 vs. 15-16 (Ref): 1.60 (1.16, 2.22)	Age	≤12 vs. 15-16 (Ref): 1.44 (1.02, 2.03)	Age, education, marital status, occupation, smoking status, drinking, hypertension, abnormal lipid, family history of diabetes, age at menopause and BMI
Au Yeung, 2017[126]	2003-2008	Diabetes: use of anti-diabetic medication, fasting glucose ≥7.0 mmol/L (126 mg/dl.)	Odds Ratio	0.65 (0.32, 1.33) per year later	Mendelian Randomization	-	-
				0.92 (0.89, 0.95) per year later	Education, recruitment phase, age, smoking, alcohol use, physical activity, job type, corresponding medications such as antihypertensive for blood pressure	-	-
Farahmand, 2017[150]	1998	Diabetes: fasting glucose≥7.0 mmol/L (126mg/dL), 2-hour glucose≥11.1 mmol/L (200 mg/dL)	Odds Ratio	<11 vs. 13-14 (Ref): 2.70 (1.40, 5.20)	Family history of diabetes, parity, education and age	<11 vs. 13-14 (Ref): 3.28 (1.50, 7.10)	Previous model + BMI
		Prediabetes: fasting glucose=100 mg/dL (5.6 mmol/L) -125 mg/dL (6.9 mmol/L) or 2-hour glucose=140 mg/dL (7.8 mmol/L) -199 mg/dL (11.0 mmol/L)		<11 vs. 13-14 (Ref): 3.74 (1.60, 8.60)		<11 vs. 13-14 (Ref): 3.56 (1.20, 10.20)	
Petersohn, 2019[157]	1999-2000	Diabetes: self-report physician diagnosis or random blood glucose>200 mg/dL	Relative Risk	-	-	0.95 (0.83, 0.98) per year later	BMI, age BMI-age interaction, family history of diabetes

Supplementary Table A-3 Summary of eligible studies for incident diabetes and/or impaired glucose tolerance

First author, year	Year at enrolment	Outcome, definition	Measures of association	Controlled variables without anthropometric adiposity measures		Controlled variables with anthropometric adiposity measures	
				Risk (95% CI)	Adjustment	Risk (95% CI)	Adjustment
He, 2010[153]	1980	Diabetes: fasting glucose≥7.8 mmol/L (≥140 mg/dL) or ≥7.0 mmol/L (≥126 mg/dL) (after 1997), 2-hour/ random glucose≥ 11.1mmol/L (≥200 mg/dL), at least one symptom related to diabetes (excessive thirst, polyuria, weight loss, hunger) or treatment with insulin/ oral hypoglycaemic medication	Odds Ratio	0.94 (0.92, 0.95) per year later; ≤11 vs. 13 (Ref): 1.21 (1.13, 1.31)	Age groups, birth weight, having been breastfed, childhood socioeconomic status, ethnicity, family history of diabetes, perceived body figure at age 10 years, the baseline factors physical activity, quintile of dietary score, alcohol consumption, smoking status, hypertension, hypercholesterolemia, menopause status, use of hormone replacement therapy, adult socioeconomic status, reproductive factors (parity, oral contraceptive use, and regularity of menstrual cycles at ages 18–22 years)	0.99 (0.97, 1.01) per year later; 13 (Ref), ≤11 vs. 13 (Ref): 1.02 (0.95, 1.10)	Previous model + BMI over the course of follow-up
	1991			0.88 (0.86, 0.91) per year later; ≤11 vs. 13 (Ref): 1.50 (1.34, 1.69)		0.97 (0.94, 1.00) per year later; ≤11 vs. 13 (Ref): (1.02, 1.29)	
Conway, 2012[133]	1997-2000	Diabetes: fasting glucose≥7 mmol/L, oral glucose tolerance test≥11.1 mmol/L and/or use of a hypoglycaemic agent	Hazard Ratio	0.95 (0.92, 0.98) per year later; 8-13 vs. 17-26 (Ref): 1.35 (1.14, 1.59)[a]	Birth cohort, education and income	0.98 (0.95, 1.01) per year later; 8-13 vs. 17-26 (Ref): 1.14 (0.95, 1.33)[a]	Previous model + participation in team sports during adolescence, BMI at baseline and BMI at age 20
Dreyfus, 2012[154]	1987-1989		Odds Ratio	8-13 vs. 17-26 (Ref): 1.27 (1.02, 1.58)		8-13 vs. 17-26 (Ref): 1.18 (0.95, 1.47)	
Elks, 2013[149]	1991	Diabetes: self-report physician diagnosis, confirmed by medical records, or local and national diabetes and pharmaceutical registers	Hazard Ratio	0.89 (0.86, 0.93) per year later; 8-11 vs. 13 (Ref): 1.70 (1.48, 1.94)	Age at recruitment, date of birth, centre, age at first full-term pregnancy, parity, menopausal status, use of oral contraceptive pill, use of hormone replacement therapy	0.96 (0.91, 1.01) per year later; 8-11 vs. 13 (Ref): 1.42 (1.18, 1.71)	Previous model + adult BMI

[a]Results were computed with the reciprocal of risk estimates at highest category

Supplementary Table A-3 Summary of eligible studies for incident diabetes and/or impaired glucose tolerance (continued)

First author, year	Year at enrolment	Outcome, definition	Measures of association	Controlled variables without anthropometric adiposity measures		Controlled variables with anthropometric adiposity measures	
				Risk (95% CI)	Adjustment	Risk (95% CI)	Adjustment
Dreyfus, 2015[130]	1985	Diabetes: fasting glucose≥7.0 mmol/L (126 mg/dL), HbA1c≥6.5%, 2-hour glucose≥11.1 mmol/L (200 mg/dL), or use of diabetes medication	Hazard Ratio	0.93 (0.86, 1.00) per year later[b], 8-11 vs. 14-17 (Ref): 1.61 (1.09, 2.37)	Age, centre, race, parental history of diabetes, education, pre-high school physical activity, high school physical activity, smoking status, oral contraceptive use, physical activity and alcohol intake	0.90 (0.86, 0.94) per year later[b], 14-17 (Ref), 8-11 vs. 14-17 (Ref): 1.33 (0.90, 1.96)	Previous model + baseline BMI
		Impaired glucose tolerance: fasting glucose=5.6 (100 mg/dL) -6.9 mmol/L, not taking diabetes medication		0.96 (0.89, 1.05) per year later[b], 8-11 vs. 14-17 (Ref): 1.50 (1.17, 1.94)		0.93 (0.88, 0.98) per year later[b], 8-11 vs. 14-17 (Ref): 1.28 (0.99, 1.62)	
LeBlanc, 2017[148]	1993-1998	Diabetes: self-report diagnosis, use of diabetes medications	Hazard Ratio	<12 vs. 12 (Ref): 1.14 (1.08, 1.20)	Age	<12 vs. 12 (Ref): 1.01 (0.95, 1.06)	Age, race, hormone therapy intervention arm membership, baseline physical activity, baseline alcohol consumption, baseline smoking history, education, baseline marital status, number of term pregnancies, family history of diabetes, years since menopause at baseline, baseline waist circumference, history of oral contraceptive use at baseline, baseline metformin use and baseline BMI
Yang, 2018[125]	2004-2008	Diabetes: Data linkage with the nationwide health insurance system	Hazard Ratio	0.96 (0.94, 0.97) per year later; 13 vs. ≥18 (Ref): 1.33 (1.24, 1.44)[a]	Education, household income, smoking status, alcohol intake, blood pressure, physical activity, menopause status, parity, age at first birth, breastfeeding duration per child, and oral contraceptive use	0.98 (0.97, 1.00) per year later	Previous model + baseline BMI and waist circumference

[a]Results were computed with the reciprocal of risk estimates at highest category
[b]Results were computed with the reciprocal of risk estimates per year early age at menarche

Supplementary Table A-3 Summary of eligible studies for incident diabetes and/or impaired glucose tolerance (continued)

First author, year	Year at enrolment	Outcome, definition	Measures of association	Controlled variables without anthropometric adiposity measures		Controlled variables with anthropometric adiposity measures	
				Risk (95% CI)	Adjustment	Risk (95% CI)	Adjustment
Pandeya, 2018[151]	1985-2009	Diabetes: self-report physician diagnosis or information from hospital patient registry data	Relative Risk	≤10 vs. 13 (Ref): 1.63 (1.40, 1.89)	Women's year of birth, age at baseline, education, smoking status at baseline, number of children, age at first birth, menopausal status/timing and hormone therapy at baseline	≤10 vs. 13 (Ref): 1.18 (1.02, 1.37)	Previous model + baseline BMI
Nanri, 2019[147]	1990, 1993	Diabetes: self-reported physician diagnosis by examining medical records	Odds Ratio	≤13 vs. ≥16 (Ref): 1.09 (0.83, 1.43)[a]	Age, study area, smoking status, alcohol consumption, family history of diabetes mellitus, total physical activity, history of hypertension, total energy intake, coffee consumption, energy-adjusted daily intake of foods or nutrients	≤13 vs. ≥16 (Ref): 1.01 (0.76, 1.33)[a]	Previous model + BMI

[a]Results were computed with the reciprocal of risk estimates at highest category

Supplementary Table A-4 Quality of eligible studies for prevalent diabetes/ impaired glucose tolerance assessed by The Newcastle-Ottawa Quality Assessment Scale for cohort studies

First author, year	1) Truly or somewhat representative of the general population	2) Selection of the non-exposed cohort from the same community as the exposed cohort	3) At least some description of assessment	4) Demonstration that the outcome was not present at the start of study	5a) Controls for age	5b) Controls for additional factors (ethnicity, diet, physical activity)	6) Assessment of outcome – oral glucose tolerance test or record linkage	7) At least 5 years follow-up for outcomes to occur	8) Adequate ≥70% of original cohort	Total (max 7 or 9)
Cooper, 2000[138]	0	1	1	1	1	0	0	1	0	5
Pierce, 2012[139]	1	1	1	1	0	0	0	1	0	5
Saquib, 2015[131]	1	1	1	NA	1	1	1	NA	0	6
Heys, 2007[140]	1	1	1	NA	1	0	1	NA	1	6
Lakshman, 2008[132]	1	1	1	NA	1	1	0	NA	0	5
Akter, 2012[141]	1	1	1	NA	1	0	1	NA	1	6
Dreyfus, 2012[154]	1	1	1	NA	1	0	1	NA	1	6
Stockl, 2012[156]	1	1	1	NA	1	1	1	NA	0	6
Qiu, 2012[142]	1	1	1	NA	1	1	1	NA	1	7
Mueller, 2014[155]	1	1	1	NA	1	0	1	NA	1	6

Supplementary Table A-4 Quality of eligible studies for prevalent diabetes/ impaired glucose tolerance assessed by The Newcastle-Ottawa Quality Assessment Scale for cohort studies (continued)

First author, year	1) Truly or somewhat representative of the general population	2) Selection of the non-exposed cohort from the same community as the exposed cohort	3) At least some description of assessment	4) Demonstration that the outcome was not present at the start of study	5a) Controls for age	5b) Controls for additional factors (ethnicity, diet, physical activity)	6) Assessment of outcome – oral glucose tolerance test or record linkage	7) At least 5 years follow-up for outcomes to occur	8) Adequate ≥70% of original cohort	Total (max 7 or 9)
Baek, 2015[143]	1	1	1	NA	1	1	1	NA	1	7
Day, 2015[33]	1	1	1	NA	1	0	0	NA	1	5
Hwang, 2015[144]	1	1	1	NA	1	1	0	NA	1	6
Lim, 2015[145]	1	1	1	NA	1	0	1	NA	1	6
Cao, 2016[134]	1	1	0	NA	1	1	1	NA	1	6
Won, 2016[152]	1	1	1	NA	1	0	0	NA	1	5
Yang, 2016[146]	0	1	1	NA	1	0	1	NA	1	5
Au Yeung, 2017[126]	1	1	1	NA	1	1	1	NA	1	7
Farahmand, 2017[150]	1	1	1	NA	1	0	1	NA	1	6
Petersohn, 2019[157]	1	1	1	NA	1	0	1	NA	1	6

Supplementary Table A-5 Quality of eligible studies for incident diabetes/ impaired glucose tolerance assessed by The Newcastle-Ottawa Quality Assessment Scale for cohort studies

First author, year	1) Truly or somewhat representative of the general population	2) Selection of the non-exposed cohort from the same community as the exposed cohort	3) At least some description of assessment	4) Demonstration that the outcome was not present at the start of study	5a) Controls for age	5b) Controls for additional factors (ethnicity, diet, physical activity)	6) Assessment of outcome – oral glucose tolerance test or record linkage	7) At least 5 years follow-up for outcomes to occur	8) Adequate ≥70% of original cohort	Total (max 9)
He 2010[153]	1	1	1	1	1	1	1	1	1	9
Conway, 2012[133]	1	1	1	1	1	1	1	1	1	9
Dreyfus, 2012[154]	1	1	1	1	1	0	1	1	1	8
Elks, 2013[149]	1	1	1	1	1	0	1	1	1	8
Dreyfus, 2015[130]	1	1	1	1	1	1	1	1	1	9
LeBlanc, 2017[148]	1	1	1	1	1	1	0	1	1	8
Yang, 2018[125]	1	1	1	1	1	1	1	1	1	9
Pandeya, 2018[151]	1	1	1	1	1	0	1	1	1	8
Nanri, 2019[147]	1	1	1	1	1	1	1	1	0	8

Supplementary Table A-6 Univariable meta-regression results and pooled RRs for T2D/IGT by study subgroups

Study-design-related factors	Non-adiposity adjusted				Adiposity adjusted			
	N	RR (95% CI)	P value[a]	R^2 (%)	N	RR (95% CI)	P value[a]	R^2 (%)
Continuous age at menarche								
Year of enrolment				0				1.3
until 2000	7	0.91 (0.89, 0.94)	-		9	0.98 (0.97, 0.99)	-	
after 2000	4	0.91 (0.87, 0.95)	0.868		3	0.94 (0.90, 0.99)	0.139	
Age at outcome assessment, years				3.1				0
<50	1	0.88 (0.86, 0.91)	-		2	0.97 (0.94, 1.00)	-	
≥50	10	0.92 (0.89, 0.94)	0.297		10	0.97 (0.95, 0.99)	0.939	
Number of variables adjusted				0				0
<5	3	0.90 (0.82, 0.98)	-		3	0.98 (0.88, 1.08)	-	
≥5	8	0.92 (0.89, 0.94)	0.617		9	0.97 (0.95, 0.98)	0.963	
Measures of association				12.2				13.2
Hazards ratio	5	0.93 (0.90, 0.97)	-		5	0.98 (0.97, 0.99)	-	
Odds ratio	5	0.90 (0.88, 0.93)	0.212		5	0.97 (0.95, 1.00)	0.762	
Relative risks	1	0.88 (0.83, 0.94)	0.246		2	0.92 (0.86, 0.98)	0.044	
Early versus later (reference) menarche								
Year of enrolment				0				11.8
until 2000	12	1.39 (1.26, 1.53)	-		12	1.14 (1.06, 1.23)	-	
after 2000	11	1.40 (1.11, 1.78)	0.809		9	1.28 (1.08, 1.51)	0.266	
Age at outcome assessment, years				0				0
<50	7	1.66 (1.13, 2.45)	-		5	1.65 (1.18. 2.29)	-	
≥50	16	1.34 (1.26, 1.48)	0.112		16	1.15 (1.07, 1.24)	0.034	
Number of variables adjusted				0				0
<5	8	1.59 (1.30, 1.95)	-		3	1.32 (0.74, 2.35)	-	
≥5	15	1.31 (1.15, 1.49)	0.248		18	1.18 (1.10, 1.27)	0.973	
Early menarche, years				27.8				0
<12	12	1.50 (1.37, 1.64)	-		10	1.20 (1.11, 1.30)	-	
<14	11	1.29 (1.03, 1.60)	0.045		11	1.20 (1.02, 1.41)	0.482	
Reference age at menarche category, years				0				0
≥12	15	1.43 (1.22, 1.67)	-		13	1.22 (1.12, 1.34)	-	
≥14	8	1.34 (1.18, 1.52)	0.702		8	1.12 (0.97, 1.30)	0.297	
Measures of association				0				0
Hazards ratio	5	1.30 (1.06, 1.60)	-		4	1.18 (0.99, 1.40)	-	
Odds ratio	16	1.43 (1.22, 1.67)	0.538		15	1.21 (1.08, 1.35)	0.817	
Relative risks	2	1.48 (1.22, 1.79)	0.559		2	1.21 (1.09, 1.36)	0.790	

[a]The reference category in meta-regression is the first subgroup in each factor
R^2 (%), % heterogeneity explained

Supplementary Table A-7 Comparisons of characteristics between excluded and included children in the ALSPAC study

	Excluded (n=3059)	Included (n=7730)	P value
Maternal characteristics			
Parity (n, %)			<0.001
0	1119 (39.1)	3407 (45.3)	
1	1064 (37.2)	2746 (36.5)	
2	455 (15.9)	1024 (13.6)	
≥3	221 (7.7)	352 (4.7)	
Highest education level (n, %)			<0.001
None/GCSE	880 (29.1)	1098 (14.2)	
Vocational	355 (11.7)	687 (8.9)	
O level	1040 (34.4)	2778 (36.0)	
A level	530 (17.5)	1947 (25.2)	
Degree	222 (7.3)	1203 (15.6)	
Active smoking during pregnancy (n, %)			<0.001
No	2041 (67.9)	6266 (82.2)	
Yes	964 (32.1)	1361 (17.8)	
Passive smoking during pregnancy (n, %)			<0.001
None	815 (30.9)	2793 (43.0)	
<1 hour per day	213 (8.1)	589 (9.1)	
≥1 hour per day	1612 (61.1)	3112 (47.9)	
Highest household socioeconomic group at 18 weeks of gestation (n, %)			<0.001
Partly skilled and unskilled	336 (12.5)	505 (6.8)	
Skilled manual and non-manual	1807 (67.4)	4829 (65.3)	
Professional, managerial and technical	540 (20.1)	2063 (27.9)	
Age at delivery, years (mean±SD)	27.07±4.85	29.02±4.50	<0.001
Age at menarche, years (mean±SD)	12.81±1.56	12.86±1.51	0.129
Pre-pregnancy BMI, kg/m^2 (mean±SD)	23.08±4.09	22.97±3.76	0.245
Child characteristics			
Sex (n, %)			<0.001
Boys	1716 (56.1)	3811 (49.3)	
Girls	1343 (43.9)	3919 (50.7)	
Breastfeeding duration (n, %)			<0.001
Never	900 (35.6)	1601 (21.2)	
<3 months	831 (32.9)	2364 (31.3)	
3-<6 months	252 (10.0)	1062 (14.1)	
≥6 months	547 (21.6)	2528 (33.5)	
Gestational age, weeks (mean±SD)	39.68±1.41	39.71±1.38	0.206
Birth weight, g (mean±SD)	3465±495	3482±474	0.100
Total energy intake, kcal (mean±SD)			
reported at age 3 years	1281±343	1250±308	<0.001
reported at age 4 years	1446±365	1407±317	<0.001
predicted at age 6 years	1635±158	1616±158	<0.001
reported at age 7 years	1884±463	1827±426	<0.001
reported at age 7.5 years	1695±322	1717±312	0.138
Total carbohydrate intake, g (mean±SD)			
reported at age 3 years	160.5±45.7	156.1±41.0	<0.001
reported at age 4 years	177.9±48.8	172.2±41.8	<0.001
predicted at age 6 years	204.6±21.1	202.0±20.8	<0.001
reported at age 7 years	233.6±60.8	225.9±54.8	<0.001
reported at age 7.5 years	216.6±45.1	219.6±43.9	0.162
Total fat intake, g (mean±SD)			
reported at age 3 years	51.1±15.0	49.4±13.6	<0.001
reported at age 4 years	60.2±16.5	58.6±14.8	<0.001
predicted at age 6 years	66.7±6.9	65.9±7.2	<0.001
reported at age 7 years	77.8±20.7	75.2±19.5	<0.001
reported at age 7.5 years	68.1±16.6	69.1±16.3	0.199
Total protein intake, g (mean±SD)			
reported at age 3 years	44.6±12.0	44.4±11.1	0.566
reported at age 4 years	50.4±12.5	49.7±11.5	0.045
predicted at age 6 years	56.0±5.2	55.6±5.6	0.021
reported at age 7 years	65.9±16.6	64.8±15.9	0.051
reported at age 7.5 years	55.4±12.8	55.7±12.7	0.705
Body fat percentage at age 11 years, % (mean±SD)	24.23±8.52	24.05±8.17	0.686

Supplementary Table A-8 Pearson's correlations between dietary intakes across ages in the ALSPAC study

	3 years	4 years	6 years (predicted)	7 years	7.5 years
Total energy intake, kcal					
3 years	1.00				
4 years	0.50	1.00			
6 years (predicted)	0.72	0.76	1.00		
7 years	0.41	0.47	0.80	1.00	
7.5 years	0.14	0.16	0.55	0.19	1.00
Carbohydrate, g					
3 years	1.00				
4 years	0.48	1.00			
6 years (predicted)	0.71	0.75	1.00		
7 years	0.39	0.44	0.79	1.00	
7.5 years	0.14	0.17	0.58	0.19	1.00
Fat, g					
3 years	1.00				
4 years	0.50	1.00			
6 years (predicted)	0.71	0.76	1.00		
7 years	0.40	0.47	0.80	1.00	
7.5 years	0.16	0.17	0.59	0.23	1.00
Protein, g					
3 years	1.00				
4 years	0.51	1.00			
6 years (predicted)	0.72	0.75	1.00		
7 years	0.42	0.49	0.81	1.00	
7.5 years	0.20	0.20	0.60	0.24	1.00

Supplementary Table A-9 Pearson correlations between puberty timing traits in boys and girls in the ALSPAC study

Boys	G2	PH2	Voice breaking	Axillary hair growth	PHV	Pubertal temp
G2	1.00					
PH2	0.32	1.00				
Voice breaking	0.14	0.15	1.00			
Axillary hair growth	0.08	0.24	0.41	1.00		
PHV	0.09	0.21	0.35	0.44	1.00	
Pubertal tempo	-0.60	-0.10	0.71	0.25	0.17	1.00

Girls	B2	PH2	Menarche	Axillary hair growth	PHV	Pubertal tempo
B2	1.00					
PH2	0.45	1.00				
Menarche	0.52	0.42	1.00			
Axillary hair growth	0.29	0.47	0.33	1.00		
PHV	0.56	0.52	0.79	0.43	1.00	
Pubertal tempo	-0.67	-0.10	0.29	-0.03	0.04	1.00

Supplementary Table A-10 Adjusted associations of total energy and macronutrient intakes predicted at age 6 years with secondary puberty timing traits in the ALSPAC study

Dietary intakes	Age at pubic hair growth		Age at axillary hair growth		Pubertal tempo	
	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value
Boys	n=2488		n=3212		n=2136	
Total energy intake (per 500kcal increase)[a]	-0.26 (-0.47, -0.05)	0.017	-0.17 (-0.37, 0.02)	0.083	-0.19 (-0.49, 0.12)	0.237
Energy partition model (per 500kcal increase)[a]						
Carbohydrates	-0.11 (-0.74, 0.51)	0.723	-0.35 (-0.93, 0.24)	0.247	-0.16 (-1.09, 0.78)	0.745
Fat	0.37 (-0.44, 1.19)	0.369	0.27 (-0.50, 1.04)	0.498	0.11 (-1.12, 1.34)	0.857
Protein	-2.94 (-5.07, -0.80)	0.007	-0.86 (-2.86, 1.15)	0.403	-1.25 (-4.46, 1.97)	0.447
Substitution of protein for carbohydrate						
Nutrient density model (per 10% increase)[b]	-0.88 (-1.56, -0.21)	0.011	-0.13 (-0.77, 0.50)	0.678	-0.32 (-1.33, 0.70)	0.541
Residual model (per 50g increase)[c]	-1.17 (-2.15, -0.19)	0.019	-0.13 (-1.05, 0.78)	0.774	-0.39 (-1.85, 1.08)	0.604
Girls	n=3002		n=3619		n=2801	
Total energy intake (per 500kcal increase)[a]	-0.19 (-0.38, -0.01)	0.045	-0.01 (-0.18, 0.18)	0.989	0.11 (-0.05, 0.28)	0.182
Energy partition model (per 500kcal increase)[a]						
Carbohydrates	-0.07 (-0.65, 0.50)	0.799	-0.26 (-0.81, 0.30)	0.364	-0.31 (-0.82, 0.20)	0.232
Fat	0.35 (-0.40, 1.10)	0.358	1.15 (0.41, 1.89)	0.002	0.41 (-0.27, 1.10)	0.237
Protein	-2.49 (-4.43, -0.56)	0.012	-2.75 (-4.63, -0.86)	0.004	0.92 (-0.83, 2.67)	0.303
Substitution of protein for carbohydrate						
Nutrient density model (per 10% increase)[b]	-0.65 (-1.24, -0.05)	0.033	-0.69 (-1.28, -0.11)	0.019	0.43 (-0.11, 0.97)	0.116
Residual model (per 50g increase)[c]	-0.99 (-1.88, -0.10)	0.030	-1.00 (-1.87, -0.13)	0.025	0.52 (-0.29, 1.33)	0.205

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration)
[b]Additionally adjusted for energy from fat (%), total energy intake (kcal)
[c]Additionally adjusted for total fat intake (g), total energy intake (kcal)

Supplementary Table A-11 Adjusted associations of total energy and macronutrient intakes at age 3-7.5 years with puberty timing

Dietary intakes	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value
Boys	n=2619		n=2215		n=3017	
Total energy intake (per 500kcal increase)[a]						
3 years old	-0.06 (-0.16, 0.04)	0.262	-0.01 (-0.08, 0.05)	0.671	-0.08 (-0.20, 0.04)	0.175
4 years old	-0.03 (-0.12, 0.07)	0.609	-0.03 (-0.10, 0.03)	0.290	-0.08 (-0.19, 0.03)	0.157
7 years old	-0.04 (-0.12, 0.03)	0.275	-0.01 (-0.06, 0.03)	0.562	-0.07 (-0.16, 0.01)	0.079
7.5 years old	-0.05 (-0.16, 0.06)	0.355	-0.08 (-0.14, -0.01)	0.025	-0.17 (-0.29, -0.04)	0.011
Heterogeneity (I^2)	0 %		0 %		0 %	
Girls	n=3204		n=2509		n=3414	
Total energy intake (per 500kcal increase)[a]						
3 years old	-0.01 (-0.11, 0.08)	0.754	-0.03 (-0.08, 0.03)	0.367	-0.01 (-0.08, 0.05)	0.703
4 years old	-0.06 (-0.15, 0.04)	0.232	-0.02 (-0.08, 0.03)	0.410	-0.03 (-0.10, 0.03)	0.317
7 years old	-0.06 (-0.12, 0.01)	0.101	-0.03 (-0.07, 0.01)	0.169	-0.04 (-0.08, 0.01)	0.159
7.5 years old	-0.22 (-0.31, -0.12)	1.85E-5	-0.11 (-0.17, -0.06)	8.69E-5	-0.09 (-0.16, -0.01)	0.034
Heterogeneity (I^2)	70 %		59 %		0 %	
Substitution of protein for carbohydrate in nutrient density model (per 10% increase)[b]						
3 years old	-0.27 (-0.58, 0.03)	0.079	-0.16 (-0.34, 0.02)	0.077	-0.14 (-0.36, 0.07)	0.182
4 years old	-0.09 (-0.41, 0.23)	0.583	-0.14 (-0.33, 0.05)	0.137	-0.15 (-0.37, 0.08)	0.206
7 years old	-0.49 (-0.81, -0.16)	0.003	-0.22 (-0.41, -0.03)	0.027	-0.27 (-0.50, -0.04)	0.022
7.5 years old	-0.29 (-0.57, -0.01)	0.046	-0.17 (-0.32, -0.02)	0.028	-0.03 (-0.22, 0.16)	0.750
Heterogeneity (I^2)	0 %		0 %		0 %	

Heterogeneity in the findings between ages was tested using fixed effect model

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration, age at dietary assessment)

[b]Additionally adjusted for energy from fat (%), total energy intake (kcal)

Supplementary Table A-12 Associations of total energy and macronutrient intakes predicted at age 6 years with puberty timing, further adjusting for adiposity in the ALSPAC study

Puberty timing	Total energy intake[a]		Substitution of protein for carbohydrate in nutrient density model[b]		Substitution of protein for carbohydrate in residual model[c]	
	Adjusted β per 500kcal increase (95% CI)	P value	Adjusted β per 10% increase (95% CI)	P value	Adjusted β per 50g increase (95% CI)	P value
Boys						
Age at genital development	-0.18 (-0.37, 0.02)	0.072	-0.15 (-0.80, 0.51)	0.662	-0.19 (-1.14, 0.75)	0.689
Age at peak height velocity	-0.08 (-0.21, 0.04)	0.184	-0.19 (-0.57, 0.20)	0.341	-0.23 (-0.78, 0.32)	0.415
Age at voice breaking	-0.29 (-0.50, -0.07)	0.009	-0.28 (-0.98, 0.42)	0.431	-0.46 (-1.48, 0.55)	0.372
Age at pubic hair growth	-0.26 (-0.47, -0.05)	0.017	-0.87 (-1.55, -0.19)	0.012	-1.15 (-2.13, -0.17)	0.021
Age at axillary hair growth	-0.17 (-0.36, 0.03)	0.092	-0.08 (-0.71, 0.55)	0.800	-0.06 (-0.98, 0.86)	0.900
Pubertal tempo	-0.18 (-0.49, 0.13)	0.255	-0.25 (-1.27, 0.76)	0.623	-0.30 (-1.77, 1.17)	0.688
Girls						
Age at breast development	-0.25 (-0.42, -0.08)	0.003	-0.71 (-1.26, -0.16)	0.012	-0.97 (-1.70, -0.05)	0.039
Age at peak height velocity	-0.14 (-0.24, -0.03)	0.013	-0.42 (-0.75, -0.09)	0.014	-0.51 (-1.00, -0.02)	0.043
Age at menarche	-0.13 (-0.26, -0.01)	0.050	-0.37 (-0.78, 0.05)	0.083	-0.50 (-1.12, 0.13)	0.118
Age at pubic hair growth	-0.18 (-0.37, 0.01)	0.053	-0.62 (-1.21, -0.03)	0.040	-0.94 (-1.82, -0.05)	0.038
Age at axillary hair growth	0.01 (-0.17, 0.19)	0.901	-0.65 (-1.22, -0.07)	0.029	-0.93 (-1.80, -0.07)	0.035
Pubertal tempo	0.10 (-0.06, 0.26)	0.227	0.38 (-0.13, 0.90)	0.147	0.45 (-0.33, 1.23)	0.260

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), body fat percentage predicted at 11 years
[b]Additionally adjusted for energy from fat (%), total energy intake (kcal)
[c]Additionally adjusted for total fat intake (g), total energy intake (kcal)

Supplementary Table A-13 Adjusted associations of total energy and macronutrient intakes at age 6 years with earlier (versus later) puberty timing in the ALSPAC study

Dietary intakes	Earlier genital/ breast development		Earlier peak height velocity		Earlier voice breaking/ Menarche	
	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Boys	n=2619		n=2215		n=3017	
Total energy intake (kcal)[a]						
Within the UK estimated average requirement	1.00		1.00		1.00	
Higher than the UK estimated average requirement	1.07 (0.90, 1.28)	0.457	1.10 (0.91, 1.33)	0.325	1.12 (0.96, 1.31)	0.159
Total energy intake (per 100kcal increase)[a]	1.05 (1.00, 1.10)	0.076	1.04 (0.98, 1.10)	0.227	1.05 (1.01, 1.10)	0.029
Substitution of protein for carbohydrate						
Nutrient density model (per 5% increase)[b]	1.19 (0.77, 1.84)	0.433	1.18 (0.76, 1.84)	0.451	1.01 (0.69, 1.48)	0.959
Residual model (per 10g increase)[c]	1.11 (0.86, 1.43)	0.417	1.08 (0.84, 1.40)	0.545	1.03 (0.83, 1.29)	0.772
Girls	n=3204		n=2509		n=3414	
Total energy intake (kcal)[a]						
Within the UK estimated average requirement	1.00		1.00		1.00	
Higher than the UK estimated average requirement	1.27 (1.07, 1.50)	0.006	1.06 (0.88, 1.27)	0.567	1.16 (0.98, 1.36)	0.080
Total energy intake (per 100kcal increase)[a]	1.04 (0.99, 1.09)	0.121	1.06 (1.00, 1.12)	0.072	1.03 (0.98, 1.08)	0.209
Substitution of protein for carbohydrate						
Nutrient density model (per 5% increase)[b]	1.85 (1.24, 2.77)	0.003	1.84 (1.17, 2.90)	0.008	1.55 (1.04, 2.30)	0.030
Residual model (per 10g increase)[c]	1.39 (1.09, 1.77)	0.008	1.33 (1.01, 1.74)	0.041	1.26 (0.99, 1.59)	0.058

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration)
[b]Additionally adjusted for energy from fat (%), total energy intake (kcal)
[c]Additionally adjusted for total fat intake (g), total energy intake (kcal)

Supplementary Table A-14 Adjusted associations of total energy and macronutrient intakes at age 6 years and tertiles of puberty timing traits in the ALSPAC study

Dietary intakes	Boys			Girls		
	T1 vs T2 (ref)	T2 vs T3 (ref)		T1 vs T2 (ref)	T2 vs T3 (ref)	
	Adjusted OR (95% CI)	Adjusted OR (95% CI)	P value[d]	Adjusted OR (95% CI)	Adjusted OR (95% CI)	P value[d]
Age at genital/ breast development						
Total energy intake (per 500kcal increase)[a]	0.41 (0.16, 1.05)	3.59 (1.32, 9.73)	0.009	2.08 (0.79, 5.44)	1.84 (0.70, 4.81)	0.878
Substitution of protein for carbohydrate						
Nutrient density model (per 5% increase)[b]	1.68 (0.41, 6.86)	1.18 (0.27, 5.10)	0.757	3.14 (0.79, 12.55)	2.93 (0.78, 11.02)	0.907
Residual model (per 10g increase)[c]	2.41 (0.47, 12.28)	1.16 (0.21, 6.29)	0.597	3.82 (0.72, 20.22)	2.71 (0.55, 13.45)	0.763
Age at peak height velocity						
Total energy intake (per 500kcal increase)[a]	0.99 (0.70, 1.39)	1.28 (0.90, 1.82)	0.390	1.20 (0.84, 1.70)	1.31 (0.92, 1.87)	0.770
Substitution of protein for carbohydrate						
Nutrient density model (per 5% increase)[b]	0.71 (0.25, 2.04)	1.31 (0.44, 3.91)	0.517	0.83 (0.27, 2.50)	3.96 (1.34, 11.70)	0.102
Residual model (per 10g increase)[c]	0.60 (0.13, 2.78)	1.27 (0.26, 6.10)	0.585	0.70 (0.14, 3.64)	5.46 (1.08, 27.65)	0.150
Age at voice breaking / menarche						
Total energy intake (per 500kcal increase)[a]	1.29 (0.97, 1.73)	1.06 (0.79, 1.42)	0.447	1.28 (0.95, 1.70)	0.99 (0.74, 1.34)	0.328
Substitution of protein for carbohydrate						
Nutrient density model (per 5% increase)[b]	0.99 (0.40, 2.47)	1.16 (0.45, 2.98)	0.851	1.13 (0.44, 2.95)	1.82 (0.71, 4.70)	0.567
Residual model (per 10g increase)[c]	1.04 (0.28, 3.89)	1.29 (0.32, 5.13)	0.859	0.99 (0.24, 4.11)	2.11 (0.51, 8.72)	0.545

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration)
[b]Additionally adjusted for energy from fat (%), total energy intake (kcal)
[c]Additionally adjusted for total fat intake (g), total energy intake (kcal)
[d]Comparisons between odds ratios in T1 vs T2 and T2 vs T3 based on Wald tests. T, tertile; vs, versus; ref, reference

Supplementary Table A-15 Adjusted associations of protein quality intakes at age 6 years with puberty timing in the ALSPAC study

Protein quality	Age at genital development		Age at peak height velocity		Age at voice breaking	
	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value
Boys[a]	n=2619		n=2215		n=3017	
Energy partition model (per 500kcal increase)[b]						
Model type 1						
Animal protein	-1.34 (-3.27, 0.59)	0.174	-0.64 (-1.78, 0.50)	0.273	-0.78 (-2.88, 1.31)	0.464
Plant protein	-0.42 (-5.23, 4.39)	0.864	-1.78 (-4.79, 1.23)	0.245	-8.58 (-13.98, -3.18)	0.002
Model type 2						
Red meat protein	0.42 (-4.40, 5.24)	0.863	-0.08 (-2.84, 2.67)	0.953	-1.56 (-6.74, 3.61)	0.554
White meat protein	-3.92 (-8.14, 0.30)	0.069	-1.57 (-4.11, 0.97)	0.227	-0.90 (-5.52, 3.72)	0.702
Dairy and egg protein	-0.20 (-3.13, 2.73)	0.893	-0.32 (-2.02, 1.38)	0.710	-0.40 (-3.55, 2.76)	0.805
Plant protein	0.24 (-4.65, 5.13)	0.924	-1.57 (-4.63, 1.49)	0.314	-8.57 (14.08, -3.05)	0.002
Nutrient density model (per 5% increase)[c]						
Model type 1						
Animal protein	-0.06 (-0.39, 0.27)	0.742	-0.12 (-0.31, 0.08)	0.242	-0.18 (-0.53, 0.18)	0.324
Plant protein	0.12 (-0.69, 0.94)	0.767	-0.29 (-0.79, 0.21)	0.254	-1.28 (-2.17, -0.39)	0.005
Model type 2						
Red meat protein	0.25 (-0.46, 0.97)	0.486	-0.02 (-0.44, 0.39)	0.908	-0.20 (-0.96, 0.56)	0.604
White meat protein	-0.44 (-1.05, 0.17)	0.156	-0.20 (-0.57, 0.16)	0.275	-0.19 (-0.86, 0.48)	0.579
Dairy and egg protein	0.15 (-0.34, 0.64)	0.536	-0.10 (-0.39, 0.18)	0.476	-0.18 (-0.70, 0.35)	0.512
Plant protein	0.26 (-0.58, 1.10)	0.545	-0.26 (-0.76, 0.25)	0.319	-1.27 (-2.17, -0.36)	0.006
Residual model (per 10g increase)[d]						
Model type 1						
Animal protein	-0.07 (-0.24, 0.10)	0.433	-0.06 (-0.16, 0.04)	0.242	-0.06 (-0.24, 0.13)	0.557
Plant protein	-0.01 (-0.44, 0.41)	0.958	-0.17 (-0.44, 0.10)	0.224	-0.66 (-1.14, -0.17)	0.008
Model type 2						
Red meat protein	0.08 (-0.31, 0.47)	0.695	-0.02 (-0.24, 0.21)	0.895	-0.14 (-0.55, 0.28)	0.527
White meat protein	-0.27 (-0.61, 0.08)	0.133	-0.13 (-0.34, 0.08)	0.211	-0.06 (-0.44, 0.32)	0.755
Dairy and egg protein	0.03 (-0.22, 0.27)	0.836	-0.03 (-0.18, 0.11)	0.652	-0.02 (-0.29, 0.25)	0.890
Plant protein	0.05 (-0.39, 0.48)	0.837	-0.15 (-0.42, 0.13)	0.299	-0.64 (-1.14, -0.15)	0.011

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration)
[b]Additionally adjusted for energy from other protein types, carbohydrate and fat (kcal)
[c]Additionally adjusted for energy from other protein types, and fat (%), total energy intake (kcal)
[d]Additionally adjusted for intakes of other protein types and fat (g), total energy intake (kcal)

Supplementary Table A-15 Adjusted associations of protein quality intakes at age 6 years with puberty timing in the ALSPAC study (continued)

Protein quality	Age at breast development		Age at peak height velocity		Age at menarche	
	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value
Girls[a]	n=3204		n=2509		n=3414	
Energy partition model (per 500kcal increase)[b]						
Model type 1						
Animal protein	-2.16 (-4.03, -0.28)	0.024	-1.56 (-2.64, -0.49)	0.004	-1.27 (-2.61, 0.07)	0.064
Plant protein	-4.74 (-9.33, -0.15)	0.043	-2.82 (-5.45, -0.20)	0.035	-2.06 (-5.31, 1.20)	0.216
Model type 2						
Red meat protein	-1.36 (-5.77, 3.04)	0.544	-2.53 (-5.06, -0.01)	0.050	-3.11 (-6.28, 0.07)	0.055
White meat protein	-2.20 (-5.88, 1.49)	0.242	-1.55 (-3.68, 0.58)	0.153	-1.17 (-3.83, 1.49)	0.387
Dairy and egg protein	-3.17 (-5.95, -0.39)	0.026	-1.30 (-2.92, 0.31)	0.114	-0.66 (-2.66, 1.33)	0.515
Plant protein	-4.97 (-9.62, -0.32)	0.036	-2.92 (-5.58, -0.27)	0.031	-2.13 (-5.42, 1.16)	0.205
Nutrient density model (per 5% increase)[c]						
Model type 1						
Animal protein	-0.45 (-0.74, -0.15)	0.003	-0.24 (-0.42, -0.07)	0.006	-0.21 (-0.42, 0.01)	0.058
Plant protein	-0.88 (-1.63, -0.14)	0.020	-0.42 (-0.86, 0.01)	0.056	-0.36 (-0.90, 0.18)	0.192
Model type 2						
Red meat protein	-0.43 (-1.09, 0.23)	0.205	-0.39 (-0.77, -0.01)	0.047	-0.50 (-0.98, -0.03)	0.038
White meat protein	-0.50 (-1.01, 0.01)	0.055	-0.26 (-0.56, 0.04)	0.093	-0.24 (-0.61, 0.13)	0.202
Dairy and egg protein	-0.56 (-1.00, -0.12)	0.012	-0.21 (-0.47, 0.05)	0.106	-0.08 (-0.39, 0.24)	0.634
Plant protein	-0.92 (-1.67, -0.16)	0.017	-0.43 (-0.88, 0.01)	0.054	-0.34 (-0.89, 0.20)	0.217
Residual model (per 10g increase)[d]						
Model type 1						
Animal protein	-0.20 (-0.37, -0.04)	0.016	-0.12 (-0.21, -0.02)	0.014	-0.10 (-0.22, 0.02)	0.091
Plant protein	-0.43 (-0.85, -0.02)	0.041	-0.22 (-0.45, 0.01)	0.066	-0.16 (-0.45, 0.12)	0.271
Model type 2						
Red meat protein	-0.15 (-0.50, 0.21)	0.425	-0.20 (-0.40, 0.01)	0.059	-0.25 (-0.51, 0.01)	0.054
White meat protein	-0.21 (-0.52, 0.09)	0.165	-0.11 (-0.29, 0.06)	0.205	-0.09 (-0.31, 0.12)	0.393
Dairy and egg protein	-0.29 (-0.53, -0.05)	0.016	-0.10 (-0.24, 0.03)	0.143	-0.05 (-0.22, 0.12)	0.574
Plant protein	-0.45 (-0.87, -0.03)	0.034	-0.24 (-0.47, 0.01)	0.050	-0.17 (-0.46, 0.12)	0.251

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration)
[b]Additionally adjusted for energy from other protein types, carbohydrate and fat (kcal)
[c]Additionally adjusted for energy from other protein types, and fat (%), total energy intake (kcal)
[d]Additionally adjusted for intakes of other protein types and fat (g), total energy intake (kcal)

Supplementary Table A-16 Comparisons of characteristics between excluded and included children in the ALSPAC study

	Excluded (n=3059)	Included (n=7730)	P value
Maternal characteristics			
Parity (n, %)			<0.001
0	1119 (39.1)	3407 (45.3)	
1	1064 (37.2)	2746 (36.5)	
2	455 (15.9)	1024 (13.6)	
≥3	221 (7.7)	352 (4.7)	
Highest education level (n, %)			<0.001
None/GCSE	880 (29.1)	1098 (14.2)	
Vocational	355 (11.7)	687 (8.9)	
O level	1040 (34.4)	2778 (36.0)	
A level	530 (17.5)	1947 (25.2)	
Degree	222 (7.3)	1203 (15.6)	
Active smoking during pregnancy (n, %)			<0.001
No	2041 (67.9)	6266 (82.2)	
Yes	964 (32.1)	1361 (17.8)	
Passive smoking during pregnancy (n, %)			<0.001
None	815 (30.9)	2793 (43.0)	
<1 hour per day	213 (8.1)	589 (9.1)	
≥1 hour per day	1612 (61.1)	3112 (47.9)	
Highest household socioeconomic group at 18 weeks of gestation (n, %)			<0.001
Partly skilled and unskilled	336 (12.5)	505 (6.8)	
Skilled manual and non-manual	1807 (67.4)	4829 (65.3)	
Professional, managerial and technical	540 (20.1)	2063 (27.9)	
Age at delivery, years (mean±SD)	27.07±4.85	29.02±4.50	<0.001
Age at menarche, years (mean±SD)	12.81±1.56	12.86±1.51	0.129
Pre-pregnancy BMI, kg/m^2 (mean±SD)	23.08±4.09	22.97±3.76	0.245
Child characteristics			
Sex (n, %)			<0.001
Boys	1716 (56.1)	3811 (49.3)	
Girls	1343 (43.9)	3919 (50.7)	
Breastfeeding duration (n, %)			<0.001
Never	900 (35.6)	1601 (21.2)	
<3 months	831 (32.9)	2364 (31.3)	
3-<6 months	252 (10.0)	1062 (14.1)	
≥6 months	547 (21.6)	2528 (33.5)	
Gestational age, weeks (mean±SD)	39.68±1.41	39.71±1.38	0.206
Birth weight, g (mean±SD)	3465±495	3482±474	0.100
Total energy intake, kcal (mean±SD)			
reported at age 3 years	1281±343	1250±308	<0.001
reported at age 4 years	1446±365	1407±317	<0.001
predicted at age 6 years	1635±158	1616±158	<0.001
reported at age 7 years	1884±463	1827±426	<0.001
reported at age 7.5 years	1695±322	1717±312	0.138
Total carbohydrate intake, g (mean±SD)			
reported at age 3 years	160.5±45.7	156.1±41.0	<0.001
reported at age 4 years	177.9±48.8	172.2±41.8	<0.001
predicted at age 6 years	204.6±21.1	202.0±20.8	<0.001
reported at age 7 years	233.6±60.8	225.9±54.8	<0.001
reported at age 7.5 years	216.6±45.1	219.6±43.9	0.162
Total fat intake, g (mean±SD)			
reported at age 3 years	51.1±15.0	49.4±13.6	<0.001
reported at age 4 years	60.2±16.5	58.6±14.8	<0.001
predicted at age 6 years	66.7±6.9	65.9±7.2	<0.001
reported at age 7 years	77.8±20.7	75.2±19.5	<0.001
reported at age 7.5 years	68.1±16.6	69.1±16.3	0.199
Total protein intake, g (mean±SD)			
reported at age 3 years	44.6±12.0	44.4±11.1	0.566
reported at age 4 years	50.4±12.5	49.7±11.5	0.045
predicted at age 6 years	56.0±5.2	55.6±5.6	0.021
reported at age 7 years	65.9±16.6	64.8±15.9	0.051
reported at age 7.5 years	55.4±12.8	55.7±12.7	0.705
Body fat percentage at age 11 years, % (mean±SD)	24.23±8.52	24.05±8.17	0.686

Supplementary Table A-17 Correlations between dietary and plasma phospholipid polyunsaturated fatty acids in the ALSPAC study

Biomarkers \ Dietary intake	Total PUFAs (g)		n-3 PUFAs (g)	
	Boys	Girls	Boys	Girls
n-6 PUFAs (μg% of total FAs)	0.19	0.16	0.02	0.03
Linoleic acid (18:2n6)	0.22	0.20	0.03	0.04
γ-Linolenic acid (18:3n6)	0.01	-0.01	-0.04	-0.03
Eicosadienoic acid (20:2n6)	0.11	0.11	0.03	0.04
Dihomo-γ-linolenic acid (20:3n6)	0.01	-0.01	-0.03	-0.01
Arachidonic acid (20:4n6)	0.05	0.02	0.00	0.03
Docosatetraenoic acid (22:4n6)	0.03	0.00	-0.11	-0.10
Docosapentaenoic acid (22:5n6)	-0.02	-0.02	-0.12	-0.08
n-3 PUFAs (μg% of total FAs)	-0.04	-0.05	0.22	0.25
α-Linolenic acid (18:3n3)	0.02	0.01	0.02	0.01
Eicosapentaenoic acid (20:5n3)	-0.11	-0.14	0.12	0.11
Docosapentaenoic acid (22:5n3)	-0.08	-0.08	-0.01	-0.01
Docosahexaenoic acid (22:6n3)	0.00	-0.01	0.28	0.32

Supplementary Table A-18 Correlations between plasma phospholipid polyunsaturated fatty acids and major monounsaturated fatty acids in the ALSPAC study

	n-6 PUFAs	18:2n6	18:3n6	20:2n6	20:3n6	20:4n6	22:4n6	22:5n6	n-3 PUFAs	18:3n3	20:5n3	22:5n3	22:6n3	MUFAs	16:1n7	18:1n9
n-6 PUFAs (μg% of total FAs)	1.00															
Linoleic acid (18:2n6)	0.92	1.00														
γ-Linolenic acid (18:3n6)	-0.04	-0.21	1.00													
Eicosadienoic acid (20:2n6)	0.40	0.29	0.09	1.00												
Dihomo-γ-linolenic acid (20:3n6)	0.38	0.17	0.34	0.53	1.00											
Arachidonic acid (20:4n6)	0.63	0.29	0.16	0.27	0.36	1.00										
Docosatetraenoic acid (22:4n6)	0.45	0.17	0.27	0.45	0.51	0.69	1.00									
Docosapentaenoic acid (22:5n6)	0.40	0.13	0.21	0.37	0.55	0.61	0.74	1.00								
n-3 PUFAs (μg% of total FAs)	0.24	0.06	0.04	0.28	0.23	0.49	0.23	0.19	1.00							
α-Linolenic acid (18:3n3)	-0.25	-0.16	-0.12	0.02	-0.21	-0.26	-0.28	-0.24	0.32	1.00						
Eicosapentaenoic acid (20:5n3)	0.04	-0.13	0.24	0.09	0.22	0.34	0.14	0.05	0.76	0.10	1.00					
Docosapentaenoic acid (22:5n3)	0.33	0.09	0.18	0.36	0.44	0.59	0.59	0.40	0.68	-0.03	0.62	1.00				
Docosahexaenoic acid (22:6n3)	0.40	0.21	-0.02	0.28	0.26	0.59	0.29	0.29	0.86	-0.09	0.53	0.52	1.00			
MUFAs (μg% of total FAs)	-0.68	-0.64	0.01	-0.12	-0.16	-0.44	-0.23	-0.15	-0.09	0.44	-0.06	-0.14	-0.31	1.00		
Palmitoleic acid (16:1n7)	-0.60	-0.60	0.26	-0.20	-0.08	-0.35	-0.18	-0.14	-0.23	-0.07	0.01	-0.16	-0.27	0.27	1.00	
Oleic acid (18:1n9)	-0.64	-0.58	-0.01	-0.12	-0.17	-0.46	-0.25	-0.16	-0.12	0.44	-0.11	-0.18	-0.33	0.99	0.16	1.00

Supplementary Table A-19 Substitution of dietary monounsaturated fat for saturate fat intake at age 6 years with puberty timing in the ALSPAC study

Dietary MUFAs intake	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value	Adjusted β (95% CI)	P value
Boys	n=2619		n=2215		n=3017	
Nutrient density model (per 5% increase)[a]	-0.32 (-1.30, 0.66)	0.527	0.14 (-0.44, 0.72)	0.643	0.08 (-0.98, 1.14)	0.881
Residual model (per 10g increase)[b]	-0.53 (-1.57, 0.50)	0.313	0.03 (-0.51, 0.24)	0.933	0.07 (-1.05, 1.19)	0.901
Girls	n=3204		n=2509		n=3414	
Nutrient density model (per 5% increase)[a]	-0.66 (-1.58, 0.25)	0.154	-0.19 (-0.72, 0.33)	0.471	-0.21 (-0.87, 0.45)	0.534
Residual model (per 10g increase)[b]	-0.67 (-1.63, 0.30)	0.175	-0.19 (-0.74, 0.36)	0.498	-0.16 (-0.85, 0.53)	0.648

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), carbohydrate intake (%), protein intake (%), monounsaturated fat intake (%), total energy intake (kcal)

[b]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), carbohydrate intake (g), protein intake (g), monounsaturated fat intake (g), total energy intake (kcal)

Supplementary Table A-20 Associations of plasma fatty acids at age 7.5 years with puberty timing, additionally adjusted for non-major and likely food sources of polyunsaturated fatty acids in the ALSPAC study

Fatty acids	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β per SD (95% CI)	P value	Adjusted β per SD (95% CI)	P value	Adjusted β per SD (95% CI)	P value
Boys	n=2619		n=2215		n=3017	
n-6 PUFAs[a]						
Total	0.01 (-0.07, 0.09)	0.868	0.03 (-0.01, 0.07)	0.206	0.01 (-0.08, 0.10)	0.900
Linoleic acid (18:2n6)	-0.01 (-0.09, 0.08)	0.904	0.03 (-0.01, 0.07)	0.176	0.01 (-0.08, 0.10)	0.811
γ-Linolenic acid (18:3n6)	0.05 (-0.02, 0.13)	0.168	-0.04 (-0.08, 0.01)	0.083	-0.03 (-0.11, 0.05)	0.510
Eicosadienoic acid (20:2n6)	-0.01 (-0.09, 0.07)	0.823	-0.01 (-0.05, 0.04)	0.949	0.01 (-0.08, 0.10)	0.822
Dihomo-γ-linolenic acid (20:3n6)	0.02 (-0.06, 0.09)	0.667	-0.03 (-0.07, 0.02)	0.211	0.01 (-0.08, 0.10)	0.790
Arachidonic acid (20:4n6)	0.02 (-0.05, 0.09)	0.559	0.02 (-0.02, 0.07)	0.324	-0.01 (-0.10, 0.07)	0.780
Docosatetraenoic acid (22:4n6)	0.04 (-0.03, 0.12)	0.242	0.03 (-0.01, 0.07)	0.201	0.04 (-0.04, 0.13)	0.328
Docosapentaenoic acid (22:5n6)	-0.03 (-0.10, 0.04)	0.345	0.04 (-0.01, 0.08)	0.108	0.04 (-0.04, 0.13)	0.307
MUFAs[a]						
Palmitoleic acid (16:1n7)	0.04 (-0.04, 0.12)	0.354	-0.05 (-0.10, -0.01)	0.025	-0.01 (-0.10, 0.08)	0.748
Oleic acid (18:1n9)	-0.02 (-0.11, 0.06)	0.551	0.01 (-0.03, 0.06)	0.553	0.03 (-0.06, 0.12)	0.460
n-6 PUFAs[b]						
Total	0.01 (-0.07, 0.09)	0.867	0.03 (-0.01, 0.07)	0.202	0.01 (-0.08, 0.10)	0.889
Linoleic acid (18:2n6)	-0.01 (-0.08, 0.08)	0.932	0.03 (-0.01, 0.07)	0.153	0.01 (-0.08, 0.10)	0.800
γ-Linolenic acid (18:3n6)	0.05 (-0.02, 0.12)	0.185	-0.04 (-0.08, 0.01)	0.064	-0.03 (-0.11, 0.05)	0.493
Eicosadienoic acid (20:2n6)	-0.01 (-0.09, 0.07)	0.831	-0.01 (-0.05, 0.04)	0.942	0.01 (-0.08, 0.10)	0.778
Dihomo-γ-linolenic acid (20:3n6)	0.01 (-0.06, 0.09)	0.715	-0.03 (-0.07, 0.01)	0.167	0.01 (-0.08, 0.10)	0.799
Arachidonic acid (20:4n6)	0.02 (-0.05, 0.09)	0.594	0.02 (-0.02, 0.06)	0.348	-0.01 (-0.10, 0.08)	0.795
Docosatetraenoic acid (22:4n6)	0.04 (-0.04, 0.11)	0.342	0.02 (-0.02, 0.06)	0.325	0.04 (-0.05, 0.13)	0.355
Docosapentaenoic acid (22:5n6)	-0.04 (-0.11, 0.03)	0.217	0.03 (-0.02, 0.07)	0.199	0.04 (-0.04, 0.13)	0.333
MUFAs[b]						
Palmitoleic acid (16:1n7)	0.04 (-0.05, 0.12)	0.392	-0.06 (-0.10, -0.01)	0.018	-0.02 (-0.11, 0.07)	0.730
Oleic acid (18:1n9)	-0.03 (-0.11, 0.05)	0.501	0.01 (-0.04, 0.05)	0.757	0.03 (-0.06, 0.12)	0.493

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), total energy intake at 6 years (kcal), red meats (g), chicken (g), fruits and vegetables (g), dairy and eggs (g) and sugar confectionary (g)
[b]Additionally adjusted for fish (g) and cereals and nuts (g)

Supplementary Table A-20 Associations of plasma fatty acids at age 7.5 years with puberty timing, additionally adjusted for non-major and likely food sources of polyunsaturated fatty acids in the ALSPAC study (continued)

Fatty acids	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β per SD (95% CI)	P value	Adjusted β per SD (95% CI)	P value	Adjusted β per SD (95% CI)	P value
Girls	n=3204		n=2509		n=3414	
n-6 PUFAs[a]						
Total	-0.01 (-0.08, 0.06)	0.726	-0.01 (-0.04, 0.4)	0.925	0.01 (-0.05, 0.05)	0.960
Linoleic acid (18:2n6)	0.01 (-0.07, 0.07)	0.947	0.01 (-0.04, 0.04)	0.821	0.01 (-0.04, 0.06)	0.660
γ-Linolenic acid (18:3n6)	0.01 (-0.06, 0.09)	0.765	-0.02 (-0.06, 0.02)	0.388	-0.04 (-0.09, 0.02)	0.196
Eicosadienoic acid (20:2n6)	-0.06 (-0.13, 0.01)	0.090	-0.03 (-0.07, 0.01)	0.121	-0.01 (-0.06, 0.03)	0.654
Dihomo-γ-linolenic acid (20:3n6)	-0.08 (-0.15, -0.01)	0.020	-0.07 (-0.11, -0.03)	5.7E-4	-0.06 (-0.11, -0.01)	0.014
Arachidonic acid (20:4n6)	-0.02 (-0.09, 0.05)	0.519	0.01 (-0.04, 0.04)	0.886	-0.01 (-0.05, 0.05)	0.875
Docosatetraenoic acid (22:4n6)	0.03 (-0.03, 0.10)	0.331	0.02 (-0.02, 0.06)	0.244	0.02 (-0.03, 0.07)	0.377
Docosapentaenoic acid (22:5n6)	0.03 (-0.04, 0.10)	0.373	0.02 (-0.02, 0.06)	0.298	0.03 (-0.02, 0.08)	0.290
MUFAs[a]						
Palmitoleic acid (16:1n7)	-0.12 (-0.19, -0.06)	3.4E-4	-0.06 (-0.10, -0.02)	0.002	-0.06 (-0.11, -0.01)	0.020
Oleic acid (18:1n9)	0.02 (-0.05, 0.09)	0.642	0.02 (-0.02, 0.06)	0.458	0.02 (-0.02, 0.07)	0.306
n-6 PUFAs[b]						
Total	0.01 (-0.08, 0.06)	0.745	-0.01 (-0.04, 0.04)	0.958	0.01 (-0.05, 0.05)	0.947
Linoleic acid (18:2n6)	0.01 (-0.07, 0.07)	0.920	0.01 (-0.03, 0.05)	0.790	0.01 (-0.04, 0.06)	0.655
γ-Linolenic acid (18:3n6)	0.01 (-0.06, 0.08)	0.788	-0.02 (-0.06, 0.02)	0.371	-0.04 (-0.09, 0.02)	0.197
Eicosadienoic acid (20:2n6)	-0.06 (-0.13, 0.01)	0.100	-0.03 (-0.06, 0.01)	0.134	-0.01 (-0.05, 0.04)	0.708
Dihomo-γ-linolenic acid (20:3n6)	-0.08 (-0.15, -0.01)	0.018	-0.07 (-0.11, -0.03)	5.2E-4	-0.06 (-0.11, -0.01)	0.015
Arachidonic acid (20:4n6)	-0.02 (-0.09, 0.05)	0.528	0.01 (-0.04, 0.04)	0.857	-0.01 (-0.05, 0.05)	0.895
Docosatetraenoic acid (22:4n6)	0.03 (-0.04, 0.09)	0.391	0.02 (-0.02, 0.06)	0.289	0.02 (-0.03, 0.07)	0.376
Docosapentaenoic acid (22:5n6)	0.03 (-0.04, 0.10)	0.431	0.02 (-0.02, 0.06)	0.338	0.03 (-0.02, 0.08)	0.289
MUFAs[b]						
Palmitoleic acid (16:1n7)	-0.12 (-0.19, -0.06)	2.8E-4	-0.06 (-0.10, -0.02)	0.002	-0.06 (-0.11, -0.01)	0.020
Oleic acid (18:1n9)	0.01 (-0.06, 0.08)	0.713	-0.03 (-0.07, 0.01)	0.161	0.02 (-0.02, 0.07)	0.329

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), total energy intake at 6 years (kcal), red meats (g), chicken (g), fruits and vegetables (g), dairy and eggs (g) and sugar confectionary (g)
[b]Additionally adjusted for fish (g) and cereals and nuts (g)

Supplementary Table A-21 Associations of plasma phospholipid fatty acids at age 7.5 years with puberty timing, additionally adjusted for body mass index in the ALSPAC study

Fatty acids	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β per SD (95% CI)[a]	P value	Adjusted β per SD (95% CI)[a]	P value	Adjusted β per SD (95% CI)[a]	P value
Boys	n=2619		n=2215		n=3017	
n-6 PUFAs						
Total	0.02 (-0.06, 0.10)	0.676	0.02 (-0.03, 0.06)	0.452	-0.01 (-0.10, 0.08)	0.793
Linoleic acid (18:2n6)	0.01 (-0.07, 0.09)	0.848	0.01 (-0.03, 0.05)	0.615	-0.01 (-0.10, 0.07)	0.822
γ-Linolenic acid (18:3n6)	0.04 (-0.03, 0.12)	0.235	-0.02 (-0.06, 0.02)	0.391	-0.01 (-0.09, 0.07)	0.837
Eicosadienoic acid (20:2n6)	-0.01 (-0.09, 0.07)	0.782	0.01 (-0.04, 0.05)	0.861	0.01 (-0.08, 0.10)	0.819
Dihomo-γ-linolenic acid (20:3n6)	0.01 (-0.07, 0.08)	0.867	-0.01 (-0.05, 0.03)	0.676	0.03 (-0.06, 0.12)	0.473
Arachidonic acid (20:4n6)	0.02 (-0.05, 0.10)	0.513	0.02 (-0.02, 0.07)	0.248	-0.01 (-0.10, 0.07)	0.782
Docosatetraenoic acid (22:4n6)	0.04 (-0.03, 0.12)	0.227	0.03 (-0.02, 0.07)	0.216	0.04 (-0.04, 0.13)	0.291
Docosapentaenoic acid (22:5n6)	-0.03 (-0.10, 0.04)	0.448	0.02 (-0.02, 0.06)	0.285	0.03 (-0.05, 0.12)	0.420
MUFAs						
Palmitoleic acid (16:1n7)	0.02 (-0.06, 0.10)	0.640	-0.02 (-0.06, 0.03)	0.432	0.02 (-0.07, 0.11)	0.630
Oleic acid (18:1n9)	-0.03 (-0.11, 0.05)	0.429	0.02 (-0.03, 0.06)	0.445	0.05 (-0.04, 0.13)	0.314
Girls	n=3204		n=2509		n=3414	
n-6 PUFAs						
Total	-0.43 (-1.04, 0.17)	0.163	-0.02 (-0.06, 0.01)	0.237	-0.02 (-0.07, 0.03)	0.408
Linoleic acid (18:2n6)	-0.05 (-0.11, 0.02)	0.155	-0.02 (-0.06, 0.01)	0.211	-0.02 (-0.06, 0.03)	0.492
γ-Linolenic acid (18:3n6)	0.04 (-0.03, 0.10)	0.278	-0.01 (-0.04, 0.04)	0.943	-0.02 (-0.07, 0.03)	0.534
Eicosadienoic acid (20:2n6)	-0.04 (-0.11, 0.02)	0.226	-0.02 (-0.05, 0.01)	0.241	-0.01 (-0.05, 0.04)	0.939
Dihomo-γ-linolenic acid (20:3n6)	-0.05 (-0.12, 0.01)	0.096	-0.05 (-0.09, -0.01)	0.008	-0.04 (-0.09, 0.01)	0.089
Arachidonic acid (20:4n6)	-0.03 (-0.09, 0.04)	0.423	0.01 (-0.03, 0.04)	0.846	-0.01 (-0.05, 0.04)	0.793
Docosatetraenoic acid (22:4n6)	-0.01 (-0.06, 0.06)	0.963	0.01 (-0.03, 0.05)	0.625	0.01 (-0.04, 0.06)	0.754
Docosapentaenoic acid (22:5n6)	-0.02 (-0.09, 0.04)	0.513	-0.01 (-0.04, 0.04)	0.937	0.01 (-0.04, 0.04)	0.907
MUFAs						
Palmitoleic acid (16:1n7)	-0.02 (-0.08, 0.04)	0.564	-0.01 (-0.04, 0.03)	0.780	-0.01 (-0.05, 0.05)	0.988
Oleic acid (18:1n9)	0.04 (-0.03, 0.10)	0.246	0.02 (-0.01, 0.06)	0.184	0.03 (-0.01, 0.08)	0.163

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), total energy intake at 6 years (kcal) and BMI at age 7.5 years (kg/m^2)

Supplementary Table A-22 Adjusted associations of dietary and plasma phospholipid n-3 polyunsaturated fatty acids with puberty timing in the ALSPAC study

	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β per SD (95% CI)[a]	P value	Adjusted β per SD (95% CI)[a]	P value	Adjusted β per SD (95% CI)[a]	P value
Dietary intake at 6 years						
Boys	n=2619		n=2215		n=3017	
n-3 PUFAs	-0.07 (-0.14, -0.01)	0.042	-0.02 (-0.06, 0.02)	0.236	-0.07 (-0.14, 0.01)	0.062
Eicosapentaenoic acid (20:5n3)	-0.07 (-0.14, -0.01)	0.038	-0.02 (-0.06, 0.02)	0.257	-0.08 (-0.15, -0.01)	0.039
Docosahexaenoic acid (22:6n3)	-0.07 (-0.14, -0.01)	0.033	-0.02 (-0.06, 0.02)	0.270	-0.07 (-0.14, 0.01)	0.064
Girls	n=3204		n=2509		n=3414	
n-3 PUFAs	-0.04 (-0.10, 0.01)	0.100	-0.01 (-0.04, 0.03)	0.721	0.01 (-0.02, 0.05)	0.472
Eicosapentaenoic acid (20:5n3)	-0.04 (-0.09, 0.02)	0.180	0.01 (-0.03, 0.03)	0.945	0.02 (-0.02, 0.06)	0.304
Docosahexaenoic acid (22:6n3)	-0.04 (-0.10, 0.01)	0.105	-0.01 (-0.04, 0.02)	0.692	0.01 (-0.02, 0.05)	0.489
Plasma phospholipid FAs at 7.5 years						
Boys	n=2619		n=2215		n=3017	
n-3 PUFAs	0.01 (-0.06, 0.08)	0.793	-0.03 (-0.08, 0.01)	0.111	-0.04 (-0.12, 0.05)	0.411
Eicosapentaenoic acid (20:5n3)	0.02 (-0.05, 0.09)	0.608	-0.02 (-0.06, 0.02)	0.222	-0.08 (-0.16, 0.01)	0.061
Docosahexaenoic acid (22:6n3)	-0.01 (-0.08, 0.07)	0.873	-0.03 (-0.08, 0.01)	0.124	-0.04 (-0.12, 0.05)	0.416
Girls	n=3204		n=2509		n=3414	
n-3 PUFAs	-0.05 (-0.11, 0.02)	0.161	-0.01 (-0.04, 0.04)	0.936	-0.02 (-0.06, 0.03)	0.476
Eicosapentaenoic acid (20:5n3)	-0.06 (-0.13, 0.01)	0.089	-0.01 (-0.04, 0.04)	0.878	-0.03 (-0.07, 0.02)	0.282
Docosahexaenoic acid (22:6n3)	-0.04 (-0.10, 0.03)	0.256	-0.01 (-0.05, 0.03)	0.573	-0.02 (-0.07, 0.02)	0.371

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), total energy intake at 6 years (kcal)

Supplementary Table A-23 Adjusted associations of monounsaturated fatty acids, saturated fatty acid categories and ratios of fatty acids at age 7.5 years with puberty timing in the ALSPAC study

	Age at genital/ breast development		Age at peak height velocity		Age at voice breaking/ menarche	
	Adjusted β per SD (95% CI)[a]	P value	Adjusted β per SD (95% CI)[a]	P value	Adjusted β per SD (95% CI)[a]	P value
Boys	n=2619		n=2215		n=3017	
MUFAs						
Vaccenic acid (18:1n7)	-0.01 (-0.09, 0.07)	0.754	0.01 (-0.04, 0.05)	0.780	0.03 (-0.05, 0.12)	0.446
Erucic acid (20:1n9)	-0.03 (-0.11, 0.05)	0.435	0.02 (-0.02, 0.06)	0.375	0.01 (-0.08, 0.09)	0.952
Nervonic acid (24:1n9)	0.04 (-0.04, 0.11)	0.337	-0.04 (-0.08, 0.01)	0.109	-0.05 (-0.13, 0.03)	0.240
SFAs						
Short-even-chain	0.01 (-0.08, 0.08)	0.968	-0.03 (-0.07, 0.01)	0.169	-0.02 (-0.11, 0.07)	0.645
Long-even-chain	0.04 (-0.04, 0.11)	0.330	0.01 (-0.03, 0.05)	0.654	-0.03 (-0.11, 0.06)	0.532
Ratios of n-6 PUFA						
Ratio: 18:3n6 / 18:2n6	0.05 (-0.03, 0.12)	0.208	-0.04 (-0.08, -0.01)	0.041	-0.02 (-0.10, 0.06)	0.678
Ratio: 20:4n6 / 20:3n6	0.01 (-0.06, 0.09)	0.756	0.04 (-0.01, 0.09)	0.070	-0.03 (-0.11, 0.06)	0.546
Ratio: 20:3n6/ 18:2n6	0.01 (-0.06, 0.09)	0.707	-0.04 (-0.08, 0.01)	0.052	0.02 (-0.06, 0.11)	0.601
Stearoyl-CoA desaturase-1						
Ratio: 16:1n7 / 16:0	0.05 (-0.03, 0.12)	0.252	-0.06 (-0.11, -0.01)	0.011	0.01 (-0.09, 0.09)	0.990
Ratio: 18:1n9 / 18:0	-0.02 (-0.09, 0.06)	0.690	0.03 (-0.01, 0.08)	0.175	0.05 (-0.04, 0.14)	0.263
Ratio: total n-6 PUFAs / total n-3 PUFAs	-0.02 (-0.09, 0.05)	0.522	0.04 (0.01, 0.09)	0.032	0.02 (-0.06, 0.09)	0.704
Ratio: total MUFAs / total SFAs	-0.02 (-0.10, 0.06)	0.623	0.02 (-0.02, 0.07)	0.304	0.05 (-0.04, 0.13)	0.284
Girls	n=3204		n=2509		n=3414	
MUFAs						
Vaccenic acid (18:1n7)	-0.07 (-0.14, 0.01)	0.064	-0.03 (-0.07, 0.01)	0.137	-0.01 (-0.06, 0.05)	0.793
Erucic acid (20:1n9)	0.03 (-0.04, 0.10)	0.341	0.02 (-0.02, 0.06)	0.382	0.02 (-0.03, 0.07)	0.436
Nervonic acid (24:1n9)	-0.04 (-0.10, 0.03)	0.298	-0.01 (-0.05, 0.03)	0.631	-0.03 (-0.08, 0.02)	0.251
SFAs						
Short-even-chain	0.03 (-0.04, 0.10)	0.399	-0.01 (-0.04, 0.04)	0.928	-0.01 (-0.06, 0.04)	0.754
Long-even-chain	0.01 (-0.05, 0.08)	0.691	0.02 (-0.02, 0.06)	0.426	-0.01 (-0.06, 0.05)	0.861
Ratios of n-6 PUFA						
Ratio: 18:3n6 / 18:2n6	-0.01 (-0.08, 0.07)	0.901	-0.02 (-0.06, 0.02)	0.317	-0.04 (-0.09, 0.02)	0.192
Ratio: 20:4n6 / 20:3n6	0.06 (-0.01, 0.13)	0.068	0.06 (0.02, 0.10)	0.001	0.04 (-0.01, 0.09)	0.070
Ratio: 20:3n6/ 18:2n6	-0.09 (-0.16, -0.02)	0.001	-0.07 (-0.11, -0.03)	5.7E-4	-0.06 (-0.11, -0.01)	0.016
Stearoyl-CoA desaturase-1						
Ratio: 16:1n7 / 16:0	-0.18 (-0.24, -0.11)	7.7E-8	-0.08 (-0.11, -0.04)	4.2E-5	-0.07 (-0.12, -0.03)	0.003
Ratio: 18:1n9 / 18:0	-0.01 (-0.08, 0.05)	0.690	0.01 (-0.03, 0.04)	0.772	0.02 (-0.02, 0.07)	0.360
Ratio: total n-6 PUFAs / total n-3 PUFAs	0.05 (-0.01, 0.12)	0.118	0.01 (-0.03, 0.04)	0.827	0.02 (-0.03, 0.06)	0.455
Ratio: total MUFAs / total SFAs	-0.04 (-0.11, 0.03)	0.310	0.01 (-0.04, 0.04)	0.916	0.01 (-0.04, 0.06)	0.652

[a]Adjusted for maternal characteristics (i.e. age at delivery, passive and active smoking during pregnancy, age at menarche, education, pre-pregnancy BMI, parity), household highest socioeconomic group, infant characteristics (i.e. birth weight, gestational age, breastfeeding duration), total energy intake at 6 years (kcal)

Supplementary Table A-24 Genetic variants associated with dihomo-γ-linolenic acid and palmitoleic acid in the EPIC-InterAct study

Chromosome	SNP	Position	Locus	Effect allele	Other allele	Effect	Standard error	P value	Frequency of effect allele
Dihomo-γ-linolenic acid (20:3n6)									
Primary instrument									
16	rs12928099	15150505	PCXDC1	A	C	0.30	0.01	2.3×10^{-196}	0.307
Secondary instruments									
8	rs721399	18259366	NAT2	T	C	0.06	0.01	1.3×10^{-8}	0.722
11	rs499974	75455021	DGAT2	A	C	-0.09	0.01	2.3×10^{-13}	0.176
Excluded due to pleiotropy									
11	rs968567	61595564	FADS1	T	C	0.50	0.01	7.2×10^{-308}	0.160
19	rs8107974	19388500	TM6SF2	A	T	0.10	0.02	1.5×10^{-8}	0.921
Palmitoleic acid (16:1n7)									
Primary instrument									
10	rs603424	102075479	SCD	A	G	-0.15	0.01	6.2×10^{-38}	0.212
Secondary instruments									
9	rs4962238	140358556	NSMF	T	C	0.11	0.02	3.3×10^{-8}	0.079
14	rs116915125	26984435	NOVA1	A	G	0.27	0.05	1.1×10^{-8}	0.015
Excluded due to pleiotropy									
2	rs1260326	27730940	GCKR	T	C	0.09	0.01	4.4×10^{-19}	0.406
4	rs1229984	100239319	ADH1A	T	C	-0.14	0.02	5.3×10^{-9}	0.046
10	rs2792736	113921159	GPAM	A	T	-0.06	0.01	4.9×10^{-8}	0.700
11	rs174566	61592362	FADS1	A	G	-0.11	0.01	1.4×10^{-29}	0.670

Supplementary Table A-25 Stages for selecting genetic variants associated with physical activity measures for Mendelian Randomisation analyses

Stages of SNP selection	Number of SNPs for each physical activity measure					
	Accelerometer-based average acceleration	Strenuous sports or other exercises	Moderate-to-vigorous intensity	Vigorous intensity	Television viewing	Computer use
Males						
Initial	4	5	8	6	152	37
Without outcome data	0	0	0	0	0	0
Proxies	0	0	0	0	0	0
Steiger-filtered	0	0	0	0	0	0
Radial analysis	-2	-2	-1	-2	-17	-5
Final	2	3	7	4	135	32
Females						
Initial	4	5	8	6	152	37
Without outcome data	0	0	0	0	-20	-7
Proxies	0	0	0	0	+18	+7
Steiger-filtered	0	-1	0	-1	-4	-2
Radial analysis	Not computable	Not computable	-3	-1	-65	-11
Final			5	4	81	24

Supplementary Table A-26 Comparisons of characteristics between excluded and included children

	Excluded (n=12,704)	Included (n=5610)	P value
Maternal characteristics			
Education (n, %)			<0.001
None/other	3329 (26.3)	715 (12.8)	
GCSE D-G	1425 (11.3)	494 (8.8)	
GCSE A-C	4295 (34.0)	1802 (32.2)	
A-level/diploma	2042 (16.1)	1235 (22.1)	
First/higher degree	1557 (12.3)	1356 (24.2)	
OECD equivalized family income (n, %)			<0.001
Q1	2965 (23.4)	548 (9.8)	
Q2	2763 (21.8)	841 (15.0)	
Q3	2546 (20.1)	1135 (20.3)	
Q4	2309 (18.2)	1411 (25.2)	
Q5	2077 (16.4)	1671 (29.8)	
Age at delivery, years	28.0±5.8	29.9±5.3	<0.001
Pre-pregnancy body mass index, kg/m^2	23.7±4.5	23.8±4.3	0.074
Child characteristics			
Sex (n, %)			<0.001
Boys	6878 (54.1)	2531 (45.1)	
Girls	5826 (45.9)	3079 (54.9)	
Ethnicity (n, %)			<0.001
White	10098 (79.5)	4915 (87.6)	
Asian	1620 (12.8)	408 (7.3)	
Black	570 (4.5)	129 (2.3)	
Mixed	416 (3.3)	158 (2.8)	
Birth weight, kg	3.34±0.59	3.40±0.56	<0.001
Body mass index at 7 years, kg/m^2	16.8±2.5	16.4±2.1	<0.001
Body-mass-index-for-age *z* scores at 7 years	0.58±1.23	0.41±1.09	<0.001
Body mass index at 11 years, kg/m^2	19.5±3.8	18.9±3.4	<0.001
Body-mass-index-for-age *z* scores at 11 years	0.66±1.28	0.48±1.19	<0.001

Supplementary Table A-27 Pearson's correlations between physical activity measures at 7 years

	Sedentary behaviour	Light intensity	Moderate intensity	Vigorous intensity	Total daily counts
Boys					
Fraction of total counts from, %					
Sedentary behaviour	1.00				
Light intensity	0.66	1.00			
Moderate intensity	-0.29	-0.28	1.00		
Vigorous intensity	-0.53	-0.88	-0.22	1.00	
Total daily counts	-0.85	-0.76	0.23	0.66	1.00
Time spent in, minutes					
Sedentary behaviour	1.00				
Light intensity	-0.37	1.00			
Moderate intensity	-0.44	0.41	1.00		
Vigorous intensity	-0.29	0.09	0.67	1.00	
Girls					
Fraction of total counts from, %					
Sedentary behaviour	1.00				
Light intensity	0.63	1.00			
Moderate intensity	-0.41	-0.46	1.00		
Vigorous intensity	-0.49	-0.87	-0.02	1.00	
Total daily counts	-0.81	-0.74	0.37	0.64	1.00
Time spent in, minutes					
Sedentary behaviour	1.00				
Light intensity	-0.41	1.00			
Moderate intensity	-0.50	0.46	1.00		
Vigorous intensity	-0.35	0.14	0.70	1.00	

Supplementary Table A-28 Spearman's correlations between puberty timing

	Growth spurt	Body hair growth	Skin changes	Voice breaking	Facial hair growth	Breast development	Categorical age at menarche	Age at menarche
Boys								
Growth spurt	1.00							
Body hair growth	0.64	1.00						
Skin changes	0.51	0.73	1.00					
Voice breaking	0.39	0.58	0.85	1.00				
Facial hair growth	0.13	0.21	0.15	0.20	1.00			
Girls								
Growth spurt	1.00							
Body hair growth	0.49	1.00						
Skin changes	0.47	0.63	1.00					
Breast development	0.60	0.67	0.56			1.00		
Age at menarche	0.36	0.43	0.43			0.51	0.94	1.00

Supplementary Table A-29 Associations of physical activity with earlier (vs. later) puberty timing in substitution models

Physical activity	Adjusted OR (95% CI)[a]	P value	Adjusted OR (95% CI)[a]	P value	Adjusted OR (95% CI)[a]	P value	Adjusted OR (95% CI)[a]	P value
Boys	Earlier growth spurt (n=1588)		Earlier body hair growth (n=788)		Earlier skin changes (n=1239)		Earlier facial hair (n=908)	
Isomovement substitution								
Fractions of counts (per 10%)								
Moderate intensity	1.07 (0.79, 1.44)	0.665	1.13 (0.71, 1.80)	0.612	1.38 (0.91, 2.08)	0.131	1.67 (0.70, 4.02)	0.251
Vigorous intensity	1.11 (0.90, 1.37)	0.343	1.47 (1.03, 2.09)	0.033	1.26 (0.94, 1.69)	0.116	1.01 (0.53, 1.93)	0.967
Total counts (per 100000)	0.87 (0.74, 1.01)	0.073	0.75 (0.57, 0.98)	0.038	0.73 (0.59, 0.92)	0.006	1.15 (0.70, 1.91)	0.577
Isotemporal substitution								
Time spent (per 10 minutes)								
Light intensity	0.99 (0.96, 1.02)	0.498	0.96 (0.91, 1.01)	0.134	0.95 (0.91, 1.00)	0.039	1.01 (0.92, 1.11)	0.829
Moderate intensity	0.95 (0.84, 1.08)	0.423	0.92 (0.75, 1.12)	0.397	1.03 (0.87, 1.23)	0.710	1.28 (0.85, 1.93)	0.232
Vigorous intensity	0.99 (0.87, 1.12)	0.850	1.08 (0.88, 1.33)	0.468	0.87 (0.73, 1.05)	0.149	0.93 (0.62, 1.41)	0.735
Girls	Earlier growth spurt (n=1503)		Earlier body hair growth (n=1246)		Earlier skin changes (n=1376)		Earlier breast development (n=1550)	
Isomovement substitution								
Fractions of counts (per 10%)								
Moderate intensity	0.80 (0.58, 1.10)	0.162	1.09 (0.71, 1.68)	0.696	1.11 (0.80, 1.55)	0.523	1.36 (0.98, 1.89)	0.063
Vigorous intensity	0.96 (0.76, 1.19)	0.688	1.13 (0.84, 1.52)	0.420	1.10 (0.87, 1.38)	0.441	0.90 (0.73, 1.12)	0.360
Total counts (per 100000)	0.90 (0.75, 1.08)	0.261	0.74 (0.58, 0.95)	0.017	0.75 (0.62, 0.91)	0.003	0.91 (0.76, 1.09)	0.302
Isotemporal substitution								
Time spent (per 10 minutes)								
Light intensity	1.00 (0.97, 1.04)	0.893	0.96 (0.92, 1.00)	0.078	0.96 (0.93, 0.99)	0.022	0.98 (0.95, 1.02)	0.275
Moderate intensity	0.88 (0.75, 1.03)	0.118	0.92 (0.75, 1.13)	0.419	0.96 (0.82, 1.13)	0.635	1.14 (0.97, 1.34)	0.121
Vigorous intensity	0.97 (0.82, 1.16)	0.765	0.93 (0.73, 1.17)	0.516	0.88 (0.73, 1.05)	0.152	0.80 (0.66, 0.95)	0.014

[a]Adjusted for maternal characteristics (age, active smoking during pregnancy, alcohol consumption during pregnancy, education, pre-pregnancy body mass index, OECD equivalized family income) and child characteristics (ethnicity, birth weight, gestational age, breastfeeding duration, mental health, dietary behaviour, regular sleep time, long-term health status and body-mass-index-for-age z scores at 7 years

Supplementary Table A-29 Associations of physical activity with earlier (vs. later) puberty timing in substitution models (continued)

Physical activity	Adjusted OR (95% CI)[a]	P value	Adjusted OR (95% CI)[a]	P value	Adjusted β (95% CI)[a]	P value
Boys	Earlier voice breaking (n=1038)					
Isomovement substitution						
Fractions of counts (per 10%)						
Moderate intensity	1.00 (0.58, 1.71)	0.996				
Vigorous intensity	1.32 (0.90, 1.96)	0.157				
Total counts (per 100000)	0.69 (0.51, 0.93)	0.015				
Isotemporal substitution						
Time spent (per 10 minutes)						
Light intensity	0.95 (0.90, 1.01)	0.118				
Moderate intensity	0.86 (0.68, 1.08)	0.192				
Vigorous intensity	1.01 (0.80, 1.28)	0.903				
Girls	Earlier menarche (T1 vs. T2)		Later menarche (T3 vs. T2)		Age at menarche (n=2904)	
Isomovement substitution						
Fractions of counts (per 10%)						
Moderate intensity	1.19 (0.93, 1.51)	0.159	1.24 (0.88, 1.74)	0.217	-0.05 (-0.18, 0.08)	0.423
Vigorous intensity	1.05 (0.89, 1.24)	0.577	1.09 (0.87, 1.38)	0.454	0.01 (-0.08, 0.10)	0.772
Total counts (per 100000)	0.83 (0.72, 0.95)	0.070	0.84 (0.69, 1.01)	0.070	0.04 (-0.04, 0.11)	0.338
Isotemporal substitution						
Time spent (per 10 minutes)						
Light intensity	0.97 (0.95, 1.00)	0.054	0.97 (0.94, 1.01)	0.135	0.01 (-0.01, 0.02)	0.668
Moderate intensity	0.99 (0.87, 1.11)	0.809	1.06 (0.90, 1.26)	0.469	0.01 (-0.06, 0.07)	0.811
Vigorous intensity	0.91 (0.80, 1.04)	0.160	0.88 (0.73, 1.06)	0.177	0.03 (-0.05, 0.10)	0.476

[a]Adjusted for maternal characteristics (age, active smoking during pregnancy, alcohol consumption during pregnancy, education, pre-pregnancy body mass index, OECD equivalized family income) and child characteristics (ethnicity, birth weight, gestational age, breastfeeding duration, mental health, dietary behaviour, regular sleep time, long-term health status and body-mass-index-for-age z scores at 7 years

Supplementary Table A-30 Associations of physical activity with body-mass- index-for-age z scores at 11 year

Physical activity	Adjusted β (95% CI)[a]	P value
Boys (n=2531)		
Single exposure model	-0.01 (-0.04, 0.02)	0.608
Total counts (per 100000)		
Compositional analysis (Log-fractions of counts)		
Light intensity	0.86 (0.37, 1.35)	5.9E-4
Moderate intensity	0.37 (-0.01, 0.72)	0.051
Vigorous intensity	0.13 (-0.11, 0.37)	0.288
Total counts (per 100000)	0.08 (0.03, 0.13)	0.001
Isomovement substitution (per 10%)		
Moderate intensity	-0.06 (-0.15, 0.03)	0.205
Vigorous intensity	-0.14 (-0.21, -0.08)	1.7E-5
Total counts (per 100000)	0.07 (0.02, 0.12)	0.004
Isotemporal substitution (per 10 minutes)		
Light intensity	0.02 (0.01, 0.03)	1.4E-4
Moderate intensity	0.01 (-0.03, 0.05)	0.727
Vigorous intensity	-0.05 (-0.09, -0.01)	0.016
Girls (n=3079)		
Single exposure model	0.01 (-0.02, 0.04)	0.446
Total counts (per 100000)		
Compositional analysis (Log-fractions of counts)		
Light intensity	0.28 (-0.19, 0.76)	0.242
Moderate intensity	0.25 (-0.05, 0.55)	0.098
Vigorous intensity	-0.08 (-0.27, 0.12)	0.427
Total counts (per 100000)	0.06 (0.01, 0.10)	0.011
Isomovement substitution (per 10%)		
Moderate intensity	0.01 (-0.06, 0.09)	0.775
Vigorous intensity	-0.10 (-0.15, -0.05)	2.6E-4
Total counts (per 100000)	0.06 (0.02, 0.10)	0.007
Isotemporal substitution (per 10 minutes)		
Light intensity	0.01 (0.01, 0.02)	0.040
Moderate intensity	0.03 (-0.01, 0.07)	0.138
Vigorous intensity	-0.05 (-0.09, -0.01)	0.029

[a]Adjusted for maternal characteristics (age, active smoking during pregnancy, alcohol consumption during pregnancy, education, pre-pregnancy body mass index, OECD equivalized family income) and child characteristics (ethnicity, birth weight, gestational age, breastfeeding duration, mental health, dietary behaviour, regular sleep time, long-term health status and body-mass-index-for-age z scores at 7 years

APPENDIX B SUPPLEMENTARY FIGURES

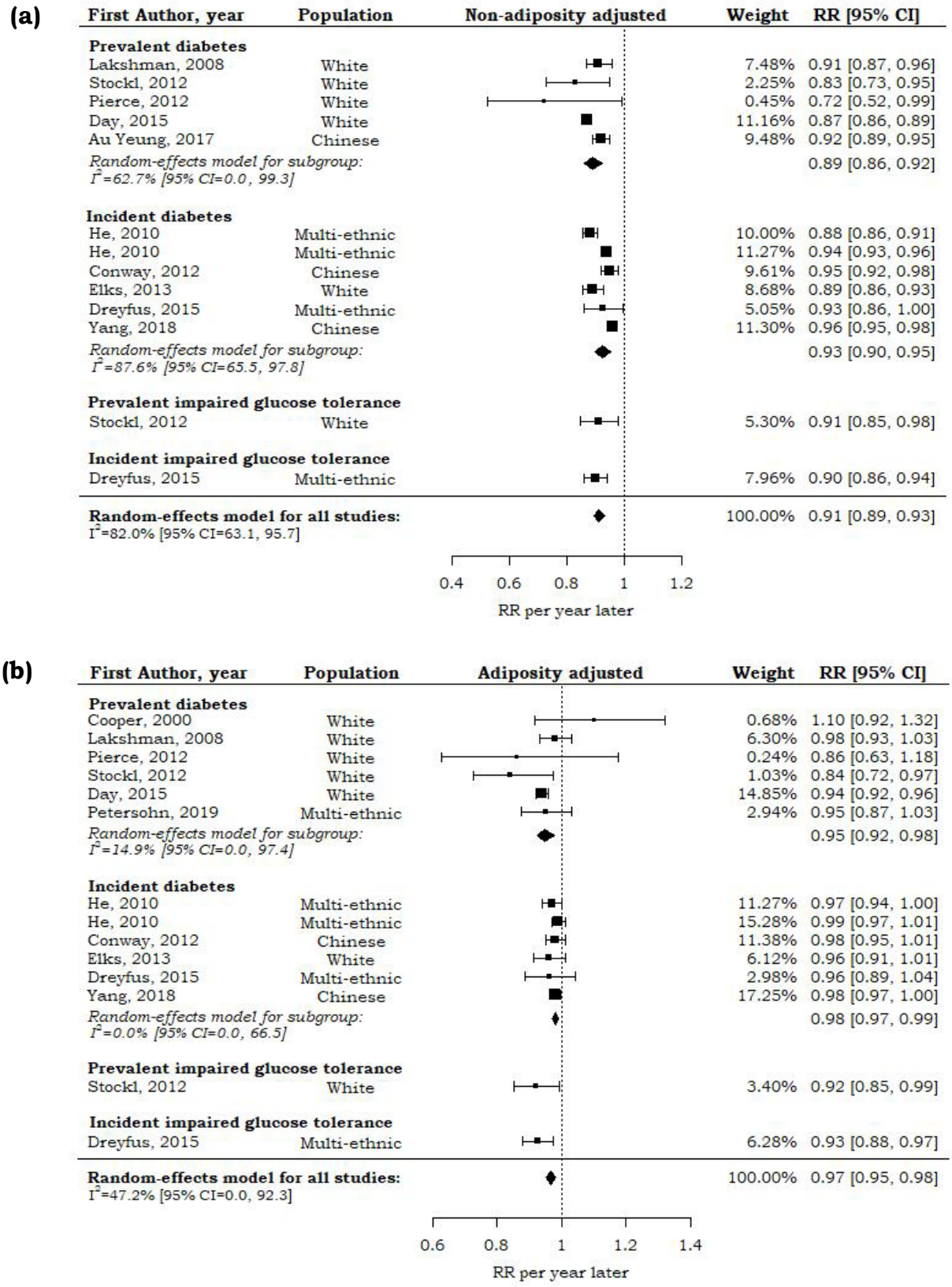
Supplementary figures related to chapters in this thesis are as follows:

Chapter 2 - **Supplementary Figure B-1** to **B-5**

Chapter 3 - Supplementary Figure B-6

Chapter 5 - Supplementary Figure B-7

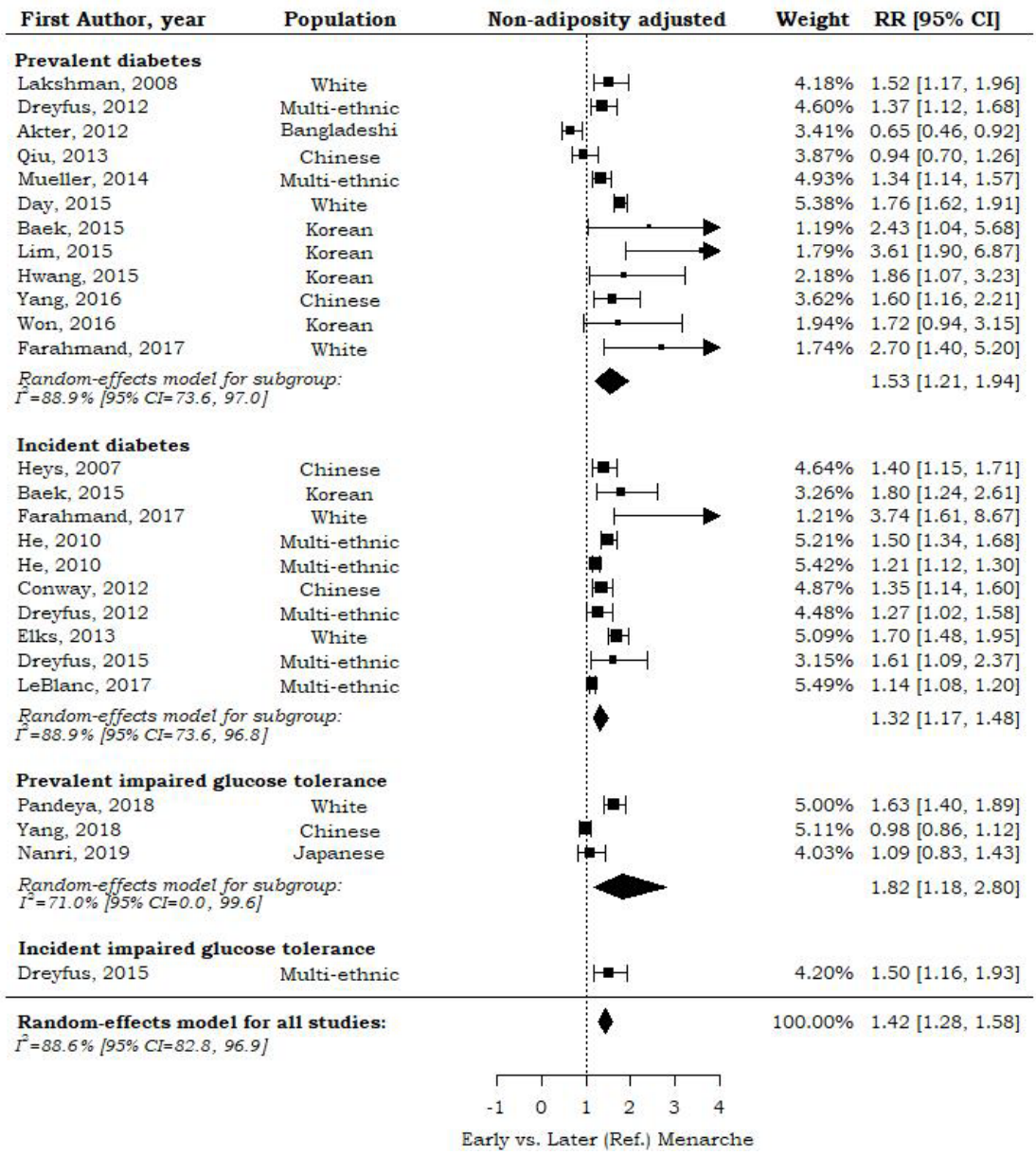
Supplementary Figure B-1 Forest plots of the associations of age at menarche with T2D or IGT



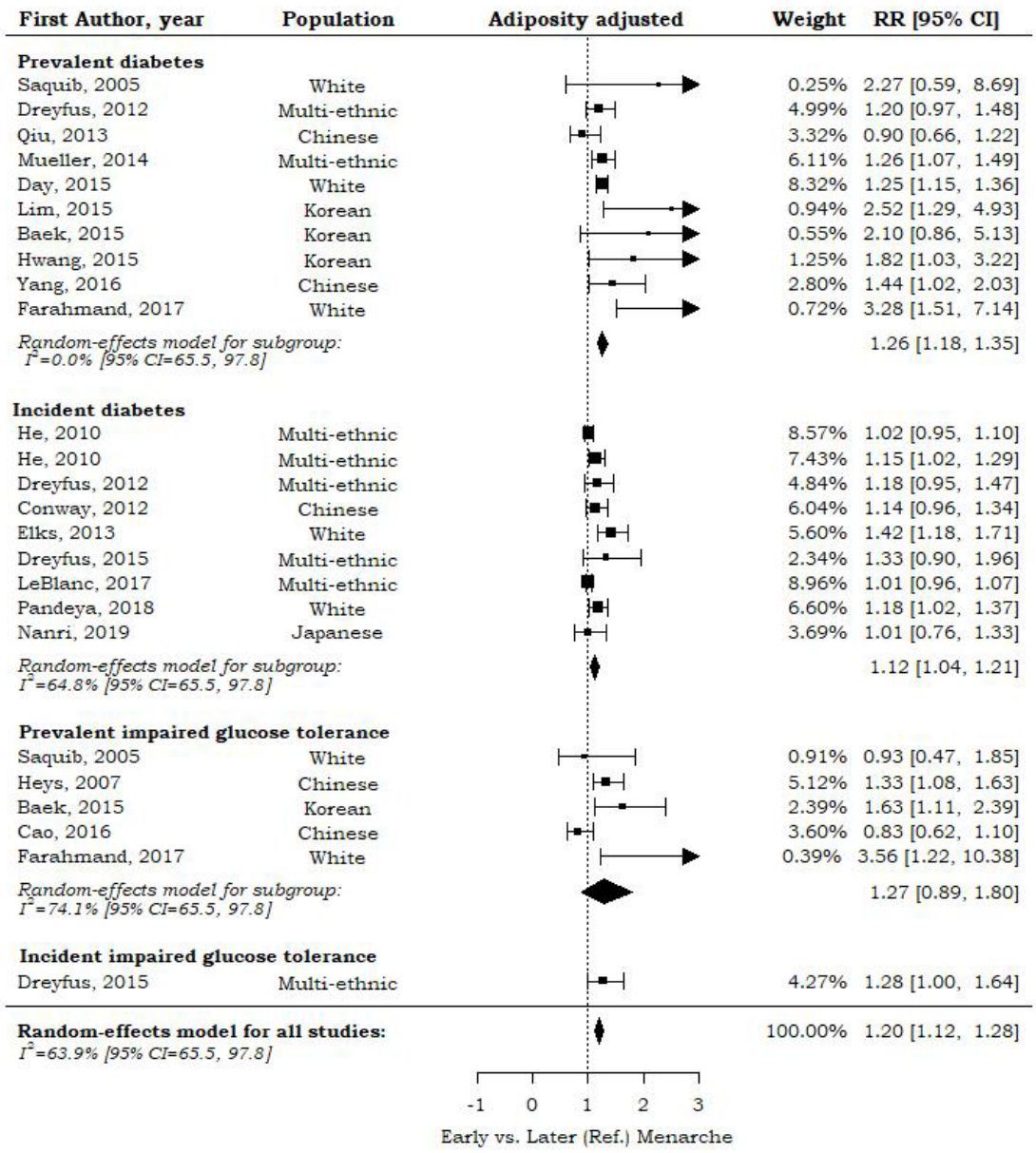
The plots illustrate the relative risks for T2D only and IGT only per each year later age at menarche, (a) without and (b) with adjustment for adiposity indicators. He et al.[153] consisted two cohort studies.

Supplementary Figure B-2 Forest plots of the associations of early versus later menarche with T2D or IGT

(a)

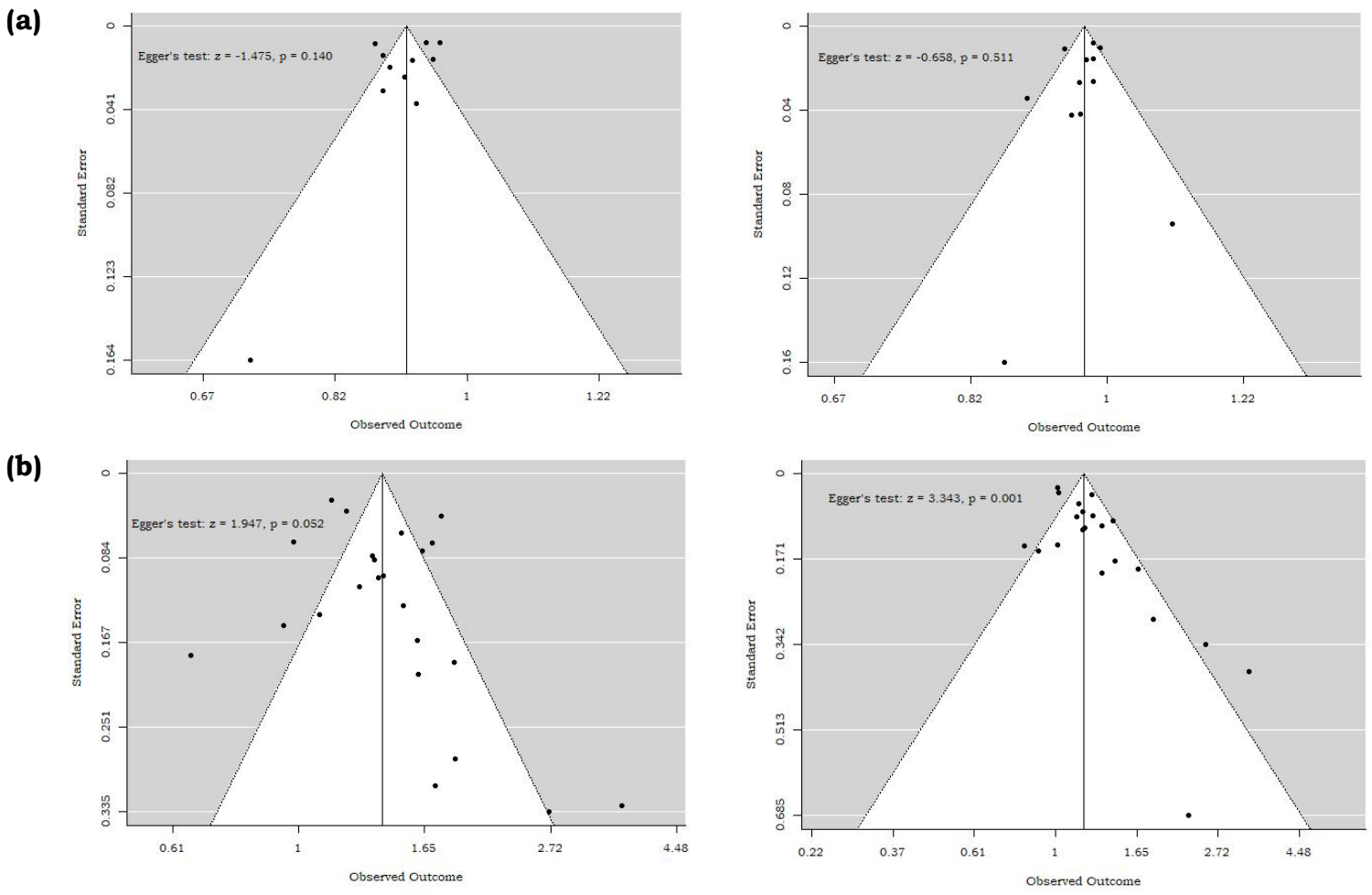


(b)



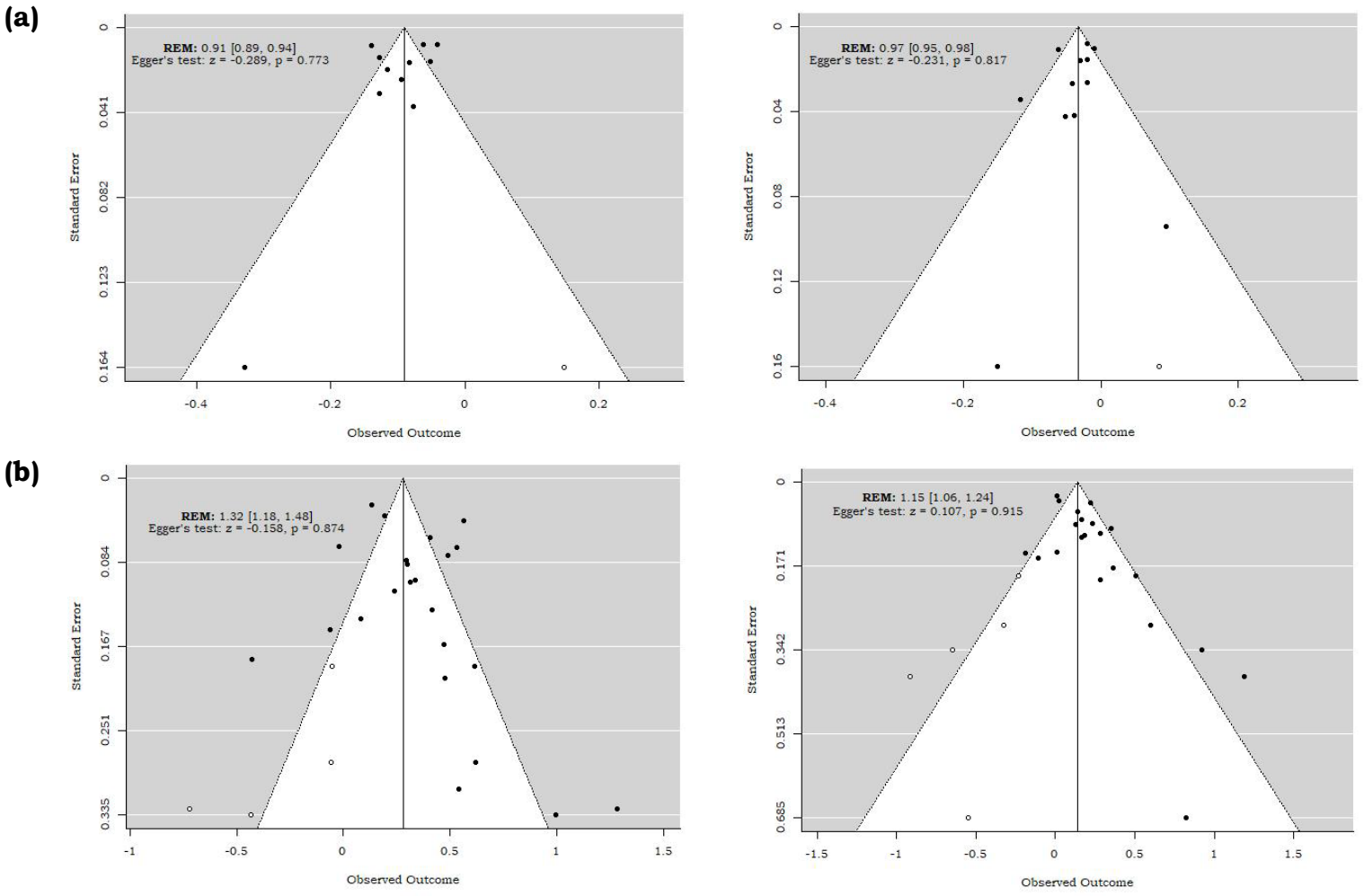
The plots illustrate the relative risks for T2D only or IGT only associated with early (versus later) menarche, (a) without and (b) with adjustment for adiposity indicators. He et al.[153] consisted two cohort studies. Ref., Reference

Supplementary Figure B-3 Funnel plots and Egger's tests for asymmetry



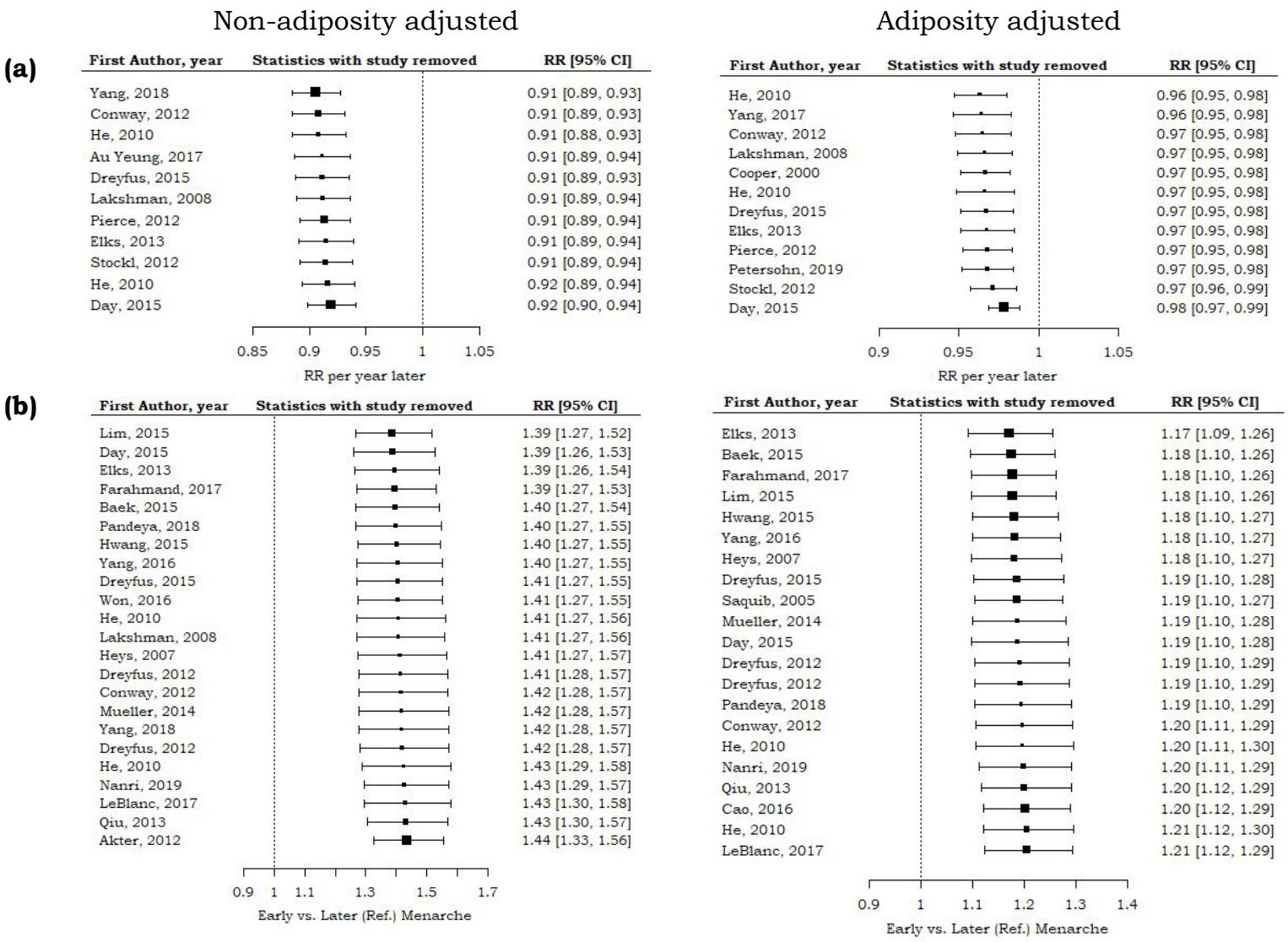
The plots show risk estimates from studies using (a) continuous age at menarche and (b) early menarche, with and without adjustment for adiposity, against a measure of study size

Supplementary Figure B-4 Funnel plots with the trim-and-fill method



The plots show filled missing studies using (a) continuous age at menarche and (b) early menarche, with and without adjustment for adiposity, which are indicated by open circles. REM, random-effects models

Supplementary Figure B-5 Forest plots of leave-one-out analysis



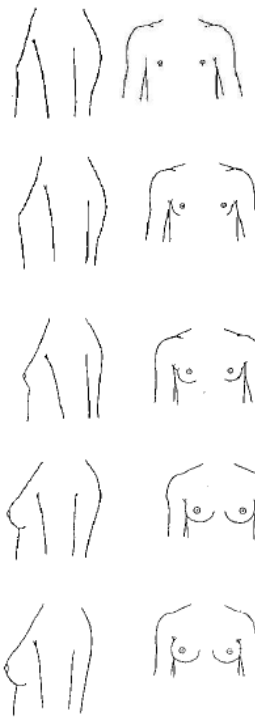
The plots show pooled estimates when each estimate in studies using (a) continuous age at menarche and (b) early menarche, with and without adjustment for adiposity, was iteratively removed. He et al.[153] consisted two cohort studies.

Supplementary Figure B-6 An adapted version of the Petersen Pubertal Development Scale

(a)

The drawings below show stages of the way the **breasts** develop. A girl can go through each of the five stages shown, although some girls skip some stages. Please look at each of the drawings. It is also important to read the descriptions.

Put a tick in the box to the right of the drawing that is **closest** to your daughter's current breast stage.



1 ☐ The nipple is raised a little in this stage. The rest of the breast is still flat.

2 ☐ This is the breast bud stage. In this stage the nipple is raised more than in stage 1. The breast is a small mound. The dark area around the nipple (areola) is larger than in stage 1.

3 ☐ The areola and the breast are both larger than in stage 2. The areola does not stick out away from the breast.

4 ☐ The areola and the nipple make up a mound that sticks up above the shape of the breast. (Note: This stage may not happen at all for some girls. Some girls develop from stage 3 to stage 5 with no stage 4.)

5 ☐ This is the mature adult stage. The breasts are fully developed. Only the nipple sticks out in this stage. The areola has moved back in the general shape of the breast.

6 ☐ Not sure

The drawings below show different amounts of **female pubic hair**. A girl can go through each of the five stages shown. Please look at each of the drawings. It is also important to read the descriptions.

Put a tick in the box to the right of the drawing that is the closest to the amount of pubic hair your daughter has.

1 ☐ There is no pubic hair

2 ☐ There is a little long, lightly coloured hair. This hair may be straight or a little curly.

3 ☐ The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a bigger area.

4 ☐ The hair is now as dark, curly, and coarse as that of an adult woman. However, the area that the hair covers is not as large as that of an adult woman. The hair has not spread out to the legs.

5 ☐ The hair now is like that of an adult woman. It also covers the same area as that of an adult woman. The hair usually forms a triangular pattern as it spreads out to the legs.

6 ☐ Not sure

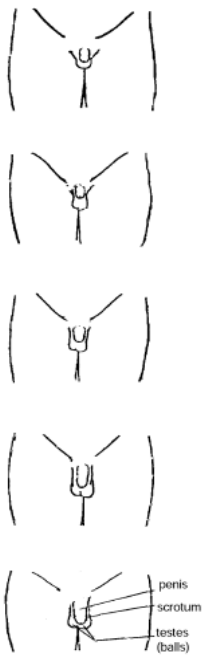
NOTE: Your daughter's pubic hair stage may or may not be the same as her stage of breast development.

(b)

Boys go through the various stages of physical development at different ages.
Some start as early as 6, others not until they are 16.
We need your help in letting us know what stage your son is at.

Please look at each of the drawings. It is also important to read the descriptions.

Put a tick in the box that is **closest** to your son's current stage.



1 ☐ The size and shape of the testes, scrotum (the sac holding the testes) and penis are about the same as when he was younger.

2 ☐ The penis is a little bit bigger. The scrotum has dropped and the skin of the scrotum has changed. The testes are bigger.

3 ☐ The penis has grown longer, the testes have grown and dropped lower.

4 ☐ The penis is longer and wider. The head of the penis is bigger, the scrotum is a darker colour and bigger. The testes are bigger.

5 ☐ The penis, scrotum and testes are the size and shape of a man's.

6 ☐ Not sure

As part of development, at some stage hair will start to grow just above the penis.

Please look at each of the drawings. It is also important to read the descriptions.

Put a tick in the box that is **closest** to the amount of pubic hair that your son has.

1 ☐ There is no hair at all.

2 ☐ There is a little soft, long, lightly coloured hair at the base of the penis. It may be straight or a little curly.

3 ☐ The hair is darker and more curled. It has spread out and thinly covers a bigger area.

4 ☐ The hair is as dark and curly as that of a man, but it hasn't spread out to the legs.

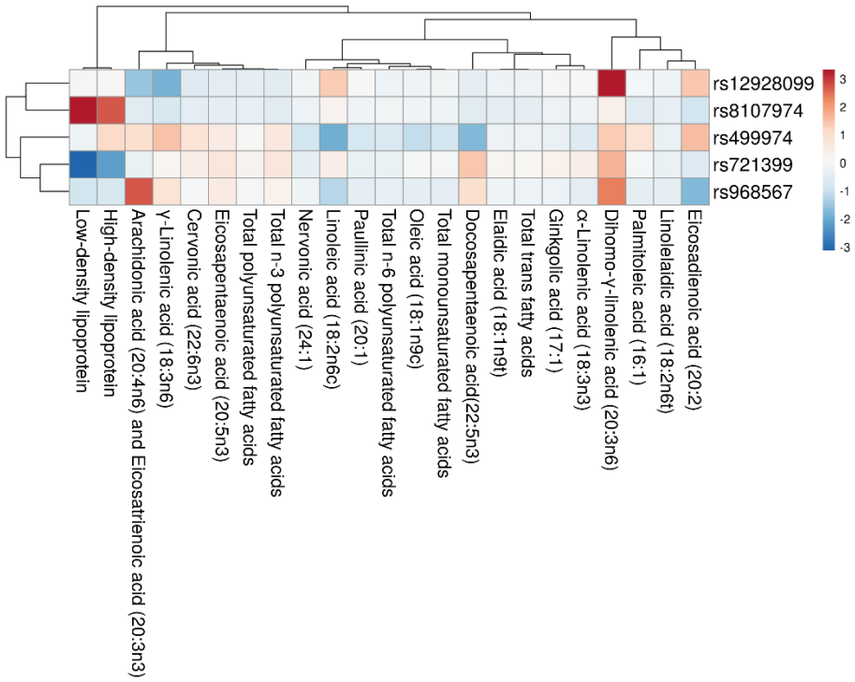
5 ☐ The hair is like that of a man. It has spread out to the legs.

6 ☐ Not sure.

The figures show the assessments of (a) breast development on the left and pubic hair growth on the right for girls and (b) genital development on the left and pubic hair growth on the right for boys.

Supplementary Figure B-7 Heatmaps of selected genetic variants against specific fatty acids in the EPIC-InterAct study and low- and high - density lipoprotein in the UK Biobank study

Dihomo-γ-linolenic acid (20:3n6)



Palmitoleic acid (16:1n7)