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Assessing causality between childhood adiposity and early puberty: A bidirectional Mendelian randomization and longitudinal study☆·☆☆

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ABSTRACT

Aims: Obesity and early puberty have been reported to be mutually causative. We investigated the causal relationship between adiposity and early puberty by performing bidirectional Mendelian randomization (MR) and longitudinal data analyses.
Methods: We used information from the Taiwan Children Health Study (3109 adolescents aged 11–12 years) with 17 body mass index (BMI)- and 10 puberty-related single-nucleotide polymorphisms (SNPs) to produce genetic instrumental variables (IVs). The two-stage least squares (2SLS) method, MR sensitivity analysis, and survival analysis were used to explore and confirm causality.
Results: Regression estimates from IVs revealed that significantly increased association of BMI with early puberty was noted (coefficients: 0.13, 0.10, and 0.09; 95% CI: 0.07–0.19, 0.02–0.19, and 0.02–0.16 for all participants, male adolescents, and female adolescents, respectively). Genetic IVs for puberty were not associated with BMI. MR sensitivity and two-sample MR analyses produced similar results. Longitudinal analysis results revealed that prepubertal overweight and obesity could predict early onset of puberty. However, after excluding children with a history of overweight and obesity at the age of 7–12 years, early puberty was not found to trigger new-onset of overweight and obesity at the age of 18 years in either sex.
Conclusions: Higher adiposity may lead to early puberty. However, the causal effects of early puberty on adiposity accumulation were not supported by our data. Targeted interventions to reduce childhood obesity are strongly recommended to prevent obesity-related comorbidities, as well as early puberty onset.

1. Introduction

Childhood obesity results in obesity in adulthood and has become a serious health issue worldwide. Moreover, the onset of puberty has been earlier since the start of the century [1]. According to large-scale studies on Chinese children, age at menarche has become 0.8 years [2] earlier and age at spermarche has become two years [3] earlier than those in the 1980s. Earlier maturing girls are more likely to be obese than nonearly maturing girls. However, the relationship between obesity and timing of puberty onset in boys has been inconsistent, depending on different ethnicities [3–5]. Although pubertal development in obese boys may be delayed rather than advanced in some ethnicities [6], several large scale multi-center Chinese studies reported that obese boys experienced early pubertal maturation as compared to non-obese ones [7–9]. One Chinese study observed that testicular volume and dehydroepiandrosterone levels were significantly higher in obese boys as compared to non-obese boys [10]. Early puberty is

Abbreviations: BIA, Bioelectrical impedance analysis; BMI, Body mass index; CI, Confidence interval; DTHM, Discrete-time hazard model; GEE, Generalized estimating equation; GnRH, gonadotropin-releasing hormone; GRS, Genetic risk scores; GWAS, Genome-wide association study; HR, Hazard ratio; IV, Instrumental variable; IVW, Inverse-variance weighted; MR, Mendelian randomization; NCGM, National Center for Genome Medicine; OR, Odds ratio; PCS, Puberty category score; SE, Standard error; SNP, Single-nucleotide polymorphism; TCHS, Taiwan Children's Health Study; TDCS, Tanner-derived composite stage; 2SLS, Two-stage least squares.

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☆☆ **Summary of the translational potential:** Using MR analysis and a longitudinal study, we confirmed the causal direction from adiposity to early puberty in both sexes. This study did not support the causality from early puberty to future adiposity accumulation. Future studies are suggested to target interventions that reduce childhood obesity to prevent early puberty onset.

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harmful to both sexes. Early puberty is a risk factor for fertility impairment [11], cardiometabolic diseases [12], breast cancer [13], and mortality [14].

Although considerable evidence supports the link between childhood obesity and early puberty in female adolescents, studies surveying the causal direction from early puberty to later onset of obesity have been highly heterogeneous. A meta-analysis reported that early menarche was associated with elevated adult BMI [15]. However, only a few of the studies in this meta-analysis included an adjustment for childhood BMI; they contained BMI measurement outcomes varying from ages 18 to 92 years. The causal direction between childhood obesity and pubertal timing remains uncertain. Multiple factors, such as birthweight, gestational weight gain, and perinatal nutritional exposure, might confound the association between obesity and early puberty. To overcome the problem of unmeasured confounders, a Mendelian randomization (MR) approach was suggested. In MR studies, causality is inferred from the association between genetic instrumental variables (IVs) that mimic the influence of modifiable exposure and outcomes. By using data from UK biobank, Gill et al. performed MR analysis and showed that earlier age at menarche causes higher adult BMI [16]. However, the causal relationships between adiposity and early puberty might vary among different ethnicities, and the causal relationships among males remain unexplored.

Clarifying the causal relationship between childhood adiposity and early puberty is critical, because determining the real underlying causes of the disease can guide proper interventions to prevent it. Besides, reports of early puberty-associated obesity in adulthood and cardiovascular diseases must be taken seriously. If the causality can be confirmed, early puberty might serve as a marker for identifying the risk of adverse cardiovascular outcomes. To determine the causal relationship between childhood obesity and early puberty, we (1) investigated the causal direction between childhood obesity and early puberty by using MR analysis and (2) used longitudinal data to investigate the causal direction from childhood obesity to early puberty as well as the effect of early puberty on the incidence of adult-onset obesity. Moreover, we explored a differential sex effect in the analyses.

2. Materials and methods

2.1. Study population

In 2007 and 2010, we conducted a nationwide "Taiwan Children Health Study (TCHS)," of 7930 nine to thirteen-year-old school children in 14 diverse Taiwanese communities over Eastern, Western, Northern and Southern parts of Taiwan. The first cohort of 5091 seventh- to eighth-grade children was enrolled in 2007, and the second cohort of 2839 fourth-grade schoolchildren was enrolled in 2010 and employed an open-cohort study design. The TCHS is a population-based cohort study focusing on obesity, pubertal development, and atopic diseases in adolescents. The analysis herein involved data from the second cohort because it contained detailed pubertal stage assessments. The detailed recruitment of participants in the TCHS was already documented [17]. Participants in the second cohort were followed up at ages 11, 12, and 18 years. Data regarding pubertal development were collected beginning at age 11. The study protocol was approved by the Institutional Review Board of National Taiwan University Hospital and complied with the principles of the Declaration of Helsinki.

2.2. Definition of early puberty timing

Pubertal staging (Tanner stages) was assessed using valid questionnaires and the Tanner-derived composite stage (TDCS) [18], which were completed by adolescents aged 11–18 years. The TDCS contains schematics of the secondary sex characteristics of puberty. We have done the validation study of the Chinese version of TDCS among 190 participating children by comparing self-report and physician inspection Tanner stages (see Supplemental Tables 1 and 2). The consistency between self-reported and physician inspection Tanner stages was high (agreement to within one Tanner stage for genital area and pubic hair distribution were observed for 94.74% and 80.53% children, with weighted kappa of 0.85 and 0.80, respectively), indicating that TDCS could be a valid tool for assessing pubertal staging. Participants were asked to select one of the drawings to represent the status of their pubic hair and breast/genital development, respectively. Early puberty was defined if adolescents reached a certain pubertal stage earlier than the median age for that stage [19]. The median ages for pubertal stages were referenced from a large Chinese population-based study [20,21]. Moreover, we defined early voice breaking for male participants and early menarche for female participants as other pubertal outcomes for early puberty. The Chinese version of the Puberty Category Score was used to evaluate age at menarche and voice breaking [22]. Early menarche was defined as age at menarche earlier than 12 years. In addition, early voice breaking was defined as age at voice breaking earlier than 13 years [23].

2.3. Assessment of adiposity and overweight

During a school visit, measurements of total body fat and fat-free mass were obtained using bioelectrical impedance analysis machines (IOI 353, Jawon Medical, Seoul, Korea). The sum of skinfold measurements at the bilateral triceps and gastrocnemius was performed using Lange calipers (Beta Technology, Santa Cruz, CA, USA). The waist to height ratio (%) was defined as waist circumference divided by height. All body composition measurements were transformed into age- and sex-specific Z-scores according to our cohort reference. BMI was calculated as weight divided by height squared (kg/m^2) and converted into age- and sex-specific percentiles according to growth charts for Taiwanese children and adolescents [24]. Subsequently, we defined overweight adolescents as those having a BMI in the 85th to 94th percentile and obese participants as those having a BMI of ≥95 percentile. New-onset overweight and obesity at the age of 18 years were defined after excluding children with a history of overweight and obesity at the age of 7–12 years.

2.4. Genotype selection and instrument establishment

Genotyping was performed using Sequenom iPLEX matrix-assisted laser desorption/ionization-time of flight mass spectrometry at the National Center for Genome Medicine platform, Taiwan. We identified candidate single-nucleotide polymorphisms (SNPs) by using the following criteria: (1) the genotyping call rate was >98% for all children; (2) SNPs exhibited a minor allele frequency of ≥5%; (3) SNPs having a linkage disequilibrium with candidate SNPs were not selected; and (4) SNPs that were reported to be associated with both obesity and puberty were excluded to avoid a pleiotropic effect. A total of 17 BMI-related SNPs were identified using the results of the meta-analyses of either Asian genome-wide association studies (GWASs) [25] or Chinese GWASs [26]. Additionally, ten puberty-related SNPs were selected based on the findings of large meta-analyses of GWASs [27]. Supplemental Tables 3 and 4 list detailed references for candidate gene selection. The details of genotyping procedures were described in our previous study [28]. Weighted genetic risk scores (GRSs) composed of BMI- and puberty-related SNPs were used as IVs.

2.5. Statistical analyses

One sample MR method, namely the two-stage least squares (2SLS) method, was first conducted. Sensitivity analysis (MR Egger, inverse-variance weighted [IVW], and median-based methods) for MR [29] was further performed to examine possible pleiotropic effects and provide robust estimation of the causality between obesity and early puberty. Individual MR estimates obtained using the 2SLS method for

early obesity and early puberty genes were pooled using the IVW method. Moreover, Egger regression was performed to detect small study bias and directional pleiotropic effects of genes. Furthermore, we used a median-based method to estimate the causal effect of each genetic variant and then calculated the median estimate. The weighted median estimate is under the assumption that genetic variants representing over 50% of the weight in the analysis are valid instruments. Additionally, we compared the effects of various body composition measurements on early puberty, early menarche, and early voice breaking.

We confirmed the temporal relationship between adiposity and early puberty by performing a longitudinal analysis. We implemented the prepubertal overweight and obesity status at the age of seven to ten years to predict early puberty outcomes by using a generalized estimating equation (GEE) baseline model. In addition, we performed a longitudinal follow-up survey at the age of 18 years to verify the temporal relationship between early pubertal maturation and new-onset overweight and obesity by using a discrete-time hazard model (DTHM). The DTHM is a survival analysis model that enables the estimation of hazard ratios (HRs) and 95% confidence intervals (CIs) for an event occurring in a discrete time framework. Multiple covariates that might confound the association between obesity and puberty were adjusted in the GEE and DTHM models, including parental education, family income, birthweight, breastfeeding, gestational age, and *in-utero* smoking. All model regression and sensitivity analysis were performed using R3.3.2.

2.6. Two-sample MR analyses

A two-sample MR method was conducted as a replica analysis using GWAS data available at the MR-Base platform (http://www.mrbase.org). In this method, estimates of the genetic association with the exposure and outcome were obtained from various study populations. Childhood BMI data and associated SNPs containing a maximum of 463,013 participants were obtained from GIANT Consortium studies. Age at menarche data was derived from UK Biobank with a total of 336,107 participants.

3. Results

3.1. Participants' characteristics

The characteristics of TCHS participants in this study are shown in Table 1. Only adolescents with complete data for body composition measurements and pubertal assessment at the age of 11–12 years were included. The average cohort follow-up rate from the age of 11 to 18 years was 64.6%. One-third of children were categorized as overweight and obese at the age of 11–12 years, and the prevalence of overweight and obesity declined to 21% at the age of 18 years; moreover, 11.6% and 24.1% of male adolescents had early pubertal maturation, and 9.8% and 6.6% of female adolescents had early pubertal maturation at the age of 11–12 years, respectively. Early voice breaking appeared in 4.1% and 12.1% of male adolescents, and early menarche appeared in 24.4% and 42.1% of female adolescents.

3.2. Adiposity increases risk of early puberty but early puberty does not lead to adiposity accumulation

Table 2 and Fig. 1 present the coefficients of the bidirectional MR analysis. The BMI GRS created from 17 BMI-related SNPs showed a positive association with BMI (coefficient: 28.6, 95% CI: 23.6–33.6, $p = 2.0 \times 10^{-16}$). The BMI GRS served as a strong instrument for adiposity, with F statistics of 126.2 [30]. Little evidence was obtained for the association of individual BMI-related SNPs with early puberty and confounders (Supplemental Tables 3 and 5). The results of the 2SLS analysis revealed that BMI was associated with the risk of early puberty in both sexes (coefficient: 0.13, 95% CI: 0.07–0.19, $p = 6.4 \times 10^{-16}$ for total participants). However, ten puberty-related SNPs were not found to be associated with Z-BMI (Supplemental Table 4) and confounders (Supplemental Table 6). The GRSs for puberty were significantly correlated with early puberty with adequate F statistics for both sexes (F statistics of 40.26; F statistics: 14.6 for male adolescents; F statistics: 11.3 for female adolescents). However, the 2SLS analysis results did not prove the causal direction from early puberty to increased BMI ($p = 0.98$). As shown in Table 3, most of the associations between body composition measurements and early puberty outcomes were significant among female adolescents. Among multiple body composition measurements, fat-free mass was the strongest predictor (OR: 1.26, 95% CI: 1.04–1.54) of early menarche among female adolescents in the IV analyses. Among MR sensitivity analyses, the IVW method provided results similar to those of the 2SLS analysis (Supplemental Table 7), indicating that no pleiotropy was detected.

Table 1
Characteristics of study children in the Taiwan Children's Health Study.

Characteristics	Children at 11 y			Children at 12 y		
	$n = 2879$			$n = 3109$		
	N with data	Distribution		N with data	Distribution	
Age, years	2879	11.09	±0.32	3109	12.08	±0.32
Male sex	2879	1435	(49.8)	3109	1568	(50.4)
Breast feeding	1900	950	(50.0)	2006	995	(49.6)
Birthweight, kg	2879	3.13	±0.46	3109	3.12	±0.47
Gestational age, weeks	2879	38.55	±2.38	3109	38.48	±2.51
In-utero smoking	2846	74	(2.6)	2556	69	(2.7)
Parental education						
High school or below	2874	1378	(47.9)	3003	1553	(51.7)
College or university	2874	1231	(42.8)	3003	1200	(39.9)
Post-graduate school	2874	265	(9.2)	3003	251	(8.4)
Family income, NTD[a]						
<600,000	2874	1277	(44.4)	3004	1439	(47.9)
600,001–1,000,000	2874	1083	(37.7)	3004	1056	(35.2)
>1,000,001	2874	514	(17.9)	3004	509	(16.9)
Adiposity measurements at that time						
Overweight and Obesity	2804	945	(33.7)	2800	1005	(35.9)
Obesity	2804	513	(18.3)	2800	574	(20.5)
BMI (kg/m^2)	2804	19.30	±0.70	2800	20.36	±0.70
Adiposity measurements at age 18 years						
Overweight and Obesity	986	211	(21.4)	1115	243	(21.8)
Obesity	986	98	(9.9)	1115	116	(10.4)
BMI (kg/m^2)	986	21.14	±3.43	1115	21.20	±3.49
Pubertal status (males)						
Early puberty	1435	166	(11.6)	1568	378	(24.1)
Early voice breaking	1435	59	(4.1)	1190	144	(12.1)
Tanner stage 1	1435	417	(29.1)	1568	185	(11.8)
Tanner stage 2	1435	793	(55.3)	1568	882	(56.3)
Tanner stage 3	1435	200	(13.9)	1568	370	(23.6)
Tanner stage 4	1435	23	(1.6)	1568	123	(7.8)
Tanner stage 5	1435	2	(0.1)	1568	8	(0.5)
Pubertal status (females)						
Early puberty	1444	142	(9.8)	1541	101	(6.6)
Early menarche	1426	348	(24.4)	1183	498	(42.1)
Tanner stage 1	1444	176	(12.2)	1541	48	(3.1)
Tanner stage 2	1444	987	(68.4)	1541	731	(47.4)
Tanner stage 3	1444	249	(17.2)	1541	632	(41.0)
Tanner stage 4	1444	30	(2.1)	1541	120	(7.8)
Tanner stage 5	1444	2	(0.1)	1541	10	(0.6)
Obesity genetic score[b]	2879	0.03	±0.01	3109	0.03	±0.01
Puberty genetic score[b]	2879	0.57	±0.14	3109	0.57	±0.14

All data are presented as means ± SD or numbers (%).
The number of participants did not add up to the total number because of missing data.
[a] NTD: New Taiwan Dollars.
[b] Obesity or puberty genetic score: The dosage of the effect allele was multiplied by a SNP-specific weights, and the weight reflects the effect of the corresponding genetic variant on the phenotype (early puberty or Z-BMI). Each weighted genetic allele was summarized into a weighted genetic score to be used as genetic IV.

Table 2
Summary of coefficients used for bidirectional Mendelian randomization analysis.

Instrumental variables	Genetic score with intermediate trait					Genetic score with outcomes				Two-stage IV analysis (Early puberty or Z-BMI)			
	Coefficient (95% CI)			*p* value	F-value	Coefficient (95% CI)			*p* value	Coefficient (95% CI)			*p* value
BMI genetic score (17 SNPs)	28.56	23.58	33.55	2.0×10^{-16}	126.20	3.67	1.93	5.40	3.5×10^{-5}	0.13	0.07	0.19	6.4×10^{-16}
Males	27.05	20.81	33.28	2.0×10^{-16}	72.31	2.58	0.10	5.05	0.04	0.10	0.002	0.19	0.04
Females	32.99	24.56	41.43	2.5×10^{-14}	58.81	2.91	0.53	5.29	0.02	0.09	0.02	0.16	0.02
Puberty genetic score (10 SNPs)	0.22	0.15	0.29	2.4×10^{-10}	40.26	2.6×10^{-3}	−0.19	0.20	0.98	0.01	−0.89	0.91	0.98
Males	0.20	0.10	0.30	1.4×10^{-4}	14.55	−0.10	−0.36	0.16	0.44	−0.52	−1.88	0.84	0.45
Females	0.15	0.06	0.23	7.7×10^{-4}	11.34	0.18	−0.13	0.49	0.24	1.24	−0.85	3.34	0.25

The results of the replication analysis of the two-sample MR analysis, performed using GIANT Consortium and UK Biobank data, showed that childhood BMI was negatively associated with age at menarche (Supplemental Table 8). Additionally, the association between age at menarche and future adulthood BMI was negative (Supplemental Table 9).

3.3. Prepubertal overweight and obesity predict early puberty onset

To confirm the causality identified in the bidirectional MR analysis, a GEE was utilized to examine whether prepubertal overweight and obesity could predict early puberty onset (Table 4). Linear regression was used to determine the strength of relationship of BMI and age at voice breaking/menarche (Supplemental Table 10). Overweight and obesity at the age of seven to ten years predicted early puberty and early menarche at the age of 11–12 years in female adolescents (overweight at ten years: OR: 1.12, 95% CI: 1.09–1.15, $p = 4.6 \times 10^{-10}$ for early puberty; OR: 1.18, 95% CI: 1.12–1.24, $p = 6.3 \times 10^{-10}$ for early menarche). In male adolescents, the causality was weaker than that in female adolescents (overweight and obesity at ten years: OR: 1.04, 95% CI: 1.00–1.08, $p = 0.04$ for early puberty; OR: 1.03, 95% CI: 1.00–1.05, $p = 0.06$ for early voice breaking). Similar findings could be observed while analyzing the correlation coefficients between prepubertal BMI and age at voice breaking/menarche (Supplemental Table 10).

3.4. Early puberty does not lead to overweight and obesity in young adulthood

We investigated the causal relationship between early puberty and incidence of overweight and obesity at the age of 18 years, after excluding children with a history of overweight and obesity at the age of 7–12 years (Table 5). Similar to the findings shown in Table 2, early puberty was not found to trigger the new onset of overweight and obesity in both sexes. In fact, all HRs concerning the incidence of overweight and obesity were below 1, indicating that early puberty might even be a protective factor for obesity at the age of 18 years.

4. Discussion

Using bidirectional MR analysis and a longitudinal cohort study, we provided robust evidence to support that childhood adiposity leads to early puberty in both sexes, whereas early puberty triggers the incidence of overweight and obesity was not confirmed in this study. In our study population, overweight and obese male participants also had an increased risk of early puberty, although the causality was weaker than that in female participants. Our results did not support the findings of a previous meta-analysis [31], indicating that any association between pubertal timing and adulthood adiposity is likely to be confounded by childhood adiposity. Our findings clarified the long-term debates on bidirectional causality between adiposity and early puberty, which might be useful for future interventional studies targeting childhood obesity prevention.

Several possible biological mechanisms have been proposed to explain the causal relationships between childhood adiposity and early puberty. Mechanisms exploring the critical fat mass in triggering pubertal onset have been widely discussed and examined, both in rodent and human studies. Insulin resistance in obese adolescents was associated with hyperinsulinemia, which is a known factor that triggers pubertal onset [32]. Besides, decreased levels of liver sex-hormone-binding globulin in obese adolescents could further result in increased sex steroid bioavailability. Increased aromatase activity in obese adolescents results in increased conversion of androgens to estrogens, contributing to early and continuous exposure to estrogens in girls [6]. In addition, hyperleptinemia plays a critical role in puberty onset. Leptin, a marker of energy abundance [33], may have direct stimulatory effects on hypothalamus to increase the gonadotropin secretion as well as accelerate gonadotropin-releasing hormone (GnRH) pulse frequency [34]. This was mainly through binding to the leptin receptor and activate kisspeptin for transmission of the regulatory effects on GnRH to stimulate luteinizing hormone and follicular stimulating hormone release [35]. The role of Leptin could be supported by human studies which reported that Leptin concentrations rise before the onset of puberty among girls [36].

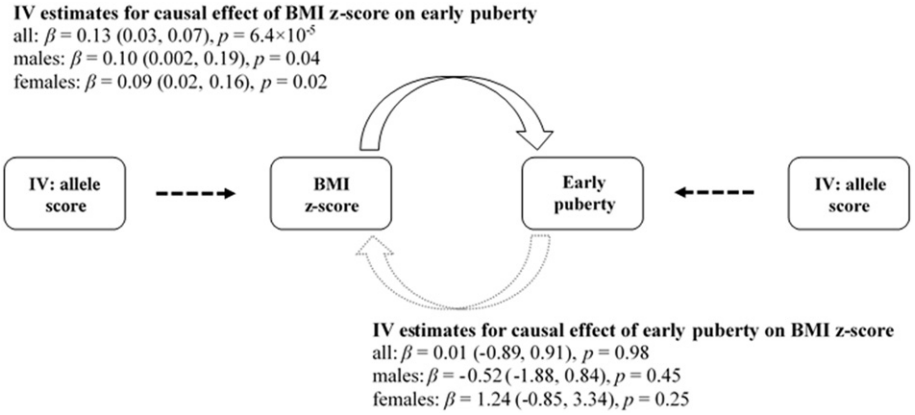

Fig. 1. Addressing the causal directions in the associations between BMI Z-score and early puberty with the use of allelic scores and bidirectional Mendelian randomization analysis.

Table 3
Instrumental variable estimates of body composition measures on pubertal outcomes in children at age 11–12 years.

Body composition measures (Z-score)	Early puberty				Early voice breaking (males)/early menarche (females)			
	OR	(95% CI)		*p* value	OR	(95% CI)		*p* value
Sum of skinfolds	1.20	1.09	1.32	2.7×10^{-4}				
Males	1.13	0.98	1.31	0.09	1.08	0.97	1.19	0.18
Females	1.15	1.04	1.27	0.01	1.19	1.00	1.41	0.04
Waist	1.16	1.08	1.25	8.6×10^{-5}				
Males	1.11	0.99	1.24	0.07	1.06	0.98	1.15	0.17
Females	1.12	1.04	1.22	0.01	1.16	1.01	1.34	0.04
Waist/height ratio	1.18	1.08	1.28	1.4×10^{-4}				
Males	1.13	0.99	1.29	0.08	1.07	0.97	1.18	0.17
Females	1.12	1.03	1.22	0.01	1.16	1.00	1.34	0.04
Total body fat	1.15	1.07	1.25	3.2×10^{-4}				
Males	1.08	0.96	1.22	0.19	1.06	0.97	1.15	0.18
Females	1.15	1.05	1.25	2.2×10^{-3}	1.19	1.02	1.38	0.02
Fat-free mass	1.17	1.08	1.28	2.1×10^{-4}				
Males	1.08	0.97	1.21	0.18	1.06	0.98	1.14	0.17
Females	1.21	1.07	1.37	3.2×10^{-3}	1.26	1.04	1.54	0.02

In the relevant literature, relationships between childhood adiposity and early pubertal maturation in males were not consistent and varied among ethnicities [9,19]. One Chinese study [3] reported a negative association between obesity and pubertal onset timing, whereas an American study [37] discovered positive relationships among boys. Another Chinese study discovered that testicular volume in obese prepubertal boys was larger than that in age-matched nonobese controls [10]. Additionally, one African adolescent longitudinal study discovered that adiposity at age 5 was associated with earlier pubertal development [38]. The possible reason of the heterogeneity of these studies is the lack of standardization for assessing genital development among males. The robust definition of early puberty among males necessitates the development of robust biomarkers. In this study, results of obesity and early puberty risk were relatively weak among males as compared to females (Tables 3 and 4 and Supplemental Table 10). However, main findings of MR analysis revealed that there's a significant increase association of BMI with early puberty. Our study provides insights into the relationship between adiposity and early puberty in male adolescents, strengthening the evidence that obesity triggers the earlier onset of puberty in Chinese populations [7]. Boys with premature pubarche, defined in one study as those having the appearance of pubic hair or axillary hair before the age of nine years, also developed hyperinsulinemia compared with normal controls. For both boys and girls, the timing of adrenarche and elevation in adrenal androgens occurs in early childhood [39]. Prepubertal adiposity may have a lasting effect on the increase in adrenal androgen, which may contribute to the timing of pubertal growth spurt. Moreover, obesity is often accompanied by inflammatory reactions that increase the cytokines and promote the synthesis of androgen, and such changes in androgen could precipitate early pubertal development among boys. Another possible mechanism may be related to Leptin [40], which is produced by fat cells and also plays a critical role in the initiation of puberty for both boys and girls [41]. More research is required to clarify detailed mechanisms underlying the effects of adiposity on pubertal development in boys.

Early pubertal timing was predictive of higher adult BMI in one meta-analysis [33] and one MR analysis [16]. This phenomenon has been postulated in women but not in men, and exact mechanisms remained unexplored. In fact, many of these included studies in the meta-analysis did not consider the effect of childhood obesity in the relationships. Another child cohort study suggested that the effect of puberty timing on adiposity could largely be confounded by prepubertal adiposity status, instead of being driven by early puberty itself [42]. The phenomenon of early puberty could originate from childhood adiposity and be traced into adulthood adiposity. And the common genetic variants with both adiposity and age at menarche still produce the possibility of pleiotropic effect in previous MR study [16]. To overcome the limitation of study designs in the relevant literature, we took advantage of our cohort by excluding those participants who had once been overweight and obese in childhood and proved that normoweight children who experienced early puberty were not likely to become obese in young adulthood. However, we cannot guarantee that these children will not become obese in their middle age. Longer follow-ups of these children with puberty until their later adulthood are suggested to determine the critical timing of obesity onset under early pubertal maturation status.

Under strong evidence from observational studies that obesity leads to early puberty in girls, only a few studies have conducted weight

Table 4
Odds ratios of early puberty at age 11–12 years, taking prepubertal overweight and obesity status at age 7–10 years as a predictor.

Prepubertal overweight status	Early puberty				Early voice breaking (males)/early menarche (females)			
	OR	(95% CI)		*p* value	OR	(95% CI)		*p* value
Overweight and obesity at age 7 years	1.08	1.05	1.11	3.7×10^{-7}				
Males	1.04	1.00	1.08	0.09	1.05	1.02	1.08	3.8×10^{-3}
Females	1.11	1.07	1.14	2.7×10^{-7}	1.24	1.17	1.31	2.8×10^{-13}
Overweight and obesity at age 8 years	1.07	1.05	1.10	1.5×10^{-6}				
Males	1.04	1.00	1.08	0.07	1.06	1.03	1.09	6.5×10^{-4}
Females	1.11	1.07	1.14	1.1×10^{-7}	1.23	1.17	1.30	2.6×10^{-13}
Overweight and obesity at age 9 years	1.09	1.06	1.11	1.0×10^{-8}				
Males	1.05	1.01	1.10	0.01	1.06	1.03	1.09	2.4×10^{-4}
Females	1.11	1.07	1.14	3.8×10^{-8}	1.19	1.13	1.25	3.9×10^{-10}
Overweight and obesity at age 10 years	1.09	1.06	1.11	1.9×10^{-9}				
Males	1.04	1.00	1.08	0.04	1.03	1.00	1.05	0.06
Females	1.12	1.09	1.15	4.6×10^{-10}	1.18	1.12	1.24	6.3×10^{-10}

Generalized estimating equations were adjusted for parental education, family income, birthweight, breastfeeding, gestational age, and *in-utero* smoking.

Table 5
Hazard ratios of incidence of overweight and obesity at age 18 years, taking early puberty status at age 11–12 years as a predictor.

Pubertal status	Overweight and obesity				Obesity			
	HR	(95% CI)		*p* value	HR	(95% CI)		*p* value
Early puberty	0.83	0.37	1.85	0.65	0.52	0.16	1.72	0.29
Males	0.86	0.33	2.29	0.77	0.43	0.10	1.83	0.25
Females	1.18	0.27	5.11	0.82	0.84	0.11	6.63	0.87
Early menarche	0.50	0.19	1.33	0.16	0.46	0.10	2.10	0.32

Discrete time hazard models were adjusted for parental education, family income, birthweight, breastfeeding, gestational age and *in-utero* smoking.

reduction interventions in obese children to determine the effects on delay in pubertal onset. One school-based weight management trial reported that weight intervention delayed menarche [43]. Moreover, the delay in menarche was produced by increased physical activity, decreased television viewing, and changes in BMI and fat distribution. Reinehr et al. [44] discovered that after one year of lifestyle intervention in overweight children with an average age of 10.3 years, puberty onset was less frequent in girls with BMI reduction compared with girls without BMI reduction. However, this finding was in contrast to that observed for boys with BMI reduction who experienced more frequent onset of puberty than boys without BMI reduction. To prevent early puberty-related future disease burden, further studies that explore the possible mechanisms and provide the critical period of time at which intervention should be targeted are required.

Our study has several strengths. The first is that it was a population-based cohort study of children with longitudinal follow-ups into young adulthood. Moreover, we adopted several new analytical techniques, such as the MR Egger, IVW, and median-based method, to validate possibilities caused by directional pleiotropy. Furthermore, two-sample MR using data from different cohorts, confirmed our findings. We confirmed the causal direction from adiposity to early puberty in both sexes. The causality from early puberty to future adiposity accumulation was not supported by this study.

The associations between different body composition measurements and early puberty outcomes among female adolescents were all significant. Among various body composition measurements, we also discovered that the strongest effect for linking early puberty among girls was fat-free mass, which is consistent with several previous studies [45,46]. One possible explanation is that the increase in intramyocellular lipid within the fat-free mass may exert sex hormone regulating effects.

This study has some limitations. First, the definition of puberty onset in males is relatively controversial, necessitating the development of robust biomarkers. Therefore, we used two valid questionnaires and two pubertal outcomes to present our analysis. Our findings were consistent in both the MR and longitudinal analyses in two pubertal outcomes. Our study was also limited by using self-reported questionnaires to define Tanner stages. However, we proved that the consistency between self-reported and physician inspection Tanner stages was high while using the Chinese version of TDCS questionnaire (see Supplemental Tables 1 and 2). This implies that TDCS could still be a valid tool for assessing pubertal staging. Additionally, our study was limited by its relatively small sample size for MR analysis; therefore, we used repeated measurements at the age of 11–12 years, which improved the validity and accuracy of measurements. Compared with large GWAS studies, the numbers of our candidate SNPs representing exposure phenotype were relatively small. However, the selected SNPs for both adiposity and puberty yielded satisfactory F statistics to serve as qualified IVs. Finally, it is possible that our findings may not be generalizable to other regions of the world, because the associations between obesity and early puberty among boys are highly-ethnicity sensitive. Our findings that obesity increases the risk of early puberty among boys have been supported by several previous Chinese studies [8,9].

The present results provide genetic evidence that higher adiposity accumulation leads to early puberty. While not supported by this study, the idea that children with early puberty may gain adiposity in later adulthood requires further testing. Our study highlighted the importance of future studies to target interventions that reduce childhood obesity to prevent early puberty onset.

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Declaration of competing interest

The authors have no conflicts of interest relevant to this article to disclose.

Author contributions

Dr. Yang-Ching Chen contributed to the conceptualization and design the study, the cohort data collection, interpretation of data, and writing. Mr. Hsien-Yu Fan assisted in the critical part of the statistical analysis, interpreted data, and revised the manuscript critically for important intellectual content. Dr. Chen Yang contributed to hypothesis generation, interpreted data, and revised the draft of the manuscript. Prof. Rong-Hong Hsieh contributed to the analysis and interpretation of data and critically revised the manuscript for intellectual content. Prof. Wen-Harn Pan critically reviewed the revised manuscript. Prof. Yungling Lee reviewed the study design, acquired and interpreted data, supervised the study, and revised the manuscript critically for valuable intellectual content. All authors approved the final manuscript for submission and publication and agree to be accountable for all aspects of the work. All authors agreed to the terms of the copyright transfer/affirmation of originality, and provide the appropriate disclosure of potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.153961.

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