Mediators Linking Obesity to Childhood Asthma: the Role of Puberty, Physical Fitness, and Pulmonary Function

Yang-Ching Chen[1], Ming-Wei Su[2], Wen-Harn Pan[3], and Yungling Lee[4]

[1]Institute of Preventive Medicine, College of Public Health, National Taiwan University
[2]EInstitute of Epidemiology and Preventive Medicine
[3]Institute of Biomedical Sciences Academia Sinica
[4]Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University

April 16, 2024

Abstract

Background: Obesity and asthma are highly associated, but the mechanisms underlying the association remain unknown. We examined five mediators linking obesity with childhood asthma: (1) pulmonary function impairment, (2) airway inflammation, (3) physical fitness, (4) sleep-disordered breathing (SDB), and (5) early puberty. Methods: A Mendelian randomization (MR) study with mediation analysis of data obtained from 5,965 children as part of the Taiwan Children Health Study. Cross-sectional regression analysis, MR two-stage least squares method, and MR sensitivity analysis were carried out to investigate each causal pathway. Prospective cohort analyses were used to confirm the findings. Results: The increased asthma risk associated with obesity was found to be mostly mediated through impaired pulmonary function, low physical fitness, early puberty. In the MR analysis, body mass index was negatively associated with FEV1/FVC and physical fitness index (β= -2.17 and -0.71; 95% CI, -3.92 to -0.42 and -1.30 to -0.13, respectively) and positively associated with early puberty (OR, 1.09; 95% CI, 1.02–1.17). High FEV1/FVC and physical fitness index reduced the risk of asthma (OR, 0.98 and 0.93; 95% CI, 0.97–0.99 and 0.88–0.98, respectively), whereas SDB and early puberty increased the risk of asthma (OR, 1.03 and 1.22; 95% CI, 1.01–1.05 and 1.05–1.42, respectively). The three main mediators were low physical fitness, impaired pulmonary function, and early puberty, with mediation proportions of 91.4%, 61.6%, and 28.3%, respectively. Temporal causality was further strengthened in prospective cohort analyses. Conclusions: Interventions promoting physical fitness and pulmonary function might effectively reduce obesity-induced asthma risk.

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Yang-Ching Chen[1,2,3,4], Yen-Tsung Huang[5], Wen-Harn Pan[6], Yungling L.Lee[6,7]

[1]Department of Family Medicine, Taipei Medical University Hospital, Taipei Medical University, Taipei, Taiwan

[2]Department of Family Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

[3]School of Nutrition and Health Sciences, College of Nutrition, Taipei Medical University, Taipei, Taiwan

[4]Graduate Institute of Metabolism and Obesity Sciences, Taipei Medical University, Taipei, Taiwan

[5]Institute of Statistical Science, Academia Sinica, Taipei, Taiwan

[6]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

[7]College of Public Health, China Medical University, Taichung, Taiwan

Short running title: Link between adiposity and asthma

Correspondence and page proofs should be sent to:

Yungling Leo Lee, MD, PhD

Institute of Biomedical Sciences, Academia Sinica, 128 Academia Road, Sec. 2, Taipei 11529, Taiwan

Tel: +886-2-2652-3013

E-mail: leolee@ibms.sinica.edu.tw

Financial disclosure: This work was supported by the National Health Research Institutes of Taiwan [NHRI-EX110-10718PC and NHRI-EX109-10606PI].

Conflicts of interest: The authors declare no conflict of interest.

Author contributions: Dr. Yang-Ching Chen collected the cohort data, interpreted the data, and wrote the manuscript. Prof. Yen-Tsung Huang and Prof. Wen-Harn Pan assisted in the critical part of the statistical analysis, interpreted data, and revised it critically for crucial intellectual content. Prof. Yungling Lee reviewed the study design, collected data, interpreted data, supervised the study, and revised the manuscript critically for valuable intellectual content. All authors approved the final manuscript as submitted and published and agree to be accountable for all aspects of the work.

Word count: 250 for abstract; 3000 for text.

Definition of abbreviations

BMI: body mass index

CI: confidence interval

CSHQ: Children's Sleep Habits Questionnaire

Fe_{NO}: fractional exhaled nitric oxide

FEV1/FVC: forced expiratory volume in 1 second/forced vital capacity

GRS: genetic risk score

IV: instrumental variable

IVW: inverse-variance weighted

MR: Mendelian randomization

OR: odds ratio

RCT: randomized controlled trial

SDB: sleep-disordered breathing

SNP: single-nucleotide polymorphism

TCHS: Taiwan Children Health Study

TDCS: Tanner-derived composite stage

2SLS: two-stage least square

ABSTRACT

Background: Obesity and asthma are highly associated, but the mechanisms underlying the association remain unknown. We examined five mediators linking obesity with childhood asthma: (1) pulmonary function impairment, (2) airway inflammation, (3) physical fitness, (4) sleep-disordered breathing (SDB), and (5) early puberty.

Methods: A Mendelian randomization (MR) study with mediation analysis of data obtained from 5,965 children as part of the Taiwan Children Health Study. Cross-sectional regression analysis, MR two-stage least squares method, and MR sensitivity analysis were carried out to investigate each causal pathway. Prospective cohort analyses were used to confirm the findings.

Results: The increased asthma risk associated with obesity was found to be mostly mediated through impaired pulmonary function, low physical fitness, early puberty. In the MR analysis, body mass index was negatively associated with FEV1/FVC and physical fitness index (β= -2.17 and -0.71; 95% CI, -3.92 to -0.42 and -1.30 to -0.13, respectively) and positively associated with early puberty (OR, 1.09; 95% CI, 1.02–1.17). High FEV1/FVC and physical fitness index reduced the risk of asthma (OR, 0.98 and 0.93; 95% CI, 0.97–0.99 and 0.88–0.98, respectively), whereas SDB and early puberty increased the risk of asthma (OR, 1.03 and 1.22; 95% CI, 1.01–1.05 and 1.05–1.42, respectively). The three main mediators were low physical fitness, impaired pulmonary function, and early puberty, with mediation proportions of 91.4%, 61.6%, and 28.3%, respectively. Temporal causality was further strengthened in prospective cohort analyses.

Conclusions: Interventions promoting physical fitness and pulmonary function might effectively reduce obesity-induced asthma risk.

Keywords: Obesity; Asthma; Mediation analysis; Mendelian randomization study; Pulmonary function

INTRODUCTION

Obesity and asthma are common in children, and obesity has been identified as a significant risk factor for asthma in several longitudinal[1] and Mendelian randomization (MR)[2] studies, but the mechanism underlying their association remains unknown. Genetic factors, environmental exposures,[3] mechanical effects (e.g., obesity-associated lung function decline), inflammation (as measured by fractional exhaled nitric oxide (Fe_{NO})), lifestyle (e.g., physical activity and diet), common comorbidities, and endocrine factors (e.g., adipokines and reproductive hormone) have all been implicated.[4] However, the relationship between obesity and airway inflammation is inconsistent;[5] and although obesity-related physical activity levels and poor physical fitness have been found to trigger the onset of asthma,[6-8] few studies have evaluated whether physical fitness mediates an increased risk of obesity-induced asthma. Similarly, comorbidities of obesity such as dyslipidemia, gastroesophageal reflux, and sleep-disordered breathing (SDB), are known to provoke asthma;[4] both SDB and asthma involve airway obstruction and share "obesity" as their significant risk factor,[9] and treatment of SDB can mitigate asthmatic symptoms.[10] However, a possible mediating role of SDB in the relationship between obesity and asthma has yet to be unexplored. Finally, though endocrine factors (which are expressed as early puberty in the current study) can be a crucial mediating pathway from obesity to asthma,[11] and a causal relationship between early puberty and asthma has been suggested,[11,12] but no estimates of mediation proportion from obesity and early puberty to asthma have been reported.

Several pathways might explain the pathophysiology underlying the association between obesity and asthma. However, past evidence is mostly limited to observational studies, whose analyses are limited by confounding factors and reverse causation. MR studies[13] use genetic variants that can divide the observed population into different subgroups according to the exposure level; therefore, it is analogous to a randomized controlled trial (RCT). Network MR studies use genetic variants as instrumental variables (IVs) to investigate mediation in causal pathways.[14]When genetic IVs are obtained for obesity and mediators in the obesity–asthma link, the direct and indirect effects of obesity on asthma can be estimated through network MR. If a mediation pathway is crucial, then an intervention focusing on reducing mediators may be as effective as weight reduction intervention in reducing asthma risk.

To improve our understanding of mechanisms and pathways that underlie obese asthma, we undertook a

network MR analysis of data from the Taiwan Children Health Study (TCHS) to study and quantify the influence of five possible mediators of obesity-induced asthma: (1) pulmonary function impairment, (2) airway inflammation, (3) low physical fitness, (4) SDB, and (5) early puberty. Then, significant mediators identified from MR analysis will be confirmed in prospective cohort analyses. Our results enable the importance of each mediator to be compared, which will inform the prioritization of future interventional studies targeting the mediating pathway of obese children, to prevent asthma.

MATERIALS AND METHODS

Study Population

The TCHS was a population-based prospective study that comprised two cohorts of children recruited from 14 communities across Taiwan. Cohort 1 was initiated in 2007 and cohort 2 in 2010; children were aged between 9 and 12 years old at the time of enrollment; and were followed until they turned 18 years old. Data collection includes parent- and children-filled questionnaires on atopy diseases, adiposity measures, pulmonary function tests, airway inflammation measures, physical fitness assessment, pubertal development record, and oral mucosa samples for DNA extraction. The cross-sectional analysis, involved 3233 children in cohort 1 and 2732 children in cohort 2, with complete data on adiposity, asthma outcomes, mediators at the age of 12 years, and genotype data. Detailed study design and recruitment strategy have been published[15] and described on the online supplementary file. Informed consent was obtained from all participating parents and children. This study was approved by the ethics committee of institute and was conducted according to the Declaration of Helsinki.

After identification of the critical mediators, we further used the annual follow-up data which assessed the mediators from age 10 to age 12 in TCHS to approve the temporal mediating causality. The analyses herein involved data beginning from 2010 Survey which contained adiposity measurement, asthma outcome, puberty stage assessment, physical fitness measurement, and pulmonary function tests. Detailed study design were described in the online supplementary material.

Assessment of Asthma Outcomes

Participants were defined to have active asthma based on an affirmative response to both of the following two questions: "Has a doctor ever diagnosed your child as having asthma?" and "Did your child ever experience difficulty breathing, or did you observe any wheezing or whistling from his or her chest in the past 12 months?"

Measurement of Mediators

Spirometry was performed as per a previously standardized protocol.[16] In our previous study, the ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) showed the strongest association with body mass index (BMI).[17] Hence, FEV1/FVC was used as a surrogate for pulmonary function performance.

Fe_{NO}, a marker of airway inflammation, was measured using a portable NO analyzer NIOX MINO® Airway Inflammation Monitor (Aerocrine AB, Solna, Sweden) according to standardized protocols.[18]

Physical fitness was assessed based on a combination of all physical fitness tests (800 m sprint, jump and reach, sit-ups, and sit-and-reach) administered during school visits using a standardized protocol.[19] Physical fitness parameters included cardiorespiratory endurance, muscular strength, endurance, and flexibility. We added them up to formulate a physical fitness index and transformed them into z-scores using age- sex-specific reference. A higher physical fitness index indicates better physical fitness.

Sleep behaviors were assessed using a parent-administrated questionnaire—the Children's Sleep Habit Questionnaire (CSHQ). The CSHQ was designed and developed to assess the sleep behaviors of school-aged children.[20] It was proven to be a valid tool for detecting the presence of SDB by comparing it with objective polysomnography-defined obstructive sleep apnea.[21]

A validated Chinese version of the Tanner-derived composite stage (TDCS)[22] was used to evaluate the Tanner stage. Children with early puberty were defined as those reaching a certain pubertal stage earlier than the median age for that stage[23] based on large-scale population-based Chinese studies.[24,25]

Other Covariates

BMI was calculated and then converted into age- and sex-specific z-scores (z-BMI).[26] Potential confounding factors such as age, sex, parental educational level, and household second-hand cigarette smoke exposure were assessed at recruitment.

Genotypes

A valid MR[13] study is predicated on three assumptions: (i) each genetic variant is associated with a risk factor; (ii) it affect the outcome only through their effect on the risk factor; and (iii) each genetic variant is independent of potential confounders. We used the following criteria to identify the candidate single-nucleotide polymorphism (SNP) for obesity and the mediators: (i) minor allele frequency of [?]5%, (ii) genotyping call rate of >98% for all children, (iii) no linkage disequilibrium with candidate SNPs, and (iv) primers designed using the National Genotyping Center of the Academia Sinica platform (*http://lims.ngc.sinica.edu.tw/service/*).[27]Genotyping was performed using Sequenom iPLEX matrix-assisted laser desorption/ionization–time of flight mass spectrometry at the National Center for Genome Medicine, Taiwan.

Using genomic information from large genome-wide association studies, we chose well-known genetic variants associated with BMI or the mediators. To address the concern of potential pleiotropic effects of genetic variants, SNPs previously reported to be associated with asthma and confounders were excluded. The online supplementary file demonstrates the details of how candidate SNPs were selected. In summary, we selected 28, 16, 4, 13, 14, and 11 SNPs for BMI, FEV1/FVC, Fe_{NO}, physical fitness, SDB, and early puberty, respectively. Weighted genetic risk scores (GRSs) composed of SNPs of BMI and the mediators were used as genetic IVs. The dosage of effect allele was multiplied by the regression coefficients of each gene on associated BMI/mediators divided by the mean value of all regression coefficients to generate GRSs.[28]

Statistical Analysis

To investigate the causal relationship between obesity, mediators, and asthma outcomes, we conducted observational, MR two-stage least squares (2SLS) and MR sensitivity analyses for all relationships (Figure 1). Observational associations were analyzed using logistic regression models for binary asthma outcome/early puberty, and linear regression models were used for continuous outcomes, such as pulmonary function, Fe_{NO}, physical fitness, and SDB scores. The MR 2SLS analysis is a well-known method in MR for binary exposure and binary outcomes.[29] The first stage involves the regression of the BMI/mediators on genetic scores, generating predicted values of BMI/mediators. The second stage involves a regression analysis of asthma on the predicted BMI/mediators. To test for directional pleiotropy, we used sensitivity analysis methods (MR inverse-variance weighted [IVW], Egger [slope], and weighted median method) to support the validity of causal inference from the MR analysis with multiple genetic variants.[30] Observational mediation analysis was implemented using a mediation package for one mediator in R software.[31] MR mediation analysis and proportion were calculated according to equations established by Burgess et al.[14] The proportion of mediation was calculated as the estimate of the indirect effect divided by the estimate of the total effect, considering one mediator at a time. All model regression and sensitivity analyses were performed using R software (vers. 3.3.2, RStudio, Inc., Boston, MA, USA).

To evaluate the longitudinal effect of obesity on mediators, and of mediators on asthma throughout the three surveys in cohort 2, we used generalized estimating equations (GEE),[32] which account for correlations between different measurements at different times in the same individual. A specific type of GEE with a Time-lag Model was used to approve temporal causality.[33] In the Time-lag Model, predictors (adiposity status in the 2010 and 2011 surveys) were modeled using data that preceded the outcome variables (mediators assessed in 2011 and 2012); the model comprised the specific time-varying nature of adiposity status. The

Time-lag Model also accounts for the temporal sequence in a possible cause-and-effect relationship.

RESULTS

This study comprised 5,965 children aged 12 years in TCHS; data of 3233 children from cohort 1 were collected in 2007, and data of 2732 children from cohort 2 were collected in 2012 (Table 1). The BMI and prevalence of active asthma were similar among the two cohorts. In total, 43.2% of the children reported exposure to household cigarette smoking, and nearly half of the children were breastfed.

Observational and MR association: obesity and asthma

Our previous bidirectional MR analysis established that adiposity causes asthma, but that asthma does not lead to adiposity accumulation.[2] In the current study, we confirmed the causal direction from obesity to asthma using TCHS cohorts 1 and 2 (Figure E1). A one-unit increase in z-BMI was associated with an elevated risk of active asthma according to both observational analysis (odds ratio [OR], 1.15; 95% confidence interval [CI], 1.04–1.28) and MR 2SLS analysis (OR, 1.07; 95% CI, 1.00–1.14). In addition, the sensitivity analysis also yielded evidence of a positive causal relationship between z-BMI and asthma.

The GRSs of the 28 BMI-SNPs were robustly correlated with z-BMI (Table E1, F-statistics = 44.41, $p < 0.001$) and were associated with active asthma (Table E1, $p = 0.04$). To prove that the individual SNP used was independent of asthma and confounders, we showed that most of the 28 BMI-SNPs were associated with z-BMI (Table E3), but that there was little evidence to support an association between individual BMI-SNPs and active asthma and confounders (Tables E3 and E4).

Observational, MR, and longitudinal association: obesity and the mediators

Figure 2 depicts the associations between BMI z-scores and mediators in both observational and MR 2SLS analyses. According to the MR analysis, a one-unit increase in the BMI z-score was associated with a decrease in FEV1/FVC ratio (β, -2.17; 95% CI, -3.92 to -0.42), physical fitness index (β, -0.71; 95% CI, -1.30 to -0.13), and an increased risk of early puberty (OR, 1.09; 95% CI, 1.02 to 1.17). Both observational and MR 2SLS analyses consistently supported the causal association of obesity with FEV1/FVC, physical fitness, and early puberty, with a similar causality direction. Although the observational association between obesity and SDB was significant (β, 0.21; 95% CI, 0.06–0.35), the MR 2SLS analysis did not support causal relationships. Furthermore, most of the results of the MR sensitivity analysis (IVW and weighted median method) confirmed the direction of causal relationships (Figure E2). No evidence supported the association of individual BMI-SNPs with the mediators (Tables E5 and E6). The GRSs of the 28 BMI-SNPs showed strong evidence of an association of obesity with low FEV1/FVC, low physical fitness, high SDB scores, and high early puberty risk (Table E1). Prospective cohort analyses supported the finding of Figure 2 that obesity was associated with a decline in FEV1/FVC, physical fitness, and increased risk of early puberty (Table 2).

Observational, MR, and longitudinal association: mediators and asthma

Both observational and MR studies supported the causal relationship between mediators and asthma (Figure 3). High FEV1/FVC and physical fitness reduced the risk of active asthma (OR, 0.98 and 0.93; 95% CI, 0.97–0.99 and 0.88–0.98, respectively), whereas SDB and early puberty increased the risk of active asthma (OR, 1.03 and 1.22; 95% CI, 1.01–1.05 and 1.05–1.42, respectively). The IVW results of the MR sensitivity analysis supported the findings of observational and MR analyses (Figure E3). However, the results of the causal effect of Fe_{NO} on asthma based on observational and MR analyses were in the opposite direction, indicating that Fe_{NO} is unlikely to mediate the association between obesity and asthma. The prospective cohort analyses also demonstrated that higher FEV1/FVC and physical fitness were associated with lower risk of active asthma (RR<1), whereas early puberty increased the risk of active asthma (RR>1) (Table 3).

The GRSs of the mediators served as an adequate indicator for the mediators, except for the GRS of Fe_{NO}. The F statistics for FEV1/FVC, Fe_{NO}, physical fitness, SDB, and early puberty were 16.25, 3.22, 10.27, 19.93, and 88.54, respectively (Figure 3). Furthermore, the GRSs of all the mediators, except for Fe_{NO},

were significantly associated with active asthma (Table E2). We did not observe strong associations of the individual SNPs of the mediators with active asthma (Tables E7–S11) and the confounders (Tables E12–E16).

Observational and MR mediation analyses

According to both observational and MR analyses, the two mediators with the highest ranking of mediation proportions were FEV1/FVC and physical fitness (Figure 4). For all the mediators, the mediation proportions of the MR analysis (Figure 4, right) were higher than that of the observational analysis (Figure 4, left). Fe_{NO} did not appear to mediate the influence of obesity on asthma, because the mediation coefficient was in the opposite direction from obesity to Fe_{NO} (negative in Figure 2) and from Fe_{NO} to active asthma (positive in Figure 3), making the indirect pathway coefficient negative; hence, the mediation proportion was unable to be calculated. Furthermore, SDB was not considered a strong mediator because the MR analysis showed that obesity-induced SDB was insignificant (Figure 2). The mediators presented as consistent causal relationships from obesity to the mediators and then from the mediators to asthma were FEV1/FVC, physical fitness, and early puberty in mediation proportions of 61.57%, 91.42%, and 28.28%, respectively, based on the MR methods; and 24.88%, 21.05%, and 10.32% based on the observational analysis. The mediation proportion implies the proportion of the mediating pathway compared with the total effect from obesity to asthma, considering one mediator at a time. Therefore, the mediation proportion from all five mediators does not necessarily have to sum to 100%. The more substantial mediation proportion indicates that the mediator might play a more critical role.

The mediation analysis assumes that the exposure and mediators had no interaction effects on the outcome.[14] We did not observe an obvious interaction effect of BMI or BMI-GRS and the mediators on predicting active asthma after correction for multiple comparisons (Table E17).

DISCUSSION

Our study has yielded new evidence for several potential mechanistic factors underlying the causative relationship between obesity and asthma. We used the network MR to examine causal mediation pathways and to confirm the temporal causality using prospective cohort analyses. Among the five mediators examined in the current study, impaired pulmonary function, low physical fitness, and early puberty were found to be robust mediators, whereas SDB was identified as a possible mediator. However, airway inflammation did not appear to be a causal mediator. Low FEV1/FVC and physical fitness were the top two leading mediation pathways. A recent interventional study wherein obese asthmatic patients were provided high-intensity pulmonary rehabilitation programs did lead to experience significant improvements in asthma control.[34] Hence, the promotion of physical fitness to improve pulmonary functions should be recommended for obese children, to prevent asthma.

According to this network MR study, obesity-induced low physical fitness and impaired pulmonary function are the main mediation pathways presenting 91.42% and 61.57% of the mediation proportion, respectively (Figure 4). Determining the causal links between physical fitness and asthma incidence is crucial in guiding clinical practice. However, there is limited proof for the causal relationship between physical fitness and the incidence of asthma. In a Danish study of school-aged children, Rasmussen et al. reported an association between poor physical fitness in childhood and incidence of asthma in young adulthood.[6] The authors then combined this result with a New Zealand-based study, and concluded that improving fitness during childhood was associated with a higher lung volume in adulthood.[8] Additionally, Ortega et al. discovered that among obese individuals, high cardiorespiratory fitness could reduce the risk of asthma by half.[35] Examining the temporal sequence of low physical fitness and asthma development in a longitudinal study is time-consuming and laborious, and testing this hypothesis in a controlled trial is not feasible. To our knowledge, this is the first MR study to investigate the causal relationship between physical fitness and asthma. Additionally, the indirect effects mediated through low physical fitness and impaired pulmonary function are much higher than the direct effects, indicating that enhancing physical fitness and pulmonary function could effectively prevent obesity-induced asthma.

Childhood obesity is a major contributor of both early puberty and asthma. In the current study, we iden-

tified a moderate degree of mediation (mediation proportion, 28.28%) for early puberty in the pathogenesis from obesity to asthma. We previously confirmed the causality between early puberty and asthma for both sexes through MR and longitudinal studies.[11] Boys and girls showed a 1.38- and 1.11-fold increased risk of asthma, respectively, with early puberty.[11] Underlying causes linking early puberty and asthma could be hormonal changes at puberty,[36]decreased pulmonary function in those with early puberty,[37] and hyperinsulinemia during the pubertal growth stage.[38] One case report stated that pharmacological therapy (such as a gonadotropin-releasing hormone analog) could be administered to prevent asthma in children experiencing early puberty.[39] To prevent obesity-induced asthma risk, clinicians must assess for the presence of early puberty.

Our study, which used a MR network analysis, adds to the existing evidence suggesting bi-directional relationships between SDB and asthma. We confirmed the causality from SDB to asthma. Moreover, we observed that SDB is a potential mediator with a moderate mediation proportion in the pathogenesis of obesity-induced asthma. Although causal relationships between SDB and asthma were never determined using a cohort study,[40] treatment of SDB, such as adenotonsillectomy, showed improved asthma outcomes.[41]Possible mechanisms linking SDB and asthma include airway edema or obstruction and systemic inflammation.[34] SDB-related hypoxia can induce reflex bronchoconstriction, which further causes asthma.[42] Additional RCTs are needed to confirm that the treatment of SDB improves pediatric asthma outcomes.

Our study has three key strengths. First, it is the large population-based study on children that involved various mediator measurements linking obesity and asthma. Second, we applied three distinct statistical analyses (observational, MR, and prospective cohort analyses) to prove the robustness of interrelations among obesity, the mediators (impaired pulmonary function, low physical fitness, and early puberty), and asthma. Moreover, several sensitivity analyses for MR results were performed to support our findings. The present study also has several limitations that should be addressed. First, SDB was defined using a questionnaire-based assessment instead of measuring it through polysomnography (PSG). This approach is common in the SDB/asthma literature,[43] where the cost involved in technology and personnel make such a large population-based study cost-prohibitive. However, the CSHQ is a well-established instrument for SDB assessment, with good performance in population-based studies as compared with the gold standard PSG.[21] Second, a lack of the association of elevated Fe_{NO} with both obesity and asthma in the MR analysis could be due to weak genetic instruments. In contrast to the genome-wide association study approach, the candidate SNP approach was limited by only a few available literatures on genetic variants relevant to Fe_{NO}.[44]

In summary, impaired pulmonary function, low physical fitness, and early puberty are prominent mediating mechanisms for obesity-induced asthma. Therefore, lifestyle interventions aimed at promoting physical fitness and pulmonary function might effectively reduce the risk of obese asthma. Assessing these promising mediators may deepen our understanding of its pathogenic features and may offer new treatment strategies for specific phenotypes of obese asthma.

Acknowledgments:

The authors thank the field workers, teachers, and other school staff who supported the data collection, as well as all the parents and children who participated in this study. We also thank the NCGM for the genotyping technical support.

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Figure Legends

Figure 1. Flow diagram depicting the associations between obesity and asthma and their respective mediators that were examined in this study

Footnotes of Figure 1: Arrows and accompanying Figures/Tables indicate the associations/mediations studied in corresponding to order of analyses in the text. *Definition of abbreviations:* BMI=body mass index; Fe_{NO}=fractional exhaled nitric oxide; FEV1/FVC = forced expiratory volume in 1 s/forced vital capacity

Figure 2. Observational and Mendelian Randomization analysis of the associations between BMI z-scores and mediators

Footnotes of Figure 2: Observational and MR two-stage least square associations were estimated in a total of 5965 children. F-statistics of BMI genetic risk scores (28 SNP) =44.41. Observational models were adjusted by age, sex, parental education, and household environmental tobacco smoking. *Definition of abbreviations:*BMI=body mass index; Fe_{NO}=fractional exhaled nitric oxide; FEV1/FVC = forced expiratory volume in 1 s/forced vital capacity

Figure 3. Observational and Mendelian Randomization analysis of associations between mediators and active asthma

Footnotes of Figure 3: Observational and MR two-stage least square associations were estimated in a total of 5965 children. F-statistics of the mediators were listed. Observational models were adjusted by age, sex, parental education, and household environmental tobacco smoking. *Definition of abbreviations:* BMI=body mass index; Fe_{NO}=fractional exhaled nitric oxide; FEV1/FVC = forced expiratory volume in 1 s/forced vital capacity

Figure 4. Mediation proportions (%) as calculated in the observational and Mendelian Randomization analyses

Footnotes of Figure 4: The proportion of mediation indicates the proportion of indirect mediation effect to total effect from obesity to asthma. Mediation proportion of the observational models were calculated by single-mediator analysis. Mediation proportion of the MR models were calculated according to equations by *Burgess et al.*Observational models were adjusted by age, sex, parental education, and household environmental tobacco smoking. *Definition of abbreviations:* BMI=body mass index; Fe_{NO}=fractional exhaled nitric oxide; FEV1/FVC = forced expiratory volume in 1 s/forced vital capacity

Table 1. Characteristics of participants aged 12 years in the Taiwan Children Health Study

Characteristics	Total	Total	Cohort 1	Cohort 1	Cohort 2	Cohort 2	P value§
N	5965	5965	3233	3233	2732	2732	
Age, yrs	12.45	0.42	12.70	0.34	12.15	0.30	<0.001
Male sex	3043	51.01	1669	51.62	1374	50.29	0.31

Characteristics	Total	Total	Cohort 1	Cohort 1	Cohort 2	Cohort 2	P value§
Breast Feeding	2480	49.50	1561	48.28	919	51.72	0.02
Parental Education							
High school or below	3440	57.67	2085	64.49	1355	49.60	<0.001
College or university	2163	36.26	1026	31.74	1137	41.62	
Post-graduate school	362	6.07	122	3.77	240	8.78	
Family income (USD)							
<20,000	3274	54.89	2021	62.51	1253	45.86	<0.001
20,001-330,000	1827	30.63	848	26.23	979	35.83	
>330,001	864	14.48	364	11.26	500	18.30	
Household cigarette smoke	2577	43.20	1525	47.17	1052	38.51	<0.001
BMI (kg/m^2) at age 12	20.49	4.16	20.57	4.23	20.40	4.06	0.11
Active Asthma	233	3.91	127	3.93	106	3.28	0.54

Definition of abbreviations: BMI=body mass index; FEV1/FVC = forced expiratory volume in 1 s/forced vital capacity; USD = United States dollars.

All data are presented as means ± standard deviation or numbers (%).

The number of participants does not add up to the total number because of missing data.

§P value indicates the difference between the two cohorts.

Table 2. Prospective cohort analyses linking obesity to three mediators (early puberty, poor physical fitness, poor pulmonary function)

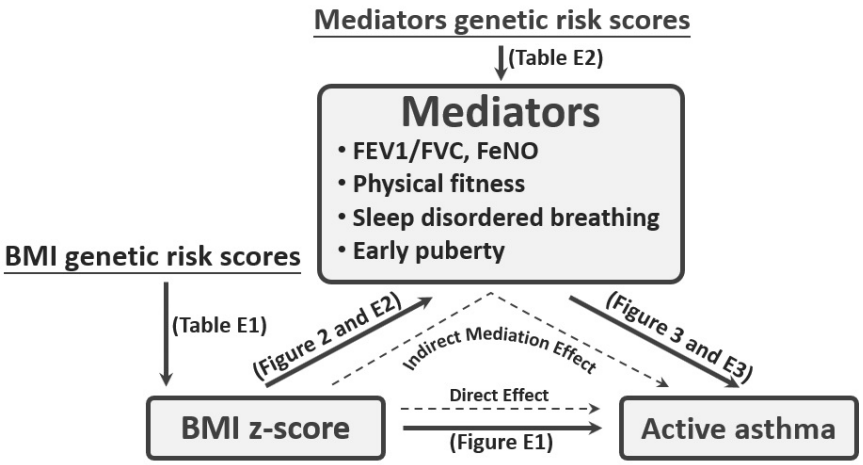
Obesity measures	**FEV1/FVC** (%)	**FEV1/FVC** (%)	**FEV1/FVC** (%)	**Physical fitness** (z-scores)	**Physical fitness** (z-scores)	**Physical fitness** (z-scores)	**Early puberty**	**Early puberty**	**Ea pu**
	β	***95%CI**	***95%CI**	β	***95%CI**	***95%CI**	**RR**	***95%CI**	***9**
Overweight	-1.24	-1.66	-0.82	-1.04	-1.22	-0.86	1.54	1.27	1.8
Obesity	-1.26	-1.79	-0.74	-1.33	-1.55	-1.11	1.57	1.25	1.9
z-BMI	-0.41	-0.55	-0.26	-0.39	-0.45	-0.33	1.26	1.18	1.3

*Models are adjusted by age, sex, parental educational level, and household cigarette smoking exposure

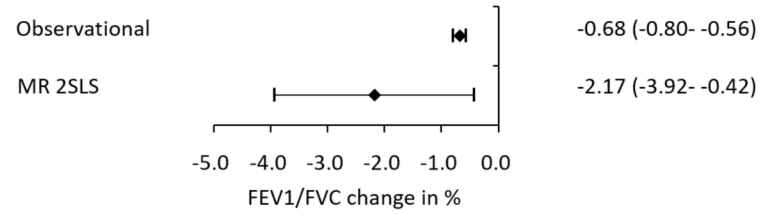
Table 3. Relative risk of active asthma for three mediators during follow-ups survey at age 11, 12 years

Active asthma	**RR**	***95%CI**	***95%CI**
FEV1/FVC (%)	0.96	0.94	0.98
Physical fitness (z-scores)	0.95	0.90	1.00
Early puberty (yes to no)	1.95	1.13	3.21

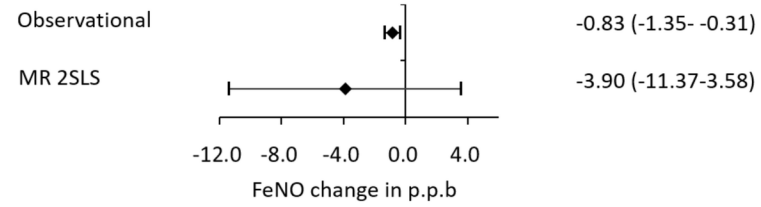
*Models are adjusted by age, sex, parental educational level, and household cigarette smoking exposure



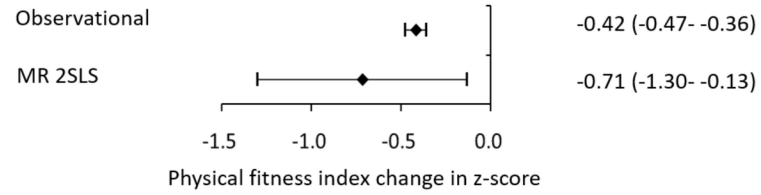
FEV1/FVC change for 1 unit increase in BMI z-score



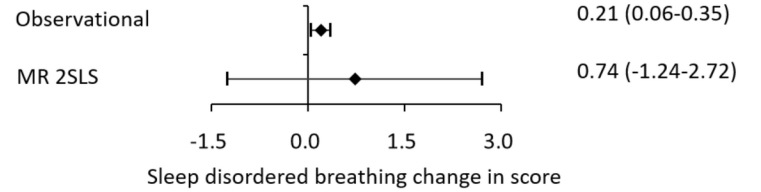
FeNO change for 1 unit increase in BMI z-score



Physical fitness change for 1 unit increase in BMI z-score



Sleep disordered breathing for 1 unit increase in BMI z-score



Early puberty for 1 unit increase in BMI z-score