Being overweight in childhood, puberty, or early adulthood: Changing asthma risk in the next generation?

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Background: Overweight status and asthma have increased during the last decades. Being overweight is a known risk factor for asthma, but it is not known whether it might also increase asthma risk in the next generation.

Objective: We aimed to examine whether parents being overweight in childhood, adolescence, or adulthood is associated with asthma in their offspring.

Methods: We included 6347 adult offspring (age, 18-52 years) investigated in the Respiratory Health in Northern Europe, Spain and Australia (RHINESSA) multigeneration study of 2044 fathers and 2549 mothers (age, 37-66 years) investigated in the European Community Respiratory Health Survey (ECRHS) study. Associations of parental overweight status at age 8 years, puberty, and age 30 years with offspring's childhood overweight status (potential mediator) and offspring's asthma with or without nasal allergies (outcomes) was analyzed by using 2-level logistic regression and 2-level multinomial logistic regression, respectively. Counterfactual-based mediation analysis was performed to establish whether observed associations were direct or indirect effects mediated through the offspring's own overweight status.

Results: We found statistically significant associations between both fathers' and mothers' childhood overweight status and offspring's childhood overweight status (odds ratio, 2.23 [95% CI, 1.45-3.42] and 2.45 [95% CI, 1.86-3.22], respectively). We also found a statistically significant effect of fathers' onset of being overweight in puberty on offspring's asthma without nasal allergies (relative risk ratio, 2.31 [95% CI, 1.23-4.33]). This effect was direct and not mediated through the offspring's own overweight status. No effect on offspring's asthma with nasal allergies was found.

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Conclusion: Our findings suggest that metabolic factors long before conception can increase asthma risk and that male puberty is a time window of particular importance for offspring's health. (J Allergy Clin Immunol 2020;145:791-9.)

Key words: *Ageing Lungs in European Cohorts study, epidemiology, multilevel mediation model, offspring, parental risk factors*

Abbreviations used
BMI: Body mass index
OR: Odds ratio
RRR: Relative risk ratio

In parallel with the increase in asthma and allergies, there has been a dramatic increase in overweight status and obesity during the last decades. More than 60% of the population in Western countries are overweight, with a body mass index (BMI) exceeding 25 kg/m^2,[1] and countries from other parts of the world follow the same trend.[2] Being overweight is a well-known risk factor for noncommunicable diseases, including cancers, cardiovascular diseases, and diabetes mellitus.[3-5] Research has also shown associations with asthma and asthma severity,[6] as well as an association between mothers' overweight status just before and during pregnancy and offspring's asthma.[7,8]

It has been known for quite some time that a mother's health behavior shortly before and during pregnancy affects her children's health. However, emerging evidence suggests that both fathers' and mothers' health behaviors could be of importance and that a sex-specific, male-line transgenerational response system could exist.[9] Existing literature suggests particularly vulnerable intergenerational exposure time windows *in utero* and just before puberty.[10,11] Until now, there have been barely any human data to support these time windows with respect to offspring's asthma, and the limited research in this field has thus far mainly investigated exposure to cigarette smoke.[12-14]

In light of emerging evidence suggesting associations between exposures in parents long before conception and adverse health outcomes in future offspring, the present study takes on an intergenerational perspective. The objectives of this study are to assess the effect of parental overweight status on offspring's asthma, taking into account different susceptibility time windows throughout parents' preconception lifespan, as well as evaluating the potential mediating role of the offspring's own overweight status.

METHODS

Study design

The Respiratory Health in Northern Europe, Spain and Australia (RHINESSA) generation study (www.rhinessa.net) examines offspring of initial participants in the European Community Respiratory Health Survey (ECRHS; www.ecrhs.org).[15]

In 1992, the ECRHS surveyed population-based random samples of adults aged 20 to 44 years (approximately 3000 per research center) in 56 study centers from 25 countries. Clinical examinations were conducted on subsamples of responders. In 7 Northern European centers (Aarhus in Denmark; Bergen in Norway; Umea, Uppsala, and Gothenburg in Sweden; Reykjavik in Iceland; and Tartu in Estonia) all responders to the 1992 postal survey were followed in a large longitudinal questionnaire study, the Respiratory Health in Northern Europe (RHINE; www.rhine.nu) study.[16] The subsamples invited for clinical examination in the ECRHS were invited to ECRHS follow-up studies. Both the ECRHS and RHINE study conducted follow-up studies in approximately 2002 (ECRHS II/RHINE II) and again in approximately 2012 (ECRHS III/RHINE III).

The Northern European ECRHS centers in the RHINE study, as well as the Spanish (Huelva and Albacete) and Australian (Melbourne) ECRHS centers, developed standardized protocols for health examination of the children (offspring generation [G1]) of study participants (parent generation [G0]), resulting in the generation study RHINESSA. Extensive questionnaire data were collected in the period 2013-2016 through 1 questionnaire deployment in each study center, and the completed adult offspring database includes 8260 offspring aged 18 years or greater. Informed consent was obtained from each participant, and all parts of the generation study (ECRHS/RHINE/RHINESSA) were approved by the appropriate regional committees of medical research ethics (https://helsebergen.no/seksjon/RHINESSA/Documents/Ethic%20Committees%20list.pdf).

Study populations

A flow chart describing the study populations in the present study is presented in Fig 1. Of the 8260 adult offspring in the RHINESSA study (G1, 42% male), 7271 offspring had a parent (G0) who had participated in the most recent RHINE/ECRHS follow-up studies in 2010-2013 (ECRHS III/RHINE III) and thus were eligible for inclusion in the present analysis. Some offspring are siblings, and the number of unique parents was 5235, of whom 45% were men (fathers) and 55% were women (mothers). The database includes 1 parent only per offspring, resulting in 2 eligible study populations for the present study: 1 population for the paternal line (3256 offspring and 2336 fathers) and 1 population for the maternal line (4015 offspring and 2899 mothers). The proportion of offspring and ECRHS/RHINE study parents (ie, mothers and fathers who themselves participated in the ECRHS/RHINE III studies) with information on key variables was 87% for the paternal line and 88% for the maternal line, resulting in net study populations of 2822 offspring (57% female and 43% male) with their 2044 fathers and 3525 offspring (58% female and 42% male) with their 2549 mothers (for population distribution across study centers, see Table E1 in this article's Online Repository at www.jacionline.org).

Definitions

Both the parents participating in the ECRHS/RHINE study and their adult offspring participating in the RHINESSA study provided information about asthma, body size, smoking history, and educational level. Offspring also provided information on their other parent (ie, the spouse of the ECRHS/RHINE parent).

Adult offspring ever having asthma was classified as follows: "ever having asthma with nasal allergies," "ever having asthma without nasal allergies," or "never having asthma." This distinction was made because asthma with allergies and asthma without allergies represent 2 asthma phenotypes: although asthma with allergies is triggered by inhaled allergens, asthma without allergies is not.[17] Even if the symptoms are similar, the underlying risk factors might differ.[18] Asthma with and without nasal allergies was defined based on answers to the following questions: "Have you ever had asthma diagnosed by a doctor?" and "Do you have any nasal allergies including hay fever?" For those who answered yes to the asthma diagnosis question, such a diagnosis had been given to them at any point before the time of study participation, and for a majority of the population (56%), the diagnosis was given after age 10 years. The ECRHS/RHINE parents' ever asthma status ("present" vs "absent") was self-reported, whereas ever having asthma in the other parent was offspring reported in the RHINESSA questionnaire.

Overweight status was identified by using a validated figural drawing scale of 9 sex-specific body silhouettes (Fig 2)[19] in RHINE III/ECRHS III for parents and in the RHINESSA study for adult offspring. To distinguish between nonoverweight and overweight-obese subjects, we used as cutoffs body silhouette 5 or greater in men and body silhouette 4 or greater in women. Using this definition, we are not able to objectively assess overweight status as defined by the World Health Organization but to identify subjects "at risk"

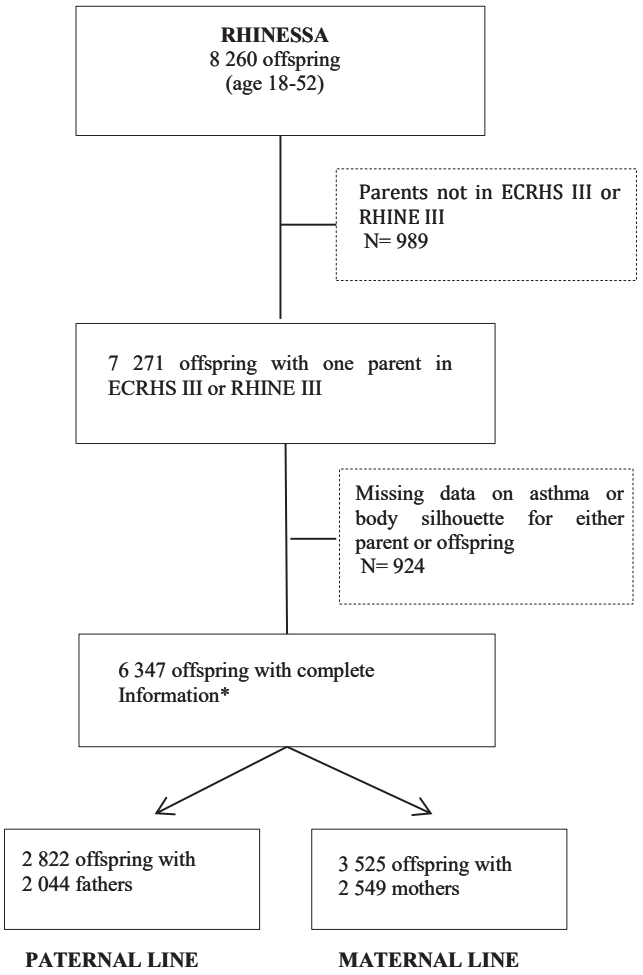

FIG 1. Study population flow chart: RHINESSA generation study. *Offspring and their participating parents with complete information on overweight status and asthma: 87% for the paternal line and 88% for the maternal line.

for overweight body size. In a recent RHINE III validation study, these cutoffs were defined as optimal for identifying overweight adults (BMI, 25-30 kg/m^2).[19] (For participating parents' body size distributions, see Fig E1 in this article's Online Repository at www.jacionline.org.) The use of self-reported body silhouettes in adults (ECRHS III/RHINE III) as a tool to reflect obesity in the past was validated against previously measured or self-reported BMI in the ECRHS and RHINE studies.[20]

ECRHS/RHINE parents' onset of overweight status was classified at 4 susceptibility time windows: age 8 years (addressing the prepuberty slow growth period time window), puberty (voice break for fathers and menarche for mothers), age 30 years before offspring conception, and age 30 years after offspring conception. In detail, "overweight at age 8 years" was present if the parent reported being overweight at age 8 years, regardless of being overweight at later susceptibility periods; "overweight in puberty" was present if he or she reported being overweight in puberty but not at age 8 years (regardless of overweight at age 30 years); "overweight at age 30 years before each offspring conception" was present if he or she reported being overweight at age 30 years but neither at age 8 years nor in puberty (regardless of overweight status after offspring conception); and "overweight at age 30 years after offspring conception" was present if he or she reported being overweight at age 30 years after offspring conception but not in the previous susceptibility time windows. The reference category was "never overweight." The offspring's overweight status at age 8 years and other parents' overweight status at age 30 years ("present" vs "absent") were both reported by the adult offspring using the same figural drawing scale described above for the ECRHS/RHINE parents.

Parents' educational level was considered "low" if equal to "primary school" (vs "secondary school" or "college or university"). An "unknown" category was used when no information on parents' educational level was available.

Statistical analysis

Offspring variables included in the analyses were the following: adult offspring ever having asthma with or without nasal allergies as the outcome and offspring's overweight status at age 8 years as the potential mediator. The parental variables included in the analyses were as follows: ECRHS/RHINE parents' overweight status as the exposure of interest, ECRHS/RHINE parents' asthma and educational level, and other parents' (reported by the adult offspring) overweight status and asthma. In addition, offspring's sex and age were included as adjustment variables of the exposure-mediator-outcome relationships.

Because of the data sparseness, we could not include offspring's sex as a potential modifier of the exposure-mediator-outcome relationships, and ECRHS/RHINE parents' ever having asthma, other parents' ever having asthma, and overweight status at age 30 years as potential modifiers of the exposure-outcome and exposure-mediator relationships. The paths investigated in the analyses are represented in Fig 3.

Exploratory analysis

Our data have a hierarchical structure because multiple adult offspring (level 1 unit) might be siblings and originate from the same ECRHS/RHINE parent (level 2 units). Furthermore, the parents are sampled from different study centers. Therefore the hypothesized relationships between the exposure-mediator and exposure-outcome were explored by using a 2-level logistic regression model and a 2-level multinomial logistic regression model (adult offspring = level 1 unit; ECRHS/RHINE parent = level 2 unit), respectively. Each model had a random intercept term at level 2 and adjustment variables as fixed effects. Furthermore, cluster-robust SEs were computed to take the correlation among parents within each of the different centers (cluster variable) into account. Exposure-mediator and exposure-outcome associations were summarized as odds ratios (ORs) and relative risk ratios (RRRs), respectively. Analyses were carried out separately within the maternal and paternal lines.

Mediation analysis

A counterfactual-based mediation analysis was carried out to establish whether the observed associations in the exploratory analysis between parents' overweight status and adult offspring's asthma are causal effects that could also be mediated through the offspring's own childhood overweight status. This approach allows us to decompose the total effect of the exposure on the outcome into the natural direct effects (ie, the effect of the exposure on the outcome through pathways that do not involve the mediator) and the natural indirect effect (ie, the effect of the exposure on the outcome caused by the effect of the exposure on the mediator).[21] The main requirement for mediation is that the indirect effect is statistically significant.[22]

At present, to the best of our knowledge, multilevel mediation models with a dichotomous mediator and a categorical outcome (with >2 unordered categories) are not included in statistical software. Therefore in our study the mediation analysis was carried out by splitting the multinomial-distributed outcome into 2 binomial-distributed outcomes ("offspring's asthma with nasal allergies" vs "no asthma" and "offspring's asthma without nasal allergies" vs "no asthma"). Furthermore, the hierarchical structure of our data was not taken into account because of the magnitude of the design effect.[23] In the mediation analysis the estimate of the natural effects was obtained by using the latent response variable mediator approach[24] with probit link, theta parameterization, and weighted least squares means and variance-adjusted estimators.[25] Non–bias-corrected bootstrap CIs (10,000 resamples) were obtained

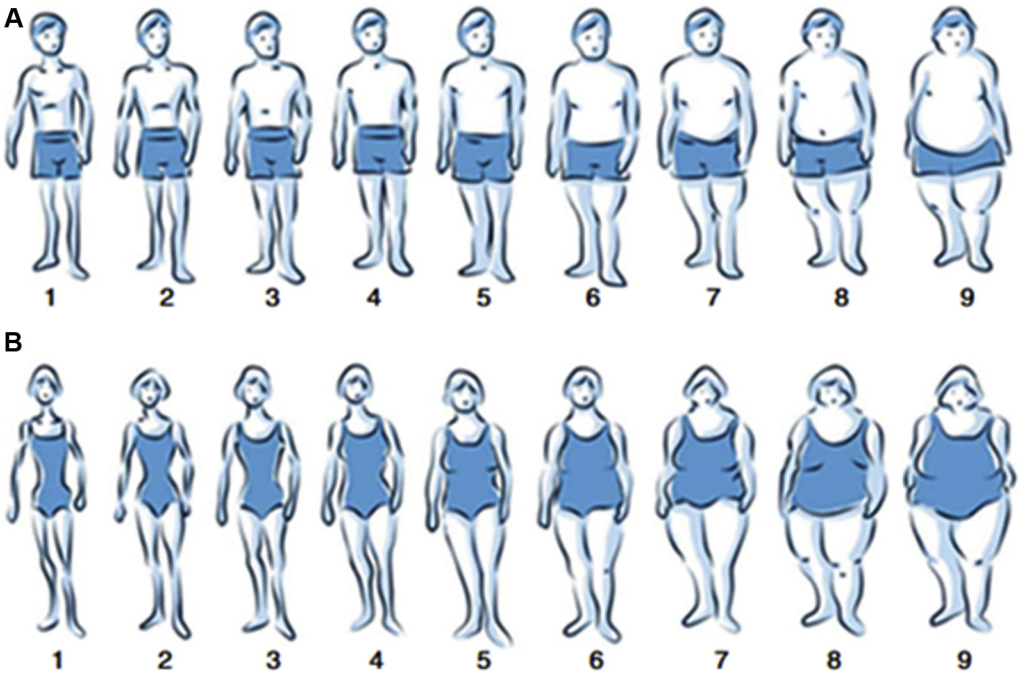

FIG 2. Figural drawing scales for men **(A)** and women **(B)** used in the ECRHS/RHINE III study and in the RHINESSA questionnaire survey. Cutoffs for overweight status were 5 or greater in men and 4 or greater in women.

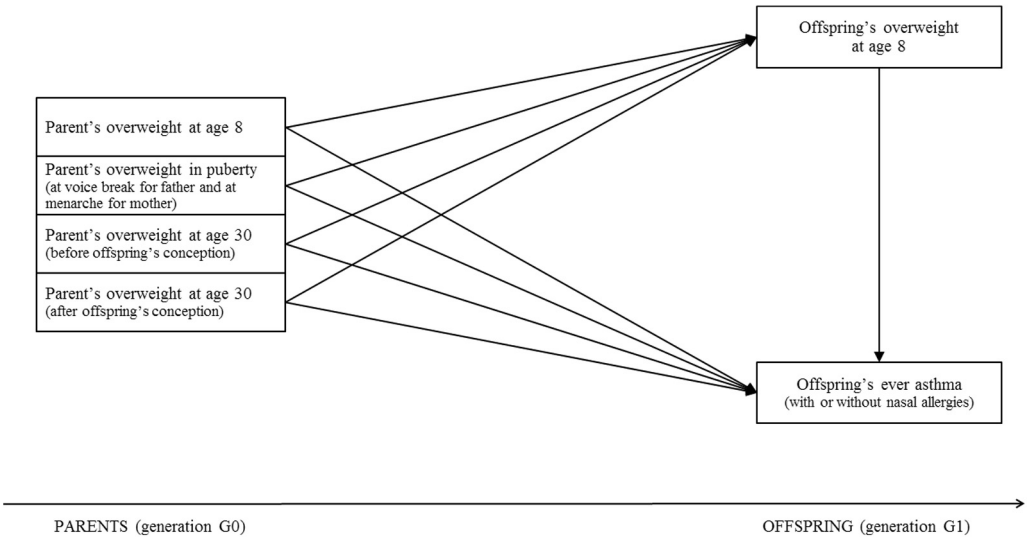

FIG 3. Schematic representation of the paths investigated within the paternal and maternal lines. Adjusted for ECRHS/RHINE parents' ever having asthma and educational level, other parents' overweight status at age 30 years and ever having asthma, and offspring's age and sex.

for the causally defined effects to take nonnormality of their estimate distribution into account. Natural effects were summarized as ORs.

Sensitivity analyses

The identification of natural effects relies on strong assumptions.[26] We checked whether the results changed in the presence of unmeasured confounding of the exposure-mediator-outcome relationship in mediation analyses.[27]

Using the Umediation R package (https://github.com/SharonLutz/Umediation), we simulated one unmeasured and normally distributed confounder ("U" variable) for the exposure-outcome, exposure-mediator, and mediator-outcome relationships, with a mean of 0 and a variance of 0.001. As inputs for Umediation, we used the coefficients of the mediation model. We carried out 4 simulation analyses splitting the categorical exposure variable ("E" variable) into 4 binary exposures (E_1, "overweight at age 8 years"; E_2, "overweight in puberty"; E_3, "overweight at age 30 years before each offspring conception"; and E_4, "overweight at age years 30 after offspring conception"; reference category: "never overweight"). Each of the 4 simulation analyses was carried out under multiple scenarios for the effects (β regression coefficients) of the unmeasured confounder U on the outcome ($\beta_{U\rightarrow O}$), the mediator (β_{Uet}), and the exposure (β_{Ueta}, i = 1…4) by fixing $\beta_{Uet} = \beta_{Uet} = \beta_{Ueta} = 0$, 1, 3, 5, 7, and 9. We specified 1000 simulation runs and 1000 Monte Carlo draws for the nonparametric bootstrap in each simulation analyses.

TABLE I. Main characteristics of the study population according to the parental line

	Paternal line	Maternal line
No. of parents	2044	2549
Parent's age (y),* median (range)	55 (37-66)	55 (39-65)
Parents' ever having asthma,* % (no.)	11.4 (233)	14.4 (367)
Parents' low education† level,* % (no.)		
Present	11.9 (243)	11.5 (293)
Unknown	4.3 (88)	4.1 (105)
Parent's overweight status,* % (no.)		
At age 8 y	9.9 (202)	21.2 (540)
In puberty (at voice break/ menarche)	2.4 (49)	7.7 (196)
At age 30 y (before offspring conception)	7.3 (149)	15.6 (398)
At age 30 y (after offspring conception)	3.0 (61)	5.0 (127)
Other parent's overweight status at age 30 y,‡ % (no.)		
Present	46.5 (950)	20.4 (520)
Unknown	1.8 (37)	4.0 (102)
Other parents' ever having asthma,‡ % (no.)		
Present	11.6 (237)	7.2 (184)
Unknown	3.2 (65)	3.5 (89)
No. of adult offspring	2822	3525
Offspring's sex (female),‡ % (no.)	57.1 (1611)	58.0 (2045)
Offspring's age (y),† median (range)	29 (18-50)	30 (18-52)
Offspring ever having asthma,‡ % (no.)		
Without nasal allergies	9.0 (254)	8.3 (293)
With nasal allergies	9.3 (262)	10.2 (360)
Offspring's overweight status at age 8 y,‡ % (no.)	13.7 (387)	15.0 (529)

*Information retrieved from ECRHS/RHINE parents.
†Educational level was considered "low" if equal to "primary school" (vs "secondary school" or "college or university").
‡Information retrieved from RHINESSA adult offspring.

Statistical analysis was carried out with Stata (version 14.2; StataCorp, College Station, Tex), Mplus (version 8.1; Muthén & Muthén, Los Angeles, Calif), and R (version 3.5.1; https://cran.r-project.org/) software.

RESULTS

Characteristics of the study population

The median age of ECRHS/RHINE parents was 55 years, whereas the median age of adult offspring was 29 years in the paternal line and 30 years in the maternal line (Table I). A majority of offspring were female (57% and 58% in the paternal and maternal lines, respectively). Adult offspring compared with parents were more likely to have ever had asthma in both the paternal (18.3% and 11.4%) and maternal (18.5% and 14.4%) lines. In the paternal and maternal lines 51% and 55% of adult offspring had at least 1 parent who had smoked during their childhood, respectively. In the paternal line 14% of offspring were overweight at age 8 years, and 23% of them had fathers who were overweight at some point in their lives. In the maternal line 15% of the offspring were overweight at age 8 years, and 50% of them had mothers who were overweight at some point (Table I).

Exploratory analysis

In the paternal line both the exposure and the potential mediator were associated with the outcome (Table II). An increased risk of adult offspring's asthma without nasal allergies was observed among offspring with ECRHS/RHINE fathers who had become overweight during puberty (RRR, 2.36 [95% CI, 1.27-4.38]) compared with fathers who had never been overweight. The strength of this association remained unaltered when the potential mediator (offspring's overweight at 8 years) was added to the model (RRR, 2.31 [95% CI, 1.23-4.33]). Offspring's overweight status at age 8 years was positively associated with adult offspring's asthma without nasal allergies (RRR, 1.50 [95% CI, 1.05-2.16]; Table II). No significant exposure-outcome and mediator-outcome associations were found for asthma with nasal allergies in adult offspring. In the maternal line (Table II) neither the exposure nor the potential mediator was significantly associated with the outcome.

In both parental lines positive associations were found between the exposure and the potential mediator (Table III). The risk of offspring's overweight status at age 8 years was greater if their parent had been overweight at the same susceptibility window (OR, 2.23 [95% CI, 1.45-3.42] and 2.45 [95% CI, 1.86-3.22], respectively, within paternal and maternal lines), if their mother had become overweight during puberty (OR, 2.13 [95% CI, 1.26-3.60]), or if their father had become overweight at age 30 years after offspring conception (OR, 1.90 [95% CI, 1.25-2.86]) compared with the offspring having mothers/fathers who had never been overweight.

Mediation analysis

Based on the associations found at the exploratory stage, a mediation analysis was conducted only for adult offspring's asthma without nasal allergies (vs no asthma) within the paternal line. We found a slight but statistically significant indirect effect of fathers' overweight status at age 8 years on adult offspring's asthma without nasal allergies mediated by offspring's overweight status at age 8 years (indirect-only mediation; Table IV). We found that the effect of fathers' onset of overweight status at voice break on the adult offspring ever having asthma without nasal allergies (OR, 2.24 [95% CI, 1.06-4.09]) did not involve the offspring's overweight status at age 8 years (direct-only nonmediation; Table IV). These results confirm the findings of the exploratory analysis (Table II). Lastly, no statistically significant indirect or direct effects were found between fathers' overweight status at age 30 years (before and after offspring conception) and adult offspring's asthma without nasal allergies (nonmediation), although the estimate for fathers' overweight status at age 30 years after conception was borderline significant (Table IV).

Sensitivity analysis

The inclusion of one unmeasured confounder U value (see Fig E2 in this article's Online Repository at www.jacionline.org) in the model had a limited effect on the estimate of the direct effects of fathers' overweight status on adult offspring ever having asthma without nasal allergies also when the U value had a very strong effect on the outcome, mediator, and exposure ($\beta_{U\rightarrow O} = \beta_{Uet} = \beta_{Ueta} > 5$, i = 1, …, 4). Indeed, as the effect increased, the proportion of simulations in which the results matched (whether the U value was included or excluded from the model) remained greater than 89%, and the average absolute difference of the direct effects remained less

TABLE II. Exploratory analysis: Association between parents' overweight status (exposure) and adult offspring ever having asthma (outcome) according to the parental line

	Variables of interest	Offspring ever having asthma without nasal allergies, RRR (95% CI)	Offspring ever having asthma with nasal allergies, RRR (95% CI)
Paternal line			
Model 1*	Parent's overweight status (vs never)		
	At age 8 y	0.86 (0.62-1.20)	0.98 (0.67-1.44)
	In puberty (at voice break/menarche)	**2.36 (1.27-4.38)**	0.74 (0.27-2.04)
	At age 30 y (before offspring conception)	0.61 (0.33-1.12)	1.32 (0.99-1.76)
	At age 30 y (after offspring conception)	0.74 (0.35-1.58)	1.16 (0.46-2.90)
Model 2*	Parent's overweight status (vs never)		
	At age 8 y	0.82 (0.60-1.13)	0.97 (0.65-1.45)
	In puberty (at voice break/menarche)	**2.31 (1.23-4.33)**	0.74 (0.27-2.06)
	At age 30 y (before offspring conception)	0.62 (0.34-1.14)	1.33 (0.99-1.76)
	At age 30 y (after offspring conception)	0.72 (0.34-1.51)	1.15 (0.46-2.85)
	Offspring's overweight status at age 8 y	**1.50 (1.05-2.16)**	1.09 (0.87-1.37)
Maternal line			
Model 1*	Parent's overweight status (vs never)		
	At age 8 y	1.05 (0.82-1.35)	0.98 (0.83-1.15)
	In puberty (at voice break/menarche)	0.91 (0.61-1.35)	0.81 (0.63-1.05)
	At age 30 y (before offspring conception)	0.82 (0.54-1.25)	1.01 (0.73-1.41)
	At age 30 y (after offspring conception)	0.82 (0.36-1.89)	0.72 (0.41-1.25)
Model 2*	Parent's overweight status (vs never)		
	At age 8 y	1.03 (0.81-1.32)	0.97 (0.84-1.12)
	In puberty (at voice break/menarche)	0.90 (0.60-1.34)	0.81 (0.62-1.05)
	At age 30 y (before offspring conception)	0.82 (0.54-1.24)	1.01 (0.73-1.41)
	At age 30 y (after offspring conception)	0.82 (0.36-1.89)	0.72 (0.41-1.25)
	Offspring's overweight at age 8 y	1.14 (0.96-1.35)	1.03 (0.80-1.32)

Statistically significant effects are indicated in boldface.
*Model 1: exposure of interest (parent's overweight) + adjusting variables (ECRHS/RHINE parents' asthma and educational level, other parents' overweight status and asthma, and offspring's sex and age); model 2: exposure of interest + potential mediator (offspring's overweight status at age 8 years) + adjusting variables.

than 0.012 (see Fig E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

In the present study we found a statistically significant effect of male onset of overweight status in puberty on asthma without nasal allergies in offspring born many years later. Mediation analysis assessed that this effect was direct and not mediated through offspring's own overweight status. Following the maternal line, we did not find any association between parental overweight status and adult offspring's asthma.

To our knowledge, this is the first study to investigate parents' overweight status long before conception and adult offspring's asthma. Although animal research and mechanistic studies have identified time windows during the lifespan in which the subject is particularly susceptible to exposures that can be transmitted to future generations in an epigenetic manner,[10,28] human data supporting the finding of such susceptibility windows are thus far scarce. Furthermore, the few studies with human data addressing this topic are limited mainly to exposure to cigarette smoke and not to onset of overweight status.[12-14] The present study suggests that the metabolic environment in male puberty might be important for offspring's health.

Paternal line

To the best of our knowledge, only 2 studies, partly in the same population as this study, have thus far investigated susceptibility time windows in fathers with regard to asthma in their adult offspring.[12,14] Svanes et al[12] reported from the RHINE study that fathers who had started smoking in early puberty (before 15 years) more than tripled the risk for early-onset asthma without nasal allergies in future offspring. Furthermore, in a recent article from the ECRHS, Accordini et al[14] showed that the onset of fathers' smoking in early puberty was a risk factor for asthma without nasal allergies in later offspring. Our finding that onset of overweight status in fathers in early puberty has a direct causal effect on asthma without nasal allergies in future offspring strengthens the hypothesis that male puberty is a time window of particular vulnerability from an intergenerational perspective. This result might substantially alter our way of thinking.

Although it is well established that *in utero* exposures are important, we have shown that it is far from the only important parental factor. Our finding supports the concept that paternal environmental exposures might lead to gametic epigenetic alterations that might affect the phenotypes of future offspring.[28] Through identifying the importance of onset of fathers' overweight status in puberty and through tying this together with new knowledge of fathers' smoking onset in puberty as a significant risk factor for adult offspring's asthma without nasal allergies,[12] our study contributes a potential game-changing new piece in the asthma puzzle: male puberty as a susceptibility time window of importance for the next generation. In recent years, studies have distinguished between different phenotypes of asthma and have studied how different phenotypes have different causative mechanisms. We encourage future studies to examine more closely what clinical asthma phenotype paternal onset of overweight status in puberty affects the most.

TABLE III. Exploratory analysis: Association between parents' overweight status (exposure) and offspring's overweight status (potential mediator) according to the parental line

	Paternal line	Maternal line
	Offspring's overweight status at age 8 y,* OR (95% CI)	Offspring's overweight status at age 8 y,* OR (95% CI)
Parent's overweight status (vs nonoverweight)		
At age 8 y	**2.23 (1.45-3.42)**	**2.45 (1.86-3.22)**
In puberty (at voice break/menarche)	1.61 (0.93-2.80)	**2.13 (1.26-3.60)**
At age 30 y (before offspring conception)	0.78 (0.46-1.34)	1.04 (0.76-1.42)
At age 30 y (after offspring conception)	**1.90 (1.25-2.86)**	0.98 (0.63-1.52)

Statistically significant effects are indicated in boldface.
*Adjusting for the following variables: ECRHS/RHINE parents' asthma and education, other parents' overweight status and asthma, and offspring's sex.

TABLE IV. Mediation analysis*: Natural effects of father's overweight status on adult offspring's asthma without nasal allergies within the paternal line

Father's overweight status (vs nonoverweight)	Natural effects	Offspring ever having asthma without nasal allergies,† OR (95% CI)
At age 8 y	Indirect	**1.03 (1.00-1.08)**
	Direct	0.83 (0.50-1.24)
	Total	0.86 (0.51-1.28)
At voice break	Indirect	1.02 (0.99-1.06)
	Direct	**2.24 (1.06-4.09)**
	Total	**2.28 (1.09-4.13)**
At age 30 y (before offspring conception)	Indirect	1.00 (0.97-1.01)
	Direct	0.65 (0.30-1.01)
	Total	0.64 (0.30-1.00)
At age 30 y (after offspring conception)	Indirect	1.02 (1.00-1.06)
	Direct	0.72 (0.22-1.42)
	Total	0.74 (0.23-1.46)

Statistically significant effects are indicated in boldface.
*The mediation model is shown in Fig 3.
†Adjusting for the following variables: ECRHS/RHINE parents' asthma and education, other parents' overweight status and asthma, and offspring's sex.

In addition, we observed that fathers' overweight status at age 8 years had an indirect-only effect on adult offspring's asthma without nasal allergies, which was mediated through offspring's own overweight status at age 8 years. This is most likely due to the strong hereditary association that we observed between fathers' overweight status at age 8 years and offspring's overweight status at age 8 years and is in agreement with previous studies showing associations between parental and offspring's weight[29] and between own overweight status and own asthma.[30]

Maternal line

Mothers' overweight status in different time periods was not associated with adult offspring's asthma in our exploratory analysis. The lack of association is not in agreement with other studies showing that maternal overweight status just before or during pregnancy is associated with offspring's asthma.[7,8] A possible explanation of this discrepancy is that these studies have not taken fathers' overweight status into account, whereas we included fathers' overweight status as a covariate in the model. However, a residual confounding effect of fathers' overweight status could be present because of the fact that fathers' overweight status was reported by the offspring and refers to a single time window (at age 30 years). In addition, associations found in previous studies could be due to in *utero* exposures. The RHINESSA study is not designed for assessment of maternal exposures during pregnancy, but it focuses on potential determinants in different time windows before conception.

Strengths and limitations

The present study has several strengths. The RHINESSA study design provides a highly efficient method for extracting detailed multigenerational information on respiratory health. In most established birth cohort studies, there is a focus exclusively on exposures in mothers, whereas in the RHINESSA study we also collected information on fathers in different time windows. For the parents, who have been followed for 20 years, information on preconception risk factors was collected retrospectively before examination of their offspring. Published validation studies from this study population on body silhouettes and overweight status,[19] on pregnancy and birth characteristics,[31] on asthma reports across generations,[32] and on the use of body silhouettes to reflect obesity in the past[20] suggest minimal recall bias in key information and high reliability of information collected across generations.

Another major strength of the present study is the statistical methods used for assessing causality among variables in different generations. The use of a counterfactual model, which has become increasingly standard for causal inference in epidemiologic and medical studies,[33] enabled us to decompose the total effect of parents' overweight status on offspring's asthma into its direct effect and the effect mediated by offspring's overweight status in causal thinking.

A third strength of this study is the use of figural drawing scales to assess body size throughout the lifespan. Although it can be difficult to recall exact body weight as far back as childhood and puberty, remembering one's image in the mirror is likely to be easier.[34] Moreover, using the same figural drawing scales for different time periods allowed for direct comparison across these periods and enabled us to construct an overweight-onset variable that enriches the quality of our study and enabled us to identify the important windows of susceptibility in parents for respiratory health in offspring.

Certain limitations need to be mentioned. First, our categorization of the outcome into asthma with nasal allergies and asthma without nasal allergies is based on self-reported questionnaires only. Our study did not include objective clinical data, such as allergy skin tests or RASTs, and therefore we were not able to define detailed clinical asthma phenotypes. However, the questions included in the RHINESSA study questionnaires are commonly used epidemiologic proxies reflecting phenotypes. If any misclassification of the outcome has occurred, it is unlikely that this would be systematically different between offspring whose fathers reported a higher or lower body silhouette in childhood or in puberty. Similarly, it is unlikely that misclassification in fathers' reports of their past body silhouettes is related to their offspring's reported asthma phenotype. Thus these potential information biases are more likely to attenuate the observed effects.

Second, we could not assess the moderating effects (interaction) of offspring's sex because of data sparseness. This is unfortunate because one might suspect a sex-specific association pattern in which paternal and maternal risk factors affect daughters and sons differently,[8,9] and sex might modify the effect of obesity on asthma.[35] However, there is not conclusive evidence of the mother-daughter and father-son intergenerational transmission of BMI being stronger.[36]

Third, we assumed that parents' overweight status and ever having asthma have an additive effect on the exposure-outcome relationship. This is a simplification of the complex interaction between body size and asthma. In fact, the association between obesity and asthma seems predominantly caused by genetic pleiotropy, meaning that these 2 conditions share genetic determinants[37] that might cause the heritability of both obesity and asthma within families. However, our study allows including other parents' overweight status and ever having asthma in the models to exclude an apparent association between parents' overweight status and offspring's asthma caused by a potential assortative mating between spouses.[36] However, the information regarding other parents was offspring reported rather than directly assessed, generating potential information bias.

Fourth, the risk for overweight status cutoff has not been validated by BMI in the childhood and puberty time windows. A validation study for these time windows is warranted but difficult to accomplish because of the wide timeframe. However, the body silhouettes have been validated by past BMI for the 30-year time point,[20] and it is likely that they will not differ substantially, even if we go further back in time. We have extrapolated the validated adult cutoffs to assess the risk for being overweight also at earlier stages in life to have comparable definitions of "risk for being overweight" across the different time windows in our analyses.

Fifth, some misclassification of asthma in the offspring is likely; however, such misclassification could not be linked to how the parent reported their past body silhouettes in a different study and would thus constitute nondifferential bias that would have attenuated the true results.

Lastly, it is possible that important confounders were not included in the models. Nevertheless, in the sensitivity analysis we found that unmeasured confounding had a limited effect on the estimated effects of fathers' overweight status on offspring's asthma without nasal allergies.

Onset of overweight status in male puberty appears to be an important risk factor for adult offspring's asthma without nasal allergies. The public health opportunities might be large: asthma in future generations might be partly prevented if we combat overweight status in today's youngest generation. Also, the identification of male puberty as a time window of particular importance for future generations provides a fundamental change in how we view the development of chronic diseases, such as asthma, and unlocks the next level of asthma research.

We thank all RHINESSA study participants and fieldworkers.

Clinical implications: Onset of overweight status in male puberty appears to be a risk factor for adult offspring's asthma. To combat being overweight in today's youngsters might partly prevent asthma in the next generation.

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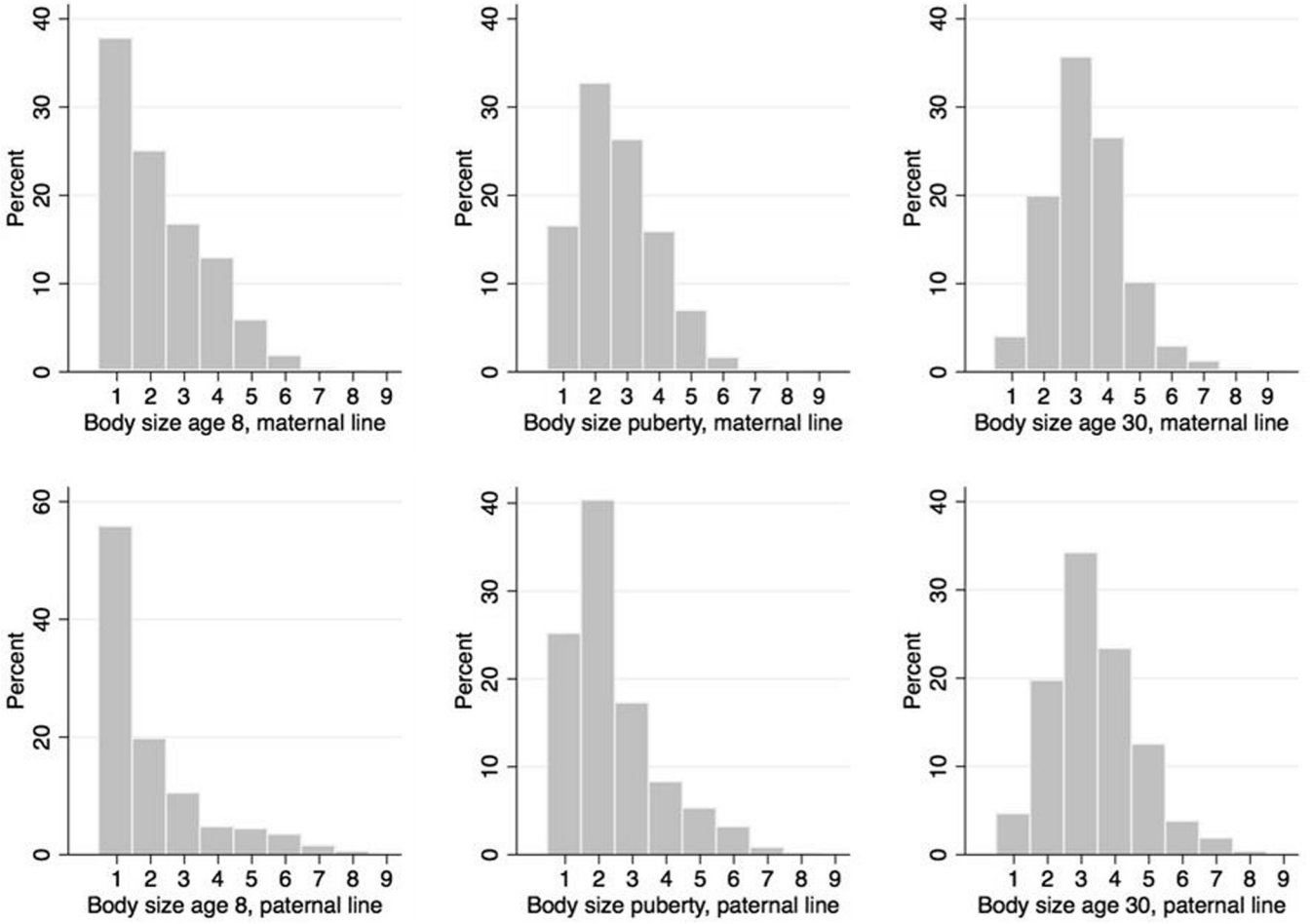

FIG E1. Body size distribution in childhood, puberty, and adulthood for participating parents in the maternal line *(3 top graphs)* and for participating parents in the paternal line *(3 bottom graphs)* in the RHINESSA generation study.

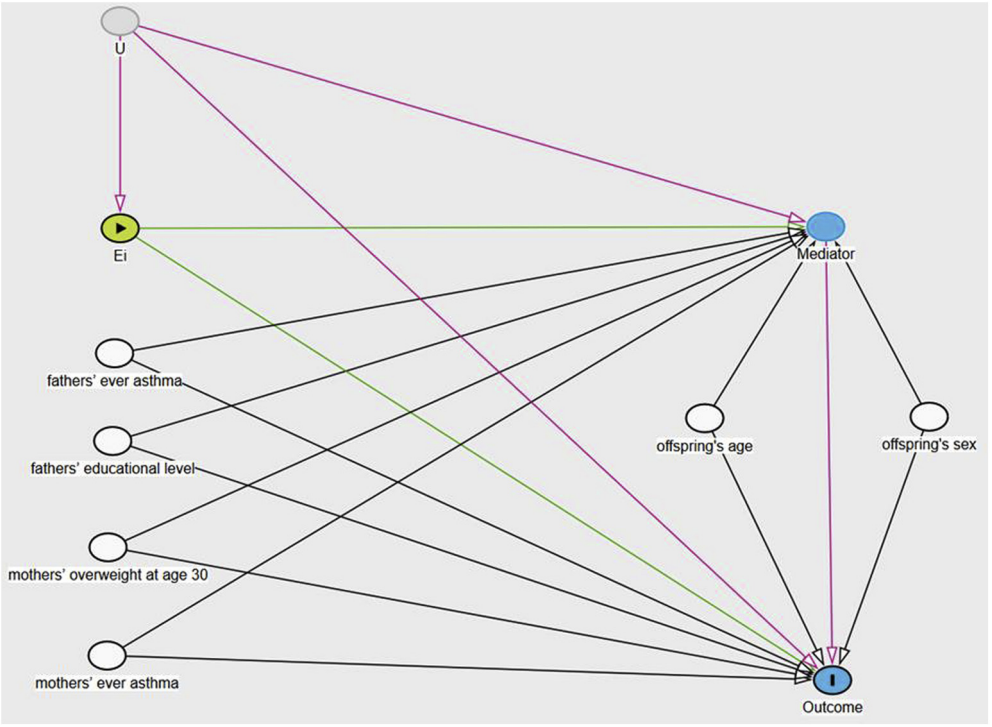

FIG E2. Directed acyclic graph showing how data were simulated for the exposure, mediator, outcome, and adjusting variables and 1 unmeasured confounder. *Ei* indicates one of the following binary exposure variables: E1, fathers' overweight status at age 8 years versus never overweight; E2, fathers' overweight status in puberty versus never overweight; E3, fathers' overweight status at age 30 years before each offspring conception versus never overweight; and E4, fathers' overweight status at age 30 years after offspring versus never overweight. *Mediator* indicates offspring's overweight status at age 8 years. *Outcome* indicates offspring ever having asthma without nasal allergies. *U* indicates the unmeasured normally distributed confounder.

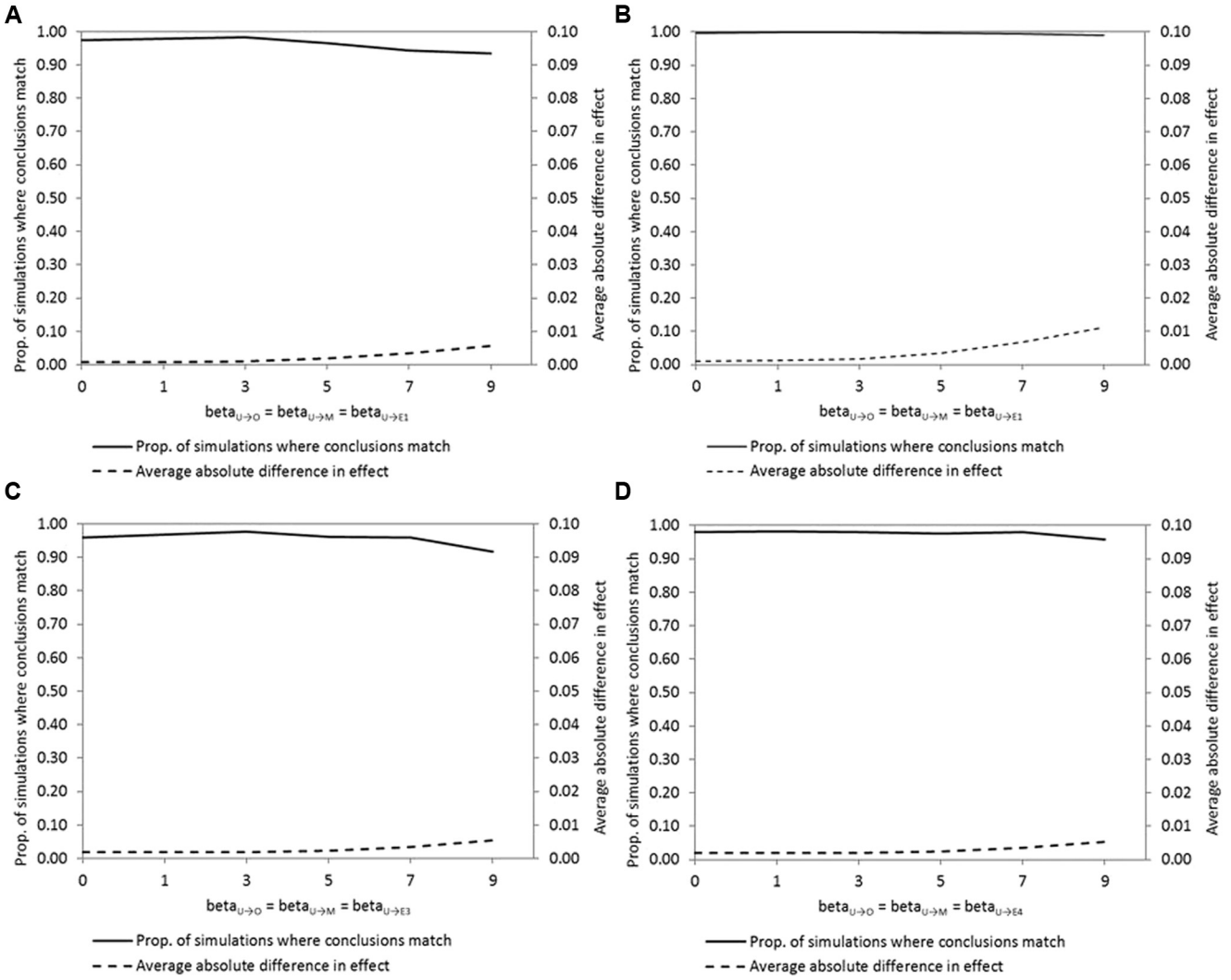

FIG E3. Proportion of simulations in which the results match and the average absolute difference for the direct effects of ECRHS/RHINE fathers' overweight status on offspring ever having asthma without nasal allergies (whether the unmeasured confounder U is included or excluded from the mediation models): **A,** E1, overweight at age 8 years versus never overweight; **B,** E2, overweight in puberty versus never overweight; **C,** E3, overweight at age 30 years before each offspring conception versus never overweight; **D,** E4, overweight at age 30 years after offspring versus never overweight.

TABLE E1. Parents and offspring in the present analysis by parental line and study center

		Paternal line		Maternal line	
Country	**Center**	**No. of fathers**	**No. of offspring**	**No. of mothers**	**No. of offspring**
Denmark	Aarhus	241	296	270	340
Spain	Albacete	20	33	23	40
	Huelva	11	18	22	43
Iceland	Reykjavik	305	393	349	449
Norway	Bergen	369	538	408	569
Sweden	Goteborg	280	378	352	465
	Umea	346	503	449	667
	Uppsala	345	492	460	662
Australia	Melbourne	50	83	52	94
Estonia	Tartu	77	88	164	196
	Total	2044	2822	2549	3525