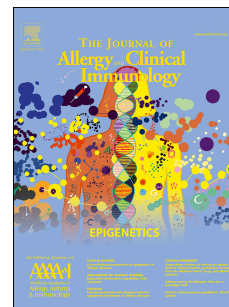


Journal Pre-proof

Overweight in childhood, puberty or early adulthood: changing the asthma risk in the next generation?

Ane Johannessen, ass. Prof., Marianne Lønnebotn, MSc, Lucia Calciano, PhD, Bryndis Benediktsdóttir, Prof., Randi Jacobsen Bertelsen, PhD, Lennart Bråbäck, PhD, Shyamali Dharmage, Prof., Karl A. Franklin, PhD, Thorarinn Gislason, Prof., Mathias Holm, PhD, Christer Janson, Prof., Deborah Jarvis, Prof., Rain Jögi, PhD, Jeong-Lim Kim, ass. Prof., Jorunn Kirkeleit, PhD, Caroline Lodge, PhD, Andrei Malinowski, Prof., Jesus Martinez-Moratalla, MD, Roy Miodini Nilsen, ass. Prof., Antonio Pereira-Vega, PhD, Francisco Gomez Real, Prof., Vivi Schlünssen, Prof., Simone Accordini, ass. Prof., Cecilie Svanes, Prof.



PII: S0091-6749(19)31172-8

DOI: <https://doi.org/10.1016/j.jaci.2019.08.030>

Reference: YMAI 14167

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 31 January 2019

Revised Date: 20 August 2019

Accepted Date: 22 August 2019

Please cite this article as: Johannessen A, Lønnebotn M, Calciano L, Benediktsdóttir B, Bertelsen RJ, Bråbäck L, Dharmage S, Franklin KA, Gislason T, Holm M, Janson C, Jarvis D, Jögi R, Kim J-L, Kirkeleit J, Lodge C, Malinowski A, Martinez-Moratalla J, Nilsen RM, Pereira-Vega A, Real FG, Schlünssen V, Accordini S, Svanes C, Overweight in childhood, puberty or early adulthood: changing the asthma risk in the next generation?, *Journal of Allergy and Clinical Immunology* (2019), doi: <https://doi.org/10.1016/j.jaci.2019.08.030>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Overweight in childhood, puberty or early adulthood: changing the asthma risk in the next generation?

Ane Johannessen, ass. Prof.^{1,2*} Marianne Lønnebotn, MSc^{1,2*}, Lucia Calciano, PhD^{3*}, Bryndis Benediktsdóttir, Prof.^{4,5}, Randi Jacobsen Bertelsen, PhD⁶, Lennart Bråbäck, PhD⁷, Shyamali Dharmage, Prof.⁸, Karl A. Franklin, PhD⁹, Thorarinn Gislason, Prof.⁵, Mathias Holm, PhD¹⁰, Christer Janson, Prof.¹¹, Deborah Jarvis, Prof.¹², Rain Jõgi, PhD¹³, Jeong-Lim Kim, ass. Prof.¹⁴, Jorunn Kirkeleit, PhD¹, Caroline Lodge, PhD¹⁵, Andrei Malinowski, Prof.¹⁶, Jesus Martinez-Moratalla, MD¹⁷, Roy Miodini Nilsen, ass. Prof.¹⁸, Antonio Pereira-Vega, PhD¹⁹, Francisco Gomez Real, Prof.^{6,20}, Vivi Schlünssen, Prof.²¹, Simone Accordini, ass. Prof.^{3**}, Cecilie Svanes, Prof.^{2,22**}.

*Equal contribution as first authors.

**Equal contribution as last authors.

¹Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

²Department of Occupational Medicine, Haukeland University Hospital, Bergen, Norway

³Unit of Epidemiology and Medical Statistics, Department of Diagnostics and Public Health, University of Verona, Verona, Italy

⁴Faculty of Medicine, University of Iceland, Reykjavik, Iceland

⁵Department of Sleep, Landspítali University Hospital, Reykjavik, Iceland

⁶Department of Clinical Science, Haukeland University Hospital, Bergen, Norway

⁷Occupational and Environmental Medicine, Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden.

⁸Allergy and Lung Health Unit, School of population and Global Health, University of Melbourne, Melbourne, Australia

⁹Department of Surgical and Perioperative Sciences, Surgery, Umeå University, Umeå, Sweden

¹⁰Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden

¹¹Department of Medical Sciences, Respiratory, Allergy & Sleep Research, Uppsala University, Uppsala, Sweden

¹²Faculty of Medicine, National Heart and Lung Institute, Imperial College, London, United Kingdom

¹³Lung Clinic, Tartu University Hospital, Tartu, Estonia

¹⁴Occupational and Environmental Medicine, Department of Public Health and Community Medicine, The Sahlgrenska Academy, University of Gothenburg, Sweden

¹⁵Department of Population Health, University of Melbourne, Melbourne, Australia

¹⁶Department of Medical Sciences, Uppsala University, Uppsala Sweden

¹⁷Servicio de Neurología del Complejo Hospitalario Universitario de Albacete (CHUA), Servicio de Salud de Castilla-La Mancha (SESCAM), Albacete, Spain

¹⁸Department of nursing, Bergen University College, Bergen, Norway

¹⁹Pneumology Section, Hospital Juan Ramón Jiménez, Huelva, Spain

²⁰Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway

²¹Department of Public Health, Aarhus University, Aarhus, Denmark

²²Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

Correspondence:

Marianne Lønnebotn, MSc, PhD-candidate

Department of Global Public Health and Primary Care, University of Bergen

5021 Bergen

Norway

Telephone: + 47 959 68 484

E-mail: marianne.lonnebotn@uib.no

Funding

European Union's Horizon 2020 research and innovation programme as part of the ALEC (Ageing Lungs in European Cohorts) study, grant no 633212. The Research Council of Norway; The Bergen Medical Research Foundation; The Western Norwegian Regional Health Authorities; The Norwegian Labor Inspection; The Norwegian Asthma and Allergy Association; The Danish Wood Foundation; The Danish Working Environment Authority; The Swedish Lung Foundation; The Swedish Asthma and Allergy Association, and The Estonian Research Council.

Conflicts of interest

Authors have no conflicts of interest.

Abstract

Background

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

Objective

We aimed to examine if parents overweight in childhood, adolescence or adulthood is associated with offspring asthma.

Methods

We included 6 347 adult offspring (age 18-52 yrs, investigated in the RHINESSA multigenerational study) of 2 044 fathers and 2 549 mothers (age 37-66 yrs, investigated in the ECRHS study). Associations of parental overweight at age eight, puberty and age 30, with offspring childhood overweight (potential mediator) and offspring asthma with or without nasal allergies (outcomes), was analyzed using two-level logistic regression and two-level multinomial logistic regression, respectively. Counterfactual-based mediation analysis was performed to establish whether observed associations were direct effects or indirect effects mediated through offspring's own overweight.

Results

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

Conclusion

Our findings suggest that metabolic factors long before conception may increase asthma risk, and that male puberty is a time window of particular importance for offspring health.

Clinical Implications

Overweight onset in male puberty appears to be a risk factor for adult offspring's asthma. To combat overweight in today's youngsters may partly prevent asthma in the next generation.

Capsule Summary

Factors long before conception may increase risk for asthma, and male puberty is a time window of particular importance. In our study, overweight onset in boys in puberty was associated with asthma in future offspring.

Keywords: Ageing Lungs in European Cohorts (ALEC) Study; epidemiology; multilevel mediation model; offspring; parental risk factors.

Abbreviations: BMI, Body Mass Index; OR, Odds Ratio; RRR, Relative Risk Ratio; CI, Confidence Interval

Introduction

In parallel with the increase in asthma and allergies, there has been a dramatic increase in overweight and obesity during the last decades. More than 60% of the population in Western countries are overweight, with a body mass index (BMI) exceeding 25kg/m^2 ¹, and countries from other parts of the world follow the same trend². Overweight is a well-known risk factor for non-communicable diseases including cancers, cardiovascular diseases and diabetes mellitus³⁻⁵. Research has also shown associations with asthma and asthma severity⁶, as well as an association between mothers' overweight 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 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 exist⁹. Existing literature suggests particularly vulnerable intergenerational exposure time-windows in utero and just before puberty^{10,11}. Until now, there is barely any human data to support these time-windows with respect to offspring asthma, and the limited research in this field has so far mainly investigated exposure to cigarette smoke¹²⁻¹⁴.

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

Methods

Study design

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

In 1992 the ECRHS study surveyed population-based random samples of adults aged 20-44 years (~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; Tartu in Estonia) all responders to the 1992 postal survey were followed in a large longitudinal questionnaire study, the Respiratory Health in Northern Europe study (RHINE, www.rhine.nu)¹⁶. The subsamples that were invited for clinical examination in ECRHS were invited to ECRHS follow-up studies. Both ECRHS and RHINE conducted follow-up studies in ~2002 (ECRHS II/RHINE II) and again in ~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 2013-16 through one questionnaire deployment in each study center and the completed adult offspring database includes 8 260 offspring aged ≥ 18 years. 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

161 medical research ethics

162 (<https://helsebergen.no/seksjon/RHINESSA/Documents/Ethic%20Committees%20list.pdf>).

163 **Study populations**

164 A flow chart describing the study populations in the present study is given in Figure 1. Of the
165 8 260 adult offspring in the RHINESSA study (G1, 42% male), 7 271 offspring had a parent
166 (G0) who had participated in the most recent RHINE/ECRHS follow up studies in 2010-13
167 (ECRHS III/RHINE III), and were thus eligible for inclusion in the present analysis. Some
168 offspring are siblings, and the number of unique parents was 5 235, of whom 45% were men
169 (fathers) and 55% were women (mothers). The database includes one parent only per
170 offspring - resulting in two eligible study populations for the present study: one population for
171 the paternal line (3 256 offspring and 2 336 fathers) and one population for the maternal line
172 (4 015 offspring and 2 899 mothers). The proportion of offspring and ECRHS/RHINE parents
173 (i.e. mothers and fathers who themselves participated in ECRHS/RHINE III) with information
174 on key variables was 87% for the paternal line and 88% for the maternal line, resulting in net
175 study populations of 2 822 offspring (57% female, 43% male) with their 2 044 fathers, and 3
176 525 offspring (58% female and 42% male) with their 2 549 mothers (for population
177 distribution across study centers, see Table E1 in the online data supplement).

178 **Definitions**

179 Both the parents participating in ECRHS/RHINE and their adult offspring participating in
180 RHINESSA provided information about asthma, body size, smoking history and educational
181 level. Offspring also provided information on their *other parent*, i.e. the spouse of the
182 ECRHS/RHINE parent.

183
184 Adult offspring ever asthma was classified as: “ever asthma with nasal allergies”, “ever
185 asthma without nasal allergies” or “never asthma”. This distinction was made because asthma

with allergies and asthma without allergies represent two asthma phenotypes: while asthma with allergies is triggered by inhaled allergens, asthma without allergies is not¹⁷. Even if the symptoms are similar, the underlying risk factors may differ¹⁸. Asthma with and without nasal allergies were defined based on answers to the 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 the age of 10 years. The ECRHS/RHINE parents’ ever asthma (“present” vs “absent”) was self-reported, whereas ever asthma in the *other parent* was offspring-reported in the RHINESSA questionnaire.

Overweight was identified using a validated figural drawing scale of nine sex-specific body silhouettes (Figure 2)¹⁹ in RHINE III / ECRHS III for the parents and in RHINESSA for the adult offspring. To distinguish between non-overweight and overweight-obese individuals we used as cut-offs figure ≥ 5 in men and ≥ 4 in women. Using this definition we are not able to objectively assess overweight as defined by the WHO, but to identify an “at risk” for overweight body size. In a recent RHINE III validation study, these cut-offs were defined as optimal for identifying overweight adults (BMI = 25-30 kg/m²)¹⁹. (For participating parents body size distributions see Figure E1 in the online data supplement). 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 studies²⁰.

ECRHS/RHINE parents’ overweight onset was classified at four susceptibility time windows: age eight (addressing the pre-puberty slow growth period time window); puberty (voice break

for fathers, menarche for mothers); age 30 before offspring conception and age 30 after offspring conception. In detail, “overweight at age eight” was present if the parent reported overweight at age eight regardless of overweight at later susceptibility periods; “overweight in puberty” if he/she reported overweight in puberty but not at age eight (regardless of overweight at age 30); “overweight at age 30 before each offspring conception” if he/she reported overweight at age 30 but neither at age eight nor in puberty (regardless of overweight after offspring conception); “overweight at age 30 after offspring conception” if he/she reported overweight at age 30 after offspring conception but not in the previous susceptibility time windows. The reference category was “never overweight”. Offspring’s overweight at age eight and *other parents’* overweight at age 30 (“present” vs “absent”) were both reported by the adult offspring, using the same figural drawing scale as 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

The offspring variables included in the analyses were the following: adult offspring’s ever asthma with or without nasal allergies as the outcome; offspring’s overweight at age eight as the potential mediator. The parental variables included in the analyses were: ECRHS/RHINE parents’ overweight as the exposure of interest; ECRHS/RHINE parents’ asthma and educational level; and *other parents’* (reported by the adult offspring) overweight 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 asthma, *other parents'* ever asthma and overweight at age 30 as potential modifiers of the exposure-outcome and exposure-mediator relationships. The paths investigated in the analyses are represented in Figure 3.

Explorative analysis

Our data have a hierarchical structure because multiple adult offspring (level 1 unit) may 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 using a two-level logistic regression model and a two-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 the adjustment variables as fixed effects. Furthermore, cluster-robust standard errors were computed in order to take the correlation among parents within each of the different centers (cluster variable) into account. The exposure-mediator and exposure-outcome associations were summarized as odds ratios (ORs) and relative risk ratios (RRRs), respectively. The 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 and adult offspring's asthma are causal effects that could be also mediated through offspring's own childhood overweight. This approach allows to decompose the total effect of the exposure on the outcome into the natural direct effects (i.e. the effect of the exposure on the outcome via pathways that do not involve the mediator) and the natural indirect effect (i.e. the effect of the

exposure on the outcome due to the effect of the exposure on the mediator)²¹. The main requirement for mediation is that the *indirect effect* is statistically significant²².

At present, to the best of our knowledge, multilevel mediation models with a dichotomous mediator and a categorical outcome (with more than two unordered categories) are not included in statistical software. Therefore, in our study, the mediation analysis was carried out splitting the multinomial-distributed outcome into two 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 due to the magnitude of the *design effect*²³. In the mediation analysis, the estimate of the natural effects was obtained by the latent response variable mediator approach²⁴ with probit link, theta parameterization and weighted least squares means and variance adjusted (WLSMV) estimators²⁵. Non-bias-corrected bootstrap confidence intervals (10,000 resamples) were obtained for the causally-defined effects in order to take non-normality of their estimate distribution into account. The natural effects were summarized as odds ratios (ORs).

Sensitivity analyses

The identification of natural effects relies on strong assumptions²⁶. We checked whether the results changed in the presence of unmeasured confounding of the exposure-mediator-outcome relationship in the mediation analyses²⁷.

Using the Umediation R package (<https://github.com/SharonLutz/Umediation>), we simulated one unmeasured normally-distributed confounder (“U” variable) for the exposure-outcome, exposure-mediator and mediator-outcome relationships, with mean 0 and variance 0.001. As inputs for Umediation, we used the coefficients of the mediation model. We carried out four

simulation analyses splitting the categorical exposure variable (“E” variable) into four binary exposures (E_1 : “overweight at age 8”, E_2 : “overweight in puberty”, E_3 : “overweight at age 30 before each offspring conception”, E_4 : “overweight at age 30 after offspring conception”; reference category: “never overweight”). Each of the four simulation analyses was carried out under multiple scenarios for the effects (beta regression coefficients) of the unmeasured confounder U on the outcome ($\beta_{U \rightarrow O}$), the mediator ($\beta_{U \rightarrow M}$) and the exposure ($\beta_{U \rightarrow E_i}$, $i=1 \dots 4$), by fixing $\beta_{U \rightarrow M} = \beta_{U \rightarrow E_i} = \beta_{U \rightarrow O} = 0, 1, 3, 5, 7$ and 9 . We specified 1000 simulation runs and 1000 Monte Carlo draws for the nonparametric bootstrap in each simulation analyses.

The statistical analysis was carried out using Stata version 14.2 (StataCorp, College Station, TX), Mplus version 8.1 (Muthén & Muthén, Los Angeles, CA) and R version 3.5.1 (<https://cran.r-project.org/>).

Results

Characteristics of the study population

Median age of ECRHS/RHINE parents was 55 years, while 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 the offspring was 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 paternal (18.3% and 11.4%) and maternal (18.5% and 14.4%) lines. In the paternal and maternal lines, 51% and 55% of the adult offspring had at least one parent who had smoked during their childhood, respectively. In the paternal line, 14% of the offspring were overweight at age eight, 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 eight, and 50% of them had mothers who were overweight at some point (Table I).

Explorative 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 to fathers who had never been overweight. The strength of this association remained unaltered when the potential mediator (offspring's overweight at eight years) was added to the model (RRR 2.31 (95%CI 1.23, 4.33)). Offspring's overweight at eight 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 the parental lines, positive associations were found between the exposure and the potential mediator (Table III). The risk of offspring's overweight at age eight was higher if their parent had been overweight at the same susceptibility window (OR 2.23 (95%CI 1.45, 3.42) and OR 2.45 (95%CI 1.86, 3.22), respectively within paternal and maternal lines), or 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 after offspring conception (OR 1.90 (95%CI 1.25, 2.86)), compared to the offspring having mothers/fathers who had never been overweight.

Mediation analysis

Based on the associations found at the explorative 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 at age eight on adult offspring's asthma without nasal allergies mediated by offspring's overweight at age eight (*indirect-only mediation*) (Table IV). We found that the effect of fathers' overweight onset at voice break on the adult offspring's ever asthma without nasal allergies (OR 2.24 (95%CI 1.06, 4.09) did not involve offspring's overweight at age eight (*direct-only non-mediation*) (Table IV). These results confirm the findings of the explorative analysis (Table II). Lastly, no statistically significant indirect or direct effects were found between fathers' overweight at age 30 (before and after offspring conception) and adult offspring's asthma without nasal allergies (*non-mediation*) – although the estimate for fathers' overweight at age 30 after conception was borderline significant (Table IV).

Sensitivity analysis

The inclusion of one unmeasured confounder U (Figure E2, online data supplement) in the model had a limited impact on the estimate of the direct effects of fathers' overweight on adult offspring's ever asthma without nasal allergies, also when U had a very strong effect on the outcome, mediator and exposure ($\beta_{U \rightarrow O} = \beta_{U \rightarrow M} = \beta_{U \rightarrow E} > 5$, $i = 1, \dots, 4$). Indeed, as

350 the effect increased, the proportion of simulations where the results matched (whether U was
351 included or excluded from the model) remained greater than 89% and the average absolute
352 difference of the direct effects remained lower than 0.012 (Figure E3, online data
353 supplement).

354

Discussion

In the present study, we found a statistical significant effect of male overweight onset 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. Following the maternal line, we did not find any association between parental overweight and adult offspring's asthma.

To our knowledge, this is the first study to investigate parents' overweight long before conception and adult offspring's asthma. Although animal research and mechanistic studies have identified time windows during the lifespan in which the individual 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 so far scarce. The few studies with human data addressing this topic are furthermore limited mainly to exposure to cigarette smoke and not to overweight onset¹²⁻¹⁴. The present study suggests that the metabolic environment in male puberty may be important for offspring health.

Paternal line

To the best of our knowledge, only two studies partly in the same population as this study have so far investigated susceptibility time windows in fathers with regard to asthma in their adult offspring^{12, 14}. Svanes et al 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 paper from the ECRHS survey, Accordini and co-workers¹⁴ 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 overweight onset in fathers' 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 may substantially alter our way of thinking. While 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 may lead to gametic epigenetic alterations that might affect the phenotypes of future offspring²⁸. Through identifying the importance of fathers' overweight onset in puberty, and through tying it together with the new knowledge of fathers' smoking onset in puberty as significant risk factor for adult offspring's asthma without nasal allergies¹², our study contributes with a potential game-changing new piece in the asthma puzzle: the 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 overweight onset in puberty affects the most.

In addition, we observed that fathers' overweight at age eight had an indirect-only effect on adult offspring asthma without nasal allergies, which was mediated through offspring's own overweight at age eight. This is most likely due to the strong hereditary association that we observed between fathers' overweight at age eight and offspring's overweight at age eight, and is in agreement with previous studies showing associations between parental and offspring weight²⁹, and between own overweight and own asthma³⁰.

Maternal line

Mothers' overweight 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 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 into account while we included fathers' overweight as a covariate in the model. However, a residual confounding effect of fathers' overweight could be present due to the fact that fathers' overweight was reported by the offspring and it refers to a single time window (at age 30). In addition, the associations found in the previous studies could be due to in *utero* exposures. RHINESSA is not designed for the 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 RHINESSA 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 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 epidemiological and medical studies ³³, enabled us to decompose the total effect of parents' overweight on offspring's asthma into its direct effect and the effect mediated by offspring's overweight in a causal thinking.

A third strength of this study is the use of figural drawing scales to assess body size throughout the life span. While it can be difficult to recall exact body weight as far back as in childhood and puberty, remembering one's image in the mirror is likely to be easier³⁴. Moreover, using the same figural drawing scales for different time periods allow 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. Firstly, 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 test or RAST, and we were therefore not able to define detailed clinical asthma phenotypes. However, the questions included in the RHINESSA questionnaires are commonly used epidemiological 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' report 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. Secondly, we could not assess the moderating effects (interaction) of offspring's sex due to data sparseness. This is unfortunate, since one may suspect a gender specific association pattern where paternal and maternal risk factors affect daughters and sons differently^{8,9} and sex might modify the effect of obesity on asthma³⁵. However, there is not a conclusive evidence of the mother-daughter and father-son intergenerational transmission of BMI being stronger³⁶. Thirdly, we assumed that parents' overweight and ever 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 two conditions share genetic determinants³⁷ that might cause the heritability of both obesity and asthma within families. However, our study allows including *other parents'* overweight and ever asthma in the models, in order to exclude an apparent association between parents' overweight and offspring's asthma due to a potential assortative mating between spouses³⁶. However, the information regarding *other parents* was offspring-reported rather than directly assessed, generating a potential information bias. Fourth, the risk for overweight cut-off 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 due to the wide time frame. However, the body silhouettes have been validated by past BMI for the 30-year time point²⁰, and it is likely that they will not differ substantially even if we go further back in time. We have extrapolated the validated adult cut-offs to assess risk for overweight also at the earlier stages in life, to have comparable definitions of "risk for 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 non-differential 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 impact on the estimated effects of fathers' overweight on offspring's asthma without nasal allergies.

Overweight onset in male puberty appears to be an important risk factor for adult offspring's asthma without nasal allergies. The public health opportunities may be large: asthma in future generations may be partly prevented if we combat overweight 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.

Authors' contributions

Study concept and design: A.J., M.L., L.C., S.A., C.S., D.J. Acquisition of data: A.J., B.B., R.J.B, L.B., S.D., T.G., M.H., C.J., R.J., A.M., J.M., A.P-V., F.G.R., V.S., C.S. Analysis and interpretation of data: A.J., M.L., L.C., S.A.,C.S. Drafting of the manuscript: A.J., M.L., L.C., S.A. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: L.C., S.A. Obtained funding: B.B., L.B., S.D., T.G., M.H., C.J., D.J., R.J., A.M., J.M., A.P-V., V.S., C.S.

Acknowledgments

We thank all RHINESSA study participants and fieldworkers. This study was funded by the European Union's Horizon 2020 research and innovation programme as part of the ALEC (Ageing Lungs in European Cohorts study, Grant Agreement No. 633212). The RHINESSA generation study also received funding by the Research Council of Norway (Grants No. 214123 and 228174), the Bergen Medical Research Foundation, the Western Norwegian Regional Health Authorities (Grants No. 912011, 911892 and 911631), the Norwegian Labour Inspection, the Norwegian Asthma and Allergy Association, the Danish Wood Foundation (Grant No. 444508795), the Danish Working Environment Authority, (Grant No. 20150067134), the Swedish Lung Foundation, the Swedish Asthma and Allergy Association, and the Estonian Research Council (Grant No. PUT562). The funding agencies had no direct role in the conduct of the study, the collection, management, statistical analysis and interpretation of the data, preparation or approval of the manuscript.

References

1. WHO. Global health observatory (GHO) data: Overweight and obesity. 2017 [cited 2017]. Available from: http://www.who.int/gho/ncd/risk_factors/overweight/en/.
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384(9945):766-81, [https://doi.org/10.1016/S0140-6736\(14\)60460-8](https://doi.org/10.1016/S0140-6736(14)60460-8)
3. Prospective Studies C, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet*. 2009;373(9669):1083-96, [https://doi.org/10.1016/S0140-6736\(09\)60318-4](https://doi.org/10.1016/S0140-6736(09)60318-4)
4. Emerging Risk Factors C, Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, et al. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet*. 2011;377(9771):1085-95, [https://doi.org/10.1016/S0140-6736\(11\)60105-0](https://doi.org/10.1016/S0140-6736(11)60105-0)
5. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet*. 2008;371(9612):569-78, [https://doi.org/10.1016/S0140-6736\(08\)60269-X](https://doi.org/10.1016/S0140-6736(08)60269-X)
6. Eder W, Ege MJ, von Mutius E. The asthma epidemic. *The New England journal of medicine*. 2006;355(21):2226-35, <https://doi.org/10.1056/NEJMra054308>
7. Harpsoe MC, Basit S, Bager P, Wohlfahrt J, Benn CS, Nohr EA, et al. Maternal obesity, gestational weight gain, and risk of asthma and atopic disease in offspring: a study within the Danish National Birth Cohort. *The Journal of allergy and clinical immunology*. 2013;131(4):1033-40, <https://doi.org/10.1016/j.jaci.2012.09.008>

- 538 8. Dumas O, Varraso R, Gillman MW, Field AE, Camargo CA, Jr. Longitudinal study of
539 maternal body mass index, gestational weight gain, and offspring asthma. *Allergy*.
540 2016;71(9):1295-304, [https://doi.org/ 10.1111/all.12876](https://doi.org/10.1111/all.12876)
- 541 9. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjöström M, et al.
542 Sex-specific, male-line transgenerational responses in humans. *European journal of human*
543 *genetics : EJHG*. 2006;14(2):159-66, [https://doi.org/ 10.1038/sj.ejhg.5201538](https://doi.org/10.1038/sj.ejhg.5201538)
- 544 10. Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence
545 for epigenetic inheritance through the male germ line. *BioEssays : news and reviews in*
546 *molecular, cellular and developmental biology*. 2014;36(4):359-71, [https://doi.org/](https://doi.org/10.1002/bies.201300113)
547 [10.1002/bies.201300113](https://doi.org/10.1002/bies.201300113)
- 548 11. Sales VM, Ferguson-Smith AC, Patti ME. Epigenetic Mechanisms of Transmission
549 of Metabolic Disease across Generations. *Cell metabolism*. 2017;25(3):559-71,
550 [https://doi.org/ 10.1016/j.cmet.2017.02.016](https://doi.org/10.1016/j.cmet.2017.02.016)
- 551 12. Svanes C, Koplin J, Skulstad SM, Johannessen A, Bertelsen RJ, Benediktsdóttir B, et
552 al. Father's environment before conception and asthma risk in his children: a multi-
553 generation analysis of the Respiratory Health In Northern Europe study. *International*
554 *journal of epidemiology*. 2017;46(1):235-45, [https://doi.org/ 10.1093/ije/dyw151](https://doi.org/10.1093/ije/dyw151)
- 555 13. Miller LL, Henderson J, Northstone K, Pembrey M, Golding J. Do grandmaternal
556 smoking patterns influence the etiology of childhood asthma? *Chest*. 2014;145(6):1213-8,
557 <https://doi.org/10.1378/chest.13-1371>
- 558 14. Accordini S, Calciano L, Johannessen A, Portas L, Benediktsdóttir B, Bertelsen RJ, et
559 al. A three-generation study on the association of tobacco smoking with asthma. *Int J*
560 *Epidemiol*. 2018;47(4):1106-17., <https://doi.org/10.1093/ije/dyy031>
- 561 15. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory
562 Health Survey. *Eur Respir J*. 1994;7(5):954-60.

- 563 16. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, et
564 al. Longterm follow-up in European respiratory health studies - patterns and implications.
565 BMC Pulm Med. 2014;14:63, [https://doi.org/ 10.1186/1471-2466-14-63](https://doi.org/10.1186/1471-2466-14-63)
- 566 17. Lafeuille MH, Gravel J, Figliomeni M, Zhang J, Lefebvre P. Burden of illness of
567 patients with allergic asthma versus non-allergic asthma. J Asthma. 2013;50(8):900-7,
568 [https://doi.org/ 10.3109/02770903.2013.810244](https://doi.org/10.3109/02770903.2013.810244)
- 569 18. Janson C, Kalm-Stephens P, Foucard T, Alving K, Nordvall SL. Risk factors
570 associated with allergic and non-allergic asthma in adolescents. Clin Respir J. 2007;1(1):16-
571 22, [https://doi.org/ 10.1111/j.1752-699X.2007.00001.x](https://doi.org/10.1111/j.1752-699X.2007.00001.x)
- 572 19. Dratva J, Bertelsen R, Janson C, Johannessen A, Benediktsdottir B, Braback L, et al.
573 Validation of self-reported figural drawing scales against anthropometric measurements in
574 adults. Public health nutrition. 2016;19(11):1944-51, [https://doi.org/](https://doi.org/10.1017/S136898001600015X)
575 [10.1017/S136898001600015X](https://doi.org/10.1017/S136898001600015X)
- 576 20. Lonnebotn M, Svanes C, Igland J, Franklin KA, Accordini S, Benediktsdottir B, et al.
577 Body silhouettes as a tool to reflect obesity in the past. PLoS One. 2018;13(4):e0195697,
578 [https://doi.org/ 10.1371/journal.pone.0195697](https://doi.org/10.1371/journal.pone.0195697)
- 579 21. Pearl J. Direct and indirect effects. Proceedings of the Seventeenth conference on
580 Uncertainty in artificial intelligence; Seattle, Washington. 2074073: Morgan Kaufmann
581 Publishers Inc.; 2001. p. 411-20.
- 582 22. Zhao XL, J.G., Jr. & Chen, Q. Reconsidering Baron and kenny: Myths and truths
583 about mediation analysis. Journal of Consumer Research. 2010;37(2):197-206, [https://doi.org/](https://doi.org/10.1086/651257)
584 [10.1086/651257](https://doi.org/10.1086/651257)
- 585 23. Mark HCL, Oi-man Kwok. Examining the Rule of Thumb of not Using Multilevel
586 Modeling: The "Design Effect Smaller Than Two" Rule. The Journal of Experimental
587 Education. 2015;83(3):423-38, <https://doi.org/10.1080/00220973.2014.907229>

- 588 24. Muthén B. Applications of causally defined direct and indirect effects in mediation
589 analysis using SEM in Mplus. 2011;1-110.
590 <https://www.statmodel.com/download/causalmediation.pdf>
- 591 25. Bandalos DL. Relative Performance of Categorical Diagonally Weighted Least
592 Squares and Robust Maximum Likelihood Estimation. Structural Equation Modelling: A
593 Multidisciplinary Journal. 2014;21(1):102-16, <https://doi.org/10.1080/10705511.2014.859510>
- 594 26. VanderWeele TJ. Bias formulas for sensitivity analysis for direct and indirect effects.
595 Epidemiology. 2010;21(4):540-51, <https://doi.org/10.1097/EDE.0b013e3181df191c>
- 596 27. Lutz SM, Thwing A, Schmiege S, Kroehl M, Baker CD, Starling AP, et al. Examining
597 the role of unmeasured confounding in mediation analysis with genetic and genomic
598 applications. BMC bioinformatics. 2017;18(1):344, [https://doi.org/10.1186/s12859-017-1749-](https://doi.org/10.1186/s12859-017-1749-y)
599 [y](https://doi.org/10.1186/s12859-017-1749-y)
- 600 28. Wei Y, Schatten H, Sun QY. Environmental epigenetic inheritance through gametes
601 and implications for human reproduction. Human reproduction update. 2015;21(2):194-208,
602 <https://doi.org/10.1093/humupd/dmu061>
- 603 29. Wardle J, Carnell S, Haworth CM, Plomin R. Evidence for a strong genetic influence
604 on childhood adiposity despite the force of the obesogenic environment. The American
605 journal of clinical nutrition. 2008;87(2):398-404, <https://doi.org/10.1093/ajcn/87.2.398>
- 606 30. Braback L, Hjern A, Rasmussen F. Body mass index, asthma and allergic
607 rhinoconjunctivitis in Swedish conscripts-a national cohort study over three decades.
608 Respiratory medicine. 2005;99(8):1010-4, <https://doi.org/10.1016/j.rmed.2005.02.004>
- 609 31. Skulstad SM, Igland J, Johannessen A, Bertelsen RJ, Lonnebotn M, Omenaas ER, et
610 al. Validation of maternal reported pregnancy and birth characteristics against the Medical
611 Birth Registry of Norway. PloS one. 2017;12(8):e0181794, [https://doi.org/](https://doi.org/10.1371/journal.pone.0181794)
612 [10.1371/journal.pone.0181794](https://doi.org/10.1371/journal.pone.0181794)

- 613 32. Kuiper IN, Svanes C, Benediktsdottir B, Bertelsen RJ, Braback L, Dharmage SC, et al.
614 Agreement in reporting of asthma by parents or offspring - the RHINESSA generation study.
615 BMC Pulm Med. 2018;18(1):122, , [https://doi.org/ 10.1186/s12890-018-0687-4](https://doi.org/10.1186/s12890-018-0687-4)
- 616 33. Hofler M. Causal inference based on counterfactuals. BMC Med Res Methodol.
617 2005;5:28, :[https://doi.org/ 10.1186/1471-2288-5-28](https://doi.org/10.1186/1471-2288-5-28)
- 618 34. Must A, Willett WC, Dietz WH. Remote recall of childhood height, weight, and body
619 build by elderly subjects. American journal of epidemiology. 1993;138(1):56-64,
620 :[https://doi.org/ 10.1093/oxfordjournals.aje.a116777](https://doi.org/10.1093/oxfordjournals.aje.a116777)
- 621 35. Wang L, Wang K, Gao X, Paul TK, Cai J, Wang Y. Sex difference in the association
622 between obesity and asthma in U.S. adults: Findings from a national study. Respir Med.
623 2015;109(8):955-62, [https://doi.org/ 10.1016/j.rmed.2015.06.001](https://doi.org/10.1016/j.rmed.2015.06.001)
- 624 36. Dolton P, Xiao M. The intergenerational transmission of body mass index across
625 countries. Economics and human biology. 2017;24:140-52, [https://doi.org/](https://doi.org/10.1016/j.ehb.2016.11.005)
626 [10.1016/j.ehb.2016.11.005](https://doi.org/10.1016/j.ehb.2016.11.005)
- 627 37. Hallstrand TS, Fischer ME, Wurfel MM, Afari N, Buchwald D, Goldberg J. Genetic
628 pleiotropy between asthma and obesity in a community-based sample of twins. J Allergy Clin
629 Immunol. 2005;116(6):1235-41, [https://doi.org/ 10.1016/j.jaci.2005.09.016](https://doi.org/10.1016/j.jaci.2005.09.016)
- 630
- 631

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

	Paternal line	Maternal line
N parents	2 044	2 549
Parent's age (years)[†], median (range)	55 (37-66)	55 (39-65)
Parent's ever asthma[†], % (N)	11.4(233)	14.4 (367)
Parent's low education[*] level[†], % (N)		
present	11.9 (243)	11.5 (293)
unknown	4.3 (88)	4.1 (105)
Parent's overweight[†], % (N)		
at age 8	9.9 (202)	21.2 (540)
in puberty (at voice break / menarche)	2.4 (49)	7.7 (196)
at age 30 (before offspring's conception)	7.3 (149)	15.6 (398)
at age 30 (after offspring's conception)	3.0 (61)	5.0 (127)
Other parent's overweight at age 30^{**}, % (N)		
present	46.5 (950)	20.4 (520)
unknown	1.8 (37)	4.0 (102)
Other parent's ever asthma^{**}, % (N)		
present	11.6 (237)	7.2 (184)
unknown	3.2 (65)	3.5 (89)
N adult offspring	2 822	3 525
Offspring's gender (female)^{**}, % (N)	57.1 (1 611)	58.0 (2 045)
Offspring's age (years)[*], median (range)	29 (18-50)	30 (18-52)
Offspring's ever asthma^{**}, % (N)		
without nasal allergies	9.0 (254)	8.3 (293)
with nasal allergies	9.3 (262)	10.2 (360)
Offspring's overweight at age 8^{**}, % (N)	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.

Table II. Explorative analysis. Association between parents' overweight (exposure) and adult offspring's ever asthma (outcome), according to the parental line.

	Variables of interest	Paternal line	
		Offspring's ever asthma without nasal allergies RRR (95% CI)	Offspring's ever asthma with nasal allergies RRR (95% CI)
Model 1[†]	Parent's overweight, (vs never)		
	at age 8	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 (before offspring's conception)	0.61 (0.33, 1.12)	1.32 (0.99, 1.76)
	at age 30 (after offspring's conception)	0.74 (0.35, 1.58)	1.16 (0.46, 2.90)
Model 2[†]	Parent's overweight, (vs never)		
	at age 8	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 (before offspring's conception)	0.62 (0.34, 1.14)	1.33 (0.99, 1.76)
	at age 30 (after offspring's conception)	0.72 (0.34, 1.51)	1.15 (0.46, 2.85)
	Offspring's overweight at 8 years	1.50 (1.05, 2.16)	1.09 (0.87, 1.37)
Maternal line			
Model 1[†]	Parent's overweight, (vs never)		
	at age 8	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 (before offspring's conception)	0.82 (0.54, 1.25)	1.01 (0.73, 1.41)
	at age 30 (after offspring's conception)	0.82 (0.36, 1.89)	0.72 (0.41, 1.25)
Model 2[†]	Parent's overweight, (vs never)		
	at age 8	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 (before offspring's conception)	0.82 (0.54, 1.24)	1.01 (0.73, 1.41)
	at age 30 (after offspring's conception)	0.82 (0.36, 1.89)	0.72 (0.41, 1.25)
	Offspring's overweight at 8 years	1.14 (0.96, 1.35)	1.03 (0.80, 1.32)

[†]Model 1: exposure of interest (parent's overweight) + adjusting variables (ECRHS/RHINE parents' asthma and education level, *other parents'* overweight and asthma, and offspring's sex and age). Model 2: exposure of interest + potential mediator (offspring's overweight at 8 years) + adjusting variables.

RRR: relative risk ratio; CI: confidence interval. The statistically significant effects are indicated in bold.

Table III. Explorative analysis. Association between parents' overweight (exposure) and offspring's overweight (potential mediator), according to the parental line.

	Paternal line	Maternal line
	Offspring's overweight at age 8 [†] OR (95% CI)	Offspring's overweight at age 8 [†] OR (95% CI)
Parent's overweight, (vs non-overweight)		
at age 8	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 (before offspring's conception)	0.78 (0.46, 1.34)	1.04 (0.76, 1.42)
at age 30 (after offspring's conception)	1.90 (1.25, 2.86)	0.98 (0.63, 1.52)

[†]Adjusting for the following variables: ECRHS/RHINE parents' asthma and education, *other parents'* overweight and asthma, and offspring's sex. OR: odds ratio; CI: confidence interval. The statistically significant effects are indicated in bold.

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

		Offspring's ever asthma without nasal allergies [†]
Father's overweight (vs non-overweight)	Natural effects	OR (95% CI)
at age 8	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 (before offspring's 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 (after offspring's conception)	Indirect	1.02 (1.00, 1.06)
	Direct	0.72 (0.22, 1.42)
	Total	0.74 (0.23, 1.46)

*The mediation model is shown in **Figure 3**.

[†]Adjusting for the following variables: ECRHS/RHINE parents' asthma and education, *other parents'* overweight and asthma, and offspring's sex.

OR: odds ratio; CI: confidence interval. The statistically significant effects are indicated in bold.

Figure legends

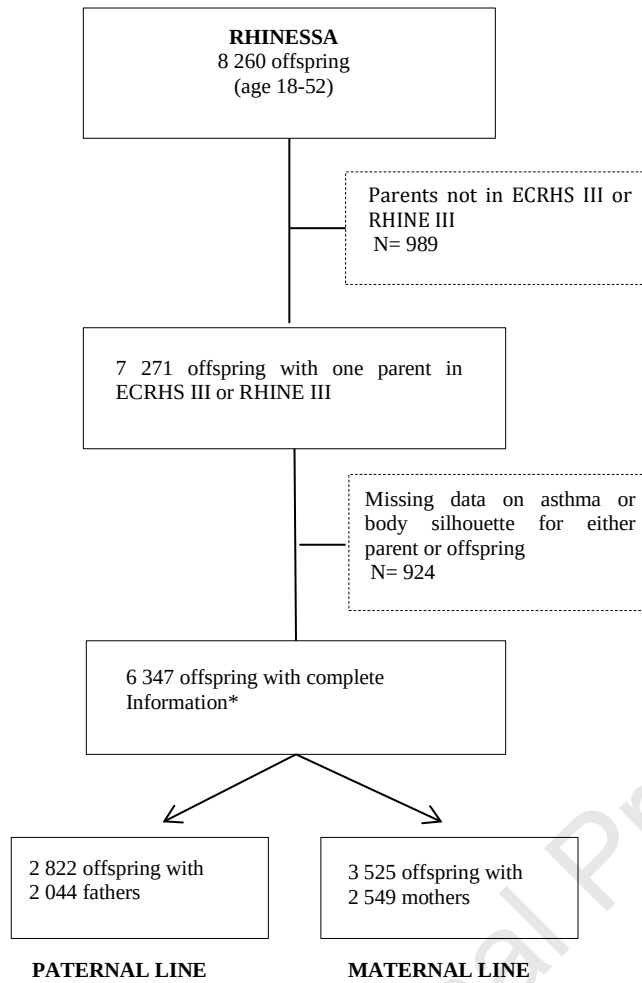
Figure 1. Study population flow chart, RHINESSA generation study.

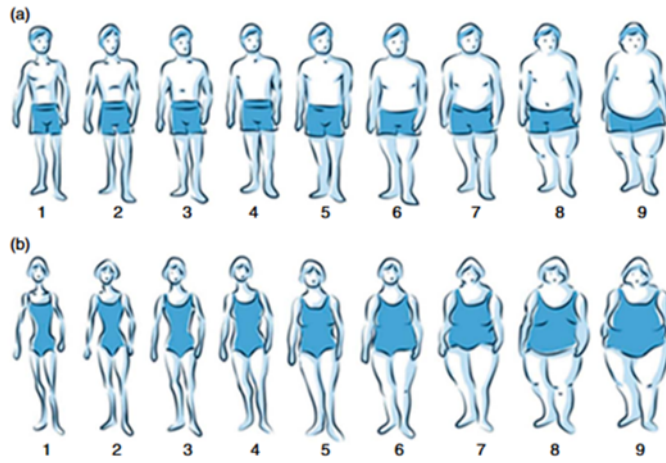
*offspring and their participating parents with complete information on overweight and asthma, 87% for paternal line, 88% for maternal line.

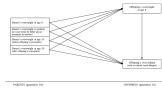
Figure 2. Figural drawing scales for (a) men and (b) women used in the ECRHS/RHINE III survey and in the RHINESSA questionnaire survey. Cut-off for overweight ≥ 5 in men and ≥ 4 in women.

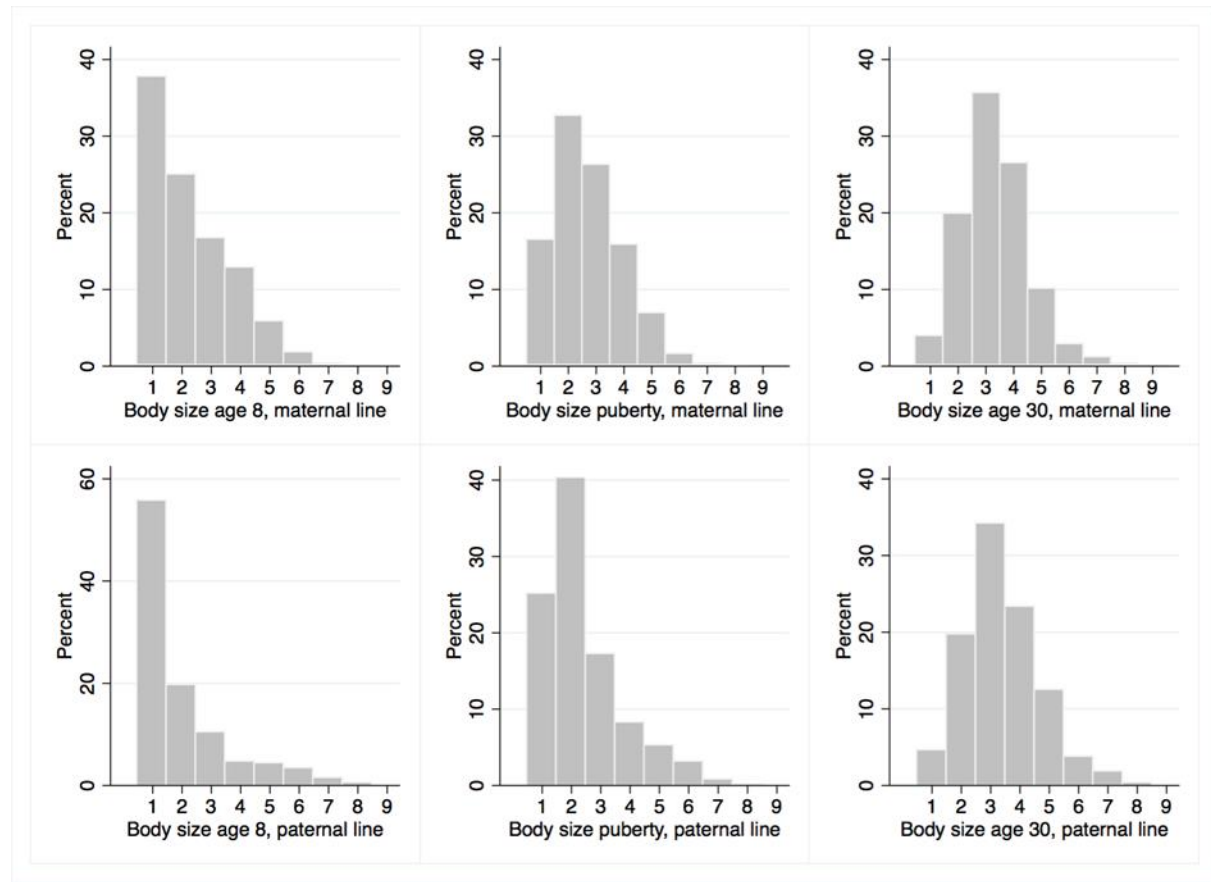
Figure 3. Schematic representation of the paths investigated within the paternal and maternal lines.

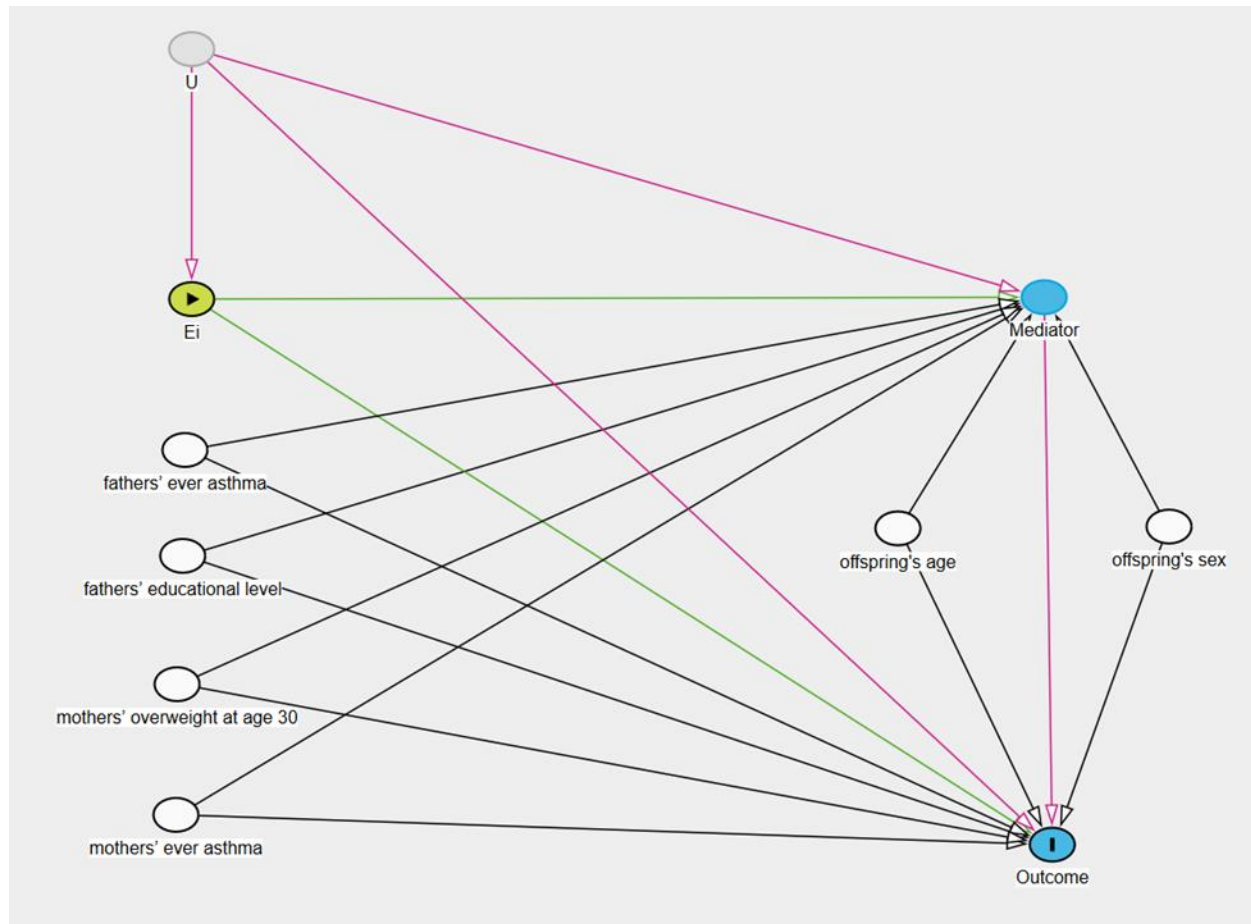
Adjusted for: ECRHS/RHINE parents' ever asthma and educational level, other parents' overweight at age 30 and ever asthma, offspring's age and sex.

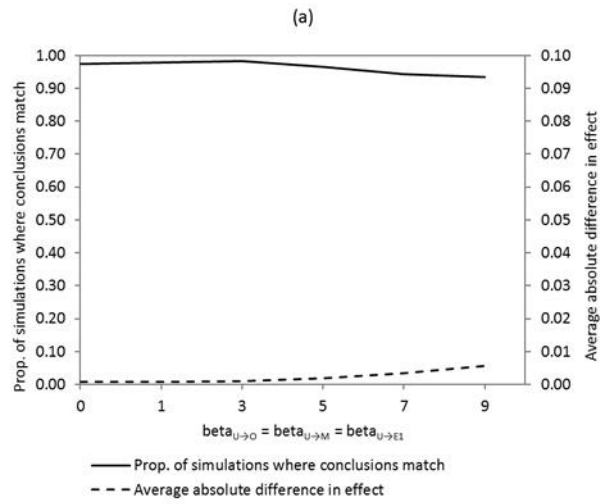


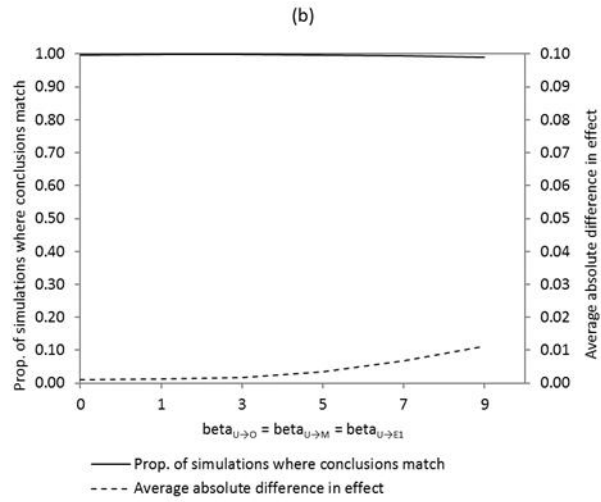


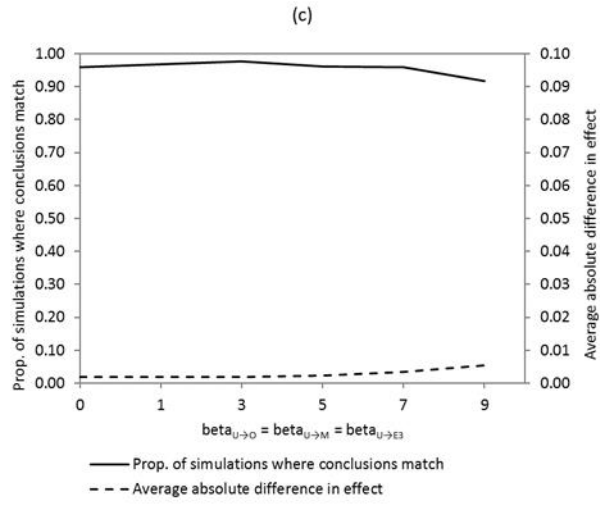


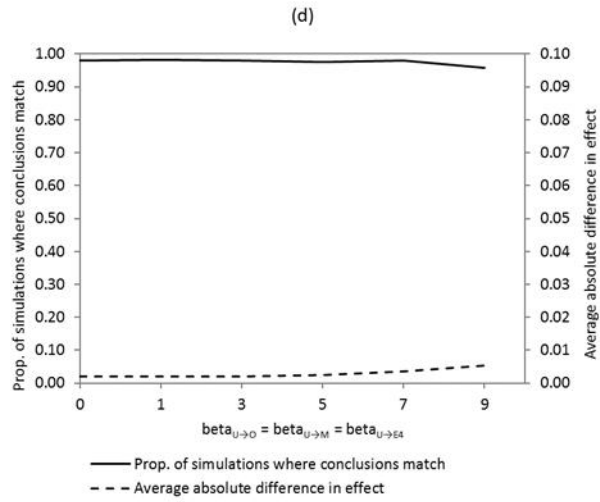












Overweight in childhood, puberty or early adulthood: changing the asthma risk in the next generation?

Johannessen A*, Lønnebotn M*, Calciano L*, Benediktsdottir B, Bertelsen RJ, Bråbäck L, Dharmage S, Franklin K, Gislason T, Holm M, Janson C, Jarvis D, Jögi R, Kim JL, Kirkeleit J, Lodge C, Malinovschi A, Martinez-Moratalla J, Nilsen RM, Real FG, Schlünssen V, Pereira-Vega A, Accordini S**, Svanes C**.

*Equal contribution as first authors.

**Equal contribution as last authors.

Online Data Supplement.

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

Country	Centre	Paternal line		Maternal line	
		N° of fathers	N°of offspring	N° of mothers	N°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	2 044	2 822	2 549	3 525

Figure legends

Figure 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), the RHINESSA generation study.

Figure E2: Directed acyclic graph (DAG) showing how the data was simulated for the exposure, the mediator, the outcome, the adjusting variables and one unmeasured confounder.

“Ei” indicates one of the following binary exposure variables: E1, fathers’ overweight at age 8 vs never overweight; E2, fathers’ overweight in puberty vs never overweight; E3, fathers’ overweight at age 30 before each offspring conception vs never overweight; E4, fathers’ overweight at age 30 after offspring vs never overweight. “Mediator” indicates offspring’s overweight at age 8. “Outcome” indicates offspring’s ever asthma without nasal allergies. “U” indicates the unmeasured normally-distributed confounder.

Figure E3: The proportion of simulations where the results match and the average absolute difference for the direct effects of ECRHS/RHINE fathers’ overweight on offspring’s ever asthma without nasal allergies (whether the unmeasured confounder U is included or excluded from the mediation models): (a) E1, overweight at age 8 vs never overweight; (b) E2, overweight in puberty vs never overweight; (c) E3, overweight at age 30 before each offspring conception vs never overweight; (d) E4, overweight at age 30 after offspring vs never overweight.