Parental body size and offspring lung health: preparing for parenthood already in childhood?

Marianne Lønnebotn

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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Scientific environment

The work in this thesis was conducted at the Department of Occupational Medicine, in the research group Respiratory Health in Northern Europe, Spain and Australia (RHINESSA) at Haukeland University Hospital. It was financed through a short-term recruitment position (1 year) in HelseVest strategic investment, and through a four-year PhD position at Centre for International Health, Department of Global Public Health and Primary Care at the University of Bergen (UiB). During the PhD-period I have been an affiliate member of the National research school in population-based epidemiology (EPINOR).

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Contents

Abbreviations

ALSPAC	Avon Longitudinal Study of Parents and Children
AUC	Area under the curve
BMI	Body mass index
ECRHS	European Community Respiratory Health Survey
RHINE	Respiratory Health In Northern Europe
RHINESSA	Respiratory Health In Northern Europe Spain and Australia
CI	Confidence interval
DAG	Directed Acyclic Graph
DNA	Deoxyribonucleic acid
DOHaD	Developmental Origins of Health and Disease
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
ncRNA	Non-coding ribonucleic acid
OR	Odds ratio
POHaD	Paternal Origins of Health and Disease
ROC	Receiver operating characteristic curve
RRR	Relative risk ratio
WHO	World Health Organization
WLSMV	Weighted least square mean and variance

Thesis at a glance

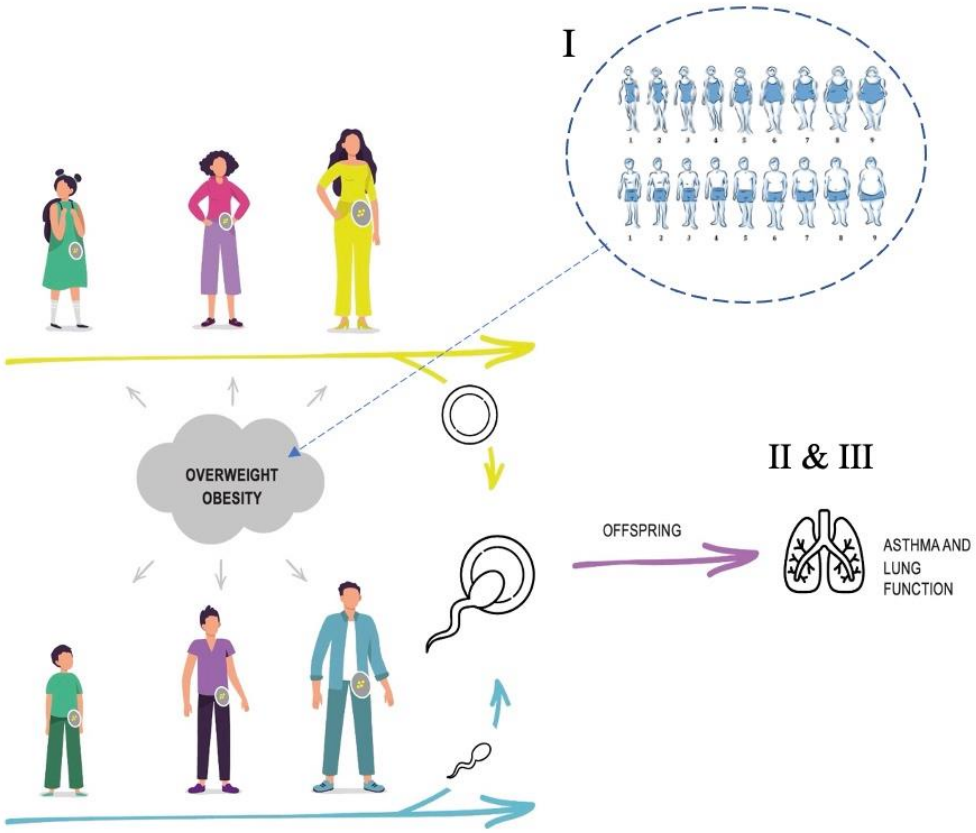

Figure 1. Overview of the three papers included in the thesis: (I) Investigation of the use of body silhouettes in adults as a tool to reflect past overweight/obesity (measured in ECRHS, reported in RHINE) Papers II & III: Multigeneration retrospective longitudinal studies, investigating parental overweight status starting at age 8, puberty or age 30 (reported in ECRHS/RHINE) in relation to offspring's asthma, with or without nasal allergies (paper II, reported in RHINESSA), or offspring lung function (paper III, measured in RHINESSA). Illustration by Nuria Baez Chocron.

Abstract

Background: In parallel with the increase in asthma and allergies there has been a dramatic increase in overweight and obesity during the last decades. Being overweight or obese is a known risk factor for asthma, and overweight and obesity are believed to be detrimental to lung function across age groups regardless of asthma status. However, potential health effects of overweight/obesity for future offspring are not well investigated. While it has been known for quite some time that a mother's health and behaviour shortly before and during pregnancy may affect her children's health, emerging evidence suggests that also parents' health and behaviours before conception – including fathers as well as mothers- could be of importance for the future health of the child. Potential effects of parental overweight for respiratory health in future offspring is not well studied and would require life course data on parental overweight/obesity. Such data are rarely available, and the use of figural body silhouettes from various ages might provide a possibility for retrospectively assessing body size at several time points in the past.

Objectives: (I) Investigate the use of body silhouettes in adults as a tool to reflect past overweight/obesity, validated against previously measured or self-reported height and weight in the European Community Respiratory Health Survey (ECRHS) and the Respiratory Health In Northern Europe (RHINE) study, respectively. (II) Examine whether mothers' and fathers' overweight in childhood, adolescence, or adulthood is associated with asthma in their offspring. (III) Investigate whether a parent's overweight in childhood, adolescence, or adulthood could be a cause of altered lung function in adult offspring.

Material and methods: (I) Data from women and men participating in the second follow up of ECRHS (N= 3041) was used to validate the selected body silhouettes against previously measured height and weight in ECRHS (recall 9-23 years). Data from women and men participating in the first follow up of RHINE (N=3410) was used to validate the selected body silhouettes against previously self-reported height and weight in the initial RHINE study (9-13 years recall). We calculated Spearman

correlations between BMI and body silhouettes and ROC-curve analyses for identifying obesity (BMI ≥30).

(II) 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 investigated in ECRHS. Associations of parental overweight status at age 8 years, puberty and age 30 years with offspring's childhood overweight status and offspring's asthma with or without nasal allergies were analysed using 2-level logistic regression and 2-level multinomial logistic regression, respectively. Counterfactual-based mediation analyses was performed to establish whether observed associations reflected direct or indirect effects mediated through offspring's own overweight status.

(III) We included 929 adult offspring (18-54 years, 54% daughters) investigated in the RHINESSA study, of 308 fathers and 388 mothers investigated in the ECRHS or RHINE follow-up studies (2011-2014). Counterfactual-based multi-group mediation analyses by offspring's sex were used to assess whether the effects of parents' overweight before puberty on adult offspring's FEV_1, FVC and FEV_1/FVC were mediated through offspring's pre-pubertal overweight and/or adult height, separately within each of the paternal and maternal lines.

Results: (I) Spearman correlations between measured BMI age 30(±2y) and body silhouettes in women and men were between 0.62 and 0.66, and correlations for self-reported BMI ranged from 0.58 to 0.70. The area under the curve for identification of obesity at age 30 using body silhouettes vs previously measured BMI at age 30(±2y) was 0.92 (95% CI 0.87, 0.97) and 0.85 (95% CI 0.75, 0.95) in women and men, respectively; for previously self-reported BMI, 0.92 (95% CI 0.88, 0.95) and 0.90 (95% CI 0.85, 0.96). (II) We found a statistically significant effect of fathers' onset of overweight in puberty for 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 of mother's overweight was associated with offspring's asthma. (III) Fathers' overweight before puberty had a negative

indirect effect, mediated through sons' height, on sons' FEV_1[beta (95% CI): -144 (-272, -23) mL] and FVC [beta (95% CI): -210 (-380, -34) mL], and a negative direct effect on sons' FVC [-262 (-501, -9) mL]. Statistically significant effects on FEV_1/FVC were not observed. In the maternal line, mothers' overweight before puberty had neither direct nor indirect effects on offspring's lung function.

Conclusions: Our study suggests that body silhouettes are a useful epidemiological tool, enabling retrospective differentiation of obesity and non-obesity in adult women and men. Further, our work suggests 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. Finally, we found that fathers' overweight starting before puberty appear to cause considerably lower FEV_1 and FVC in their future sons. These effects could be partly mediated through sons' adult height, but not through his pre-pubertal overweight.

Implications: We have shown that the metabolic environment in male prepuberty might influence the health of the next generation. Closer scientific attention to male puberty in relation to future generations health may have profound implications and open new opportunities for targeted public health strategies. We speculate that while intervening in the prepuberty age window in one generation we might improve the health of two generations.

List of Publications

The thesis is based on the following three original papers:

I. Lønnebotn M, Svanes C, Igland J, Franklin KA, Accordini S, Benediktsdóttir B, Bentouhami H, Blanco JAG, Bono R, Corsico A, Demoly P, Dharmage S, Dorado Arenas S, Garcia J, Heinrich J, Holm M, Janson C, Jarvis D, Leynaert B, Martinez-Moratalla J, Nowak D, Pin I, Raherison-Semjen C, Sánchez-Ramos JL, Schlünssen V, Skulstad SM, Dratva J, Gómez Real F. Body silhouettes as a tool to reflect obesity in the past. *PLoS One* 2018 Apr 25;13(4)

II. Johannessen A[*], Lønnebotn M[*], Calciano L[*], Benediktsdóttir B, Bertelsen RJ, Bråbäck L, Dharmage S, Franklin KA, Gislason T, Holm M, Janson C, Jarvis D, Jogi R, Kim JL, Kirkeleit J, Lodge C, Malinovschi A, Martinez-Moratalla J, Nilsen RM, Pereira-Vega A, Gómez Real F, Schlünssen V, Accordini S, Svanes C. Being overweight in childhood, puberty, or early adulthood: changing asthma risk in the next generation? *J Allergy Clin Immunol.* 2020 Mar;145(3):791-799

III. Lønnebotn M[*], Calciano L[*], Johannessen A, Jarvis D, Abramson MJ, Benediktsdóttir B, Bråbäck L, Franklin KA, Godoy R, Holm M, Janson C, Jogi NO, Kirkeleit J, Malinovschi A, Pereira-Vega A, Schlünssen V, Dharmage SC, Accordini S, Gómez Real F, Svanes C. Parental prepubertal overweight and offspring lung function. (Submitted, August 2021)

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1. Introduction

It is generally accepted that a mother's intrauterine environment plays a key role in child health. However, emerging evidence suggests that there are mechanisms whereby the father's environment before conception could also impact the health of future generations [1-4]. Environmental factors such as smoking, and overweight can cause genetic and epigenetic changes in sperm that are transmissible to offspring [2-5]. One outcome in which parental exposures might play an important role is offspring asthma. Despite decades of research, our knowledge of the causes of asthma is still lacking an overall explanation and there are no proven strategies for prevention.

In parallel with the increase in asthma and allergies, there has been a dramatic increase in overweight and obesity. Among children and adolescents, the worldwide prevalence of overweight and obesity has risen from just 4% in 1975 to just over 18% in 2016, according to WHO [6]. Numerous epidemiological studies have demonstrated associations between overweight/obesity and asthma on a personal level [7, 8], as well as an association of mothers' overweight just before and during pregnancy in relation to offspring's asthma [9, 10]. However, the underlying mechanisms for these associations are not fully understood.

In the 1970s Forsdahl found that poor living conditions in childhood and adolescence could be associated with an increased risk for heart disease later in life [11]. In the 1980s, Barker and colleagues introduced the fetal origins hypothesis and the Forsdahl/Barker Developmental Origins of Health and Disease (DOHaD) hypothesis evolved from epidemiological studies of infant and adult mortality [12, 13]. Intrauterine and early life has been accepted as important susceptibility windows for environmental exposure and disease later in life and this concept has had an enormous impact on health policy and healthcare programs for mother- and childcare. Furthermore, there are substantial epidemiological and experimental evidence supporting the concept of developmental origins of adult lung disease and impaired lung function [14-17] and, moreover, indicating that early life exposures may also be important to future outcomes in the offspring [13]. A new paradigm on the role of the

male germ line, the paternal origins of health and disease (POHaD), has been introduced the last years [2, 5, 18, 19] to stress the need for more research on the role of the father in relation to offspring health.

Based on recent evidence that epigenetic changes may be heritable, it is relevant to ask what role the metabolic environment and condition of previous generations may have in lung health and disease in future generations. There is a need for increased knowledge about exposures throughout the lifespan and across generations. In this thesis we want to investigate what role overweight at different time points in one generation may have in asthma and lung function in the next. To accomplish this, we need to know if parent-reported body silhouettes can be used as a valid tool to report overweight/obesity back in time. The use of body silhouettes will allow us to investigate start of overweight/obesity in different susceptibility windows in parents, from childhood to adulthood, and possible associations with future offspring lung health.

In the following section's brief introductions are provided of the outcomes, exposure and key terms used in this thesis.

1.1 Asthma

Asthma is a heterogeneous disease affecting 1-18% of the population in different countries, characterized by variable symptoms of wheeze, shortness of breath, chest tightness and/or cough, and by variable expiratory airflow limitation [20]. Asthma varies considerably across the life course and affect all age groups, but often starts in childhood [21]. Asthma is a common condition in adults and carries a huge economic, morbidity and mortality burden in both developing and developed countries. Most studies, which have determined the prevalence of asthma symptoms using the same methodology, in the same community, at different timepoints have reported that asthma prevalence has increased in recent decades [22].

There are sex-differences in asthma in different age groups. Among children males are more commonly affected by asthma than females and after puberty the risk in females is greater than that in males [23-25]. In the ECRHS cohort, Leynaert et al. [23] found that females are more affected by non-allergic asthma after the age of 35 years than males. These observations are only partially explained by women's smaller airway calibre or hormonal differences [21, 23].

Asthma phenotyping began decades ago with the concepts of extrinsic (allergic) and intrinsic (nonallergic) asthma, introduced by Rackemann in 1947, and thus described the first phenotypes of asthma [26]. A phenotype is defined as the "observable properties of an organism that are produced by the interactions of the genotype and the environment" [27, 28]. The concept of asthma phenotypes is evolving from one that was focused on clinical characteristics to one that links underlying biology to phenotype. Over the recent decades, cluster analyses have confirmed that asthma is more of a heterogeneous disorder rather than just a single disease [27].

The rapid global increase in asthma prevalence makes it unlikely that genetic factors alone can account for a substantial proportion of asthma cases, although genetic susceptibility to changing environmental exposures may play an important role [29, 30]. The realization that genetics cannot "explain" asthma and allergic disease has shifted interest toward alternative sources of phenotypic variance, primarily the environment and development and the role of epigenetic processes in asthma, epigenetic marks being strongly influenced by the environment [29, 31].

1.2 Lung function

Lung function is a predictor of future morbidity and mortality in the general population [32]. To assess lung function and diagnose and monitor respiratory disease in children and adults, spirometry is a well-established clinical tool [33]. The primary signal measured in spirometry is either volume or flow as a function of time. The main measurements used to describe lung function are the forced vital capacity (FVC) which represents the total volume delivered during a forced expiration starting

from full inspiration, and the forced expiratory volume in one second (FEV_1), representing the volume delivered in the first second of an FVC manoeuvre and their ratio, FEV_1/FVC [33]. Spirometry is most useful in the evaluation of obstructive airway disorders, like asthma, where abnormal findings are reversible or in chronic obstructive pulmonary disease (COPD) where the findings are classically irreversible. It is less helpful in assessing restrictive airway diseases [33].

It is well established that trajectories of lung function during childhood are already established at birth [34]. It is believed that lung function either tracks or deteriorates, but never improves after the preschool years [35-38]. Thus, lung function in adult life is critically dependent on in utero and postnatal lung development. Furthermore, evidence suggests that lung function, as measured by the FEV_1 or FVC, has a large heritable component, and that genetic factors explain 50% of the phenotypic variance for the FEV_1 and up to two thirds of that for the FEV_1/FVC ratio [34, 39]. Genome wide association studies have discovered that there are genetic variants associated with the FEV_1 that are also associated with myocardial infarction, cancer, and height. Evidence suggests that the FEV_1 level is controlled by a very large number of biologic pathways, most of which remain to be identified, with genetic variation in each pathway having small effects on phenotypic expression [39]

Forced vital capacity (FVC) is increasingly recognised as an important parameter beyond its diagnostic and prognostic role in restrictive lung disease, and an important predictor of all-cause mortality in the absence of chronic respiratory conditions [40]. Epidemiological evidence is pointing to environmental exposures and genes affecting lung development as risk factors for low FVC later in life. Early life and genetic effects that manifest in childhood will influence the individual FVC life trajectory [40]. There are substantial epidemiological and experimental evidence supporting the concept of developmental origins of adult lung disease and impaired lung function [14-16], and there are strong evidence of an association of birth weight with adult FVC [35]. Weight gain can affect lung function through mechanical effects on lungs. Furthermore, weight gain can impair lung function by inflammatory processes, as

adipose tissue is a source of inflammatory mediators that can damage lung tissue and reduce airway diameter [32, 41].

1.3 Parental overweight and obesity

Overweight and obesity are recognized as major risk factors for personal asthma in both children and adults [7, 42-45]. Furthermore, it is thought to be detrimental to lung function across age groups and regardless of asthma status [41]. Research has also shown associations between mothers' overweight status just before and during pregnancy and offspring's asthma [9, 10]. While associations between obesity in women and offspring health are currently being extensively documented, paternal obesity is seldom included in epidemiological designs to investigate influences from parental lifestyles. If the paternal BMI is taken into consideration, it is often reported by the mother and it is the paternal BMI around the time of pregnancy.

To our knowledge there is no existing literature on parental overweight starting in childhood or around puberty in relation to offspring lung health. In this project we will use parental report of previous body size, using figural drawing scales depicting body size from lean to obese, to obtain proxies for BMI. This gives us the opportunity to collect data on previous body size, from childhood to adulthood.

Overweight and obesity in relation to puberty

Puberty is a developmental stage marked by the gradual transition from childhood to adulthood and it is characterized by the maturation of testes in boys, and ovaries in girls [46]. The mechanisms controlling the normal onset of puberty are complicated and do involve hormonal, genetic, environmental, and nutritional factors. These factors can affect physical growth and timing of puberty, independently or in concert [46, 47]. The association between fat mass and timing of puberty is well established in girls, with most of the previous research reporting early pubertal development among obese and overweight girls. In boys there have been fewer studies conducted and their findings are conflicting [46, 47]. However, there are studies showing a tendency towards early voice break with increasing BMI [48-50]. Childhood obesity, improved health,

endocrine disrupting chemicals and prenatal exposures have been studied as potential causal factors [46].

1.4 Epigenetics and susceptibility windows

The term "epigenetics" has now been applied quite widely in biology to describe a range of biological processes and phenomenon. Epigenetics are heritable characteristics that affect gene expression without altering DNA sequence in contrast to genetics [51]. Epigenetic processes are in general changeable, cell-type and tissue specific and can occur in response to environmental factors, such as obesity, diet and toxins, and other factors. The main types of epigenetic regulatory mechanisms are DNA methylation, histone modifications, and noncoding RNAs [52]. It is hypothesized that transmission of information occurs through epigenetic variation in sperm, oocytes, or both sets of gametes [51].

Several animal and human studies demonstrate that periconceptional and *in utero* maternal environmental exposures affect the risk for disease development in subsequent generations. However, only a very few studies have provided evidence for the inheritance of epigenetic information through the male germ line [3, 5, 53]. Results from animal research suggest particularly vulnerable susceptibility windows *in utero*, just before puberty, and at each reproductive cycle [2, 4]. Until now, there have been barely any human data to support these time windows with respect to offspring's asthma and lung function, and the limited research in this field has thus far mainly investigated exposure to cigarette smoke [54-58].

1.5 Evidence for inter-and transgenerational inheritance in humans

In the Överkalix cohort, Pembrey and colleagues found that variation in food supply during the early life of paternal grandparents was associated with variation in mortality rate in their grandchildren [59, 60]. They also found sex-specific differences in transmissions. The paternal grandfather's food supply was associated with the mortality rate of his grandsons only, while the early life food supply of the paternal grandmother was associated with the mortality rate of granddaughters only. This was only found when the exposure occurred before puberty, suggesting that a reprogramming of gametes might be involved.

In the ECRHS cohort, asthmatic and allergic disease status was measured at three time points over twenty years in the parent generation [61]. In the third study wave, parents reported on offspring allergies. Stronger associations of offspring allergies with parental asthmatic and allergic disease assessed before conception, as compared to after birth of the child, was found. Disease activity in the parents might induce changes that are transmissible to their offspring, possibly explained by epigenetic inheritance rather than by shared environment or genetics alone [61].

Higher asthma risk has been shown in persons whose maternal grandmother smoked when pregnant, independent of maternal smoking [56, 58, 62, 63]. Other studies have found associations with grandmothers' smoking and grandchildren's respiratory outcomes through the paternal line [54, 55]. Svanes et al. investigated pre-conception risks in the RHINE cohort and found no effect of maternal smoking prior to conception on offspring asthma, while an effect of smoking in pregnancy was confirmed, as in previous studies [54, 55]. However, fathers' smoking before conception was associated with offspring asthma, and the association was particularly strong if the father had started smoking before age 15 years. In the ALSPAC study they found that sons of fathers who smoked before age 11 years had increased body fat, which also points to early puberty as an important window of susceptibility [64]. Accordini et al. [56] showed that fathers' smoking during early adolescence and grandmothers' and mothers' smoking during pregnancy may independently increase asthma risk in offspring.

Furthermore, they found that lung function was lower among offspring of fathers who started to smoke before age 15 years [57].

1.6 Epidemiology and causal inference

Epidemiology is the study of how disease is distributed in populations and the factors that influence or determine this distribution [65]. The specific objective for epidemiology is to identify etiology or causal factors of a disease and the relevant risk factors. Causal inference is the examination of causal associations to estimate the causal effect of an exposure on an outcome, and it has a central role in public health; the determination that an association is causal indicates the possibility for intervention [66, 67].

The classic framework for causal thinking in public health was articulated by Sir Austin Bradford Hill in the 1950s and 1960s and was developed to identify the causes of diseases and particularly to determine the role of smoking in lung cancer. Since the 1970s the field has progressed forward towards what is termed the modern approach, based on the counterfactual or potential outcomes framework [68].

The challenge of determining causation in public health has always been shaped by the limitations of the available data, the understanding of the underlying biological or sociological processes, and the ability to intervene in the real world. Randomized experiments are often unethical, impractical and too lengthy for decision making and causal inferences for public health are usually derived from observational studies [68]. Causal inference studies require a clearly articulated hypothesis, careful attention to minimizing selection and information bias, and a deliberate and rigorous plan to control for confounding [66].

Literature review completed August 2021.

2. Aims of thesis

2.1 Main objective

The aim of this thesis was to investigate the influence of parental overweight status on lung health in their future offspring, and to identify pre-conception time windows of importance for offspring health. We hypothesized that causes of asthma and allergy in offspring could be linked to parents' overweight long before conception, and that parents' overweight could cause lower lung function in offspring, possibly through epigenetic mechanisms (Figure 1). An additional aim of this thesis was to validate the use of recalled body silhouettes for past overweight/obesity, which would be needed to address the aims above. Data from the European Community Respiratory Health Survey (ECRHS), the Respiratory Health In Northern Europe (RHINE) study and the Respiratory Health In Northern Europe, Spain and Australia (RHINESSA) generation study was used to explore the hypothesis and address the aims of the thesis.

2.2 Specific objectives

Paper I. To validate the use of self-reported body silhouettes in adults as a tool to reflect past overweight/obesity.

Paper II. To examine whether parents' overweight in childhood, adolescence or adulthood was associated with asthma in their offspring, and to assess if observed associations were direct or mediated through the offspring's own overweight status.

Paper III. To examine whether parents overweight starting before or after puberty (but prior to conception) was associated with adult offspring's lung function (FEV_1, FVC or FEV_1/FVC), and to assess if observed associations were direct or mediated through offspring's pre-pubertal overweight and/or adult height.

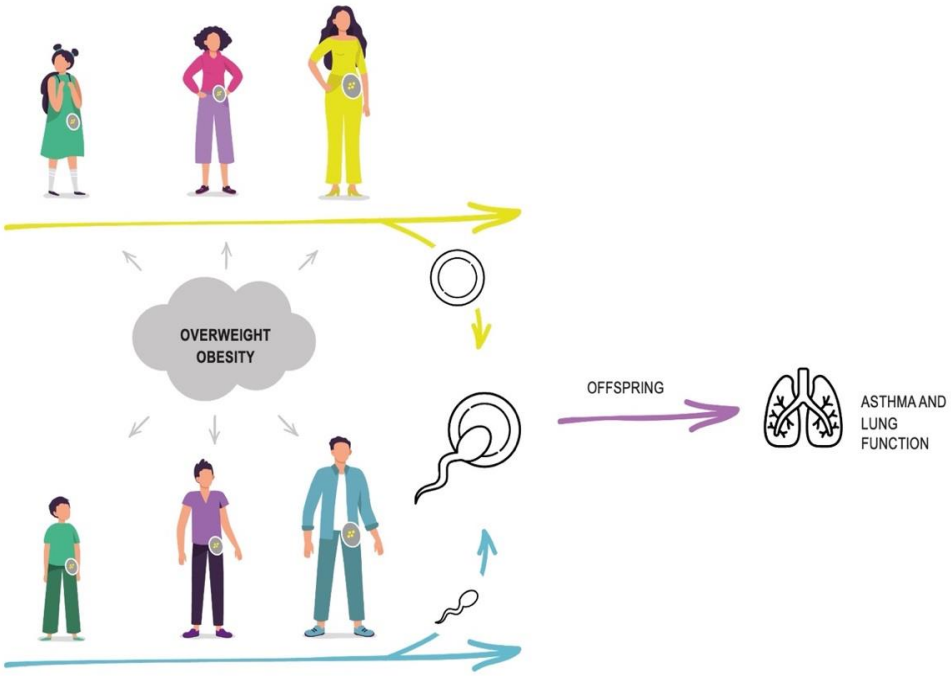

Figure 1. Maternal and paternal lifeline with possible susceptible time windows (age 8, puberty (menarche/voice-break) and age 30), before conception of the offspring, for environmental influence of overweight and obesity on asthma and lung function in the offspring. Illustration by Nuria Baez Chocron.

3. Material and methods

Table 1. Overview of material and methods used in papers I-III

	Paper I	Paper II	Paper III
Aims	To investigate the use of self-reported body silhouettes in adults as a tool to reflect past overweight/obesity.	Examine whether overweight in paternal or maternal childhood, adolescence or adulthood was causally associated with asthma in their offspring.	To examine whether paternal or maternal overweight starting before or after puberty was causally associated with adult offspring's lung function.
Design	Validation study. Retrospective cohort analysis.	Multigeneration retrospective longitudinal analysis.	Multigeneration retrospective longitudinal analysis.
Data source	ECRHS/RHINE	ECRHS/RHINE/RHINESSA	ECRHS/RHINE/RHINESSA clinical investigation
Study population	3041 participants from ECRHS with measured height and weight. 3410 participants from RHINE with self-reported height and weight.	6347 adult offspring from RHINESSA of 2044 fathers and 2549 mothers who participated in ECRHS/RHINE III.	929 adult offspring from RHINESSA of 308 fathers and 388 mothers who participated in ECRHS/RHINE III.
Exposures	Self-reported body silhouettes in ECRHS/RHINE III.	Paternal or maternal overweight in childhood, adolescence, or adulthood.	Paternal or maternal overweight starting before or after puberty.
Outcomes	Correlation with previously measured (ECRHS I or II) or self-reported height and weight (RHINE II).	Adult offspring ever asthma with or without nasal allergies.	Adult offspring lung function, FEV_1, FVC or FEV_1/FVC ratio.
Covariates	Sex for stratification, and age for differentiated validation for age 30 and 45.	Parents' asthma and education level; other parents' (reported by offspring) overweight status and asthma; offspring sex and age.	Parents' education level; offspring age and smoking history; offspring sex used to separate the observations into subgroups (sons and daughters).
Statistics	Spearman correlations. Receiver- Operating characteristics (ROC) curves with calculation of Area Under the Curve (AUC). Specificity and sensitivity according to Youden Index.	2-level logistic regression. 2-level multinomial logistic regression. Counterfactual-based mediation analysis with offspring overweight at age 8 years as potential mediator.	Counterfactual-based-multi-group mediation analyses by offspring's sex (potential moderator). Potential mediators were offspring pre-puberty overweight and adult height. Sensitivity analyses.

3.1 Data sources

This thesis is based on questionnaire and clinical data from adult offspring [G1] in the RHINESSA generation study. Parent information [G0] is retrieved from the European Community Respiratory Health Survey (ECRHS) and the Respiratory Health In Northern Europe (RHINE) study (Paper II and III). Paper I, a validation study, is based on questionnaire and clinical data from the ECRHS and the RHINE study. Data sources are explained in more detail in paragraph 3.2.

3.2 Study-populations and design

RHINESSA is a cohort dedicated to study asthma, allergy, and lung health across generations and throughout the lifespan (Figure 2). The aim is to understand how our lifestyle and environment influence not only our own health, but also the health over several generations. These multi-generation data are used to identify ages of susceptibility which may be of importance for future health, in one or in several generations. The RHINESSA study consists of offspring (mostly adults, but for some centres (Bergen, Tartu, and Melbourne) also adolescents and children) of parents from two large cohort studies of respiratory health in adults: the ECRHS survey [69] and the RHINE study [70]. ECRHS I, stage 1 was an international postal questionnaire survey, mainly carried out in 1990 to 1992 including randomly selected individuals from general populations in 54 centres in 16 countries, www.ecrhs.org. In the ECRHS I stage 2, smaller random and symptomatic samples were invited to a clinical examination in 1992-94. The ECRHS I aimed at describing variations in exposure to risk factors and their association with asthma and allergy. Responders from 29 centres, in 14 countries were invited to a clinical follow up 10 and 20 years after baseline (ECRHS II and III), with a third follow up (ECRHS IV) planned for 2021.

The RHINE study was a follow up of the ECRHS I questionnaire-stage 1 by using extensive postal questionnaires in two stages (RHINE II in 2000 and RHINE III in 2010) with a third follow up (RHINE IV) planned for 2021. RHINE, www.rhine.nu consists of the seven Northern European centres (Reykjavik (Iceland), Bergen

(Norway), Umeå, Uppsala, and Gothenburg (Sweden), Aarhus (Denmark) and Tartu (Estonia).

The Northern European centres in RHINE and three centres from the ECRHS (Huelva and Albacete (Spain) and Melbourne (Australia)) developed standardized protocols for health examination of the children (offspring generation [G1] of study participants (parent generation [G0]), resulting in the generation study RHINESSA (RHINESSA English- Helse Bergen helse-bergen.no). All eligible offspring from participants in the RHINE centres and the Spanish/Australian ECRHS centres were invited to participate in the questionnaire part of RHINESSA, while offspring of participants from the ten centres who had taken part in the clinical part of ECRHS were in addition invited to a clinical examination in RHINESSA.

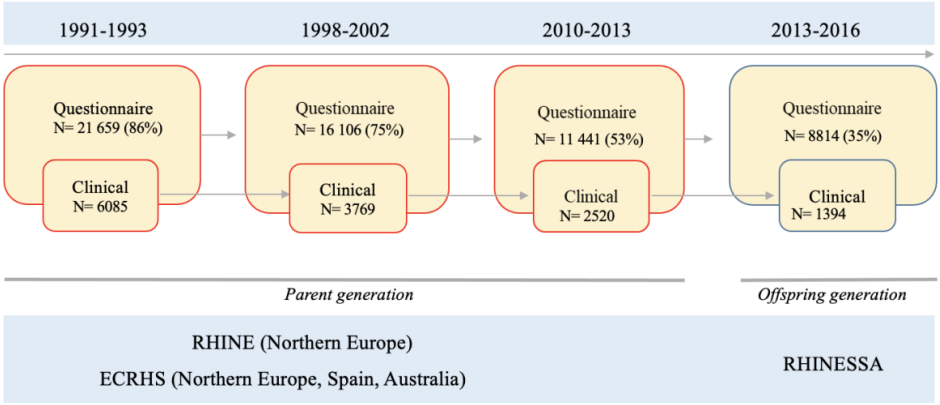

Figure 2. Flowchart of the RHINESSA study population. The RHINESSA study population consists of offspring from participants from the Northern European RHINE centres in Iceland (Reykjavik), Norway (Bergen), Sweden (Uppsala, Umeå, Gothenburg), Denmark (Aarhus) and Estonia (Tartu) and the ECRHS centres in Australia (Melbourne) and Spain (Huelva, Albacete).

Participants in RHINESSA received questionnaires between 2013 and 2015, where they provided information regarding themselves, in addition to their parents and their offspring. In addition, extensive questionnaire and lung function data were collected

among participants in the clinical part in the period 2013-2016. The study protocols were harmonised with the ECRHS protocols.

Study populations used in the respective scientific papers

Data from ECRHS and RHINE were used for the validation of body silhouettes in paper I (Figure 3) For paper II and III we used data from RHINESSA generation study (Figure 4).

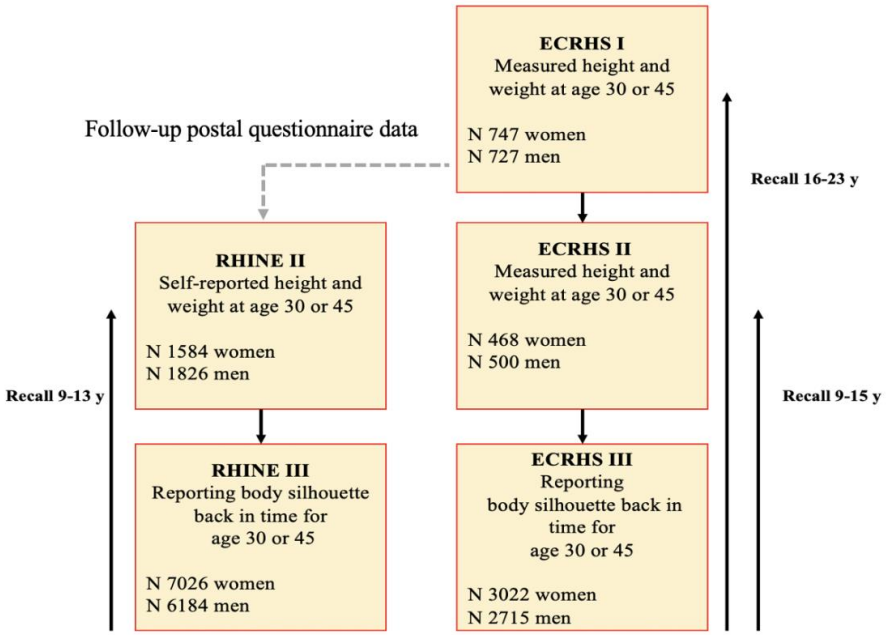

Figure 3. Flow chart paper I, study population with participation in the RHINE III or ECRHS III study, reporting body silhouette at age 30 or 45, and with self-reported or objectively measured height and weight at age 30(+/-2 years) or 45 (+/-2 years) in RHINE II or ECRHS I or II.

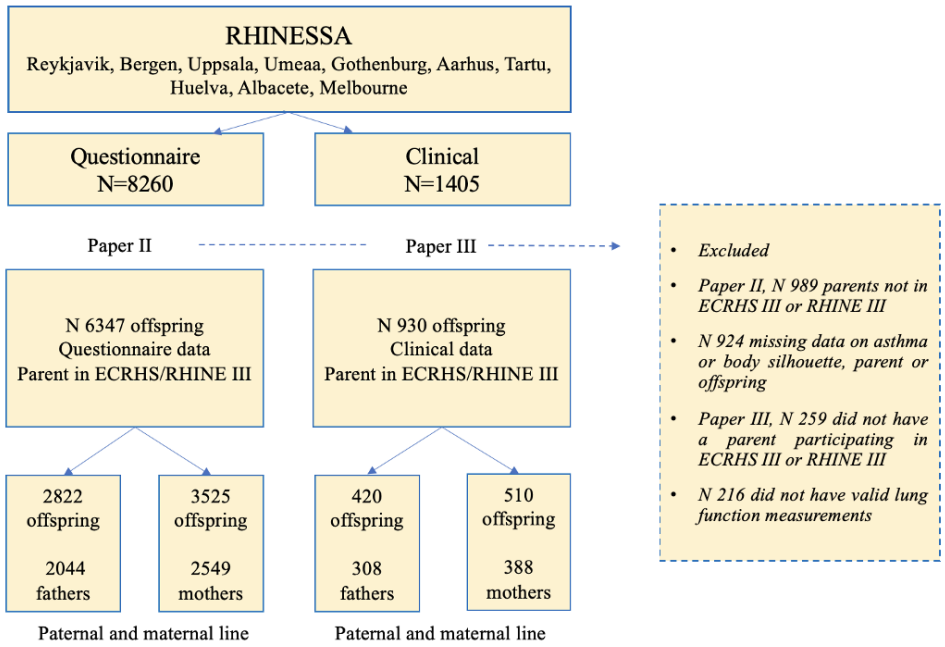

Figure 4. Study population flow chart, Paper II and III, RHINESSA generation study.

3.3 Questionnaires

Analyses in this thesis were based on questionnaire data from the ECRHS survey, the RHINE and the RHINESSA study. For all the RHINE centres, postal questionnaires were used for data collection. For the ECRHS centres, interview-based questionnaires were used. For RHINESSA, the questionnaires were web-based in all centres. Questionnaires included questions on respiratory symptoms and allergies, smoking habits, education, general health, and comorbidities. Body silhouettes (Figure 5) were introduced in ECRHS III (Appendix A) and RHINE III and used in RHINESSA. The participants in RHINESSA also answered questions on "the other parent's" (the parent not participating themselves in ECRHS or RHINE) body silhouette at age 30 and smoking habits (Appendix B, RHINESSA adult offspring main questionnaire).

For paper I, we used interview-based questionnaire data from the ECRHS study centres in South Antwerp, Antwerp City (Belgium), Hamburg, Erfurt (Germany), Paris, Grenoble, Montpellier, Bordeaux (France), Barcelona, Galdakao, Albacete, Oviedo, Huelva (Spain), Ipswich, Norwich (UK), Melbourne (Australia), Reykjavik (Iceland), Tartu (Estonia), Bergen (Norway), Gothenburg, Umeå, Uppsala (Sweden), Aarhus (Denmark) and self-reported questionnaire data from the RHINE study for the seven Northern European centres (RHINE II and III).

For paper II (Figure 4) we used self-reported questionnaire data from RHINESSA adult offspring, and questionnaire data from ECRHS III and RHINE III parents.

For paper III (Figure 4) clinical and questionnaire data from RHINESSA adult offspring was used, and questionnaire data from ECRHS III and RHINE III parents.

3.4 Spirometry

Participants in the clinical part of the RHINESSA study performed spirometry with reversibility-testing in the period between 2013 and 2016. The maximum pre-and post-bronchodilator forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were recorded at the best of at least two manoeuvres that met the American Thoracic Society criteria for reproducibility [71]. Spirometry was performed using the The EasyOne Spirometer, according to standardised protocols and by trained personnel. The participants performed up to eight manoeuvres until adequate flow volume loops were produced, while seated and wearing a nose clip.

3.5 Exposure variables

Paternal and maternal overweight at different timepoints in life was the primary exposures in paper II and III.

3.5.1 Defining overweight

Overweight status was defined by using a validated figural drawing scale of 9 sex-specific body silhouettes (Figure 5) [72]. Parents participating in the ECRHS III or

RHINE III study were asked to tick the figural scale that best described their figure at specific time points including age 8 years, voice break/menarche and age 30 years. To distinguish between no overweight or overweight-obese subjects, we used as cut offs body silhouette 5 or greater in men and body silhouette 4 or greater in women.

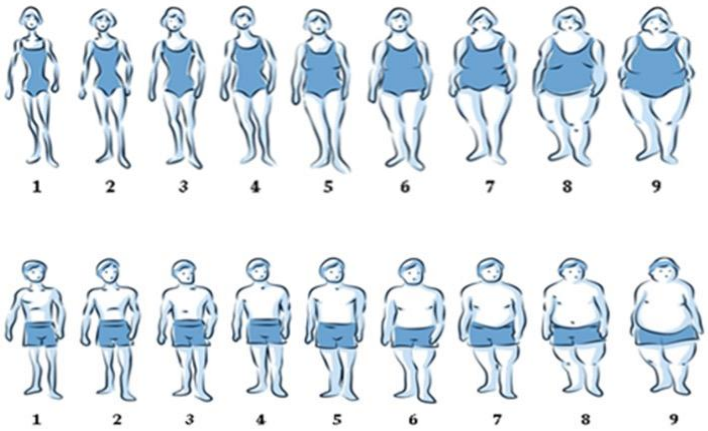

Figure 5. Body silhouettes for women and men introduced in the ECRHS III and RHINE III study and in the RHINESSA questionnaire survey. Cutoffs for overweight status were 5 or greater in men and 4 or greater in women.

In paper II, parents' overweight status was classified at four susceptibility 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' (the parent not participating in ECRHS/RHINE) 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.

In paper III, parents' overweight status was defined as: *"overweight before puberty"*, i.e., at age 8 years and/or in puberty (voice break for fathers and menarche for mothers), *"overweight at age 30 years but not before puberty"* i.e., at age 30 years but neither at age 8 years and/or in puberty, and *"never overweight"*, i.e., neither at age 8 years nor in puberty nor at age 30 years. RHINESSA offspring's "overweight before puberty" (present vs absent), i.e., at age 8 years and/or in puberty, were reported by the adult offspring using the same figural drawing scale described above for their parents.

3.6 Outcomes and covariates

Outcomes

Self-reported body silhouettes and BMI

In paper I, self-reported body silhouettes were validated against previously measured or self-reported height and weight. Subjects who had A: reported their body silhouettes at age 30 or 45 in ECRHS III or RHINE III, and B: who were aged 30(±2) years or age 45(±2) years when they participated in previous study phases, so that they had had their height and weight measured or reported at those ages (measured in ECRHS I or II, reported in RHINE II) were included. We included ±2 years around age 30 and 45 to increase sample size, assuming that major changes in body size are unlikely to occur over two years.

Adult offspring ever asthma

The outcomes in paper II were offspring ever asthma with or without nasal allergies. 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". Asthma with and without 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?".

Adult offspring lung function

In paper III, the outcomes were adult offspring lung function, using measured pre-bronchodilator FEV_1, FVC and FEV_1/FVC. At RHINESSA clinical examinations, the maximum pre-and post- bronchodilator forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were recorded as the best of at least two manoeuvres that met the American Thoracic Society criteria for repeatability [33].

Susceptibility windows

We investigated several susceptibility windows in fathers and mothers when investigating risk for disease in the next generation. Being overweight at age 8, at puberty or at age 30, before or after offspring conception, was investigated in paper II. In paper III, paternal or maternal overweight at age 8 and/or puberty or at age 30 years was investigated.

Covariates

Potential adjusting variables and confounders were considered and discussed based on knowledge from previous literature and identified by using direct acyclic graphs (DAGs). A DAG is a graph in which unidirectional arrows are used to represent known causal effects, based on prior knowledge [66].

In paper I all analyses were stratified by sex. No covariates were taken into consideration. In paper II the following covariates were included: participating parents' asthma and education level, the other parents' (reported by the adult offspring) overweight status at age 30 and asthma, offspring sex and age. In paper III, parents' education level and offspring's age and smoking history were evaluated as adjusting variables. Offspring's sex was used to separate the observations into subgroups (sons and daughters) in paper III.

3.7 Statistical analyses

Spearman correlation coefficients and box plots, paper I

In the validation of self-reported body silhouettes against previously measured or self-reported height and weight, the strength of the monotonic relationship between the variables was estimated in terms of Spearman correlation coefficients and box plots, showing median BMI and interquartile range for each body silhouette. When evaluating the relationship between two variables, it is important to determine how the variables are related. In a monotonic relationship, the variables tend to move in the same relative direction, but not necessarily at a constant rate. Spearman correlation coefficient is a nonparametric statistical measure of the strength of a monotonic relationship [73]. Correlation is an effect size, and the strength of the correlation is often interpreted as "weak", "moderate", or "strong". Cutoff points are arbitrary and inconsistent and should be used with good judgment [73]. Box plots graphically depicting groups of numerical data through their quartiles, showing the distribution of measured or self-reported BMI by figural scale.

ROC-curves and AUC-values, paper I

To investigate the body silhouette's ability to correctly classify the participants according to the body mass index cut-offs we used non-parametric Receiver-operating characteristic (ROC) curves and calculations of Area under the curve (AUC) with the body silhouettes specified as ordinal classification variables. The optimal cut-off was defined as the cut-off resulting from the best trade-off between specificity and sensitivity according to the Youden Index, which is defined as sensitivity + specificity -1 [74]. ROC-curves are probability curves, where the sensitivity is plotted against the specificity and AUC tells how much the model is capable of distinguishing between classes. Sensitivity (the true positive rate) and specificity (the true negative rate) are statistical measures of the performance of a binary classification test [75]. To investigate how the number of recall years in the ECRHS cohort affected the association between BMI and body silhouettes, we

performed additional analyses stratified on number of recall years between report of body silhouette and measurement of BMI.

Schematic representation of the paths investigated in paper II

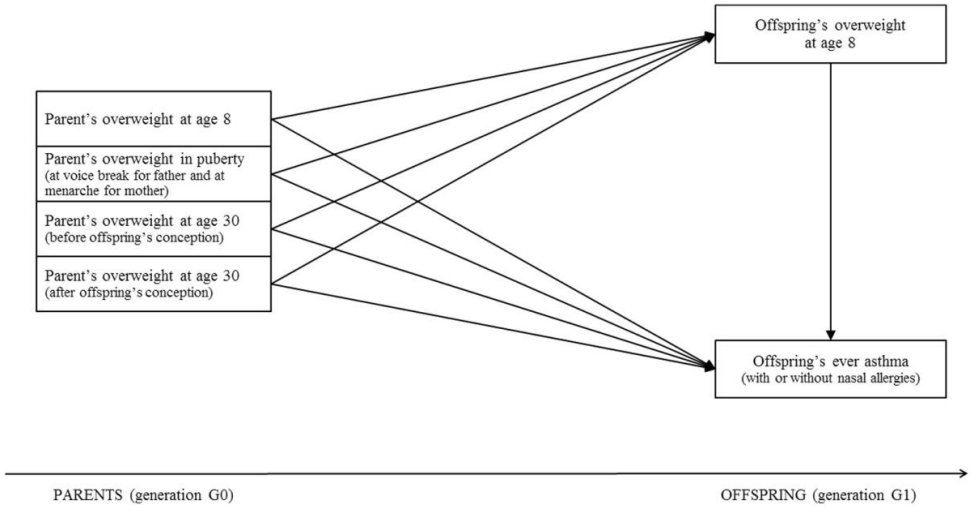

Figure 6. Schematic representation of the paths investigated within the paternal and maternal lines. Adjusted for ECRHS/RHINE parents' ever having asthma and educational level, other parents' overweight status at age 30 years and ever having asthma, and offspring's age and sex.

Regression analysis, paper II

In paper II we used regression analysis to investigate the association between parents' overweight status at different time points and adult offspring ever having asthma in the maternal and paternal line (Figure 6). 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). Children with the same parents tend to be more alike in their physical and mental characteristics than individuals chosen at random from the population at large. Furthermore, the parents are sampled from different study centres. 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 Standard Errors (SE) were computed to take the correlation among parents within each of the different centres (cluster variable) into account. Cluster-robust SEs are designed to allow for correlation between observations within clusters. Failure to control for within-cluster error correlation can lead to very misleadingly small standard errors, and consequent misleading narrow confidence intervals, large t-statistics, and low p-values [76]. 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.

Counterfactual mediation analyses, paper II and III

Counterfactual or potential outcome is a key concept in the causal effect literature [77]. This approach allows us 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 through pathways that do not involve the mediator) and the natural indirect effect (i.e. the effect of the exposure on the outcome caused by the effect of the exposure on the mediator) [78]. A counterfactual mediation analysis was carried out in paper II to establish whether the observed associations in the exploratory analysis between parents' overweight status at different time points and adult offspring's asthma were causal effects that could also be mediated through the offspring's own childhood overweight status [79]. At present, to 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 paper II, the mediation analysis was carried out by 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 considered because of the magnitude of the design effect [80]. In the mediation analysis the estimate of the natural effects was obtained by using the latent response variable mediator approach [79] with probit link, theta parameterization, and weighted least squares means and variance-adjusted

estimators [79]. Non-bias corrected bootstrap Cis (10 000 resamples) were obtained for the causally defined effects to take nonnormality of their estimate distribution into account. Natural effects were summarized as ORs.

In paper III counterfactual-based mediation analyses were carried out to investigate the pathways among parents' overweight before puberty and offspring's lung function. Mediation was combined with moderation (moderated mediation) to determine whether the indirect effect varied across levels of the moderator variable, being offspring sex [81] (Figure 7).

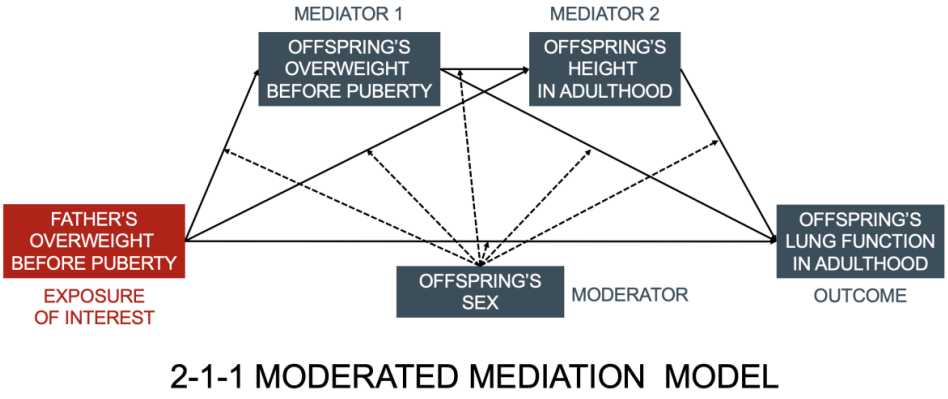

Figure 7. Moderated mediation model, Paper III. The exposure of interest is at level 2 (parent, G0), the mediators and the outcome are at level 1 (offspring, G1), with offspring sex as a moderator, a 2-1-1 moderated mediation model.

Two multi-group mediation models were used within the paternal and maternal lines. Model 1 (Figure 8) included offspring's FEV_1 and FVC as the normally distributed, correlated, parallel outcomes. Model 2 (Appendix C) included offspring's FEV_1/FVC as the normally distributed outcome.

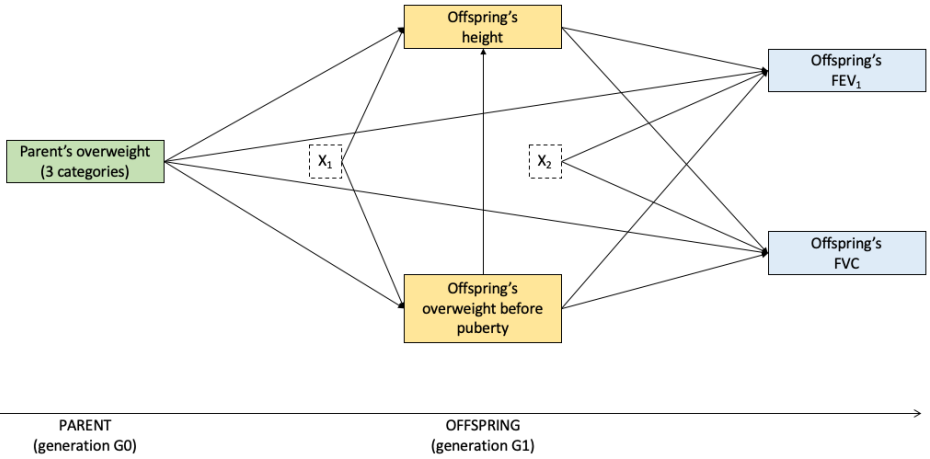

Figure 8. Graphical representation of the mediation model for FEV_1 and FVC in sons or daughters within the paternal or maternal lines (Model 1). The green box represents the exposure of interest, the yellow boxes the mediators and the blue boxes the outcomes. The dotted boxes represent the set of potential confounders and adjusting variables of the mediators (X_1: parents' education level) and of the outcomes (X_2: parents' education level and offspring's age and their own smoking history).

In both models' fathers' or mothers' overweight before puberty was the exposure of interest and a serial causal chain of the two mediators (offspring's overweight before puberty and offspring's adult height) was assumed, since we hypothesized a causal order between the mediators. In addition, parents' education level was the potential confounding variable of the exposure-mediator, mediator-mediator, and mediator-outcome relationships; offspring's age and their own smoking history were analysed as adjustment variables of the exposure-outcome and mediator-outcome relationships. The moderator (offspring's sex) does not appear in the model as a variable, but it was used to separate the observations into subgroups (sons and daughters).

Due to non-normality of offspring's overweight before puberty, the magnitude of the natural (counterfactual-based) direct and indirect effects was computed based on the

latent response variable mediator approach with probit link, theta parameterization and weighted least square mean and variance adjusted (WLSMV) estimators [79] with robust standard errors (father or mother= cluster variable). WLSMV estimator yields probit regression coefficients for the effects on the latent mediator (offspring's overweight before puberty) and linear regression coefficients for the effects on offspring's adult height, FEV_1, FVC and FEV_1/FVC. Centre clustering of our data was not considered (intraclass correlation <0.1) due to the complexity of the relationships.

The natural direct and indirect effects of the exposure on the outcomes were summarised as differences in offspring's expected lung function values. The natural direct effect is the difference in offspring's expected lung function value when the exposure is present, but offspring's height and/or offspring's overweight change from their expected value when the exposure is absent to their expected value when the exposure is present. Non-bias corrected bootstrap Confidence Intervals (CI) (10,000 resamples) were obtained for the causally defined effects to take their asymmetric distribution into account. To test the difference of direct and indirect effects between sons and daughters, the non-bias -corrected bootstrap CI for the group difference in the direct and indirect effects [82] was computed.

Sensitivity analyses in paper II and III

The identification of natural effects relies on strong assumptions [83]. In paper II, we checked whether the results changed in the presence of unmeasured confounding of the exposure-mediator-outcome relationship in mediation analyses [84]. 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 offspring

conception"; E_4, "overweight at age 30 years after offspring conception"; reference category "never overweight").

In paper III, we assessed whether the estimated direct and indirect effects changed after the inclusion of up to two unmeasured confounders for the exposure-outcome, exposure-mediator, and mediator-outcome relationships. Furthermore, we assessed how our results changed when offspring's post-bronchodilator lung function measurements were used as outcomes.

Statistical analyses were performed using Stata versions 14.0 – 16 (Stata Statistical Software, Statacorp, College station, TX: Statacorp LLC) and , Mplus version 8.1/8.6 (Muthén & Muthén, Los Angeles, CA) and R 3.5.1 (https://cran.r-project.org) and 3.6.1 (www.R-project.org).

3.8 Ethical considerations

A written informed consent was obtained from each participant prior to participation. All parts of the generation study (ECRHS, RHINE, RHINESSA) were approved by the appropriate regional committees of medical research ethics [85] and data collection complied with the principles of the Helsinki Declaration [86]. We considered the risk and inconveniences for participants to be minimal, due to focus on questionnaire data and only low-risk clinical examinations, including lung function testing. The participants were informed that withdrawal from the study was possible at any time, and the participants did not receive any kind of economic benefit.

Data management

Appropriate Data Protection measures have been taken to ensure safe storage of information. Data protection has been highly prioritized to avoid non-authorized access and misuse. The overall study database was stored on a designated research server at the Haukeland University Hospital, in accordance with hospital regulations for research [87]. The server is developed by the IT department at the hospital for secure

processing of sensitive personal data for research purposes. The storage system adheres to the “Norwegian Code of conduct for information security in the healthcare and care services sector” [88] and ensures that confidentiality, integrity, and availability are preserved when processing sensitive personal data.

4. Summary of main results

4.1 Validation of body silhouettes

We investigated the use of body silhouettes in adults as a tool to reflect overweight/obesity in the past. We used large population-based samples to analyse to what extent self-reported body silhouettes correlated with the previously measured (9-23 years) height and weight from both measured (in ECRHS I or II, N= 3041) or self-reported (in RHINE II, N= 3410) height and weight. We calculated Spearman correlation between body mass index (BMI) and body silhouettes and ROC-curve analyses for identifying obesity (BMI>30) at ages 30 and 45 years.

Our analyses showed that reported past body silhouettes correlated with BMI as measured or self-reported at the corresponding ages. Spearman correlations between measured BMI age 30 (±2y) or 45 (±2y) and body silhouettes in women and men were between 0.62 and 0.66 and correlations for self-reported BMI were between 0.58 and 0.70. Furthermore, the retrospective body silhouettes made it possible to differentiate between obese and non-obese persons at previous ages with an acceptable validity. The area under the curve for identification of obesity at age 30 using body silhouettes vs previously measured BMI at age 30 (±2y) was 0.92 (95% CI 0.87, 0.97) and 0.85 (95% CI 0.75, 0.95) in women and men, respectively; for previously self-reported BMI, 0.92 (95% CI 0.88, 0.95) and 0.90 (95% CI 0.85, 0.96).

Longer recall times weakened the correlations to some extent, while the age at the time of recalled body size was of larger importance for detecting obesity. These results were equal for both women and men, and the findings were consistent when validated against measured as well as self-reported anthropometric data. We found no support for a common cut-off for both genders and all ages that could be used to accurately separate between obese and non-obese participants.

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

We aimed to examine whether parents being overweight in childhood, adolescence, or adulthood was associated with asthma in their offspring. We included 6347 adult offspring (age, 18-52 years) investigated in the RHINESSA multigeneration study of 2044 fathers and 2549 mothers (age, 37-66 years) investigated in the ECRHS III/RHINE III studies. Associations of parental overweight status at age 8 years, puberty, and age 30 years (before or after conception of the offspring) with offspring's childhood overweight status (potential mediator) and offspring's asthma, with or without nasal allergies was analysed 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.

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

4.3 The effect of parental overweight before puberty on lung function in the offspring

We investigated the causal associations to estimate the causal effects of parents' overweight on adult offspring's lung function within the paternal and maternal line. In this analysis we included 929 adult offspring (age 18-54 years, 54% daughters) of 308 fathers and 388 mothers (age, 40-66 years). Counterfactual-based multi-group mediation analyses by offspring's sex (potential moderator) were used to assess whether the effects of parents' overweight before puberty on adult offspring's FEV_1, FVC or FEV_1/FVC were mediated through offspring's prepubertal overweight and/or adult height (potential mediators) within the paternal and maternal lines. Unknown confounding was addressed by simulation models.

We found that fathers' overweight with start before puberty had a negative direct effect on sons' adult height [beta (95%CI): -3.42 (-6.18, -0.57) cm], and an indirect effect, mediated through sons' height, on sons' FEV_1[beta (95% CI): -144 (-272, -23) mL] and FVC [beta (95% CI): -210 (-380, -34) mL], and a negative direct effect on sons' FVC [-262 (-501, -9) mL]. Furthermore, fathers' overweight before puberty had a positive direct effect on daughters' overweight before puberty [beta (95%CI): 0.83 (0.32, 1.45)], which in turn had a negative direct effect on their own height in adulthood [beta (95%CI): -1.17 (-2.28, -0.09) cm]. Statistically significant effects on FEV_1/FVC were not observed. In the maternal line, mothers' overweight before puberty had neither direct nor indirect effects on offspring's lung function.

Our findings suggest that fathers' overweight starting before puberty may cause lower FEV_1 and FVC in their future sons. The effects could be partly mediated through sons' adult height but not through his prepubertal overweight.

5. Discussion

In this chapter the methodological strengths and limitations of the three papers included in the thesis are discussed. Furthermore, the main findings of the papers are discussed.

5.1 Methodological considerations

5.1.1 Study design

In this thesis we use data from three large prospective cohort studies, ECRHS, RHINE and RHINESSA, aiming to study respiratory health over time and across generations. A cohort study is a longitudinal observational study which usually aims to investigate causes of disease or factors related to health outcomes, and often starts with an unselected group of individuals which are followed up over a period of time [75]. We used exposure data from these cohort studies in a retrospective manner. Typical for a cohort study design, parental risk factor data were obtained for all individuals in the study independent of offspring health outcomes and recorded prospectively and before health outcomes in the offspring were recorded; retrospective because parental information on body silhouettes partly referred to body silhouettes back in time.

The RHINESSA study design provides a highly efficient method for providing detailed information on multiple generations, mostly, but not only, on respiratory health. In established birth cohort studies, there is often a focus exclusively on exposures in mothers, whereas in the RHINESSA study information on fathers at different time points is also available since the cohort investigated offspring of persons well-characterised over twenty years of child-bearing age. However, information on parent's puberty was collected retrospectively, as the first examination of the parental cohorts included persons aged 20-44 years.

The parents' cohort was investigated at baseline and two later study waves. In paper I, we use measurements from the first two study waves, and relate these to a tool

available from the third study wave that recorded previous body silhouettes including the time points of the two former study waves.

In papers II and III, we use measurements from two different generations, the parent generation (G0) who participated in the third study wave of ECRHS or RHINE and the adult offspring generation (G1) from RHINESSA. These data form a longitudinal data design (Figure 9).

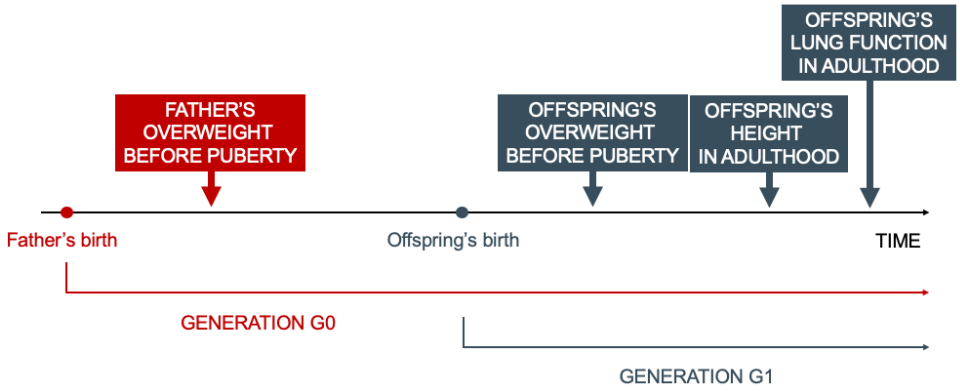

Figure 9. Data on exposure in parents (G0) precede data from adult offspring (G1) on the outcome and thus form a longitudinal data design.

Multi-centre, multi-generation studies, as these are, present challenges regarding possible methodological differences between the different study centres, standardisation of data collection over time, between generations and study centres, and therefore potential heterogeneity in the data. These factors would be random regarding studied risk factor-outcome associations and may result in attenuation of observed associations and less reliable negative results. On the other hand, results that are consistent across centres and generations may be more likely to reflect homogeneous biological processes than heterogeneous socio-cultural processes. To minimize the chance of discrepancies, standardised protocols and questionnaires were used in all centres and were harmonised across the three different study cohorts. Another challenge with multigenerational data is the need for statistical methods that

can consider the different levels of data, as well as nested data (multiple adult offspring might be siblings and originate from the same parent and parents are sampled from different study centres).

5.1.2 Study populations

Participants in ECRHS were originally recruited randomly from the general population living at the respective study sites, with an overall response rate of 78% [89, 90]. Paper I was based on data from ECRHS I, II and III and RHINE II and III (Figure 3). In RHINE, 86% responded at baseline. In the first follow-up in 1999-2000, 75% answered a new questionnaire and in the second follow-up in 2010-12, 53% of those who had participated in both the two previous stages, responded [70].

The long-term follow-up and non-response in RHINE have been investigated [70]. More men than women were lost to follow-up, as well as those who were youngest at baseline, and they found comparable patterns of long-term participation and loss to follow-up. They concluded that estimates from the follow-up populations should be interpreted with some caution in future reports from RHINE since they tended to be slightly lower than for the total baseline population. On the other hand, exposure-outcome associations were mainly unchanged by loss to follow-up and overall, the analysis indicated high validity in the data from RHINE.

Offspring were identified through national registries in the Northern European countries, while offspring's contact details were obtained from the parents participating in the ECRHS III survey for the Spanish and Australian centres. The participation rate in RHINESSA was rather low, only 38% of the eligible offspring of ECRHS/RHINE participants agreed to participate in RHINESSA. However, the offspring population was not severely skewed in any direction. The distribution of demographic characteristics did not seem to differ substantially from that of a general population in the same age range [91].

Our results should be generalisable to adult women and men living in urban western populations comparable to the investigated study sites in Europe and Australia. The multi-centre structure is a strength in terms of larger external validity: while the

largest number of study participants are from relatively homogeneous Nordic countries, the Estonian, Spanish, and Australian study centres contribute to socio-cultural diversity in the study population.

5.1.3 Validation of questionnaire data

The cross-cultural validity in reporting respiratory symptoms in the ECRHS has been assessed previously [92]. The multilingual translations and backtranslations of the ECRHS respiratory symptoms questionnaire's showed high internal consistency, suggesting that international comparisons were not importantly affected by errors due to cross-cultural variations in reporting of symptoms. One may assume that this also applies to the RHINE questionnaire, since it is a part of the ECRHS.

5.1.4 Body silhouettes

Slightly modified body silhouettes from the Stunkard body images (Figure 5) were introduced in the third follow-up of the ECRHS and RHINE studies [72]. One limitation with the self-reported body silhouettes is that individual differences in misconception of body size cannot be captured by our analysis. Further, there has been some criticism of the coarse and ordinal nature of the body silhouette scale. Their ordinal and fixed scale forces people to decide on one figure or the other, even though they might feel they are in between two figural scales. Another limitation is that we have not been able to validate overweight status by BMI for the childhood and puberty susceptibility windows.

5.1.5 Assessment of outcomes

Asthma with or without nasal allergies

Our categorization of the outcome into asthma with nasal allergies and asthma without nasal allergies (paper II) is based on self-reported questionnaires only. Our study did not include objective clinical data, such as allergy skin tests or serum specific IgE allergy testing, 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. Asthma with and without 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?”

In paper II, 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 [10, 59] and sex might modify the effect of obesity on asthma [93].

5.1.6 Spirometry and lung function

Lung function in terms of Forced Expiratory Volume in 1 second (FEV_1) and Forced Vital Capacity (FVC) were measured by spirometry testing. To accomplish good reliability, the spirometry testing in the RHINESSA study was performed according to the American Thoracic Society (ATS) criteria for repeatability and standardisation of spirometry [33], and both standard operating procedures and standardized equipment were used. The procedure was performed by trained personnel that followed detailed instructions to ensure measurements were performed in the same way in all participants in all study centres. Participants were asked to perform up to eight spirometry tests to accomplish acceptable and reproducible measures. A manoeuvre free from error was defined as acceptable, while a manoeuvre without excessive variability from other manoeuvres, was defined as being reproducible. To achieve reproducibility, three acceptable manoeuvres were needed and the two highest values for FVC and FEV_1 should not vary more than 200 millilitres from each other. In addition, calibration checks on the equipment were performed daily according to recommended standards.

5.1.7 Bias and confounding

To draw valid scientific conclusions, it is of great importance to collect data in a way that minimises systematic errors. Systematic errors (biases) that can distort the estimation of epidemiological measures could be missing data bias, selection bias, response bias, information bias, recall bias, confounding and misclassification.

Missing data bias

Our study samples in paper I were drawn from the source populations of participants in ECRHS and RHINE, based on defined inclusion and exclusion criteria. Complete case analysis for the main variables was used. In paper II and III, our study samples were drawn from the source populations of participants in the three different cohort studies, ECRHS, RHINE and RHINESSA, based on defined inclusion and exclusion criteria. We used complete information on exposures, mediators, and outcomes and in paper II, an "unknown" category was used for parent education when information was missing.

Discarding incomplete cases, leads to a loss of information which in turn could lead to selection bias. In the RHINE III survey population, 93% filled in current figural scales. Differences between responders and non-responders to figural scales in RHINE was investigated by Dratva et al [72]. They found that older, less educated, and male participants were less inclined to fill in the figural scales. Selection bias due to age, sex and education cannot be totally excluded, but it is unlikely that the parents' reporting of body silhouette, even before data was collected in the offspring, should affect the offspring reporting of own asthma.

Selection bias and response bias

Nonresponse of study subjects could be systematic and thereby lead to selection bias. The response rate in RHINESSA is 35% and this could influence the possibility for selection bias, however, it is unlikely that selection bias should cause spurious associations between parental overweight long before conception of the offspring and reported asthma and measured lung function in offspring, years later.

In the RHINESSA/RHINE/ECRHS studies the distributions of sex, smoking habits, educational level, and asthma status in parents with participating offspring, as compared to parents with non-participating offspring, were examined to explore potential response bias [91]. The two groups were found to be very similar, although offspring who did participate in RHINESSA more often had a parent with asthma, a parent who were non-smoker and a female parent. In addition, slightly less parents with higher education had offspring who participated in RHINESSA [91].

Information bias

There will nearly always be some errors in the information gathered from or about study participants. In paper I, it is likely that there are minor errors in measurements of height and weight, and errors in how people report their body silhouette. However, it is unlikely that measurement error in height/weight would be related to how participants reported their body silhouette back in time several years later. With the retrospective reporting of body silhouette, misconception of own body size could occur, but it is unlikely that the reporting of body silhouette is dependent on potential measurement errors several years earlier.

In papers II and III, if misclassification of the respiratory outcomes has occurred, it is unlikely that this would be systematically different between offspring whose fathers reported a higher or lower body size in childhood or in puberty. It is unlikely that misclassification in fathers' reports of their past body silhouettes is related to their offspring's reported asthma phenotype or to their offspring's objectively measured lung function. Furthermore, the paternal data are self-reported, and not reported by others (offspring, partner) which is often the case in birth cohort studies mostly focused on maternal exposures. This will reduce information bias. In paper II, we assumed that parents' overweight status and ever having asthma had an additive effect on the exposure-outcome relationship. This might be a simplification of the complex interaction between body size and asthma. In fact, the association between obesity and asthma seems partly caused by genetic pleiotropy, meaning that these two conditions share genetic determinants [94] 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 [95]. The information regarding "the other parent" was reported by the offspring rather than directly assessed, generating a potential for information bias. It seems unlikely that misclassification in offspring reported parental overweight would be differential regarding offspring asthma or measured lung function while it could possibly be

differential regarding offspring reported own body silhouette, which was an adjustment variable. This is unlikely to have influenced the main results.

Recall bias

For the parents who have been followed for 20 years, information on preconception risk factors was collected retrospectively before examination of their offspring, including data on parental puberty, hence reducing recall-bias. Published validation studies from this study population on body silhouettes and overweight status in RHINE [72], on pregnancy and birth characteristics [96] and on asthma report across generations [91], suggest minimal recall bias in key information and high reliability of information collected across generations.

Confounding paper II and III

In the analyses of exposure-mediator-outcome relationships in paper II, we included as adjustment variables asthma and educational level of the exposed parent (the ECRHS/RHINE participant), overweight status and asthma of the other parent (as reported by the adult offspring), and offspring sex and age. ECRHS/RHINE parent's educational level is the only variable that could be considered a confounder, potentially associated with both the exposure (parental overweight) and the outcome (offspring asthma) – but only for the analyses of parental overweight at age 30 years, not in childhood/adolescence. Offspring's sex is a potential modifier of the exposure-mediator-outcome relationships. ECRHS/RHINE parents' ever asthma, other parents' ever asthma and overweight at age 30 are potential modifiers of the exposure-outcome and exposure-mediator relationships. Because of the data sparseness, these interaction terms could not be included in the models. Therefore, the potential modifiers have been included as adjusting variables of the exposure-mediator-outcome relationships. The inclusion of these as modifiers could have contributed to even more detailed understanding of the relationship between parental overweight and offspring asthma, however, including these as adjustment variables rather than modifiers will not have caused spurious associations. To be noted, our analyses include several variables from both parents and the offspring, which is often not the case i.e.in analyses of maternal asthma or maternal age as associated with offspring

outcomes. In the analyses of exposure-mediator-outcome relationships in paper III, parents' education level and offspring's age and own smoking history was included as potential confounders and adjusting variables (Figure 8). Offspring's sex was included as moderator to gain more understanding by separating the observations for sons and daughters. However, unmeasured confounding may still be present and sensitivity analyses were performed to assess whether estimated effects changed after inclusion of one (paper II) or up to two unmeasured confounders (paper III).

The identification of natural direct effects, with the aim of approaching an interpretation of statistically causal associations, relies on strong assumptions [83]. A set of assumptions needs to be fulfilled for the effects to be interpreted as causal, and the plausibility of these assumptions needs to be considered [77]. The main requirement for mediation is that the indirect effect is statistically significant under the assumption of no unmeasured confounding [97]. In an observational study setting, this assumption will often not be satisfied, and it is important to investigate the potential impact of unmeasured confounding on effect estimates. We therefore explored whether the results of the exposure-mediator-outcome relationship in mediation analyses changed in the presence of unmeasured confounding [84](Figure 10). These sensitivity analyses revealed that unmeasured confounding had only a limited effect on the estimated direct effects of fathers' overweight status on offspring asthma (paper II), also when the U value (unmeasured confounding) had a very strong effect on the outcome, mediator, and exposure. In paper III, U mediation analyses were conducted for sons' FEV_1 and FVC in the paternal line. The analyses found that the inclusion of two unmeasured confounders in the model had only a limited impact on the direct and indirect effects of fathers' overweight before puberty on adult sons' FEV_1 and FVC, also in the scenario that each unmeasured confounder should have a very strong effect on the outcome, mediator, and exposure. One cannot exclude the possibility that there still may be unmeasured confounders, such as genetic or environmental factors in any of the two generations. However, our probabilistic simulations on the impact of unmeasured confounding suggest that this was not the case and support a causal interpretation of our results.

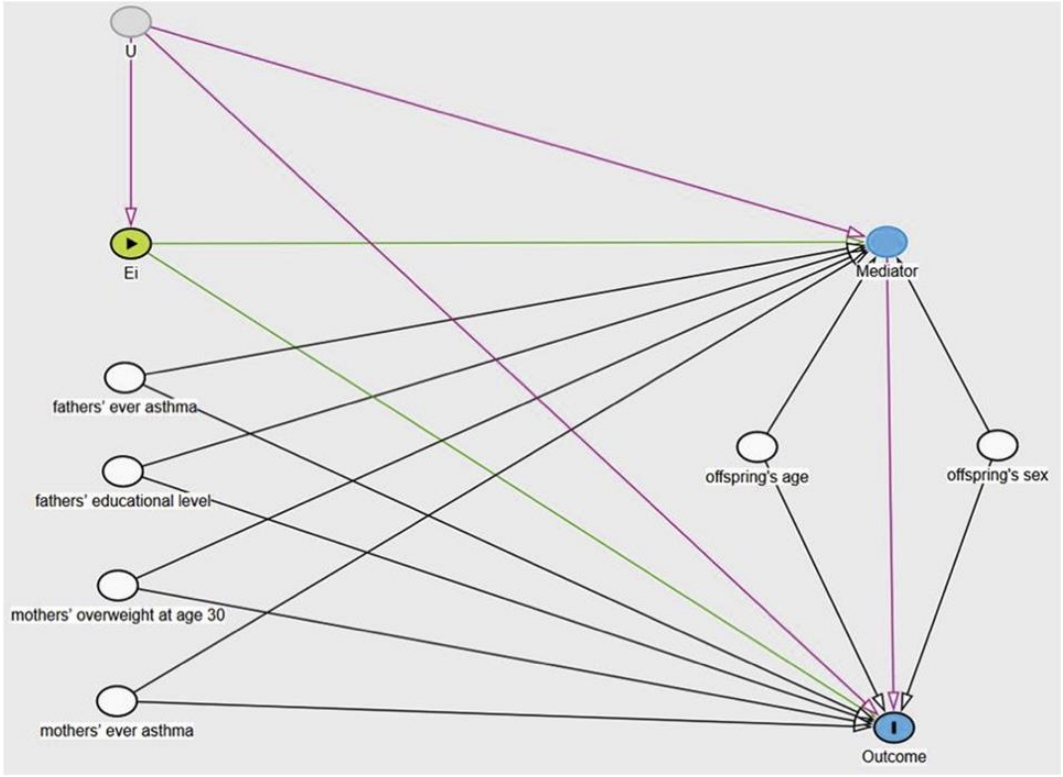

Figure 10. (Paper II) Directed acyclic graph showing how data were simulated for the exposure, mediator, outcome, and adjusting variables and one unmeasured confounder. Ei indicates one of the following binary exposure variables: E1, fathers' overweight status at age 8 years versus never overweight; 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 conception versus never overweight. Mediator indicates offspring's overweight status at age 8 years. Outcome, indicate offspring ever having asthma without nasal allergies. U indicates the unmeasured normally distributed confounder.

5.2 Discussion of main results

5.2.1 Validation of body silhouettes

We found that reported past body silhouettes were moderately correlated with BMI as measured (ECRHS) or self-reported (RHINE) at the corresponding ages, and that the retrospective body silhouettes made it possible to differentiate between obese and non-obese persons at previous ages with an acceptable validity. To our knowledge, our study was the first study to validate the use of self-reported past body silhouettes against previously measured or self-reported height and weight during adulthood in a large study population, and secondly to study the importance of recall time. Several studies have shown high correlation between objectively measured or self-reported current height and weight and body silhouettes [72, 98-100], but for the use of reporting body size back in time, we have only found two previous studies; these showed results in agreement with those from our study: Must et al [101] asked elderly men and women aged 71-76 years to select body silhouettes reflecting their childhood and adolescent years. With a recall time of several decades, they found that selected body sizes were reasonably well correlated with measured BMI at age 20 years ($r^{Pearsson}$=0.53 in men and 0.66 in women) and in childhood, at age 10 years ($r^{Pearsson}$=0.66 in men and 0.65 in women). A study by Koprowski et al [102] investigated the ability of women with an average age of 17 years to recall body size at the age of menarche using body silhouettes and found good correlation between actual BMI at the time of menarche and recalled body silhouette ($r^{Pearsson}$=0.77).

We found no significant gender differences, but the discriminatory capability of the body silhouettes to identify obesity was slightly better in women than in men, both regarding measured and self-reported BMI. Must et al [101] also suggested a somewhat stronger correlation in women, and they found gender differences in over-*versus* underestimation of high school weight. In our analysis, the associations of recalled body silhouette with measured or self-reported height and weight did not significantly differ according to recall time, although correlations appeared somewhat weaker for the longer recall time. This agrees with the findings of Must et al [101] of overall moderate correlations even in the remote past.

We found no support for a common cut-off for both genders and all ages that could be used to accurately separate between obese and non-obese participants. There was an overlap of the BMI-range associated with each body silhouette. This overlap supports the idea that body silhouettes represent gradations in body size rather than clear-cut categories. In general, the retrospective body silhouettes seem to be best used as an ordinal measure of body size and probability of obesity, rather than a measure to strictly define BMI-groups.

The use of body silhouettes to define overweight in paper II and III

One purpose of introducing body silhouettes into the ECRHS and RHINE surveys was to assess anthropometric status at specific time points in the past and thereby provide a history of body size earlier in life, often missing in epidemiological studies of adults. Dratva et al [72] validated reported current body silhouette with measured current BMI in a sub-sample of RHINE participants with data on measured height, weight, and waist circumference (WC). Their aim was to investigate the predictive power of body silhouettes to identify individuals at metabolic risk. They found that subjects "at risk", as defined by BMI and WC, could be identified with high accuracy using this instrument. This was the case for both women and men, although a higher correlation for women than for men have consistently been observed in other studies [103-105].

The cut-offs that we used to define overweight status (non-overweight or overweight-obese subjects) in paper II and III were based on this validation study by Dratva et al [72], supported by our validation study for the use of body silhouettes back in time. In our study we found that spearman correlations between measured height and weight and retrospectively reported body silhouettes in women and men, were between 0.62 and 0.66 and correlations for self-reported height and weight were between 0.58 and 0.70. Mean objectively measured BMI in both ECRHS I and II was between 24.0 and 24.9, in women. This corresponded to a median reported body silhouette figure #4. Figure #4 correlates with the cut-off for detection of current overweight status in women in the study by Dratva et al [72], although the BMI is slightly lower than the definition of overweight (BMI >25). In men, mean objectively measured BMI in

ECRHS I and II was between 25.4 and 26.5, and corresponded to a median reported body silhouette figure #4. Their measured BMI corresponds to being overweight (BMI >25), and in the validation by Dratva et al. figure #5 was found to best detect current overweight status. Furthermore, in our validation study on the reporting of retrospective body silhouette against measured height and weight, figure #5 in women and figure #6 in men had the best discriminatory capability to detect risk of obesity. This corresponds to the finding in the validation of reporting of current body silhouette against measured height and weight by Dratva et al [72]. The participants reporting their current or past body silhouette in these two validation studies were approximately the same age (mean age 51-55) at participation.

Unfortunately, we were not able to validate the risk for overweight or obesity status by BMI in the childhood and puberty windows. Must et al[101] did find reasonably well correlation between measured BMI at age 10 years and reported body silhouettes more than 55 years later, although in a relatively small study population (n=181). A validation study for these time windows in a larger population is warranted but difficult to accomplish due to the wide time frame. However, we validated the body silhouettes by using past BMI for the 30-year time point, and it is likely that the cut-offs will not differ substantially even if we go further back in time.

5.2.2 Parental overweight and offspring asthma

We found a direct effect of fathers' overweight at voice break on adult offspring's ever asthma without nasal allergies. In addition, we found an indirect effect of fathers' overweight at age 8 years on adult offspring's asthma without nasal allergies, mediated by offspring's own childhood overweight. This indirect effect is most likely due to the strong hereditary association that we observed between fathers' childhood overweight and offspring childhood overweight and agrees with previous studies showing associations between parental and offspring's weight [106] and between own overweight status and own asthma [107]. 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,

although the estimate for fathers' overweight status at age 30 years after conception was borderline significant.

Since we had no information on skin prick tests or other clinical examinations relevant for the allergic status in our study, we decided to use the terms "asthma with nasal allergies" and "asthma without nasal allergies", instead of allergic and non-allergic asthma in our paper. "Asthma with nasal allergies" and "asthma without nasal allergies" to some degree represent different asthma phenotypes: while asthma with allergies is triggered by inhaled allergens, asthma without allergies is not [108]. Further, even if the symptoms are similar, the underlying risk factors may differ [109]. Little is known on non-allergic asthma in the general population. A study from the ECRHS cohort by Leynaert et al.[23] suggest that non-allergic asthma may be more severe and difficult to control than allergic asthma, and that women might be at increased risk of non-allergic asthma. We could not assess the moderating effects of offspring's sex because of data sparseness, which is unfortunate as one might suspect a sex-specific association pattern in which paternal and maternal risk factors affect daughters and sons differently. Overweight and obesity have been found to be more strongly associated with non-allergic asthma than with allergic asthma [7, 27], and we found no associations of parental overweight with offspring asthma with nasal allergies.

To the best of our knowledge, only two studies, partly in the same population as this study, have thus far investigated susceptibility time windows in fathers regarding asthma risk in their adult offspring [54, 56]. Svanes et al [54] 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 [56] showed that in ECRHS onset of fathers' smoking in early puberty was a risk factor for asthma without nasal allergies in later offspring. Early smoking in fathers has also been found to be related to increased risk of obesity in their sons, in the ALSPAC study in the UK [64]. We did not take fathers smoking into consideration in our analyses, since

we considered fathers smoking to be another exposure and not a confounder for his body silhouette in the puberty time-window and adult offspring asthma.

Mothers' overweight status in the different time periods was not associated with adult offspring's asthma in our analyses. Other studies have found an association of maternal overweight status just before or during pregnancy with offspring's asthma [9, 10]. However, our study could not specifically address this time window. Further, these studies have not taken father's overweight status into account, whereas we included fathers' overweight status at age 30 years as a covariate in the model. Residual confounding effect of fathers' overweight status could be present because fathers' overweight status was reported by the offspring and refers to a single time window (at age 30 years).

5.2.3 Parental overweight and offspring lung function

Fathers' overweight before puberty reduced both sons' and daughters' height in adulthood compared to fathers who had never been overweight, although only statistically significant in sons. Moreover, the mediated effect through lower adult-attained height on offspring FEV_1 and FVC was only found in his sons. In our statistical model we hypothesized that offspring prepuberty overweight could affect own adult height. This was based on existing literature that adult height might be influenced by the timing of pubertal events and that onset of puberty could be related to overweight and obesity [110-113]. This was only found in daughters, in the paternal line. Further, we found a positive direct effect of fathers' prepuberty overweight on daughters' overweight before puberty, which in turn had a negative direct effect on daughters' own height in adulthood. Despite this association, fathers' prepuberty overweight was neither directly nor indirectly associated with daughters' lung function. Height is partly genetically inherited, but independent of a shared postnatal environment, epigenetic mechanisms could possibly affect offspring growth and height [114]. In addition, height is of particular importance when respiratory health is considered since lung function is related to lung growth and lung volume [112]. Our finding, that height mediates the relationship between fathers' prepuberty overweight and lung function outcomes in their sons, raise a broader issue around the common

practice of adjusting for height in analyses using absolute lung function values. It would be inappropriate to adjust for height or to use transformations of lung function outcomes (e.g., z-score or % predicted) that inherently take differences in the attained height into account [115], and more appropriate to investigate height as a mediating variable.

Fathers' prepuberty overweight had a negative indirect effect on male offspring FEV_1. We also found a direct effect on FEV_1 in sons, although it did not reach a statistical significance. Further, fathers' overweight before puberty had a negative direct effect on male offspring FVC, meaning an effect through pathways that did not involve the two mediators, offspring pre-pubertal overweight or offspring adult height. It has been shown that early life factors and genetic effects that manifest in childhood will influence the individual's FEV_1 and FVC life trajectory over the life-span, pointing to environmental exposures and genes affecting lung development as risk factors for low FEV_1 and FVC in later life [34, 40]. Further, FVC is an important predictor of all-cause mortality in asymptomatic adults without chronic respiratory conditions [116].

In a study by Accordini et al.[57] from the RHINESSA cohort, causal effects of tobacco smoking on offspring lung function in the paternal line was investigated and they found that fathers' smoking initiation in prepuberty may cause lower lung function in offspring. This is the only other human study we know of that investigate exposure in paternal prepuberty in relation to offspring lung function. In our analyses, paternal smoking was not taken into consideration as a confounder since paternal smoking was not a confounder for overweight/obesity in the prepuberty window in relation to lung function in the next generation. Smoking and overweight were both found to be associated with offspring lung function, these represent two different exposures, and one might also speculate whether these would interact in relation to lung function outcomes in the next generation.

For our main analyses, pre-bronchodilator lung function measurements were used. We also had data on post-bronchodilator measurements for most of the offspring. Our

sensitivity analysis showed that the indirect effect of fathers' overweight before puberty on FEV_1 and FVC in sons remained statistically significant when post-bronchodilator measurements were used.

In the paternal line we did not observe statistically significant effects of fathers' overweight before puberty on offspring's FEV_1/FVC. In the maternal line, mothers' overweight status in different time periods was not associated with offspring adult height, and no direct and indirect effects between mothers' overweight status and adult offspring's lung function (FEV_1, FVC or FEV_1/FVC) were found.

5.2.4 Did we forget the fathers?

There is substantial epidemiological and experimental evidence supporting the concept of developmental origins of adult lung disease and impaired lung function [14-17], and there is strong evidence of an association of birth weight with adult FVC [35]. The effect of birth weight on adult FVC is in line with the Developmental Origins of Health and Disease (DOHaD) hypothesis, stating that early life exposures may be important to future outcomes in an individual [13], although, this finding might be mediated by adverse early-life factors affecting birth weight.

Early life factors have traditionally focused on how maternal exposures might influence offspring health and risk of disease, with the *in utero* environment as the one particular susceptibility window [13]. Nevertheless, paternal influences acting on the gamete environment long before conception of the child could possibly affect foetal conditions and development *in utero*, a function of the placenta and foetal growth [3, 117-119]. Several animal models demonstrate that the sperm epigenome functions as a vector to transfer preconception messages from the paternal environment to the offspring [120-122]. A rodent study demonstrated that male offspring from obese fathers had reduced birth weight and a growth deficit phenotype was observed from birth to 6 months of age [123]. However, the paternal contribution of early exposures is often overlooked in human studies and empirical evidence on paternally transmitted effects in humans is still limited [2, 18]. The possibility that environmental impact can be transmitted to offspring through epigenetic marks has

contributed to a proposed new field: that of Paternal Origins of Health and Disease (POHaD) [19].

5.2.5 Puberty as a potential susceptibility window

We have demonstrated causal associations between fathers' overweight status before puberty and male offspring respiratory health. It seems biologically plausible that male prepuberty could be a time window of susceptibility. Exposures in fathers' prepuberty might influence male germ cells, given the extensive epigenetic reprogramming taking place in the spermatocyte precursors at this age [2, 124]. Germ cells develop differently in males and females, which could explain the differences in effects through the maternal and paternal line, and at which time window susceptibility is present for various exposures.

We have defined overweight status at puberty based on the reporting of body silhouettes back in time, at voice break. Voice break is considered a late sign of puberty, occurring towards the end of the transition. In the 1946 British Birth Cohort Study voice-breaking status was assessed in boys at age 14 years (mean 14.5, range 14.3-15.2 years) [50]. At age 14 years, 26.8% of 2001 males had no voice breaking, 37.1% were starting, and 36.0% had complete voice breaking. Even if overweight is reported to start at age 8 years or after age 8 years, but is present at voice break, that should make it possible for us to "catch" the prepuberty susceptibility window. However, investigating the prepubertal window as a susceptibility window for future health can be challenging. First, one needs the data. Most established birth cohorts do have more data on mothers than on fathers, and for both gender there is usually limited data back in time. Secondly, investigating data over two generations make it even more challenging, since the human reproductive cycle spans decades. Dependent on the exposure of interest, randomizing exposures during parents' puberty in humans would be ethically challenging, and it would be too time consuming and costly to wait and see possible consequences in their future offspring. Thus, animal models can be useful, as they allow to study exposures in highly controlled settings and relate the outcomes in offspring within a relatively short time frame. Data from animal models has clearly demonstrated that epigenetic processes

can be involved in transmission of parental exposures to their offspring [120-124]. However, in mice, puberty last for only a few days, which makes it hard to investigate prepuberty in animal models. Despite these challenges there exist valuable scientific advancements from both animal and human studies, epigenetic research, and methodological work that support the hypothesis that male adolescence may be an important susceptibility window for the health and disease in the next generation [2, 54, 56, 64, 125].

5.2.6 Causal inference with observational data

Methods of causal inference can greatly increase the information that can be obtained from observational studies. One of Hill's criteria that is essential to causal inference is temporality [126]. In our analyses, using data from two generations, with the exposure in the parent generation and the outcome in the offspring, we know that the exposure precedes the outcome. Further, a major strength in this thesis is the statistical approach used for assessing causality among variables in different generations. The use of counterfactual models, which has increasingly become a standard for causal inference in epidemiologic and medical studies [77, 127] enabled us to decompose the total effect of parents' overweight status on offspring's asthma or attained lung function into its direct effect and the effect mediated by offspring's overweight status or overweight status and height, respectively. This disentanglement of the various biological pathways can guide the interpretation of the statistically causal associations that we find, supported by existing evidence to build upon biological plausibility. It is possible that unmeasured confounders, such as genetic and other environmental factors in two generations, may be important. However, probabilistic simulations on the impact of unmeasured confounding support our results. 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, as well as on sons' FEV_1 and FVC in the paternal line.

6. Conclusions and implications

Conclusions

When investigating parental overweight long before conception of the offspring, as related to lung health in the offspring in adulthood, we found evidence that 1) male onset of overweight starting between childhood and puberty appeared to increase asthma without nasal allergies in offspring, and 2) that fathers' overweight starting before puberty appeared to cause lower FEV_1 and FVC in male offspring. By using mediation analyses we were able to show that the effect of male onset of overweight in puberty on offspring asthma, was direct and not mediated through offspring's own overweight status. Further, we showed that the observed association between a father's overweight before puberty and adult offspring lung function involved mediation through lower attained adult height in his sons, but not through his sons' own overweight before puberty. In the maternal line, we could not identify associations between mother's overweight and adult offspring's asthma or lung function.

To define parent's overweight or obesity in the past, we used participant-reported body silhouettes. We validated this tool and found that body silhouettes can be a satisfactory correlate for past body size in adulthood and a useful epidemiological tool to differentiate retrospectively between obesity and non-obesity in women and men; the probability of being obese increased with increasing body silhouette. This validated instrument made it possible to investigate overweight status in different susceptibility windows in the maternal and paternal lines in relation to lung health in the next generation.

Our analyses were based on data from a multi-generation study; thus, parents had provided data on their body silhouettes and other relevant variables, and extensive information was obtained from investigation of the offspring, including detailed assessment of asthma and respiratory symptoms as well as measurements of lung function. The analyses were performed using advanced statistical models that accounted for potential confounding variables in each generation as well as one or

two unknown confounders, and for the multi-level structure of a dataset that included multiple siblings, study centres, and two generations.

Implications

Although it is well established that *in utero* exposures are important for offspring health, we have shown that this is not the only exposure time of importance for offspring health. Our findings support the concept that the metabolic environment in male prepuberty might influence the health of the next generation. Addressing the prepubertal time window is however a challenge, in both human and mechanistic studies. Human epidemiologic studies investigating such susceptibility windows are scarce and are challenged by the temporal difficulties with covering two or more generations in humans. Furthermore, there is more data available on offspring's health in relation to mothers' environment and exposures than on fathers, and for both future mothers and fathers there is scarce data on the prepuberty/puberty time window. A closer scientific attention to male puberty in relation to future generations health may have profound implications and open new opportunities for targeted public health strategies. This could also help to balance the public perception of intergenerational harms. A focus on both male and female importance for the health of future offspring could lessen the burden on pregnant women. We speculate that one might improve the health of two generations by targeting the prepuberty time window in one generation- an age window that is key for both.

7. Future perspectives

Our findings support the concept that paternal environmental exposures in prepuberty might lead to gametic epigenetic alterations that might affect the phenotypes of future offspring. Mechanistic research in humans is needed to explore this further. It would be interesting to attempt to explore the potential role of non-coding RNAs in multigenerational effects through the male germ line. However, there are more limitations and challenges when it comes to human studies, compared to animal studies. In animal studies you could explore primordial germ cells in relation to parents' exposures and characteristics in future offspring.

Life course data on overweight and obesity may enrich the quality of epidemiologic studies of the related health consequences. However, such data is most often lacking. In the absence of previously measured height and weight, alternative methods that can capture weight history are needed. Validation studies of body silhouettes for the childhood and adolescence time windows would be very advantageous but still difficult to accomplish due to the wide time frame. For the future, it would be valuable if studies with BMI information on children and adolescents performed a follow up on retrospect body silhouettes at age 8 years and puberty for validation by previously collected BMI data for the same time windows.

Regarding mother and childcare, public health policies have given priority to the vulnerability window around pregnancy and nursing, which is indeed an important window. There is however reasonably convincing evidence that the metabolic environment prior to puberty also might influence both the individual's own health and the health of the next generation. This calls for an increased focus and research effort on how to reach the youngsters, both male and female, and how to induce beneficial behaviours and choices, even long before they become parents. This involves public school health systems, urban planning adapted for physical activity, and a focus on the availability of healthy food for everyone, across the socioeconomic scale. These are not novel suggestions, as they have been suggested many times, but they are not easy to implement. With more evidence and focus on the proposed

susceptibility window in male prepuberty, it might be easier to reach the policy makers and health strategy planners. There is already in the society a large burden on the individual to improve own health and now also that of future generations. It is therefore of key importance that intervention towards better health should not be only a matter of personal achievement, but of the society. Policy makers and health strategy planners must be informed about the potential opportunity for efficient interventions. Improving health in childhood is likely to benefit not only the children themselves, but also their future offspring. We must prepare for parenthood already in childhood.

8. References

1. Pembrey M, Saffery R, Bygren LO, Network in Epigenetic E, Network in Epigenetic E. Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research. J Med Genet 2014; 51:563-72.
2. Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. Bioessays 2014; 36:359-71.
3. Soubry A, Murphy SK, Wang F, Huang Z, Vidal AC, Fuemmeler BF, et al. Newborns of obese parents have altered DNA methylation patterns at imprinted genes. Int J Obes (Lond) 2015; 39:650-7.
4. Sales VM, Ferguson-Smith AC, Patti ME. Epigenetic Mechanisms of Transmission of Metabolic Disease across Generations. Cell Metab 2017; 25:559-71.
5. Houfflyn S, Matthys C, Soubry A. Male Obesity: Epigenetic Origin and Effects in Sperm and Offspring. Curr Mol Biol Rep 2017; 3:288-96.
6. WHO. Global health observatory (GHO) data: overweight and obesity. 2017.
7. Peters U, Dixon AE, Forno E. Obesity and asthma. J Allergy Clin Immunol 2018; 141:1169-79.
8. Miethe S, Karsonova A, Karaulov A, Renz H. Obesity and asthma. J Allergy Clin Immunol 2020; 146:685-93.
9. 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. J Allergy Clin Immunol 2013; 131:1033-40.
10. Dumas O, Varraso R, Gillman MW, Field AE, Camargo CA, Jr. Longitudinal study of maternal body mass index, gestational weight gain, and offspring asthma. Allergy 2016; 71:1295-304.
11. Forsdahl A. Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? Br J Prev Soc Med 1977; 31:91-5.
12. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 1989; 2:577-80.
13. Barker DJ. The fetal and infant origins of adult disease. BMJ 1990; 301:1111.
14. Krauss-Etschmann S, Bush A, Bellusci S, Brusselle GG, Dahlen SE, Dehmel S, et al. Of flies, mice and men: a systematic approach to understanding the early life origins of chronic lung disease. Thorax 2013; 68:380-4.
15. Lannero E, Wickman M, Pershagen G, Nordvall L. Maternal smoking during pregnancy increases the risk of recurrent wheezing during the first years of life (BAMSE). Respir Res 2006; 7:3.
16. Bekkers MB, Elstgeest LE, Scholtens S, Haveman-Nies A, de Jongste JC, Kerkhof M, et al. Maternal use of folic acid supplements during pregnancy, and childhood respiratory health and atopy. Eur Respir J 2012; 39:1468-74.

17. Svanes C, Sunyer J, Plana E, Dharmage S, Heinrich J, Jarvis D, et al. Early life origins of chronic obstructive pulmonary disease. Thorax 2010; 65:14-20.
18. Soubry A. POHaD: why we should study future fathers. Environ Epigenet 2018; 4:dvy007.
19. Soubry A. Epigenetics as a Driver of Developmental Origins of Health and Disease: Did We Forget the Fathers? Bioessays 2018; 40.
20. Report Global Strategy for Asthma Management and Prevention, 2021.] Available from https://ginasthma.org.
21. Trivedi M, Denton E. Asthma in Children and Adults-What Are the Differences and What Can They Tell us About Asthma? Front Pediatr 2019; 7:256.
22. Asher MI, Garcia-Marcos L, Pearce NE, Strachan DP. Trends in worldwide asthma prevalence. Eur Respir J 2020; 56.
23. Leynaert B, Sunyer J, Garcia-Esteban R, Svanes C, Jarvis D, Cerveri I, et al. Gender differences in prevalence, diagnosis and incidence of allergic and non-allergic asthma: a population-based cohort. Thorax 2012; 67:625-31.
24. Almqvist C, Worm M, Leynaert B, working group of GALENWPG. Impact of gender on asthma in childhood and adolescence: a GA2LEN review. Allergy 2008; 63:47-57.
25. Vink NM, Postma DS, Schouten JP, Rosmalen JG, Boezen HM. Gender differences in asthma development and remission during transition through puberty: the TRacking Adolescents' Individual Lives Survey (TRAILS) study. J Allergy Clin Immunol 2010; 126:498-504 e1-6.
26. Rackemann FM. A working classification of asthma. Am J Med 1947; 3:601-6.
27. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012; 18:716-25.
28. Merriam-Webster's Collegiate Dictionary 11th edn: Merriam-Webster, Inc.,2008.
29. DeVries A, Vercelli D. Epigenetic Mechanisms in Asthma. Ann Am Thorac Soc 2016; 13 Suppl 1:S48-50.
30. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. Immunol Rev 2011; 242:10-30.
31. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet 2007; 8:253-62.
32. Peralta GP, Marcon A, Carsin AE, Abramson MJ, Accordini S, Amaral AF, et al. Body mass index and weight change are associated with adult lung function trajectories: the prospective ECRHS study. Thorax 2020; 75:313-20.
33. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005; 26:319-38.
34. Martinez FD. Early-Life Origins of Chronic Obstructive Pulmonary Disease. N Engl J Med 2016; 375:871-8.
35. Saad NJ, Patel J, Burney P, Minelli C. Birth Weight and Lung Function in Adulthood: A Systematic Review and Meta-analysis. Ann Am Thorac Soc 2017; 14:994-1004.

36. Lima Rda C, Victora CG, Menezes AM, Barros FC. Respiratory function in adolescence in relation to low birth weight, preterm delivery, and intrauterine growth restriction. Chest 2005; 128:2400-7.
37. Cheung YB, Karlberg JP, Low L, Ip M. Birth weight and adult lung function in China. Thorax 2001; 56:85.
38. Hancox RJ, Poulton R, Greene JM, McLachlan CR, Pearce MS, Sears MR. Associations between birth weight, early childhood weight gain and adult lung function. Thorax 2009; 64:228-32.
39. Obeidat M, Hao K, Bosse Y, Nickle DC, Nie Y, Postma DS, et al. Molecular mechanisms underlying variations in lung function: a systems genetics analysis. Lancet Respir Med 2015; 3:782-95.
40. Minelli C, Dean CH, Hind M, Alves AC, Amaral AF, Siroux V, et al. Association of Forced Vital Capacity with the Developmental Gene NCOR2. PLoS One 2016; 11:e0147388.
41. Forno E, Han YY, Mullen J, Celedon JC. Overweight, Obesity, and Lung Function in Children and Adults-A Meta-analysis. J Allergy Clin Immunol Pract 2018; 6:570-81 e10.
42. Akinbami LJ, Fryar CD. Current Asthma Prevalence by Weight Status Among Adults: United States, 2001-2014. NCHS Data Brief 2016:1-8.
43. Mamun AA, Lawlor DA, Alati R, O'Callaghan MJ, Williams GM, Najman JM. Increasing body mass index from age 5 to 14 years predicts asthma among adolescents: evidence from a birth cohort study. Int J Obes (Lond) 2007; 31:578-83.
44. Zhang Z, Lai HJ, Roberg KA, Gangnon RE, Evans MD, Anderson EL, et al. Early childhood weight status in relation to asthma development in high-risk children. J Allergy Clin Immunol 2010; 126:1157-62.
45. Weinmayr G, Forastiere F, Buchele G, Jaensch A, Strachan DP, Nagel G, et al. Overweight/obesity and respiratory and allergic disease in children: international study of asthma and allergies in childhood (ISAAC) phase two. PLoS One 2014; 9:e113996.
46. Alotaibi MF. Physiology of puberty in boys and girls and pathological disorders affecting its onset. J Adolesc 2019; 71:63-71.
47. Wood CL, Lane LC, Cheetham T. Puberty: Normal physiology (brief overview). Best Pract Res Clin Endocrinol Metab 2019; 33:101265.
48. Juul A, Magnusdottir S, Scheike T, Prytz S, Skakkebaek NE. Age at voice break in Danish boys: effects of pre-pubertal body mass index and secular trend. Int J Androl 2007; 30:537-42.
49. Busch AS, Hollis B, Day FR, Sorensen K, Aksglaede L, Perry JRB, et al. Voice break in boys-temporal relations with other pubertal milestones and likely causal effects of BMI. Hum Reprod 2019; 34:1514-22.
50. Ong KK, Bann D, Wills AK, Ward K, Adams JE, Hardy R, et al. Timing of voice breaking in males associated with growth and weight gain across the life course. J Clin Endocrinol Metab 2012; 97:2844-52.
51. Soubry A, Guo L, Huang Z, Hoyo C, Romanus S, Price T, et al. Obesity-related DNA methylation at imprinted genes in human sperm: Results from the TIEGER study. Clin Epigenetics 2016; 8:51.

52. Bohacek J, Mansuy IM. Molecular insights into transgenerational non-genetic inheritance of acquired behaviours. Nat Rev Genet 2015; 16:641-52.
53. Soubry A, Schildkraut JM, Murtha A, Wang F, Huang Z, Bernal A, et al. Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. BMC Med 2013; 11:29.
54. Svanes C, Koplin J, Skulstad SM, Johannessen A, Bertelsen RJ, Benediktsdottir B, et al. Father's environment before conception and asthma risk in his children: a multi-generation analysis of the Respiratory Health In Northern Europe study. Int J Epidemiol 2017; 46:235-45.
55. Miller LL, Henderson J, Northstone K, Pembrey M, Golding J. Do grandmaternal smoking patterns influence the etiology of childhood asthma? Chest 2014; 145:1213-8.
56. Accordini S, Calciano L, Johannessen A, Portas L, Benediktsdottir B, Bertelsen RJ, et al. A three-generation study on the association of tobacco smoking with asthma. Int J Epidemiol 2018; 47:1106-17.
57. Accordini S, Calciano L, Johannessen A, Benediktsdottir B, Bertelsen RJ, Braback L, et al. Prenatal and prepubertal exposures to tobacco smoke in men may cause lower lung function in future offspring: a three-generation study using a causal modelling approach. Eur Respir J 2021.
58. Li YF, Langholz B, Salam MT, Gilliland FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. Chest 2005; 127:1232-41.
59. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, et al. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 2006; 14:159-66.
60. Bygren LO, Kaati G, Edvinsson S. Longevity determined by paternal ancestors' nutrition during their slow growth period. Acta Biotheor 2001; 49:53-9.
61. Bertelsen RJ, Rava M, Carsin AE, Accordini S, Benediktsdottir B, Dratva J, et al. Clinical markers of asthma and IgE assessed in parents before conception predict asthma and hayfever in the offspring. Clin Exp Allergy 2017; 47:627-38.
62. Magnus MC, Haberg SE, Karlstad O, Nafstad P, London SJ, Nystad W. Grandmother's smoking when pregnant with the mother and asthma in the grandchild: the Norwegian Mother and Child Cohort Study. Thorax 2015; 70:237-43.
63. Lodge CJ, Braback L, Lowe AJ, Dharmage SC, Olsson D, Forsberg B. Grandmaternal smoking increases asthma risk in grandchildren: A nationwide Swedish cohort. Clin Exp Allergy 2018; 48:167-74.
64. Northstone K, Golding J, Davey Smith G, Miller LL, Pembrey M. Prepubertal start of father's smoking and increased body fat in his sons: further characterisation of paternal transgenerational responses. Eur J Hum Genet 2014; 22:1382-6.
65. Gordis L. Epidemiology, 5th edition. Philadelphia: Elsevier Saunders; 2014.
66. Lederer DJ, Bell SC, Branson RD, Chalmers JD, Marshall R, Maslove DM, et al. Control of Confounding and Reporting of Results in Causal Inference

Studies. Guidance for Authors from Editors of Respiratory, Sleep, and Critical Care Journals. Ann Am Thorac Soc 2019; 16:22-8.
67. Rothman KJ, Greenland S. Causation and causal inference in epidemiology. Am J Public Health 2005; 95 Suppl 1:S144-50.
68. Glass TA, Goodman SN, Hernan MA, Samet JM. Causal inference in public health. Annu Rev Public Health 2013; 34:61-75.
69. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J 1994; 7:954-60.
70. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, et al. Longterm follow-up in European respiratory health studies - patterns and implications. BMC Pulm Med 2014; 14:63.
71. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med 1995; 152:1107-36.
72. Dratva J, Bertelsen R, Janson C, Johannessen A, Benediktsdottir B, Braback L, et al. Validation of self-reported figural drawing scales against anthropometric measurements in adults. Public Health Nutr 2016; 19:1944-51.
73. Schober P, Boer C, Schwarte LA. Correlation Coefficients: Appropriate Use and Interpretation. Anesth Analg 2018; 126:1763-8.
74. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. Biom J 2005; 47:458-72.
75. Janet L Peacock. PJP. Oxford handbook of medical statistics. Oxford NewYork: Oxford University Press Inc.; 2011.
76. A. Colin Cameron DM. A practioner's Guide to Cluster-Robust Inference. Journal of Human Resources 2015; 50:317-73.
77. Muthén BO, Muthén, L. K, Asparouhov, T. Regression and Mediation Analysis Using Mplus. Los Angeles: Muthén & Muthén; 2016.
78. Pearl J. Direct and indirect effects. Proceedings of the Seventeenth conference on Uncertainty in artificial intelligence. Seattle (WA): Morgan Kaufmann Publishers; 2001.
79. Applications of causally defined direct and indirect effects in mediation analysis using SEM in Mplus. [Cited 2021 29.08.] Available from https://www.statmodel.com/download/causalmediation.pdf.
80. Mark H, Oi-Man K. Examining the Rule of Thumb of Not Using Multilevel Modeling: The «Design Effect Smaller Than Two» Rule. J Exp Educ 2015; 83:423-38.
81. Preacher KJ, Rucker DD, Hayes AF. Addressing Moderated Mediation Hypotheses: Theory, Methods, and Prescriptions. Multivariate Behav Res 2007; 42:185-227.
82. Ryu E, Cheong J. Comparing Indirect Effects in Different Groups in Single-Group and Multi-Group Structural Equation Models. Front Psychol 2017; 8:747.
83. VanderWeele TJ. Bias formulas for sensitivity analysis for direct and indirect effects. Epidemiology 2010; 21:540-51.
84. Lutz SM, Thwing A, Schmiege S, Kroehl M, Baker CD, Starling AP, et al. Examining the role of unmeasured confounding in mediation analysis with genetic and genomic applications. BMC Bioinformatics 2017; 18:344.

85. Overview of ethics committees and approval numbers of RHINESSA centres. Availabel online:.] Available from https//helse-bergen.no/seksjon/RHINESSA/Documents/Ethic%20Commitees%20list.pdf.
86. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310:2191-4.
87. Haukeland University Hospital regulations for research. Available online:.] Available from https://helse-bergen.no/fag-og-forsking/forsking/forskingsrutinar#malar.
88. Norwegian Code of conduct for inforamtion security in the healthcare and care services sector. Available online:.] Available from https://ehelse.no/normen/documents-in-english.
89. Janson C, Anto J, Burney P, Chinn S, de Marco R, Heinrich J, et al. The European Community Respiratory Health Survey: what are the main results so far? European Community Respiratory Health Survey II. Eur Respir J 2001; 18:598-611.
90. European Community Respiratory Health, Survey (ECRHS) homepage.] Available from http://www.ecrhs.org/.
91. Kuiper IN, Svanes C, Benediktsdottir B, Bertelsen RJ, Braback L, Dharmage SC, et al. Agreement in reporting of asthma by parents or offspring - the RHINESSA generation study. BMC Pulm Med 2018; 18:122.
92. Sunyer J, Basagana X, Burney P, Anto JM. International assessment of the internal consistency of respiratory symptoms. European Community Respiratory Health Study (ECRHS). Am J Respir Crit Care Med 2000; 162:930-5.
93. Wang L, Wang K, Gao X, Paul TK, Cai J, Wang Y. Sex difference in the association between obesity and asthma in U.S. adults: Findings from a national study. Respir Med 2015; 109:955-62.
94. Hallstrand TS, Fischer ME, Wurfel MM, Afari N, Buchwald D, Goldberg J. Genetic pleiotropy between asthma and obesity in a community-based sample of twins. J Allergy Clin Immunol 2005; 116:1235-41.
95. Dolton P, Xiao M. The intergenerational transmission of body mass index across countries. Econ Hum Biol 2017; 24:140-52.
96. Skulstad SM, Igland J, Johannessen A, Bertelsen RJ, Lonnebotn M, Omenaas ER, et al. Validation of maternal reported pregnancy and birth characteristics against the Medical Birth Registry of Norway. PLoS One 2017; 12:e0181794.
97. Zhao X, Lynch, J.G. Jr., Chen Q. Reconsidering Baron and Kenny: myths and thruths about meidation analysis. J Consumer Res 2010; 37:197-206.
98. Tehard B, van Liere MJ, Com Nougue C, Clavel-Chapelon F. Anthropometric measurements and body silhouette of women: validity and perception. J Am Diet Assoc 2002; 102:1779-84.
99. Munoz-Cachon MJ, Salces I, Arroyo M, Ansotegui L, Rocandio AM, Rebato E. Overweight and obesity: prediction by silhouettes in young adults. Obesity (Silver Spring) 2009; 17:545-9.

100. Bulik CM, Wade TD, Heath AC, Martin NG, Stunkard AJ, Eaves LJ. Relating body mass index to figural stimuli: population-based normative data for Caucasians. Int J Obes Relat Metab Disord 2001; 25:1517-24.
101. Must A, Willett WC, Dietz WH. Remote recall of childhood height, weight, and body build by elderly subjects. Am J Epidemiol 1993; 138:56-64.
102. Koprowski C, Coates RJ, Bernstein L. Ability of young women to recall past body size and age at menarche. Obes Res 2001; 9:478-85.
103. Fitzgibbon ML, Blackman LR, Avellone ME. The relationship between body image discrepancy and body mass index across ethnic groups. Obes Res 2000; 8:582-9.
104. Madrigal H, Sanchez-Villegas A, Martinez-Gonzalez MA, Kearney J, Gibney MJ, Irala J, et al. Underestimation of body mass index through perceived body image as compared to self-reported body mass index in the European Union. Public Health 2000; 114:468-73.
105. Martinez JA, Kearney JM, Kafatos A, Paquet S, Martinez-Gonzalez MA. Variables independently associated with self-reported obesity in the European Union. Public Health Nutr 1999; 2:125-33.
106. Wardle J, Carnell S, Haworth CM, Plomin R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am J Clin Nutr 2008; 87:398-404.
107. Braback L, Hjern A, Rasmussen F. Body mass index, asthma and allergic rhinoconjunctivitis in Swedish conscripts-a national cohort study over three decades. Respir Med 2005; 99:1010-4.
108. Lafeuille MH, Gravel J, Figliomeni M, Zhang J, Lefebvre P. Burden of illness of patients with allergic asthma versus non-allergic asthma. J Asthma 2013; 50:900-7.
109. Janson C, Kalm-Stephens P, Foucard T, Alving K, Nordvall SL. Risk factors associated with allergic and non-allergic asthma in adolescents. Clin Respir J 2007; 1:16-22.
110. Reinehr T, Roth CL. Is there a causal relationship between obesity and puberty? Lancet Child Adolesc Health 2019; 3:44-54.
111. Mahmoud O, Granell R, Tilling K, Minelli C, Garcia-Aymerich J, Holloway JW, et al. Association of Height Growth in Puberty with Lung Function. A Longitudinal Study. Am J Respir Crit Care Med 2018; 198:1539-48.
112. Yousefi M, Karmaus W, Zhang H, Roberts G, Matthews S, Clayton B, et al. Relationships between age of puberty onset and height at age 18 years in girls and boys. World J Pediatr 2013; 9:230-8.
113. Marcovecchio ML, Chiarelli F. Obesity and growth during childhood and puberty. World Rev Nutr Diet 2013; 106:135-41.
114. Dodd JM, Du Plessis LE, Deussen AR, Grivell RM, Yelland LN, Louise J, et al. Paternal obesity modifies the effect of an antenatal lifestyle intervention in women who are overweight or obese on newborn anthropometry. Sci Rep 2017; 7:1557.
115. Campbell B, Simpson JA, Bui DS, Lodge CJ, Lowe AJ, Matheson MC, et al. Early menarche is associated with lower adult lung function: A longitudinal

cohort study from the first to sixth decade of life. Respirology 2020; 25:289-97.
116. Burney PG, Hooper R. Forced vital capacity, airway obstruction and survival in a general population sample from the USA. Thorax 2011; 66:49-54.
117. Binder NK, Beard SA, Kaitu'u-Lino TJ, Tong S, Hannan NJ, Gardner DK. Paternal obesity in a rodent model affects placental gene expression in a sex-specific manner. Reproduction 2015; 149:435-44.
118. McPherson NO, Fullston T, Aitken RJ, Lane M. Paternal obesity, interventions, and mechanistic pathways to impaired health in offspring. Ann Nutr Metab 2014; 64:231-8.
119. Isganaitis E, Suehiro H, Cardona C. Who's your daddy?: paternal inheritance of metabolic disease risk. Curr Opin Endocrinol Diabetes Obes 2017; 24:47-55.
120. Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ. Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. Nature 2010; 467:963-6.
121. McPherson NO, Bakos HW, Owens JA, Setchell BP, Lane M. Improving metabolic health in obese male mice via diet and exercise restores embryo development and fetal growth. PLoS One 2013; 8:e71459.
122. McPherson NO, Owens JA, Fullston T, Lane M. Preconception diet or exercise intervention in obese fathers normalizes sperm microRNA profile and metabolic syndrome in female offspring. Am J Physiol Endocrinol Metab 2015; 308:E805-21.
123. Lecomte V, Maloney CA, Wang KW, Morris MJ. Effects of paternal obesity on growth and adiposity of male rat offspring. Am J Physiol Endocrinol Metab 2017; 312:E117-E25.
124. Kobayashi H, Sakurai T, Miura F, Imai M, Mochiduki K, Yanagisawa E, et al. High-resolution DNA methylome analysis of primordial germ cells identifies gender-specific reprogramming in mice. Genome Res 2013; 23:616-27.
125. Hammer B, Kadalayil L, Boateng E, Buschmann D, Rezwan FI, Wolff M, et al. Preconceptional smoking alters spermatozoal miRNAs of murine fathers and affects offspring's body weight. Int J Obes (Lond) 2021; 45:1623-7.
126. Fedak KM, Bernal A, Capshaw ZA, Gross S. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerg Themes Epidemiol 2015; 12:14.
127. Hofler M. Causal inference based on counterfactuals. BMC Med Res Methodol 2005; 5:28.

9. Papers I-III

I

PLOS ONE

RESEARCH ARTICLE

Body silhouettes as a tool to reflect obesity in the past

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Abstract

Life course data on obesity may enrich the quality of epidemiologic studies analysing health consequences of obesity. However, achieving such data may require substantial resources.

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We investigated the use of body silhouettes in adults as a tool to reflect obesity in the past. We used large population-based samples to analyse to what extent self-reported body silhouettes correlated with the previously measured (9–23 years) body mass index (BMI) from both measured (European Community Respiratory Health Survey, N = 3 041) and self-reported (Respiratory Health In Northern Europe study, N = 3 410) height and weight. We calculated Spearman correlation between BMI and body silhouettes and ROC-curve analyses for identifying obesity (BMI $\geq$30) at ages 30 and 45 years. Spearman correlations between measured BMI age 30 (±2y) or 45 (±2y) and body silhouettes in women and men were between 0.62–0.66 and correlations for self-reported BMI were between 0.58–0.70. The area under the curve for identification of obesity at age 30 using body silhouettes *vs* previously measured BMI at age 30 (±2y) was 0.92 (95% CI 0.87, 0.97) and 0.85 (95% CI 0.75, 0.95) in women and men, respectively; for previously self-reported BMI, 0.92 (95% CI 0.88, 0.95) and 0.90 (95% CI 0.85, 0.96). Our study suggests that body silhouettes are a useful epidemiological tool, enabling retrospective differentiation of obesity and non-obesity in adult women and men.

Introduction

Obesity has emerged as one of the most prevalent risk factors for non-communicable diseases [1]. Many studies investigating the association between obesity and disease are based on simple measurements of height and weight at one time in life. Life course data on obesity may enrich the quality of epidemiologic studies of the related health consequences [2, 3]. However, many studies have not collected such data during the life course of the study participants. Figural body silhouettes from various ages might provide a possibility for retrospectively assessing obesity at several time points in the past. One possibility for assessing weight or body size in the past is to use figural body silhouettes from various ages. Sorensen et al introduced nine figural body silhouettes in 1983, from extremely lean to extremely obese, as an easy to-administer self-reported measure of body image [4]. The figural scales have been used in several studies as an adjunct to objective measured or self-reported height and weight or to assess body satisfaction [5–7]. We introduced body silhouettes (Fig 1), slightly modified from the Stunkard body images [4], in the third follow-up of the population-based cohort studies European Community Respiratory Health Survey (ECRHS) [8, 9] and Respiratory Health In Northern Europe (RHINE) study [10, 11].

Several studies have shown high correlation between objectively measured or self-reported current height and weight and body silhouettes [6, 7, 12] and we have recently reported that it is possible to define obesity and current body mass index (BMI) with high reliability from body silhouettes [13]. There is, however, little knowledge concerning the validity of assessing obesity in the past using self-reported recalled body silhouettes, beyond two small studies from selected populations assessing recall of body silhouettes, reflecting their child and adolescent years [14, 15]. Large population-based studies investigating recall of body silhouettes in adults are lacking.

We investigated the use of body silhouettes as a tool to reflect past obesity in adult men and women. We used large population-based samples to analyse to what extent self-reported body silhouettes correlated with BMI obtained from both measured and self-reported height and weight in previous surveys, conducted 9–23 years before the reporting of the body silhouettes.

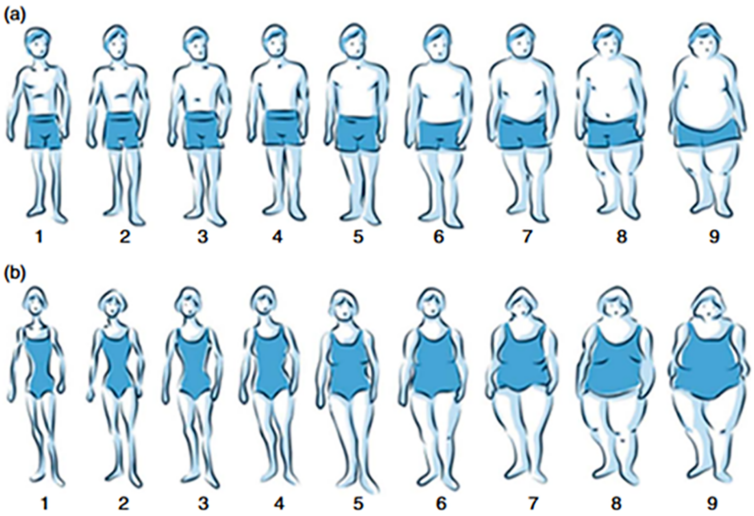

Fig 1. Body silhouettes for a) men and b) women introduced in the ECRHS III and RHINE III study. a) men, tick off your silhouette at ages: current, 8 years, voice break, 30 years, 45 years and 55 years. **b)** women, tick off your silhouette at ages: current, 8 years, menarche, 30 years, 45 years and menopause.

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Materials and methods

Study design

European Community Respiratory Health Survey. In 1991–1993, the European Community Respiratory Health Survey (ECRHS, www.ecrhs.org) first stage postal screening questionnaire was administered to population-based samples aged 20–44 years from 56 centres. The samples were randomly selected from the national population registers [8]. The ECRHS II was a follow-up study of the participants in the clinical phase of ECRHS I, performed in 29 centres between 1999 and 2002 [9]. Participants underwent the same clinical examination as in the first survey. ECRHS III is the third wave of data collection in 29 centres. It was conducted in 2011–13.

Respiratory Health In Northern Europe study. In seven Northern European centres (Reykjavik, Iceland; Bergen, Norway; Umeå, Uppsala and Gothenburg, Sweden; Aarhus, Denmark; and Tartu, Estonia) all responders to the first postal survey (ECRHS I) were followed at two time-points (RHINE II and RHINE III) in a large longitudinal questionnaire study, the Respiratory Health In Northern Europe study RHINE, www.rhine.nu).

Study population

In the current study, we included persons who had A: reported their body silhouettes at age 30 or 45 in ECRHS III or RHINE III, and B: who were aged 30 (±2) years or age 45 (±2) years when they participated in previous study phases, so that they had had their height and weight measured or reported at those ages (measured in ECRHS I or II, reported in RHINE II).

We included ±2 years around age 30 and 45 to increase sample size, making the assumption that major changes in body size are unlikely to occur over two years.

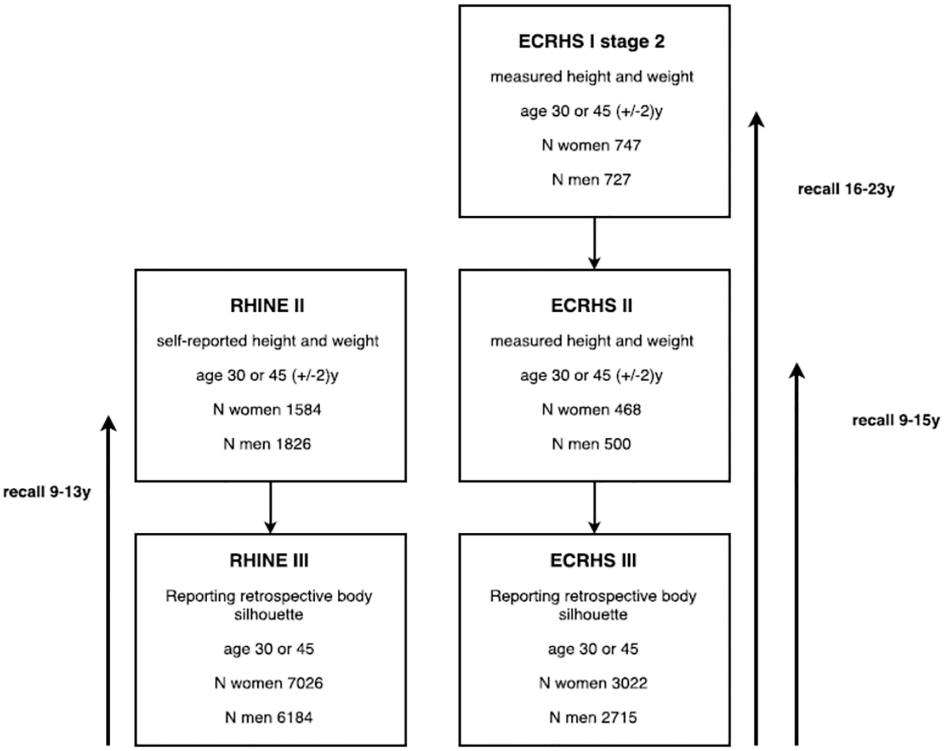

Fig 2. Flow chart, study population with participation in the RHINE III or ECRHS III study, reporting body silhouette at age 30 or 45, and with self-reported or objectively measured height and weight at age 30(±2y) or 45(±2y) in RHINE II or ECRHS I or II.

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Data from ECRHS were used to validate the selected body silhouettes against BMI from measured height and weight. Among the 3 022 women and 2 715 men in ECRHS III who reported their body silhouette at age 30 or 45, 468 women and 500 men were 30 (±2) years or 45(±2) years in ECRHS II when they had their height and weight measured. In addition, 747 women and 727 men were age 30 (±2) years or 45(±2) years in ECRHS I when height and weight was measured (Fig 2). Among the 7 026 women and 6 184 men in RHINE III who reported their body silhouette at age 30 or 45, 1 584 women and 1 826 men were 30 (±2) years or 45 (±2) years in RHINE II when they reported their height and weight (Fig 2).

Ethics

Medical research ethics committees in each study centre approved the study protocols, according to the Helsinki declaration, and all participants gave their written consent (S1 Resource).

Body silhouettes

The figural body silhouettes (Fig 1) introduced in ECRHS III and RHINE III were designed specifically for the survey by Alejandro Villén-Real [13]. These are based on Stunkard´s body

image scales [4], but slightly modified with blue colour and marked clothes. Participants were asked to tick the figural scale that best described their figure at specific time points including the ages 30 and 45 years.

Statistical methods

All analyses were stratified by sex and validations were done separately for the body silhouettes at age 30 and 45 against objectively measured height and weight for the ECRHS participants and self-reported height and weight for the RHINE participants. The strength of the monotonic relationship between body silhouettes and BMI was estimated in terms of Spearman correlation coefficients and box plots showing median BMI and interquartile range for each body silhouette. Obesity was defined as BMI $\geq$ 30 kg/m^2 according to WHO criteria. The body silhouette's ability to classify the participants correctly according to the body mass index cut-offs was investigated by non-parametric Receiver-operating characteristic curves (ROC) and calculation of Area under the curve (AUC) with the body silhouettes specified as ordinal classification variables. The optimal cut-off was defined as the cut-off resulting from the best trade-off between specificity and sensitivity according to the Youden Index, which is defined as sensitivity + specificity -1 [16]. All analyses were performed using Stata SE 14.0.

In order to investigate how the number of recall years in the ECRHS cohort affected the association between BMI and body silhouettes, we performed additional analyses stratified on number of recall years between report of body silhouette and measurement of BMI.

Results

In ECRHS III 1 215 women and 1 227 men reported their recalled body silhouette at age 30 or 45 years and had their BMI measured at that age in ECRHS I or II. Mean age and BMI were slightly higher in men than in women and the median reported body silhouette in ECRHS III was silhouette #5 for both women and men (Table 1). With increasing figural scale, from scale 1 (extremely lean) to scale 9 (extremely obese), the measured BMI (median, 25th and 75th percentiles; measured values) increased in both women and men, and for both ages 30 and 45 years (Fig 3). The same was found for self-reported BMI (Fig 4). From body silhouette #5 and upwards, the majority of the respondents were overweight or obese according to BMI derived from height and weight measured in ECRHS I or II (S1 Fig).

Validation against measured BMI in women and men

Despite a recall interval of 9–15 years, there was a relatively strong correlation between self-reported past body silhouettes and previously measured BMI in women at ages 30 and 45, with

Table 1. Characteristics of the study population reporting body silhouettes in ECRHS III and in RHINE III.

	ECRHS III		*RHINE III*	
	Women	**Men**	**Women**	**Men**
n	1215	1227	1584	1826
Age, mean (SD)	54.2 (7.5)	55.3 (7.4)	46.0 (6.6)	49.9 (7.5)
Height in meter, mean	1.63	1.77	1.68	1.81
Weight, kg, mean (SD)	71.1 (14.6)	86.3 (14.9)	69.6 (13.4)	86.7 (14.1)
BMI, mean (SD)	26.7 (5.3)	27.7 (4.4)	24.8 (4.7)	26.5 (4.0)
Current body silhouette, median	5	5	4	5

Abbreviations: ECRHS, European community respiratory health survey; RHINE, Respiratory Health In Northern Europe.

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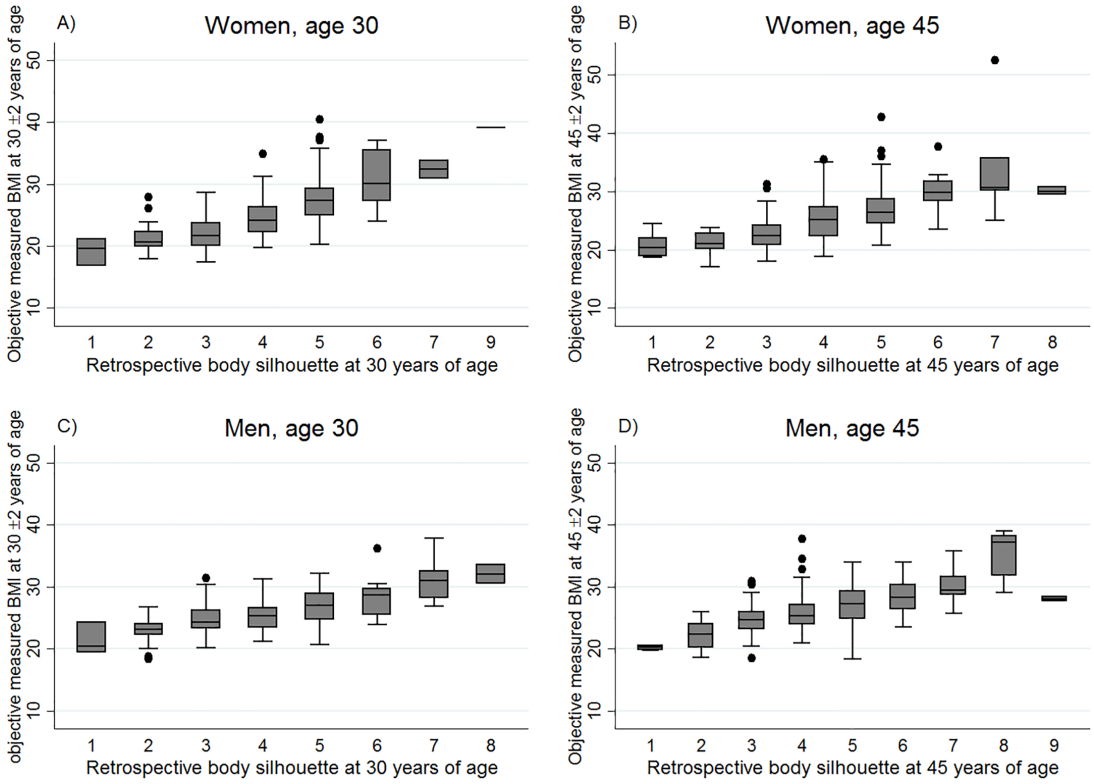

Fig 3. Box-and-whisker plots showing the distribution of measured BMI by figural scale (reported in ECRHS III), according to sex, in European adults aged 30 (±2)years or 45(±2) years in ECRHS II,: **A)** n 172, BMI range 16.7–40.3; no reporting on figure #8; **B)** n 296, BMI range 17.0–52.5, no reporting on figure #9; **C)** n 138, BMI range 18.4–37.9, no reporting on figure #9; **D)** n 362, BMI range 18.3–39.1. The bottom and top edge of the box represent the first and third quartiles (interquartile range); the line within the box represents the median; the ends of the bottom and top whiskers represent the upper and lower adjacent values; and the dots represent outliers (ECRHS, the European Community Respiratory Health Survey).

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a Spearman correlation of 0.64 and 0.62, respectively. With a more extended recall interval of 16–23 years, the correlation at ages 30 and 45 decreased somewhat to 0.49 and 0.57, respectively (Table 2.)

The Spearman correlation between measured BMI and self-reported past body-silhouettes in men showed approximately similar results as for women, although with a higher correlation for the shorter recall time and a somewhat lower correlation for the longer recall time (Table 2).

Among obese women (BMI≥30) at age 30 years, the ROC analysis yielded an AUC value of 0.92, (95% CI 0.87, 0.97) when the recall time was 9 to 15 years (Table 3; S2 Fig), and the AUC value was 0.88, (95% CI 0.80, 0.96) when the recall time was 16–23 years (S2 Fig). In women, body silhouette #5 had the best trade-off between sensitivity and specificity for obesity at age 30 with 16% misclassification at 9–15 year recall, i.e. 16% of participants were either not obese and classified as obese or they were obese and classified as not obese. For 16–23 year recall, 12% were misclassified when body silhouette #5 was used as cut-off for classification of obesity

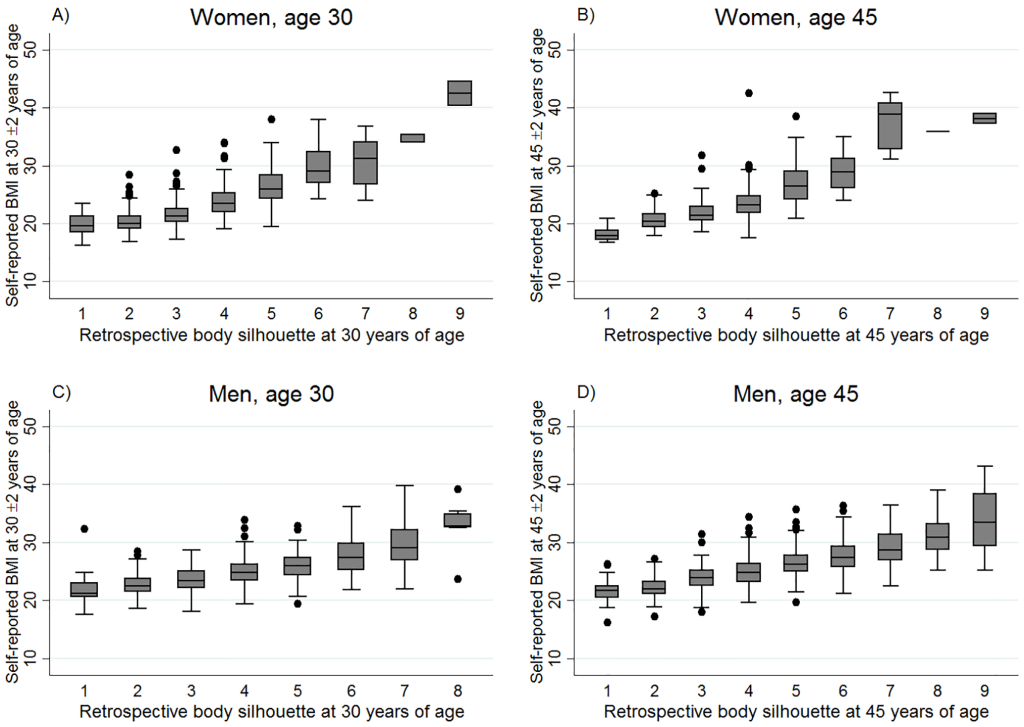

Fig 4. Box-and-whisker plots showing the distribution of self-reported BMI by figural scale, according to sex, in North-European adults aged 30(±2) years or 45(±2) years in RHINE II, reporting body silhouette in RHINE III: **A)** n 1145; **B)** n 439; **C)** n 829; **D)** n 997. The bottom and top edge of the box represent the first and third quartiles (interquartile range); the line within the box represents the median; the ends of the bottom and top whiskers represent the upper and lower adjacent values; and the dots represent outliers (RHINE, the Respiratory Health in Northern Europe study).

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(Table 3). The AUC for detecting obesity in women at age 45 was somewhat lower than for detecting obesity at age 30, with AUC 0.82, (95% CI 0.75, 0.89) with a recall of 9–15 years and 0.80, (95% CI 0.72, 0.89) at a recall 16–23 years. Body silhouette #5 had the best trade-off between sensitivity and specificity to detect obesity in women age 45 with a recall of 9–15 years. For a recall of 16–23 years, body silhouette #4 had the best trade-off. The misclassification was 20% at a recall of 9–15 years and 50% at a recall of 16–23 years (Table 3).

In men, the AUC values for detecting obesity at age 30 were 0.85, (95% CI 0.75, 0.95) with a recall time of 9–15 years, and 0.78, (95% CI 0.63, 0.93) for a recall time of 16–23 years (Table 3; S2 Fig). For 9–15 year recall, body silhouette #6 had the best trade-off between sensitivity and specificity for obesity, with a 16% misclassification. For 16–23 year recall, body silhouette #5 had the best cut-off, with 19% misclassification (Table 3).

To detect obesity in men at age 45, the AUC did not vary by recall time, AUC 0.79, (95% CI 0.73, 0.85) with a recall of 9–15 years and AUC 0.80, (95% CI 0.72, 0.87) with at recall time of 16–23 years (Table 3). Body silhouette #5 had the best trade-off between sensitivity and specificity to detect obesity in men age 45, regardless of recall time, with a misclassification of 37–40% (Table 3).

Table 2. Correlation between objectively measured (ECRHS I or II) or self-reported (RHINE II) height and weight and body silhouettes reported in ECRHS III and RHINE III.

Reporting of body silhouette (BS) ECRHS III, measured BMI	*Age*	*n*	*Mean BMI (SD)*	*Median reported BS*	*Spearman correlation**	*p-value***
Women						
Recall time, 9-15y	30±2y	172	24.3 (4.6)	4	0.64	p <.001
(ECRHS II)	45±2y	296	24.9 (4.4)	4	0.62	p <.001
Recall time, 16-23y	30±2y	442	22.7 (3.8)	3	0.49	p <.001
(ECRHS I)	45±2y	305	24.0 (4.1)	4	0.57	p <.001
Men						
Recall time, 9-15y	30±2y	138	25.9 (3.6)	4	0.66	p <.001
(ECRHS II)	45±2y	362	26.5 (3.7)	4	0.63	p <.001
Recall time, 16-23y	30±2y	365	24.3 (3.2)	3	0.54	p <.001
(ECRHS I)	45±2y	362	25.4 (3.3)	4	0.48	p <.001
Reporting of body silhouette (BS) RHINE III, self-reported BMI	*Age*	*n*	*Mean BMI (SD)*	*Median reported BS*	*Spearman correlation**	*p-value***
Women						
Recall time, 9-13y	30±2y	1145	23.1 (4.1)	4	0.70	p <.001
	45±2y	439	23.9 (5.0)	4	0.68	p <.001
Men						
Recall time, 9-13y	30±2y	829	24.7 (3.1)	4	0.58	p <.001
	45±2y	997	25.7 (3.3)	4	0.63	p <.001

Abbreviations: ECRHS, European community respiratory health survey; RHINE, Respiratory Health In Northern Europe; BS, Body Silhouette.

*Spearman correlations between objectively measured (ECRHS I or II) or self-reported (RHINE II) height and weight and reported body silhouettes in ECRHS III or RHINE III.

**test for significance of Spearman correlation.

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Validation against self-reported BMI in women and men

Validating past body silhouettes against self-reported height/weight data gave approximately similar results as the validation against measured height/weight. In RHINE III 1 584 women and 1 826 men reported their historical body silhouette at age 30 or 45 years and had 9–13 years earlier self-reported their height and weight in RHINE II. In RHINE III mean age and BMI was higher in men than in women and median reported body silhouette was #4 for women and #5 for men (Table 1). For women, the Spearman correlation between self-reported BMI in RHINE II and reporting of body silhouette age 30 (RHINE III), was 0.70. At age 45, spearman correlation was 0.68 (Table 2). For men, the spearman correlation was 0.58 between self-reported BMI (RHINE II), and reporting of body silhouette age 30 (RHINE III), and 0.68 for age 45. The recall time was 9–13 years in the RHINE study (Table 2).

In women, the AUC from ROC curve analyses was 0.92, (95% CI 0.88, 0.95) at age 30 (±2y), and 0.87, (95% CI 0.79–0.94) at age 45(±2y). Body silhouette #5 had the best trade-off between sensitivity and specificity for obesity at ages 30 and 45 years, with 14% and 17% respectively, being misclassified (Table 3).

For men, the AUC from ROC curve analyses were 0.90, (95% CI 0.85, 0.96) at age 30(±2y), and 0.84, (95% CI 0.80, 0.88) at age 45(±2y). Body silhouette #6 had the best trade-off between sensitivity and specificity for obesity at ages 30 and 45 years, with 9% and 18%, respectively, being misclassified (Table 3).

Table 3. Discriminatory capabilities of body silhouettes for identifying obesity (BMI≥30) retrospectively, according to sex and recall time in women and men age 30(±2) or 45(±2) years in ECRHS I or II with objectively measured height and weight and in RHINE II with self-reported height and weight. Results of ROC curve analysis.

Reporting of body silhouette ECRHS III	*Age*	*n*	*AUC (95% CI)*	*OC**	*Youden Index*	*Sensitivity, % (95% CI)*	*Specificity, % (95% CI)*	*Correctly classified %*
Women								
Recall 9-15y	30±2y	172	0.92 (.87, .97)	5	.715	88.2 (.64, .99)	83.2 (.76, .89)	83.7
(ECRHS II)	45±2y	296	0.82 (.75, .89)	5	.511	72.7 (.54, .87)	78.3(.73, .83)	77.7
Recall 16-23y	30±2y	442	0.88 (.80, .96)	5	.633	75.0 (.48, .93)	88.3 (.85, .91)	87.8
(ECRHS I)	45±2y	305	0.80 (.72, .89)	4	.416	96.4 (.82, .99)	45.1 (.39, .51)	49.8
Men								
Recall 9–15 y	30±2y	138	0.85 (.75, .95)	6	.533	66.7 (.41, .87)	86.7(.79, .92)	84.1
(ECRHS II)	45±2y	362	0.79 (.73, .85)	5	.433	85.2 (.73, .93)	59.1(.73, .83)	63.0
Recall 16–23 y	30±2y	365	0.78 (.63, .93)	5	.548	73.3 (.45, .92)	81.4(.45, .92)	81.1
(ECRHS I)	45±2y	362	0.80 (.72, .87)	5	.463	77.4 (.82, .99)	68.9 (.64, .74)	69.6
Reporting of body silhouette RHINE III	*Age*	*n*	*AUC (95% CI)*	*OC**	*Youden Index*	*Sensitivity, % (95% CI)*	*Specificity, % (95% CI)*	*Correctly classified %*
Women								
Recall 9-13y	30±2y	1145	0.92 (.88, .95)	5	.715	85.5 (.74, .93)	86.0 (.84, .88)	86.0
	45±2y	439	0.87 (.79, .94)	5	.679	85.7 (.70, .95)	82.2 (.78, .86)	82.5
Men								
Recall 9-13y	30±2y	829	0.90 (.85, .96)	6	.689	77.3 (.62, .89)	91.6 (.89, .93)	90.8
	45±2y	997	0.84 (.80, .88)	6	.505	67.9 (.74, .93)	82.6 (.80, .85)	81.4

Abbreviations: AUC, Area Under the Curve; BMI, Body Mass Index; ECRHS, European community respiratory health survey; RHINE, Respiratory Health In Northern Europe.

*OC, optimal sensitivity and specificity criterion in relation to body silhouette.

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Discussion

This analysis of large population-based cohorts of adult women and men showed that reported past body silhouettes correlated with BMI as measured (reported) at the corresponding ages, and that the retrospective body silhouettes made it possible to differentiate between obese and non-obese persons at previous ages with an acceptable validity. Longer recall times weakened the correlations to some extent, while the age at the time of recalled body size was of larger importance for detecting obesity. These results were the same for both women and men, and the findings were consistent when validated against measured as well as self-reported anthropometric data.

This is the first study to validate the use of self-reported body silhouettes against previously measured or self-reported height and weight during adulthood in a large study population, and to study the importance of recall time. The concept that body silhouettes may be a valid tool to recall body size back in time is supported by two previous studies. Must et al [14] asked elderly men and women aged 71–76 years (n 181) to select body silhouettes reflecting their childhood and adolescent years. With a recall time of several decades they found that selected body sizes were reasonably well correlated with measured BMI age 20 ($r^{Pearsson}$ = 0.51 in men and 0.64 in women). A study by Koprowski et al [15] investigated the ability of women with an average age of 17 years (n = 132) to recall body size at the age of menarche using body silhouettes, and found good correlation between actual BMI at the time of menarche and body silhouette ($r^{Pearsson}$ = 0.77). Thus, good correlation between body silhouettes and measured

height and weight is a consistent finding in these studies of elderly and adolescent body size and our study of adult body sizes.

In our study there were no significant gender differences, but the discriminatory capability of the body silhouettes to identify obesity was slightly better in women than in men, both with regard to measured and self-reported BMI. Must et al [14] also suggested a somewhat stronger correlation in women, and they found gender differences in over- *vs* underestimation of high school weight. There is no previous literature addressing potential differences relating to recall time. In our analysis, the associations of recalled body silhouette with measured/reported height and weight did not significantly differ according to recall time, although correlations appeared somewhat weaker for the longer recall time. This agrees with the findings of Must et al [14] of overall moderate correlations even in the remote past. However, our analyses suggest that obesity was better discriminated at age 30 years than at age 45 years, a higher percentage of both women and men were "correctly classified" as obese at age 30. We speculate that this could be related to age 45 years being an age of transition and a more unstable weight.

The body silhouettes showed the best discriminatory capability for obese women and men age 30(±2y) with short recall time. The ROC curve analyses showed that for both women and men age 30(±2y), more than 80% were correctly classified when the optimal cut-off value according to the Youden index was applied. For some groups, however, the sensitivities were quite low when the optimum cut-off was used. There is therefore, no support for a common cut-off that could be used to accurately separate between obese and non-obese participants, for both genders and all ages. There was an overlap of the BMI- range associated with each body silhouette, as the body silhouettes represent gradations in body size. In general, the retrospective body silhouettes are best used as an ordinal measure of body size and probability of obesity, rather than a measure to strictly define BMI groups.

The differences in associations with measured and self-reported height and weight were minimal. We observed a slightly higher percentage of correctly classified individuals using self-reported data than when using objectively measured height and weight. This might be due to the fact that both body silhouettes and weight and height were self-reported in RHINE, and participants who have a tendency to over- or under-report past body size might possibly also have a tendency to previously have over- or under-reported weight.

This study's strong point is the high numbers of participant with data on measured or self-reported height and weight at two time points in the past, in both women and men. Participants were originally recruited randomly from the general population living at the respective study sites. The multi-centre design is also a strong asset. Our results should thereby be generalizable to adult women and men living in areas comparable to the investigated study sites in Europe and Melbourne, Australia. The tool would need to be validated for use in other populations.

As to the limitations of the self-reported body silhouettes, individual differences in misconception of body size cannot be captured by our analysis. Further, there has been some criticism of the coarse and ordinal nature of the body silhouette scale. Their ordinal and fixed scale forces people to decide on one figure or the other, even though they might feel they are in between two figural scales. This may contribute to the variation in BMI within each figural scale [17, 18].

Most studies investigating the association between obesity and disease are based on single measurements of height and weight at one time in life. Life course data on obesity may enrich the quality of epidemiologic studies of the related health consequences [2, 3]. In the absence of previously measured height and weight, alternative methods for assessment are needed that can capture key features of weight history. The use of body silhouettes through the lifespan may be a simple and inexpensive epidemiological tool to obtain this information.

In conclusion, our study suggests that body silhouettes can be a satisfactory correlate for past body size in adulthood and a useful epidemiological tool to differentiate retrospectively between non-obesity or obesity in women and men. There was no specific cut-off for the body silhouettes that can be used to define obese persons, but the probability of being obese increased with increasing body silhouette.

Supporting information

S1 Fig. Distribution of retrospectively self-reported body silhouettes in relation to measured height and weight in ECRHS I or II (different **colours** illustrating the different BMI-categories; y-axis showing percent of BMI-category in each bar; x-axis showing the different body silhouettes by number).
(TIF)

S2 Fig. ROC-curves, discriminatory capabilities of body silhouettes for identifying obesity retrospectively, according to sex and recall time in women and men age 30(±2) years in the European Community Respiratory Health Survey (ECRHS I/II) with objectively measured height and weight; A) women 30(±2)y, recall time 9–15 y, sensitivity = 0.88, specificity = 0.83, AUC = 0.92; **B)** women 30(±2)y, 16-23y recall time, sensitivity = 0.75, specificity = 0.88, AUC = 0.88; **C)** men 30(±2)y, recall time 9-15y, sensitivity = 0.67, specificity = 0.87, AUC = 0.85; **D)** men 30(±2)y, 16-23y recall time, sensitivity = 0.73, specificity = 0.81, AUC = 0.78 (ROC, receiver-operating characteristic; AUC, area under the curve).
(TIF)

S1 Resource. Ethics committees and approval numbers ECRHS/RHINE.
(DOCX)

S2 Resource. Funding. Sources for the local ECRHS and RHINE studies.
(DOCX)

Acknowledgments

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References

1. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet. 2011 Feb 12; 377 (9765):557–67. https://doi.org/10.1016/S0140-6736(10)62037-5 PMID: 21295846.
2. Global BMIMC, Di Angelantonio E, Bhupathiraju Sh N, Wormser D, Gao P, Kaptoge S, et al. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. Lancet. 2016 Aug 20; 388(10046):776–86. https://doi.org/10.1016/S0140-6736(16)30175-1 PMID: 27423262.
3. Stokes A, Ni Y. Validating a summary measure of weight history for modeling the health consequences of obesity. Annals of epidemiology. 2016 Dec; 26(12):821–6 e2. https://doi.org/10.1016/j.annepidem.2016.10.005 PMID: 27894565.
4. Sorensen TI, Stunkard AJ, Teasdale TW, Higgins MW. The accuracy of reports of weight: children's recall of their parents' weights 15 years earlier. Int J Obes. 1983; 7(2):115–22. PMID: 6862758.
5. Romieu I, Escamilla-Nunez MC, Sanchez-Zamorano LM, Lopez-Ridaura R, Torres-Mejia G, Yunes EM, et al. The association between body shape silhouette and dietary pattern among Mexican women. Public Health Nutr. 2012 Jan; 15(1):116–25. https://doi.org/10.1017/S1368980011001182 PMID: 21875454.
6. Tehard B, van Liere MJ, Com Nougue C, Clavel-Chapelon F. Anthropometric measurements and body silhouette of women: validity and perception. J Am Diet Assoc. 2002 Dec; 102(12):1779–84. PMID: 12487540.
7. Munoz-Cachon MJ, Salces I, Arroyo M, Ansotegui L, Rocandio AM, Rebato E. Overweight and obesity: prediction by silhouettes in young adults. Obesity (Silver Spring). 2009 Mar; 17(3):545–9. https://doi.org/10.1038/oby.2008.541 PMID: 19057522.
8. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. The European respiratory journal. 1994 May; 7(5):954–60. PMID: 8050554.
9. European Community Respiratory Health Survey IISC. The European Community Respiratory Health Survey II. The European respiratory journal. 2002 Nov; 20(5):1071–9. PMID: 12449157.
10. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, et al. Longterm follow-up in European respiratory health studies—patterns and implications. BMC pulmonary medicine. 2014; 14:63. https://doi.org/10.1186/1471-2466-14-63 PMID: 24739530.
11. Gomez Real F, Perez Barrionuevo L, Franklin K, Lindberg E, Bertelsen RJ, Benediktsdottir B, et al. The Association of Gum Bleeding with Respiratory Health in a Population Based Study from Northern Europe. PloS one. 2016; 11(1):e0147518. https://doi.org/10.1371/journal.pone.0147518 PMID: 26808490.

12. Bulik CM, Wade TD, Heath AC, Martin NG, Stunkard AJ, Eaves LJ. Relating body mass index to figural stimuli: population-based normative data for Caucasians. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity. 2001 Oct; 25 (10):1517–24. https://doi.org/10.1038/sj.ijo.0801742 PMID: 11673775.

13. Dratva J, Bertelsen R, Janson C, Johannessen A, Benediktsdottir B, Braback L, et al. Validation of self-reported figural drawing scales against anthropometric measurements in adults. Public Health Nutr. 2016 Aug; 19(11):1944–51. https://doi.org/10.1017/S136898001600015X PMID: 26879067.

14. Must A, Willett WC, Dietz WH. Remote recall of childhood height, weight, and body build by elderly subjects. American journal of epidemiology. 1993 Jul 01; 138(1):56–64. PMID: 8333427.

15. Koprowski C, Coates RJ, Bernstein L. Ability of young women to recall past body size and age at menarche. Obesity research. 2001 Aug; 9(8):478–85. https://doi.org/10.1038/oby.2001.62 PMID: 11500528.

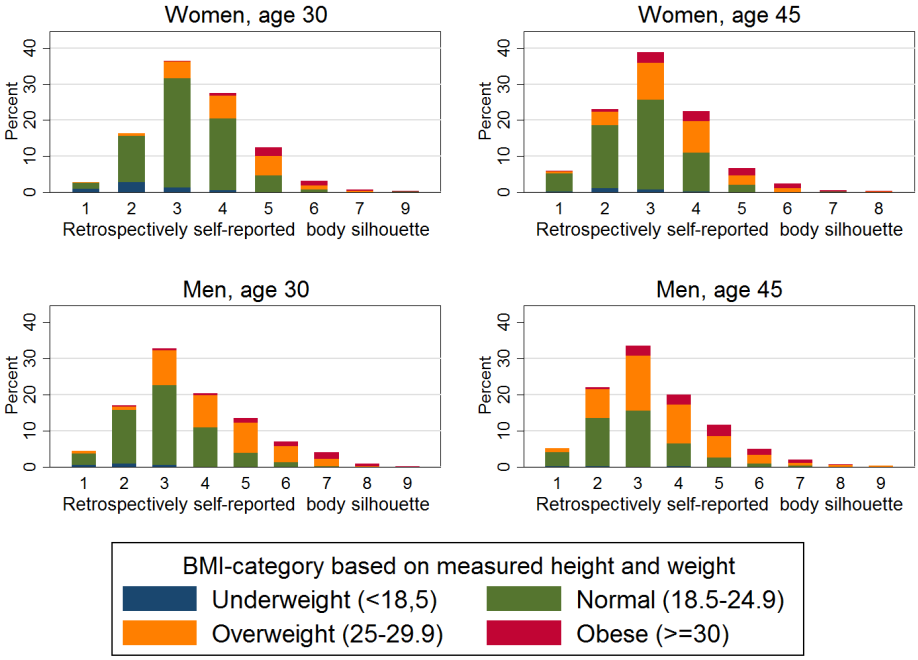
16. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. Biometrical journal Biometrische Zeitschrift. 2005 Aug; 47(4):458–72. PMID: 16161804.

17. Kaufer-Horwitz M, Martinez J, Goti-Rodriguez LM, Avila-Rosas H. Association between measured BMI and self-perceived body size in Mexican adults. Annals of human biology. 2006 Sep-Dec; 33(5–6):536–45. PMID: 17381052.

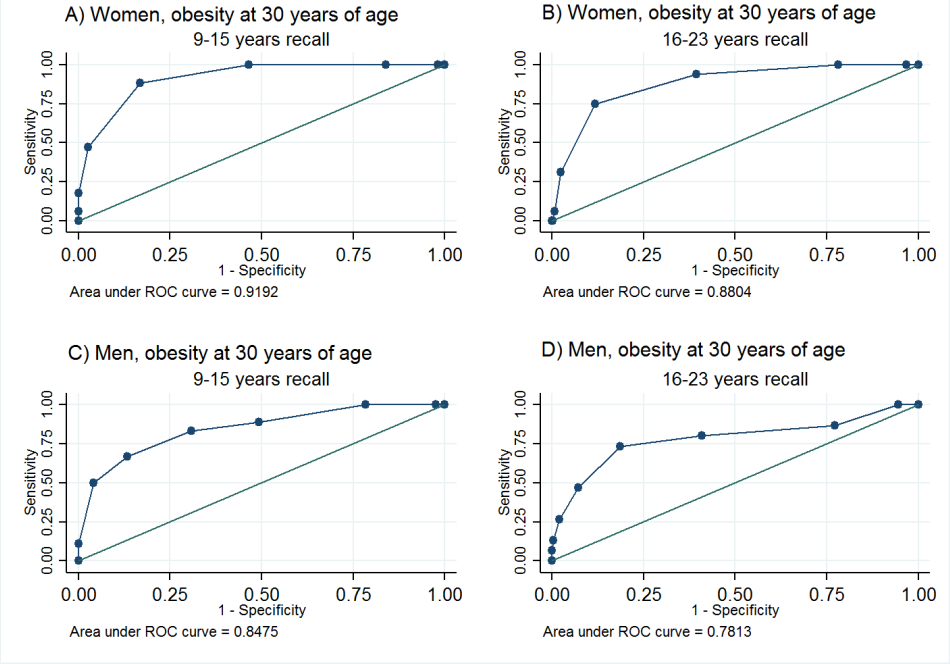
18. Dratva J, Bertelsen R, Janson C, Johannessen A, Benediktsdottir B, Braback L, et al. Validation of self-reported figural drawing scales against anthropometric measurements in adults. Public Health Nutr. 2016 Feb 16:1–8. https://doi.org/10.1017/S136898001600015X PMID: 26879067.

Supplementary materials

S1 Fig: Distribution of retrospectively self-reported body silhouettes in relation to measured height and weight in ECRHS I or II (different **colours** illustrating the different BMI-categories; y-axis showing percent of BMI-category in each bar; x-axis showing the different body silhouettes by number).



S2 Fig: ROC-curves, discriminatory capabilities of body silhouettes for identifying obesity retrospectively, according to sex and recall time in women and men age 30(±2) years in the European Community Respiratory Health Survey (ECRHS I/II) with objectively measured height and weight; A) women 30(±2)y, recall time 9–15 y, sensitivity = 0.88, specificity = 0.83, AUC = 0.92; **B)** women 30(±2)y, 16-23y recall time, sensitivity = 0.75, specificity = 0.88, AUC = 0.88; **C)** men 30(±2)y, recall time 9-15y, sensitivity = 0.67, specificity = 0.87, AUC = 0.85; **D)** men 30(±2)y, 16-23y recall time, sensitivity = 0.73, specificity = 0.81, AUC = 0.78 (ROC, receiver-operating characteristic; AUC, area under the curve).



II

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"

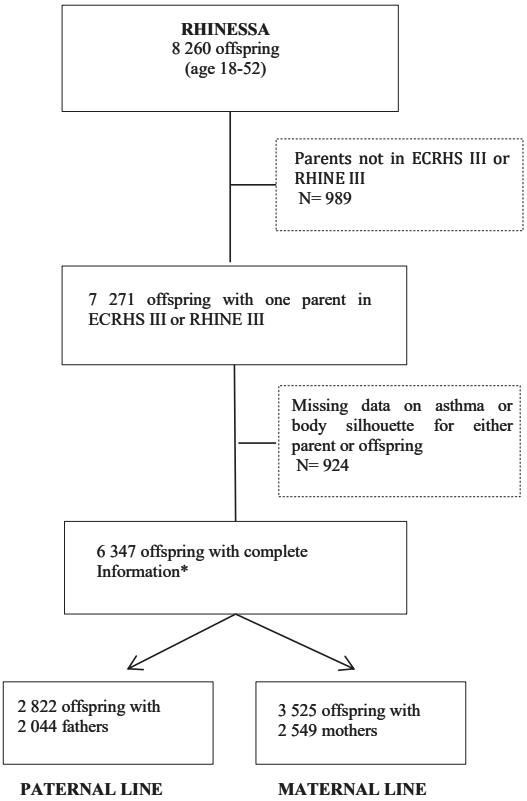

FIG 1. Study population flow chart: RHINESSA generation study. *Offspring and their participating parents with complete information on overweight status and asthma: 87% for the paternal line and 88% for the maternal line.

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

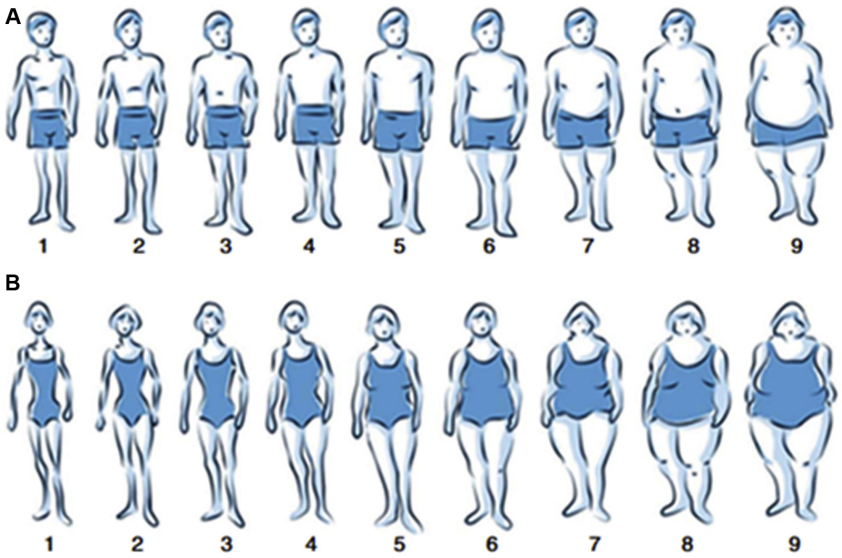

FIG 2. Figural drawing scales for men **(A)** and women **(B)** used in the ECRHS/RHINE III study and in the RHINESSA questionnaire survey. Cutoffs for overweight status were 5 or greater in men and 4 or greater in women.

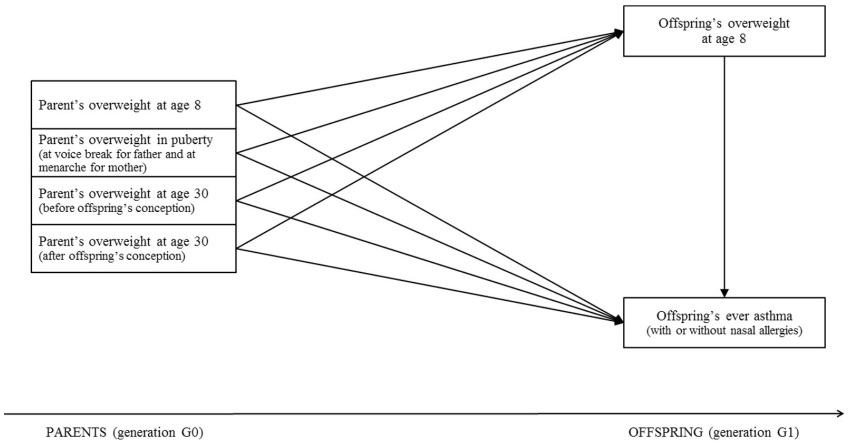

FIG 3. Schematic representation of the paths investigated within the paternal and maternal lines. Adjusted for ECRHS/RHINE parents' ever having asthma and educational level, other parents' overweight status at age 30 years and ever having asthma, and offspring's age and sex.

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.

REFERENCES

1. World Health Organization. Global health observatory (GHO) data: overweight and obesity. 2017. Available at: http://www.who.int/gho/ncd/risk_factors/overweight/en/. Accessed November 10, 2018.
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;384:766-81.
3. Prospective Studies Collaboration, 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;373:1083-96.
4. Emerging Risk Factors Collaboration, 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;377:1085-95.
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;371:569-78.
6. Eder W, Ege MJ, von Mutius E. The asthma epidemic. N Engl J Med 2006;355:2226-35.
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. J Allergy Clin Immunol 2013;131:1033-40.
8. Dumas O, Varraso R, Gillman MW, Field AE, Camargo CA Jr. Longitudinal study of maternal body mass index, gestational weight gain, and offspring asthma. Allergy 2016;71:1295-304.
9. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, et al. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 2006;14:159-66.
10. Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. Bioessays 2014;36:359-71.
11. Sales VM, Ferguson-Smith AC, Patti ME. Epigenetic mechanisms of transmission of metabolic disease across generations. Cell Metab 2017;25:559-71.
12. Svanes C, Koplin J, Skulstad SM, Johannessen A, Bertelsen RJ, Benediktsdottir B, et al. Father's environment before conception and asthma risk in his children: a multi-generation analysis of the Respiratory Health In Northern Europe study. Int J Epidemiol 2017;46:235-45.
13. Miller LL, Henderson J, Northstone K, Pembrey M, Golding J. Do grandmaternal smoking patterns influence the etiology of childhood asthma? Chest 2014;145:1213-8.
14. Accordini S, Calciano L, Johannessen A, Portas L, Benediktsdottir B, Bertelsen RJ, et al. A three-generation study on the association of tobacco smoking with asthma. Int J Epidemiol 2018;47:1106-17.
15. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J 1994;7:954-60.
16. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, et al. Longterm follow-up in European respiratory health studies—patterns and implications. BMC Pulm Med 2014;14:63.
17. Lafeuille MH, Gravel J, Figliomeni M, Zhang J, Lefebvre P. Burden of illness of patients with allergic asthma versus non-allergic asthma. J Asthma 2013;50:900-7.
18. Janson C, Kalm-Stephens P, Foucard T, Alving K, Nordvall SL. Risk factors associated with allergic and non-allergic asthma in adolescents. Clin Respir J 2007;1:16-22.
19. Dratva J, Bertelsen R, Janson C, Johannessen A, Benediktsdottir B, Braback L, et al. Validation of self-reported figural drawing scales against anthropometric measurements in adults. Public Health Nutr 2016;19:1944-51.
20. Lonnebotn M, Svanes C, Igland J, Franklin KA, Accordini S, Benediktsdottir B, et al. Body silhouettes as a tool to reflect obesity in the past. PLoS One 2018;13:e0195697.
21. Pearl J. Direct and indirect effects. Proceedings of the Seventeenth conference on Uncertainty in artificial intelligence. Seattle (WA): Morgan Kaufmann Publishers; 2001. p. 411-20.
22. Zhao X, Lynch JG Jr, Chen Q. Reconsidering Baron and Kenny: myths and truths about mediation analysis. J Consumer Res 2010;37:197-206.
23. Mark HCL, Oi-man K. Examining the rule of thumb of not using multilevel modeling: the "design effect smaller than two" rule. J Exp Educ 2015;83:423-38.
24. Muthén B. Applications of causally defined direct and indirect effects in mediation analysis using SEM in Mplus. Available at: https://www.statmodel.com/download/causalmediation.pdf. Accessed November 22, 2018.
25. Bandalos DL. Relative performance of categorical diagonally weighted least squares and robust maximum likelihood estimation. Structural Equation Modelling 2014;21:102-16.
26. VanderWeele TJ. Bias formulas for sensitivity analysis for direct and indirect effects. Epidemiology 2010;21:540-51.

27. Lutz SM, Thwing A, Schmiege S, Kroehl M, Baker CD, Starling AP, et al. Examining the role of unmeasured confounding in mediation analysis with genetic and genomic applications. BMC Bioinformatics 2017;18:344.
28. Wei Y, Schatten H, Sun QY. Environmental epigenetic inheritance through gametes and implications for human reproduction. Hum Reprod Update 2015;21:194-208.
29. Wardle J, Carnell S, Haworth CM, Plomin R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am J Clin Nutr 2008;87:398-404.
30. Braback L, Hjern A, Rasmussen F. Body mass index, asthma and allergic rhinoconjunctivitis in Swedish conscripts-a national cohort study over three decades. Respir Med 2005;99:1010-4.
31. Skulstad SM, Igland J, Johannessen A, Bertelsen RJ, Lonnebotn M, Omenaas ER, et al. Validation of maternal reported pregnancy and birth characteristics against the Medical Birth Registry of Norway. PLoS One 2017;12:e0181794.
32. Kuiper IN, Svanes C, Benediktsdottir B, Bertelsen RJ, Braback L, Dharmage SC, et al. Agreement in reporting of asthma by parents or offspring - the RHINESSA generation study. BMC Pulm Med 2018;18:122.
33. Hofler M. Causal inference based on counterfactuals. BMC Med Res Methodol 2005;5:28.
34. Must A, Willett WC, Dietz WH. Remote recall of childhood height, weight, and body build by elderly subjects. Am J Epidemiol 1993;138:56-64.
35. Wang L, Wang K, Gao X, Paul TK, Cai J, Wang Y. Sex difference in the association between obesity and asthma in U.S. adults: findings from a national study. Respir Med 2015;109:955-62.
36. Dolton P, Xiao M. The intergenerational transmission of body mass index across countries. Econ Hum Biol 2017;24:140-52.
37. Hallstrand TS, Fischer ME, Wurfel MM, Afari N, Buchwald D, Goldberg J. Genetic pleiotropy between asthma and obesity in a community-based sample of twins. J Allergy Clin Immunol 2005;116:1235-41.

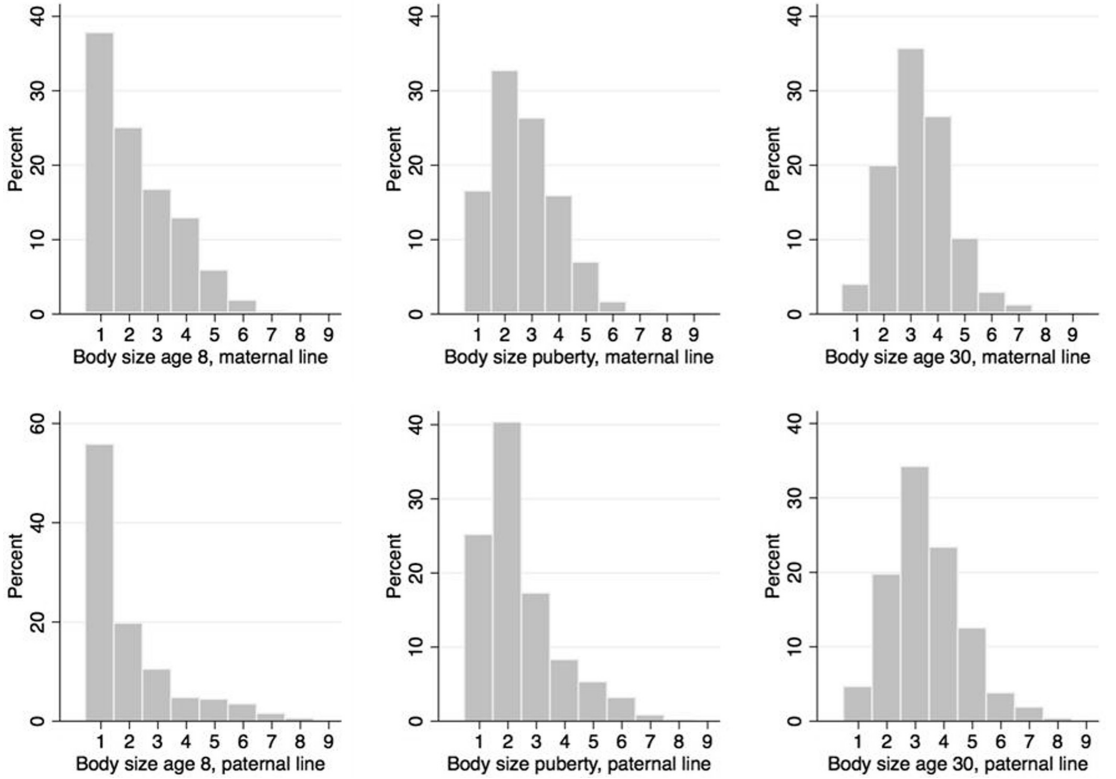

FIG E1. Body size distribution in childhood, puberty, and adulthood for participating parents in the maternal line *(3 top graphs)* and for participating parents in the paternal line *(3 bottom graphs)* in the RHINESSA generation study.

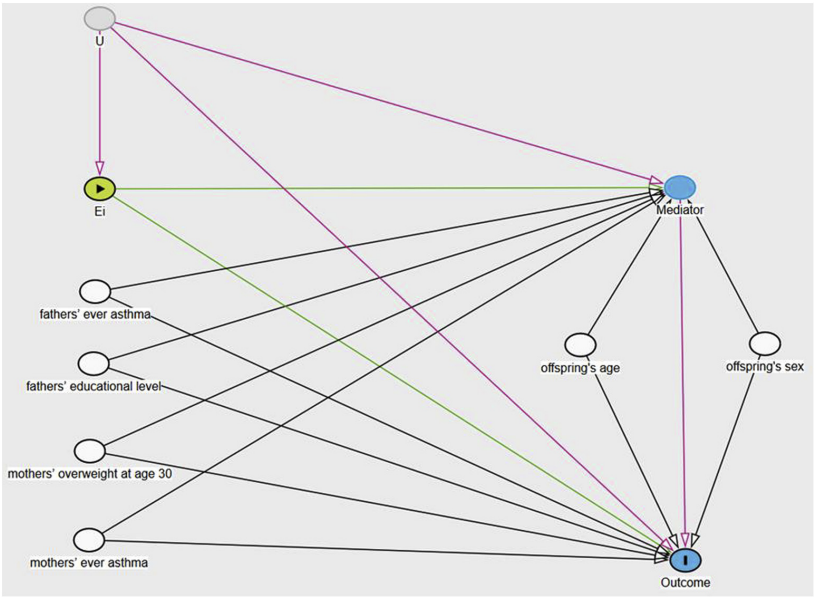

FIG E2. Directed acyclic graph showing how data were simulated for the exposure, mediator, outcome, and adjusting variables and 1 unmeasured confounder. *Ei* indicates one of the following binary exposure variables: E1, fathers' overweight status at age 8 years versus never overweight; E2, fathers' overweight 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.

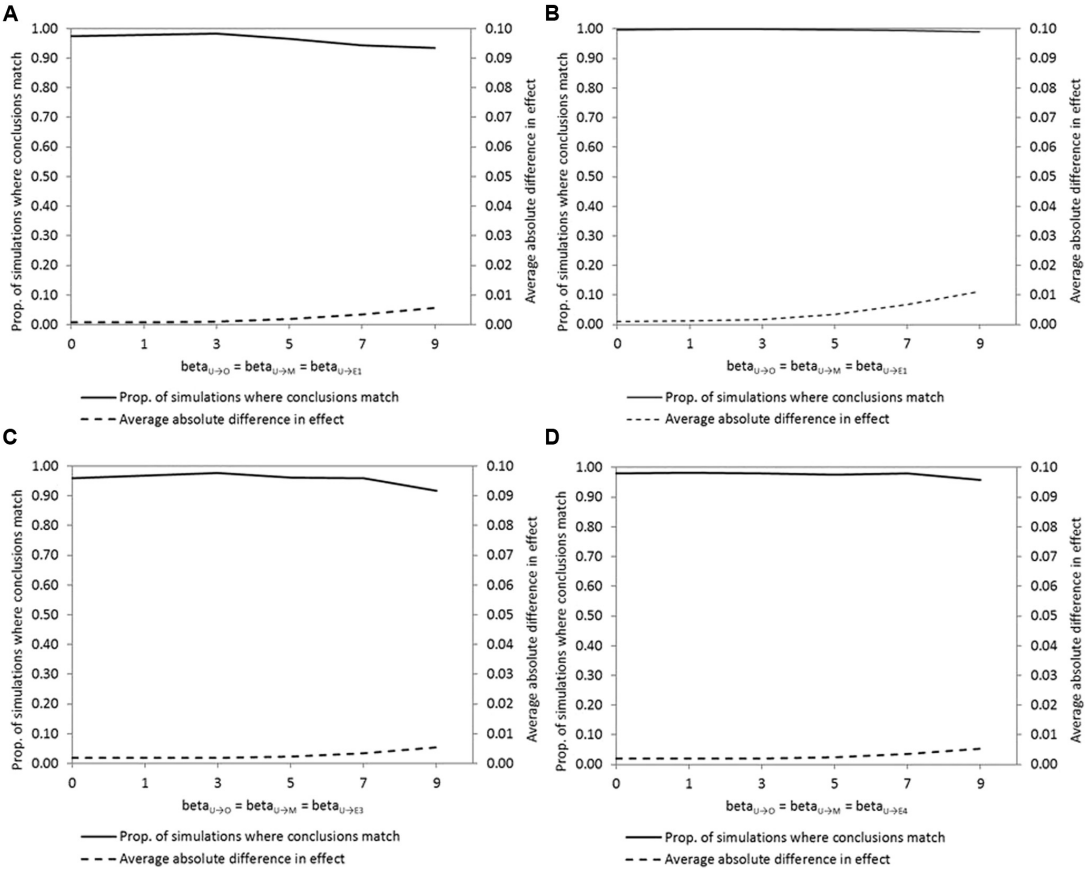

FIG E3. Proportion of simulations in which the results match and the average absolute difference for the direct effects of ECRHS/RHINE fathers' overweight status on offspring ever having asthma without nasal allergies (whether the unmeasured confounder U is included or excluded from the mediation models): **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

III

Parental prepubertal overweight and offspring lung function.

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Conflict of interest

Michael J Abramson holds investigator initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research. He undertook an unrelated consultancy for and received assistance with conference attendance from Sanofi. He also received an unrelated speaker's fee from GSK. All other authors declare no competing interests.

Words count: 3657

Abstract

Background

In a recent study we found that fathers' but not mothers' onset of overweight in puberty was causally associated with increased asthma risk in adult offspring. Potential impact on offspring's adult lung function, a key marker of general and respiratory health, has not been studied.

Objective

We investigated causal associations to estimate the causal effects of parents' overweight on adult offspring's lung function within the paternal and maternal lines.

Methods

We included 929 adult offspring (age 18-54 years, 54% daughters) of 308 fathers and 388 mothers (age 40-66 years). Counterfactual-based multi-group mediation analyses by offspring's sex (potential moderator) were used to assess whether the effects of parents' overweight before puberty on adult offspring's FEV_1, FVC and FEV_1/FVC were mediated through offspring's pre-pubertal overweight and/or adult height (potential mediators) within the paternal and maternal lines. Unknown confounding was addressed by simulation models.

Results

Fathers' overweight before puberty had a negative indirect effect, mediated through sons' height, on sons' FEV_1 [beta (95%CI): -144 (-272, -23) mL] and FVC [beta (95%CI): -210 (-380, -34) mL], and a negative direct effect on sons' FVC [beta (95%CI): -262 (-501, -9) mL]; statistically significant effects on FEV_1/FVC were not observed. Mothers' overweight before puberty had neither direct nor indirect effects on offspring's lung function.

Conclusion

Fathers' overweight starting before puberty appears to cause lower FEV_1 and FVC in their future sons. The effects were partly mediated through sons' adult height but not through sons' prepubertal

overweight. Scientific attention to male puberty may open new opportunities for targeted public health strategies.

Clinical implications

Fathers' overweight starting before puberty may negatively affect lung function in their future sons, partly mediated by reduced height in sons. Scientific attention to male puberty may open new opportunities for targeted public health strategies.

Keywords: lung function; overweight; prepuberty; paternal line; causal inference; intergenerational; counterfactual based mediation analysis; RHINE; ECRHS; RHINESSA.

Abbreviations: ECRHS, European Community Respiratory Health Survey; RHINE, Respiratory Health In Northern Europe; RHINESSA, Respiratory Health In Northern Europe, Spain and Australia; FEV_1, Forced Expiratory Volume in 1 second; FVC, Forced Vital Capacity; WLSMV, Weighted Least Square Mean and Variance; CI, Confidence Interval; BMI, Body Mass Index

Introduction

The paternal environment and exposures before conception on offspring health in humans has largely been neglected, despite animal models showing that the paternal environment plays a key role in non-genetic inheritance across generations through the male germ line [1]. There are several potential windows of susceptibility during the lifespan of the father, in which environmental effects could impact the epigenetic profile of his gametes, paternal prepuberty being one of them [2-5]. Human epidemiologic studies investigating such susceptibility windows are scarce, challenged by the temporal difficulties with covering two or more generations in humans. Furthermore, there is more data available on offsprings' health in relation to mothers' environment and exposures than on fathers.

A recent study from the Respiratory Health in Northern Europe, Spain & Australia (RHINESSA) cohort found that fathers', but not mothers', self-reported onset of overweight in puberty was associated with increased asthma risk in future adult offspring [6]. Three other studies from the same cohort found that fathers', but not mothers', smoking onset in puberty was associated with offspring's asthma and reduced lung function [7-9]. Overweight and obesity are thought to be detrimental to lung function across age groups regardless of asthma status [10], and reduced lung function is a strong predictor of morbidity and mortality from non-communicable diseases, including chronic respiratory diseases later in life [11]. The global increase in overweight [12], in parallel with the increase of asthma in children and adolescents, makes it important to further explore the effect of overweight on adverse lung health.

Studies suggest that childhood overweight and obesity influences growth patterns and pubertal development [13, 14], and that height might be influenced by the timing of pubertal events [15]. Height is of particular importance when respiratory health is considered, because it is related to lung growth and lung volumes [16].

In the present study, we investigated causal associations to estimate the causal effects of parents' overweight before puberty (generation G0) on adult offspring's lung function (generation G1) within the paternal and maternal lines, by examining both the potential mediating effect of offspring's overweight before puberty and adult height, and the potential moderating effect of offspring's sex on these relationships.

Methods

Study design

The European Community Respiratory Health Survey (ECRHS, www.ecrhs.org) is an international, population-based, cohort study of respiratory health in subjects aged 20-44 years at the time of recruitment (ECRHS I; 1992-1994) in 56 study centers from 25 countries (approximately 3000 subjects per study center) [17, 18]. In the 7 Northern European centres (Aarhus in Denmark; Tartu in Estonia; Reykjavik in Iceland; Bergen in Norway; Gothenburg, Umeå and Uppsala in Sweden) 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 [19]**.** 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) [20].

The Respiratory Health in Northern Europe, Spain and Australia (RHINESSA, www.rhinessa.net) generation study examines offspring of the participants in 10 ECRHS/RHINE centres [in addition to the 7 Northern European centres, two centres in Spain (Albacete and Huelva) and one in Australia (Melbourne)]. Extensive questionnaire and lung function data were collected in clinical centres in the period 2013-2016. The RHINESSA study protocols were harmonised with the ECRHS protocols.

Informed consent was obtained from each participant, and the appropriate regional committees of medical research ethics approved each survey of the ECRHS, RHINE and RHINESSA studies (https://helsebergen.no/seksjon/RHINESSA/Documents/Ethic%20Committees%20list.pdf).

Study population

A flow chart describing the study population is presented in **Figure 1**. Of the 1405 adult offspring (aged 18 years or greater) who participated in the clinical stage of the RHINESSA study, 1025 had a parent who had participated in the most recent ECRHS/RHINE follow-up studies (2010-2013). The database includes 1 parent only per offspring, resulting in 2 eligible study populations for the

present analysis: 1 population for the paternal line (439 offspring and 322 fathers) and 1 population for the maternal line (586 offspring and 434 mothers). The number of offspring with valid lung function measurements and complete information on key variables was 420 for the paternal line and 510 for the maternal line, originating from 308 fathers and 388 mothers, respectively. These offspring and parents were included in our analysis. Distribution by RHINESSA study centre in the paternal and maternal lines is shown in online supplementary **Table E1.**

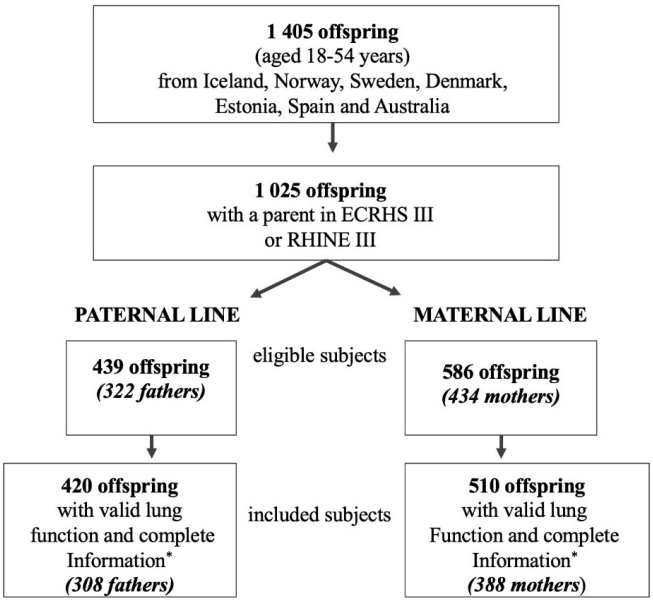

Figure 1. *Study population RHINESSA clinical stage flow chart. *Offspring with information on own body silhouette and height and on body silhouettes of their participating parent.*

Lung function and definitions

At RHINESSA clinical examinations, the maximum pre- and post bronchodilator forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were recorded as the best of at least two manoeuvres that met the American Thoracic Society criteria for repeatability [21]. At the same occasion, offspring's height was objectively measured.

Overweight status was defined by using a validated figural drawing scale of 9 sex-specific body silhouettes [22] (**Figure 2**). Parents in the ECRHS/RHINE III study were asked to tick the figural scale that best described their figure at specific time points including age 8 years, voice break/menarche and age 30 years. Participants were classified as overweight if their self-reported body silhouettes were equal to or higher than figure 4 for female and figure 5 for male. In detail, ECRHS parents' overweight status was classified as *"overweight before puberty"*, i.e. at age 8 years and/or in puberty (voice break for fathers and menarche for mothers), *"overweight at age 30 years but not before puberty"*, i.e. at age 30 years but neither at age 8 years nor in puberty, and *"never overweight"*, i.e. neither at age 8 years nor in puberty nor at age 30 years. RHINESSA offspring's *"overweight before puberty"* (present vs absent), i.e. at age 8 years and/or in puberty, were reported by the adult offspring using the same figural drawing scale described above for their parents. Offspring reported their own smoking history (never or ever smoker).

ECRHS/RHINE parents' education level was categorized as "low" if less than or equal to the minimum school-leaving age [23].

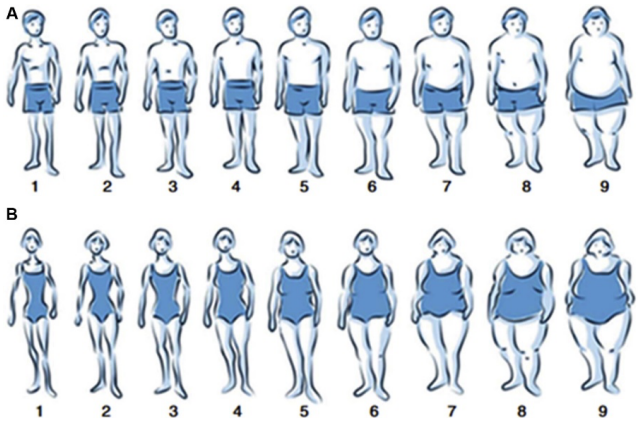

Figure 2. *Body silhouettes for men (A) and women (B) used in the ECRHS/RHINE III studies and in the RHINESSA questionnaire survey. Cutoffs for overweight status were 5 or greater in men, and 4 or greater in women.*

Statistical analysis

Counterfactual-based mediation analyses [24] were carried out to investigate the pathways among parents' overweight before puberty and offspring's lung function. The mediation analysis allows decomposing the total effect of the exposure on the outcome into the natural indirect effect (i.e. acting through the mediators) and the natural direct effect (i.e. not acting through the mediators). The main requirement for mediation is that the indirect effect is statistically significant under the assumption of no-unmeasured-confounding. In our analysis, mediation was combined with moderation (moderated mediation) to determine whether the indirect effect varied across levels of the moderator variable [25].

Two multi-group mediation models [26] were used within the paternal and maternal lines. *Model 1* included offspring's FEV_1 and FVC as the normally distributed, correlated, parallel outcomes. *Model 2* included offspring's FEV_1/FVC as the normally distributed outcome. In both models, fathers' or mothers' overweight before puberty was the exposure of interest and a serial causal chain of the two mediators (offspring's overweight before puberty and offspring's height) was assumed, since we hypothesized a causual order between the mediators. In addition, parents' education level was the potential confounding variable of the exposure-mediator, mediator-mediator and mediator-outcome relationships; offspring's age and their own smoking history were analysed as adjustment variables of the exposure-outcome and mediator-outcome relationships. The moderator (offspring's sex) was used to separate the observations into subgroups (sons and daughters). **Figure 3** (*model 1)* and online **Figure E1** (*model 2)* provide a graphic depiction of all directional paths of the hypothesized models.

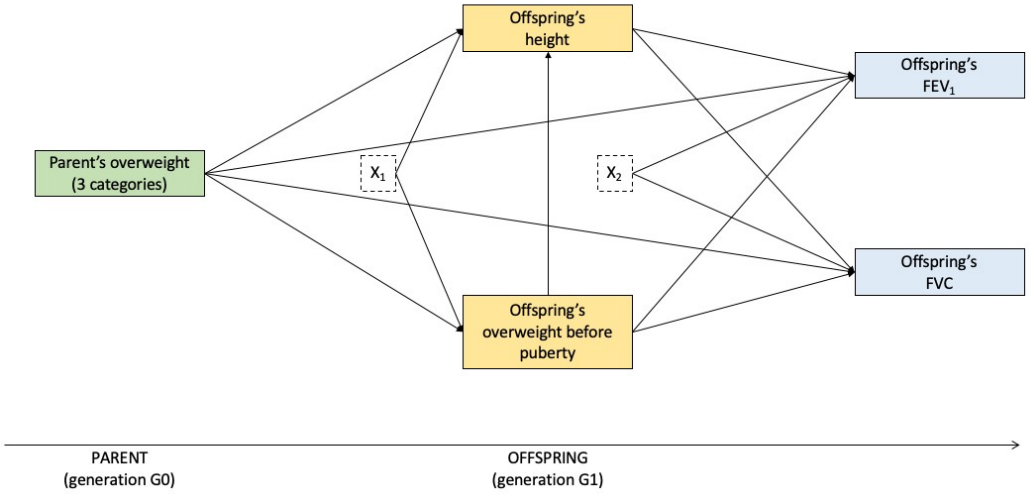

Figure 3. *Graphical representation of the mediation model for FEV_1 and FVC in sons or daughters within the paternal or maternal lines (Model 1). The green box represents the exposure of interest, the yellow boxes the mediators and the blue boxes the outcomes. The dotted boxes represent the set of potential confounders and adjusting variables of the meidators (X_1: parents' education level) and of the outcomes (X_2: parents' education level and offspring's age and their own smoking history).*

Due to non-normality of offspring's overweight before puberty, the magnitude of the natural (counterfactual-based) direct and indirect effects was computed based on the latent response variable mediator approach with probit link, theta parameterization and weighted least square mean and variance adjusted (WLSMV) estimators [27] with robust standard errors (father or mother = cluster variable). WLSMV estimator yielded probit regression coefficients for the effects on the latent mediator (offspring's overweight before puberty) and linear regression coefficients for the effects on offspring's adult height, FEV_1, FVC and FEV_1/FVC. Centre clustering of our data was not considered (*intraclass correlation* <0.1) due to the complexity of the relationships. The natural direct and indirect effects of the exposure on the outcomes were summarised as differences in offspring's expected lung

function values. The natural direct effect is the difference in offspring's expected lung function value for the change in exposure status, keeping offspring's height and/or offspring's overweight at their expected value when the exposure is absent. The natural indirect effect is the difference in offspring's expected lung function value when the exposure is present, but offspring's height and/or offspring's overweight change from their expected value when the exposure is absent to their expected value when the exposure is present. Non-bias-corrected bootstrap 95% Confidence Intervals (95%CI) (10,000 resamples) were obtained for the causally-defined effects in order to take their asymmetric distribution into account. In order to test the difference (Δ) of direct and indirect effects between sons and daughters, the non-bias-corrected bootstrap CI for the group difference in the direct and indirect effects [26] was computed.

Sensitivity analyses

Using the Umediation R package (https://github.com/SharonLutz/Umediation), we assessed whether the estimated direct and indirect effects change after the inclusion of up to two unmeasured confounders for the exposure-outcome, exposure-mediator and mediator-outcome relationships (see the **online supplementary material**).

Furthermore, we assessed how our results changed when offspring's post-bronchodilator lung function measurements (available from 191 sons and 209 daughters in the paternal line, and from 222 sons and 255 daughters in the maternal line) were used as outcomes.

STATA 16 (StataCorp, College Station, TX), Mplus 8.6 (Muthén & Muthén, Los Angeles, CA), and R 3.6.1 (www.R-project.org) were used for the statistical analyses.

Results

Main characteristics of the study subjects

The median age of ECRHS fathers and mothers was 56 and 55 years, respectively. The median age of adult offspring was 29 years (53.1% daughters) in the paternal line and 31 years (54.5% daughters) in the maternal line (**Table 1**)**.** Twelve percent of the 308 fathers reported being overweight before puberty, compared to 25% of the 388 mothers. Furthermore, 10.4% of the fathers and 22.9% of the mothers reported overweight at age 30 years, starting after puberty. Overweight before puberty was reported by 21.7 % of the offspring in the paternal line and by 20.4% of the offspring in the maternal line, and more frequently by female offspring (p-value<0.001). In both paternal and maternal lines, daughters had pre- and post- bronchodilator FEV_1 and FVC values that were statistically significantly lower (p-value <0.001), and pre- and post- bronchodilator FEV_1/FVC ratios that were statistically significantly higher (p-value <0.001) than sons. Ever smoking was reported by 25.2% and 32.6% of the offspring in the paternal and maternal lines, respectively (**Table 1**)**.**

Mediation analysis

Paternal line

Fathers' overweight before puberty had a negative direct effect on sons' adult height [beta (95%CI): -3.42 (-6.18, -0.57) cm], and sons' adult height in turn had a positive direct effect on their own FEV_1 [beta (95%CI): 42 (31, 53) mL] and FVC [beta (95%CI): 61 (48, 74) mL] (**Table 2**). Furthermore, fathers' overweight before puberty had a positive direct effect on daughters' overweight before puberty [beta (95%CI): 0.83 (0.32, 1.45)], which in turn had a negative direct effect on their own height in adulthood [beta (95%CI): -1.17 (-2.28, -0.09) cm] (**Table 2**).

Fathers' overweight before puberty had indirect (**Table 3**) but not direct (**Table 2**) effects on offspring's FEV_1 (*indirect-only mediation*), compared to fathers who had never been overweight.

Specifically, this indirect effect occurred in sons through their own adult height [beta (95%CI): -144 (-272, -23) mL] but not in daughters (**Table 3**). However, no moderated mediation by offspring's sex was observed because the difference in indirect effects between sons and daughters [Δ (95%CI): -81 (-230, 54) mL] was not statistically significant.

In addition, fathers' overweight before puberty had both a negative direct effect (**Table 2**) and a negative indirect effect through offspring's height on offspring's FVC (**Table 3**) (*complementary mediation)*, compared to fathers who had never been overweight. The direct effect on offspring's FVC was moderated by offspring's sex [Δ (95%CI): -340 (-642, -36) mL] and it was statistically significant in sons [beta (95%CI): -262 (-501, -9) mL] but not in daughters. The indirect effect was statistically significant only in sons [beta (95%CI): -210 (-380, -34) mL] and no moderated mediation by offspring's sex was observed [Δ (95%CI): -123 (-319, 69) mL].

We did not observe statistically significant effects of fathers' overweight before puberty on offspring's FEV_1/FVC (online **Table E2** and **Table E3**).

Maternal line

Mothers' overweight before puberty had neither direct nor indirect effects (mediated by offspring' overweight before puberty and/or offspring' adult height) on offspring's lung function (*nonmediation*; **Table 4** and **Table 5**; online **Table E4** and **Table E5**).

Sensitivity analyses

Umediation analysis was only conducted for sons' FEV_1 and FVC in the paternal line. The inclusion of two unmeasured confounders (online **Figure E2**) in the model had a limited impact on the direct and indirect effects of fathers' overweight before puberty on adult sons' FEV_1 and FVC, also when each unmeasured confounder had a very strong effect on the outcome, mediator and exposure. In fact, when $beta_{U\rightarrow E}$, $beta_{U\rightarrow M}$ and $beta_{U\rightarrow O}$ were set less than or equal to five, the

proportion of simulations where the results matched (whether U_1 and U_2 were included or excluded from the models) was greater than 80.1% and the average absolute difference of the average effects was lower than 0.176.

When offspring's post-bronchodilator values were used, the indirect effect of fathers' overweight before puberty on FEV_1 [beta (95%CI): -165 (-312, -29) mL] and FVC [beta (95%CI): -223 (-410, -41) mL] in sons remained statistically significant (**Table E6**). Nonmediation in the maternal line (**Table E7**).

Discussion

Using statistical models for causal inference, we found that fathers' overweight may negatively affect adult sons' lung function. Our results suggest that the sons of fathers who were overweight before puberty may have lower pre-bronchodilator FEV_1 and FVC values compared to the sons of fathers who were never overweight. The causal association of fathers' overweight before puberty with pre-bronchodilator FEV_1 and FVC was, respectively, fully (indirect-only effect) and partially (involving both direct and indirect effects) mediated through sons' adult height, but not through sons' own overweight before puberty. In the maternal line, no direct and indirect effects between mothers' overweight status and adult offspring's lung function were found. To our knowledge, this is the first study investigating parental overweight long before conception and adult offspring's lung function. Our results support the concept that the metabolic environment in males prepuberty might influence the health of the next generation.

Using mediation analyses, we were able to show that the observed association between a father's overweight before puberty and adult offspring's lung function involved mediation through lower adult-attained height in his sons.

The historical Överkalix study showed that grandfathers' diet during pre-puberty affected longevity of grandchildren in a sex-specific way [28]. The ALSPAC study in the UK showed that early onset smoking in fathers was related to increased risk of obesity in their sons [29]. These data suggest the importance of early life exposures in men, the involvement of epigenetic mechanisms and sex-specific outcomes. Johannessen et al [6] reported from the RHINESSA study that onset of overweight in paternal puberty was an important risk factor for their adult offspring's asthma without nasal allergies. No associations were found in the maternal line, which corresponds to our findings in this analysis.

In our statistical models, we hypothesized that offspring's overweight in prepuberty could affect own height in adulthood. Based on existing literature, adult height might be influenced by the timing of pubertal events and onset of puberty could be related to overweight and obesity [13, 15, 16, 30]. In our analysis, we found a positive direct effect of fathers' overweight in prepuberty on daughters' overweight before puberty, which in turn had a negative direct effect on their own height in adulthood. Despite this association, fathers' overweight in prepuberty was neither directly or indirectly associated with daughters' lung function in adulthood.

Fathers' overweight before puberty reduced both sons' and daughters' height in adulthood, compared to fathers who had never been overweight, although only statistical significant in sons. Furthermore, the mediated effect through lower adult-attained height on offspring's FEV_1 and FVC was only found in sons. In the maternal line, mothers' overweight status in different time periods was not associated with offspring's height in adulthood. Height is genetically inherited, but independent of genetics and a shared postnatal environment, paternal influences acting through epigenetic mechanisms could possibly affect offspring's growth and height [31].

Height is of particular importance when respiratory health is considered because it is related to lung growth and lung volume [16]. Our findings raise a broader issue around the common practice of adjusting for height in analyses using absolute lung function values. In our analyses, height mediated the relationship between fathers' prepuberty overweight and lung function outcomes in their sons. Therefore, it would be inappropriate to adjust for height or to use transformations of lung function outcomes (e.g. z-score or % predicted) that inherently take differences in the attained height into account [32].

Fathers' overweight before puberty had both a negative indirect and direct effect on male offspring's FVC (the direct effect meant an effect through pathways that did not involve the two mediators, offspring's pre-pubertal overweight or offspring's height). Furthermore, fathers' overweight in prepuberty had a negative indirect effect on male offspring's FEV_1. We also found a

direct effect on FEV_1 in sons, although it did not reach statistical significance. FVC is an important predictor of all-cause mortality in asymptomatic adults without chronic respiratory conditions [11]. Furthermore, it has been shown that early life factors and genetic effects that manifest in childhood would influence the individual's whole FEV_1 and FVC life trajectories, pointing to environmental exposures and genes affecting lung development as risk factors for low FEV_1 and FVC in later life [33, 34]. There is substantial epidemiological and experimental evidence supporting the concept of developmental origins of adult lung disease and impaired lung function [35], and there is strong evidence of an association between birth weight and adult FVC [36].

The effect of birth weight on adult FVC is in line with the Barker's hypothesis that early life exposures may be important to future outcomes in an individual [37], although this finding might be mediated by adverse early-life factors affecting birth weight. A rodent study demonstrated that male offspring from obese fathers had reduced birth weight and a growth deficit phenotype was observed from birth to 6 months of age [38]. The investigation of early life factors has traditionally been focused on how maternal exposures might influence offspring's health and risk of disease, with the *in utero* environment as one particular susceptibility window[37]. Nevertheless, paternal influences acting on the gamete environment long before conception of the child could possibly affect foetal conditions and development *in utero*, a function of the placenta and foetal growth [3, 39, 40].

Strengths and limitations

The present study has several strengths. The RHINESSA study design gives us comprehensive information on two generations. In most established birth cohort studies, the focus is mainly on exposures in the mothers. In the RHINESSA study we also have detailed information on fathers, including data on parental puberty, the latter are rarely available in multigeneration human cohorts. Furthermore, the paternal data were self-reported, and not reported by others (offspring, partner) like in many birth cohort studies mostly focused on maternal exposures. One limitation is that we lack information on age of paternal pubertal timing, we only have their reporting of body silhouette

"at voice breaking". In addition, the information reported by fathers and mothers was collected retrospectively before examination of their offspring. However, published validation studies from this study population on body silhouettes and overweight status [22], and on the use of body silhouettes to reflect obesity in the past, validated against previously measured or self-reported BMI in the ECRHS and RHINE studies [41], suggests minimal recall bias in key information. Furthermore, offspring's lung function and height was objectively measured at the clinical visit and strengthens the data.

A major strength of the present study is the use of figural drawing scales to assess body size throughout the lifespan. Despite the figural drawing scale not allowing objective assessment of the overweight status as defined by the World Health Organization, it identified subjects "at risk" for overweight body size. In fact, the cutoffs for the bodysilhouettes were defined as optimal for identifying overweight adults ($25 \leq$ BMI < 30 kg/m^2) in a recent validation study [22]. Moreover, using the same figural drawing scales for different time periods allowed for direct comparison across these periods and enabled us to identify the important window of susceptibility in parents for respiratory health in offspring. One limitation is that we were not able to validate overweight status by BMI for the childhood and puberty susceptibility windows. However, the body silhouettes have been validated by past BMI for the 30-year time-point [41].

Another strength of this study is the statistical approach used for assessing causal associations to estimate causal effects across two generations. Counterfactual mediation models enabled us to simultaneously investigate all the mediation paths (via multiple ordered mediators) in the same model. It is possible that unmeasured confounders (such as genetic and other environmental factors in the two generations) may be important. However, probabilistic simulations on the impact of unmeasured confounding support our results.

Conclusions

Fathers' overweight starting before puberty may negatively affect male adult offspring's lung function. The effects seem to be mediated by offspring's height, but not offspring's overweight status before puberty. We did not find causal associations in the maternal line. Our findings support the concept that the metabolic environment in males prepuberty might influence the health of the next generation. Increased scientific attention to male puberty in relation to future generations' health may have profound implications and open new opportunities for targeted public health strategies. We speculate that one might improve the health of two generations while intervening in one generation – in an age window that is key for both.

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Table 1. Main characteristics of parents and offspring in the paternal and maternal lines.

		Paternal line				Maternal line			
Generation		Total	Sons	Daughters	p-value‡	Total	Sons	Daughters	p-value‡
Parent (G0)	Number of parents	308	165	143	-	388	197	191	-
	Age (years), median (range)	56 (40-66)	55 (40-66)	56 (40-65)	0.311	55 (40-66)	55 (40-66)	55 (40-66)	0.869
	Low education level†, % (n)	8.4 (26)	8.5 (14)	8.4 (12)	0.571	10.1 (39)	8.6 (17)	11.5 (22)	0.219
	Overweight status, % (n)								
	before puberty	12.0 (37)	12.1 (20)	11.9 (17)	0.747	25.0 (97)	24.9 (49)	25.1 (48)	0.910
	at age 30 years but not before puberty	10.4 (32)	9.1 (15)	11.9 (17)		22.9 (89)	23.9 (47)	22.0 (42)	
	never	77.6 (239)	78.8 (130)	76.2 (109)		52.1 (202)	51.2(101)	52.9(101)	
Offspring (G1)	Number of adult offspring	420	197	223	-	510	232	278	-
	Age (years), median (range)	29 (18-51)	30 (18-47)	28 (18-51)	0.122	31 (18-54)	31 (18-46)	30 (18-54)	0.797
	Height (cm), mean (sd)	174.1 (8.98)	181 (6.5)	168 (6.3)	<0.001	173.9 (9.44)	181 (6.4)	168 (6.6)	<0.001
	Pre-bronchodilator FEV_1 (mL), mean (sd)	3798 (761)	4387 (629)	3277 (405)	<0.001	3780 (814)	4430 (620)	3238 (500)	<0.001
	Pre-bronchodilator FVC (mL), mean (sd)	4692 (969)	5490 (727)	3987 (492)	<0.001	4675 (1028)	5537 (761)	3957 (568)	<0.001
	Pre-bronchodilator FEV_1/FVC%, mean (sd)	81.3 (6.0)	80.0 (6.0)	82.4 (5.8)	<0.001	81.1 (5.9)	80.2 (5.9)	81.9 (5.9)	<0.001
	Post-bronchodilator FEV_1* (mL), mean (sd)	3918 (790)	4531 (648)	3359 (394)	<0.001	3915 (832)	4577 (630)	3339 (488)	<0.001
	Post -bronchodilator FVC* (mL), mean (sd)	4673 (978)	5460 (750)	3954 (481)	<0.001	4679 (1039)	5526 (785)	3942 (561)	<0.001
	Post-bronchodilator FEV_1/FVC%*, mean (sd)	84.2 (5.7)	83.1 (5.6)	85.2 (5.6)	<0.001	84.0 (5.5)	83.1 (5.6)	84.8 (5.4)	<0.001
	Overweight before puberty, % (n)	21.7 (91)	12.7 (25)	29.6 (66)	<0.001	20.4 (104)	17.7 (41)	22.7 (63)	0.186
	Ever smoking, % (n)	25.2 (106)	28.4 (56)	22.4 (50)	0.177	32.6 (166)	30.2 (70)	34.5 (96)	0.299

† Less than or equal to the minimum school-leaving age.
* Available from 191 sons and 209 daughters in the paternal line, and from 222 sons and 255 daughters in the maternal line.
‡ P-values obtained by using two-sample t test, two-sample test on the equality of medians, $\chi 2$ test or Fisher exact test.

Table 2. Natural direct effects* on offspring's pre-bronchodilator FEV_1 and FVC (model 1) within the paternal line.

	Offspring's overweight before puberty‡ beta (95%CI)		Offspring's adult height (cm) beta (95%CI)		Offspring's FEV_1 (mL) beta (95%CI)		Offspring's FVC (mL) beta (95%CI)	
	Sons	Daughters	Sons	Daughters	Sons	Daughters	Sons	Daughters
Fathers' overweight (*vs* never)								
before puberty	0.56 (-0.19, 1.19)	**0.83 (0.32, 1.45)**	**-3.42 (-6.18, -0.57)**	-2.11 (-4.58, 0.65)	-164 (-355, 45)	26 (-155, 187)	**-262†** **(-501, -9)**	78† (-138, 283)
at age 30 years but not before puberty	0.07 (-4.26, 0.88)	-0.02 (-0.79, 0.57)	-0.17 (-3.56, 6.85)	-0.2 (-2.72, 1.87)	-15 (-313, 504)	51 (-101, 209)	-43 (-363, 632)	64 (-109, 252)
Offspring's overweight before puberty (*vs* absent)	-	-	0.37 (-1.16, 1.99)	**-1.17 (-2.28, -0.09)**	36 (-64, 139)	32 (-35, 97)	54 (-51, 170)	48 (-29, 122)
Offspring's height in adulthood (cm)	-	-	-	-	**42 (31, 53)**	**30 (21, 40)**	**61†** **(48, 74)**	**41†** **(31, 53)**

*Difference (beta) in offspring's expected lung function value for the change in exposure status, keeping offspring's height and/or offspring's overweight at their expected value when the exposure is absent. Model 1 also includes the potential confounders and adjusting variables of the mediators (fathers' low education level) and of the outcomes (fathers' low education level and offspring's age and ever smoking). Beta is a probit regression coefficient for the effect on the latent mediator (offspring's overweight before puberty) and a linear regression coefficient for the effect on offspring's adult height, FEV_1 and FVC.

‡ Offspring's overweight before puberty was considered as a continuous latent mediator variable.

† The difference of direct effects between sons and daughters is statistically significant at $p < 0.05$.

95%CI: 95% confidence interval. The statistically significant effects are indicated in **bold.**

Table 3. Natural indirect effects* on offspring's pre-bronchodilator FEV_1 and FVC (model 1) within the paternal line.

		Offspring's FEV_1 (mL) beta (95%CI)		Offspring's FVC (mL) beta (95%CI)	
	Indirect effects	Sons	Daughters	Sons	Daughters
Father's overweight (*vs* never)					
before puberty	via offspring's overweight	20 (-41, 111)	26 (-30, 99)	30 (-30, 143)	40 (-24, 126)
	via offspring's height	**-144 (-272, -23)**	-64 (-146, 19)	**-210 (-380, -34)**	-87 (-202, 26)
	via offspring's overweight and height	9 (-31, 69)	-29 (-83, 1)	13 (-43, 99)	-40 (-111, 2)
at age 30 years but not before puberty	via offspring's overweight	3 (-426, 69)	-1 (-35, 33)	4 (-555, 79)	-1 (-47, 43)
	via offspring's height	-7 (-152, 267)	-6 (-86, 58)	-10 (-218, 395)	-8 (-118, 77)
	via offspring's overweight and height	1 (-229, 47)	1 (-26, 34)	2 (-336, 66)	1 (-36, 46)

*Difference (beta) in offspring's expected lung function value when the exposure is present, but offspring's height and/or offspring's overweight change from their expected value when the exposure is absent to their expected value when the exposure is present. Model 1 also includes the potential confounders and adjusting variables of the mediators (fathers' low education level) and of the outcomes (fathers' low education level and offspring's age and ever smoking).
95%CI: 95% confidence interval. The statistically significant effects are indicated in **bold.**

Table 4. Natural direct effects* on offspring's pre-bronchodilator FEV_1 and FVC (model 1) within the maternal line.

	Offspring's overweight before puberty‡ beta (95%CI)		Offspring's adult height (cm) beta (95%CI)		Offspring's FEV_1 (mL) beta (95%CI)		Offspring's FVC (mL) beta (95%CI)	
	Sons	Daughters	Sons	Daughters	Sons	Daughters	Sons	Daughters
Mothers' overweight (*vs* never)								
before puberty	0.59 (-0.31, 0.82)	0.52 (-0.09, 0.71)	0.1 (-3.54, 1.13)	-0.12 (-3.25, 0.82)	115 (-182, 199)	160 (-39, 213)	124 (-237, 229)	110 (-117, 170)
at age 30 years but not before puberty	0.54 (-0.34, 0.78)	0.3 (-0.45, 0.5)	1.41 (-2.33, 2.5)	1.1 (-2.4, 2.04)	98 (-228, 200)	186 (-47, 244)	116 (-263, 236)	180 (-87, 253)
Offspring's overweight before puberty (*vs* absent)	-	-	1.38 (-0.77, 1.96)	0.57 (-1.31, 1.09)	114 (-69, 165)	82 (-36, 113)	135 (-69, 192)	83 (-51, 117)
Offspring's height in adulthood (cm)	-	-	-	-	**53 (34, 59)**	**42 (29, 46)**	**75† (53, 82)**	**53† (38, 58)**

* Difference (beta) in offspring's expected lung function value for the change in exposure status, keeping offspring's height and/or offspring's overweight at their expected value when the exposure is absent. Model 1 also includes the potential confounders and adjusting variables of the mediators (mothers' low education level) and of the outcomes (mothers' low education level and offspring's age and ever smoking). Beta is a probit regression coefficient for the effect on the latent mediator (offspring's overweight before puberty) and a linear regression coefficient for the effect on offspring's adult height, FEV_1 and FVC.

‡ Offspring's overweight before puberty was considered as a continuous latent mediator variable.

† The difference of direct effects between sons and daughters is statistically significant at $p < 0.05$.

95%CI: 95% confidence interval. The statistically significant effects are indicated in **bold.**

Table 5. Natural indirect effects* on offspring's pre-bronchodilator FEV_1 and FVC (model 1) within the maternal line.

		Offspring's FEV_1 (mL) beta (95%CI)		Offspring's FVC (mL) beta (95%CI)	
	Indirect effects	Sons	Daughters	Sons	Daughters
Mother's overweight (*vs* never)					
before puberty	via offspring's overweight	34 (-24, 70)	27 (-10, 49)	40 (-29, 81)	28 (-14, 51)
	via offspring's height	4 (-160, 48)	-4 (-117, 30)	6 (-236, 69)	-5 (-149, 37)
	via offspring's overweight and height	19 (-12, 37)	5 (-16, 12)	27 (-18, 54)	6 (-21, 15)
at age 30 years but not before puberty	via offspring's overweight	26 (-29, 57)	13 (-19, 29)	32 (-33, 67)	15 (-19, 34)
	via offspring's height	61 (-104, 112)	39 (-87, 74)	91 (-151, 163)	50 (-110, 93)
	via offspring's overweight and height	20 (-9, 42)	9 (-8, 21)	29 (-14, 60)	12 (-11, 27)

*Difference (beta) in offspring's expected lung function value when the exposure is present, but offspring's height and/or offspring's overweight change from their expected value when the exposure is absent to their expected value when the exposure is present. Model 1 also includes the potential confounders and adjusting variables of the mediators (mothers' low education level) and of the outcomes (mothers' low education level and offspring's age and ever smoking).
95%CI: 95% confidence interval. The statistically significant effects are indicated in **bold.**

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References

1. Soubry A. Epigenetics as a Driver of Developmental Origins of Health and Disease: Did We Forget the Fathers? Bioessays 2018; 40.
2. Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. Bioessays 2014; 36:359-71.
3. Soubry A, Murphy SK, Wang F, Huang Z, Vidal AC, Fuemmeler BF, et al. Newborns of obese parents have altered DNA methylation patterns at imprinted genes. Int J Obes (Lond) 2015; 39:650-7.
4. Sales VM, Ferguson-Smith AC, Patti ME. Epigenetic Mechanisms of Transmission of Metabolic Disease across Generations. Cell Metab 2017; 25:559-71.
5. Wei Y, Schatten H, Sun QY. Environmental epigenetic inheritance through gametes and implications for human reproduction. Hum Reprod Update 2015; 21:194-208.
6. Johannessen A, Lonnebotn M, Calciano L, Benediktsdottir B, Bertelsen RJ, Braback L, et al. Being overweight in childhood, puberty, or early adulthood: Changing asthma risk in the next generation? J Allergy Clin Immunol 2020; 145:791-9 e4.
7. Accordini S, Calciano L, Johannessen A, Portas L, Benediktsdottir B, Bertelsen RJ, et al. A three-generation study on the association of tobacco smoking with asthma. Int J Epidemiol 2018; 47:1106-17.
8. Svanes C, Koplin J, Skulstad SM, Johannessen A, Bertelsen RJ, Benediktsdottir B, et al. Father's environment before conception and asthma risk in his children: a multi-generation analysis of the Respiratory Health In Northern Europe study. Int J Epidemiol 2017; 46:235-45.
9. Accordini S, Calciano L, Johannessen A, Benediktsdottir B, Bertelsen RJ, Braback L, et al. Prenatal and prepubertal exposures to tobacco smoke in men may cause lower lung function in future offspring: a three-generation study using a causal modelling approach. Eur Respir J 2021.

10. Forno E, Han YY, Mullen J, Celedon JC. Overweight, Obesity, and Lung Function in Children and Adults-A Meta-analysis. J Allergy Clin Immunol Pract 2018; 6:570-81 e10.
11. Burney PG, Hooper R. Forced vital capacity, airway obstruction and survival in a general population sample from the USA. Thorax 2011; 66:49-54.
12. World Health Organization. Global health observatory (GHO) data: overweight and obesity. 2017.] Available from http://www.who.int/gho/ncd/risk_factors/overweight/en/.
13. Reinehr T, Roth CL. Is there a causal relationship between obesity and puberty? Lancet Child Adolesc Health 2019; 3:44-54.
14. Alotaibi MF. Physiology of puberty in boys and girls and pathological disorders affecting its onset. J Adolesc 2019; 71:63-71.
15. Mahmoud O, Granell R, Tilling K, Minelli C, Garcia-Aymerich J, Holloway JW, et al. Association of Height Growth in Puberty with Lung Function. A Longitudinal Study. Am J Respir Crit Care Med 2018; 198:1539-48.
16. Yousefi M, Karmaus W, Zhang H, Roberts G, Matthews S, Clayton B, et al. Relationships between age of puberty onset and height at age 18 years in girls and boys. World J Pediatr 2013; 9:230-8.
17. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J 1994; 7:954-60.
18. Janson C, Chinn S, Jarvis D, Burney P. Determinants of cough in young adults participating in the European Community Respiratory Health Survey. Eur Respir J 2001; 18:647-54.
19. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, et al. Longterm follow-up in European respiratory health studies - patterns and implications. BMC Pulm Med 2014; 14:63.
20. Jarvis D, Newson R, Janson C, Corsico A, Heinrich J, Anto JM, et al. Prevalence of asthma-like symptoms with ageing. Thorax 2018; 73:37-48.

21. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005; 26:319-38.

22. Dratva J, Bertelsen R, Janson C, Johannessen A, Benediktsdottir B, Braback L, et al. Validation of self-reported figural drawing scales against anthropometric measurements in adults. Public Health Nutr 2016; 19:1944-51.

23. Hill M. Social policy: a comparative analysis. London: Prentice-Hall/Harvester Wheatsheaf; 1996.

24. Muthén BO, Muthén, L.K., Asparouhov, T. Regression and Mediation Analysis Using Mplus. Los Angeles: Muthén & Muthén; 2016.

25. Preacher KJ, Rucker DD, Hayes AF. Addressing Moderated Mediation Hypotheses: Theory, Methods, and Prescriptions. Multivariate Behav Res 2007; 42:185-227.

26. Ryu E, Cheong J. Comparing Indirect Effects in Different Groups in Single-Group and Multi-Group Structural Equation Models. Front Psychol 2017; 8:747.

27. Muthén B. Applications of Causally Defined Direct and Indirect Effects in Mediation Analysis using SEM in Mplus. 2011.

28. Bygren LO, Kaati G, Edvinsson S. Longevity determined by paternal ancestors' nutrition during their slow growth period. Acta Biotheor 2001; 49:53-9.

29. Northstone K, Golding J, Davey Smith G, Miller LL, Pembrey M. Prepubertal start of father's smoking and increased body fat in his sons: further characterisation of paternal transgenerational responses. Eur J Hum Genet 2014; 22:1382-6.

30. Marcovecchio ML, Chiarelli F. Obesity and growth during childhood and puberty. World Rev Nutr Diet 2013; 106:135-41.

31. Dodd JM, Du Plessis LE, Deussen AR, Grivell RM, Yelland LN, Louise J, et al. Paternal obesity modifies the effect of an antenatal lifestyle intervention in women who are overweight or obese on newborn anthropometry. Sci Rep 2017; 7:1557.

32. Campbell B, Simpson JA, Bui DS, Lodge CJ, Lowe AJ, Matheson MC, et al. Early menarche is associated with lower adult lung function: A longitudinal cohort study from the first to sixth decade of life. Respirology 2020; 25:289-97.

33. Martinez FD. Early-Life Origins of Chronic Obstructive Pulmonary Disease. N Engl J Med 2016; 375:871-8.

34. Minelli C, Dean CH, Hind M, Alves AC, Amaral AF, Siroux V, et al. Association of Forced Vital Capacity with the Developmental Gene NCOR2. PLoS One 2016; 11:e0147388.

35. Krauss-Etschmann S, Bush A, Bellusci S, Brusselle GG, Dahlen SE, Dehmel S, et al. Of flies, mice and men: a systematic approach to understanding the early life origins of chronic lung disease. Thorax 2013; 68:380-4.

36. Saad NJ, Patel J, Burney P, Minelli C. Birth Weight and Lung Function in Adulthood: A Systematic Review and Meta-analysis. Ann Am Thorac Soc 2017; 14:994-1004.

37. Barker DJ. The fetal and infant origins of adult disease. BMJ 1990; 301:1111.

38. Lecomte V, Maloney CA, Wang KW, Morris MJ. Effects of paternal obesity on growth and adiposity of male rat offspring. Am J Physiol Endocrinol Metab 2017; 312:E117-E25.

39. Binder NK, Beard SA, Kaitu'u-Lino TJ, Tong S, Hannan NJ, Gardner DK. Paternal obesity in a rodent model affects placental gene expression in a sex-specific manner. Reproduction 2015; 149:435-44.

40. McPherson NO, Fullston T, Aitken RJ, Lane M. Paternal obesity, interventions, and mechanistic pathways to impaired health in offspring. Ann Nutr Metab 2014; 64:231-8.

41. Lonnebotn M, Svanes C, Igland J, Franklin KA, Accordini S, Benediktsdottir B, et al. Body silhouettes as a tool to reflect obesity in the past. PLoS One 2018; 13:e0195697.

ONLINE SUPPLEMENTARY MATERIAL

Title: Parental prepubertal overweight and offspring lung function

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Table E1. Distribution by RHINESSA study centre in the paternal and maternal lines.

		Paternal line		Maternal line	
Country	**Centre**	**N° of offspring**	**N° of fathers**	**N° of offspring**	**N° of mothers**
Denmark	Aarhus	18	17	2	2
Estonia	Tartu	24	21	66	51
Iceland	Reykjavik	41	33	61	50
Norway	Bergen	182	119	168	118
Sweden	Goteborg	20	19	32	30
	Umea	34	27	44	33
	Uppsala	39	32	45	39
Spain	Albacete	9	5	10	8
	Huelva	12	7	32	19
Australia	Melbourne	41	28	50	38
	Total	**420**	**308**	**510**	**388**

SENSITIVITY ANALYSIS

Using the Umediation R package (https://github.com/SharonLutz/Umediation), we assessed whether the estimated direct and indirect effects change after the inclusion of up to two unmeasured confounders for the exposure-outcome, exposure-mediator, and mediator-outcome relationships. We simulated two normally distributed unmeasured confounders (U_1 and U_2) with mean of 0 and variance of 0.001, within a single-exposure, single-mediator, and single-outcome framework. As inputs for Umediation, we used the coefficients of the mediation analysis. The simulation analyses were carried out under multiple scenarios for the effects (beta regression coefficients) of the unmeasured confounder "*U_1*" on the outcome ($beta_{U\rightarrow O}$), the mediator ($beta_{U\rightarrow M}$), and the exposure ($beta_{U\rightarrow E}$) by fixing $beta_{U\rightarrow E} = beta_{U\rightarrow M} = beta_{U\rightarrow O} = 0$, 1, 3, 5, 7, and 9. For this purpose, pre-bronchodilator FEV_1 and FVC were expressed in decilitre. We repeated the simulations by adding "*U_2*" to the models under the same assumptions. We specified 1000 simulation runs and 1000 Monte Carlo draws for the nonparametric bootstrap.

Table E2. Natural direct effects* on offspring's pre-bronchodilator FEV_1/FVC (model 2) within the paternal line.

	Offspring's overweight before puberty‡ beta (95%CI)		Offspring's adult height (cm) beta (95%CI)		Offspring's FEV_1/FVC beta (95%CI)	
	Sons	Daughters	Sons	Daughters	Sons	Daughters
Fathers' overweight (*vs* never)						
before puberty	0.55 (-0.19, 1.19)	0.83 (0.32, 1.45)	**-3.41 (-6.18, -0.57)**	-2.11 (-4.59, 0.64)	0.97 (-1.9, 4.14)	-0.84 (-2.94, 1.19)
at age 30 years but not before puberty	0.07 (-4.26, 0.88)	-0.02 (-0.79, 0.57)	-0.28 (-3.56, 6.85)	-0.2 (-2.73, 1.86)	0.24 (-3.97, 3.57)	-0.02 (-2.71, 2.44)
Offspring's overweight before puberty	-	-	0.38 (-1.17, 1.99)	**-1.16 (-2.28, -0.09)**	-0.13 (-1.4, 1.05)	-0.2 (-1.17, 0.79)
Offspring's height in adulthood (cm)	-	-	-	-	-0.12 (-0.23, 0.01)	-0.09 (-0.21, 0.02)

*Difference (beta) in offspring's expected lung function value for the change in exposure status, keeping offspring's height and/or offspring's overweight at their expected value when the exposure is absent. Model 2 also includes the potential confounders and adjusting variables of the mediators (fathers' low education level) and of the outcomes (fathers' low education level and offspring's age and ever smoking). Beta is a probit regression coefficient for the effect on the latent mediator (offspring's overweight before puberty) and a linear regression coefficient for the effect on offspring's adult height and FEV1/FVC.

‡ Offspring's overweight before puberty was considered as a continuous latent mediator variable.

95%CI: 95% confidence interval. The statistically significant effects are indicated in bold.

Table E3. Natural indirect effects* on offspring's pre-bronchodilator FEV_1/ FVC (model 2) within the paternal line.

		Offspring's FEV_1/FVC beta (95%CI)	
	Indirect effects	Sons	Daughters
Father's overweight (*vs* never)			
before puberty	via offspring's overweight	-0.07 (-1.11, 0.68)	-0.17 (-1.18, 0.73)
	via offspring's height	0.4 (-0.05, 0.95)	0.2 (-0.08, 0.68)
	via offspring's overweight and height	-0.02 (-0.21, 0.08)	0.09 (-0.03, 0.33)
at age 30 years but not before puberty	via offspring's overweight	-0.01 (-1.54, 3.16)	0 (-0.37, 0.45)
	via offspring's height	0.03 (-0.77, 0.52)	0.02 (-0.19, 0.35)
	via offspring's overweight and height	0 (-0.13, 0.64)	0 (-0.12, 0.1)

*Difference (beta) in offspring's expected lung function value when the exposure is present, but offspring's height and/or offspring's overweight change from their expected value when the exposure is absent to their expected value when the exposure is present. Model 2 also includes the potential confounders and adjusting variables of the mediators (fathers' low education level) and of the outcomes (fathers' low education level and offspring's age and ever smoking).
95%CI: 95% confidence interval. The statistically significant effects are indicated in bold.

Table E4. Natural direct effects* on offspring's pre-bronchodilator FEV_1/FVC (model 2) within the maternal line.

	Offspring's overweight before puberty‡ beta (95%CI)		Offspring's adult height (cm) beta (95%CI)		Offspring's FEV_1/FVC beta (95%CI)	
	Sons	Daughters	Sons	Daughters	Sons	Daughters
Mothers' overweight (*vs* never)						
before puberty	0.16 (-0.42, 0.67)	0.23 (-0.15, 0.58)	-1.74 (-3.91, 0.44)	-1.73 (-3.55, 0.20)	0.39 (-1.33, 2.09)	1.58 (0.02, 3.15)
at age 30 years but not before puberty	0.13 (-0.45, 0.62)	-0.05 (-0.53, 0.37)	-0.43 (-2.70, 1.79)	-0.67 (-2.74, 1.44)	0.01 (-2.01, 2.02)	0.87 (-0.86, 2.59)
Offspring's overweight before puberty	-	-	0.33 (-0.99, 1.59)	-0.37 (-1.49, 0.73)	-0.12 (-1.16, 0.97)	0.22 (-0.64, 1.12)
Offspring's height in adulthood (cm)	-	-	-	-	**-0.14 (-0.25, -0.02)**	-0.01 (-0.12, 0.10)

*Difference (beta) in offspring's expected lung function value for the change in exposure status, keeping offspring's height and/or offspring's overweight at their expected value when the exposure is absent. Model 2 also includes the potential confounders and adjusting variables of the mediators (mothers' low education level) and of the outcomes (mothers' low education level and offspring's age and ever smoking). Beta is a probit regression coefficient for the effect on the latent mediator (offspring's overweight before puberty) and a linear regression coefficient for the effect on offspring's adult height and FEV1/FVC.

‡ Offspring's overweight before puberty was considered as a continuous latent mediator variable.

95%CI: 95% confidence interval. The statistically significant effects are indicated in bold.

Table E5. Natural indirect effects* on offspring's pre-bronchodilator FEV_1/ FVC (model 2) within the maternal line.

		Offspring's FEV_1/FVC beta (95%CI)	
	Indirect effects	Sons	Daughters
Mother's overweight (*vs* never)			
before puberty	via offspring's overweight	-0.02 (-0.39, 0.37)	0.05 (-0.21, 0.38)
	via offspring's height	0.24 (-0.06, 0.70)	0.02 (-0.19, 0.26)
	via offspring's overweight and height	-0.01 (-0.08, 0.06)	0.00 (-0.02, 0.03)
at age 30 years but not before puberty	via offspring's overweight	-0.02 (-0.39, 0.31)	-0.01 (-0.31, 0.21)
	via offspring's height	0.06 (-0.28, 0.42)	0.01 (-0.13, 0.15)
	via offspring's overweight and height	-0.01 (-0.09, 0.04)	0.00 (-0.02, 0.02)

*Difference (beta) in offspring's expected lung function value when the exposure is present, but offspring's height and/or offspring's overweight change from their expected value when the exposure is absent to their expected value when the exposure is present. Model 2 also includes the potential confounders and adjusting variables of the mediators (mothers' low education level) and of the outcomes (mothers' low education level and offspring's age and ever smoking).
95%CI: 95% confidence interval. The statistically significant effects are indicated in bold.

Table E6. Natural indirect effects* on offspring's post-bronchodilator FEV_1 and FVC (model 1) or FEV_1/ FVC (model 2) within the paternal line.

		Offspring's FEV_1 (mL) beta (95%CI)		Offspring's FVC (mL) beta (95%CI)		Offspring's FEV_1/FVC beta (95%CI)	
	Indirect effects	Sons	Daughters	Sons	Daughters	Sons	Daughters
Father's overweight (*vs* never)							
before puberty	via offspring's overweight	1 (-77, 86)	17 (-40, 86)	8 (-81, 114)	28 (-37, 111)	-0.04 (-1.19, 0.86)	-0.21 (-1.14, 0.65)
	via offspring's height	**-165 (-312, -29)**	-71 (-163, 21)	**-223 (-410, -41)**	-96 (-221, 27)	0.34 (-0.11, 0.96)	0.23 (-0.07, 0.77)
	via offspring's overweight and height	10 (-37, 89)	-23 (-77, 6)	14 (-49, 120)	-32 (-102, 9)	-0.02 (-0.23, 0.09)	0.08 (-0.03, 0.31)
at age 30 years but not before puberty	via offspring's overweight	0 (-233, 118)	-4 (-48, 29)	1 (-339, 102)	-7 (-58, 33)	-0.01 (-1.87, 2.80)	0.05 (-0.43, 0.62)
	via offspring's height	0 (-153, 317)	15 (-86, 90)	0 (-210, 429)	20 (-118, 117)	0.00 (-0.7, 0.45)	-0.05 (-0.33, 0.36)
	via offspring's overweight and height	1 (-262, 48)	6 (-14, 51)	2 (-358, 65)	8 (-19, 68)	0.00 (-0.11, 0.54)	-0.02 (-0.19, 0.05)

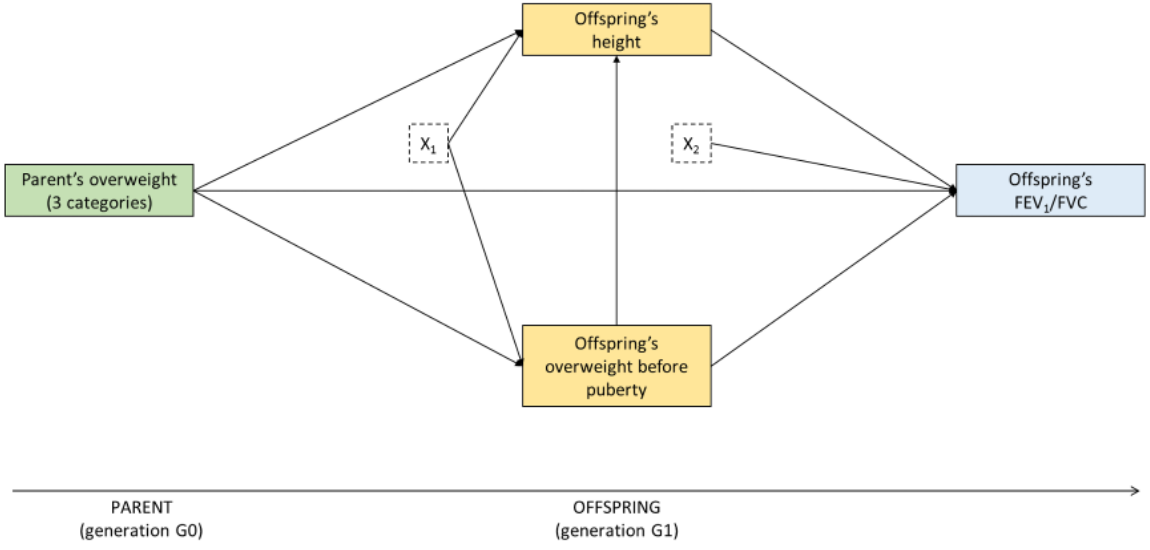
*Difference (beta) in offspring's expected lung function value when the exposure is present, but offspring's height and/or offspring's overweight change from their expected value when the exposure is absent to their expected value when the exposure is present. Model 1 and model 2 also include the potential confounders and adjusting variables of the mediators (fathers' low education level) and of the outcomes (fathers' low education level and offspring's age and ever smoking).
95%CI: 95% confidence interval. The statistically significant effects are indicated in bold.

Table E7. Natural indirect effects* on offspring's post-bronchodilator FEV_1 and FVC (model 1) or FEV_1/ FVC (model 2) within the maternal line.

		Offspring's FEV_1 (mL) beta (95%CI)		Offspring's FVC (mL) beta (95%CI)		Offspring's FEV_1/FVC beta (95%CI)	
	Indirect effects	Sons	Daughters	Sons	Daughters	Sons	Daughters
Mother's overweight (*vs* never)							
before puberty	via offspring's overweight	7 (-35, 55)	7 (-13, 39)	8 (-42, 67)	9 (-15, 48)	-0.01 (-0.41, 0.40)	-0.02 (-0.31, 0.22)
	via offspring's height	-77 (-179, 19)	-67 (-138, 3)	-109 (-256, 26)	-86 (-176, 4)	0.23 (-0.05, 0.70)	0.12 (-0.06, 0.41)
	via offspring's overweight and height	3 (-18, 30)	-4 (-23, 7)	5 (-25, 43)	-5 (-29, 9)	-0.01 (-0.10, 0.06)	0.01 (-0.02, 0.05)
at age 30 years but not before puberty	via offspring's overweight	6 (-38, 51)	-3 (-34, 20)	7 (-44, 60)	-4 (-45, 23)	-0.01 (-0.40, 0.37)	0.01 (-0.20, 0.31)
	via offspring's height	-22 (-129, 79)	-18 (-102, 64)	-31 (-182, 115)	-23 (-128, 81)	0.06 (-0.27, 0.43)	0.03 (-0.14, 0.24)
	via offspring's overweight and height	3 (-14, 33)	2 (-13, 18)	4 (-20, 47)	2 (-17, 23)	-0.01 (-0.10, 0.05)	0.00 (-0.04, 0.03)

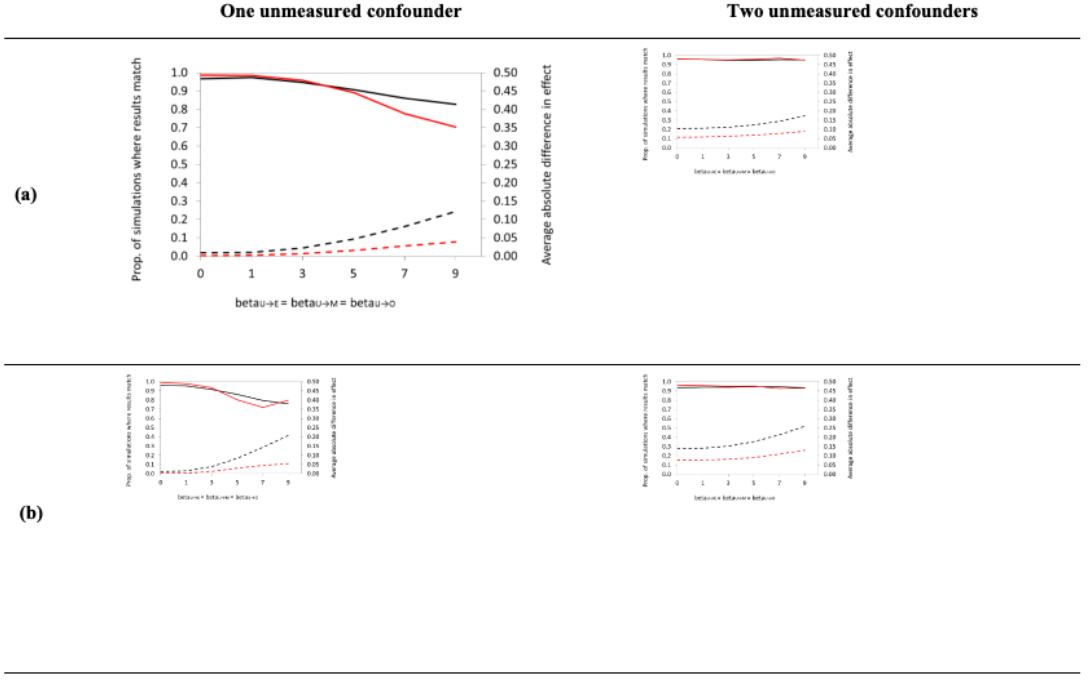
*Difference (beta) in offspring's expected lung function value when the exposure is present, but offspring's height and/or offspring's overweight change from their expected value when the exposure is absent to their expected value when the exposure is present. Model 1 and model 2 also include the potential confounders and adjusting variables of the mediators (mothers' low education level) and of the outcomes (mothers' low education level and offspring's age and ever smoking).
95%CI: 95% confidence interval. The statistically significant effects are indicated in bold.

Model 2. Graphical representation of the mediation model for FEV_1/FVC in sons or daughters within the paternal or maternal lines.



The green box represents the exposure of interest, the yellow boxes the mediators and the blue boxes the outcomes. The dotted boxes represent the set of potential confounders and adjusting variables of the mediators (X_1: parent's low education level) and of the outcomes (X_2: parent's low education level and offspring's age and smoking).

Figure E2. Proportion of Monte Carlo simulations where results match (solid line) and average absolute difference (dashed line) in the average direct (black line) and indirect (red line) effects of fathers' overweight start in prepuberty on sons' lung function (whether one or two unmeasured confounders are included or excluded from the models). Outcomes: (a) FEV_1 and (b) FVC.



SUPPLEMENTARY INFORMATION ON THE EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY (ECRHS)

ECRHS III

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Coordination: The coordination was funded through the Medical Research Council (92091).

Ethics approval: Ethics approval was obtained by all centres from the appropriate ethics committees: **Tartu:** Research Ethics Committee of the University of Tartu, Estland; **Reykjavik:** National Biotecs Committe of Iceland (NBCI) (N° VSNb2011090016/03.11); **Bergen:** Universitetet i Bergen, Regional komité for medisinsk og helsefaglig forskningsetikk, Vest-Norge (REK Vest) (N° 2010/759); **Albacete:** Comité de Ética e Investigación de Complejo Hospitalario de Albacete (N° 04/09); **Huelva**: Comisión de Investigación del Hospital Juan Ramón Jiménez de Huelva (N° 20090417); **Swedish centres:** Ethics Committee at the Medical Faculty, Uppsala University (N° 1999/313 and 2010/068); **Århus:** De videnskabsetiske komiteer for region Midtjylland (M-20110106).

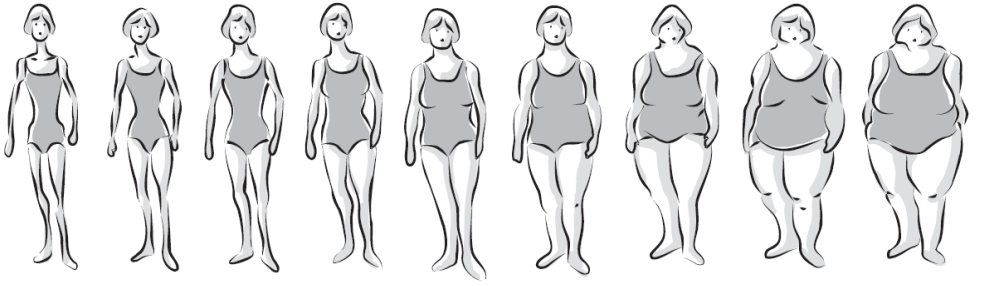
Appendix A. Body shape questionnaire ECRHS III

ECRHS III BODY SHAPE QUESTIONNAIRE

Centre ID

Your body shape

1. What picture best describe your body shape at each age (women only)?



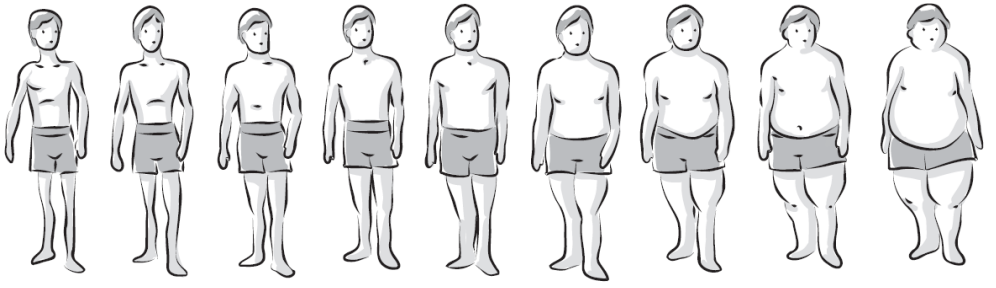
1.1	current									
1.2	age 8									
1.3	when you had your first period									
1.4	age 30									
1.5	age 45									
1.6	when you had your menopause *(12 months without a period)*									
		1	2	3	4	5	6	7	8	9

2 What picture best describes your biological mother at

											Don't know
2.1	age 30										
2.2	age 45										

Your body shape

3. What picture best describe your body shape at each age (men only)?



		1	2	3	4	5	6	7	8	9
3.1	current	☐	☐	☐	☐	☐	☐	☐	☐	☐
3.2	age 8	☐	☐	☐	☐	☐	☐	☐	☐	☐
3.3	at age voice broke	☐	☐	☐	☐	☐	☐	☐	☐	☐
3.4	age 30	☐	☐	☐	☐	☐	☐	☐	☐	☐
3.5	age 45	☐	☐	☐	☐	☐	☐	☐	☐	☐
3.6	age 55	☐	☐	☐	☐	☐	☐	☐	☐	☐

4 What picture best describes your biological father at

											Don't know
4.1	age 30	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4.2	age 45	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Appendix B. Questionnaire from the RHINESSA adult offspring study

Lungehelseundersøkelsens Generasjonsstudie

– translated «The lung health investigation's Generation Study"
Name chosen in order to be as similar as possible to

Airways symptoms and allergic symptoms

1. Have you had wheezing or whistling in your chest at any time **in the last 12 months?** ☐No ☐Yes

If NO go to question 2, if YES:

1.1. Have you been at all breathless when the wheezing noise was present? ..... ☐No ☐Yes

1.2. Have you had this wheezing or whistling when you did not have a cold?..... ☐No ☐Yes

2. Have you woken up with a feeling of tightness in your chest at any time **in the last 12 months?** ..... ☐No ☐Yes

3. Have you been woken by an attack of shortness of breath at any time **in the last 12 months?** ..... ☐No ☐Yes

4. Have you been woken by an attack of coughing at any time **in the last 12 months?**... ☐No ☐Yes

5. Have you had an attack of asthma **in the last 12 months**? ..... ☐No ☐Yes

6. Are you currently taking any medicine (including inhalers, aerosols or tablets) for asthma?..... ☐No ☐Yes

7. Do you have any nasal allergies including hay fever?..... ☐No ☐Yes

8. What is your date of birth? (day/month/year) ..... ____dd ____mm_____yyyy

9. What is today's date? (day/month/year) ..... ____dd ____mm _____yyyy

10. Gender ☐Man ☐Woman

11. How tall are you? ..... _____cm

12. How much do you weigh? ..... _____ kg

13. In recent years, have you been troubled by a protracted cough?..... ☐No ☐Yes

14. Do you usually bring up phlegm or do you have phlegm in your lungs which you have difficulty bringing up? ☐No ☐Yes

If NO to question 13 and 14 go to question 15, if YES:

14.1. Do you cough or bring up phlegm in this way almost every day for at least three months every year? ..... ☐No ☐Yes

14.2. Have you had periods of this kind for at least two years in a row? ☐No ☐Yes

15. Do you have or have you ever had asthma?..... ☐No ☐Yes

If NO go to question 16, if YES:

15.1. Have you ever had asthma diagnosed by a doctor?..... ☐No ☐Yes

15.2. How old were you when you first experienced asthma symptoms? _____years

15.3. How old were you when you last experienced asthma symptoms?..... _____years

16. Has a doctor ever told you that you have chronic obstructive pulmonary disease (COPD)? ☐No ☐Yes

17. Have you been woken by an attack of shortness of breath at any time in **the last 3 days**? ☐No ☐Yes

18. Have you been woken by an attack of coughing at any time in **the last 3 days**? ☐No ☐Yes

19. Have you had wheezing or whistling in your chest in **the last 3 days**? ☐No ☐Yes

20. Have you **ever** had wheezing or whistling in your chest? ☐No ☐Yes

If NO go to question 21, if YES:

20.1 How old were you when you first noticed wheezing or whistling in your chest? ..... _____ years

21. Have you ever experienced nasal symptoms such as nasal congestion, rhinorrhoea (runny nose) and/or sneezing attacks without having a cold? ☐No ☐Yes

If No go to question 22, if YES:

21.1. How old were you when you experienced such nasal symptoms for the first time? _____ years

21.2. Have you had such nasal symptoms in **the last 12 months**?..... ☐No ☐Yes

21.3. Has this nose problem been accompanied by itchy or watery eyes? ☐No ☐Yes

21.4. In which months of the year did this nose problem occur?

January / February ..... ☐
March / April ..... ☐
May / June..... ☐
July / August ..... ☐
September / October..... ☐

November / December…… ☐

22. Have you ever had eczema or any kind of skin allergy? ☐No ☐Yes

If NO go to question 23,if YES:

22.1. How old were you when you first had eczema or skin allergy? _____ years

23. Have you ever had an itchy rash that was coming and going for at least 6 months? ☐No ☐Yes

If NO go to question 24, if YES:

23.1. Have you had this itchy rash in **the last 12 months**? ☐No ☐Yes

23.2. Has this itchy rash at any time affected any of the following places:
the folds of the elbows, behind the knees, in front of the ankles, under the buttocks or around the neck, ears or eyes? ☐No ☐Yes

23.3. Has this itchy rash affected your hands at any time in **the last 12 months?** ☐No ☐Yes

24. Have you ever had an illness or truoble caused by eating **a particular** food or foods? ☐No ☐Yes

If NO go to question 25, if YES:

24.1. Have you nearly always had the same illness or trouble after eating this type of food? ☐No ☐Yes

If NO go to question 25, if YES:

24.2. What type of food was this (*list up to three foods*)?

_____

24.3. Did this illness or truoble include:

24.3.1. a rash or itchy skin? ☐No ☐Yes

24.3.2. diarrhea or vomiting? ☐No ☐Yes

24.3.3. runny or stuffy nose? ☐No ☐Yes

24.3.4. severe headaches? ☐No ☐Yes

24.3.5. breathlessness? ☐No ☐Yes

24.4. How soon after eating this food did/do you get the first sympoms?

Less than half an hour	½ - 1 hour	1-2 hours	2-4 hours	More than 4 hours
☐	☐	☐	☐	☐

24.5. How old were you when you **first** had this attack? _____years

24.6. How old were you when you **last** had this attack? _____years

Smoking habits

25. Do you smoke? (this applies even if you only smoke the odd cigarette/cigar or pipe every week) ☐No ☐Yes

26. Did you smoke previously?..... ☐No ☐Yes

If NO to question 25 and 26 go to question 27, if YES:

26.1. How much do or did you smoke? *(give an average)*

Cigarettes/day	Cigars/week	Pkts pipe tobacco/week

26.2. How old were you when you started smoking? _____ years

26.3. For how long have you smoked? (applies to both smokers and ex-smokers) _____ years

26.4. If you are an ex- smoker, how old were you when you stopped smoking? _____years

27. Do you use moist snuff, nicotine patches, or other products containing nicotine? ☐No ☐Yes

28. Did you use moist snuff, nicotine patches, or other products containing nicotine previously? ☐No ☐Yes

If NO to question 27 and 28 go to question 30, if YES:

29. What kind of nicotine-containing product do /did you use?

29.1. Moist Snuff ☐No ☐Yes

If you use/have used moist snuff:

29.1.1. How old were you when you started using moist snuff? _____ years

29.1.2. For how long have you been using moist snuff? (applies to both current users and past users) _____ years

29.1.3. If you did use moist snuff previously, how old were you when you stopped using it? _____ years

29.2. Nicotine patches/ gum /tablets ☐No ☐Yes

If you have been using nicotine patches/gum/tablets:

29.2.1. For how long have you used nicotine patches/gum/tablets: _____ months

Childhood and family

30. What term best describes **the place you lived most of the time before the age of 5 years**? (tick one box only)

Farm with livestock	Farm without livestock	Village in rural area	Small town	Suburb of city	Inner city
☐	☐	☐	☐	☐	☐

30.1. What term best describes the place **your father** lived as a child? (tick one box only)

Farm with livestock	Farm without livestock	Village in rural area	Small town	Suburb of city	Inner city	Don't know
☐	☐	☐	☐	☐	☐	☐

30.2. What term best describes the place **your mother** lived as a child? (tick one box only)

Farm with livestock	Farm without livestock	Village in rural area	Small town	Suburb of city	Inner city	Don't know
☐	☐	☐	☐	☐	☐	☐

30.3. What term best describes the place **your grandparents'** lived as a child? (tick one box for each grandparent)

	Farm	Village in rural area	Small town	Inner city	Don't know
Father's father	☐	☐	☐	☐	☐
Father's mother	☐	☐	☐	☐	☐
Mother's father	☐	☐	☐	☐	☐
Mother's mother	☐	☐	☐	☐	☐

31. How many persons, including yourself, lived in your home when you were 5 years old (where you lived most of the time)? (number)……

32. Did you have a serious respiratory infection before the age of five years?....☐No ☐ Yes ☐ Don't know

33. Did your father ever smoke regularly during your childhood? .....☐No ☐ Yes ☐ Don't know

34. Did your mother ever smoke regularly during your childhood? .....☐No ☐ Yes ☐ Don't know

If NO / DON'T KNOW go to question 35, if YES:

34.1. Did your mother smoke when she was pregnant with you? ☐No ☐ Yes ☐ Don't know

35. Did other people (other than parents) smoke regularly at home during your childhood?..... ☐No ☐ Yes ☐ Don't know

36. How often did you take cod liver oil when you were a child? (tick one box only)

Never	Rarely	Every week	Daily
☐	☐	☐	☐

37. How often did you eat fresh fruits and berries when you were a child? (tick one box only)

Never	Rarely	Every week	Almost daily	Almost daily in the autumn season
☐	☐	☐	☐	☐

38. How often did you eat potatoes or vegetables that **you or your family had cultivated** when you were a child? (tick one box only)

Never	Rarely	Almost weekly in the growing season	Almost daily in the growing season
☐	☐	☐	☐

39. Was there a cat in your home?

39.1. During your first year of life ☐No ☐ Yes ☐ Don't know

39.2. When you were aged 1 to 4 years ☐No ☐ Yes ☐ Don't know

39.3. When you were aged 5- 15 years ☐No ☐ Yes ☐ Don't know

40. Was there a dog in your home?

40.1. During your first year of life ☐No ☐ Yes ☐ Don't know

40.2. When you were aged 1 to 4 years ☐No ☐ Yes ☐ Don't know

40.3. When you were aged 5- 15 years ☐No ☐ Yes ☐ Don't know

41. What was the highest level of education your mother has/had? (tick one box only)

Primary school (up to the minimum school leaving age)..... ☐

Secondary school / technical school (past the minimum age)..... ☐

College or university ..... ☐

42. What was the highest level of education your father has/had? (tick one box only)

Primary school (up to the minimum school leaving age)..... ☐

Secondary school / technical school (past the minimum age)..... ☐

College or university ..... ☐

43. Did your biological parents ever suffer from any of the following:

	Mother (tick box if YES)	Father (tick box if YES)
Asthma	☐	☐
Chronich bronchitis, emphysema and/or COPD	☐	☐
Heart disease	☐	☐
Hypertension	☐	☐
Stroke	☐	☐
Diabetes	☐	☐
Cancer	☐	☐

44. Do you have any biological children? ☐No ☐ Yes

If NONE go to question 45, if you have (had) biological children:

44.1. How many children? NUMBER_____

44.2. Please write the years when your biological children were born, and tick "YES" if they have had any of the following:

	Year of birth	Girl/ boy	Asthma before 10 years	Asthma after 10 years	Hayfever/ Rhinitis	Atopic eczema/ skin allergies
Child 1						
Child 2						
Child 3						
Child 4						
Child 5						
Child 6						

Education and occupation

45. Please mark the educational level which best describes your level: *(tick one box only)*)

Primary school ..... ☐

Secondary school/technical school..... ☐

College or University ..... ☐

46. Which is your current or most recent work or occupation?

Employed	Self- employed	Homemaker	Student	Unemployed	Other
☐	☐	☐	☐	☐	☐

47. Do you currently have /have you ever had paid work?..... ☐No ☐Yes

*Please **do not** include occupations of shorter duration than three months.*
*Please **do** include part time jobs of 20 or more hours per week.*
If NO go to question 54, if YES:

48. Which is your current or most recent work or occupation? *(please use capital letters)*

.....

48.1. How many years have you worked / did you work in this occupation? .....years

49. Does being at your current workplace ever cause breathing problems (chest tightness, wheezing, coughing)? ☐No ☐Yes

50. In your current job, are you regularly exposed to vapours, gas, dust or fumes? ☐No ☐Yes

51. Have you ever changed job because the job affected your breathing?..... ☐No ☐Yes

52. Have you ever changed job because of hay fever or nasal symptom?..... ☐No ☐Yes

53. Have you ever changed job because of eczema or skin disease?..... ☐No ☐Yes

In-door environment

54. Do you keep a cat? ☐No ☐Yes

If NO go to 55,if YES:

54.1. Is your cat (are your cats) allowed inside the house? ☐No ☐Yes

54.2. Is your cat (are your cats) allowed in the bedroom? ☐No ☐Yes

55. Do you keep a dog? ☐No ☐Yes

If NO go to question 56, if YES:

55.1. Is your dog (are your dogs) allowed inside the house? ☐No ☐Yes

55.2. Is your dog (are your dogs) allowed in your bedroom? ☐No ☐Yes

56. In which type of accommodation do you live? *(tick one box only)*

Detached house ☐

Semidetached or terraced house ☐

Apartment ☐

Other ☐

57. When did you move to your current home?..... Year_____

58. Have you ever moved house because of breathing problems?..... ☐No ☐Yes

59. When was your present home built?..... Year _____

60. Does tobacco smoking take place in your present home? *(tick one box only)*

Yes, every day	Yes, frequently 1-4 times/week	Yes, sometimes 1-3 times/month	No, never
☐	☐	☐	☐

61. Have any of the following been identified in your home in **the last 12 months:**

61.1. Water leakage or water damage indoors in walls, floor or ceilings?..... ☐No ☐Yes

61.2. Bubbles or yellow discoloration on plastic floor covering, or black discoloration of parquet floor? ..... ☐No ☐Yes

61.3. Visible mould growth indoors on walls, floor or ceilings..... ☐No ☐Yes

62. Have you seen any signs of damp, water leakage or mould in your home at any time **in the last 10 years**? ..... ☐No ☐Yes

63. Have you noticed the odour of mould or mildew (not from food) in your home at any time **in the last 12 months**?..... ☐No ☐Yes

General health

64. Have you had a course of antibiotics in **the last 12 months**?..... ☐No ☐Yes
(*i.e. Apocillin, Azitromax, Imacillin) LIST the three most commonly used antibiotics in your country*

64.1. If YES, how many courses of antibiotics..... (number) _____

65. Have you had a course of antibiotics in **the last 14 days**?..... ☐No ☐Yes

66. Does your gum bleed when you brush your teeth? *(tick one box only)*

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

67. How often do you usually brush your teeth? *(tick one box only)*

2 times/day or more	Once daily	Less than daily
☐	☐	☐

68. How frequently do you exercise? *(give an average, tick one box only)*

Never	Less than once a week	Once a week	2-3 times a week	Almost every day
☐	☐	☐	☐	☐

If you do such exercise as frequently as one or more times a week:

68.1. How hard do you push yourself? *(tick one box only)*

I take it easy without breaking into a sweat or losing my breath..... ☐
I push myself so hard that I lose my breath and break into a sweat..... ☐
I push myself to near-exhaustion..... ☐

68.2. How long does each session last? *(give an average, tick one box only)*

Less than 15 minutes ..... ☐
16-30 minutes ..... ☐

30 minutes to 1 hour ..... ☐

More than 1 hour ..... ☐

Sleep and daytime symptoms

69. How often has it occurred **in the last months** *(circle one number for each question):*

1: *Never or almost never*	**2**: *Less than once a week*	**3**: *Once or twice a week*	**4**: *3- 5 nights/days a week*	**5**: *Almost every day or night*

69.1. ... that you snore loudly and disturbingly?.....	1	2	3	4	5
69.2. ...that you have heartburn or belching when you have gone to bed?	1	2	3	4	5
69.3. ... that you have difficulty in getting to sleep at night?...	1	2	3	4	5
69.4. ... that you wake up repeatedly during the night?.....	1	2	3	4	5
69.5. ... that you perspire heavily during the night?	1	2	3	4	5
69.6. ... that you feel drowsy in the daytime?	1	2	3	4	5
69.7. ...that you wake up too early and have difficulty In getting to sleep again?.....	1	2	3	4	5

70. How long time do you usually sleep per night? _____Hours _____Minutes

Other diseases

71. Has a doctor or health professional ever told you that you have?

71.1. Diabetes? ☐No ☐Yes

If NO go to question 71.2, if YES:

71.1.1. How old were you when you were diagnosed with diabetes? _____years

71.1.2. What treatment are you currently using for diabetes? *(tick one box only)*

Insulin	Tablets	Both insulin and tablets	Only diet
☐	☐	☐	☐

71.1.3. Which type of diabetes do/did you have:

☐ Type 1 ☐ Type 2 ☐ Only in pregnancy ☐ Don't know

71.2. Psoriasis? ☐No ☐Yes

If NO go to question 71.3, if YES::

71.2.1. How old were you when you were diagnosed with psoriasis? _____years

71.3. Bechterew's disease? ☐No ☐Yes

If NO go to question 71.4, if YES:

71.3.1. How old were you when you were diagnosed with Bechterew's disease? _____years

71.4. Rheumatiod arthritis? ☐No ☐Yes

If NO go to question 71.5, if YES:

71.4.1. How old were you when you were diagnosed with rheumatoid arthritis? _____years

71.5. Ulcerous Colitis? ☐No ☐Yes

If NO go to question 71.6, if YES:

71.5.1. How old were you when the disease started? ..... _____years

71.6. Crohn's disease? ☐No ☐Yes

If NO go to question 71.7, if YES:

71.6.1. How old were you when the disease started? ..... _____years

71.7. Sleep apnea? ☐No ☐Yes

If NO go to question 71.8, if YES:

71.7.1. How old were you when you were diagnosed with sleep apnea? _____years

71.7.2. What treatment are you currently using for sleep apnea? (more than one box may apply)

CPAP	Oral appliance (bite splint)	Other
☐	☐	☐

71.8. Hypertension (high blood pressure)? ☐No ☐Yes

If NO go to question 71.9, if YES:

71.8.1. How old were you when you were diagnosed with hypertension (high blood pressure)? _____years

71.8.2. Are you currently taking any medication for hypertension (high blood pressure)? ☐No ☐Yes

71.9. Heart infarction or angina pectoris? ☐No ☐Yes

If NO go to question 72, if YES:

71.9.1. Have you ever been treated in hospital because of heart infarction or angina pectoris? ☐No ☐Yes

If NO go to question 72, if YES:

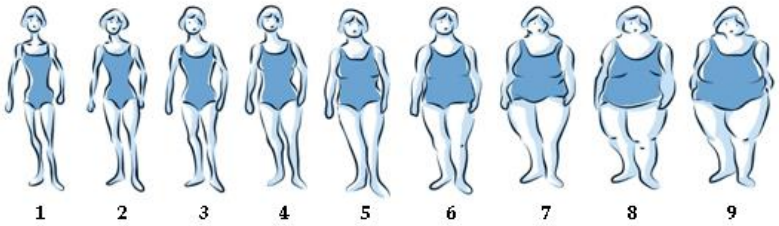
71.9.2. How old were you when you were treated in hospital (for the first time) for heart infarction or angina pectoris? _____years

Body shape

72. Gender: ☐Man ☐Woman

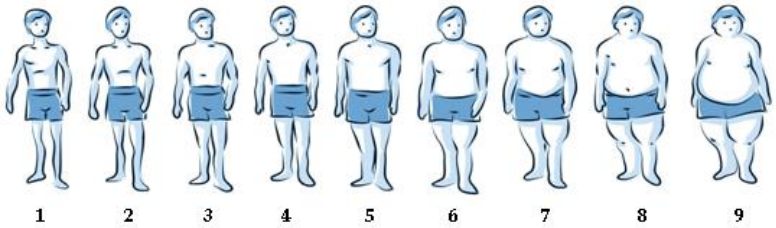
What picture best describes your body shape at each age
(tick one box only for each age/ period you have reached)

72.1. WOMEN



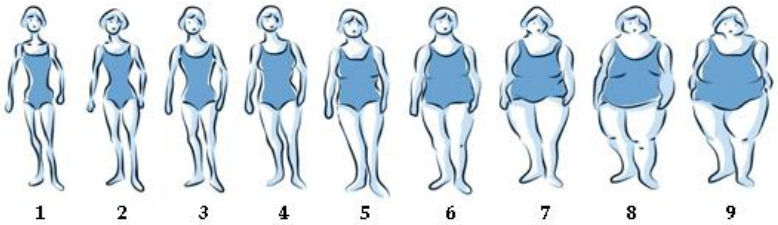
Current	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 8 years	☐	☐	☐	☐	☐	☐	☐	☐	☐
At first menstruation	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 20	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 30	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 45	☐	☐	☐	☐	☐	☐	☐	☐	☐

72.2. MEN



Current	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 8 years	☐	☐	☐	☐	☐	☐	☐	☐	☐
At voice break	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 20	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 30	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 45	☐	☐	☐	☐	☐	☐	☐	☐	☐

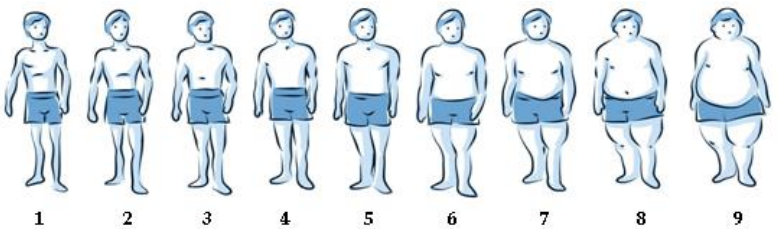
73. What picture best describes the body shape of your biological mother at



Don't know

Age 30	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 45	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

74. What picture best the body shape of your biological father at



Don't know

Age 30	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Age 45	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Food and drinks

75. How many days each week do you usually eat/ drink the following:

	Never	Rarely	1 day a week	2 days a week	3 days a week	4 days a week	5 days a week	6 days a week	7 days a week
Meat or sausage	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fish	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vegetables	☐	☐	☐	☐	☐	☐	☐	☐	☐
Raw vegetables, salad, vegetable juice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Potatoes	☐	☐	☐	☐	☐	☐	☐	☐	☐
Potatoes or vegetables you or your family have cultivated yourselves	☐	☐	☐	☐	☐	☐	☐	☐	☐
Olive oil	☐	☐	☐	☐	☐	☐	☐	☐	☐
Citrus fruit or citrus fruit juice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any fruit (except citrus fruit)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Milk (not including milk you have in tea or coffe)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soft drinks	☐	☐	☐	☐	☒	☐	☐	☐	☐
Dark (not white) bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Food heated in plastic container in microwave	☐	☐	☐	☐	☐	☐	☐	☐	☐
Unpasteurized milk?	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beer or wine	☐	☐	☐	☐	☐	☐	☐	☐	☐
Naturally fermented foods	☐	☐	☐	☐	☐	☐	☐	☐	☐

76. To collect data on outdoor exposures in places you have lived, we would like to ask for your address history. Some countries provide address information through registries, others do not.

Which country do you live in?

If you live in NORWAY, SWEDEN, DENMARK or SWITZERLAND:

Your country provides address history through registries.
Thank you for participation in this survey.

If you live in AUSTRALIA, ICELAND, SPAIN, or ESTONIA:

76.1. We would like to know where you have lived since January 1990.
Please give the address, including postcode, of all homes you have lived in **for at least one year since 1990, starting with your current address**

House number	**Street name**	**City**	**Postcode**	**Moved in**	**Lived there until (YEAR)**
					current

Norwegian consent form
To be signed before submitting the *postal* questionnaire

Respondent number

Project title	Project number
The Lung Health Investigation's Generation study	

Project leader

Department/hospital

Participation in the study is voluntary. If you want to participate, you have to sign this consent form. If you agree to participate, you can at any time and without giving a reason, withdraw your consent. Further, this will not have any consequences for your future contact with the health care system.

If you want to withdraw, or have any questions about the study, you can contact the project leader.

I would like to participate in this study

Name in capitals

Date	Signed
__ __ /__ __ / 20 __ __	

Thank you for your help!

Consent form - translation for web:

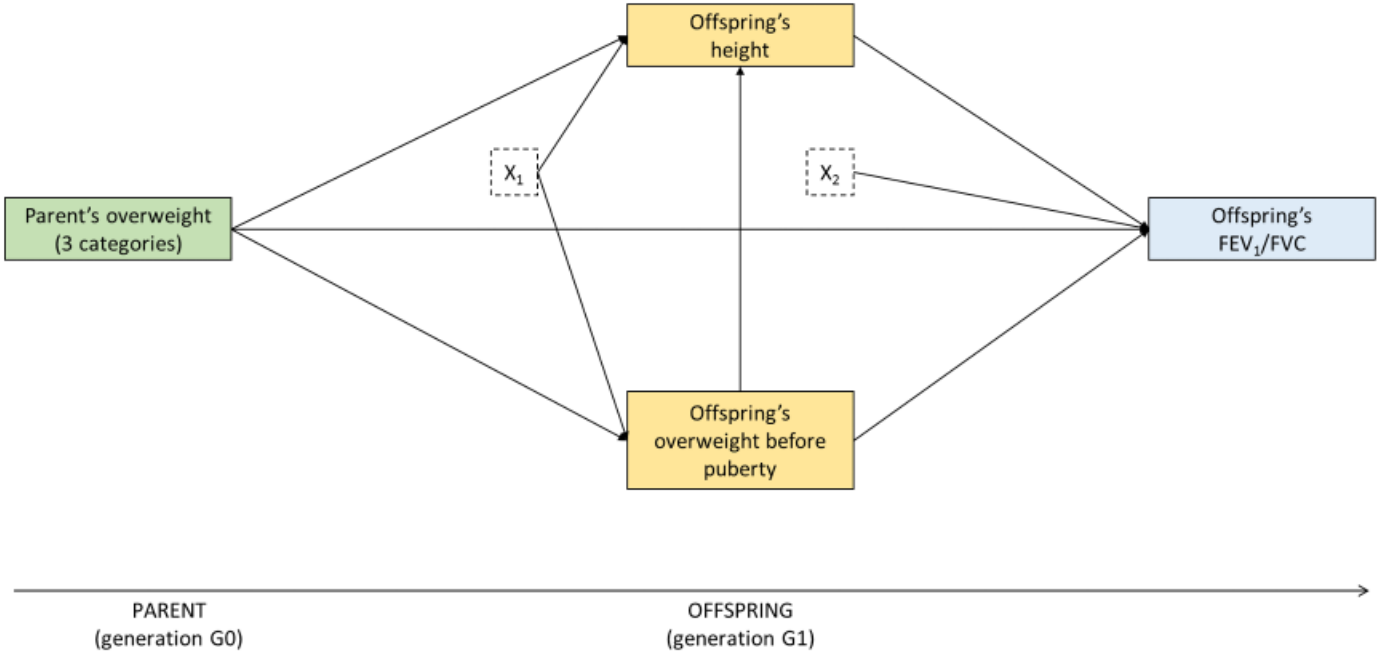
Participation in the study is voluntary. If you want to participate, you have to sign this consent form by ticking 'yes' at the bottom of this page. If you agree to participate, you can at any time and without giving a reason, withdraw your consent. Further, this will not have any consequences for your future contact with the health care system.

If you want to withdraw, or have any questions about the study, you can contact the project leader.

I would like to participate in this study:

Appendix C. Model 2, paper III

Model 2. Graphical representation of the mediation model for FEV_1/FVC in sons or daughters within the paternal or maternal lines.



The green box represents the exposure of interest, the yellow boxes the mediators and the blue boxes the outcomes. The dotted boxes represent the set of potential confounders and adjusting variables of the mediators (X_1: parent's low education level) and of the outcomes (X_2: parent's low education level and offspring's age and smoking).

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