Cured meat intake is associated with worsening asthma symptoms.

Zhen Li, Marta Rava, Annabelle Bédard, Orianne Dumas, Judith Garcia-Aymerich, Bénédicte Leynaert, Christophe Pison, Nicole Le Moual, Isabelle Romieu, Valérie Siroux, et al.

Manuscript Title: Cured meat intake is associated with worsening asthma symptoms

Corresponding author:

Name: Zhen Li

Address: Inserm U1168, Hopital Paul Brousse, Batiment Leriche, 16, avenue Paul Vaillant Couturier, 94807 Villejuif, France

Tel: +33 1 45 59 53 58

Fax: +33 1 45 59 51 69

E-mail: zhen.li@inserm.fr

Authors' full names and affiliations:

Zhen Li,[1,2,3] Marta Rava,[1,4] Annabelle Bédard,[1,2] Orianne Dumas,[1,2] Judith Garcia-Aymerich,[5,6,7] Bénédicte Leynaert,[8] Christophe Pison,[9] Nicole Le Moual,[1,2] Isabelle Romieu,[10] Valérie Siroux,[11,12,13] Carlos A. Camargo Jr,[14] Rachel Nadif,[1,2*] Raphaëlle Varraso[1,2*]

*These authors contributed equally to this work

[1]Inserm, U1168, VIMA: Aging and chronic diseases. Epidemiological and public health approaches, F-94807, Villejuif, France

[2]Univ. Versailles St-Quentin-en-Yvelines, UMR-S 1168, F-78180, Montigny le Bretonneux, France

[3]Univ. Paris-Sud, France

[4]Spanish National Cancer Research Centre (CNIO), Genetic & Molecular Epidemiology Group, Human Cancer Genetics Program, Madrid, Spain

[5]Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain

[6]Universitat Pompeu Fabra (UPF), Barcelona, Spain

[7]CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

[8]Inserm UMR1152, Physiopathologie et épidémiologie des maladies respiratoires, équipe

Epidémiologie, Paris, France

[9]Clinique Universitaire de Pneumologie, Pôle Thorax et Vaisseaux, CHU Grenoble, Grenoble, France; Inserm1055, Grenoble, France; Université Alpes Grenoble, France

[10]Instituto Nacional De Salud Publica, Mexico; currently at the International Agency for Research on Cancer, Lyon, France.

[11]Inserm, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Grenoble, France

[12]Univ. Grenoble Alpes, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, F-38000 Grenoble, France

[13]CHU de Grenoble, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, F-38000 Grenoble, France

[14]Department of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, USA

Key words: asthma, asthma symptom score, cured meat, body mass index, mediation analysis

Key messages:

What is the key question?

Is high cured meat intake, a risk factor for several diseases, associated with worsening asthma symptoms in adults? Does body mass index (BMI) play as a mediator in this association?

What is the bottom line?

Correctly accounting for the BMI as a mediator, this paper shows that high cured meat intake was associated with worsening asthma symptoms over time, through a direct effect and to a lesser extent an effect mediated by BMI.

Why read on?

This paper is not only the first prospective study on the association between cured meat intake and asthma symptoms, it is also the first application of newly developed methods to estimate the role of BMI as a mediator in the diet-asthma association.

Conclusion for Twitter feed: Higher cured meat intake was associated with worsening asthma symptoms over time, directly and indirectly through BMI.

Word count: 3647

Number of figures: 3

Number of tables: 2

Number of references: 43

Number of online supplementary files: 1

ABSTRACT (244 words, 250 max)

Background Cured meat intake – a recent carcinogenic factor – may increase the risk of chronic obstructive pulmonary disease, but its association with asthma remains unknown. Though body mass index (BMI) is a likely risk factor for asthma, its role in the diet-asthma association as a mediator has never been studied. We investigated the association between cured meat intake and worsening asthma symptoms in adults, and the role of BMI as a potential mediator.

Methods Using data from the French prospective EGEA study (baseline: 2003-2007; follow-up: 2011-2013), we applied a mediation analysis in the counterfactual framework, a marginal structural model (MSM), to estimate the direct effect of baseline cured meat intake (<1, 1-3.9, ≥4 servings/week) on change in asthma symptom score (worsening or not), and the indirect effect mediated by BMI.

Results Among the 971 participants (mean age 43 years; 49% men; 42% with asthma), 20% reported worsening asthma symptoms during the mean follow-up time of seven years. Using the MSM, we reported a positive direct effect of cured meat intake on worsening asthma symptoms (multivariable OR=1.76, 95% CI 1.01 to 3.06 for ≥4 vs. <1 serving/week). We also reported an indirect effect mediated by BMI (OR=1.07; 95%CI 1.01 to 1.14), accounting for 14% of the total effect.

Conclusions Higher cured meat intake was associated with worsening asthma symptoms over time, through a direct effect and to a lesser extent an effect mediated by BMI. This research extends the effect of diet on asthma in adults.

Main Text (3579 words, 3500 max)

INTRODUCTION

Cured meat, an important component of the Western diet, has recently been classified as carcinogenic by the World Health Organization.[1] High cured meat intake is a risk factor not only for cancer,[2] but also for several chronic diseases [3,4] and all-cause mortality.[3] The deleterious health effects of high cured meat intake have been increasingly observed. Regarding lung health, frequent cured meat intake is associated with lung cancer,[2,5,6] decreased lung function [7,8] and chronic obstructive pulmonary disease (COPD) symptoms, [6,7] exacerbations [9] or incidence.[10,11] As cured meat is known for its high-nitrite content [12] which may lead to nitrosative stress related airway inflammation – one of the several physiological processes involved in asthma,[13] it is reasonable to posit that cured meat intake is an independent risk factor for asthma. To our knowledge, only two studies – both performed in the United States – have investigated the association between cured meat intake and the risk of adult-onset asthma, and they reported no significant association after adjustment for body mass index (BMI) and other confounders.[10,11]

Since obesity is a likely risk factor for both incidence and exacerbations [14–16] of asthma, one challenge when investigating the association between diet and asthma is to properly account for BMI. As diet affects BMI, it is plausible that BMI lies in the causal pathway between diet and asthma.[17] Thus the classical way, in which we consider BMI as a confounder in the analysis and adjust for BMI, may over adjust the diet-asthma association due to the likely mediating role of BMI.[17,18] Recently, mediation analyses in the counterfactual framework have been proposed to disentangle direct and indirect effects.[19–21]

Asthma is a complex disease, with strong clinical phenotypic heterogeneity and temporal phenotypic variability. Whereas the doctor-diagnosed incidence of asthma is widely

used, there have been increasing concerns about its limitations, particularly due to the dichotomous assumption of asthma.[22] Recently, an asthma symptom score has been proposed to study risk factors for asthma in longitudinal studies.[23,24] Being a multi-categorical measure, the score provides more power to detect risk factors for asthma incidence and asthma control.[23,24] Moreover, the asthma symptom score detects the change in asthma symptoms over time, reflecting either asthma incidence, or captures the temporal variability of the disease among participants with asthma - remission, relapse or persistence.[23] Given that previous studies showed weak association between dietary factors and adult-onset asthma incidence,[25] the asthma symptom score might be particularly relevant to studying the role of dietary factors as an etiological risk factor and as a disease modifier.[26]

In the current study, we aimed to investigate the association between cured meat intake and the change in asthma symptom score in a French prospective study of adults, and to assess the role of BMI as a mediator using counterfactual approach to mediation analysis.

METHODS

Study sample

The EGEA study (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy) is a longitudinal study with an initial group of asthma cases, their first-degree relatives, and a group of controls, followed-up over 20 years (https://egeanet.vjf.inserm.fr/index.php/fr/). Protocol and descriptive characteristics of the EGEA study have been previously published [27,28]. Briefly, 2047 participants from 5 cities in France were enrolled between 1991 and 1995. Between 2003 and 2007, this population was contacted for the second survey (EGEA2). Out of 2002 participants alive, 1845 completed a short self-administered questionnaire and 1601 had a complete medical examination (among whom 715 (42%) ever had asthma). As a follow-up study of EGEA2, the third survey (EGEA3) was performed between 2011 and 2013 using a self-completed questionnaire, and 1558 questionnaires were returned.

Since diet was measured at EGEA2 only, we used data collected at EGEA2 as baseline and EGEA3 as the follow-up. Out of the 1601 participants from EGEA2, 35 adolescents (less than 16 years-old) were firstly excluded. Among adults (n=1566), we excluded 330 participants who did not fill the food frequency questionnaire, had an inadequate assessment of diet, or had an implausibly high or low total energy intake.[29] Ten participants with missing data for the asthma symptom score were further excluded. Among the 1226 remaining, 185 (15%) were lost to follow-up; compared with participants lost to follow-up (n=185), participants followed at EGEA3 (n=1041) were less likely to be men or smokers, and were similar regarding age, BMI, educational level, physical activity, and asthma status (online supplemental table 1). After excluding 70 participants with missing data for the asthma symptom score assessed at EGEA3 out of the 1041, the final study sample included 971 participants (figure 1).

Among adults, excluded participants (n=595) were similar compared with participants included in analysis (n=971) regarding age, BMI, physical activity, sex, smoking and asthma status, but they were more likely to have a lower educational level (online supplemental table 2).

Ethical approval was obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris). All participants signed written informed consent.

Dietary intakes

At EGEA2, we used a 118-item food frequency questionnaire based on a French validated dietary questionnaire [30] to assess average dietary intakes during the previous 12 months. These 118 items were grouped into 46 food groups and average intake was calculated for each group.[29] The French food composition table from the SU.VI.MAX survey was used to calculate the nutrients intakes and total energy intake (kcal/day).[31] The cured meat group included 3 items: ham (2 slices/portion), sausage (1/portion) and dried sausage (2 slices/portion). According to the distribution of cured meat intake, exposure was categorized in 3 classes: <1 serving/week (lowest; 19%), 1-3.9 servings/week (medium; 48%) and ≥4 servings/week (highest; 33%). The group with the lowest intake was considered as "non-exposed" and was taken as the reference group.

To account for the overall remaining effect of diet in the cured meat-asthma association, analyses were adjusted for dietary patterns derived from all food groups except cured meat. In the EGEA study, two dietary patterns were previously identified using confirmatory factor analysis (CFA) on the 46 food groups: 1) a Prudent pattern characterized by a high intake of vegetables, fruits, oils, whole grains cereals and fish, and 2) a Western pattern loaded by a high consumption of prepared meal, French fries, cured meat, condiments, alcohol, beer/cider,

sandwiches and potatoes.[29] In the current analysis, we derived a "modified Western pattern" and a Prudent pattern using CFA on 45 food groups (all food groups except cured meat, online supplemental table 3). The individual scores obtained from CFA for the two dietary patterns were used as potential confounders in analysis.

Asthma symptom score

The asthma symptom score has been proposed as a continuous measure to study risk factors for asthma in longitudinal studies. Compared with dichotomous definition of asthma, increased power in analysis identifying risk factors was suggested when using the asthma symptoms score. [23,24] In the EGEA study, the asthma symptom score was calculated both at EGEA2 and EGEA3. Ranging from 0 to 5, the score is based on the number of respiratory symptoms during the past 12 months reported in the questionnaire: 1) breathless while wheezing, 2) woken up with chest tightness, 3) attack of shortness of breath at rest, 4) attack of shortness of breath after exercise, and 5) woken by attack of shortness of breath. Change in asthma symptom score was categorized as "stable or improved" if EGEA3 score - EGEA2 score ≤ 0, and "worsening" if EGEA3 score - EGEA2 score > 0.

Body mass index

Weight and height of participants were measured at EGEA2. Body mass index (kg/m^2) at EGEA2 was calculated as weight/height^2 and was categorized into 5 classes: <22.5, 22.5-24.9, 25-27.4, 27.5-29.9, ≥30.

Other variables

Age, sex, smoking status (never and ever smoking), educational level, and physical activity were assessed at baseline (EGEA2). Educational level was categorized into 2 classes

depending on whether the participants had higher education (university or equivalent). Leisure-time physical activity was assessed in metabolic equivalents per week (METs/w) taking into account a variety of intensive activities (such as jogging, aerobic, swimming, cycling, tennis, soccer, digging in the garden), moderate activities (such as cleaning, walking, golf, slight gardening), and light activities (such as reading, watching TV, office work, driving, eating). Average physical activity was estimated by summing the product of average active time per week and the metabolic equivalent value of each activity.

Due to the case-control design of the EGEA study, the study population was enriched with participants with asthma. To take into account this study design, ever asthma was considered as a covariate in analysis. Ever asthma was defined by being recruited as an asthma case in chest clinics at EGEA1 or responding positively to at least one of the two questions at EGEA1 or EGEA2: 1)“Have you ever had attacks of breathlessness at rest with wheezing?” and 2)“Have you ever had asthma attacks?” Never asthma was defined by absence of asthma at EGEA1 and EGEA2.

Statistical analysis

We used a direct acyclic graph (DAG) to represent our mediation model (figure 2). Age (continuous), sex, smoking status (never/ever), educational level (low/high (i.e., university or equivalent)), leisure-time physical activity (expressed in metabolic equivalents/week, continuous), dietary patterns, total daily energy intake, and asthma status at EGEA2 were considered as potential confounders.

Several methods with practical implementation have recently been proposed to disentangle the natural direct and indirect effects in the counterfactual framework.[19–21] In the current analysis, we applied the marginal structural model proposed by Lange and colleagues, [20] which has been described as a simple approach and can be implemented in

standard software for almost any variable type. Detailed implementation of this method was described in the online supplementary methods. As previously described,[19,20] several conditions are required to apply such methods, and we assumed that they were satisfied (online supplementary methods).

To evaluate how much of the total effect was due to the effect of BMI, we calculated the "proportion explained" by BMI as $(OR_{TE}-OR_{NDE}) / (OR_{TE}-1)$, where OR_{TE} and OR_{NDE} were respectively the odds ratio of total effect and natural direct effect.[32] The total effect was calculated as $OR_{NDE} \times OR_{NIE,}$ where OR_{NIE} denoted the natural indirect odds ratio.

We tested potential interactions between cured meat intake and BMI in the association with asthma.[20] In addition, since cured meat intake is usually higher in men than in women,[33,34] and often associated with smoking habit,[35] we conducted sensitivity analyses stratified on sex and smoking status, and formally tested interactions of cured meat intake with sex and smoking. Furthermore, considering the case-control design of EGEA study, we also performed a stratified analysis on asthma status at EGEA2.

As a sensibility analysis, we replicated our results using another method of mediation analysis in the counterfactual framework, a 2-stage regression model, which provides estimation of total effect and standard errors using bootstrapped samples [19] (see online supplementary methods for details).

All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Description of population

The study sample was comprised of 971 participants. The mean age was 43 years old and 49% were men. At EGEA2, 42% participants had ever asthma, 51% were never smokers, 35% were overweight and 9% were obese. The median cured meat intake was 2.5 (Q1-Q3: 1.5 - 4.5) servings/week.

Baseline characteristics of the participants according to cured meat intake are presented in table 1. Compared with participants with the lowest intake (<1 serving/week) of cured meat, participants with the highest intake (≥4 serving/week) were younger ($p<0.001$), more likely to be men ($p<0.001$) and smokers ($p=0.04$), to report a higher total energy intake ($p<0.001$), and to have ever asthma ($p=0.03$); as expected, they also consumed more sodium ($p<0.001$) and saturated fat ($p<0.001$). Besides, participants who had a higher intake of cured meat were more likely to be in a higher BMI category ($p= 0.01$).

Table 1 Characteristics for participants at baseline (EGEA2) according to cured meat intake (n=971)

	Cured meat intake			p Value
	<1 s/w	1-3.9 s/w	≥4 s/w	
	(n = 181)	*(n =462)*	*(n=328)*	
Age, y	45 ± 16	44 ± 16	40 ± 16	<0.001
Men, n (%)	60 (33)	221 (48)	195 (59)	<0.001
BMI, kg/m^2	23.7 ± 3.8	24.5 ± 4.1	24.6 ± 4.4	0.07
BMI, kg/m^2, n (%)				0.01
<22.5	85 (47)	172 (37)	131 (40)	
22.5-24.9	40 (22)	128 (28)	71 (22)	
25-27.4	34 (19)	77 (17)	54 (16)	
27.5-29.9	13 (7)	44 (9)	32 (10)	
≥30	9 (5)	41 (9)	40 (12)	
Smoking (ever), n (%)	82 (45)	209 (45)	179 (55)	0.03
Higher education, n (%)	87 (48)	254 (55)	153 (47)	0.06
Total energy intake, kcal/day	2187 ± 593	2357 ± 606	2657 ± 650	<0.001
Leisure-time physical activity, METs/w	44 ± 16	45 ± 17	45 ± 17	0.72
Ever asthma at EGEA2, n (%)	68 (38)	184 (40)	156 (48)	0.04
Asthma symptom score at EGEA2, n (%)				0.11
0	86 (47)	231 (50)	142 (43)	
1	39 (22)	116 (25)	70 (21)	
2 - 5	56 (31)	115 (25)	116 (36)	
Modified Western pattern*	-1.5 ± 3.3	-0.5 ± 3.3	1.6 ± 4.4	<0.001
Prudent pattern*	0.5 ± 2.4	0 ± 1.9	-0.2 ± 2.4	0.002
Foods/nutrients intake				
Cured meat, serving/day	0.7 ± 0.4	2.3 ± 0.7	6.2 ± 3.5	<0.001
Fruits, serving/day	3.9 ± 2.8	3.7 ± 2.8	3.6 ± 3.0	0.47
Vegetables, serving/day	3.9 ± 2.1	3.7 ± 2.0	3.8 ± 2.2	0.83
Whole grain cereals, serving/day	0.6 ± 1.0	0.5 ± 0.8	0.4 ± 0.8	0.07
Fish, serving/day	0.4 ± 0.3	0.4 ± 0.3	0.5± 0.4	0.18
Vitamin C, mg/day	252 ± 136	240 ± 124	248 ± 133	0.50
Sodium, g/day	3.2 ± 1.1	3.5 ± 1.1	4.3 ± 1.2	<0.001
Total fat, g/day	88 ± 27	100 ± 29	115 ± 33	<0.001
Saturated fat, g/day	33 ± 10	38 ± 12	45 ± 13	<0.001

Data are expressed as mean ± SDs, or number of subjects (%). Overall p values were calculated from ANOVA for continuous variables, from Mantel-Haenszel chi-square test for ordinal categorized variables (BMI classes and asthma symptom score), and from Pearson chi-square test for other categorized variables.

*Modified Western pattern, Prudent pattern: identified using confirmatory factor analysis (CFA). Mean individual scores obtained from CFA were shown.

s/w, serving/week; METs/w, metabolic equivalents per week.

1

Cured meat intake and change in asthma symptom score

At EGEA3, after a mean follow-up time of seven years (ranging from five to nine years), 513 participants (53%) reported the same asthma symptom score as EGEA2 (stable), 191 (20%) reported more symptoms (worsening), and 267 (27%) had less symptoms (improved). The proportion of participants with worsening asthma symptoms was 14%, 20% and 22% respectively among those who ate <1, 1-3.9 and ≥4 servings/week of cured meat (p for trend=0.04).

Results of mediation analyses using the marginal structural model are presented in table 2. Among all the participants, after adjustments for age, sex, smoking, educational level, physical activity, dietary patterns, total energy intake, and asthma status, we observed a positive direct effect of cured meat intake on worsening asthma symptoms: the odds ratio (OR) was 1.76 (95% CI: 1.01, 3.06) when comparing the highest to the lowest intake. We also reported a positive indirect effect mediated by BMI: the adjusted OR was 1.07 (95% CI: 1.01, 1.14) when comparing the highest to the lowest intake (figure 3). The percentage mediated by BMI of the association was 14%. No interaction was observed between cured meat intake and BMI (p for interaction=0.90) in the associations with asthma. We formally tested interactions for total effect between cured meat intake with sex (p for interaction=0.50), smoking status (p for interaction=0.60), and asthma status (p for interaction=0.50). When we performed stratified analyses according to sex, smoking, and asthma status, results were similar in all subgroups (OR>1), although the power was limited (table 2).

Table 2 Direct and indirect effects of cured meat intake on worsening asthma symptoms between EGEA 2 and 3, using the marginal structural model (n=971)

	No. stable or improved/ worsening			Direct effect* OR (95%CI)			Indirect effect* OR (95%CI)		
	<1 s/w	1-3.9 s/w	≥4 s/w	<1 s/w	1-3.9 s/w	≥4 s/w	<1 s/w	1-3.9 s/w	≥4 s/w
All participants	155/26	370/92	255/73	1.00	1.56 (0.93, 2.61)	1.76 (1.01, 3.06)	1.00	1.04 (1.00, 1.09)	1.07 (1.01, 1.14)
Subgroups									
Sex									
Men	51/9	184/37	153/42	1.00	1.04 (0.43, 2.50)	1.31 (0.54, 3.20)	1.00	1.04 (0.96, 1.12)	1.06 (0.96, 1.17)
Women	104/17	186/55	102/31	1.00	1.97 (1.03, 3.77)	2.00 (0.96, 4.16)	1.00	1.05 (1.00, 1.11)	1.09 (1.00, 1.18)
Smoking									
Never	87/12	206/45	113/36	1.00	1.61 (0.73, 3.54)	2.47 (1.03, 5.92)	1.00	1.08 (0.98, 1.19)	1.11 (0.99, 1.24)
Ever	68/14	162/47	142/37	1.00	1.52 (0.76, 3.06)	1.25 (0.60, 2.61)	1.00	1.02 (0.99, 1.04)	1.05 (0.98, 1.11)
Asthma									
Ever	57/11	135/49	118/38	1.00	2.21 (1.00, 4.88)	2.16 (0.93, 5.04)	1.00	1.08 (1.00, 1.16)	1.13 (1.01, 1.27)
No	98/15	235/43	137/35	1.00	1.27 (0.64, 2.49)	1.63 (0.80, 3.33)	1.00	1.02 (0.97, 1.08)	1.04 (0.97, 1.11)

*For all participants, we adjusted for age, sex, smoking status, educational level, leisure-time physical activity, modified Western and Prudent patterns, total energy intake, and asthma status at EGEA2; for subgroup analyses, we adjusted for all potential confounders except the stratifying variable.

s/w, serving/week.

Analyses using another method of mediation analysis in the counterfactual framework yielded similar results to those obtained through the marginal structural model (online supplemental table 5 and 6).

DISCUSSION

In this French prospective study of adults, using mediation analysis in the counterfactual framework, we reported a positive association between high cured meat intake (≥4 serving/week) and worsening asthma symptoms over time. While the indirect effect mediated through BMI accounted for only 14% of this association, the direct effect explained a greater proportion, suggesting a deleterious role of cured meat independent of BMI.

Diet is highly influenced by culture as well as geographical, environmental, and socioeconomic factors. In our study, cured meat food group included three typical French cured meats - ham, sausages, and dried sausage. The mean intakes of cured meats in the EGEA study were similar to those reported by the French National Individual Survey on Food Consumption 2006-2007.[34] The EGEA results are in agreement with previous researches indicating a harmful association between cured meat intake and different measures of lung health in several countries, where the types and preparation of cured meats may be different.[5–9] However, two large prospective American studies,[10,11] with different types of cured meat (mainly bacon, hot dogs and sausages), reported that cured meat intake may increase the risk of newly diagnosed COPD, but not the risk of incident asthma, after adjustment for BMI and other potential confounders. This different finding may result from the role of BMI in the association (confounder vs. mediator) or the assessment of respiratory phenotypes (incidence vs. change in asthma symptoms).

In the present study, BMI was taken into account as a mediator rather than a confounder. The modification of dietary habits, the obesity epidemic, and the decrease in physical activity, have been suggested to play a role in the increase of asthma worldwide during the last decades.[36] Overweight and obesity reflect an imbalance between energy provision (i.e. intake of calories) and expenditure (i.e. physical activity), so the interrelations between these factors make it difficult to disentangle their separate roles on asthma, and to

identify confounders and mediators in the diet-asthma association.[17] To date, the adjustment for BMI has been widely used when investigating the role of nutritional factors in obesity-related diseases. Previous studies have consistently suggested a dose-dependent association between BMI and the risk of asthma incidence and control,[14–16] indicating that obesity is a likely mediator in the diet-asthma association. The traditional BMI-adjusted method could over adjust the association and lead to biased results. Mediation analysis in the counterfactual framework allows taking into account BMI correctly in such association. Moreover, it provides a quantitative measure of the proportion mediated through a given mediator, which could help to better understand the observed association. To the best of our knowledge, this is the first application of newly developed methods for mediation analysis to estimate the association between cured meat intake and asthma.

Accounting for BMI as a mediator, we reported an overall positive association between high cured meat intake and worsening asthma symptoms. The indirect effect mediated through BMI accounted for only 14% of the total effect, supporting the hypothesis that the cured meat-asthma association is related to other mechanisms. Several potential mechanisms were proposed by previous studies involving biological markers. First, cured meats are rich in nitrite, which may lead to nitrosative stress [13] and oxidative stress [37] related lung damage and asthma. Second, the positive relation between cured meat intake and C-reactive protein [38] indicated that cured meat might increase the systemic inflammation, which may have an influence on asthma. In addition, the high content of salt and saturated fat in cured meat might also contribute in part to the association with asthma, though existing evidence has been mainly for childhood-onset asthma.[39] Further studies need to be carried out to improve our understanding regarding potential mechanistic processes.

Besides the BMI issue (mediator vs. confounder), we have used, unlike the previous studies,[10,11] an asthma symptom score instead of incident doctor-diagnosed adult-onset

asthma. Studies on incidence assume that the disease is a true dichotomous entity, which might be inappropriate in asthma for several reasons: 1) asthma is a complex disease, with strong clinical phenotypic heterogeneity and temporal phenotypic variability in both adults and children; [40, 41] 2) asthma develop over a long period of time with preclinical status difficult to be defined; and 3) asthma has low incidence in adults and different epidemiological definitions of asthma exist. These characteristics of asthma might lead to misclassification and biased association estimates.[22] Without making the dichotomous assumption of the asthma status, the asthma symptom score captures temporal variability of asthma and allows assessment of the disease progression in longitudinal studies,[23] and it is a more powerful tool to investigate risk factors for asthma. Furthermore, the asthma symptom score was shown to have increased specificity and positive predictive value when compared to self-report ever asthma or bronchial hyperresponsiveness;[23] and the asthma symptom score at baseline has been associated with markers of asthma but not those of COPD at follow-up.[24] In participants with asthma, worsening symptoms capture relapse or exacerbation of the disease, and in participants without asthma, worsening symptoms coincide with the asthma incidence and activity. While the asthma symptom score was developed and validated in a general population,[23, 24] our results were obtained in a population among which 42% had ever asthma. Although previous research has suggested that diet could be a modifier on asthma control rather than an etiological risk factor for incidence of asthma,[26] we did not observe interaction between cured meat intake and asthma status at EGEA2 on the association with worsening symptoms. The stratified analysis according to asthma status at EGEA2 showed similar effects in participants with ever asthma. Further studies are needed to clarify potential difference related to asthma status in the effect of cured meat intake on worsening asthma symptoms.

Because people consume complex meals rather than isolated food/nutrients that might interact with each other, the association between cured meat intake itself and the asthma symptom score is difficult to assess and may be partly due to the confounding by other food groups.[25] Therefore we adjusted the associations for scores of dietary patterns. The total, direct and indirect effects remained after adjustment for these dietary scores. We acknowledge that findings might not be generalizable to populations with completely different diet habits, such as infrequent cured meat intake and very different food consumptions. The association between cured meat intake and the asthma symptom score may also be due, in part, to a residual confounding by cigarette smoking.[42] To minimize this possibility, we performed a stratified analysis according to smoking status and yielded comparable results in never smokers, although we faced a statistical power issue. Additionally, since cured meat intake has been suggested as a risk factor for COPD,[10,11] the potential overlap between COPD and asthma might contribute to the association between high cured meat intake and asthma. Nevertheless, the overlap between COPD and asthma is less likely in the current study because: 1) comparable results were obtained among never smokers, 2) the EGEA study was initiated as a case-control study on asthma with cases recruited in hospital, with both childhood asthma and adult-onset asthma,[27,28] and 3) the use of the asthma symptom score reduces the possibility of this overlap.[24] We acknowledge that our definition of "non-exposed" group (<1 serving/week) was not ideal. Due to the limited number of non-eaters of cured meat in the EGEA study, we faced a statistical power issue in subgroup analyses. We acknowledge that 15% of the participants were lost to follow-up. However, most of the characteristics were similar among included and lost to follow-up participants, excepted for sex and smoking (as expected, more men and more smokers were lost to follow-up). We also acknowledge that deterministic approach was used to deal with missing data for asthma symptom score (7% participants). Similar to any

other observational study, though we were able to adjust for many potential confounders due to the extended information available in our study, the validity of the results relies on the assumption of no unmeasured confounding.[43] Besides, we acknowledge that the recall bias could exist as data from questionnaires were used.

In summary, correctly accounting for BMI as a potential mediator in the diet-asthma association, higher cured meat intake was associated with worsening asthma symptoms over time, through not only an indirect effect mediated by BMI but also a greater direct effect. This research extends the deleterious effect of cured meat in health, as well as the effect of diet on asthma in adults, and provides a novel analysing approach regarding the role of BMI in the diet-asthma association.

Acknowledgements We thank Pr. Serge Hercberg who provided us the nutrients database from the SU.VI.MAX survey; Katelle Joly, Anne Forhan and Peggy Drouillet for computation of the nutrients in the EGEA2 survey. We thank also all those who participated in the setting of the study, the EGEA cooperative group. They are as follows. Coordination: V Siroux (epidemiology, PI since 2013); F Demenais (genetics); I Pin (clinical aspects); R Nadif (biology); F Kauffmann (PI 1992-2012). Respiratory epidemiology: Inserm U700, Paris: M Korobaeff (Egea1), F Neukirch (Egea1); Inserm U707, Paris: I Annesi-Maesano (Egea1-2); Inserm CESP/U1018, Villejuif: F Kauffmann, N Le Moual, R Nadif, MP Oryszczyn (Egea1-2), R Varraso; Inserm U823, Grenoble: V Siroux. Genetics: Inserm U393, Paris: J Feingold; Inserm U946, Paris: E Bouzigon, F Demenais, MH Dizier; CNG, Evry: I Gut (now CNAG, Barcelona, Spain), M Lathrop (now Univ McGill, Montreal, Canada). Clinical centers: Grenoble: I Pin, C Pison; Lyon: D Ecochard (Egea1), F Gormand, Y Pacheco; Marseille: D Charpin (Egea1), D Vervloet (Egea1-2); Montpellier: J Bousquet; Paris Cochin: A Lockhart (Egea1), R Matran (now in Lille); Paris Necker: E Paty (Egea1-2), P Scheinmann (Egea1-2); Paris-Trousseau: A Grimfeld (Egea1-2), J Just. Data and quality management: Inserm ex-U155 (Egea1): J Hochez; Inserm CESP/U1018, Villejuif: N Le Moual; Inserm ex-U780: C Ravault (Egea1-2); Inserm ex-U794: N Chateigner (Egea1-2); Grenoble: J Quentin-Ferran (Egea1-2).

Contributors ZL, RN and RV: designed and conducted the research; BL, NLM, CP, VS, RN and RV: provided essential reagents or provided essential materials; ZL, MR, AB, OD, JG-A, VS, CAC, RN and RV: analyzed data or performed statistical analysis; ZL, RN and RV: wrote the manuscript and had primary responsibility for final content; all authors read, edited and approved the final manuscript.

Funding This research was funded in part by Merck Sharp & Dohme (MSD), the GA2LEN project, Global Allergy and Asthma European Network, and Conseil scientifique AGIR pour les maladies chroniques, National Hospital program of clinical research (PHRC-national).

Ethics approval Ethical approval was obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris).

Competing interests None declared.

REFERENCES

1 WHO | Q&A on the carcinogenicity of the consumption of red meat and processed meat. WHO. http://www.who.int/features/qa/cancer-red-meat/en/ (accessed 13 Jan2016).

2 Cross AJ, Leitzmann MF, Gail MH, et al. A Prospective Study of Red and Processed Meat Intake in Relation to Cancer Risk. PLoS Med 2007;4:e325.

3 Pan A, Sun Q, Bernstein AM, et al. Red meat consumption and mortality: results from 2 prospective cohort studies. Arch Intern Med 2012;172:555–63.

4 Micha R, Wallace SK, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. Circulation 2010;121:2271–83.

5 Lam TK, Cross AJ, Consonni D, et al. Intakes of Red Meat, Processed Meat, and Meat Mutagens Increase Lung Cancer Risk. Cancer Res 2009;69:932–9.

6 Yang L, Lu X, Deng J, et al. Risk factors shared by COPD and lung cancer and mediation effect of COPD: two center case–control studies. Cancer Causes Control 2015;26:11–24.

7 Jiang R, Paik DC, Hankinson JL, et al. Cured Meat Consumption, Lung Function, and Chronic Obstructive Pulmonary Disease among United States Adults. Am J Respir Crit Care Med 2007;175:798–804.

8 Okubo H, Shaheen SO, Ntani G, et al. Processed meat consumption and lung function: modification by antioxidants and smoking. Eur Respir J 2014;43:972–82.

9 Batlle J de, Mendez M, Romieu I, et al. Cured meat consumption increases risk of readmission in COPD patients. Eur Respir J 2012;40:555–60.

10 Jiang R, Camargo CA, Varraso R, et al. Consumption of cured meats and prospective risk of chronic obstructive pulmonary disease in women. Am J Clin Nutr 2008;87:1002–8.

11 Varraso R, Jiang R, Barr RG, et al. Prospective Study of Cured Meats Consumption and Risk of Chronic Obstructive Pulmonary Disease in Men. Am J Epidemiol 2007;166:1438–45.

12 Lijinsky W. N-Nitroso compounds in the diet. Mutat Res 1999;443:129–38.

13 Sugiura H, Ichinose M. Nitrative stress in inflammatory lung diseases. Nitric Oxide 2011;25:138–44.

14 Boulet L-P. Asthma and obesity. Clin Exp Allergy 2013;43:8–21.

15 Beuther DA, Sutherland ER. Overweight, Obesity, and Incident Asthma. Am J Respir Crit Care Med 2007;175:661–6.

16 Rodrigo GJ, Plaza V. Body mass index and response to emergency department treatment in adults with severe asthma exacerbations: a prospective cohort study. Chest 2007;132:1513–9.

17 Bédard A, Dumas O, Kauffmann F, et al. Potential confounders in the asthma–diet association: how causal approach could help? Allergy 2012;67:1461–3.

18 Weinberg CR. Toward a clearer definition of confounding. Am J Epidemiol 1993;137:1–8.

19 Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. Psychol Methods 2013;18:137–50.

20 Lange T, Vansteelandt S, Bekaert M. A Simple Unified Approach for Estimating Natural Direct and Indirect Effects. Am J Epidemiol 2012;176:190–5.

21 Imai K, Keele L, Tingley D. A general approach to causal mediation analysis. Psychol Methods 2010;15:309–34.

22 Pekkanen J, Sunyer J. Problems in using incidence to analyze risk factors in follow-up studies. Eur J Epidemiol 2008;23:581–4.

23 Pekkanen J, Sunyer J, Anto JM, et al. Operational definitions of asthma in studies on its aetiology. Eur Respir J 2005;26:28–35.

24 Sunyer J, Pekkanen J, Garcia-Esteban R, et al. Asthma score: predictive ability and risk factors. Allergy 2007;62:142–8.

25 Varraso R. Nutrition and asthma. Curr Allergy Asthma Rep 2012;12:201–10. doi:10.1007/s11882-012-0253-8

26 Varraso R, Camargo CA. Can dietary interventions improve asthma control? Prim Care Respir J 2012;21:367.

27 Kauffmann F, Dizier MH, Pin I, et al. Epidemiological study of the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy: phenotype issues. Am J Respir Crit Care Med 1997;156:S123–9.

28 Kauffmann F, Dizier MH, Annesi-Maesano I, et al. EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy)--descriptive characteristics. Clin Exp Allergy J Br Soc Allergy Clin Immunol 1999;29 Suppl 4:17–21.

29 Varraso R, Garcia-Aymerich J, Monier F, et al. Assessment of dietary patterns in nutritional epidemiology: principal component analysis compared with confirmatory factor analysis. Am J Clin Nutr 2012;96:1079–92.

30 Bonifacj C, Gerber M, Scali J, et al. Comparison of dietary assessment methods in a southern French population: use of weighed records, estimated-diet records and a food-frequency questionnaire. Eur J Clin Nutr 1997;51:217–31.

31 Hercberg S, Preziosi P, Briançon S, et al. A primary prevention trial using nutritional doses of antioxidant vitamins and minerals in cardiovascular diseases and cancers in a general population: the SU.VI.MAX study--design, methods, and participant

characteristics. SUpplementation en VItamines et Minéraux AntioXydants. Control Clin Trials 1998;19:336–51.

32 Hafeman DM. “Proportion Explained”: A Causal Interpretation for Standard Measures of Indirect Effect? Am J Epidemiol 2009;170:1443–8.

33 Daniel CR, Cross AJ, Koebnick C, et al. Trends in meat consumption in the United States. Public Health Nutr 2011;14:575–83.

34 Etude individuelle Nationale sur les Consommations Alimentaires 2006-2007. https://www.anses.fr/fr/content/inca-2-les-r%C3%A9sultats-dune-grande-%C3%A9tude (accessed 8 Jun2015).

35 Margetts BM, Jackson AA. Interactions between people’s diet and their smoking habits: the dietary and nutritional survey of British adults. BMJ 1993;307:1381–4.

36 Devereux G. The increase in the prevalence of asthma and allergy: food for thought. Nat Rev Immunol 2006;6:869–74.

37 Holguin F. Oxidative stress in airway diseases. Ann Am Thorac Soc 2013;10 Suppl:S150–7.

38 Ley SH, Sun Q, Willett WC, et al. Associations between red meat intake and biomarkers of inflammation and glucose metabolism in women. Am J Clin Nutr 2014;:ajcn.075663.

39 Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? The Lancet 2015;386:1075–85.

40 Boudier A, Curjuric I, Basagaña X, et al. Ten-year follow-up of cluster-based asthma phenotypes in adults. A pooled analysis of three cohorts. Am J Respir Crit Care Med 2013;188:550–60.

41 Henderson J, Granell R, Heron J, et al. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. Thorax 2008;63:974–80.

42 Polosa R, Thomson NC. Smoking and asthma: dangerous liaisons. Eur Respir J 2013;41:716–26.

43 Hernan MA, Robins JM. Estimating causal effects from epidemiological data. J Epidemiol Community Health 2006;60:578–86.

Figures:

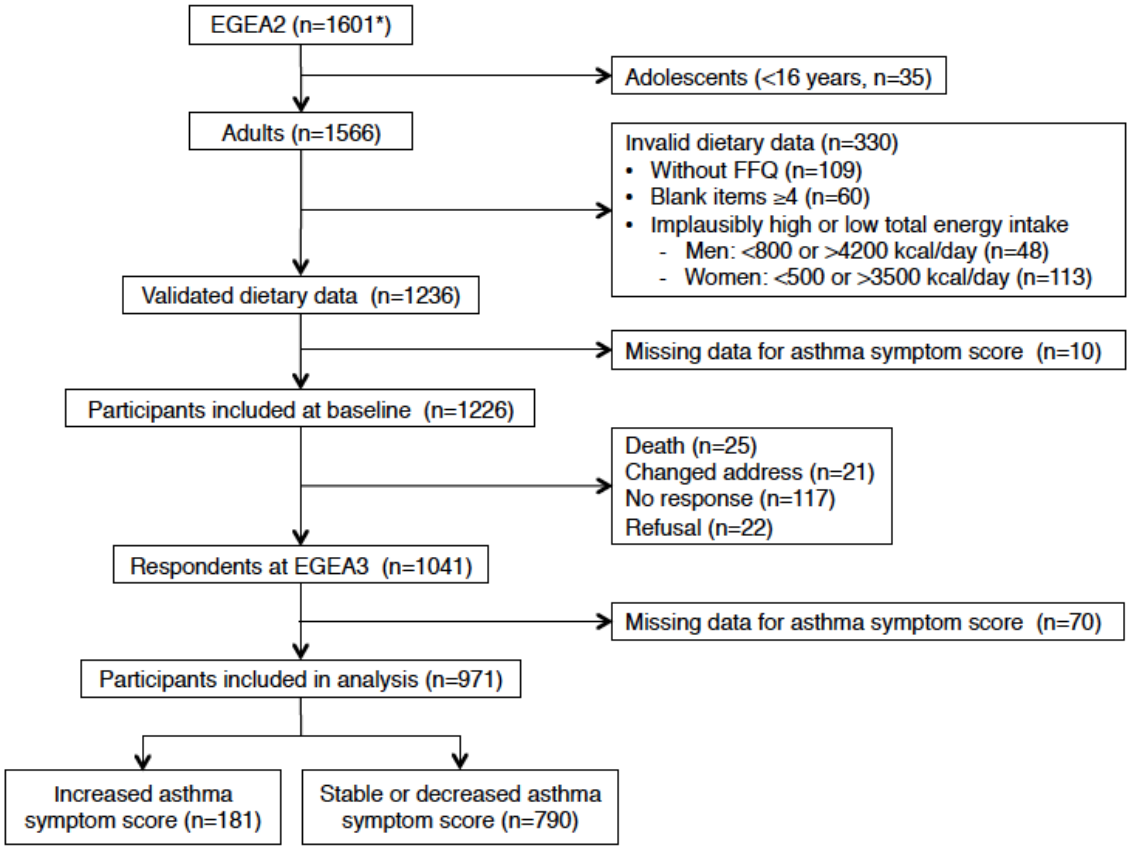

Figure 1 Flowchart for inclusion of participants. *Among the 1601 participants, 715 (45%) had ever asthma. FFQ, food frequency questionnaire.

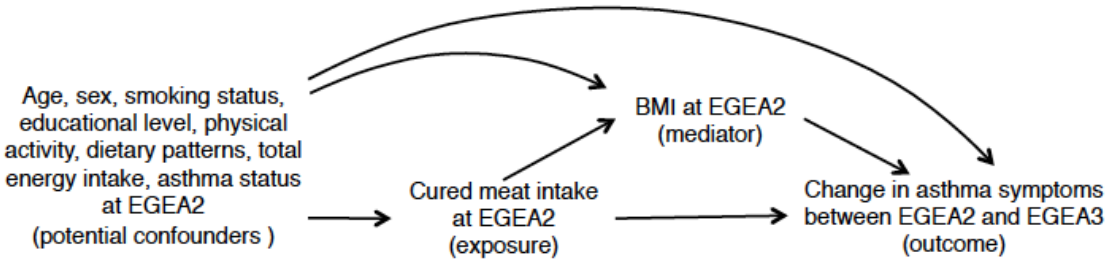

Figure 2 Direct acyclic graph of the proposed mediation model.

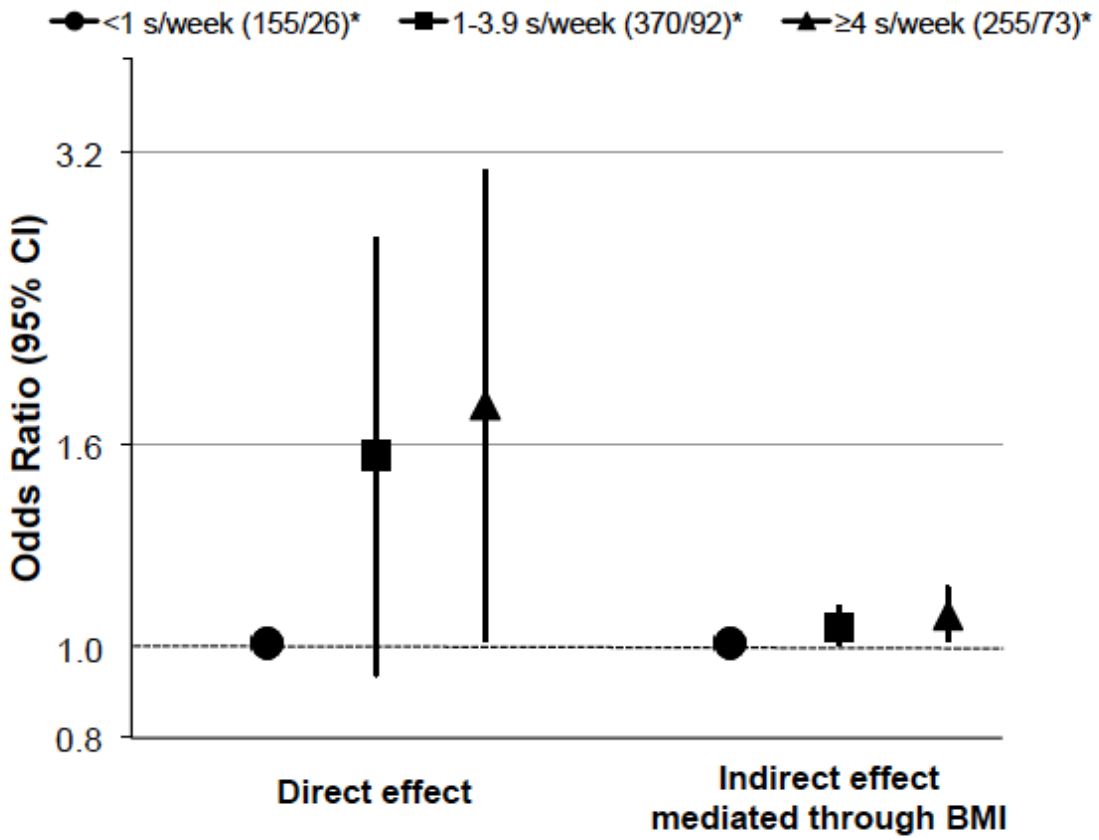

Figure 3 Direct and indirect effects of cured meat intake on worsening asthma symptoms between EGEA2 and 3, using the marginal structural model (n=971). We adjusted for age, sex, smoking status, educational level, leisure-time physical activity, modified Western and Prudent patterns, total energy intake, and asthma status at EGEA2. *Numbers of participants (stable or improved/worsening). s/week, serving/week.

Online Supplementary Material

Supplementary Methods

Statistical analysis-mediation analyses

Several methods with practical implementation have recently been proposed to disentangle the natural direct and indirect effects in the counterfactual framework.[1–3]. Using such analyses, we assumed the following conditions were satisfied for the application of counterfactual approach to mediation analysis: no unmeasured confounders for the associations between: 1) cured meat and asthma score, 2) BMI and asthma score, 3) cured meat and BMI, as well as 4) no BMI-asthma score confounders affected by cured meat.[1,2]

The marginal structural model is implemented in several steps as previously described in detail.[1] Briefly, we first created a new data set by repeating each observation three times including a new variable A^*, which was each time set to one of the three possible categories of the cured meat intake (exposure, denoted by A). Next, a generalized linear regression model was applied to the new dataset to estimate the association between BMI (mediator, denoted by M) and cured meat intake, first using the original variable A, and then the new variable A*. Using predicted probabilities from the models with A and A*, the individual stabilized weights were calculated as $W_i^c = P(M = M_i|A = A_i^*, C = C_i)/$ $P(M = M_i|A = A_i, C = C_i)$, where C denoted all the confounders. Finally, the following weighted logistic regression model was applied to estimate the association between the change in asthma symptom score and cured meat intake: $logit\left[E\left[Y_{a,M_{a^*}}\right]\right] = c_0 + c_1 a + c_2 a^* + c_3 C$. The odds ratio of the natural direct effect was estimated by $exp[c_1(a - a^*)]$, and the natural indirect effect by $exp[c_2(a - a^*)]$.

As a sensibility analysis, in addition to the marginal structural model, [1] we applied a 2-stage regression model proposed by Valeri and VanderWeele, which provides estimation of total effect and standard errors using bootstrapped samples. [2] For the 2-stage regression model, a macro statement [2] was applied. Cured meat intake was regrouped into two classes: <1 serving/week and ≥1 serving/week, because a three-class exposure was not supported in the SAS macro. The following linear regression model was implemented in the first stage to assess the association between BMI and cured meat intake: $E(M|A = a, M = m, C = c) = \beta_0 + \beta_1 a + \beta_2' c$; and in the second stage, the following logistic regression model was implemented to assess the association between the change in asthma symptom score and cured meat intake: $logit[P(Y = 1|A = a, M = m, C = c)] = \theta_0 + \theta_1 a + \theta_2 \mathrm{m} + \theta_3' \mathrm{c}$. Confidence intervals (95%) were estimated from 1000 bootstrapped samples. As the 2-stage regression approach was performed using the exposure as a two-classes variable, we also applied the marginal structural model using cured meat intake as a dichotomous variable.

All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina).

Supplementary Results

In addition to baseline characteristics of the participants according to cured meat intake (table 1, main text), baseline characteristics according to change in asthma symptoms are presented in the Supplemental Table 4.

As a sensibility analysis, we replicated our results using another method of mediation analysis in the counterfactual framework. Analyses using the 2-stage

regression model yielded similar results to those obtained through the marginal structural model (Supplemental Table 5 and 6).

Supplemental Table 1 Comparison of characteristics between participants followed at EGEA3 (n=1041) and participants lost to follow-up (n=185)

	Participants		*P*
	Followed at EGEA3	Lost to follow-up	
	(n =1041)	*(n = 185)*	
Age, y	43 ± 16	41 ± 17	0.07
Men, n (%)	508 (49)	117 (63)	<0.001
BMI, kg/m^2	24.4 ± 4.2	24.8 ± 4.5	0.38
Smoking (ever), n (%)	514 (49)	107 (58)	0.04
Higher education*, n (%)	519 (50)	83 (45)	0.20
Leisure time physical activity, METs/week	45 ± 17	47 ± 18	0.11
Ever asthmatic at EGEA2, n (%)	439 (42)	90 (49)	0.10
Cured meat intake	3.2 ± 2.9	3.2 ± 2.1	0.83

Data are presented as mean ± SDs, or number of subjects (%). BMI: body mass index; METs/w: metabolic equivalents per week. *Higher education: college education or equivalent.

Supplemental Table 2 Comparison of characteristics between included (n=971) and excluded (n=595) adult participants

	Participants		*P*
	Included *(n = 971)*	Excluded *(n = 595)*	
Age, y	43 ± 16	43 ± 17	0.47
Men, n (%)	476 (49)	297 (50)	0.73
BMI, kg/m^2	24.4 ± 4.2	24.7 ± 4.5	0.27
Smoking (ever), n (%)	470 (49)	313 (53)	0.08
Higher education*, n (%)	494 (51)	220 (43)	0.002
Leisure time physical activity, METs/week	45 ± 17	45 ± 18	0.87
Ever asthmatic at EGEA2, n (%)	408 (42)	272 (46)	0.15

Data are presented as mean ± SDs, or number of subjects (%). BMI: body mass index; s/w: serving/week; METs/w: metabolic equivalents per week. *Higher education: college education or equivalent.

Supplemental Table 3 Factor-loading matrix for the major factors (dietary patterns) using confirmatory factor analysis in EGEA2 study (n=1236)

	Factor 1 (Prudent pattern)	Factor 2 Modified Western pattern)
Fruity vegetables	0.62	0.20
Leafy vegetables	0.59	
Other vegetables	0.58	
Fruits with flavonoids	0.55	
Root vegetables	0.54	0.32
Fruits with ß-carotene	0.48	
Citrus fruits	0.42	
Cruciferous vegetables	0.40	
Olive oil	0.35	
Other oil	0.32	
White fish	0.26	
Dried Legumes	0.27	0.25
Whole grains products	0.23	
Blue fish	0.23	0.23
Tea	0.20	
Other fruits	0.20	
Prepared meal		0.54
Condiments		0.45
French fries	-0.28	0.45
Pods and peas		0.33
Alcohol		0.31
Potatoes		0.30
Beer / cider		0.30

Sandwiches	-0.28	0.28
Snack	-0.23	0.27
Cakes		0.26
Soda	-0.28	0.27
Shellfish		0.21
Nuts and seeds		0.21

45 food groups (all food groups except cured meat) were included in analysis. Factor loadings represent the correlation between factor scores and intakes of food groups. The food groups with factor loadings < 0.20 for both factors were excluded. Absolute values < 0.20 were not listed in the table for simplicity; those with loadings of 0.30 or greater are underlined.

Supplemental Table 4 Characteristics for participants at baseline (EGEA2) according to change in asthma symptoms

	Change in asthma symptoms		
	Stable or improved *(n = 780)*	Worsening *(n =191)*	p Value
Age, y	42 ± 16	45 ± 16	0.04
Men, n (%)	388 (50)	88 (46)	0.36
BMI, kg/m^2	24.2 ± 3.9	25.0 ± 5.0	0.001
BMI, kg/m^2, n (%)			0.001
<22.5	323 (41)	65 (34)	
22.5-24.9	191 (25)	48 (25)	
25-27.4	141 (18)	24 (13)	
27.5-29.9	66 (8)	23 (12)	
≥30	59 (8)	31 (16)	
Smoking (ever), n (%)	406 (52)	93 (51)	0.39
Higher education, n (%)	410 (53)	84 (44)	0.03
Total energy intake, kcal/day	2418 ± 638	2464 ± 662	0.38
Leisure-time physical activity, METs/w	45 ± 17	45 ± 17	0.79
Ever asthma at EGEA2, n (%)	310 (40)	98 (51)	0.004
Asthma symptom score at EGEA2, n (%)			0.02
0	364 (47)	95 (50)	
1	180 (23)	45 (24)	
2 - 5	236 (30)	51 (26)	
Modified Western pattern*	0.0 ± 3.8	0.2 ± 4.0	0.46
Prudent pattern*	0.0 ± 2.2	0.2 ± 2.3	0.47
Foods/nutrients intake			
Fruits, serving/day	3.7 ± 2.9	3.8 ± 2.8	0.46
Vegetables, serving/day	3.7 ± 2.0	3.9 ± 2.2	0.37
Whole grain cereals, serving/day	0.5 ± 0.9	0.4 ± 0.8	0.12
Fish, serving/day	0.4 ± 0.3	0.4 ± 0.3	0.28
Vitamin C, mg/day	242 ± 127	259 ± 138	0.11
Sodium, g/day	3.7 ± 1.2	3.8 ± 1.3	0.26

Total fat, g/day	102 ± 32	105 ± 32	0.24
Saturated fat, g/day	39 ± 13	41 ± 13	0.23

Data are expressed as mean ± SDs, or number of subjects (%). Overall P values were calculated from ANOVA for continuous variables, from Mantel-Haenszel chi-square test for ordinal categorized variables (BMI classes and asthma symptom score), and from Pearson chi-square test for other categorized variables.

Supplemental Table 5 Total, direct and indirect effects of cured meat intake on worsening asthma symptoms between EGEA 2 and 3, using the 2-stage regression model (n=971)

	No. stable or improved/ worse		Total effect* OR (95% CI)	Direct Effect* OR (95% CI)	Indirect effect* OR (95% CI)
	<1 s/w	≥1 s/w			
All participants	155/26	625/165	1.80 (1.08, 2.95)	1.70 (1.02, 2.76)	1.06 (1.01, 1.14)
Subgroups					
Sex					
Men	51/9	337/79	1.70 (0.67, 4.36)	1.59 (0.62, 4.23)	1.07 (0.97, 1.22)
Women	104/17	288/86	2.06 (1.08, 3.95)	1.94 (1.01, 3.68)	1.06 (1.00, 1.16)
Smoking					
Never	87/12	319/81	2.24 (1.04, 4.77)	1.97 (0.92, 4.17)	1.14 (1.02, 1.32)
Ever	68/14	304/84	1.76 (0.80, 3.85)	1.73 (0.80, 3.70)	1.02 (0.97, 1.09)
Asthma					
Ever	57/11	253/87	2.50 (1.09, 5.63)	2.22 (0.96, 5.06)	1.13 (1.01, 1.32)
No	98/15	372/78	1.61 (0.80, 3.07)	1.57 (0.80, 3.04)	1.03 (0.96, 1.11)

*For all participants, we adjusted for age, sex, smoking status, educational level, leisure-time physical activity, modified western and prudent patterns, total energy intake, and asthma status at EGEA2; for subgroup analyses, we adjusted for all potential confounders except the stratifying variable. Reference: <1 serving/week. Estimates and confidence intervals (CIs) obtained using 1000 bootstrapped samples.

s/w, serving/week.

Supplemental Table 6 Direct and indirect effects of dichotomous cured meat intake on worsening asthma symptoms between EGEA 2 and 3, using the marginal structural model (n=971)

	No. Stable or improved/worse		Direct effect* OR (95% CI)	Indirect effect* OR (95% CI)
	<1 s/w	≥1 s/w		
All participants	155/26	625/165	1.63 (1.00, 2.67)	1.05 (1.01, 1.11)
Subgroups				
Sex				
Men	51/9	337/79	1.15 (0.50, 2.62)	1.05 (0.96, 1.14)
Women	104/17	288/86	1.99 (1.07, 3.70)	1.06 (1.00, 1.13)
Smoking				
Never	87/12	319/81	1.87 (0.88, 3.97)	1.09 (0.99, 1.21)
Ever	68/14	304/84	1.43 (0.74, 2.79)	1.03 (0.99, 1.07)
Asthma				
Ever	57/11	253/87	2.09 (0.97, 4.47)	1.10 (1.01, 1.20)
No	98/15	372/78	1.41 (0.71, 2.66)	1.03 (0.97, 1.09)

s/w, serving/week.

*For all participants, we adjusted for age, sex, smoking status, educational level, leisure-time physical activity, modified western and prudent patterns, total energy intake, and asthma status at EGEA2; for subgroup analyses, we adjusted for all potential confounders except the stratifying variable. Reference: <1 serving/week

References

1 Lange T, Vansteelandt S, Bekaert M. A Simple Unified Approach for Estimating Natural Direct and Indirect Effects. *Am J Epidemiol* 2012;**176**:190–5.

2 Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods* 2013;**18**:137–50.

3 Imai K, Keele L, Tingley D. A general approach to causal mediation analysis. *Psychol Methods* 2010;**15**:309–34.