Online Supplementary Material

Cured meat intake is prospectively associated with worsening asthma symptoms in adults, partly mediated through BMI

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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: $logit\left[E\left[Y_{a,M_{a^*}}\right]\right] = c_0 + c_1a + c_2a^* + c_3C$. 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: $logit[P(Y = 1|A = a, M = m, C = c)] = \theta_0 + \theta_1 a + \theta_2 m + \theta_3' c$. 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 *(n =1041)*	Lost to follow-up *(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.