The mediating role of inflammation in the association between cigarette smoking and intima-media thickness: the Guangzhou biobank cohort study

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Abstract

Background: Cigarette smoking is associated with thicker carotid intima-media thickness (IMT), probably partly through inflammatory pathways. However, to what extent does inflammation mediate the smoking-carotid atherosclerosis association remains unclear. We investigated the mediating effect of inflammation on the association between cigarette smoking and carotid IMT, and quantified the respective contributions of inflammatory markers to this association. Methods: A total of 1,752 participants from Guangzhou Biobank Cohort Study-Cardiovascular Disease Sub-cohort (GBCS-CVD) were included. Using causal mediation analysis under the counterfactual framework, we decomposed total effects of cigarette smoking on IMT into indirect effects (through inflammatory response) and direct effects (not through inflammatory response). Results: After adjusting for traditional risk factors, the indirect effects of per 109/L increment in leukocyte and granulocyte, per mg/L increment in high-sensitivity C-reactive protein (hs-CRP) and per mg/dl increment in fibrinogen on carotid IMT was 0.0028 mm (95% confidence interval (CI), 0.0011, 0.0047), 0.0019 mm (95% CI, 0.0006, 0.0034), 0.0017 mm (95% CI, 0.0006, 0.003) and 0.001 mm (95% CI, 0.0001, 0.0021), respectively. No evidence for a mediating role of lymphocyte was found. The proportion of the smoking-IMT association mediated by leukocyte, granulocyte, hs-CRP and fibrinogen was 12.57% (95% CI, 8.50%, 22.11%), 8.50% (95% CI, 5.76%, 15.09%), 7.64% (95% CI, 5.20%, 13.79%) and 4.48% (95% CI, 3.04%, 8.03%), respectively. Restricting data analysis to men showed similar results. Conclusions: The effects of cigarette smoking on IMT were partly mediated by leukocyte, hs-CRP and fibrinogen. The mediating role of leukocyte was likely mainly driven by higher granulocyte.

Background
Cigarette smoking causes the development and progression of atherosclerosis [1, 2], but the underlying mechanisms have yet to be confirmed [3, 4]. There are several potential mechanisms that could be relevant, i.e., inducing endothelial dysfunction, modifying lipid profile, and increasing inflammatory response and thrombosis [5, 6]. Thickening of carotid intima-media thickness (IMT) is a surrogate marker of early atherosclerosis and predicts the development of cardiovascular disease (CVD), independent of traditional CVD risk factors [7, 8]. While there was general agreement with the adverse effects of cigarette smoking and high inflammation on carotid IMT [1, 2, 9, 10], whether and to what extent inflammation mediates the smoking-atherosclerosis association remains unclear.

Fibrinogen is a marker of inflammation and thrombosis. It has been associated with a higher risk of CVD [11] and thicker IMT in some [12, 13], but not all studies [14, 15]. Previous studies showed that smokers had higher levels of fibrinogen than non-smokers [16, 17], and smoking might modify the association of fibrinogen with carotid IMT [9]. However, whether fibrinogen mediates the association between smoking and carotid atherosclerosis remains unclear.

As much evidence, including our previous papers from the Guangzhou Biobank Cohort Study (GBCS) [18, 19], has shown the detrimental effects of smoking on carotid IMT [1, 2], knowledge on the underlying mechanisms will be helpful for intervention strategies against smoking-induced atherosclerosis. We took advantage of the well-established GBCS using causal mediation analysis [20, 21] to investigate the mediating effects of several common inflammatory markers on the association between cigarette smoking and carotid IMT.

Methods

Study populations

The GBCS is a three-way collaboration among the Guangzhou Number12 Hospital in
Guangzhou, China, and the Universities of Hong Kong and Birmingham. The study aims to examine the effects of genetic, environmental, lifestyle, and occupational factors on health problems and chronic diseases. The Guangzhou Biobank Cohort Study-Cardiovascular Disease Sub-cohort (GBCS-CVD), nested in the GBCS, included 1,996 participants (992 (49.7%) men and 1,004 (50.3%) women) examined during September 2006 to December 2007. A detailed description of the CVD Sub-cohort has been published elsewhere [22, 23].

**Ethical approval**

The study received ethical approval from the Guangzhou Medical Ethics Committee of the Chinese Medical Association, Guangzhou, China. All participants provided written informed consent before participation. All methods were performed in accordance with the relevant guidelines and regulations.

**Exposure measurement**

A computer-assisted standardized questionnaire was used to collect information of demographic characteristics, socioeconomic position, occupational exposure, family and personal disease history, and lifestyle factors including smoking status, alcohol use, and physical activity measured by the International Physical Activity Questionnaire (IPAQ). Reliability of the questionnaire was evaluated by recalling 200 randomly selected participants for re-interview and the results were satisfactory [24]. Body height, weight, waist and hip circumference, and blood pressure were measured following standardized procedure. Body mass index (BMI) was calculated by weight (kg) divided by the square of body height (m²). Venous blood samples were collected after fasting for at least 8 hours for assay of lipids, fibrinogen and high-sensitivity C-reactive protein (hs-CRP) levels. Assay of complete blood count including leukocyte count and differential (granulocyte and lymphocyte count) was performed in an automated hematology analyzer (KX-21, SYMEX,
Japan).

**Study outcome**

Carotid intima-media thickness (IMT) were measured by carotid B-mode color ultrasonography using ALT HDI 3000 mainframe with a high-resolution, linear array scanner (medium frequency 7.5MHz) by a specialist physician [25]. The operators were registered ultrasound doctors who had a professional certificate for color Doppler ultrasound measurement awarded by the Ministry of Health of China. All scans were performed following a predetermined, standardized scanning protocol for the right and left carotid arteries using images of the far wall of the distal 10mm of the common carotid arteries. Three scanning angles, with the image focused on the posterior wall, were recorded from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface. Carotid IMT measurements were performed off-line with the use of automated image analysis software. All scans were analyzed by the same physician, blinded to participants’ information. Details of our carotid IMT measurement and research findings have been reported elsewhere [26, 27].

**Mediation analysis**

To estimate the contribution of inflammation to the association between smoking and IMT, we used causal mediation analysis under the counterfactual framework, which can decompose the averaged total effects (ATE) into indirect (average causal mediation effect, ACME) and direct effects (average direct effect, ADE) [28]. Let $M_i(t)$ denotes the potential value of a mediator of interest for unit $i$ under the positive exposure status $T_i = t$, $Y_i(t, m)$ denotes the potential outcome if the positive exposure status and mediating variables equal to $t$ and $m$. As only one outcome was considered in the current study, the observed mediating and outcome variables were represented as $M_i = M_i(T_i)$ and $Y_i = Y_i\{T_i, M_i(T_i)\}$,
respectively. Following the potential outcomes notation, the indirect effects ($\delta_i$) and direct effects ($\gamma$) for each unit $i$ and each treatment status $t = (0, 1)$ were defined as $(t) = Y_i \{ t, M_i (1) \} - Y_i \{ t, M_i (0) \}$ and $(t) = Y_i \{ 1, M_i(t) \} - Y_i \{ 0, M_i (t) \}$, respectively, and the ACME was defined as $= E [Y_i \{ t, M_i (1) \} - Y_i \{ t, M_i (0) \}]$. Similarly, the ADE was defined as $= E [Y_i \{ 1, M_i(t) \} - Y_i \{ 0, M_i(t) \}]$. As it is implausible to observe a counterfactual outcome $Y_i \{ t, M_i(1 - t) \}$ with one observational unit, the estimation of ACME and ADE requires an additional assumption known as sequential ignorability (SI) [21]. The SI assumes that, firstly, given the observed pretreatment confounders, the treatment assignment is ignorable, and sequentially, given the actual treatment status and pretreatment confounders, the observed mediating status is ignorable. The causal effects could be estimated as function of the sensitivity parameter $\rho$ [20, 29]. The equation between $\rho$ and the coefficients of determination as below, $\rho^2 = \rho_M^2 - \rho_Y^2$, where $\rho_M$ and $\rho_Y$ represent the percentage of residual variance that is explained by the unmeasured confounders in the mediator and in the outcome, and $\rho_M$ represent the proportion of variance that is explained by the unobserved confounder in the mediator and in the outcome. Values of $\rho$ different from zero may imply that the SI assumption is violated, indicating that estimation of the mediation effect may be biased. In this study, we used the mediation package in Stata for the mediation analysis [30].

**Statistical analysis**

Continuous variables were presented as means (standard deviations (SD), or medians (25th to 75th quartiles) for variables that were not normally distributed. Categorical variables were presented as frequencies (percentages). Chi-square tests or analysis of variance (ANVOA) were used to compare participants’ baseline characteristics by cigarette smoking status (never, former, and current smoking). The average IMT of the left and right common carotid artery was used for data analysis. Cigarette smoking was considered as
the exposure variable, inflammation indicated by leukocyte and differential count (granulocyte and lymphocyte count), hs-CRP and fibrinogen were considered as potential mediators, and IMT was considered as the study outcome. All tests of significance were two-tailed, with \( p < 0.05 \) as statistically significant.

Results

Of 1,996 participants in the GBCS-CVD sub-cohort, 1,752 with complete information of interest were included in the current study, including 869 (49.6%) women and 883 (50.4%) men. Table 1 shows that 1,210 (69.06%) were never, 237 (13.53%) were former, and 305 (17.51%) were current smokers. Former smokers were older than never or current smokers \( (p < 0.001) \). Most former and current smokers were men (98.31%, 97.38%, respectively). Compared to never smokers, current smokers were less physically active, had more with manual occupation and alcohol use, and had lower levels of BMI and LDL cholesterol (all \( p < 0.05 \)). They also had higher levels of leukocyte, lymphocyte, granulocyte, hs-CRP, fibrinogen and triglycerides, and a thicker IMT (all \( p < 0.001 \)).

Table 2 shows that, after adjustment for sex, age, occupation, education, physical activity, alcohol use, triglycerides and LDL cholesterol, the association of cigarette smoking with IMT was significantly mediated by leukocyte, granulocyte, hs-CRP and fibrinogen. The indirect effect of leukocyte, granulocyte, hs-CRP and fibrinogen on carotid IMT was 0.0028 mm (95% confidence interval (CI), 0.0011, 0.0047), 0.0019 mm (95% CI, 0.0006, 0.0034), 0.0017 mm (95% CI, 0.0006, 0.003) and 0.001 mm (95% CI, 0.0001, 0.0021), respectively. The proportion of the association between cigarette smoking and IMT mediated by leukocyte, granulocyte, hs-CRP and fibrinogen was 12.57% (95% CI, 8.50%, 22.11%), 8.50% (95% CI, 5.76%, 15.09%), 7.64% (95% CI, 5.20%, 13.79%) and 4.48% (95% CI, 3.04%, 8.03%), respectively. No evidence for a mediating role of lymphocyte was found.

As few women (4.31%) smoked in our study population, we conducted sensitivity analyses
by excluding women and found similar results (Table 3). In men, the proportion of the cigarette smoking-IMT association mediated by leukocyte, granulocyte, hs-CRP and fibrinogen was 13.07% (95% CI, 8.96%, 22.78%), 9.04% (95% CI, 6.20%, 16.01%), 6.77% (4.69%, 12.04%) and 7.46% (95% CI, 5.16%, 13.26%), respectively (Table 3).

Results of sensitivity analyses support the robustness of the mediation results. Table 4 shows that for the point estimate of the ACME by leukocyte to be zero, ρ value should at least be 0.0768, which means that the direction of ACME by leukocyte would remain positive unless the ρ value higher than 0.0768. The products of the determination coefficients at which ACME by leukocyte being zero were *=0.0059 and =0.0042. This means ACME would be positive unless the confounders explained at least 7.7% of residual variance (square root of 0.0059) in the mediator and in the outcome, though other combinations are possible. Similarly, ACME would be positive unless the omitted confounders explained more than 6.5% of the original variance (square root of 0.0042) in the mediator and in the outcome.

Discussion

Our study provided for the first time a quantitative assessment of respective contributions of several common inflammatory factors to the association between cigarette smoking and IMT based on a well-conducted population-based study. We found that of the selected inflammatory markers including leukocyte and subtypes, hs-CRP and fibrinogen, leukocyte counts mediated the effects of smoking on IMT by a greatest proportion (12.57%), followed subsequently by hs-CRP (7.64%) and fibrinogen (4.48%). Furthermore, we found that the mediating effect of leukocyte might be mainly due to granulocyte rather than lymphocyte.

Previous studies showed that cigarette smoking increased multiple inflammatory markers
including leukocyte counts and differential (neutrophil, lymphocyte, monocyte counts), hs-CRP, fibrinogen and interleukin-6 [31-33]. In addition, cigarette smoking was associated with development and progression of carotid atherosclerosis, probably partly through the inflammation pathway [34]. Accumulating evidence has shown a positive association between inflammation and carotid atherosclerosis. A meta-analysis based on 20 large prospective cohort studies within the PROG-IMT (individual progression of carotid intima media thickness as a surrogate of vascular risk) collaboration involving 49,097 participants from the general population showed positive associations between three markers of inflammation (leukocyte count, hs-CRP, fibrinogen) and carotid common artery (CCA) IMT at baseline [10]. However, no evidence for an association between baseline inflammatory markers with IMT progression after adjusting for traditional risk factors was found, probably because of imprecise measurements of IMT progression during only a few years (an average follow-up of 3.9 years). A large longitudinal study showed that baseline leukocyte count was significantly associated with thickening of IMT over a 9-year follow up period [35]. Similarly, some cross-sectional studies also reported a positive association between leucocyte count and IMT in middle-aged free-living men [36] and in patients with diabetes [37, 38]. Thus, the evidence above supports the pathological pathways from smoking to atherosclerosis through inflammation. However, we found only one study investigating the mediating effect of inflammation on the association between smoking and atherosclerosis. This study showed that the detrimental effects of smoking on progression of carotid IMT did not change after adjusting for hs-CRP and WBC, suggesting hs-CRP and WBC did not mediate the association [39]. However, in this longitudinal study with 16 years of follow-up, smoking status and inflammatory markers assessed at baseline might change during the follow-up. Misclassification of both exposure and mediating variables was likely to explain the null mediation results. Our findings based on accurate
measurement may minimize such misclassification bias and extend our understanding of
the mechanisms for the smoking-induced atherosclerosis.

Results of the association between fibrinogen and IMT have been less consistent. The
Coronary Artery Risk Development in Young Adults (CARDIA) study [13] showed that
elevated fibrinogen concentrations were independently associated with thicker IMT.
Another study of participants free of clinical cardiovascular disease also reported a
positive association between plasma fibrinogen concentrations and carotid IMT [40].
However, in a study of patients with atherosclerosis, no association between fibrinogen
and IMT was found [14]. Our mediation analysis showed that fibrinogen was significantly
associated with carotid IMT, and might explain in part the association between cigarette
smoking and carotid IMT. The results were similar after excluding women. Some possible
mechanisms have been suggested. Cytokines such as interleukin-6 significantly increase
fibrinogen transcription, a possible source of these cytokines is the recruitment of
macrophages to the lungs due to damage caused by smoking [41]. Other possible
explanations include the effects on plaque composition, blood viscosity, endothelial and
smooth muscle cell activation, platelet aggregation and activation, and immune cell
recruitment [42, 43], although the exact mechanisms warrant further investigation.

The strengths of this study include the comprehensive measurement and adjustment of
potential confounding factors, and the application of a mediation analysis in quantifying
mediation effect due to inflammation. There are some limitations to consider in
interpreting our results. First, the causal inference for the associations between smoking
and inflammation, as well as inflammation and carotid IMT might not be confirmed in the
current cross-sectional analysis. However, much evidence for the causal associations
between smoking and inflammation, and between inflammation and carotid IMT have been
consistently reported in previous studies [34, 44, 45]. Thus, the causal pathways from
smoking to atherosclerosis through inflammation is biologically plausible. Secondly, inflammatory factors were measured only at a single time point, which might not reflect a long-term inflammatory response. Third, some inflammatory factors associated with cardiovascular disease in previous studies, such as interleukin 6 or interleukin-1β [46, 47], were not measured in our study. Fourth, the calculation of ACME and ADE effects requires a SI assumption, which is a strong assumption in a non-trial setting. Although adjusting for potential confounders might to some extent mitigate this concern, this assumption cannot be perfectly tested (i.e., it is nonrefutable) by using observational data [48]. We conducted a sensitivity analysis to examine the violation of the SI assumption and found that the probability for the violation (indicating by the value of $p$ and for the ACME to be zero) was low, supporting the robustness of the mediation results. Further studies in other settings to replicate our results are warranted. Finally, due to financial constraints, not all GBCS participants had IMT measured, and the sample size in the current analysis was relatively small.

Conclusion

In conclusion, our study showed that the effects of cigarette smoking on IMT were partly mediated by leukocyte, high sensitivity C-reactive protein and fibrinogen. Of the inflammatory factors, leukocyte appeared to mediate much of the effect of smoking-induced atherosclerosis, and the mediating role was mainly driven by granulocyte rather than lymphocyte.

List Of Abbreviations

**ACME**: Average causal mediation effect

**ADE**: Average direct effect

**ATE**: Averaged total effects
**Declarations**

**Ethics approval and consent to participate**

The GBCS was approved by Guangzhou Medical Ethics Committee of the Chinese Medical Association. All participants provided written, informed consent before participation.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Data that support findings are restricted to researchers who have permission from the Guangzhou biobank cohort study, and so are not publicly available. The data that support this analysis may be available on reasonable request from the corresponding author.

**Competing interests**

The authors have no competing interests

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Authors' contributions

TW, LX, THL, GNT, WSZ, FZ, YLJ, CQJ, and KKC have substantial contributions to conception and design, acquisition of funding, data and interpretation of data; TW, LX, CQJ and THL analysed the data, TW, LX, CQJ, THL, GNT and KKC drafted the article, LX, CQJ, THL and KKC revised it critically for important intellectual content. All authors read and approved the final manuscript.

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Tables

Table 1 Demographic characteristics of the participants by smoking status in 1,752 participants in GBCS-CVD.

| Smoking status | Never (n=1210) | Former (n=237) | Current (n=305) | p-value |
|----------------|----------------|----------------|-----------------|---------|
| Age, y, mean (SD) | 57.99 (6.41) | 63.39 (7.00) | 60.51 (6.27) | <0.001 |
| Men, n (%) | 353 (29.17) | 233 (98.31) | 297 (97.38) | <0.001 |
| Occupation, n (%) | | | | 0.023 |
| Manual | 281 (23.22) | 70 (29.54) | 92 (30.16) | |
| Non-manual | 464 (38.35) | 93 (39.24) | 115 (37.7) | |
| Others | 465 (38.43) | 74 (31.22) | 98 (32.13) | |
| Education, n (%) | | | | 0.086 |
| ≤Primary | 302 (24.96) | 70 (29.54) | 95 (31.15) | |
| Middle school | 744 (61.49) | 142 (59.92) | 180 (59.02) | |
| ≥College | 164 (13.55) | 25 (10.55) | 30 (9.84) | |
### Alcohol drinking, n (%)

|          | Current   | Former | Never     |
|----------|-----------|--------|-----------|
|          | 658 (54.74) | 169 (71.61) | 223 (73.36) |
|          | <0.001    |        |           |

|          | 27 (2.25) | 13 (5.51) | 10 (3.29) |

### IPAQ Physical activity, n (%)

|          | High      | Moderate | Low       |
|----------|-----------|----------|-----------|
|          | 759 (62.73) | 337 (27.85) | 114 (9.42) |
|          | 143 (60.34) | 71 (29.96) | 23 (9.7)  |
|          | 148 (48.52) | 115 (37.7) | 42 (13.77) |
|          | <0.001     |          |           |

### BMI, kg/m², mean (SD)

|          | 23.75 (2.98) | 24.02 (2.83) | 23.16 (3.12) |
|          | 0.002       |          |           |

### Leukocyte, \( \times 10^9/l \), mean (SD)

|          | 6.12 (1.46) | 6.65 (1.69) | 7.23 (1.59) |
|          | <0.001      |          |           |

### Lymphocyte, \( \times 10^9/l \), mean (SD)

|          | 2.12 (0.58) | 2.11 (0.56) | 2.35 (0.67) |
|          | <0.001      |          |           |

### Granulocyte, \( \times 10^9/l \), mean (SD)

|          | 3.63 (1.15) | 4.06 (1.49) | 4.33 (1.22) |
|          | <0.001      |          |           |

### hs-CRP, mg/l, geometric mean (95% CI)

|          | 1.32 (1.25-1.41) | 1.79 (1.55-2.07) | 1.58 (1.40-1.79) |
|          | <0.001          |          |           |

### Fibrinogen, mg/dl, mean (SD)

|          | 296.99 (77.16) | 304.77 (104.35) | 314.56 (90.07) |
|          | <0.001         |          |           |

### Triglycerides, mmol/L, geometric mean (95% CI)

|          | 1.50 (1.46-1.55) | 1.68 (1.57-1.79) | 1.56 (1.46-1.66) |
|          | <0.001          |          |           |
| Mediators          | Indirect effect (ACME)         | Direct effect (ADE)         |
|-------------------|--------------------------------|-----------------------------|
|                   | Estimate (95% CI)              | Estimate (95% CI)           |
| Leukocyte, ×10^9/l | 0.0028 (0.0011, 0.0047)        | 0.0195 (0.0092, 0.0293)     |
| Lymphocyte, ×10^9/l| 0.0009 (-0.0003, 0.0024)       | 0.0213 (0.0111, 0.0311)     |
| Granulocyte, ×10^9/l| 0.0019 (0.0006, 0.0034)       | 0.0204 (0.0102, 0.0302)     |
| hs-CRP, mg/l      | 0.0017 (0.0006, 0.003)        | 0.0206 (0.0104, 0.0303)     |
| Fibrinogen, mg/dl | 0.0010 (0.0001, 0.0021)       | 0.0213 (0.0111, 0.031)      |

The mediation analysis models were adjusted for age, sex, drinking status, education, occupation, BMI, physical activity, low density lipoprotein cholesterol and triglycerides.

Abbreviations: GBCS-CVD, The Guangzhou biobank cohort study-cardiovascular disease sub-cohort; SD, standard deviation; CI, confidence interval; IPAQ, international physical activity questionnaire; BMI, body mass index; LDL, low density lipoprotein; hs-CRP, high sensitivity C-reactive protein; IMT, intima-media thickness.
sub-cohort; ACME, average causal mediation effect; ADE, average direct effect; CI, confidence interval; IMT, intima-media thickness; hs-CRP, high sensitivity C-reactive protein.

Table 3 Association between smoking and carotid IMT (mm) with mediation by leukocyte, lymphocyte, granulocyte, hs-CRP, fibrinogen in 883 men in GBCS-CVD.

| Mediators | Indirect effect (ACME) | Direct effect (ADE) |
|-----------|------------------------|---------------------|
|           | Estimate (95% CI)       | Estimate (95% CI)   | 0.025 |
| Leukocyte, ×10⁹/l | 0.0033 (0.0007, 0.0063) | 0.0222 (0.0105, 0.0336) | 0.025 |
| Lymphocyte, ×10⁹/l | 0.0008 (-0.0012, 0.0029) | 0.0247 (0.0131, 0.036) | 0.025 |
| Granulocyte, ×10⁹/l | 0.0023 (0.0003, 0.0046) | 0.0232 (0.0116, 0.0345) | 0.025 |
| hs-CRP, mg/l | 0.0017 (0.0003, 0.0036) | 0.0238 (0.0123, 0.035) | 0.025 |
| Fibrinogen, mg/dl | 0.0019 (0.0003, 0.0039) | 0.0236 (0.0121, 0.0348) | 0.025 |

The mediation analysis models were adjusted for age, drinking status, education, occupation, BMI, physical activity, low density lipoprotein cholesterol and triglyceride.

Abbreviations: GBCS-CVD, The Guangzhou biobank cohort study-cardiovascular disease sub-cohort; ACME, average causal mediation effect; ADE, average direct effect; CI, confidence interval; IMT, intima-media thickness; hs-CRP, High sensitivity C-reactive protein.

Table 4 Sensitive analysis examining the violation of sequential ignorability assumption.
|                |          |
|----------------|----------|
| Leukocyte, $\times 10^9/l$ | 0.0768   |
| Lymphocyte, $\times 10^9/l$  | 0.0343   |
| Granulocyte, $\times 10^9/l$ | 0.0712   |
| hs-CRP, mg/l              | 0.1015   |
| Fibrinogen, mg/dl          | 0.0501   |

**Abbreviations:** ACME, average causal mediation effect; $: \text{ proportion of the unexplained variance explained by the unobserved confounder in the mediator;}$ $: \text{ proportion of the unexplained variance explained by the unobserved confounder in the outcome;}$ $: \text{ proportion of variance that is explained by the unobserved confounder in the mediator;}$ $: \text{ proportion of variance that is explained by the unobserved confounder in the outcome.}$