Lipids in Health and Disease

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The mediating role of inflammation in the association between cotinine levels and remnant cholesterol: a cross-sectional study

Tianjie Lai[1†], Zhihao Su[1,2†], Gaoqiang Tian[1], Jingui Sun[1] and Konghe Hu[1*]

Abstract

Background Remnant Cholesterol (RC) has emerged as a significant risk factor for cardiovascular disease. However, the factors influencing RC levels remain incompletely understood. This research investigates smoking—a major modifiable risk factor—to elucidate its impact on RC levels and examine the mediating role of inflammation in this relationship.

Methods Using NHANES data from 1999 to 2018, this study analyzed the association between serum cotinine levels (a biomarker of smoking intensity) and RC in 8,829 participants aged 20 years and older. Through complex sampling design and adjustment for multiple covariables, we examined both linear and nonlinear relationships using linear regression models, restricted cubic splines (RCS), and subgroup analyses. Additionally, mediation analyses evaluated the role of inflammatory markers—neutrophils (NEU), monocytes (MON), lymphocytes (LYM), and platelets (PLT)—in this association.

Results The high cotinine exposure group demonstrated significantly elevated RC levels (β = 2.256, 95% CI: 1.401–3.112, $p < 0.001$) compared to the no/minimal exposure group. This positive association was particularly pronounced in females (p for interaction < 0.05). Restricted cubic spline analysis demonstrated a nonlinear, N-shaped relationship (p for nonlinearity < 0.05), with RC levels reaching their peak at cotinine concentrations of approximately 172 ng/mL. In the mediation analysis, inflammatory markers showed significant mediating effects: NEU (28%), LYM (14.1%), PLT (9.5%), and MON (6.9%) of the total effect.

Conclusion A significant positive association exists between cotinine and RC levels, moderated by sex. Inflammatory markers, particularly NEU, partially mediate this association.

Highlights

- Serum cotinine levels are positively associated with remnant cholesterol.
- Cotinine-remnant cholesterol association is pronounced in females.
- An n-shaped relationship is observed between cotinine and remnant cholesterol, with remnant cholesterol peaking at cotinine concentrations of approximately 172 ng/mL.

[†]Tianjie Lai and Zhihao Su contributed equally to this work.

*Correspondence:
Konghe Hu
hkhgksq@126.com
Full list of author information is available at the end of the article

• Inflammatory markers mediate the cotinine-remnant cholesterol association; neutrophils show the strongest effect (28% of the total effect).

Keywords Remnant cholesterol (RC), Cotinine; cross-sectional study, NHANES, Biomarker, Cardiovascular disease

Introduction

Remnant cholesterol (RC), defined as the cholesterol content within triglyceride-rich lipoprotein (TRL) remnants, primarily originates from chylomicrons and very low-density lipoproteins (VLDL) following lipoprotein lipase hydrolysis [1, 2]. Recent evidence demonstrates that RC exhibits comparable or greater precision in cardiovascular event prediction compared to conventional markers such as LDL-C or HDL-C [3, 4]. RC shares pathogenic mechanisms with LDL-C, including the ability to penetrate the arterial endothelium and bind to proteoglycans, thereby promoting atherosclerosis through cholesterol accumulation, plaque formation, and inflammatory processes [5, 6]. Notably, elevated RC levels may increase mortality risk and recurrent event rates even among cardiovascular patients receiving lipid-lowering therapy [7, 8]. Although RC plays a pivotal role in cardiovascular disease prediction and prognosis, no clinical consensus exists regarding the management of high RC (HRC). Consequently, identifying modifiable factors that influence elevated RC levels has emerged as a critical research priority.

Among modifiable factors, smoking stands as one of the most preventable major risk factors for cardiovascular disease and mortality [9, 10]. Cigarette smoke emits numerous harmful compounds, including nicotine, polycyclic aromatic hydrocarbons, and heavy metals [11–13].

Cotinine, the principal metabolite of nicotine, serves as a highly specific and sensitive biomarker for quantifying recent tobacco exposure [12, 14]. Substantial epidemiological and experimental evidence has established that smoking disrupts lipid metabolism through multiple pathways, thereby increasing cardiovascular disease risk and associated mortality [15–17]. Specifically, smoking elevates total cholesterol (TC), triglyceride (TG), and LDL-C levels while reducing cardioprotective HDL-C concentrations [18, 19]. However, current research has predominantly examined smoking's effects on conventional lipid markers, with a limited investigation into its impact on RC. Given the crucial role of RC in cardiovascular disease pathogenesis and smoking's significance as a major modifiable risk factor, elucidating the relationship between smoking and RC could reveal novel approaches for HRC prevention.

Materials and methods

Data source and study population

This study utilized data from the National Health and Nutrition Examination Survey (NHANES) spanning 1999–2018, encompassing 8,829 participants aged 20 and above. NHANES, a comprehensive, multi-stage, probability-sampled national health survey led by the National Center for Health Statistics (NCHS), aims to assess the health and nutritional status of the non-institutionalized U.S. population. The participant selection process is illustrated in Fig. 1. Exclusion criteria comprised: 1) missing data for Cotinine and variables required to calculate RC; 2) subjects lacking data in other covariable modules; and 3) participants with zero weighting.

Measures

Primary outcome

Remnant cholesterol (RC) was the primary outcome of this study. Participants underwent an 8-h overnight fast before blood collection. TC was measured enzymatically, while HDL-C quantification involved either heparin-manganese precipitation or immunoassay techniques. The Friedewald equation was used to calculate LDL-C. A validated formula determined RC levels. All procedures adhered to the NHANES Laboratory Manual, guaranteeing standardization. This methodological approach strengthened the investigation into smoking's effects on lipid profiles.

$$Remnant\ Cholesterol = TotalCholesterol - (Low-DensityLipoproteinCholesterol + High-DensityLipoproteinCholesterol)$$

Primary exposure

Serum cotinine, a main metabolite of nicotine, was measured as the primary exposure variable in this study, serving as a sensitive and specific biomarker to reflect smoking status. Serum cotinine was determined by isotope dilution combined with APCI-MS/MS and HPLC. Methyl-D3-cotinine internal standard was added to the alkaline serum sample for SLE extraction. After C18 HPLC separation, the extract was monitored by

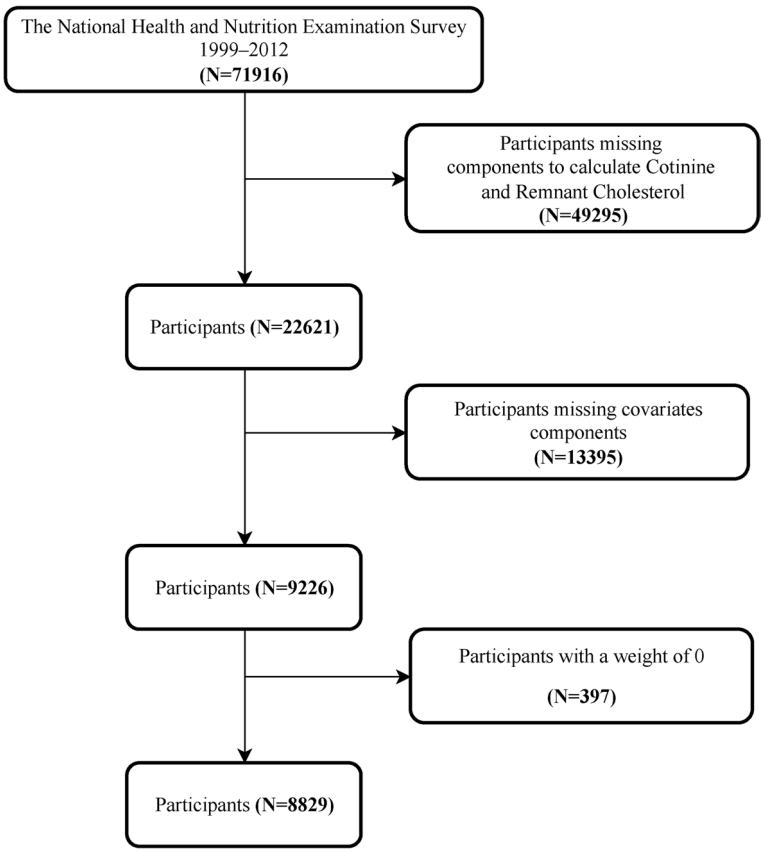

Fig. 1 Flowchart of the participants' selection

APCI-MS/MS, and the m/z 80 daughter ion of the m/z 177 quasi-molecular ion was used to identify cotinine [20]. Detailed processing steps can be found in the Laboratory/Medical Technology Procedure Manual on the NHANES official website. According to previous studies and literature, serum cotinine levels are divided into three groups: No/Minimal exposure (< 0. 05 ng/mL), Low exposure (0. 05–2. 99 ng/mL), High exposure (≥ 3. 00 ng/mL) [21].

Definitions of covariables

Several covariables were included in this study. These were organized into the following categories:

1. Demographic characteristics
   - Age (years)
   - Sex (male/female)
   - Race (non-Hispanic white, non-Hispanic black, other/multiracial)
2. Socioeconomic factors
   - Educational attainment (high school or below, college graduate or above)
   - Income level (poor, not poor)
3. Lifestyle factors
   - Smoking status (never, former, now)
   - Alcohol use (drinkers, non-drinkers)
   - Physical activity (MET × min/week)
4. Health Status indicators
   - Body Mass Index (BMI, kg/m^2)
   - Cancer (yes/no)
   - Heart disease (yes/no)
   - Diabetes (yes/no)

The specific measurement methods for the above variables are as follows: The history of cancer, heart disease, and diabetes in participants was determined through individual interviews using questionnaires. Income level was assessed using the ratio of family income to poverty (PIR), where PIR < 1 was defined as poor and PIR ≥ 1 was

not poor. Smoking status was assessed based on two questions: " Smoked at least 100 cigarettes in life?" and "Do you now smoke cigarettes? " Based on the responses, participants were categorized as never, former, and now. Alcohol use is determined by the response to "Had at least 12 alcoholic drinks/lifetime?", with "yes" classified as a drinker and "no" as a non-drinker. Physical activity levels were assessed using the World Health Organization Global Physical Activity Questionnaire (GPAQ), and weekly activity levels were converted to metabolic equivalent minutes (MET) according to WHO guidelines.

Statistical analysis

The analyses considered NHANES' complex multistage sampling design and incorporated sample weights, stratification, and clustering. Participants were stratified by serum cotinine concentrations. Continuous variables were reported as mean [95% CIs], while categorical variables as percentages [95% CIs]. Wilcoxon rank-sum tests and Rao & Scott chi-square tests assessed group differences for continuous and categorical variables, respectively. We employed weighted linear regression with four progressively adjusted models to analyze the cotinine-RC relationship. Model 1 was unadjusted; Model 2 adjusted for primary NHANES sampling factors (age, sex, and race); Model 3 further adjusted for BMI, educational attainment, physical activity, cancer, and diabetes, while Model 4 included adjustments for all covariable. The covariables for Model 3 were selected based on a greater than 10% change in the regression coefficient of cotinine levels when added to or removed from the unadjusted and fully adjusted models. Additionally, cotinine was categorized into quintiles, and trend tests evaluated the consistency of cotinine-RC associations across increasing quintiles. Restricted cubic spline analysis assessed potential nonlinear relationships between cotinine and RC after covariable adjustment, with nonlinearity evaluated by likelihood ratio tests. Subgroup analysis was conducted to investigate factors that may influence the association between cotinine and RC. Mediation analysis was conducted using a "mediation" package of R software to assess inflammatory markers as potential mediators of the effect between cotinine and RC. In all analyses, statistical significance was set at $P < 0.05$ (two-tailed). All analyses were conducted using R version 4.3.1.

Results

Characteristics of participants

Our study analyzed 8,829 individuals, categorized based on Cotinine exposure (Table 1). Cotinine exposure levels are strongly associated with smoking status. In the high-exposure group, 76% of the individuals were current smokers, while only 11% were never smokers. In contrast, in the no/minimal exposure group, only 0.4% of the individuals were current smokers, and 70% were never-smokers. Participants with higher Cotinine exposure exhibited distinct characteristics: they were generally younger, had lower BMI, were more likely to be male, consumed alcohol more frequently, and had lower levels of education and income. In addition, these participants have a relatively low prevalence of cancer and diabetes, but their level of physical activity is high.

Association between cotinine and RC

Weighted multivariable linear regression analysis revealed a significant positive association between cotinine and RC in all models ($p < 0.05$) (Table 2). The fully adjusted Model 4 revealed that participants in the high cotinine exposure group exhibited significantly elevated RC levels ($\beta = 2.256$, 95% CI: 1.401–3.112, $p < 0.001$) compared to the no/minimal exposure group. In contrast, the low exposure group showed no statistically significant difference in RC levels ($\beta = 0.3071$, 95% CI: -0.5821–1.196, $p = 0.5$) compared to the no/minimal exposure group.

Trend tests of association between cotinine and RC

Participants were stratified into quintiles based on serum cotinine concentrations for trend analysis (Fig. 2). Although there are some fluctuations between quintiles, all four models demonstrate a clear overall upward trend in RC concentrations as cotinine levels increase across quintiles (Q1 to Q5). This positive dose–response relationship is statistically significant in all models (p for trend < 0.001).

RCS analysis of the association between cotinine and RC

We employed weighted RCS analysis to examine the nonlinear relationship between cotinine and RC, adjusting for all covariable. Figure 3 reveals a significant non-linear "n-shaped" association between cotinine and RC (P-Nonlinear < 0.05). The RC effects initially increased with rising cotinine levels, reached a peak, and then decreased. The inflection point was identified at a cotinine level of 172 ng/ml. Further stratified RCS analyses demonstrated consistent n-shaped associations between cotinine and RC across all subgroups (Supplementary file S2).

Subgroup analysis of the relationship between cotinine and RC

We conducted a stratified analysis of the relationship between cotinine and RC across different subgroups to investigate potential variations in this association among populations with diverse characteristics (Table 3). A statistically significant difference was observed in the subgroup of sex (p for interaction < 0.05). Specifically, in the

Table 1 Characteristics of participants

Characteristic	Cotinine (*N* = 8829)			
	No/Minimal exposure, *N* = 4272[ab]	Low exposure, *N* = 2246[ab]	High exposure, *N* = 2311[ab]	*P* Value[c]
Age, years	48 [47, 49]	44 [43, 45]	41 [40, 42]	**< 0.001**
BMI (kg/m^2)	28.0 [28]	28.8 [29]	27.5 [27, 28]	**< 0.001**
Sex, %				**< 0.001**
Male	44 [42, 45]	55 [52, 57]	63 [61, 66]	
Female	56 [55, 58]	45 [43, 48]	37 [34, 39]	
Race, %				**< 0.001**
Other/multiracial	17 [15, 19]	16 [14, 19]	13 [11, 16]	
Non-Hispanic black	6.3 [5.2, 7.5]	13 [11, 15]	12 [10, 14]	
Non-Hispanic white	76 [74, 79]	71 [68, 74]	75 [72, 78]	
Alcohol use, %				**< 0.001**
Non drinker	13 [11, 16]	9.3 [7.8, 11]	2.2 [1.6, 3.0]	
Drinker	87 [84, 89]	91 [89, 92]	98 [97, 98]	
Smoke status, %				**< 0.001**
Never	70 [68, 72]	64 [61, 66]	11 [10, 13]	
Former	29 [27, 32]	33 [31, 36]	12 [11, 14]	
Now	0.4 [0.21, 0.58]	2.9 [2.2, 3.9]	76 [74, 79]	
Education attainment, %				**< 0.001**
High school or below	26 [24, 29]	40 [37, 43]	55 [52, 58]	
College graduate or above	74 [71, 76]	60 [57, 63]	45 [42, 48]	
Income level, %				**< 0.001**
Poor	6.7 [5.6, 8.1]	12 [9.9, 13]	18 [16, 20]	
Not poor	93 [92, 94]	88 [87, 90]	82 [80, 84]	
Cancer, %	9.6 [8.6, 11]	6.3 [5.2, 7.6]	5.3 [4.5, 6.1]	**< 0.001**
Heart disease, %	6.6 [5.9, 7.4]	7.5 [6.3, 9.0]	6.8 [5.7, 8.1]	0.4
Diabetes, %	7.3 [6.4, 8.3]	6.1 [5.1, 7.3]	5.5 [4.6, 6.6]	**0.032**
Physical activity (MET × min/week)	2,299 [2,067, 2,531]	2,570 [2,288, 2,851]	3,311 [2,920, 3,702]	**0.002**

[a] Mean; %

[b] *CI* Confidence Interval

[c] Wilcoxon rank-sum test for complex survey samples; chi-squared test with Rao & Scott's second-order correction

highest quintile group of cotinine, the RC level for the female group (β = 3.22, 95% CI: 1.79–4.65) was notably higher than that of the male group (β = 2.22, 95% CI: 0.73–3.71), indicating a more pronounced association between cotinine and RC in females. This association did not show significant differences across other subgroups such as age, race, income level, alcohol use, educational attainment, cancer, heart disease, and diabetes (p for interaction > 0.05). This indicates that the association between cotinine and RC is relatively stable within these subgroups.

The mediating role of inflammatory biomarkers in the association between cotinine and RC

The mediation analysis results (Fig. 4 and Supplement Table S1) demonstrated a significant total effect between cotinine and RC (total effect = 1.189, $p < 0.0001$). All four inflammatory markers partially mediated this association ($p < 0.01$). Among them, NEU exhibited the strongest mediation effect, accounting for 28% of the total effect (mediation effect = 0.333, $p < 0.0001$), followed by LYM at 14.1% (mediation effect = 0.167, $p < 0.0001$). PLT and MON demonstrated smaller mediating effects, contributing 9.5% (mediation effect = 0.113, $p < 0.0001$) and 6.9% (mediation effect = 0.082, $p = 0.0080$) to the total effect, respectively.

Discussion

This study provides the first systematic investigation of the relationship between smoking intensity, measured by serum cotinine levels, and RC. Our findings reveal that blood cell inflammatory markers mediate smoking-induced elevations in RC levels. The positive association

Table 2 Association between Cotinine and RC

Characteristic[a]	Model 1			Model 2			Model 3			Model 4		
	Beta	**95% CI[b]**	***p*-value**	**Beta**	**95% CI[b]**	***p*-value**	**Beta**	**95% CI[b]**	***p*-value**	**Beta**	**95% CI[b]**	***p*-value**
Cotinine-strata												
No/Minimal exposure	Ref	Ref		Ref	Ref		Ref	Ref		Ref	Ref	
Low exposure	0.3411	-0.5853, 1.267	0.5	0.9703	0.0332, 1.907	**0.043**	0.3185	-0.5748, 1.212	0.5	0.3071	-0.5821, 1.196	0.5
High exposure	1.629	0.8298, 2.427	**< 0.001**	2.425	1.566, 3.283	**< 0.001**	2.291	1.439, 3.143	**< 0.001**	2.256	1.401, 3.112	**< 0.001**

[a] Models:

Model 1: Not adjusted

Model 2: Adjusted Age, Sex, Race

Model 3: Adjusted Age, Sex, BMI, Race, Education attainment, MET, Cancer, Diabetes

Model 4: Adjusted Age, Sex, BMI, Race, Income level, Education attainment, Alcohol use, MET, Cancer, Heart disease, Diabetes

[b] *CI* Confidence Interval

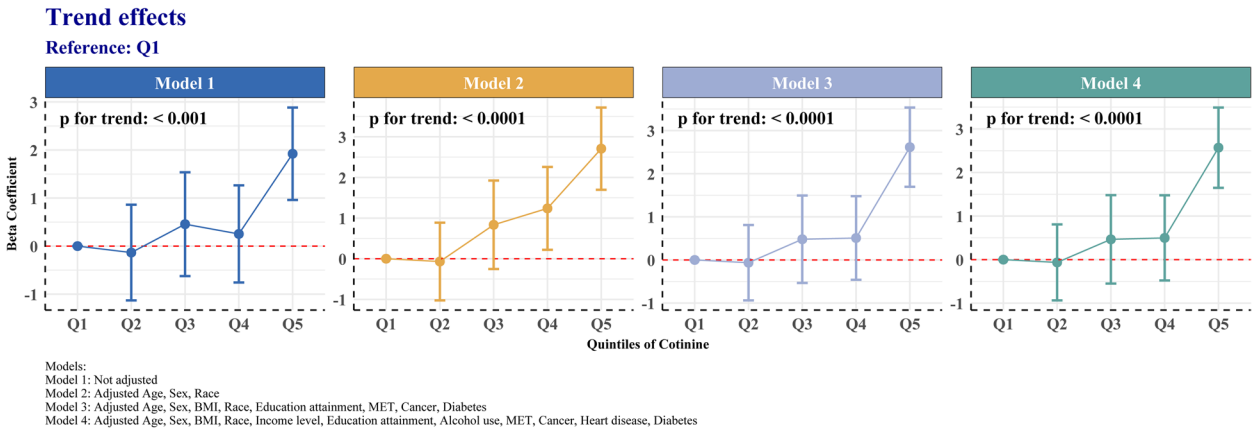

Fig. 2 Trend tests of association between cotinine and RC

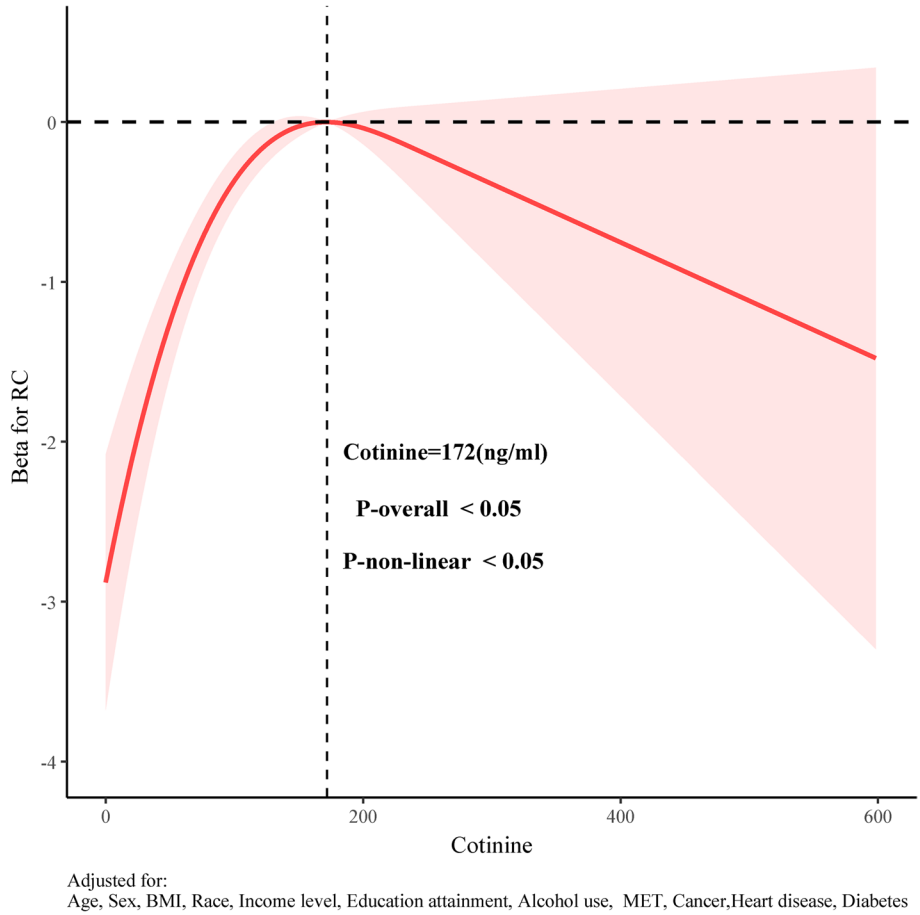

Fig. 3 RCS analysis of the association between cotinine and RC

between cotinine and RC was particularly pronounced in females, demonstrating significant effect modification. Notably, we identified a nonlinear, N-shaped relationship between cotinine and RC levels, with a critical inflection point at 172 ng/mL. Furthermore, inflammatory cells—NEU, LYM, MON, and PLT—significantly mediated this association, suggesting a mechanistic link between smoking and RC metabolism.

Serum cotinine levels demonstrate a dose-dependent relationship with cardiovascular disease risk [22]. Both

Table 3 Subgroup analysis of the relationship between cotinine and RC

Characteristic[b]	Q1	Q2	Q3	Q4	Q5	p-int[a]
Age strata						0.76
< 60 years	ref	-0.35(-1.41, 0.72)	0(-1.20, 1.21)	0.09(-1.03, 1.21)	2.05(1.01, 3.09)	
≥ 60 years	ref	0.34(-1.03, 1.70)	1.5(-0.52, 3.51)	1.44(-0.55, 3.43)	2.75(0.97, 4.53)	
Sex						**0.02**
Male	ref	-0.05(-1.37, 1.28)	0.8(-0.77, 2.38)	1.15(-0.41, 2.71)	2.22(0.73, 3.71)	
Female	ref	-0.1(-1.32, 1.12)	-0.02(-1.22, 1.18)	-0.49(-1.73, 0.75)	3.22(1.79, 4.65)	
Race						0.73
Other/multiracial	ref	0.11(-1.77, 1.98)	0.25(-1.74, 2.25)	0.7(-1.33, 2.72)	1.08(-1.36, 3.52)	
Non-Hispanic black	ref	-0.17(-2.56, 2.21)	0.12(-2.42, 2.65)	-0.47(-2.88, 1.93)	1.53(-0.90, 3.96)	
Non-Hispanic white	ref	-0.18(-1.19, 0.84)	0.52(-0.73, 1.78)	0.52(-0.73, 1.77)	2.86(1.76, 3.96)	
Alcohol use						0.55
Non drinker	ref	0.69(-1.11, 2.50)	0.05(-1.68, 1.78)	0.91(-1.67, 3.49)	4.32(0.74, 7.90)	
Drinker	ref	-0.23(-1.13, 0.67)	0.5(-0.66, 1.65)	0.37(-0.69, 1.43)	2.39(1.39, 3.39)	
Income level						0.58
Poor	ref	1.01(-2.59, 4.62)	-0.88(-4.47, 2.71)	0.98(-2.73, 4.70)	2.78(-1.01, 6.57)	
Not poor	ref	-0.17(-1.03, 0.70)	0.6(-0.51, 1.70)	0.45(-0.63, 1.53)	2.59(1.62, 3.55)	
Education attainment						0.05
High school or below	ref	-0.13(-1.87, 1.62)	-0.02(-1.84, 1.80)	-1.02(-2.82, 0.79)	1.71(-0.03, 3.46)	
College graduate or above	ref	-0.17(-1.14, 0.81)	0.49(-0.64, 1.62)	1.2(-0.13, 2.52)	2.98(1.81, 4.16)	
Cancer						0.84
No	ref	0.03(-0.86, 0.92)	0.44(-0.66, 1.54)	0.46(-0.56, 1.49)	2.6(1.56, 3.64)	
Yes	ref	-0.72(-4.19, 2.74)	0.32(-3.41, 4.04)	0.69(-3.18, 4.57)	1.06(-3.24, 5.36)	
Heart disease						0.25
No	ref	-0.1(-1.01, 0.81)	0.61(-0.42, 1.64)	0.62(-0.36, 1.61)	2.49(1.58, 3.40)	
Yes	ref	0.01(-3.30, 3.32)	-1.03(-4.74, 2.67)	-1.48(-5.41, 2.45)	2.54(-1.54, 6.63)	
Diabetes						0.79
No	ref	-0.07(-0.95, 0.82)	0.57(-0.49, 1.63)	0.47(-0.48, 1.42)	2.72(1.79, 3.64)	
Yes	ref	-0.38(-4.71, 3.95)	-1.67(-7.90, 4.56)	1.8(-2.53, 6.14)	0.35(-3.64, 4.34)	

[a] p-int: p for interaction

[b] Adjusted for: Age, Sex, BMI, Race, Income level, Education attainment, Alcohol use, MET, Cancer, Heart disease, Diabetes

cross-sectional and prospective studies have established that elevated cotinine levels are associated with increased cardiovascular events and their severity [16, 23]. Smoking-induced alterations in lipid profiles accelerate atherosclerosis development [15, 24], with elevated RC particularly associated with arterial disease [25] and cardiovascular mortality [26]. Our findings identify smoking as a significant modifiable factor affecting RC levels, suggesting that smoking cessation or reduction may serve as a therapeutic target for RC management and cardiovascular risk reduction. The observed positive association between cotinine and RC levels provides additional evidence linking smoking to adverse cardiovascular outcomes. Mechanistically, cotinine—nicotine's primary metabolite—may influence RC levels through multiple pathways: enhanced oxidative stress [27], inflammatory activation [28, 29], endothelial dysfunction [30], musculoskeletal degeneration [31, 32], and dysregulation of hepatic lipid metabolism [33, 34].

Female participants exhibited heightened sensitivity to cotinine level fluctuations [35]. This enhanced sensitivity in females may be attributed to estrogen-mediated acceleration of nicotine metabolism, as reported in previous research [36]. Estrogen also directly modulates lipid metabolism by elevating HDL cholesterol while reducing LDL cholesterol, potentially explaining our observed sex-specific differences [37].

The significant positive associations between cotinine levels and inflammatory biomarkers (LYM, NEU, MON, and PLT) are consistent with findings from a Korean study of adult smokers [38]. Their research demonstrated elevated white blood cell counts in smokers compared

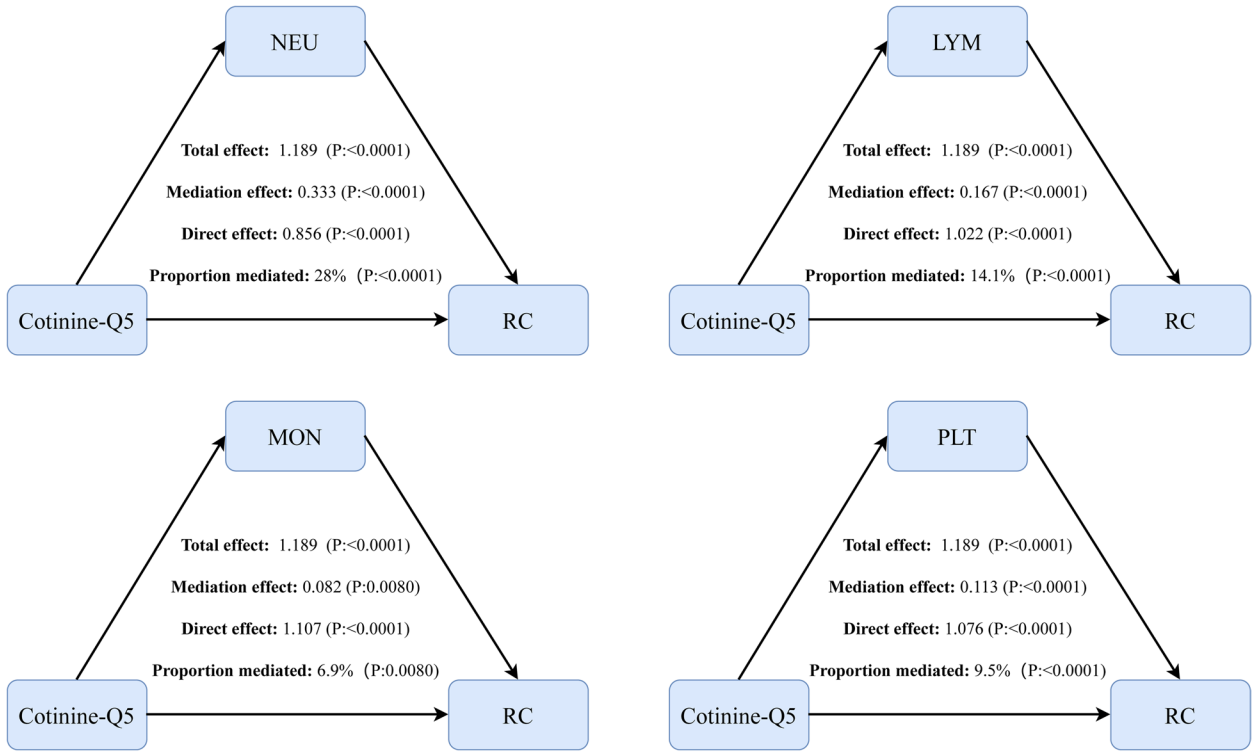

Fig. 4 The mediating role of inflammatory biomarkers in the association between cotinine and RC

to non-smokers, with counts increasing proportionally to cotinine concentrations [38]. Chronic tobacco smoke exposure induces low-grade systemic inflammation and promotes widespread vascular damage through multiple pathways [27]. While its cardiovascular and respiratory effects are well-reported, the impact of systemic inflammation on hepatic function warrants particular attention. Cotinine exerts hepatic effects primarily through p-38 MAPK/AP-1 and ROS/STAT-3 signaling pathway activation, triggering endothelial damage and upregulating pro-inflammatory IL-6 expression. Smokers exhibit markedly elevated IL-6 levels compared to non-smokers [28], with increased IL-6 serving as a potential harbinger of hepatocellular injury. Additionally, smoking-induced carboxyhemoglobin elevation reduces blood oxygen-carrying capacity, stimulating erythropoietin secretion. This leads to increased red blood cell mass, concurrent erythrocyte destruction, and disrupted iron metabolism, culminating in hepatocyte iron accumulation, oxidative stress, and liver damage. The resultant hepatocellular dysfunction directly impairs lipoprotein metabolism, manifesting as blood lipid abnormalities [39]. While current evidence preliminarily supports the hypothesis that cotinine influences RC levels via lipid metabolism pathway disruption, comprehensive investigation is needed to fully elucidate the relationship between residual cholesterol and cotinine levels.

These findings suggest that early intervention strategies in high-risk populations may help control RC levels [40, 41]. Our study reveals a positive association between cotinine and RC levels, providing potentially valuable insights for clinical application. Cotinine could potentially serve as a reference biomarker for RC risk assessment, contributing to the prevention and management of RC elevation. Given the heightened sensitivity to cotinine effects observed in female subjects, the implementation of sex-specific strategies in RC management warrants consideration. Furthermore, the observed relationship with inflammatory markers suggests that blood cell count monitoring could offer complementary information, especially in heavy smokers. While these findings provide promising initial evidence for developing targeted strategies in smoking-related RC management, further research would be valuable to fully understand their clinical applications.

Strengths and limitations

A primary strength of this study lies in its utilization of a large cohort of non-hospitalized participants, significantly enhancing both statistical power and the generalizability of findings. Nevertheless, several limitations warrant consideration. The cross-sectional nature of the NHANES data precludes establishing causality between smoking and RC levels, underscoring the importance of future prospective studies. While we adjusted for multiple confounding variables, the reliance on self-reported data introduces potential recall bias, and unmeasured confounders may persist. Although serum cotinine provides an objective measure of smoking status, it may not fully capture long-term smoking patterns. Furthermore, the absence of longitudinal follow-up data prevents the assessment of smoking's sustained effects on RC levels.

Conclusions

A significant positive association exists between cotinine and RC levels, moderated by sex. Inflammatory markers, particularly NEU, partially mediate this association.

Abbreviations

RCS	Restricted Cubic Spline
RC	Remnant cholesterol
TC	Total cholesterol
TG	Triglyceride
VLDL	Very low-density lipoproteins
LDL-C	Low-density lipoproteins-cholesterol
HDL-C	High-density lipoproteins-cholesterol
CIs / CI	Confidence Intervals
PIR	Ratio of family income to poverty
NCHS	National Center for Health Statistics
NEU	Neutrophils
MON	Monocytes
LYM	Lymphocytes
PLT	Platelets

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02372-x.

Supplementary Material 1.

Authors' contributions

T.L. and Z.S. are co-first authors, jointly leading research conceptualization, statistical analysis, result interpretation, data visualization, and initial manuscript drafting. G.T., J.S., and K.H. ensured data integrity and analysis accuracy, with K.H. conducting a comprehensive study review. All authors contributed to manuscript revisions and approved the final version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This research complied with the Helsinki Declaration and used NHANES data which has undergone ethical review by NCHS. As a secondary analysis of this approved dataset following STROBE guidelines, no additional ethical approval was required.

Consent for publication

Written informed consent for publication was obtained from all participants.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Spine Surgery, The Affiliated Yuebei People's Hospital of Shantou University Medical College, No. 108, Huimin South Road, Wujiang District, Shaoguan City, Guangdong Province, P.R. China. [2]First Clinical Medical College, Guangdong Medical University, Zhanjiang, China.

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