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Association between serum cotinine level and prevalence of non-alcoholic fatty liver disease: a cross-sectional study from the Third National Health and Nutrition Examination Survey

Huafeng Shen¹, Jennifer L Peng², Sucharat Tayarachakul², and Suthat Liangpunsaku^{2,3,4}¹Division of Gastroenterology and Hepatology, Department of Medicine, University of Iowa, Iowa City, Iowa, USA²Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University, Indianapolis, Indiana, USA³Roudebush Veterans Administration Medical Center, Indianapolis, Indiana, USA⁴Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA

Abstract

The data on the effect of smoking on non-alcoholic fatty liver disease (NAFLD) has been controversial. The aim of this study was to investigate if an association exists between serum cotinine level (a tobacco biomarker) and NAFLD prevalence in the general US population. We conducted a cross-sectional analysis of data from the Third National Health and Nutrition Examination Survey (NHANES III). We included 11,003 adults aged 20–74 years who underwent ultrasonography. Of those, 4036 were identified as having NAFLD and 6967 were recognized as controls. The percentage of current smokers was significantly lower in subjects with NAFLD compared with those in controls (21.5% vs 26.0%, $p < 0.01$). After adjustment for potential confounders, there was no association between current or former smokers with NAFLD. Additionally, no associations were observed between the levels of serum cotinine and NAFLD. No association between serum cotinine levels at each quartile level and NAFLD was observed regardless of smoking status. In this large US population-based study, we did not find an association between NAFLD and self-reported smoking status or measured serum cotinine level.

Correspondence to: Dr Suthat Liangpunsaku, Associate Professor of Medicine, Biochemistry and Molecular Biology, Division of Gastroenterology and Hepatology, 550 N. University Blvd, UH 4100, Indianapolis, IN 46202, USA; sliangpu@iupui.edu.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide.¹ It comprises the spectrum of fatty liver diseases including simple steatosis, steatohepatitis, and cirrhosis.¹ In a sense, NAFLD represents another component of metabolic syndrome (MetS)^{2,3} since not only is obesity a shared major risk factor but decreased insulin sensitivity is also commonly observed in both. Additionally, the strong association of NAFLD with features of MetS⁴ such as obesity, central fat distribution, diabetes, dyslipidemia, and hypertension, provide further support for this hypothesis. Several reports and a meta-analysis have demonstrated smoking contributes to MetS.^{5–7} Slagter *et al*⁷ investigated the relationship between smoking and the individual components of MetS. The most notable differences between smokers and non-smokers were observed in the levels of high-density lipoprotein (HDL) and triglycerides. This finding was seen in men and women, irrespective of their body mass index (BMI).⁷ Since MetS is a known risk factor for NAFLD, several studies were conducted to determine the association between smoking and NAFLD. However, the results have been conflicting.^{8,9} In a community-based study of 8580 Chinese subjects, the authors found that both passive and heavy active smoking were associated with NAFLD.⁹ Conversely, no association was observed in a cross-sectional study from Mexico in which the routine health examination results from 933 subjects were examined.⁸

A plausible explanation for these contradicting results could lie in the reliability of the questionnaire used to determine smoking status.^{10,11} Smoke exposure can be assessed with levels of serum cotinine, which is a metabolite of nicotine often applied as a surrogate measurement of smoke exposure due to its longer half-life than nicotine.¹² The half-life of cotinine is about 24 hours compared with nicotine's half-life of 30 min.¹² Its levels, when used in combination with self-reported status, can confirm the history of smoke exposure.

As part of the Third National Health and Nutrition Examination Survey (NHANES III), ultrasonographic examinations of the abdomen were performed and the presence or absence of hepatic steatosis was determined through a standardized algorithm.¹³ Given the conflicting association between NAFLD and smoking as demonstrated by the aforementioned self-reported questionnaires, we conducted this population-based study using data from NHANES III, with a focus on levels of serum cotinine, to further clarify the relationship between these two conditions.

METHODS

The Third National Health and Nutrition Examination Survey

The NHANES III (1988–1994) is a complex, multistage, stratified, clustered, probability sample study designed to obtain information regarding nutritional status and health of the US population.¹⁴ The survey included questionnaires, physical examination, and laboratory tests. The study was approved by the Center for Disease Control and Prevention's Institutional Review board.

Study population

During the survey period, 16,115 subjects who were 20–74 years old completed the interview, physical examination, laboratory tests, and liver ultrasound examination. After excluding subjects without ultrasound images and those with ungradable degree of hepatic steatosis from ultrasound (n=2259), a total of 13,856 subjects were eligible for further study. We also excluded 2852 subjects with hepatic conditions other than NAFLD (ie, alcoholic liver disease, viral hepatitis, iron overload) and one subject without smoking status information. A total of 11,003 participants constituted the study cohort (figure 1).

Data collection

Baseline demographic data on age, race or ethnicity, sex, income, and education level were obtained using a questionnaire. Information on alcohol consumption, smoking, underlying medical conditions, physical activity, and drug use was obtained in a household interview. During the physical examination, height and body weight were measured and BMI was calculated. Blood pressure was recorded either at the household interview or during physical examination.

Race or ethnicity was categorized as ‘non-Hispanic blacks’, ‘non-Hispanic whites’, ‘Mexican-American’, and ‘others’. Education was categorized as 8, 9 to 12, and 12 years, based on completed years of schooling. Economic status was categorized as \$15,000, \$15,001 to \$25,000, and \$25,000, according to the household income.

Smoking status was categorized as ‘never’, ‘current’, and ‘former’. Current smokers were those who smoked daily or occasionally and reported having smoked more than 100 cigarettes in their lifetime. Former smokers were defined as those who reported having smoked >100 cigarettes during their lifetime but no longer smoked at all at the time of enrollment. Those who smoked <100 cigarettes during their lifetime were classified as ‘having never smoked’.¹⁵ Heavy alcohol use was defined as an average of >20 g/day for men and >10 g/day for women.¹⁶

Physical activity was calculated based on a self-reported history of participating in one of the following activities during the past month: swimming, jogging, walking, running, aerobics, bicycling, weight lifting, and any gardening/yard work, dancing, and calisthenics. Based on the information, a physical activity intensity score was calculated using the ratio of metabolic rate achieved during physical activity compared with that during resting. The physical activity intensity score was then categorized as inactive (score <3.5), moderately active (score 3.6–14.9), and active (score >15.0).¹⁷

Insulin resistance was defined by a homeostasis of model assessment score >3.0.¹⁸ Elevated serum aminotransferases was defined as alanine aminotransferase (ALT) >40 U/L or aspartate aminotransferase (AST) >37 U/L (for men), and ALT or AST >31 U/L (for women). Transferrin saturation was considered to be elevated when its level was <50%.¹⁹ Serum cotinine levels were measured using an isotope dilution, liquid chromatography, and tandem mass spectrometry method. The limit of detection using this method is 0.05 ng/mL. Subjects with serum cotinine levels below the limit of detection were assigned a level of

0.035 ng/mL. In our study, serum cotinine levels were determined and divided into quartiles for each smoking status (never-smokers, former smokers, and current smokers).

Definitions of NAFLD and controls

Ultrasonography examinations of the liver and gallbladder were performed in participants aged 20–74 years with a Toshiba (Tustin, California, USA) SSA-90A machine using a 3.75 and 5.0 MHz transducer.¹³ The liver was graded as normal, mild, moderate, or severe hepatic steatosis based on the following information: (1) Degree of the brightness of liver parenchyma, (2) Presence of liver-to-kidney contrast, (3) Presence of echogenic walls in the small intrahepatic vessels, (4) Presence of deep beam attenuation, and (5) Definition of the gallbladder walls.¹³

In the absence of a consensus definition, NAFLD in our study was defined as the presence of mild-to-severe hepatic steatosis on ultrasonography without other causes of chronic liver diseases (such as iron overload, defined by transferrin saturation > 50%, positive hepatitis B and C serology, and heavy alcohol consumption). Healthy controls were defined as those without any evidence of steatosis on ultrasonography and any evidence of chronic liver diseases.

Statistical analyses

All analyses were weighted to account for unequal probabilities of selection, based on the complexity of the survey data and the study design. Demographic and clinical differences among study participants according to smoking status were compared using the one-way analysis of variance or χ^2 test, as appropriate. Serum cotinine was log-transformed to reduce the skewness in its distribution. Unconditional multivariate logistic regression was used to assess the association between smoking status/serum cotinine and NAFLD. The first model included age, sex, ethnicity, socioeconomic status, BMI, and physical activity. The second model included age, sex, ethnicity, socioeconomic status, BMI, physical activity, insulin resistance, systolic/diastolic blood pressure, and serum triglycerides and HDL. We also conducted stratified analyses based on self-reported smoking status. All analyses were performed using SAS V.9.3 (SAS Institute, Cary, North Carolina, USA). A p value < 0.05 (two-sided) was considered statistically significant.

RESULTS

Demographic and clinical characteristics of the study participants

The schematic diagram on the selection of participants is shown in figure 1. Of the 11,003 participants included in the main analyses, 4036 (37%) were identified as having NAFLD and 6967 (63%) as controls. Subjects with NAFLD were older (48.5 vs 43 years, $p < 0.01$), had higher systolic (129.1 vs 121.6 mm Hg, $p < 0.01$) and diastolic (77.1 vs 73.5 mm Hg, $p < 0.01$) blood pressures; higher serum triglycerides (2.2 vs 1.5 mmol/L, $p < 0.01$), higher total cholesterol (5.5 vs 5.3 mmol/L, $p < 0.01$), higher circulating glycosylated hemoglobin (6.0% vs 5.5%, $p < 0.01$), higher serum AST (24.4 vs 20.1 U/L, $p < 0.01$), and higher serum ALT (23.5 vs 15.7 U/L, $p < 0.01$). Subjects with NAFLD were more likely to be men (53.1% vs 41.6%, $p < 0.01$), obese (49.5% vs 22.4%, $p < 0.01$), and Mexican-American (38.5% vs

28%, $p < 0.01$). Selected demographic and clinical characteristics stratified by smoking status are summarized in table 1. Severe hepatic steatosis was found in 7%, 9.9%, and 5.8% among non-smokers, former smokers, and current smokers, respectively ($p < 0.01$).

Association between smoking status, serum cotinine levels and NAFLD

Using the questionnaires, 51.1% and 27.5% of NAFLD subjects were non-smokers and former smokers, respectively; whereas 53.1% of controls were non-smokers and 20.9% were former smokers. The percentage of current smokers was significantly lower in subjects with NAFLD compared with those in controls (21.5% vs 26.0%, $p < 0.01$). The medians of serum cotinine levels were 0.32 ng/mL in those with NAFLD and 0.37 ng/mL in controls ($p < 0.01$; table 2).

Associations of self-reported smoking status and serum cotinine levels with NAFLD are summarized in table 2. After adjustment for potential confounders in the model 1, there was no association between current (OR 0.92, 95% CI 0.83 to 1.03) and former smokers (OR 1.11, 95% CI 0.99 to 1.24) with NAFLD. No associations were observed between the levels of serum cotinine (OR 1.000, 95% CI 0.999 to 1.000) or its log-transformed levels (OR 0.991, 95% CI 0.977 to 1.006) with NAFLD. Similar results were observed in the model 2 (table 2).

The association of serum cotinine divided by quartile levels and NAFLD, stratified by smoking status is shown in table 3. No association between serum cotinine levels at each quartile level and NAFLD was observed regardless of smoking status in the logistic regression analyses for both models. The trend analysis demonstrated the inverse association between serum cotinine levels and NAFLD among current smokers (p for trend < 0.01).

DISCUSSION

In this population-based study, we found no association between NAFLD with self-reported smoking status and serum cotinine level. Active tobacco smoking is a known risk factor for several chronic diseases such as cancer, cardiovascular disease, and diabetes.^{20–22} Smoking also has an impact on liver diseases as it has shown to be a risk factor for advanced fibrosis in those with hepatitis C infection.²³ The long-term morbidity and mortality from chronic hepatitis C infection are expected to decline due to highly effective, direct-acting antiviral agents.²⁴ However, the health and economic burdens from NAFLD will continue due to the worsening epidemics of obesity and MetS.²⁵ Therefore, identification of risk-modifying factors associated with NAFLD is of the utmost importance, as it may have an impact on the natural course of this disease.

MetS, through a combination of unfavorable health factors such as dyslipidemia, visceral obesity, and hypertension, is strongly associated with NAFLD. Smoking has also been associated with MetS.⁵⁷²⁶ Tobacco use is associated with increasing waist circumference, dyslipidemia, and hypertension which suggests a role for smoking on insulin resistance.⁵²⁷²⁸ A recent population-based study of 59,467 individuals showed that smoking is associated with a higher risk of MetS in both genders and in all BMI classes.⁷ Moreover, the increased risk was stronger for heavy smokers but not as significant among light and former smokers.⁷

Given the link between smoking and MetS, a known risk factor for NAFLD, several previous studies attempted to determine the relationship between smoking and NAFLD.⁸⁹ A study from China observed a positive association between active smoking and NAFLD.⁹ Furthermore, active tobacco smoking and an elevated BMI had a synergistic effect on NAFLD prevalence.⁹ However, another study from Mexico found contradicting results.⁸ It is important to note these studies used the self-reported questionnaires to determine the smoking status of the participants. In our study, we found self-reported former smokers had a mean serum cotinine level of 24.6 ng/mL. Therefore, there exists the concern of reporting bias that participants may tend to report less than the amount they actually smoke. It is important to note that we observed that among current smokers, the prevalence of NAFLD was lower in subjects with higher levels of cotinine (OR 0.62, 95% CI 0.49 to 0.79) in the crude analysis. This finding is likely due to significantly lower BMI among NAFLD subjects with serum cotinine in the fourth quartile range (25.2 ± 5.0 kg/m²) than that in the first quartile (27.8 ± 6.1 kg/m², $p < 0.01$). However, in the multivariate analyses adjusting for potential confounders, we found no association between serum cotinine levels at each quartile level and NAFLD.

NHANES III provided an opportunity for detailed analysis on the relationships between tobacco smoking and NAFLD. First, this is a population-based study representing the US population. It included comprehensive information on anthropometric measurements, laboratory results, and abdominal ultrasound and, thus, allowed us to determine the presence of MetS as well as NAFLD. Additionally, the use of self-reported questionnaires with NHANES III provided objective evidence of smoking exposure with levels of serum cotinine, a metabolite of nicotine which can be used as a marker of smoke exposure due to its longer half-life than nicotine.¹² In this population-based study, we found no association between NAFLD with self-reported smoking status and serum cotinine level. It is known that the overall lifestyle of smokers is generally different from that of non-smokers.²⁹ Despite the adjustments for physical activity in our analyses, we still did not find an association between smoking exposure and NAFLD.

In conclusion, in this large US population-based study, we did not find a relationship between NAFLD and self-reported smoking status or serum cotinine levels. Despite the strength of the sample size and the study cohort representing the US population, we acknowledge several limitations. NHANES III does not provide histological data which limited our ability to further categorize NAFLD by histological subtypes. Furthermore, ultrasound is not the most sensitive modality to characterize steatosis or diagnose NAFLD. However, it is the most practical screening modality for a large population-based study when obtaining liver biopsies is not feasible. Though we failed to demonstrate smoking as a possible risk-modifying factor associated with NAFLD, tobacco smoking is a well-known risk factor for several chronic diseases such as cancer, cardiovascular disease, and diabetes. Thus, interventions to promote successful smoking cessation should still be implemented as preventative measures.

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Significance of this study

What is already known about this subject?

- ▶ Non-alcoholic fatty liver disease (NAFLD) is associated with obesity and metabolic syndrome.
- ▶ Several reports and a meta-analysis have shown that smoking is linked to metabolic syndrome.
- ▶ The association between smoking and NAFLD is debatable.

What are the new findings?

- ▶ In this large US population-based study, we did not find an association between NAFLD and self-reported smoking status or measured serum cotinine level.

How might these results change the focus of research or clinical practice?

- ▶ Though we failed to demonstrate smoking as a possible risk-modifying factor associated with NAFLD, tobacco smoking is a known risk factor for several chronic diseases such as cancer, cardiovascular disease, and diabetes. Thus, interventions should still be implemented to promote successful smoking cessation.

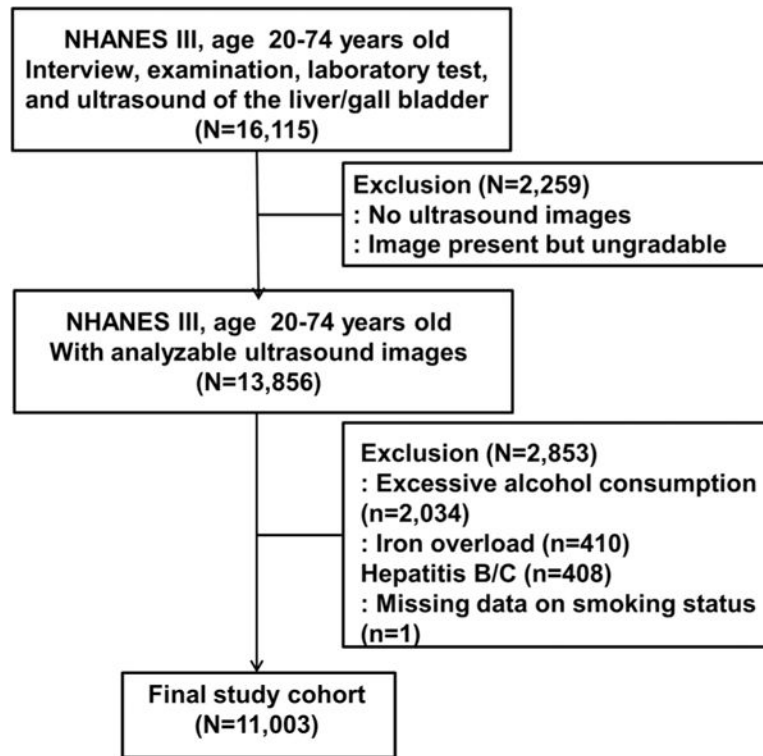


Figure 1. Schematic diagram on the selection of the study participants. NHANES III, Third National Health and Nutrition Examination Survey.

Table 1

Selected demographic and clinical characteristics of the study participants *

	Non-smokers (N=5761)	Former smokers (N=2563)	Current smokers (N=2679)	p Value [†]
Age (years.)	41.8 (0.2)	52.2 (0.3)	42.2 (0.3)	<0.01
Men (n, %)	1833 (31.8)	1534 (59.9)	1344 (50.2)	<0.01
Race or ethnicity (n, %)				
Non-Hispanic white	1739 (30.2)	1227 (47.9)	986 (36.8)	<0.01
Non-Hispanic black	1726 (30.0)	555 (21.7)	940 (35.1)	
Mexican-American	1979 (34.4)	706 (27.5)	664 (24.8)	
Other	317 (5.5)	75 (2.9)	89 (3.3)	
Annual household income (n, %)				
\$15,000	1670 (32.1)	649 (27.6)	983 (40.1)	<0.01
\$15,001–\$25,000	1205 (23.1)	551 (23.5)	598 (24.4)	
>\$25,000	2331 (44.8)	1148 (48.9)	871 (35.5)	
Education (n, %)				
8 years	1289 (22.5)	636 (24.9)	614 (23.1)	<0.01
9–12 years	2604 (45.5)	1113 (43.6)	1527 (57.5)	
>12 years	1828 (32.0)	801 (31.4)	516 (19.4)	
Body mass index (n, %)				
<25 kg/m ²	2121 (36.9)	752 (29.4)	1181 (44.1)	<0.01
25–30 kg/m ²	1901 (33.1)	982 (38.4)	888 (33.2)	
30 kg/m ²	1726 (30.0)	821 (32.1)	606 (22.7)	
Physical activity (n, %)				
Inactive	1907 (33.1)	749 (29.2)	920 (34.3)	<0.01
Moderately active	2548 (44.2)	1300 (50.7)	1264 (47.2)	
Active	1306 (22.7)	514 (20.1)	495 (18.5)	
Diabetes [‡] (n, %)	777 (13.5)	456 (17.8)	356 (13.3)	<0.01
Hypertension [‡] (n, %)	1396 (24.5)	858 (33.7)	602 (22.7)	<0.01
Hypercholesterolemia [‡] (n, %)	928 (32.5)	656 (40.7)	383 (34.9)	<0.01
Glycosylated hemoglobin (%)	5.5 (0.02)	5.8 (0.03)	5.6 (0.02)	<0.01
Insulin resistance [§] (n, %)	2127 (37.0)	1102 (43.0)	847 (31.7)	<0.01
Systolic BP (mm Hg)	121.7 (0.2)	128.6 (0.4)	122.0 (0.4)	<0.01
Diastolic BP (mm Hg)	73.7 (0.1)	76.2 (0.2)	73.7 (0.2)	<0.01
Serum triglycerides (mmol/L)	1.5 (0.02)	1.9 (0.03)	1.7 (0.03)	<0.01
Serum total cholesterol (mmol/L)	5.2 (0.02)	5.6 (0.02)	5.2 (0.02)	<0.01
Serum LDL cholesterol (mmol/L)	3.3 (0.02)	3.5 (0.03)	3.3 (0.03)	<0.01
Serum HDL cholesterol (mmol/L)	1.3 (0.005)	1.3 (0.008)	1.2 (0.008)	<0.01
Serum alanine aminotransferase (U/L)	17.7 (0.2)	18.3 (0.3)	16.6 (0.3)	<0.01
Serum aspartate aminotransferase (U/L)	21.2 (0.2)	22.0 (0.2)	20.1 (0.2)	<0.01
Serum γ -glutamyltransferase (U/L)	28.3 (0.5)	33.6 (0.9)	32.2 (0.9)	<0.01
Serum uric acid (μ mol/L)	300.4 (1.2)	335.0 (1.8)	310.1 (1.7)	<0.01

	Non-smokers (N=5761)	Former smokers (N=2563)	Current smokers (N=2679)	p Value [†]
Serum cotinine (ng/mL) [¶]	0.17±0.44	0.22±0.72	210.0±221.0	<0.01
Hepatic steatosis (n, %)				
None	3700 (64.2)	1454 (56.7)	1813 (67.7)	<0.01
Mild	795 (13.8)	341 (13.3)	339 (12.7)	
Moderate	862 (15.0)	514 (20.1)	372 (13.9)	
Severe	404 (7.0)	254 (9.9)	155 (5.8)	

* Values are numbers (percentages) for categorical variables and means (SEs) for continuous variables.

[†] From one-way ANOVA for continuous variables and χ^2 test for categorical variables.

[‡] Self-reported doctor diagnosis.

[§] Insulin resistance was defined as HOMA>3.0.

[¶] Values are medians±IQR.

ANOVA, analysis of variance; BP, blood pressure; HDL, high-density lipoprotein; HOMA, homeostasis of model assessment score; LDL, low-density lipoprotein.

Table 2

Association of smoking status, serum cotinine level and NAFLD

Tobacco smoking	Controls* (n=6967)	NAFLD* (n=4036)	OR (95% CI)		
			Crude	Model 1 [†]	Model 2 [‡]
Self-reported smoking status					
Never-smokers	3700 (53.1)	2061 (51.1)	1.00	1.00	1.00
Former smokers	1454 (20.9)	1109 (27.5)	1.37 (1.25 to 1.51)	1.11 (0.99 to 1.24)	1.08 (0.96 to 1.22)
Current smokers	1813 (26.0)	866 (21.5)	0.86 (0.78 to 0.95)	0.92 (0.83 to 1.03)	0.92 (0.82 to 1.04)
Serum cotinine level (ng/mL)	0.37 (58.20)	0.32 (11.41)	0.999 (0.999 to 1.000)	1.000 (0.999 to 1.000)	1.000 (0.999 to 1.000)
Log-transformed cotinine level (ng/mL)	-1.01 (6.34)	-1.15 (4.84)	0.971 (0.959 to 0.983)	0.991 (0.977 to 1.006)	0.989 (0.974 to 1.004)

* Values are numbers (percentages) for categorical variables and medians±IQR for continuous variables.

[†] Unconditional logistic regression model, adjusted for age, sex, ethnicity, socioeconomic status, body mass index, physical activity.[‡] Unconditional logistic regression model, adjusted for age, sex, ethnicity, socioeconomic status, body mass index, physical activity, IR, systolic/diastolic blood pressure, serum triglycerides and HDL cholesterol.

HDL, high-density lipoprotein; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

Table 3

Association of serum cotinine divided by quartile levels and NAFLD, stratified by smoking status

Serum cotinine level (ng/mL)	OR (95% CI)				p for trend*
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Non-smokers					
Crude	1.00	0.99 (0.85 to 1.16)	0.92 (0.79 to 1.08)	0.97 (0.83 to 1.13)	0.49
Model 1 [†]	1.00	1.06 (0.89 to 1.27)	0.98 (0.82 to 1.17)	1.07 (0.89 to 1.29)	
Model 2 [‡]	1.00	1.00 (0.84 to 1.20)	0.95 (0.79 to 1.14)	1.02 (0.84 to 1.24)	
Former smokers					
Crude	1.00	0.79 (0.63 to 1.00)	0.93 (0.75 to 1.17)	0.85 (0.67 to 1.06)	0.34
Model 1	1.00	0.89 (0.69 to 1.15)	1.07 (0.82 to 1.39)	1.13 (0.86 to 1.48)	
Model 2	1.00	0.84 (0.64 to 1.10)	1.01 (0.77 to 1.33)	1.03 (0.78 to 1.37)	
Current smokers					
Crude	1.00	0.86 (0.69 to 1.09)	0.75 (0.59 to 0.95)	0.62 (0.49 to 0.79)	<0.01
Model 1	1.00	0.97 (0.75 to 1.26)	0.94 (0.72 to 1.23)	0.88 (0.66 to 1.17)	
Model 2	1.00	1.05 (0.80 to 1.39)	1.07 (0.81 to 1.43)	0.98 (0.73 to 1.32)	

* Cochran-Armitage trend test.

[†] Unconditional logistic regression model, adjusted for age, sex, ethnicity, socioeconomic status, body mass index, physical activity.[‡] Unconditional logistic regression model, adjusted for age, sex, ethnicity, socioeconomic status, body mass index, physical activity, IR, systolic/diastolic blood pressure, serum triglycerides and HDL cholesterol.

HDL, high-density lipoprotein; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.