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Association between snus use and lipid status in Swedish men

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ABSTRACT

Snus is a common tobacco product in Sweden, but the cardiovascular risk profile for snus users is less known than for cigarette smokers. We examined the association of snus use with lipid status, particularly in comparison to non-tobacco use and cigarette smoking, using data from 5930 men in the Northern Sweden MONICA study. Tobacco use was self-reported in 1986 to 2014 (24.4% used snus) and blood samples were collected at the same time. Harmonized analyses on non-high-density lipoprotein (non-HDL) cholesterol, HDL cholesterol, and triglycerides were conducted in 2016 to 2018. Three hundred eighty-one snus users had also been examined more than once, allowing us to study the effect of discontinued use (achieved by 21.0%). In multivariable linear regression models, snus use was associated with higher HDL cholesterol and triglyceride concentrations compared to non-tobacco use (p values ≤ 0.04), and it was associated with higher HDL cholesterol concentrations and lower triglyceride concentrations compared to cigarette smoking (p values ≤ 0.02). Snus use was not associated with non-HDL cholesterol concentrations, irrespective of the comparison group (p values ≥ 0.07). There was no indication that higher intensity of snus use led to a worse lipid profile, given that high-consumers had higher HDL cholesterol concentrations than low-consumers (p value = 0.02), or that discontinuation of snus use led to a better lipid profile, given that continued users had lower triglyceride concentrations than discontinued users (p value = 0.03). Further studies are needed to confirm or refute our findings.

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Introduction

Lipid status is a major risk factor for cardiovascular disease (CVD), with higher risks associated with increased concentrations of total cholesterol [1], low-density lipoprotein (LDL) cholesterol [2], triglycerides [3], and apolipoprotein B [4] and lower risks associated with increased concentrations of high-density lipoprotein (HDL) cholesterol [2] and apolipoprotein A1 [4]. Increased concentrations of non-HDL cholesterol—that is, the difference between total and HDL cholesterol, thereby representing the cholesterol content in all proatherogenic lipoproteins [5]—have also been strongly associated with a higher risk of CVD [6].

Snus is a common tobacco product in Sweden, especially popular among adult men, among whom it was used by more than 20% in a national survey in 2020 [7]. The product is normally placed under the lip, either in loose or portion-packed form. In comparison to cigarette smoking, which has been causally linked to several CVD outcomes [8], the effects of snus use on cardiovascular health are less clear [9]. With that said, recent observational studies from Sweden do indicate that snus use might be associated with an increased risk of certain CVD outcomes, including incident heart failure [10], fatal myocardial infarction [11], and fatal stroke [12], as well as with an increased overall and cardiovascular mortality [13]. It has also been observed that post-myocardial infarction mortality seems to be lower in people who discontinue their snus use [14]. A potential explanation for the observed exposure-outcomes associations could be non-beneficial alterations in lipid metabolism (increased non-HDL cholesterol and decreased HDL cholesterol) by the nicotine content in snus [15,16]; that is, the same explanation that has been theorized for the rather well-established effect of cigarette smoking on lipid status [17–20]. Previous studies from Sweden on the association between snus use and lipid status have shown inconsistent results, with either null findings [21,22] or slightly increased triglyceride concentrations in current snus users [23,24].

The aim of this study was to examine the association of snus use and discontinuation of snus use with lipid status, particularly in comparison with non-tobacco use and cigarette smoking, using data from the Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study.

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Subjects and methods

Study population

The Northern Sweden MONICA study consists of seven population-based surveys (in 1986, 1990, 1994, 1999, 2004, 2009, and 2014; $n = 12{,}069$) that used a random sampling design, stratified for age (25 to 64 years in 1986 and 1990; 25 to 74 years in 1994 to 2014) and sex [25–27]. Each participant filled in a questionnaire on lifestyle habits, underwent a clinical examination, and had blood samples drawn. The participation rate decreased over time, from 81% in 1986 to 63% in 2014. In 1999, the participants who had been sampled in 1986 to 1994 were invited for follow-up measurements (using the same questionnaire and clinical examination as in the main surveys; participation rate 81%; $n = 4136$).

The current study was restricted to men from the main surveys in 1986 to 2014 for our primary analyses on tobacco use ($n = 5930$) and to men who were or had been tobacco users in the follow-up survey in 1999 for our secondary analyses on discontinuation of tobacco use ($n = 836$). Women were not included because of the very low number of female snus users (around 5%, of whom [i] 73% were sampled from 2004 and onwards and [ii] 72% reported current or past use of cigarettes).

The Northern Sweden MONICA study has been covered by multiple ethical approvals from the Regional Ethical Committee at Umeå University (Sweden) from its initiation up until 2014; and the current study was approved by the Swedish Ethical Review Authority (2021-00100). The recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) initiative were followed whenever applicable [28].

Assessment of tobacco use and other variables

On each questionnaire (available in Swedish at www.umu.se/forskning/projekt/monica-studien/monica-undersokningen-i-norra-sverige2/), the participants answered to a number of questions related to their current and previous use of snus and cigarettes. In general, the questions on cigarette smoking were more detailed than the questions on snus use (e.g. the questionnaire [i] differentiated between daily and non-daily use of cigarettes but not of snus and [ii] collected information on time since smoking cessation but not on time since snus cessation). To validate the self-reported tobacco habits, plasma concentrations of cotinine (the predominant nicotine metabolite) were measured in a subsample of the 1990 survey ($n = 321$ subjects; 46.4% men) [21]. Cotinine concentrations were below 12 ng/mL in all non-tobacco users and above 55 ng/mL in almost all tobacco users.

The participants also answered questions on their educational level, physical activity, alcohol consumption (of strong beer [amount of alcohol 5.4%], wine [amount of alcohol 12 to 13%], and liquor/spirits [amount of alcohol 40%]), diagnosis of diabetes, and use of lipid-lowering drugs (not included in the questionnaire in 1986). Height and weight were measured during the clinical examination, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Laboratory procedures and lipid biomarkers

Blood samples were drawn after the participants had fasted for at least 4 h and were then stored at −80 °C. Total cholesterol concentrations (mmol/L) were measured in connection with each survey and without freezing. The assay method was enzymatic-based from 1986 to 1994 (BM Monotest Cholesterol CHOD-PAP; Boehringer Mannheim GmbH, Germany) and dry chemistry-based from 1999 to 2014 (Vitros 950, Kodak Echtachem, USA). The total cholesterol measurement was accredited by the national accreditation body in Sweden (Swedac), with a coefficient of variation of 3.6% at 3.9 mmol/L and 3.1% at 6.7 mmol/L [29]. The other lipid biomarkers (i.e. LDL and HDL cholesterol, triglycerides, and apolipoprotein B and A1) were measured between 2016 and 2018 at the Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) laboratory in Germany [30]. The measurement unit, assay method, and coefficient of variation for each biomarker are shown in Supplementary Table 1. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol, and the apolipoprotein B/A1-ratio was calculated as the ratio between the two biomarkers.

The primary outcomes of this study were non-HDL cholesterol, triglycerides, and HDL cholesterol (as general proxies for non-beneficial and beneficial lipids, respectively) and the secondary outcomes were total and LDL cholesterol, apolipoprotein B and A1, and apolioprotein B/A1-ratio.

Statistical analysis

Statistical significance was set at a 2-sided p value less than 0.05. Analyses were performed using Stata version 14 (StataCorp LP, College Station, USA).

Main survey sample

A total of 5930 men were eligible and included in the main survey sample. Missing data on survey and clinical examination variables ranged from 0.2 to 2.4% and on blood sample variables from 0.4 to 3.9% (see Supplementary Table 2 for details). Missing data were handled by using multiple imputations by chained equations (30 data sets were created and combined using Rubin's rule) [31]. In a sensitivity analysis, we repeated the analyses by using complete case data.

Linear and quantile regression models were used to calculate mean and median (10th and 20th percentile; 80th and 90th percentile) values of lipid biomarkers by tobacco use (never use of snus and cigarettes [reference], past use of snus or cigarettes, current use of snus, or current use of cigarettes [including non-daily use]). Current snus users with combined cigarette use (and vice versa, for cigarette users) were kept for the integrity of the imputation model, and included in the regression model as a separate category, but no statistical inference was drawn on them since our aim was to differentiate between snus use and cigarette use.

In subgroup analyses, current snus users were further categorized according to past cigarette use (no or yes) and intensity of snus use (<4 or ≥4 cans/week). One can of snus contains around 20 to 42 grams of tobacco (with the nicotine content ranging from 0.8 to 3.0%). For comparative reasons, current cigarette smokers were also further categorized according to intensity of use (<13 or ≥13 cigarettes/day [median value and corresponding to roughly 4 packs/week]).

Potential confounders were decided upon a priori based on their possible associations with tobacco use as well as with lipid biomarker concentrations. Quantile regression models (continuous variables) and logistic regression models (categorical variables), adjusted for age (<35, 35–44, 45–54, 55–64, and ≥65 years), were used to calculate p values of the potential confounders by tobacco use (for the overall exposure variable as well as for each level of the exposure variable).

The multivariable regression models for the association between tobacco use and lipid biomarker concentrations included the following potential confounders: age (continuous, years), calendar year (continuous, year), educational level (university or non-university), BMI (continuous, kg/m^2), alcohol consumption (never, less than once/week, or at least once/week), and diagnosis of diabetes (no or yes). Since there was evidence of non-linear associations of age and BMI with lipid status, we modelled these covariates by using 3-knot restricted cubic splines (knot placement at the 10th, 50th, and 90h percentile) [32]. (See Supplementary Text 1 for additional details on the parametric model assumptions.)

In a sensitivity analysis, we further adjusted for (and restricted by) lipid-lowering drug use (no or yes) by (i) restricting the analysis to the surveys in 1990 to 2014 (n = 5116; since there was no question on lipid-lowering drug use in 1986) and (ii) assuming that no one in the survey in 1986 used lipid-lowering drugs (since the first statin, lovastatin, was not approved for commercial use until 1987 [33]). Another sensitivity analysis was restricted to the surveys in 1990 to 2009 and further adjusted for physical activity (almost none, light-effort ≥1 h/week, or high-effort ≥1 h/week) (n = 4370; since that variable was only measured in a standardized way in 1990 to 2009). To avoid repeated imputations—and since the results of the imputed and complete case data were almost identical in the main model—the above-mentioned sensitivity analyses were based on complete case data.

Separate regression models by calendar period (1986 to 1994 and 1999 to 2014; representing the eras when cigarettes were more common than snus [1986 to 1994] and snus was more common than cigarettes [1999 to 2014]) were performed as subgroup analyses. To obtain p values of interaction, we included an interaction term between tobacco status and calendar period and tested its coefficients equal to zero.

Follow-up survey sample

A total of 381 snus users and 455 cigarette users from 1986, 1990, and 1994 were eligible and included in the follow-up survey sample in 1999. Multiple imputation was used to handle missing data (n = 30 data sets), and linear and quantile regression models were used to calculate mean and median values of lipid biomarkers by discontinuation of snus (no or yes) and cigarettes (no or yes). The multivariable model was adjusted for the same variables as in the multivariable model of the main survey sample, with the addition of lipid-lowering drug use and physical activity.

Results

Main survey sample

In total, 5930 men (median age 50.0 years) were included for analysis, of whom 13.7% were sampled in the 1980s, 43.6% in the 1990s, 30.1% in the 2000s, and 12.6% in the 2010s. Based on complete data (n = 5840 and 5906 for snus and cigarette use, respectively), the proportion of current snus use increased over time (from 19.3 to 30.0%), while that of current cigarette use decreased over time (from 30.9 to 11.8%). Snus use became more common than cigarette smoking from 1999 and onwards (Supplementary Figure 1).

Survey characteristics according to tobacco use are shown in Table 1. Compared with never tobacco users, current snus users seemed more likely to be younger, less educated, and less physically active as well as more likely to use lipid-lowering drugs, to consume alcohol, and to have a diagnosis of diabetes and a BMI of 30 kg/m^2 or more (all p values < 0.01, except for lipid-lowering drugs and diabetes [p value = 0.09 and 0.13, respectively]). Compared with current cigarette smokers, current snus users seemed more likely to be younger, well-educated, and more physically active as well as less likely to consume alcohol but more likely to use lipid-lowering drugs and to have a BMI of 30 kg/m^2 or more (all p values ≤ 0.04, except for alcohol consumption [p value = 0.09]). More than half of the current snus users had smoked cigarettes in the past (median time from smoking cessation to blood sampling 13.0 [interquartile range 6.0, 22.0] years). With respect to intensity of use, 69.0% of snus users consumed less than 4 cans per week and 31.0% consumed at least 4 cans per week.

The mean and 5th to 95th percentile concentrations of total cholesterol, non-HDL cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apolipoprotein B, and apolipoprotein A1 are presented in Supplementary Table 3 (entire study population) and Supplementary Figure 2 (by tobacco use).

Table 2 shows the association of non-HDL cholesterol, HDL cholesterol, and triglycerides with tobacco use (based on the linear regression model). After adjustment for potential confounders, and compared to never tobacco use, current snus use had a statistically significant positive association with HDL cholesterol and triglycerides (p values ≤ 0.04) and a borderline statistically significant positive association with non-HDL cholesterol (p value = 0.07). Compared to never tobacco use, current cigarette smoking had a statistically significant positive association with non-HDL cholesterol and triglycerides (p values ≤ 0.01). In

Table 1. Characteristics of the study population with complete data on snus and cigarette use according to tobacco use.

Characteristics	Tobacco use[a,b]				p value[c]
	Never	Past	Current, snus	Current, cigarettes	
Participants (n)	2000	1559	1109	850	—
Tobacco use (%)[d]					
Past use of snus	—	48.6	—	23.2	—
Past use of cigarettes	—	82.6	55.4	—	—
Covariates[e]					
Age (median, years)	49.0 (36.0–61.0)	56.0 (45.0–64.0)	46.0 (36.0–58.0)	50.0 (39.0–59.0)	<0.01
University education (%)	22.8 (21.0–24.7)	18.6 (16.6–20.9)	15.4 (13.4–17.7)	11.7 (9.8–14.1)	<0.01
Body mass index ≥30 kg/m^2 (%)	15.3 (13.8–17.0)	16.4 (14.6–18.3)	19.9 (17.6–22.4)	13.3 (11.1–15.8)	<0.01
Alcohol consumption at least once/week (%)[f]	39.9 (37.7–42.1)	47.1 (44.5–49.8)	45.9 (42.9–48.9)	49.9 (46.5–53.2)	<0.01
High-effort physical activity ≥1 h/week (%)[g]	39.8 (37.4–42.2)	36.8 (33.8–39.9)	31.7 (28.7–34.9)	23.1 (19.9–26.7)	<0.01
Use of lipid-lowering drugs (%)[h]	6.6 (5.6–7.9)	9.0 (7.8–10.4)	8.3 (6.7–10.3)	5.5 (4.0–7.5)	<0.01
Diagnosis of diabetes (%)	3.7 (3.0–4.7)	5.1 (4.2–6.2)	5.0 (3.8–6.6)	4.9 (3.6–6.6)	0.23

[a]Five thousand eight hundred thirty-one participants had complete data on snus and cigarette use, of whom 313 were current users of both snus and cigarettes (not included in the table).
[b]Never = no history of snus or cigarette use; past = past history of snus or cigarette use; current, snus = current snus use; and current, cigarettes = current cigarette use (including non-daily).
[c]p values for overall associations were obtained *via* quantile and logistic regression models (for continuous and categorical variables, respectively) and by testing the coefficients of the exposure variable jointly equal to zero.
[d]Percentages might sum up to more than 100%, since some participants had used both snus and cigarettes.
[e]All values, except age, were standardized to the age distribution (<35, 35–44, 45–54, 55–64, and ≥65 years) of the entire study population. Numbers in parentheses represent interquartile ranges (continuous variables) or 95% confidence intervals (categorical variables).
[f]Included questions on strong beer (amount of alcohol 5.4%), wine (amount of alcohol 12 to 13%), and liquor/spirits (amount of alcohol 40%).
[g]Based on data from 1990 to 2009 (n = 4370), since the surveys in 1986 and 2014 had a vastly different question on leisure time physical activity.
[h]Based on data from 1990 to 2014 (n = 5116), since the survey in 1986 had no question on lipid-lowering drugs.

Table 2. Mean differences in non-HDL cholesterol, HDL cholesterol, and triglycerides by tobacco use and based on multiple imputed data sets (n = 30).

Mean difference (95% CI)[b]	Tobacco use[a]			
	Never	Past	Current, snus	Current, cigarettes
Non-HDL cholesterol (mmol/L)				
Age-adjusted model	Ref.	0.13 (0.05, 0.21)	0.04 (-0.05, 0.13)	0.26 (0.17, 0.36)
Multivariable-adjusted model	Ref.	0.07 (-0.01, 0.15)	0.08 (-0.01, 0.16)	0.12 (0.02, 0.21)
HDL cholesterol (mmol/L)				
Age-adjusted model	Ref.	−0.00 (-0.03, 0.03)	0.03 (0.00, 0.06)	−0.01 (-0.05, 0.02)
Multivariable-adjusted model	Ref.	0.01 (-0.02, 0.03)	0.03 (0.00, 0.06)	−0.01 (-0.04, 0.02)
Triglycerides (mmol/L)				
Age-adjusted model	Ref.	0.08 (0.01, 0.15)	0.12 (0.05, 0.19)	0.25 (0.16, 0.33)
Multivariable-adjusted model	Ref.	0.05 (-0.02, 0.11)	0.08 (0.01, 0.16)	0.26 (0.17, 0.34)

CI confidence interval; *HDL* high-density lipoprotein.
[a]Never = no history of snus or cigarette use; past = past history of snus or cigarette use; current, snus = current snus use; and current, cigarettes = current cigarette use (including non-daily).
[b]Estimated from a linear regression model adjusted for age (continuous using 3-knot restricted cubic splines, years), calendar year (continuous, year), educational level (university or non-university), body mass index (continuous using 3-knot restricted cubic splines, kg/m^2), alcohol consumption (never, less than once/week, or at least once/week), and diagnosis of diabetes (no or yes). The lipid biomarkers were not adjusted for each other.

a direct comparison with current cigarette smokers, current snus users had lower triglyceride concentrations (mean difference −0.17 [95% confidence interval (CI) −0.26, −0.08] mmol/L) and higher HDL cholesterol concentrations (mean difference 0.04 [95% CI 0.01, 0.08] mmol/L). Even though the overall interpretation of the results did not change in the median regression model, it should be noted that none of the snus-lipid biomarker associations was statistically significant in that model (Supplementary Table 4). Additional results from the quantile regression models—that is, by using more extreme percentiles (10th and 20th; 80th and 90th)—are shown in Supplementary Tables 5,6.

The multivariable linear regression-based associations of non-HDL cholesterol, HDL cholesterol, and triglycerides with current snus use were consistent in the subgroup analysis by past cigarette use (data not shown). In the subgroup analysis by intensity of use, there was no indication that higher intensity of snus use led to a worse lipid profile, given that men who consumed at least 4 cans per week had higher HDL cholesterol concentrations than those who consumed less than 4 cans per week (p value = 0.02) (Supplementary Figure 3, upper panel). In contrast, current cigarette users who smoked at least 13 cigarettes per day had a worse lipid profile than those who smoked less than 13 cigarettes per day, exemplified by higher concentrations of non-HDL cholesterol and triglycerides (p value = 0.06 and 0.04, respectively) (Supplementary Figure 3, lower panel). The snus-lipid biomarker associations were similar, although with slight differences in magnitude and statistical significance (at least partly related to random variability

due to differences in sample size; as shown in Supplementary Table 7), in the sensitivity analyses that were (i) based on complete data (sample size reduction 7.0 to 31.5%) and (ii) further adjusted for lipid-lowering drug use and physical activity (sample size reduction 8.7 to 32.8%) (Table 3). However, the positive association with non-HDL cholesterol concentrations became stronger after restriction of the analyses to non-users of lipid-lowering drugs (sample size reduction 15.1 to 27.5%) (Table 3). (Nota bene: Corresponding data for cigarette smoking are shown in Supplementary Table 8.) There was no evidence of an interaction between snus use (p value ≥ 0.13) or cigarette smoking (p value ≥ 0.44) and calendar period in relation to non-HDL cholesterol, HDL cholesterol, and triglyceride concentrations (Supplementary Figure 4).

The multivariable linear regression-based associations of total and LDL cholesterol, apolioprotein B and A1, and apolioprotein B/A1-ratio with tobacco use are presented in Figure 1. Compared to never tobacco use, current snus use had a statistically significant positive association with total cholesterol (p value = 0.01) and a borderline statistically significant positive association with LDL cholesterol and apolipoprotein B (p value = 0.09 and 0.06, respectively), while current cigarette smoking had a statistically significant positive association with total and LDL cholesterol, apolioprotein B, and apolipoprotein B/A1-ratio (p values ≤ 0.02). In a direct comparison with current cigarette smoking, current snus use had a statistically significant inverse association with apolipoprotein B and apolipoprotein B/A1-ratio (p values < 0.01) and a non-statistically significant inverse association with LDL cholesterol concentrations (p value = 0.11).

Follow-up survey sample

In total, 381 snus users (median age 53.0 years) and 455 cigarette users (median age 54.0 years) from the main surveys in 1986, 1990, and 1994 were included for analysis of the follow-up survey in 1999. Of the snus users, 21.0% had discontinued their use (of whom none had started to smoke cigarettes), while 44.6% of the cigarette smokers had discontinued their use (of whom 18.7% had started to use snus). (Nota bene: Age-specific discontinuation percentages of the two tobacco habits are available in Supplementary Figure 5.) No information was available on the time from snus cessation to blood sampling; however, the median time from smoking cessation to blood sampling was 6.0 (interquartile range 2.0, 11.0) years, with 11.0% of the participants quitting their habit in proximity to the blood sampling (during the same year or the preceding year). Follow-up survey characteristics by discontinuation of tobacco products are shown in Supplementary Table 9.

In general, men who had continued to use snus seemed to have a better lipid profile than those who had discontinued snus (Figure 2, upper panel). Of note was the large difference in triglyceride concentrations in the linear regression model (mean difference −0.33 [95% CI −0.63, −0.04] mmol/L for continued snus use]), which, however, seemed to be mainly driven by outlier data (as the corresponding estimate in the median regression model was

Table 3. Complete case sensitivity analyses of the mean and median differences in non-HDL cholesterol, HDL cholesterol, and triglycerides in current snus users compared with never tobacco users.

	Lipid biomarker (mmol/L)[a]					
	Non-HDL cholesterol		HDL cholesterol		Triglycerides	
Complete case analysis	Mean	Median	Mean	Median	Mean	Median
Survey in 1986 to 2014[b]						
Multivariable model[c]	0.07	0.05	**0.03**	0.01	**0.09**	**0.06**
Multivariable model[c] + adjustment for lipid-lowering drug use[d]	0.08	0.06	**0.03**	0.01	**0.09**	**0.06**
Multivariable model[c] + exclusion of lipid-lowering drug users[d]	**0.10**	0.09	**0.03**	0.01	**0.09**	**0.06**
Survey in 1990 to 2014[e]						
Multivariable model[c]	**0.10**	0.07	**0.04**	0.01	**0.10**	**0.06**
Multivariable model[c] + adjustment for lipid-lowering drug use[f]	**0.11**	0.09	**0.03**	0.01	**0.09**	**0.06**
Multivariable model[c] + exclusion of lipid-lowering drug users[f]	**0.14**	**0.12**	**0.04**	0.02	**0.10**	**0.08**
Survey in 1990 to 2009[g]						
Multivariable model[c]	**0.12**	**0.12**	0.02	0.01	**0.11**	**0.09**
Multivariable model[c] + adjustment for physical activity[h]	**0.12**	**0.11**	0.02	0.01	**0.10**	0.06

HDL high-density lipoprotein.
[a]Bold text denotes statistically significant findings (p value < 0.05). Estimates were derived from linear and quantile regression models.
[b]n = 5504–5406, 5511–5413, and 5512–5414 for non-HDL cholesterol, HDL cholesterol, and triglycerides, respectively, in the multivariable models.
[c]Adjusted for the same variables as the multivariable model in Table 2.
[d]Further adjusted for (or restricted by) lipid-lowering drug use (no or yes [n = 398]) under the assumption that that no one in the survey in 1986 used lipid-lowering drugs.
[e]n = 4771–4673, 4778–4680, and 4779–4681 for non-HDL cholesterol, HDL cholesterol, and triglycerides, respectively, in the multivariable models.
[f]Further adjusted for (or restricted by) lipid-lowering drug use (no or yes [n = 398]).
[g]n = 4059–3987, 4063–3991, and 4064–3991 for non-HDL cholesterol, HDL cholesterol, and triglycerides, respectively, in the multivariable models.

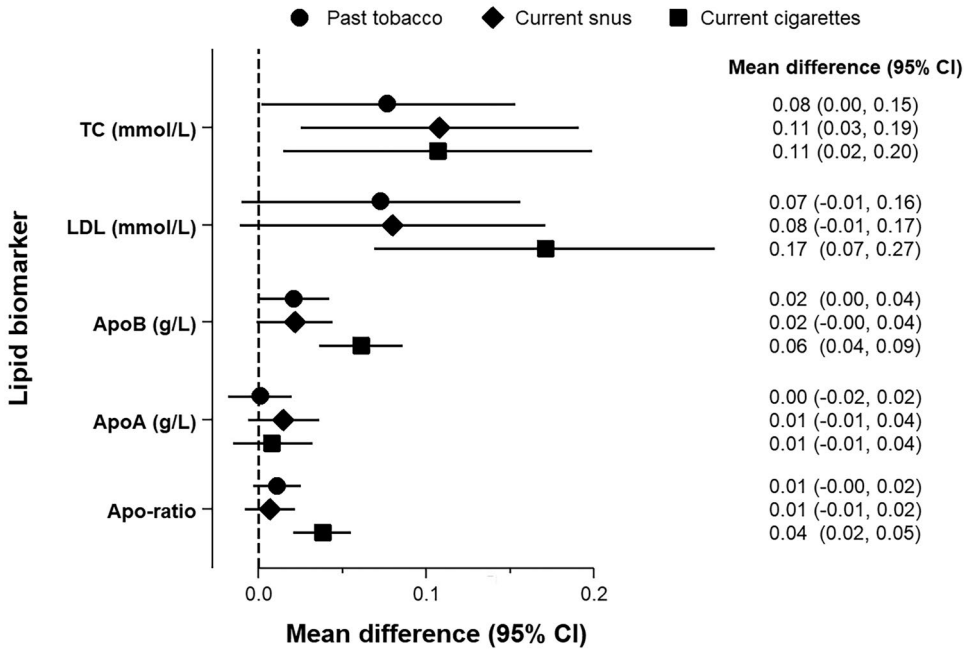

Figure 1. Mean differences in total cholesterol (TC), low-density lipoprotein cholesterol (LDL), apolipoprotein B (ApoB), apolipoprotein A1 (ApoA), and apolipoprotein B/A1-ratio (Apo-ratio) by tobacco use and based on multiple imputed data sets ($n = 30$). The *solid markers* represent the linear regression-estimated differences (*solid lines* 95% confidence intervals [CI]) for past tobacco use, current snus use, and current cigarette smoking compared to never tobacco use. The estimates were adjusted for the same variables as the multivariable model in Table 2. The lipid biomarkers were not adjusted for each other. The *dashed lines* represent the cut-off for statistical significance (p value < 0.05).

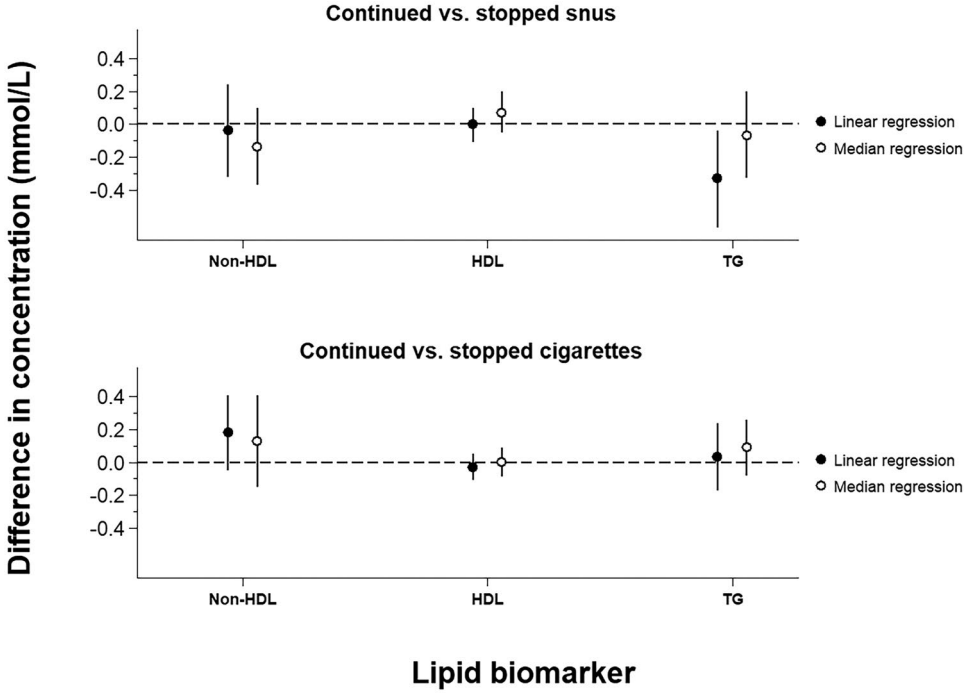

Figure 2. Differences in non-high-density lipoprotein (HDL) cholesterol, HDL cholesterol, and triglycerides (TG) in men who used snus and/or cigarettes in 1986 to 1994 by their continued use in 1999 (yes vs. no; based on multiple imputed data sets [$n = 30$]). The *solid dots* and the *hollow dots* represent the estimated differences (*solid lines* 95% confidence intervals) from a linear regression model and a quantile regression model, respectively. The estimates were adjusted for the same variables as the multivariable model in Table 2, with the addition of lipid-lowering drug use (no or yes) and physical activity (almost none, light-effort ≥1 h/week, or high-effort ≥1 h/week). The lipid biomarkers were not adjusted for each other. The *dashed lines* represent the cut-off for statistical significance (p value < 0.05).

−0.07 [95% CI −0.33, 0.20] mmol/L). In contrast, men who had continued to smoke cigarettes seemed to have a worse lipid profile than those who had discontinued cigarettes (Figure 2, lower panel), even though none of the associations were statistically significant (p values ≥ 0.12). A similar pattern was observed for the other lipid biomarkers (Supplementary Figure 6), with mostly lower concentrations of non-beneficial lipids (total and LDL

cholesterol, apolipoprotein B, and apolipoprotein B/A1-ratio) and higher concentrations of beneficial lipids (apolipoprotein A1) among continued snus users (and vice versa, for continued cigarette smokers).

Discussion

In this cross-sectional study, which was based on data from seven population-based surveys and included almost 6000 Swedish men, we observed that snus users had slightly increased concentrations of some lipid biomarkers (i.e. total cholesterol, HDL cholesterol, and triglycerides). However, there was no indication that higher intensity of snus use led to a worse lipid profile or that discontinuation of snus use led to a better lipid profile (the latter analysis based on participants with repeated examinations). In contrast, cigarette smokers had increased concentrations of several lipid biomarkers (i.e. total cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, and apolioprotein B as well as a higher apolipoprotein B/A1-ratio), with indications of a worse lipid profile by intensity of smoking and a better lipid profile by discontinuation of smoking (the latter analysis based on participants with repeated examinations).

Previous research from Sweden on the association between snus use and lipid status is fairly consistent with our findings. In one study ($n = 391$), Wallenfeldt et al. found increased concentrations of triglycerides—but not of other lipid biomarkers—among male snus users [23]. Similarly, in a larger study by Norberg et al. ($n = 16{,}492$; 46.6% men), an isolated increase in triglyceride concentrations was observed among snus users [24]. Null-findings with respect to changes in lipid biomarkers were found in studies by Eliasson et al. ($n = 1266$; 47.7% men) [21] and Byhamre et al. ($n = 880$; 51.9% men) [22]. Our findings for cigarette smoking are also consistent with the available literature, in which cigarette smoking has been shown to lead to an impaired lipid profile in several studies [17–20].

Snus use is a very common habit among Swedish men and has increased over time (from 17% in 1989 to 22% in 2020, according to national survey data) [7]. In general, and especially in comparison to cigarette smoking [8], the epidemiological evidence of an association between snus use and CVD is not overly strong [9]. Nonetheless, snus use has been positively associated with incidence and, particularly, mortality of CVD in a number of studies [10–14]. A theoretical mechanism behind the observed associations could be lipid-altering effects of the nicotine content in snus. Animal studies have shown that nicotine (given orally or intravenously) increases the concentrations of total cholesterol and LDL cholesterol and decreases the concentration of HDL cholesterol [15,16]; that is, lipid alterations that are major risk factors for CVD [1–6]. However, in our study, snus users had increased concentrations of both non-beneficial (i.e. total cholesterol and triglycerides) and beneficial (i.e. HDL cholesterol) lipids, not to mention that the magnitudes of the associations were rather small. It is, therefore, possible that the observed associations for snus use were due to unmeasured or residual confounding. One important variable for which we had no data was eating habits. A previous Swedish study reported that snus users were less likely to have regular breakfast habits and to be daily consumers of fruit and berries—two eating habits that are generally part of a lipid-lowering diet [34]—compared with never tobacco users [35]. The possibility of an association-by-confounding is further strengthened by our findings that (i) men who consumed at least 4 cans per week seemed to have a better lipid profile than those who consumed less than 4 cans per week and (ii) men who had continued to use snus seemed to have a better lipid profile than those who had discontinued to use snus.

Taken together, and in the light of previous research on the association between snus use and lipid status [21–24], it seems as if other mechanisms than lipid alterations are likely to constitute the potential link between snus use and CVD. With that said, a lipid-altering effect of snus use is still possible and should not be marginalized (even if small in magnitude), especially considering its widespread use in Sweden. It should also be noted that a large proportion of our main survey sample was recruited prior to or in the early stages of the lipid-lowering drug era [33], which hindered us to include lipid-lowering drug use in the multiple imputed analyses of that sample. Indeed, in the complete case analyses that were restricted to non-users of lipid-lowering drugs, there were indications of an underestimation of the association with non-HDL cholesterol concentrations by snus use in our main model (although the exact degree of which is hard to estimate, considering the large sample size differences and the possibility of bias introduced by the complete case methodology [36]).

Cigarette smoking was positively associated with all non-beneficial lipids in our study (i.e. total cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, apolioprotein B, and apolipoprotein B/A1-ratio). There were also indications of an intensity and discontinuation effect of cigarette smoking on lipid status. A lipid-altering effect of cigarette smoking is, therefore, most likely stronger than one of snus use. In a direct comparison with snus users, cigarette smokers had increased triglyceride and apolipoprotein B concentrations, higher apolipoprotein B/A1-ratio, and decreased HDL cholesterol concentrations. Hence, at least in humans, any lipid-altering mechanism by tobacco use could be more related to combustion products of nicotine rather than to nicotine per se.

Most epidemiological studies have suffered from decreased participation rates during the last decades [37]—an issue to which the Northern Sweden MONICA study is no exception (81% in 1986, 63% in 2014). In theory, if the decreased participation rates were strongly related to both tobacco use and lipid status, the exposure-outcome associations in our study could be skewed by selection bias. However, as detailed by Eriksson et al. who compared the characteristics of participants and non-participants in the surveys in 1986 to 2009 [26], the between-group difference in regular cigarette smoking was rather stable over time (9.3% in 1986, 3.8% in 1990, 5.8% in 1994, 6.0% in 1999, 6.6% in 2004, and 5.0% in 2009; with non-participants being more likely

to smoke in all six surveys). (Nota bene: No similar data are available for snus use.)

The major strength of our study was the large and population-based study sample (with respect to the main survey sample), located in a country and region where snus use is a very common habit among men. Additional strengths were the availability of repeated examinations in a subsample of the tobacco users (which allowed us to examine the discontinuation effects of snus and cigarettes), the availability of a large number of lipid biomarkers, the adjustment for several potential confounders, and the use of multiple regression methods (parametric and non-parametric) and sensitivity and subgroup analyses.

Apart from the previously mentioned possibility of selection bias and unmeasured or residual confounding, a number of additional limitations must be mentioned. First, and in contrast to the main survey sample, the follow-up survey sample was rather small and had a low proportion of discontinued snus users (around one-fifth of those who had used snus in a prior survey), which might have affected the possibility to detect statistically significant differences for some of the lipid biomarkers. It should, however, be noted that the discontinuation percentage was highly similar to that in other Swedish studies (ranging from 19.6 to 27.2%) [14,38]. Second, the use of tobacco products was self-reported and some degree of misclassification due to social desirability is expected. However, the answers to the tobacco questions in the Northern Sweden MONICA study were in good agreement with plasma concentrations of cotinine (the primary metabolite of nicotine) in a previous validation study [21]. In addition, the questions on snus use were less comprehensive than those on cigarette smoking, which hindered a more detailed dose-response analysis of snus use (in the main survey sample) and an assessment of the time between snus cessation and blood sampling (in the follow-up survey sample). Third, we only had one point of measurement for the lipid biomarkers (except for in the subsample with repeated examinations), leaving the possibility of diurnal, weekday, and intra-individual variations in lipid status [39,40]. However, such variations should not differ by the use of tobacco products and should, therefore, not have affected our results in a systematic way. Fourth, with respect to the measurement of total cholesterol, another limitation could be changes in the assay method (in 1999) or drift in measurements over time (each assay method was used for at least 9 years). However, as discussed in detail by Eriksson et al. [29] and Eliasson et al. [41], the total cholesterol measurements in the Northern Sweden MONICA study were checked by international and national accreditation bodies and the measured concentrations were corrected if needed. Finally, with respect to the measurement of the other lipid biomarkers, the frozen blood samples were stored for up to 30 years before the harmonized analyses were conducted at the BiomarCaRE laboratory. The literature on the long-term stability of lipid biomarkers is contradictory and different studies have shown increased, decreased, or stable concentrations over time (as summarized by Zivkovic et al. [42]). Any systematic bias due to a long storage time should, however, have had a marginal effect on the comparison between never tobacco use and current snus use, given that the prevalence of both habits increased over time (with a never tobacco-to-current snus ratio of 1.51:1 in 1986 and 1.14:1 in 2014). In contrast, the comparison between never tobacco use and current cigarette smoking would be more sensitive to such systematic bias (with a never tobacco-to-current cigarette ratio of 0.93:1 in 1986 and 2.89:1 in 2014).

In summary, we observed that snus users had slightly increased concentrations of some lipid biomarkers (e.g. total cholesterol, HDL cholesterol, and triglycerides), but without indications of a worse lipid profile by intensity of use or a better lipid profile by discontinuation of use, questioning the causality of these associations. Further studies are needed to confirm or refute our findings, preferably with adjustment for dietary factors and conducted in the lipid-lowering drug era.

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Authors' contributions

ME and SS: acquired the data; VO: performed the statistical analyses; MLB and VO: drafted the manuscript; and MLB and VO: primarily responsible for the final content. All authors participated in the study design, interpreted the results, reviewed and revised the manuscript, and approved the final version.

Disclosure statement

Abbott Laboratories provided test reagents for the laboratory analyses within the frame of the BiomarCaRE project. Abbott Laboratories had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript. SS has received speakers' honoraria from Jansen and has participated in advisory boards for Novartis (outside of the submitted work). The remaining authors have no conflicts of interest to declare.

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Data availability statement

The data that support the findings of this study are available from Umeå University, Sweden, but restrictions apply to the availability of

these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of the MONICA study steering committee at the Unit of Biobank Research, Umeå University, Umeå, Sweden.

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