Published in final edited form as:

Prostaglandins Leukot Essent Fatty Acids. 2016 September ; 112: 24–31. doi:10.1016/j.plefa.2016.08.004.

Smoking and Red Blood Cell Phospholipid Membrane Fatty Acids

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Abstract

Smoking is associated with lower n-3 long chain polyunsaturated fatty acids (LCPUFA) concentrations; however, limited studies have accounted for dietary PUFA intake or whether tobacco dose or smoking duration influences this association. We measured red blood cell phospholipid (RBC) membrane concentrations of fatty acids in 126 current smokers, 311 former smokers, and 461 never smokers using gas liquid chromatography and tandem mass spectrometry. Smokers had lower RBC membrane percentages of total n-3 LCPUFAs compared to former smokers or never smokers (median percent: 5.46, [interquartile range (IQR) 4.52, 6.28] versus 6.39; [IQR: 5.18, 7.85] versus 6.59; [IQR 5.34, 8.01]) ($p < 0.001$) and this association remained after adjusting for dietary PUFA intake. Duration of smoking and cigarettes per day were not associated with RBC membrane n-3 LCPUFA differences. Smoking is associated with lower n-3 LCPUFA RBC membrane percentages and this association was not influenced by diet or smoking dose or duration.

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Disclosure of potential conflict of interest

The authors report no conflict of interests to disclose.

Keywords

Tobacco use; fatty acid; unsaturated; oxidative stress

1. Introduction

Smoking is the leading cause of preventable death within the United States and an estimated 1 in 5 men and 1 in 7 women are currently smoking [1, 2]. Smoking directly effects the development of atherosclerosis and is a strong and independent risk factor for cardiovascular diseases [3, 4]. The underlying mechanisms relating smoking to atherosclerosis are still being elucidated and are likely mediated through several factors such as detrimental changes in serum lipids, enhanced platelet activation and thrombosis formation, direct endothelial damage, and oxidative stress [5–8]. Several studies have suggested that a relative deficiency of n-3 long-chain polyunsaturated fatty acids (LCPUFAs) may also be a risk factor for cardiovascular disease, although evidence from dietary supplement interventions have been mixed [9, 10]. The effects of smoking on circulating and tissue levels of n-3 LCPUFA levels are incompletely understood.

Prior studies have reported that smokers have lower circulating n-3 LCPUFAs compared to non-smokers [11–14]. It has been consistently reported that both plasma and red blood cell (RBC) membrane eicosapentaenoic acid (EPA: C20:n5-3) and docosahexaenoic acid (DHA: C22n6-3) are lower in active smokers compared to non-smokers. The mechanisms behind this relative n-3 LCPUFA deficiency are not known; however, possible reasons include 1) dietary differences between smokers and non-smokers; 2) preferential lipid peroxidation of n-3 LCPUFA versus n-6 LCPUFAs; 3) tobacco-related changes in endogenous n-3 LCPUFA metabolism; and 4) changes in fatty acid absorption. The dietary habits of smokers compared to non-smokers have been described and conflicting data exist regarding total PUFA intake with much fewer studies reporting specifically on n-3 LCPUFA intake [12, 15–18]. To our knowledge there is only one published study which measured RBC membrane PUFAs and adjusted for dietary intake of fatty acids [11]. Thus, it is still unclear to what effect diet might impact the circulating differences in PUFAs seen in smokers.

In this study we determine the association between circulating RBC membrane fatty acids and smoking status in 126 smokers, 311 former smokers, and 461 never smokers with self-reported dietary intakes. Additionally, we determined the association between smoking related behaviors, such as cigarettes per day or duration of smoking, and RBC n-3 LCPUFAs. Finally we report on the effect of smoking on measures of n-6 and n-3 LCPUFA lipid peroxidation, specifically urinary F₂- and F₃-isoprostanes.

2. Material And Methods

2.1 Study Population

Study methods for TCPS have been published elsewhere [19, 20]. Briefly, eligible participants, aged 40 to 75 years, were identified from patients scheduled for a colonoscopy at the Vanderbilt Gastroenterology Clinic between 1 February 2003 and 31 April 2010 and

the Veterans' Affairs Tennessee Valley Health System between 21 August 2003 and 30 May 2007. Patients with genetic CRC syndromes (such as hereditary non-polyposis CRC or familial adenomatous polyposis) or a previous history of inflammatory bowel disease, adenomatous polyps, or any cancer other than non-melanoma skin cancers were excluded from the study. Most of the participants were recruited at the time of the colonoscopy (n = 11,863). For potential participants who were missed at the time of the colonoscopy, recruitment occurred after the procedure (n = 722), which occurred in 6% of subjects and resulted when study staff members were not available to meet the participant at recruitment. These subjects did not contribute a blood or urine sample and were not eligible for the current study. Thus, of 12,585 eligible individuals, 7,621 subjects provided a written informed consent and participated in at least one component of the study (61%). Cases were classified into three case groups: single small adenomas, multiple small adenomas, and advanced adenomas. Controls were matched to case groups by age (within 5 years), gender, race (white/non-white). Additional matching criteria included at least one of the following criteria: sample collection date (within 90 days or season), study site (Vanderbilt University Medical Center/Veterans Affairs hospital), and regular use of NSAIDs (current; former or never). This sample was constructed as part of a case-control study of RBC membrane fatty acids and adenoma risk [21]. For the current study, we included only polyp-free controls as prior studies have reported differences in plasma and RBC fatty acid content in colorectal adenomas and cancer patients compared to controls [22–28]. We measured RBC fatty acid content in 977 polyp-free controls, of which 975 had information on smoking status. We excluded 37 samples in which we had an insufficient quantity of RBCs to measure membrane fatty acids. In addition, we excluded 40 samples whose total RBC membrane phospholipid content was less than 1000 µg/ml because in these samples some of the lesser abundant fatty acids are not detected, thereby artificially increasing the proportions of the more common RBC membrane fatty acids. Our analytic samples included 126 current smokers, 311 former smokers, and 461 never smokers. In this sample urine was available for 613 (68%) participants.

A standardized telephone interview was conducted by trained interviewers following colonoscopy to obtain information on medication use, demographics, medical history, smoking history, family history, reproductive history, anthropometry, and lifestyle. Interviewers were blind to the results of the colonoscopy. Participants also completed a self-administered mail survey using a semi-quantitative 108-item food frequency questionnaire (FFQ) which was developed to capture diet in the Southeastern United States and which used the NHANES III database [29]. Although this FFQ has previously been found to have moderate correlations to biomarkers of carotenoids and α -tocopherol, it has not been validated for fatty acid intake [30]. The FFQ also contains 5 items that survey eating habits and 13 items for capturing vitamin and supplement use. Usual dietary intake of PUFAs was estimated using US Department of Agriculture food composition tables and presented in grams per day.

TCPS participants were asked "Have you ever smoked at least 100 cigarettes in your lifetime?" Individuals marking "no" skipped the following 5 questions regarding smoking characteristics. Participants marking "yes" were then asked to report their age when they first started smoking on a regular basis which was defined as 1 cigarette a day for 6 months

in a row. Those who reported that they had never smoked regularly were asked to skip the questions regarding smoking characteristics. Participants were then asked “Do you still smoke cigarettes?” Those indicating “yes” then answered the following question “On average, about how many cigarettes do you smoke per day?” Subjects who responded “no” to “Do you still smoke cigarettes” were directed to an additional set of questions requesting information on the age of smoking cessation, and cigarettes per day smoked prior to quitting. All participants who reported smoking were asked to report the greatest number of cigarettes per day regularly smoked.

The study was approved by the Vanderbilt University Institutional Review Board, the Veterans’ Affairs Institutional Review Board, and the Veterans’ Affairs Research and Development Committee.

2.2 Laboratory assays

Participants recruited prior to colonoscopy were asked to donate a 20 mL fasting blood sample. The blood was drawn into an EDTA, ACD tube, and serum tubes. Whole blood in the EDTA tube was separated into plasma, buffy coats (white cells), and red blood cells, while viable lymphocytes were retained in the ACD tube. Participants were also asked to donate a cup of spot urine sample. Samples were processed within 6 hours of collection and stored for future analyses in -80°C freezer. RBC membrane phospholipid fatty acid concentrations were determined by gas chromatography. Briefly, the method was as follows. Total lipids were extracted from 200 μL of double-washed packed red blood cells using the method described by Folch et al [31]. Phospholipids were isolated using thin layer chromatography on Silica Gel 60 A plates and fatty acids methylated using BF_3 /methanol [32]. The methylated fatty acids were analyzed by gas chromatography using an HP 7890A gas chromatograph equipped with flame ionization detectors and a capillary column (SP2380, 0.25 mm $\times$ 30 m, 0.20 μm film, Supelco, Bellefonte, PA). Helium was used as a carrier gas. Fatty acid methyl esters were identified by comparing the retention times to those of known standards. Inclusion of the internal standard, dipentadecanoyl phosphatidylcholine (C15:0), permitted quantitation of the amount of phospholipid in the sample. Fatty acid values are presented as percentage of total RBC membrane phospholipid fatty acid content. The lowest level of detection for individuals’ fatty acids is less than 0.4–0.5% of the total profile. The inter-assay coefficients of variation less than 10% for all measured fatty acids with the exception of adrenic acid (24.6%), n-6 DPA (42%), N-3 DPA (12%), and DHA (12%). The intra-assay coefficient of variation was less than 10% for all measured fatty acids with the exception of adrenic acid (12%) and n-6 DPA (26%).

N-6 PUFAs included: linoleic acid (LA: 18:2n-6), di-homo- γ -linoleic acid (DGLA: 20:3n-6), arachidonic acid (ARA: 20:4n-6), adrenic acid (ADA: 22:4n-6), and docosapentaenoic acid (n-6 DPA: 22:5n-6). Total n-6 PUFA was constructed by summing the values of all five n-6 PUFAs. N-3 PUFAs were eicosapentaenoic acid (EPA: 20:5n-3), docosapentaenoic acid (DPA: 22:5n-3) and docosahexaenoic acid (DHA: 22:6n-3). We were unable to detect any α -linolenic acid (18:3n-3) within the RBC membrane which is similar to prior studies and likely reflects the fact that α -linolenic acid predominately undergoes β -

oxidation after consumption [33, 34]. Total n-3 PUFA was estimated by summing the values of all three detected n-3 PUFAs.

Urinary F2- and F3- IsoP were measured using GC/NICI-MS using a validated method previously described [35–38]. Briefly, GC/NICI-MS was carried out on an Agilent 5973 Inert Mass Selective Detector that was coupled with an Agilent 6890n Network GC computer system (Santa Clara, CA). The column temperature was programmed from 190° to 300°C at 20°C per minute. The metabolite was chemically synthesized and converted to an ¹⁸O₂-labeled derivative as an internal standard. Data are expressed after correction for urinary creatinine concentrations and are reported as nanograms per milligram creatinine. Employing this assay, the lower limit of detection of F₂-IsoPs was in the range of 4 pg. Normal human urine levels are 1.6 ± 0.6 ng/mg Cr (mean + 1 S.D.) in our laboratory. The assay inter- and intraday variability were both less than 10%. The assay has a precision of +/– 6% and an accuracy of 96%.

2.3 Statistical Analysis

We stratified participants based on their self-reported smoking status (current, former, never). Current smoker were defined as individuals reporting regular smoking within the 12 months prior to their colonoscopy. Former smokers were defined as individuals who reported regular smoking which had stopped for at least 12 months prior to their colonoscopy. Individuals who had never smoked regularly were defined as never smokers. We compared the distribution of demographic characteristics such as age, sex, race, body mass index (BMI), study site location, alcohol status, educational status, self-reported exercise activity, menopausal status, and self-reported dietary fatty acid intake between participants based on their smoking status. Continuous variables are presented as median along with the interquartile range. Wilcoxon Rank Sum tests were used for the continuous variables and chi-square tests were used for categorical variables. We compared self-reported dietary fatty acid intake by smoking status using regression modeling adjusting for age (continuous), sex (female, male), race (white, black, other), body mass index (continuous), alcohol use (current, former, never), educational status (some high school, graduated high school, some college or college graduate, graduate or professional school), regular exercise over past 10 years (yes, no), study site (Vanderbilt University Medical Center, Veterans Affairs Medical Center), postmenopausal (yes, no), and total energy intake (continuous).

We conducted multivariate linear regression models with the dependent variable being RBC membrane fatty acid percentage and the independent variable being smoking status. RBC membrane fatty acids are presented as percentages of total RBC phospholipid membrane fatty acids. RBC membrane eicosapentaenoic acid was significantly skewed so the value was log transformed for modeling. Models were adjusted for the same covariates described above along with self-reported total dietary intake of palmitic acid, stearic acid, oleic acid, linoleic acid, α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid (all continuous).

We conducted subgroup analyses in current smokers based on the following self-reported smoking characteristics: number of cigarettes smoked per day (< 20 cigarettes per day; 20

to < 30 cigarettes per day; 30 cigarettes per day), duration of smoking (< 30 years; 30 years to < 40 years; 40 years), and pack-years smoking (< 10 pack-years; 10 to < 30 pack-years smoking; 30 pack-years smoking). Models were adjusted for the same covariates described above. We also constructed additional models including the smoking characteristics as a continuous variable.

Values for urinary isoprostanes were right skewed and were log transformed. We present geometric means and constructed linear regression models to calculate p-values using transformed values. We calculated Spearman Partial Correlation Coefficient to determine the correlation between isoprostane levels and self-reported cigarettes per day adjusted for age and sex. Analyses were performed using SAS version 9.4.

3. Results

3.1. Smoking and baseline characteristics

Compared to former smokers, current smokers were younger, had lower educational levels, reported less regular exercise and were more likely to be post-menopausal. (Table 1) Compared to never smokers, current smokers were less likely to be women, more likely to use alcohol, had lower educational levels, and reported less regular exercise. Former smokers, when compared to never smokers, were older, less likely to be women, had a higher body mass index, were more likely to use alcohol, and had lower educational levels.

3.2 Smoking and self-reported diet

Current smokers reported similar dietary intakes of total calories, total fat, total saturated fat, palmitic acid, total monounsaturated fats, oleic acid, arachidonic acid, α -linolenic acid, EPA and DHA compared to former smokers (Table 2). Current smokers reported higher intakes of stearic acid and lower intakes total PUFA and linoleic acid compared to former smokers. Current smokers reported higher total intake of total saturated fats, palmitic acid, and stearic acid compared to never smoker, but no statistically significant differences were found with any of the fatty acids. Former smokers, when compared to never smokers, reported higher total fat intake, total PUFA intake, and linoleic acid intake.

3.3 Smoking and RBC membrane fatty acids

In unadjusted analyses, current smokers had higher RBC membrane percentages of palmitic acid and stearic acid compared to former and never smokers (Table 3). Current smokers compared to former and never smokers had lower RBC membrane percentages EPA, n-3 DPA, DHA, and total n-3 LCPUFAs. No differences were found between RBC membrane fatty acid content and former or never smokers. No differences were seen in any group with respect to RBC membrane percentages of oleic acid, linoleic acid, di-homo- γ -linolenic acid, arachidonic acid, adrenic acid, and n-6 DPA. In multivariable-adjusted analyses, current smokers, compared to former/never smokers, had higher RBC membrane fatty acid percentages of palmitic acid and stearic acid and lower RBC membrane fatty acid percentages of arachidonic acid, adrenic acid and for all n-3 LCPUFAs after adjusting for demographic and dietary confounders (Table 4). In current smokers, age at initiation of

smoking, cigarettes smoker per day, duration of smoking, and pack-years smoked was not associated with any changes in RBC membrane fatty acid percentages (data not shown).

3.4 Smoking and isoprostanes

No differences were found between urinary IsoP levels in former and never smokers; therefore, these analyses for these two groups were combined. We found that urinary F₂ and F₃-IsoP levels were higher in smokers compared to former/never smokers (geometric means for F₂-IsoPs = 23.9, 95% CI 3.88, 147.05 [n = 86] versus 4.78, 95% CI 4.39, 5.22 [n = 527], p-value <0.0001, and for F₃-IsoPs = 1.58, 95% CI 1.10, 2.27 [n = 73] versus 1.19, 95% CI 1.18, 1.20 [n = 504], p-value <0.0001, current smokers versus former/never, respectively). Urinary F₂-IsoP was not correlated to reported cigarettes per day in current smokers (r = 0.17, 95% CI -0.05, 0.37, p-value = 0.12). Urinary F₃-IsoP were also not correlated to reported cigarettes per day in current smokers (r = 0.20, 95% CI -0.03, 0.42, p-value = 0.09).

4. Discussion And Conclusions

We found that current smokers reported similar dietary intakes of EPA and DHA compared to former smokers or never smokers. In concordance with earlier studies, we found that current smokers, when compared to former smokers and never smokers, had lower RBC membrane n-3 LCPUFA percentages. These differences in RBC membrane n-3 LCPUFA remained after adjusting for self-reported dietary intake. In current smokers, we found no association with age of initiation of smoking, duration of smoking, or cigarettes per day and RBC membrane fatty acids. Two markers of n-6 and n-3 PUFA oxidation were increased in smokers but were not correlated with cigarettes per day in current smokers.

Limited prior studies have specifically reported on n-3 LCPUFA intake and smoking status. Subar et al. using data from the National Health and Nutrition Examination Survey found smokers reported lower intakes of fish; however, the differences were not statistically significant [18]. The INTERMAP study included 4680 participants and found lower reported n-3 LCPUFA intake for smokers compared to never smokers in US men only [15]. No differences were found in smokers from China, Japan, or the United Kingdom. In US women, a difference was found between smokers and former smokers with respect to dietary n-3 LCPUFA. Most prior studies have only compared differences in dietary total PUFA intake and have reported smokers consuming lower amounts of total PUFA. Our findings vary from earlier studies in that we found no difference in dietary total PUFAs intake between smokers and never smokers [15–17]. Reasons for this discrepancy could be related to differing populations' samples and different methodologies to assess dietary intake. Given our smaller sample size of smokers we could have been unable to identify subtle differences in self-reported n-3 LCPUFA intake.

We found that RBC membrane n-3 LCPUFA percentages were lower in smokers compared to never smokers and former smokers even after adjusting for dietary intake of n-3 PUFAs. Our results are similar to prior studies that have reported lower serum or RBC n-3 LCPUFAs in smokers [11–14, 39–43]. Similar to our findings, stronger association have been seen with DHA as opposed to EPA. We found no difference between RBC membrane n-3 LCPUFA

levels and former and never smokers suggesting that these differences may correct with smoking cessation. This correction could be related to changes in absorption or metabolisms or increased dietary intake of n-3 LCPUFA. Thompson et al has reported that long-term quitters report increases in fish intake; [44] however, how much dietary differences in n-3 LCPUFA contributes towards circulating differences in smokers is still an unanswered question [11, 12]. Unexpectedly, we found no association between smoking dose or duration and RBC membrane n-3 LCPUFA status. Smoking may have an effect acutely on fatty acid absorption and metabolism and most of the smokers in our study had been smoking multiple years [45]. As such, homeostatic mechanisms may already be in place to maintain tissue levels of n-3 LCPUFAs in the setting of enhanced oxidation. Longitudinal studies of smoking might be better designed to address the effects of smoking initiation on n-3 LCPUFA tissue levels.

We found higher levels of F2 IsoPs in smokers compared to former and never smokers supporting prior studies, which have found increased lipid peroxidation in smokers [46–48]. We also found levels of n-3 PUFA peroxidation products (F3-IsoPs) to also be higher in smokers compared to former and never smokers. Our results differ from Puri et al who found no evidence of increased n-3 lipid peroxidation in smokers [49]. This study however had a much smaller sample size (11 smokers and 18 non-smokers) and utilized expired ethane as a marker of lipid oxidation. As with our RBC membrane findings we did not find a correlation between cigarette dose and urinary isoprostanes; however this could be a consequence of the study design and sample size. Similarly to tissue levels of n-3 LCPUFAs, smoking's effects on isoprostane levels appears acutely [46].

Most, but not all studies have found smokers with lower plasma and RBC membrane n-3 LCPUFAs. In general, it has been suggested that lipid peroxidation likely contributed towards these differences however changes in fatty acid metabolisms might also impact tissue levels [11–14, 39–43, 50]. Differences in dietary intake of PUFAs have been identified as associated with smoking status, however in our study and the studies of others; adjustment for dietary intake does not seem to alter these effects [11]. Human studies have found that smoking does not appear to impact the absorption of the 18C n-3 PUFA linolenic acid and in fact may increase bioavailability; however, whether smoking might impact EPA or DHA absorption is unknown [45]. Pawlosky et al found in a human feeding study, that smoking did not enhance the conversion of linolenic acid to eicosapentaenoic acid [45]. In addition, in smokers a higher percentage of EPA was utilized for DPA which could suggest enhanced elongase activity in smokers.

Understanding how smoking impacts n-3 LCPUFA status is important as emerging data is beginning to suggest that n-3 LCUFA relative deficiencies could have a role in behavior. In animal models, n-3 PUFA deficiencies result in structural changes in nervous tissue which impacts dopaminergic and serotonergic systems and correction of these deficiencies can reverse these changes [51–54]. In particular, n-3 PUFA deficiency can result in hypofunctioning of the dopamine mesocorticolimbic pathways which are related to reward and dependence [55, 56]. Nicotine results in an elevation of dopamine in the nucleus accumbens, which is associated with the pleasurable sensations related to nicotine use [57, 58]. As such, it has been hypothesized that correcting the hypofunctioning dopaminergic

system through n-3 LCPUFA supplementation might reduce the symptoms of withdrawal associated with smoking cessation and reduce nicotine cravings [59]

Recently, two small scale, double-blind, randomized controlled trials have found that supplementation with n-3 LCPUFA reduces signs of nicotine dependence [60, 61]. One could speculate that through tobacco-related reduction in n-3 LCPUFAs, neurohumoral changes only serve to contribute toward making it even more difficult to stop smoking. These two small scale studies suggest supplementation could be useful and requires further study.

This study has several strengths. First, the large sample size of participant who have been well-characterized with regards to smoking status and have RBC membrane fatty acids measured along with urinary isoprostanes. Few studies have been able to account for dietary exposures in relation to smoking and circulating PUFAs. Weaknesses include self-reported smoking and dietary measures. In addition, coefficients of variation were large for the very-long chain n-6 PUFAs. Although we did not use any biochemical measures to validate smoking, our isoprostane results mirror clinical studies [46]. In addition the study was cross-sectional and no assessment of causality can be made.

In conclusion, RBC membrane n-3 LCPUFA levels appear to be lower in smokers compared to never and former smokers and these differences are not explained by dietary intake differences. The differences are not related to smoking dose or duration in current smokers; however, cigarettes per day are directly correlated to levels of lipid peroxidation. Future work investigating the effect of correction of n-3 LCPUFA deficiencies in smokers on smoking cessation is warranted.

Acknowledgments

Grant Support

This study was supported through the National Institute of Health grants P50CA95103, R01CA97386, R01CA143288, R01CA160938, and R01HL106845. Surveys and sample collection, processing, and preparation for this study were conducted by the Survey and Biospecimen Shared Resource, which is supported in part by P30CA068485. Fatty acid measurements were made in the Diabetes Research Training Center Lipid Sub core, supported in part by DK 020593. The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. A portion of the participants were studied as the result of resources and the use of facilities at the VA Tennessee Valley Healthcare System.

The authors would like to acknowledge Carla Harris, Judy Brown, and Ilhan Mahamed Eli who performed the fatty acid bioassays with in DRTC Lipid Core.

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Highlights

- Smokers have lower red blood cell phospholipid membrane n-3 long-chain polyunsaturated fatty acid (PUFA) concentrations compared to former or never smokers
- Differences in dietary intake of n-3 long-chain PUFAs are unlikely to explain these differences
- Number of cigarettes smoked per day or duration of smoking does not seem to impact red blood cell n-3 long-chain PUFA concentrations

Table 1

Participant Characteristics Stratified by Smoking Status¹

	Smoking Status			
	Current (n = 126)	Former (n = 311)	Never (n = 461)	
Age in years, median (IQR)	55.6 (51.4, 58.2)	57.5 (53.8, 62.7)	55.3 (50.2, 60.3)	P -value ² <0.0001
Sex, female, n (%)	22 (17.5)	45 (14.5)	168 (36.4)	P -value ² <0.0001
Race, n (%)				
White	114 (90.5)	285 (91.6)	423 (91.8)	
African-American	12 (9.5)	20 (6.4)	27 (5.9)	0.87
Other	0 (0)	6 (1.9)	11 (2.4)	
Body mass index in kg/m ² , median (IQR)	27.5 (25.0, 30.4)	28.6 (25.0, 30.4)	27.2 (24.0, 30.5)	0.0002
Study site, n (%)				
Vanderbilt University	47 (37.3)	160 (51.5)	372 (80.7)	
Veterans Affairs Hospital	79 (62.7)	151 (48.6)	89 (19.3)	0.007
Alcohol use, n (%)				
Current	30 (23.1)	74 (23.9)	70 (15.2)	
Former	45 (35.7)	114 (36.8)	55 (12.0)	0.97
Never	51 (40.5)	122 (39.4)	335 (72.8)	<0.0001
Educational status, n (%)				
Less than high school	58 (47.2)	96 (30.9)	62 (13.5)	
Graduated high school	47 (38.2)	101 (32.5)	115 (25.1)	
Some college	9 (7.3)	52 (16.7)	120 (26.1)	<0.0001
Graduate or professional school	9 (7.3)	62 (19.9)	162 (35.3)	<0.0001
Regular exercise, n (%)	48 (38.1)	178 (57.2)	274 (59.7)	0.0003
Postmenopausal status, n (%) ⁵	17 (77.3)	32 (71.1)	112 (66.7)	0.01
				0.31
				0.57

¹ P -value calculated using Wilcoxon Rank Order Sum Test for continuous variables and Chi-square for ordinal variables² Comparing current smokers to former smokers

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- ³ Comparing current smokers to never smokers
- ⁴ Comparing former smokers to never smokers
- ⁵ n = 235

Table 2

Participant Self-Reported Dietary Intake Stratified by Smoking Status[/]

	Smoking Status				P-value ²	P-value ³	P-value ⁴
	Current (n = 126)	Former (n = 311)	Never (n = 461)				
Total calories in kcal/day, median (IQR) ⁵	2200 (1589, 3042)	2106 (1593, 3042)	2026 (1450, 2610)	0.78	0.30	0.27	
Total fat intake in g/d, median (IQR)	83.0 (54.1, 121.0)	80.8 (58.1, 121.1)	72.5 (49.9, 99.1)	0.84	0.06	0.04	
Total saturated fat intake in g/d, median (IQR)	26.2 (17.3, 41.8)	24.7 (17.5, 37.7)	22.7 (15.6, 31.8)	0.06	0.002	0.14	
Palmitic acid (16:0) in g/d, median (IQR)	14.4 (9.31, 22.0)	13.4 (9.43, 20.2)	12.3 (8.43, 17.0)	0.10	0.005	0.07	
Stearic acid (18:0) in g/d, median (IQR)	6.97 (4.50, 10.8)	6.25 (4.44, 9.64)	5.66 (3.85, 8.00)	0.05	0.001	0.11	
Total monounsaturated fat intake in g/d, median (IQR)	30.9 (19.8, 45.0)	30.7 (22.0, 46.4)	27.2 (18.6, 37.5)	0.88	0.10	0.07	
Oleic acid (18:1n-9) in g/d, median (IQR)	29.0 (18.4, 42.0)	28.5 (20.5, 43.5)	25.4 (17.1, 34.9)	0.98	0.16	0.10	
Total PUFA intake in g/d, median (IQR)	16.7 (11.0, 24.6)	17.7 (12.8, 26.1)	16.1 (11.0, 22.6)	0.02	0.42	0.03	
n-6 PUFAs							
Linoleic acid (18:2n-6) in g/d, median (IQR)	14.6 (9.4, 21.3)	15.7 (11.1, 22.7)	14.2 (9.67, 19.8)	0.02	0.24	0.05	
Arachidonic acid (20:4n-6) in g/d, median (IQR)	0.10 (0.05, 0.14)	0.09 (0.07, 0.14)	0.08 (0.05, 0.12)	0.68	0.76	0.58	
n-3 PUFAs							
α-linolenic acid (18:3n-3) in g/d, median (IQR)	1.37 (0.88, 2.22)	1.30 (0.94, 1.99)	1.17 (0.79, 1.65)	0.65	0.08	0.11	
Eicosapentaenoic acid (20:5n-3) in g/d, median (IQR)	0.017 (0.006, 0.048)	0.025 (0.009, 0.053)	0.025 (0.010, 0.050)	0.30	0.41	0.95	
Docosahexaenoic acid (22:6n-3) in g/d, median (IQR)	0.042 (0.014, 0.104)	0.052 (0.023, 0.102)	0.051 (0.023, 0.094)	0.35	0.36	0.77	

[/] P-value calculated using logistic regression adjusting for age, sex, race, educational level, and total energy intake

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- ²Comparing current smokers to former smokers
- ³Comparing current smokers to never smokers
- ⁴Comparing former smokers to never smokers
- ⁵IQR = interquartile range

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Erythrocyte Phospholipid Red Blood Cell Fatty Acid Concentrations Stratified by Smoking Status[†]

Table 3

	Smoking Status				P-value ²	P-value ³	P-value ⁴
	Current (n = 126)	Former (n = 311)	Never (n = 461)				
Saturated fatty acids							
Palmitic acid (16:0)	26.7 (24.8, 28.9) ⁵	25.7 (24.6, 27.3)	25.5 (24.5, 27.5)	0.004	0.002	0.68	
Stearic acid (18:0)	17.5 (16.5, 18.8)	17.0 (16.2, 18.4)	17.0 (16.1, 18.2)	0.009	0.002	0.53	
Monounsaturated fatty acids							
Oleic acid (18:1)	14.4 (13.3, 15.8)	14.5 (13.5, 15.9)	14.6 (13.4, 16.0)	0.92	0.97	0.97	
Polysaturated fatty acids							
n-6							
Linoleic acid (18:2n-6)	11.1 (9.86, 12.1)	11.2 (10.2, 12.5)	11.3 (10.3, 12.2)	0.28	0.12	0.50	
Di-homo- γ -linolenic acid (20:3n-6)	1.60 (1.31, 1.90)	1.56 (1.34, 1.82)	1.59 (1.35, 1.86)	0.55	0.84	0.49	
Arachidonic acid (20:4n-6)	17.2 (14.4, 18.7)	16.9 (14.8, 18.5)	16.9 (14.8, 18.6)	0.96	0.95	0.97	
Adrenic acid (22:4n-6)	3.75 (3.03, 4.32)	3.64 (2.81, 4.31)	3.64 (2.73, 4.28)	0.97	0.63	0.56	
Docosapentaenoic acid (22:5n-6)	0.70 (0, 0.92)	0.67 (0.38, 0.84)	0.64 (0.29, 0.82)	0.31	0.08	0.27	
Total n-6 PUFA	35.1 (31.4, 36.9)	34.5 (31.7, 36.5)	34.6 (31.2, 36.7)	0.64	0.53	0.79	
n-3							
Eicosapentaenoic acid (20:5n-3)	0 (0, 0.36)	0.35 (0, 0.60)	0.41 (0, 0.63)	<0.0001	<0.0001	0.21	
Docosapentaenoic acid (22:5n-3)	1.96 (1.49, 2.37)	2.20 (1.80, 2.59)	2.27 (1.86, 2.53)	<0.0001	<0.0001	0.96	
Docosahexaenoic acid (22:6n-3)	3.18 (2.59, 4.03)	3.90 (2.94, 4.96)	4.04 (3.05, 5.23)	<0.0001	<0.0001	0.22	
Total n-3 PUFA	5.46 (4.52, 6.28)	6.39 (5.18, 7.85)	6.59 (5.34, 8.01)	<0.0001	<0.0001	0.24	

Smoking Status					P- value ³	P- value ⁴
Current (n = 126)	Former (n = 311)	Never (n = 461)				
Total n-6 to n-3 ratio					<0.0001	0.21
6.60 (5.62, 7.69)	5.60 (4.42, 6.68)	5.39 (4.26, 6.55)				

¹ P-value calculated using Wilcoxon Rank Order Sum Test

² Comparing current smokers to former smokers

³ Comparing current smokers to never smokers

⁴ Comparing former smokers to never smokers

⁵ Values expressed as percentage of individual fatty acid over total fatty acid; median and interquartile range

Table 4
Multivariable-adjusted RBC membrane fatty acid levels in current vs. former/never smokers

	Age adjusted				Model 1 ¹				Model 2 ²			
	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value
Saturated fatty acids												
Palmitic acid (16:0)	1.64	0.71, 2.58	0.0006	1.77	0.78, 2.76	0.0005	1.73	0.73, 2.74	0.0007	1.73	0.73, 2.74	0.0007
Stearic acid (18:0)	0.47	0.08, 0.85	0.02	0.46	0.06, 0.87	0.02	0.42	0.004, 0.83	0.05	0.42	0.004, 0.83	0.05
Monounsaturated fatty acids												
Oleic acid (18:1)	0.30	-0.14, 0.73	0.19	0.24	-0.22, 0.70	0.30	0.26	-0.20, 0.73	0.29	0.26	-0.20, 0.73	0.29
Polyunsaturated fatty acids												
n-6												
Linoleic acid (18:2n-6)	-0.31	-0.66, 0.04	0.08	-0.18	-0.54, 0.18	0.33	-0.14	-0.51, 0.23	0.45	-0.14	-0.51, 0.23	0.45
Di-homo- γ -linolenic acid (20:3n-6)	-0.01	-0.11, 0.09	0.84	-0.02	-0.13, 0.09	0.69	-0.008	-0.12, 0.10	0.89	-0.008	-0.12, 0.10	0.89
Arachidonic acid (20:4n-6)	-0.57	-1.38, 0.24	0.17	-0.87	-1.73, -0.02	0.05	-0.91	-1.78, -0.05	0.04	-0.91	-1.78, -0.05	0.04
Adrenic acid (22:4n-6)	-0.14	-0.40, 0.12	0.29	-0.35	-0.63, -0.08	0.01	-0.38	-0.66, -0.11	0.006	-0.38	-0.66, -0.11	0.006
Docosapentaenoic acid (22:5n-6)	0.05	-0.03, 0.14	0.23	-0.008	-0.10, 0.08	0.85	-0.02	-0.11, 0.06	0.67	-0.02	-0.11, 0.06	0.67
Total n-6 PUFA	-0.64	-1.83, 0.55	0.29	-1.23	-2.48, 0.02	0.05	-1.26	-2.52, -0.003	0.05	-1.26	-2.52, -0.003	0.05
n-3												
Eicosapentaenoic acid (20:5n-3)	-0.38	-0.54, -0.22	<0.0001	-0.27	-0.43, -0.11	0.001	-0.26	-0.42, -0.10	0.002	-0.26	-0.42, -0.10	0.002
Docosapentaenoic acid (22:5n-3)	-0.34	-0.51, -0.17	0.0001	-0.35	-0.52, -0.17	0.0002	-0.32	-0.51, -0.14	0.0006	-0.32	-0.51, -0.14	0.0006
Docosahexaenoic acid (22:6n-3)	-0.94	-1.29, -0.58	<0.0001	-0.72	-1.08, -0.35	0.0001	-0.66	-1.03, -0.24	0.0004	-0.66	-1.03, -0.24	0.0004
Total n-3 PUFA	-1.52	-2.06, -0.97	<0.0001	-1.26	-1.82, -0.70	<0.0001	-1.15	-1.72, -0.59	<0.0001	-1.15	-1.72, -0.59	<0.0001

	Age adjusted			Model 1 ¹			Model 2 ²		
	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value
Total n-6 to n-3 ratio	1.28	0.51, 2.05	0.001	1.07	0.27, 1.88	0.009	0.96	0.14, 1.78	0.02

¹Model 1 adjusted for age, sex, race, body mass index, study site, alcohol use, exercise status, aspirin use, educational status, and post-menopausal status

²Model 2 adjusted for age, sex, race, body mass index, study site, alcohol use, exercise status, aspirin use, educational status, post-menopausal status, and total energy intake, dietary intake of palmitic acid, stearic acid, oleic acid, linoleic acid, arachidonic acid, α -linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid.