

Altered Saturated and Monounsaturated Plasma Phospholipid Fatty Acid Profiles in Adult Males with Colon Adenomas

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Abstract

Background: Altered lipid metabolism and plasma fatty acid (FA) levels are associated with colorectal cancer. Obesity and elevated waist circumference (WC) increase the likelihood of developing precancerous colon adenomas.

Methods: Venous blood was collected from 126 males, ages 48 to 65 years, who received routine colonoscopies. Plasma phospholipid (PPL) FAs were isolated, derivatized, and then analyzed using gas chromatography. ORs and 95% confidence intervals were determined using polytomous logistic regression after adjusting for confounding factors [i.e., age, smoking, WC, and body mass index (BMI)].

Results: PPL palmitic acid (PA) was inversely correlated with the presence of colon adenomas ($P = 0.01$). For each unit increase in palmitoleic acid (OR, 3.75; $P = 0.04$) or elaidic acid (OR, 2.92; $P = 0.04$), an individual was more likely to have adenomas

relative to no colon polyps. Higher enzyme activity estimates (EAE) of stearoyl-CoA desaturase-1 (SCD-1; $P = 0.02$) and elongation of very long chain fatty acids protein-6 (ELOVL-6; $P = 0.03$) were associated with an individual being approximately 1.5 times more likely to have an adenoma compared with no polyps.

Conclusions: PPL FAs and EAEs, which have previously been associated with colorectal cancer, are significantly different in those with adenomas when compared with those without polyps. PPL PA, elaidic acid, and SCD-1 and ELOVL-6 EAEs are associated with adenomas independent of BMI and WC.

Impact: PPL PA, elaidic acid, and SCD-1 and ELOVL-6 EAEs are associated with adenomas even after adjusting for obesity-related risk factors and may function as novel biomarkers of early colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev*; 25(3): 1–9. ©2015 AACR.

Introduction

Colorectal cancer is the third most prevalent cancer among men and women in the United States (1). Risk factors for colorectal cancer include obesity, waist circumference (WC), age, smoking, physical inactivity, inflammatory bowel disease, and a family history of colorectal cancer or adenomas (2). As much as 70% of the risk of developing colorectal cancer has been attributed to modifiable risk factors, including diet (3). Consequently, dietary intake of varying amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) has been an area of active research in the pathology and prevention of colorectal cancer.

Several specific FAs are associated with colorectal cancer. For example, a higher erythrocyte oleic acid (OA) to stearic acid (SA) ratio has been associated with colorectal cancer (4). Also, colorectal cancer is associated with higher levels of plasma phospholipid (PPL) SFAs, in particular palmitic acid (PA; ref. 5). Among PUFAs, dietary consumption of greater amounts of omega-3 PUFAs and lesser amounts of omega-6 PUFAs are typically associated with a decreased risk for developing colorectal cancer (6).

Diets higher in MUFAs and lower in SFAs also potentially prevent colorectal cancer (7). Blood FAs associated with colorectal cancer may originate from dietary intake as well as from endogenous synthesis through lipid metabolism.

Altered lipid metabolism is also suspected to play a role in colon carcinogenesis during the transformation of colorectal polyps to colorectal cancer (7–9). Dietary SFAs can be desaturated and elongated through the action of various enzymes. Stearoyl-CoA desaturase-1 (SCD-1) and elongation of very long chain fatty acids protein-6 (ELOVL-6) are the rate-limiting enzymes controlling metabolic shifts toward production of long-chain MUFAs. Upregulation of SCD-1, the desaturase responsible for converting PA and SA into MUFAs, has been linked to colorectal cancer (9). MUFAs influence cellular apoptosis and are believed to play a role in the mutagenesis of tumors in several types of cancer, including colorectal cancer (8, 10). However, FAs and the enzymes that regulate the endogenous production of long-chain MUFAs have not been sufficiently investigated in relation to precancerous colon adenomas. In addition, the complex mechanisms by which dietary FAs and lipid metabolism influence the development of colorectal cancer continue to be investigated.

The formation of adenomas precedes the onset of colorectal cancer, with removal of adenomas significantly decreasing the risk of developing colorectal cancer (11). Determining the levels of specific PPL FAs associated with the presence of adenomas could lead to the identification of blood-based biomarkers useful for early colorectal cancer screening, increasing opportunities for preventative interventions. PPLs are reflective of endogenous and exogenous sources of FAs and have been used to measure colorectal cancer risk in relation to FA intake (5, 12). Limitations in

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accurately measuring dietary intake combined with the need to assess endogenous lipid synthesis dictate that the direct analysis of PPLs is necessary in order to accurately determine the association between plasma FAs and colon carcinogenesis. Therefore, in this study, we sought to identify specific PPL levels of SFAs, cis-MUFAs, and trans-MUFAs associated with the presence of colorectal adenomas.

Materials and Methods

Study population and clinical parameters

Healthy male subjects ($n = 126$, >96% Caucasian) 48 to 65 years of age were enrolled as previously reported (13). Individuals were excluded for medical conditions associated with increased colorectal cancer risk (13). Immediately after enrollment, trained staff collected anthropometric measurements and venous blood of study participants (13). Smoking status was assessed as "ever smoked" or "never smoked." Each individual received a full colonoscopy as previously described (14). Serum and plasma fractions were separated from blood and stored at -80°C .

PPL extraction, isolation, and analysis

In brief, approximately 200 mg plasma per subject was weighed and extracted using a modified Rose and Oaklander extraction (15). PPLs were isolated using Isolute-XL SPE aminopropyl columns (500 mg; Biotage) as described by Agren and colleagues (16). Fatty acid methyl esters (FAME) were prepared as previously described (17, 18). PPL FAMEs were analyzed using HS-Omega-3 Index methodology at OmegaQuant Analytics, LLC as previously described (19). The coefficient of variation for PPL extraction, isolation, and PPL FA analysis is less than 7% for the 11 FAs presented.

Statistical analyses

Frequencies, means, and SDs were calculated for descriptive analyses (Table 1). Each FA was expressed as a percentage of total PPL. Means were obtained for the PL FAs (Fig. 1). PPL FA enzyme activity estimates (EAE) were calculated as the ratio of product-to-substrate. SCD-1 EAE was calculated in two ways (20): SCD n-7 index ($\text{SCD n-7} = \text{palmitoleic (POA)}/\text{PA}$), and SCD n-9 index ($\text{SCD n-9} = \text{OA}/\text{SA}$). A variation of the ELOVL-6 EAE was calculated as $\text{ELOVL-6} = \Sigma [\text{SA} + \text{OA}]/\text{PA}$ (21, 22). The total PPL SFA, cis-MUFA, trans-MUFA were calculated as follows: total PPL SFA was calculated as $\Sigma \text{PA} + \text{SA} + \text{arachidic} + \text{behenic} + \text{lignoceric}$; total PPL cis-MUFA was calculated as $\Sigma \text{POA} + \text{eicosaenoic} + \text{nervonic (NA)}$; total PPL trans-MUFA was calculated as $\Sigma \text{palmitelaidic} + \text{elaidic}$. Spearman correlations were performed since several variables were not normally distributed. These correlations, presented in Table 2, were conducted using only the 106 individuals that had adenomas or no polyps.

Table 1. Characteristics of study population^a

	Overall	No polyp	Hyperplastic	Adenoma
	$n = 126$	$n = 69$	$n = 20$	$n = 37$
Age (years)	57 ± 5	57 ± 5	57 ± 4	57 ± 5
Ever smoked (% total) ^b	31	15	4	12
BMI (kg/m^2)	30 ± 5	28 ± 4	29 ± 5	32 ± 6
WC (inches)	41 ± 6	40 ± 6	42 ± 4	44 ± 6

^aParticipants ($n = 126$) were male, >96% Caucasian; values expressed as mean \pm SD.

^bData missing for 22 participants.

Multiple imputation (seed = 20121119, imputations = 7) was used to impute all missing smoking data (23). The factors—smoking, PA, SA, arachidic, behenic, lignoceric, POA, OA, NA, palmitelaidic, and elaidic—were used in the imputation algorithm of missing values. Eicosaenoic acid was removed from the imputation algorithm due to a high correlation with elaidic acid.

The Wilcoxon–Mann–Whitney test was performed to compare the PPL FA composition of participants with adenomas to that of those with no polyps. Polytomous logistic regression models for categorical outcome data were used to determine OR and 95% confidence intervals (CI) for the likelihood of having an adenoma relative to no polyps. Categories were defined as polyp severity: (i) Individuals with no colon polyps and (ii) individuals with ≥ 1 adenoma. Individuals with polyps not classified as adenomas were excluded from statistical analyses. In all polytomous logistic regression models, polyp severity was analyzed categorically as the dependent variable with the reference category defined as individuals with no colon polyps. The ORs for ELOVL-6, SCD n-7, and palmitelaidic acid have been calculated on the basis that there is a unit change of 0.01 for the respective beta coefficient for each given parameter. All models were adjusted for age and smoking status except where noted.

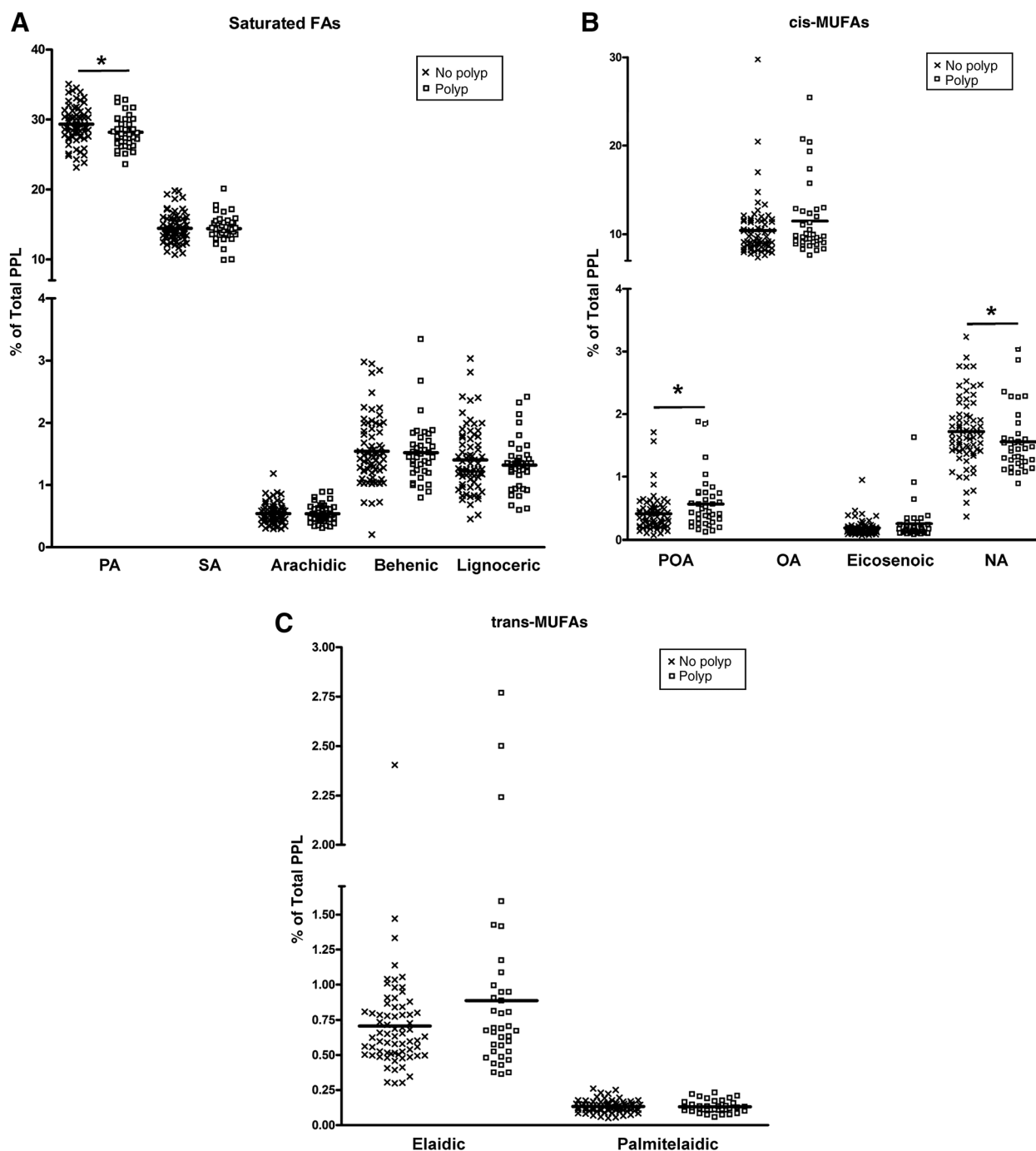
Due to high correlation (>0.9 , data not shown) between body mass index (BMI) and WC, these anthropometric measurements could not be analyzed in the same model. Two additional models were run, the first with the addition of BMI and the second with the addition of WC. These models are referred to as model 2 and model 3, respectively (Table 3). FAs were analyzed as continuous (Tables 2 and 3, and Fig. 1) and categorical independent variables (Fig. 2). FAs were categorized into tertiles (with lowest tertile as reference) for adenomas relative to no polyps. Test for trend was carried out across tertiles for the FAs of interest. Because smoking data were imputed, multiple imputation analyze (Proc MI ANALYZE) was used to determine the results from analysis of the imputed datasets. *P* values were considered statistically significant if $P \leq 0.05$ and a statistical trend if $0.05 < P \leq 0.09$. Statistical analyses were conducted using SAS version 9.3.

Results

Participant characteristics are displayed in Table 1. As previously reported (13), 37 (29.4%) participants had adenomas, whereas 69 (54.8%) had no polyps. Seventeen (13.5%) participants had ≥ 3 polyps including at least one adenoma. Both BMI and WC increased with polyp severity, as previously reported (13).

The PPL FA proportions are presented in Fig. 1. PPL PA was significantly lower, and total SFAs tended ($P = 0.0684$) to be lower in those with adenomas compared with those without polyps. The PPL cis-MUFA POA was significantly higher in individuals with adenomas, whereas PPL cis-MUFA NA was significantly lower in the adenoma group compared with those with no colon polyps. The percentage of total trans-MUFAs in PPLs did not differ between the groups.

Elongating and desaturating EAEs were positively associated with polyp severity. SFAs are enzymatically desaturated to form cis-MUFAs. Both SFAs and cis-MUFAs can be enzymatically elongated to form longer chain products. SCD n-7, SCD n-9, and ELOVL-6 EAEs are noninvasive methods to assess FA metabolism (24), calculated as the FA product-to-precursor ratio for respective EAE. We observed that SCD n-7 was significantly elevated ($P =$

**Figure 1.**

FA content of PPLs. (A) saturated FAs, (B) cis-MUFAs, and (C) trans-MUFAs. The symbol "X" represents PPL FA levels of individuals with no polyps, and "□" represents PPL FA levels of individuals with adenomas. The solid lines indicate the mean. FAs are expressed as a percentage of total PPL FAs. Asterisk indicates $P \leq 0.05$, calculated by the Wilcoxon-Mann-Whitney nonparametric U test.

0.0163) in those with adenomas compared with those with no colon polyps. However, SCD n-9 did not differ ($P = 0.5868$) between individuals with no polyps and those with adenomas. ELOVL-6 was significantly elevated (0.0105) in those with adenomas compared with those with no colon polyps.

Several PPL FAs measured were significantly correlated with polyp severity and with other SFAs and MUFAs (Table 2). Polyp

severity was not correlated with PPL palmitelaidic, elaidic, or total trans-MUFA. Polyp severity was inversely correlated with PPL PA and NA (Table 2). Also, polyp severity was positively correlated with PPL POA, SCD n-7, and ELOVL-6.

Colon polyps and several PPL FAs were correlated with confounding factors, such as age, smoking status, BMI, and WC (data not shown). Polytomous logistic regression was performed to

Table 2. Spearman correlation between FAs and polyp severity^a

	PA	SA	Arachidic	Behenic	Lignoceric	Total SFA ^b	POA	OA	Eicosenoic	NA	Total Cis-MUFA ^c	Palmitelaidic	Elaidic	Total Trans-MUFA ^d	SCD n-7 ^e	SCD n-9 ^f	ELOVL-6 ^g
Polyp severity	-0.245	0.042	0.003	-0.033	-0.051	-0.185	0.195	0.124	0.094	-0.205	0.102	-0.033	0.127	0.110	0.228	0.048	0.255
PA	0.011	0.665	0.973	0.740	0.603	0.058	0.045	0.207	0.340	0.035	0.296	0.738	0.195	0.260	0.019	0.624	0.008
SA	0.023	-0.005	-0.042	-0.035	0.752	0.337	-0.041	-0.352	-0.238	-0.087	-0.087	-0.083	-0.193	-0.190	0.216	-0.044	-0.768
Arachidic	0.816	0.957	0.672	0.718	<0.0001	0.0004	0.675	0.0002	0.014	0.378	0.400	0.047	0.051	-0.282	0.783	0.209	0.031
Behenic	0.078	0.150	-0.053	0.551	<0.0001	0.004	<0.0001	0.127	0.0003	<0.0001	0.064	-0.046	0.014	0.011	-0.095	-0.079	0.079
Lignoceric	0.428	0.549	0.423	0.247	0.011	0.386	0.630	0.099	<0.0001	0.516	0.640	0.888	0.915	0.424	0.331	0.424	0.422
Total SFA	<0.0001	<0.0001	<0.0001	0.011	0.666	0.310	-0.199	-0.225	0.117	0.239	-0.156	0.011	0.094	0.092	-0.192	-0.236	-0.010
POA	<0.0001	0.001	0.041	0.001	0.041	0.021	0.021	0.231	0.013	0.111	0.914	0.340	0.348	0.048	0.015	0.918	0.918
OA	0.192	-0.083	-0.008	0.157	0.274	0.025	-0.032	-0.008	-0.015	-0.073	0.005	-0.001	<0.0001	0.704	0.661	0.924	0.341
Eicosenoic	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019
NA	0.255	0.336	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Total Cis-MUFA	0.008	0.244	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012
Palmitelaidic	0.338	0.463	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
Elaidic	0.259	0.208	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033
Total trans-MUFA	0.257	0.188	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
SCD n-7	0.592	0.066	0.503	0.503	0.503	0.503	0.503	0.503	0.503	0.503	0.503	0.503	0.503	0.503	0.503	0.503	0.503
SCD n-9	0.159	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.103

Abbreviations: SCD n-7, stearoyl-CoA desaturase n-7 EAE; SCD n-9, stearoyl-CoA desaturase n-9 EAE.

^aCorrelations were conducted using only the 106 individuals that had no polyps or adenomas. Numbers in gray rows indicate spearman correlation coefficient, and numbers listed directly below, in white rows, indicate corresponding *P* value. *P* values bolded if significant (*P* ≤ 0.05) and italicized if 0.05 > *P* ≥ 0.09.^bTotal SFA calculated as the Σ PA + SA + arachidic + behenic + lignoceric.^cTotal Cis-MUFA calculated as the Σ POA + OA + eicosenoic + NA.^dTotal trans-MUFA calculated as the Σ palmitelaidic + elaidic.^eSCD n-7 calculated as the ratio of POA/PA.^fSCD n-9 calculated as the ratio of OA/SA.^gELOVL-6 calculated as the ratio of Σ [SA + OA]/PA.

Table 3. Association of FAs and EAEs, as continuous variables, with having adenomas relative to no colon polyps^a

Variable	Model 1		Model 2		Model 3	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
C16:0 Palmitic (PA)	0.830 (0.701–0.982)	0.0303	0.718 (0.582–0.886)	0.0020	0.756 (0.623–0.917)	0.0045
C18:0 Stearic (SA)	0.973 (0.806–1.174)	0.7737	0.917 (0.748–1.123)	0.4008	0.914 (0.747–1.119)	0.3851
C20:0 Arachidic	0.901 (0.058–13.943)	0.9403	1.053 (0.058–19.211)	0.9721	0.978 (0.059–16.274)	0.9877
C21:0 Behenic	0.923 (0.400–2.131)	0.8504	0.916 (0.383–2.196)	0.8449	0.936 (0.400–2.188)	0.8778
C24:0 Lignoceric	0.732 (0.306–1.754)	0.4842	0.925 (0.366–2.335)	0.8686	0.848 (0.345–2.085)	0.7192
Total SFA ^b	0.902 (0.803–1.013)	<i>0.0811</i>	0.837 (0.730–0.960)	0.0112	0.847 (0.742–0.967)	0.0144
C16:1 Palmitoleic (POA)	3.750 (1.079–13.036)	0.0376	2.442 (0.679–8.783)	0.1716	2.652 (0.739–9.519)	0.1347
C18:1 Oleic (OA)	1.097 (0.981–1.226)	0.1048	1.106 (0.983–1.245)	0.0932	1.103 (0.981–1.239)	0.1002
C20:1 Eicosenoic	1.020 (0.995–1.045)	0.1161	1.023 (0.997–1.050)	<i>0.0794</i>	1.024 (0.998–1.051)	<i>0.0741</i>
C24:1 Nervonic (NA)	0.568 (0.260–1.243)	0.1570	0.743 (0.327–1.690)	0.4792	0.745 (0.329–1.686)	0.4794
Total Cis-MUFA ^c	1.088 (0.981–1.206)	0.1103	1.099 (0.985–1.226)	0.0901	1.100 (0.987–1.226)	<i>0.0864</i>
C16:1 Palmitelaidic ^d	0.982 (0.894–1.078)	0.7017	1.013 (0.916–1.119)	0.8073	1.006 (0.912–1.108)	0.9116
C18:1 Elaidic	2.915 (1.030–8.246)	0.0438	3.111 (1.031–9.388)	0.0440	3.224 (1.060–9.801)	0.0391
Total Trans-MUFA ^e	2.708 (1.000–7.337)	<i>0.0501</i>	2.990 (1.029–8.687)	0.0441	3.066 (1.050–8.955)	0.0405
SCD n-7 ^{d,f}	1.538 (1.068–2.215)	0.0207	1.383 (0.960–1.992)	<i>0.0819</i>	1.410 (0.977–2.035)	<i>0.0664</i>
SCD n-9 ^g	2.229 (0.724–6.864)	0.1623	2.846 (0.850–9.534)	<i>0.0899</i>	2.739 (0.822–9.132)	0.1010
ELOVL-6 ^{d,h}	1.358 (1.039–1.775)	0.0250	1.467 (1.090–1.973)	0.0114	1.405 (1.059–1.865)	0.0184

Abbreviations: SCDn-7, stearoyl-CoA desaturase n-7 EAE; SCDn-9, stearoyl-CoA desaturase n-9 EAE.

^aModels defined as: Model 1: adenoma = FA + age + smoking. Model 2: adenoma = FA + age + smoking + BMI. Model 3: adenoma = FA + age + smoking + WC. FAs expressed as a percentage of total phospholipids. P values bolded if significant ($P \leq 0.05$) and italicized if $0.05 > P \geq 0.09$.^bTotal SFA calculated as the Σ PA + SA + arachidic + behenic + lignoceric.^cTotal Cis-MUFA calculated as the Σ POA + OA + eicosenoic + NA.^dORs for palmitelaidic, SCD n-7, and ELOVL-6 have been calculated on the basis that there is a unit change of 0.01 for the respective beta coefficient for each given parameter.^eTrans-MUFA calculated as the Σ palmitelaidic + elaidic.^fSCD n-7 calculated as the ratio of POA/PA.^gSCD n-9 calculated as the ratio of OA/SA.^hELOVL-6 calculated as the ratio of Σ [SA + OA]/PA.

determine which PPL FAs and EAEs were significantly associated with adenomas after adjusting for these confounding factors (Table 3). Model 1 included PPL FA and was adjusted for age and smoking. To account for the potential contribution of BMI or visceral adiposity (WC) to the likelihood of having an adenoma, two additional models were tested. Model 2 included PPL FA and was adjusted for BMI, in addition to age and smoking. Model 3 included PPL FA and was adjusted for WC, in addition to age and smoking.

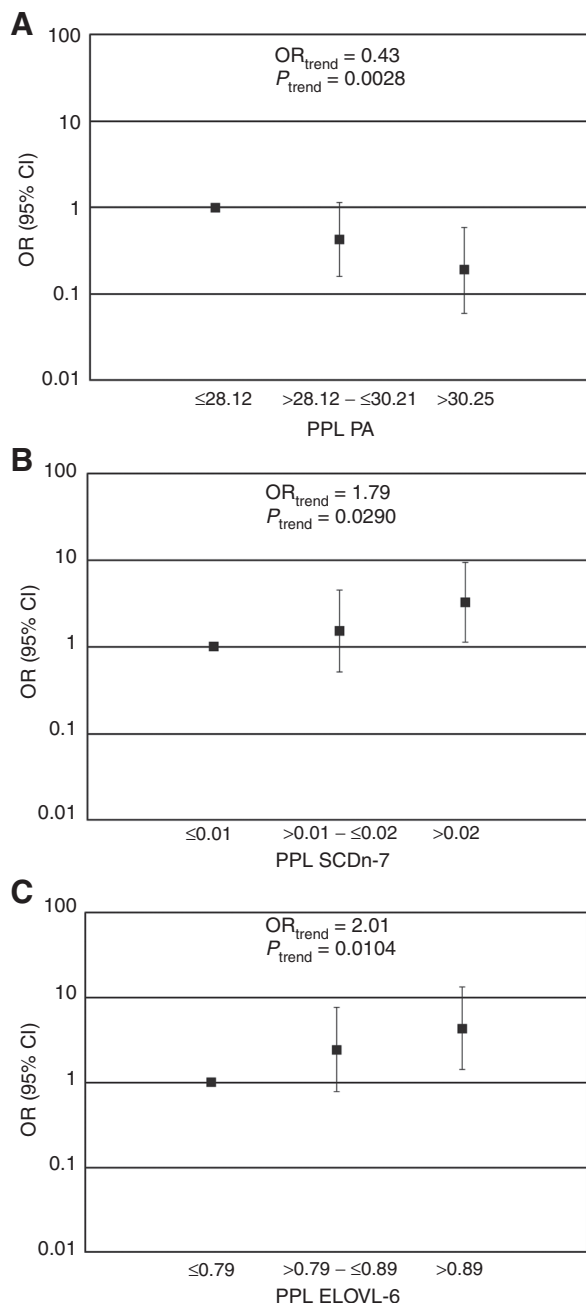
The odds that an individual whose PPL contained high levels of PA would have an adenoma were significantly lower than those of an individual whose PPL contained low levels of PA. This was consistent across all three models (Table 3). Specifically, for each unit increase in PPL PA, individuals were 0.83 (95% CI, 0.70–0.98) times as likely in model 1, 0.72 (95% CI, 0.58–0.89) times as likely in model 2, and 0.76 (95% CI, 0.62–0.92) times as likely in model 3 to have adenomas rather than no colon polyps. PPL SA, arachidic, behenic, and lignoceric acid showed no association with adenomas in these three models. However, for each unit increase in total PPL SFAs, individuals tended to be 0.90 (95% CI, 0.80–1.01) times as likely to have adenomas in model 1, and individuals were 0.84 (95% CI, 0.73–0.96) and 0.85 (95% CI, 0.74–0.97) times as likely to have adenomas compared with no colon polyps when adjusted for BMI or WC, respectively.

Some MUFAs were significantly associated with the presence of adenomas (Table 3). In model 1, for each unit increase in PPL POA, an individual was 3.75 (95% CI, 1.08–13.04) times more likely to have an adenoma compared with no colon polyps, but there were no significant associations after adjusting for BMI (model 2) or WC (model 3). PPL elaidic acid, a C18:1 trans-MUFA, was highly associated with an increased likelihood of

adenoma presence in all three models analyzed. Specifically, for each unit increase in PPL elaidic acid, individuals were 2.92 (95% CI, 1.03–8.25) times more likely in model 1, 3.11 (95% CI, 1.03–9.39) times more likely in model 2, and 3.22 (95% CI, 1.06–9.80) times more likely in model 3, to have adenomas relative to no colon polyps (Table 3). PPL palmitelaidic acid, a C16:1 trans-MUFA, was not significantly associated with adenomas. For each unit increase in PPL total trans-MUFA, calculated as the Σ elaidic + palmitelaidic, individuals tended to be 2.71 (95% CI, 1.00–7.34) times more likely to have adenomas in model 1, and individuals were 2.99 (95% CI, 1.03–8.69) and 3.07 (95% CI, 1.05–8.96) times more likely to have adenomas rather than no colon polyps in model 2 and model 3, respectively (Table 3).

Each unit increase in SCD n-7 was associated with individuals being 1.54 (95% CI, 1.07–2.22) times more likely to have adenomas than no polyps, and individuals with high SCD n-7 tended to be 1.38 (95% CI, 0.96–1.99) and 1.41 (95% CI, 0.98–2.04) times more likely to have adenomas rather than no polyps in models 2 and 3, respectively (Table 3). Unit increases in ELOVL-6 were associated with adenomas in all three models analyzed. Specifically, for each unit increase in ELOVL-6, individuals were 1.36 (95% CI, 1.04–1.78) times more likely in model 1, 1.47 (95% CI, 1.09–1.97) times more likely in model 2, and 1.41 (95% CI, 1.06–1.87) times more likely in model 3 to have adenomas relative to no colon polyps (Table 3).

Next, we separated several of our highly significant FAs and EAEs into tertiles, providing insight into the specific PPL FA ranges that were most likely to be associated with the presence of adenomas (Fig. 2). For each tertile increase in PPL PA, individuals were 0.43 (95% CI, 0.25–0.75) times as likely to have adenomas rather than no colon polyps (Fig. 2A). Categorical increases for

**Figure 2.**

Associations of PPL FA and EAEs, as tertiles, with having adenomas relative to no colon polyps. (A) PA, (B) SCD n-7, and (C) ELOVL-6. The symbol "■" represents the OR, and error bars indicate lower and upper CIs, respectively. Both test for exposure and test for trend models adjusted for age and smoking. PA is expressed as a percentage of total PPL FAs. SCD n-7, stearyl-CoA desaturase n-7 EAE.

POA, total SFA, elaidic, and total trans-MUFA showed no significant association with adenomas. However, for each tertile increase in SCD n-7, the calculated ratio of POA/PA, an individual was 1.79 (95% CI, 1.06–3.03) times more likely to have at least one adenoma rather than no polyps (Fig. 2B). The association of ELOVL-6 with colon adenomas was similar to the association of

SCD n-7 with colon adenomas. For each tertile increase in ELOVL-6, individuals were 2.01 (95% CI, 1.18–3.42) times more likely to have an adenoma rather than no polyps (Fig. 2C).

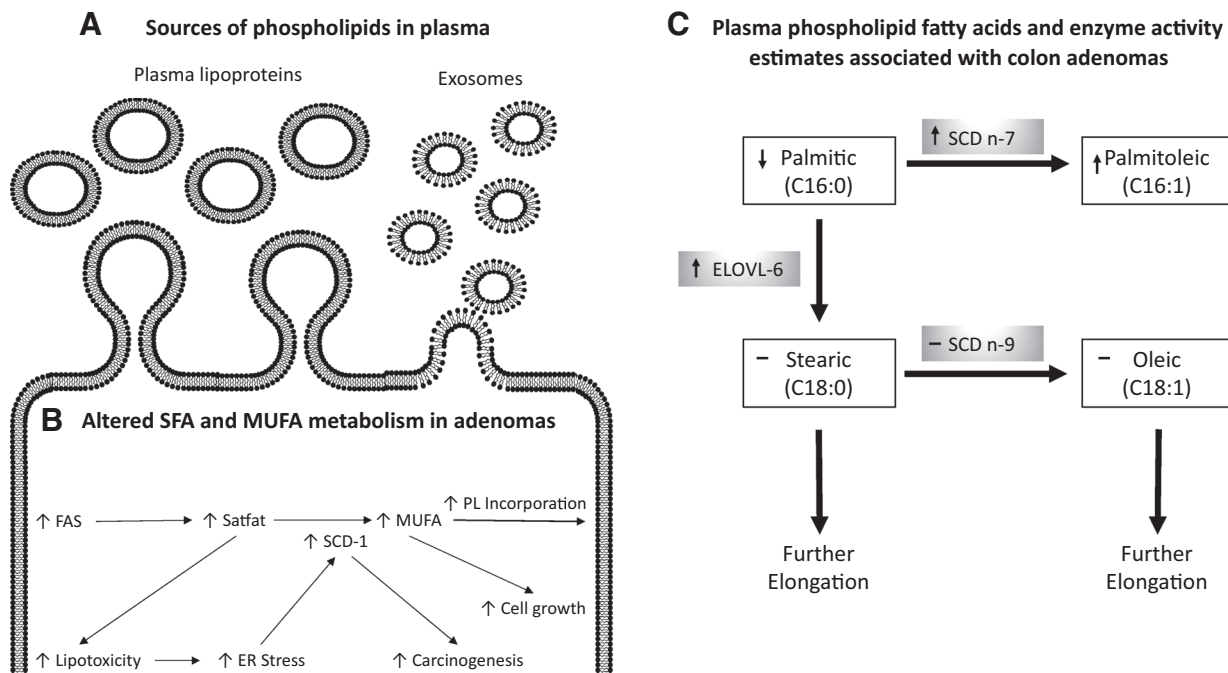
Discussion

This study characterized PPL FA profiles associated with the presence of adenomas in adult males. Specifically, we report that adenomas are positively associated with PPL elaidic, POA, total trans-MUFAs, as well as SCD n-7 and ELOVL-6 EAEs. PPL PA was inversely associated with the presence of adenomas. These data indicate that specific PPL FAs and EAEs are associated with adenomas even after adjusting for obesity, smoking, age, and elevated WC, which are factors known to increase colorectal cancer risk (2).

The PPL FA compartment is an ideal location for biomarker identification. Not only is the PPL FA compartment easily accessible to clinicians through a blood draw or simple blood spot using cards treated to prevent oxidation, but the PPL FA compartment also contains PL from sources such as plasma lipoproteins (25) and plasma microvesicle exosomes (ref. 26; Fig. 3A). Because PLs are endogenously synthesized, proportional differences in PPL FAs likely reflect cellular FA metabolism (ref. 27; Fig. 3B). If cellular FA metabolism is changed during the formation of adenomas, then new FA metabolites would be detectable in the PPL fraction. However, PL FA proportions in individuals also may reflect dietary FA intake (28), in addition to altered lipid metabolism (28) associated with colon carcinogenesis (8).

The ability to easily measure changes in cellular FA metabolism is important in the identification of biomarkers of colorectal polyp formation because colon adenomas are associated with changes in FA metabolism. For instance, colon adenomas are positively associated with fatty acid synthase (FAS) expression (29), which increases SFA synthesis, in particular PA synthesis (30). Endogenous FA synthesis occurs in the smooth endoplasmic reticulum (ER), where the enzymes ELOVL-6 and SCD-1 enzymes are located (31). Elevated intracellular concentrations of SFAs are associated with increased lipotoxicity and ER stress (32–34). The positive association of cellular stress responses and carcinogenesis is well documented (reviewed in detail in ref. 35). Thus, our observation that higher SCD n-7 and ELOVL-6 EAEs are associated with the presence of adenomas may be indicative of a cellular stress response to the process of carcinogenesis.

Aside from cellular stress, FA metabolism also increases during mitogenesis. Mitogenic factors associated with adenomas increase SCD-1 expression (36), which in turn increases *de novo* production of MUFAs such as POA (37). In order for cell division to occur, cells must double their membrane FA content (38). In particular, there is an increased demand for MUFA incorporation into PL membranes (8, 37). Therefore, changes in FA metabolism (i.e., FAS, ELOVL-6, and SCD-1) associated with increased cellular proliferation (i.e., adenomas) may be detectable by identifying specific proportions of PPL FAs and EAEs. Higher plasma SCD EAEs are associated with an increased risk of several cancers (39–41). What remains unclear is whether the resulting metabolites specifically participate in the process of carcinogenesis or if they are merely a by-product of the metabolism of abnormal cells. The visual representation of the PA pathway in Fig. 3C incorporates results from our logistic regressions that demonstrate significant associations between

**Figure 3.**

Relationship between cellular FA metabolic pathways and observed associations of FAs and EAs with colorectal adenomas. (A) lipoproteins and exosomes are the most abundant sources of PPL FAs. (B) increased FAS increases intracellular SFAs. These SFAs have lipotoxic effects causing SCD-1 expression to increase. Higher concentrations of SFAs and expression SCD-1 increase MUFA production leading to increased PL MUFA incorporation and cellular enlargement. (C) visual representation of PA metabolic pathway. PPL FAs appear in white boxes, and EAs appear in gray boxes. The arrow in each box indicates the direction of the association between the substrate and the likelihood of having an adenoma compared with having no colon polyps. – indicates no observed association. SCD n-7, stearoyl-CoA desaturase n-7 EAE; SCD n-9, stearoyl-CoA desaturase n-9 EAE.

the presence of adenomas and the PPL FAs and EAs associated with PA metabolism. Taken together, our data suggest that the observed associations are likely the result of altered desaturation and elongation of PA during carcinogenesis.

PA is desaturated by SCD-1 to form the cis-MUFA POA. We used two separate estimates of SCD-1 activity, SCD n-7, and SCD n-9. We observed that SCD n-7 EAE was positively associated with adenomas, and there was no association of SCD n-9 with adenomas. An increase in the proportion of plasma POA is positively associated with risk of future all-cause cancer mortality (42). Higher levels of PPL POA are indicative of increased *de novo* synthesis, because dietary POA is rapidly oxidized after absorption resulting in negligible effects of dietary POA on the lipid profile (43). Plasma SCD n-7 EAE positively correlates with SCD-1 enzyme activity measured in biopsied tissues, but SCD n-9 EAE does not (44). Aside from PA, no other SFAs analyzed (SA, arachidic, behenic, or lignoceric) had significant associations with adenomas. We speculate that the inverse association between PPL PA and adenomas reflects underlying changes in PA metabolism such as increased desaturation.

Our cross-sectional study was conducted in a population of males ($n = 126$, >96% Caucasian, ages 48–65) to identify associations between colon polyps and PPL FAs or EAs. We recognize that the generalizability of these observations is limited. Therefore, studies need to be conducted prospectively in larger, more diverse populations. In addition, we report that PPL FA-based EAs of ELOVL-6 and SCD n-7 are associated with adenomas. These EAs have yet to be extensively validated and may not fully represent enzyme kinetics in adenomas.

Thus, reported differences in EAs could be related to other factors (i.e., diet, preferential FA uptake, etc.) rather than enzyme activities as we did not directly collect or assess dietary intake in this study. To our knowledge, no research group has sought to establish a preliminary range of PPL FA or EAE levels associated with colorectal adenomas. Our research suggests that specific levels of PPL FAs and EAs may be useful as novel biomarkers of colon carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.S. Comstock, J.I. Fenton

Development of methodology: C.A. Pickens, J.I. Fenton

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.A. Pickens, S.S. Comstock, J.I. Fenton

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.A. Pickens, A. Lane-Elliott, S.S. Comstock, J.I. Fenton

Writing, review, and/or revision of the manuscript: C.A. Pickens, A. Lane-Elliott, S.S. Comstock, J.I. Fenton

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Lane-Elliott, J.I. Fenton

Study supervision: C.A. Pickens, J.I. Fenton

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