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

C. Austin Pickens, Ami Lane-Elliot, Sarah S. Comstock, and Jenifer I. Fenton

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.

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 though 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 PPI 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. PPIs 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
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; Bioatage) 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).

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 (SCD n-7) = palmitoleic (POA)/PA, and SCD n-9 index (SCD n-9) = OA/SA. A variation of the ELOVL-6 EAE was calculated as ELOVL-6 = Σ [SA + OA]/PA (21, 22). The total PPL SFA, cis-MUFA, trans-MUFA were calculated as follows: total PPL SFA was calculated as Σ PA + SA + arachidic + behenic + lignoceric; total PPL cis-MUFA was calculated as Σ POA + eicosanoic + nervonic (NA); total PPL trans-MUFA was calculated as Σ palmitelaidic + 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.

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 EAs 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 were noninvasive methods to assess FA metabolism (24), calculated as the FA product-to-precursor ratio for respective EA. We observed that SCD n-7 was significantly elevated (P =
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

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.
Table 2. Spearman correlation between FAs and polyp severity

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<th>SA</th>
<th>Arachidic</th>
<th>Behenic</th>
<th>Lignoceric</th>
<th>Total SFA</th>
<th>POA</th>
<th>Elaeoceroic</th>
<th>NA</th>
<th>Total Cis-MUFA</th>
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Abbreviations: SCD n-7, stearoyl-CoA desaturase n-7 EAE; SCD n-9, stearoyl-CoA desaturase n-9 EAE.

*Correlations were conducted using only the 166 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.

1Total SFA calculated as the Σ PA + SA + arachidonic + behenic + lignoceric.

2Total Cis-MUFA calculated as the Σ POA + OA + eicosenoic + NA.

3Total trans-MUFA calculated as the Σ palmitelaidic + elaidic.

4SCD n-7 calculated as the ratio of POA/PA.

5SCD n-9 calculated as the ratio of OA/SA.

6ELOVL-6 calculated as the ratio of Σ [3A + 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 as model 1, 0.72 (95% CI, 0.58–0.89) times as likely as model 2, and 0.76 (95% CI, 0.62–0.92) times as likely as model 3 to have adenomas rather than no 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, 0.87–0.93) times as likely as model 3 to have adenomas rather than no polyps. PPL SA, arachidic, behenic, and lignoceric acid showed no association with adenomas in these three models.

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, 1.16–1.78) 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
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-MUFA, 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 SCD n-7 and 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).

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, stearoyl-CoA desaturase n-7 EAE.
the presence of adenomas and the PPL FAs and EAEs 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 EAEs. 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 EAEs of ELOVL-6 and SCD n-7 are associated with adenomas. These EAEs have yet to be extensively validated and may not fully represent enzyme kinetics in adenomas.

Thus, reported differences in EAEs 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 EAEs may be useful as novel biomarkers of colon carcinogenesis.

**Disclosure of Potential Conflicts of Interest**
No potential conflicts of interest were disclosed.

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