Title: Altered saturated and monounsaturated plasma phospholipid fatty acid profiles in adult males with colon adenomas

Authors: C. Austin Pickens¹, Ami Lane-Elliot¹, Sarah S. Comstock¹, and Jenifer I. Fenton¹.

¹Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI;

Running Title: Associations between fatty acids and adenomas.

Key Words: Palmitic acid, Colon adenomas, Biomarker, Fatty acid metabolism, Fatty acid desaturation

Financial support: Research supported in part by the National Cancer Institute 1R03CA142000 (J. I. Fenton) and the Clinical and Translational Sciences Institute at MSU.

Corresponding Author: Jenifer I. Fenton. 469 Wilson Rd, Rm 208B, Michigan State University, East Lansing, MI 48824. Phone: (517) 353-3342; Fax: (517) 353-8963 Email: imigjeni@msu.edu.

Disclosure: No authors report a conflict of interest.

Word count: 3145

Table count: 3

Figure count: 3

Reference count: 44

Article type: Research article

Abbreviations: BHT, Butylated hydroxytoluene; BMI, Body mass index; CC, Colon cancer; CI, Confidence interval; CRC, Colorectal cancer; EAE, Enzyme activity estimate; Elovl, Elongation of very long chain fatty acid; FA, Fatty acid; FAME, Fatty acid methyl ester; FAS, Fatty acid synthase; MUFA, Monounsaturated fatty acid; NA, nervonic acid; OA, Oleic acid; OR, Odds ratio; PA, Palmitic acid; PL, phospholipid; POA, Palmitoleic acid; PPL, Plasma phospholipid; PUFA, Polyunsaturated fatty acid; SA, Stearic acid; SCD, Stearoyl-CoA desaturase; SFA, Saturated fatty acid; SNP, Single nucleotide polymorphism; WC, Waist circumference
Abstract

Background: Altered lipid metabolism and plasma fatty acid (FA) levels are associated with colorectal cancer (CRC). 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. Odds ratios (ORs) and 95% confidence intervals were determined using polytomous logistic regression after adjusting for confounding factors (i.e. age, smoking, WC, and 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 (EAEs) of stearoyl-CoA desaturase-1 (SCD-1, p = 0.02) and elongation of very long chain-6 (Elovl-6, p = 0.03) were associated with an individual being approximately 1.5 times more likely to have an adenoma compared to no polyps.

Conclusions: PPL FAs and EAEs, which have previously been associated with CRC, are significantly different in those with adenomas when compared to 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 CRC risk.
Introduction

Colorectal cancer (CRC) is the third most prevalent cancer among men and women in the US (1). Risk factors for CRC include obesity, waist circumference (WC), age, smoking, physical inactivity, inflammatory bowel disease, and a family history of CRC or adenomas (2). As much as 70% of the risk of developing CRC 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 CRC.

Several specific FAs are associated with CRC. For example, a higher erythrocyte oleic acid (OA) to stearic acid (SA) ratio has been associated with CRC (4). Also, CRC is associated with higher levels of plasma phospholipid (PPL) SFAs, in particular palmitic acid (PA) (5). Among PUFAs, dietary consumption of greater amounts of omega-3 PUFAs and lesser amounts of omega-6 PUFAs is typically associated with a decreased risk for developing CRC (6). Diets higher in MUFAs and lower in SFAs also potentially prevent CRC (7). Blood FAs associated with CRC may originate from dietary intake as well as from endogenous synthesis though lipid metabolism.

Altered lipid metabolism also is suspected to play a role in colon carcinogenesis during the transformation of colorectal polyps to CRC (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 acid protein-6 (Elov6) are the rate-limiting enzymes controlling metabolic shifts towards production of long chain MUFAs. Upregulation of SCD-1, the desaturase responsible for converting PA and SA into MUFAs, has been linked to CRC (9). MUFAs influence cellular apoptosis and are believed to play a role in the mutagenesis of tumors
in several types of cancer, including CRC (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. Additionally, the complex mechanisms by which dietary FAs and lipid metabolism influence the development of CRC continue to be investigated.

The formation of adenomas precedes the onset of CRC, with removal of adenomas significantly decreasing the risk of developing CRC (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 CRC screening, increasing opportunities for preventative interventions. PPLs are reflective of endogenous and exogenous sources of FAs and have been used to measure CRC risk in relation to FA intake (5, 12). Limitations in 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 CRC 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.

**Plasma Phospholipid 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, Charlotte, NC) as described by Agren et al (16). Fatty acid methyl esters (FAMEs) were prepared as previously described (17, 18). PPL FAMEs were analyzed using HS-Omega-3 Index® methodology at OmegaQuant Analytics, LLC (Sioux Falls, SD) as previously described (19). The coefficient of variation for PPL extraction, isolation, and PPL FA analysis is less than 7% for the eleven FAs presented.

**Statistical analyses**

Frequencies, means, and standard deviations were calculated for descriptive analyses (Table 1). Each FA was expressed as a percentage of total PPL. Means were obtained for the PL FAs (Figure 1). PPL FA enzyme activity estimates (EAE) were calculated as the ratio of product-to-substrate. SC-D-1 EAE was calculated in two ways (20): SC-D n-7 index (SC-D n-7) = palmitoleic (POA) / PA, and SC-D n-9 index (SC-D n-9) = OA / SA. A variation of the Elovl-6 EAE was calculated as Elovl-6 = \( \sum [SA + OA] / PA \) (21, 22). The total PPL SFA, cis-MUFA, trans-MUFA were calculated as follows: total PPL SFA was calculated as \( \sum PA + SA + arachidic + behenic + lignoceric \); total PPL cis-MUFA was calculated as \( \sum POA + eicosaenoic + nervonic (NA) \); total PPL trans-MUFA was calculated as \( \sum \) 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.

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, elaidic—were used in the imputation algorithm of missing values. Eicosenoic 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 odds ratios (OR) and 95% confidence intervals (CI) for the likelihood of having an adenoma relative to no polyps. Categories were defined as polyp severity: 1) Individuals with no colon polyps, and 2) 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 odds ratios 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 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 (Table 2 and 3, and Figure 1) and categorical independent variables (Figure 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 was 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 (Cary, NC).

**Results**

Participant characteristics are displayed in Table 1. As previously reported (13), 37 (29.4%) participants had adenomas while 69 (54.8%) had no polyps. Seventeen (13.5%) participants had $\geq 3$ 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 Figure 1. PPL PA was significantly lower and total SFAs tended ($p=0.0684$) to be lower in those with adenomas compared to those without polyps. The PPL cis-MUFA POA was significantly higher in individuals with adenomas, while PPL cis-MUFA NA was significantly lower in the adenoma group compared to 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 non-invasive methods to assess FA metabolism (24), calculated as the FA product-to-precursor ratio for respective EAE. We observed SCD n-7 was significantly elevated ($p=0.0163$)
in those with adenomas compared to 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 to 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 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 3 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 to 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 to 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 3 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 \( \Sigma \) elaidic + palmitelaidic, individuals tended 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 3 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 (Figure 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 (Figure 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 (Figure 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 (Figure 2C).

Discussion

This study characterized PPL FA profiles associated with the presence of adenomas in adult males. Specifically, we report 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 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 CRC 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 (26) (Figure 3A). Since PLs are endogenously synthesized, proportional differences in PPL FAs likely reflect cellular FA metabolism (27) (Figure 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 associated (28) with colon carcinogenesis (8).

The ability to easily measure changes in cellular fatty acid 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 ER, where the enzymes ELOVL-6 and SCD-1 enzymes are located (31). Elevated intracellular concentrations of SFAs are associated with increased lipotoxicity and endoplasmic reticulum (ER) stress (32-34). The positive association of cellular stress responses and carcinogenesis are well documented [reviewed in detail (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 Figure 3C incorporates results from our logistic regressions that demonstrate significant associations between the presence of adenomas and the PPL FAs and EAEs associated with PA metabolism. Taken together, our data suggests 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 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 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 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 specific levels of PPL FAs and EAEs may be useful as novel biomarkers of colon carcinogenesis.

Acknowledgments

We thank Catherine Belcher for assistance with sample processing, and Emily Davidson for figure editing.
References

Table 1. Characteristics of study population

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>No Polyp</th>
<th>Hyperplastic</th>
<th>Adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>126</td>
<td>69</td>
<td>20</td>
<td>37</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 ± 5</td>
<td>57 ± 5</td>
<td>57 ± 4</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>Ever Smoked (% total)</td>
<td>31</td>
<td>15</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30 ± 5</td>
<td>28 ± 4</td>
<td>29 ± 5</td>
<td>32 ± 6</td>
</tr>
<tr>
<td>WC (inches)</td>
<td>41 ± 6</td>
<td>40 ± 6</td>
<td>42 ± 4</td>
<td>44 ± 6</td>
</tr>
</tbody>
</table>

a Participants (n = 126) were male, > 96% Caucasian; Values expressed as mean ± standard deviation.

b Data missing for 22 participants.

BMI: body mass index; WC: waist circumference.
Table 2. Spearman correlation between fatty acids and polyp severity \(^a\)

<table>
<thead>
<tr>
<th></th>
<th>PA</th>
<th>SA</th>
<th>Arachidic</th>
<th>Behenic</th>
<th>Lignoceric</th>
<th>Total SFA (^b)</th>
<th>POA</th>
<th>OA</th>
<th>Eicosenoic</th>
<th>NA</th>
<th>Total Cis-MUFA (^c)</th>
<th>Palmitelaidic</th>
<th>Elaidic</th>
<th>Total Trans-MUFA (^d)</th>
<th>SCD n-7 (^e)</th>
<th>SCD n-9 (^f)</th>
<th>Elov-6 (^g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyp severity</td>
<td>-0.245</td>
<td>0.042</td>
<td>0.003</td>
<td>-0.033</td>
<td>-0.051</td>
<td>-0.153</td>
<td>0.195</td>
<td>0.124</td>
<td>0.094</td>
<td>-0.205</td>
<td>0.102</td>
<td>-0.033</td>
<td>0.127</td>
<td>0.110</td>
<td>0.228</td>
<td>0.048</td>
<td>0.255</td>
</tr>
<tr>
<td>PA</td>
<td>0.011</td>
<td>0.665</td>
<td>0.973</td>
<td>0.740</td>
<td>0.603</td>
<td>0.058</td>
<td>0.045</td>
<td>0.207</td>
<td>0.340</td>
<td>0.035</td>
<td>0.296</td>
<td>0.738</td>
<td>0.195</td>
<td>0.260</td>
<td>0.019</td>
<td>0.624</td>
<td>0.008</td>
</tr>
<tr>
<td>OA</td>
<td>0.023</td>
<td>-0.005</td>
<td>-0.042</td>
<td>-0.035</td>
<td>0.752</td>
<td>0.337</td>
<td>-0.041</td>
<td>-0.352</td>
<td>-0.238</td>
<td>-0.087</td>
<td>-0.085</td>
<td>-0.103</td>
<td>-0.190</td>
<td>0.216</td>
<td>-0.044</td>
<td>-0.768</td>
<td></td>
</tr>
<tr>
<td>Eicosenoic</td>
<td>0.048</td>
<td>-0.205</td>
<td>0.228</td>
<td>-0.584</td>
<td>-0.028</td>
<td>0.180</td>
<td>-0.346</td>
<td>-0.078</td>
<td>0.878</td>
<td>0.047</td>
<td>0.004</td>
<td>0.002</td>
<td>0.031</td>
<td>0.209</td>
<td>0.026</td>
<td>0.655</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NA</td>
<td>0.428</td>
<td>0.125</td>
<td>0.587</td>
<td>&lt;0.0001</td>
<td>0.004</td>
<td>&lt;0.0001</td>
<td>0.127</td>
<td>0.003</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>0.772</td>
<td>0.063</td>
<td>0.109</td>
<td>0.063</td>
<td>&lt;0.0001</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>Arachidic</td>
<td>0.549</td>
<td>0.423</td>
<td>0.247</td>
<td>-0.058</td>
<td>0.047</td>
<td>0.161</td>
<td>0.453</td>
<td>0.064</td>
<td>-0.046</td>
<td>0.014</td>
<td>0.111</td>
<td>0.915</td>
<td>0.331</td>
<td>0.422</td>
<td>0.014</td>
<td>0.422</td>
<td></td>
</tr>
<tr>
<td>Behenic</td>
<td>0.666</td>
<td>0.310</td>
<td>-0.199</td>
<td>-0.225</td>
<td>0.117</td>
<td>0.239</td>
<td>-0.156</td>
<td>0.011</td>
<td>0.094</td>
<td>0.092</td>
<td>-0.192</td>
<td>-0.236</td>
<td>-0.010</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Lignoceric</td>
<td>0.192</td>
<td>0.001</td>
<td>0.041</td>
<td>0.231</td>
<td>0.013</td>
<td>0.111</td>
<td>0.914</td>
<td>0.340</td>
<td>0.348</td>
<td>0.048</td>
<td>0.155</td>
<td>0.0000</td>
<td>0.015</td>
<td>0.918</td>
<td>0.0000</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Total SFA</td>
<td>0.049</td>
<td>0.400</td>
<td>0.932</td>
<td>0.109</td>
<td>0.005</td>
<td>0.800</td>
<td>0.746</td>
<td>0.938</td>
<td>0.878</td>
<td>0.434</td>
<td>0.958</td>
<td>0.947</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POA</td>
<td>0.338</td>
<td>0.002</td>
<td>0.007</td>
<td>0.004</td>
<td>0.0003</td>
<td>0.262</td>
<td>0.531</td>
<td>0.445</td>
<td>0.991</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>OA</td>
<td>0.634</td>
<td>-0.088</td>
<td>-0.239</td>
<td>0.598</td>
<td>-0.173</td>
<td>-0.275</td>
<td>-0.273</td>
<td>0.988</td>
<td>0.572</td>
<td>-0.037</td>
<td>0.704</td>
<td>0.704</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eicosenoic</td>
<td>0.019</td>
<td>0.917</td>
<td>&lt;0.0001</td>
<td>0.957</td>
<td>0.089</td>
<td>0.127</td>
<td>&lt;0.0001</td>
<td>0.014</td>
<td>0.014</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>0.255</td>
<td>0.336</td>
<td>-0.151</td>
<td>0.191</td>
<td>0.166</td>
<td>-0.044</td>
<td>0.205</td>
<td>0.424</td>
<td>0.008</td>
<td>0.0004</td>
<td>0.0004</td>
<td>0.0004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elaidic</td>
<td>0.008</td>
<td>0.0004</td>
<td>0.122</td>
<td>0.050</td>
<td>0.089</td>
<td>0.657</td>
<td>0.035</td>
<td>&lt;0.0001</td>
<td>0.006</td>
<td>0.012</td>
<td>0.012</td>
<td>0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cis-MUFA</td>
<td>0.244</td>
<td>-0.028</td>
<td>-0.074</td>
<td>-0.062</td>
<td>-0.210</td>
<td>0.155</td>
<td>0.016</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitelaidic</td>
<td>0.012</td>
<td>0.777</td>
<td>0.451</td>
<td>0.528</td>
<td>0.031</td>
<td>0.112</td>
<td>0.874</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elaidic</td>
<td>0.338</td>
<td>0.463</td>
<td>-0.173</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>0.076</td>
<td>0.868</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Trans-MUFA</td>
<td>0.614</td>
<td>0.086</td>
<td>0.120</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.076</td>
<td>0.382</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCD n-7</td>
<td>0.592</td>
<td>0.666</td>
<td>&lt;0.0001</td>
<td>0.159</td>
<td>0.103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCD n-9</td>
<td>0.592</td>
<td>0.666</td>
<td>&lt;0.0001</td>
<td>0.159</td>
<td>0.103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Correlations 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. EAE, enzyme activity estimate; NA, nervonic acid; OA, oleic acid; PA, palmitic acid; SA, stearic acid; SFA, saturated fatty acids; SCD n-7, stearoyl-CoA desaturase n-7 EAE; SCDn-9, stearoyl-CoA desaturase n-9 EAE. Elov-6: elongation of very long chain fatty acids-6 EAE.

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

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

Total Trans-MUFA calculated as the Σ palmitelaidic + elaidic.

SCDn-7 calculated as the ratio of POA / PA.

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

Elov-6 calculated as the ratio of Σ [ SA + OA ] / PA.
Table 3. Association of fatty acids and enzyme activity estimates, as continuous variables, with having adenomas relative to no colon polyps a

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
<th>Model 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P-value</td>
<td>OR (95%CI)</td>
<td>P-value</td>
<td>OR (95%CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>C16:0 Palmitic (PA)</td>
<td>0.830 (0.701, 0.982)</td>
<td>0.0303</td>
<td>0.718 (0.582, 0.886)</td>
<td>0.0200</td>
<td>0.756 (0.623, 0.917)</td>
<td>0.0045</td>
</tr>
<tr>
<td>C18:0 Stearic (SA)</td>
<td>0.973 (0.806, 1.174)</td>
<td>0.7737</td>
<td>0.917 (0.748, 1.123)</td>
<td>0.4008</td>
<td>0.914 (0.747, 1.119)</td>
<td>0.3851</td>
</tr>
<tr>
<td>C20:0 Arachidic</td>
<td>0.901 (0.058, 13.943)</td>
<td>0.9403</td>
<td>1.053 (0.058, 19.211)</td>
<td>0.9721</td>
<td>0.978 (0.059, 16.274)</td>
<td>0.9877</td>
</tr>
<tr>
<td>C21:0 Behenic</td>
<td>0.923 (0.400, 2.131)</td>
<td>0.8504</td>
<td>0.916 (0.383, 2.196)</td>
<td>0.8449</td>
<td>0.936 (0.400, 2.188)</td>
<td>0.8778</td>
</tr>
<tr>
<td>C24:0 Lignoceric</td>
<td>0.732 (0.306, 1.754)</td>
<td>0.4842</td>
<td>0.925 (0.366, 2.335)</td>
<td>0.8686</td>
<td>0.848 (0.345, 2.085)</td>
<td>0.7192</td>
</tr>
<tr>
<td>Total SFA b</td>
<td>0.902 (0.803, 1.013)</td>
<td>0.0811</td>
<td>0.837 (0.730, 0.960)</td>
<td>0.0112</td>
<td>0.847 (0.742, 0.967)</td>
<td>0.0144</td>
</tr>
<tr>
<td>C16:1 Palmitoleic (POA)</td>
<td>3.750 (1.079, 13.036)</td>
<td>0.0376</td>
<td>2.442 (0.679, 8.783)</td>
<td>0.1716</td>
<td>2.652 (0.739, 9.519)</td>
<td>0.1347</td>
</tr>
<tr>
<td>C18:1 Oleic (OA)</td>
<td>1.097 (0.981, 1.226)</td>
<td>0.1048</td>
<td>1.106 (0.983, 1.245)</td>
<td>0.0932</td>
<td>1.103 (0.981, 1.239)</td>
<td>0.1002</td>
</tr>
<tr>
<td>C20:1 Eicosenoic</td>
<td>1.020 (0.995, 1.045)</td>
<td>0.1161</td>
<td>1.023 (0.997, 1.050)</td>
<td>0.0794</td>
<td>1.024 (0.998, 1.051)</td>
<td>0.0741</td>
</tr>
<tr>
<td>C24:1 Nervonic (NA)</td>
<td>0.568 (0.260, 1.243)</td>
<td>0.1570</td>
<td>0.743 (0.327, 1.690)</td>
<td>0.4792</td>
<td>0.745 (0.329, 1.686)</td>
<td>0.4794</td>
</tr>
<tr>
<td>Total Cis-MUFA c</td>
<td>1.088 (0.981, 1.206)</td>
<td>0.1103</td>
<td>1.099 (0.985, 1.226)</td>
<td>0.0901</td>
<td>1.100 (0.987, 1.226)</td>
<td>0.0864</td>
</tr>
<tr>
<td>C16:1 Palmitelaidic d</td>
<td>0.982 (0.894, 1.078)</td>
<td>0.7017</td>
<td>1.013 (0.916, 1.119)</td>
<td>0.8073</td>
<td>1.006 (0.912, 1.108)</td>
<td>0.9116</td>
</tr>
<tr>
<td>C18:1 Elaidic</td>
<td>2.915 (1.030, 8.246)</td>
<td>0.0438</td>
<td>3.111 (1.031, 9.388)</td>
<td>0.0440</td>
<td>3.224 (1.060, 9.801)</td>
<td>0.0391</td>
</tr>
<tr>
<td>Total Trans-MUFA e</td>
<td>2.708 (1.000, 7.337)</td>
<td>0.0501</td>
<td>2.990 (1.029, 8.687)</td>
<td>0.0441</td>
<td>3.066 (1.050, 8.955)</td>
<td>0.0405</td>
</tr>
<tr>
<td>SCD n-7 d,f</td>
<td>1.538 (1.068, 2.215)</td>
<td>0.0207</td>
<td>1.383 (0.960, 1.992)</td>
<td>0.0819</td>
<td>1.410 (0.977, 2.035)</td>
<td>0.0664</td>
</tr>
<tr>
<td>SCD n-9 g</td>
<td>2.229 (0.724, 6.864)</td>
<td>0.1623</td>
<td>2.846 (0.850, 9.534)</td>
<td>0.0899</td>
<td>2.739 (0.822, 9.132)</td>
<td>0.1010</td>
</tr>
<tr>
<td>Elovl-6 d,h</td>
<td>1.358 (1.039, 1.775)</td>
<td>0.0250</td>
<td>1.467 (1.090, 1.973)</td>
<td>0.0114</td>
<td>1.405 (1.059, 1.865)</td>
<td>0.0184</td>
</tr>
</tbody>
</table>

a Models defined as: Model 1: adenoma = fatty acid + age + smoking. Model 2: adenoma = fatty acid + age + smoking + BMI. Model 3: adenoma = fatty acid + age + smoking + waist circumference. Fatty acids expressed as percent of total phospholipids. P-values bolded if significant (p ≤ 0.05) and italicized if 0.05 ≥ p ≥ 0.09. EAE, enzyme activity estimate; MUFA, monounsaturated fatty acid; SFA, saturated fatty acids; SCDn-7, stearoyl-CoA desaturase n-7 EAE; SCDn-9, stearoyl-CoA desaturase n-9 EAE; Elovl-6, elongation of very long chain fatty acids-6 EAE.

b Total SFA calculated as the ∑ PA+ SA + arachidic + behenic + lignoceric.

c Total Cis-MUFA calculated as the ∑ POA+ SA + arachidic + behenic + eicosenoic + NA.

d Odds ratios 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.

e Trans-MUFA calculated as the ∑ palmitelaidic + elaidic.

f SCDn-7 calculated as the ratio of POA / PA.

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

h Elovl-6 calculated as the ratio of ∑ [SA+ OA] / PA.
FIGURE LEGEND:

Figure 1. Fatty acid (FA) content of plasma phospholipids (PPLs). (A) Saturated FAs (SatFAs), (B) Cis-monounsaturated FAs (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 percent of total PPL FAs. A “*” indicates p ≤ 0.05, calculated by Wilcoxon-Mann-Whitney nonparametric U-test. NA, nervonic acid; OA, oleic acid; PA, palmitic acid; POA, palmitoleic acid; SA, stearic acid.

Figure 2. Associations of plasma phospholipid (PPL) fatty acid and enzyme activity estimates (EAEs), as tertiles, with having adenomas relative to no colon polyps. (A) Palmitic acid (PA), (B) ELOVL-6, and (C) SCDn-7. The symbol “■” represent the odds ratio and error bars indicate lower and upper confidence intervals, respectively. Both test for exposure and test for trend models adjusted for age and smoking. PA is expressed as a percent of total PPL FAs. Elovl-6, elongation of very long chain fatty acids-6 EAE; PA, palmitic acid; SCDn-7, stearoyl-CoA desaturase n-7 EAE.

Figure 3. Relationship between cellular fatty acid (FA) metabolic pathways and observed associations of FAs and enzyme activity estimates (EAEs) with colorectal adenomas. (A) Lipoproteins and exosomes are the most abundant sources of plasma phospholipid (PPL) FAs. (B) Increased fatty acid synthase (FAS) increases intracellular saturated fatty acids (SFAs). These SFAs have lipotoxic effects causing stearoyl-COA desaturase-1 (SCD-1) expression to increase. Higher concentrations of SFAs and expression SCD-1, increase monounsaturated FA (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 EAEs 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 to having no colon polyps. A “–“ indicates no observed association. Elovl-6, elongation of very long chain fatty acids-6 EAE; ER, endoplasmic reticulum; SCDn-7, stearoyl-CoA desaturase n-7 EAE; SCDn-9, stearoyl-CoA desaturase n-9 EAE.
Figure 1

A.

Saturated FAs

% of total PPL

PA SA Arachidic Behenic Lignoceric

× no polyp □ polyp

Downloaded from cebp.aacrjournals.org on April 28, 2017. © 2015 American Association for Cancer Research.
Figure 1 C.

trans-MUFAs

% of total PPL

Elaidic  Palmitelaidic

no polyp  polyp
Figure 2

A. 

\[ \text{OR_{wind} = 0.43} \]
\[ P_{\text{wind}} = 0.0028 \]

\[
\begin{align*}
\text{PPL PA} \\
\leq 28.12 & > 28.12 \text{ to } \leq 30.21 & > 30.25
\end{align*}
\]

B. 

\[ \text{OR_{read} = 1.79} \]
\[ P_{\text{read}} = 0.0290 \]

\[
\begin{align*}
\text{PPL SCDn-7} \\
\leq 0.01 & > 0.01 \text{ to } \leq 0.02 & > 0.02
\end{align*}
\]

C. 

\[ \text{OR_{wind} = 2.01} \]
\[ P_{\text{wind}} = 0.0104 \]

\[
\begin{align*}
\text{PPL Elowd-6} \\
\leq 0.79 & > 0.79 \text{ to } \leq 0.89 & > 0.89
\end{align*}
\]
A. Sources of phospholipids in plasma

Plasma Lipoproteins

Exosomes

B. Altered SFA and MUFA metabolism in adenomas

↑ FAS → ↑ Satfat → ↑ MUFA → ↑ PL Incorporation

↑ SCD-1 → ↑ Cell growth

↑ Lipotoxicity → ↑ ER Stress → ↑ Carcinogenesis

C. Plasma phospholipid fatty acids and enzyme activity estimates associated with colon adenomas

↓ Palmitic (C16:0) → ↑ SCD n-7 → ↑ Palmitoleic (C16:1)

↑ Elovl-6 → ↓ Stearic (C18:0) → ↓ SCD n-9 → ↓ Oleic (C18:1)

Further Elongation

Further Elongation
Altered saturated and monounsaturated plasma phospholipid fatty acid profiles in adult males with colon adenomas

C. Austin Pickens, Ami Lane-Elliot, Sarah S. Comstock, et al.

Cancer Epidemiol Biomarkers Prev Published OnlineFirst December 31, 2015.