Long-chain ω-6 plasma phospholipid polyunsaturated fatty acids and association with colon adenomas in adult men: a cross-sectional study

Charles A. Pickens, Mariana de Fátima Albuquerque Pereira and Jenifer I. Fenton

Dietary lipid intake can be associated with an increased risk for colorectal cancer depending on its composition. Carcinogenesis alters lipid metabolism to facilitate cell growth and survival. For instance, metabolites of polyunsaturated fatty acids (PUFAs) are associated with increasing colon cell proliferation. Moreover, precancerous colon lesions (i.e. adenomas) increase the risk for colorectal cancer. In this study, we investigated associations between plasma PUFAs and the number of colon polyps and polyp type (i.e. hyperplastic and adenoma). Healthy male participants ($n = 126$) of 48–65 years of age were recruited before a routine colonoscopy screening. Plasma phospholipid (PPL) PUFAs were isolated by means of solid phase extraction and methylated. Fatty acid methyl esters were analyzed using gas chromatography. Factor analysis was used to cluster PUFAs into groups, and then generated factors and individual PUFAs were analyzed using polytomous logistic regression. In our age-adjusted and smoking-adjusted polytomous logistic regression, for each unit increase in PPL docosatetraenoic acid (DTA), individuals were 1.43 (1.00–2.06) and 1.33 (0.99–1.80) times more likely to have hyperplastic polyps and adenomas rather than no polyps, respectively. In our factor analysis, high PPL ω-6 PUFA and trans-fatty acid loading scores were associated with increased odds of adenoma presence rather than no polyps. Increases in long-chain PPL ω-6 PUFAs are associated with an increased risk for adenomas. As relative levels of DTA increase in PPLs, individuals had increased odds of having hyperplastic polyps and adenomas. Elevated conversion of ω-6 PUFAs to longer-chain ω-6s such as DTA may indicate altered PUFA metabolism at the tissue level. ***European Journal of Cancer Prevention*** **00:000–000 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.**

European Journal of Cancer Prevention 2016, **00**:000–000

Keywords: adenoma, colon, colon polyps, fatty acids, males, phospholipids, polyunsaturated fatty acids

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan, USA

Correspondence to Jenifer I. Fenton, PhD, MPH, MS, Department of Food Science and Human Nutrition, Michigan State University, 469 Wilson Road, Room 208B, East Lansing, MI 48824, USA
Tel: +1 517 353 3342; fax: +1 517 353 8963; e-mail: imigjeni@msu.edu

Received 31 December 2015 **Accepted** 31 August 2016

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the USA and the fourth most common cancer worldwide (Shim *et al.*, 2005). Factors associated with increased CRC risk include diet, age, smoking, and colon polyps (Haggar and Boushey, 2009). In particular, dietary lipid intake is suggested to play a major role in colorectal carcinogenesis (Methy *et al.*, 2008), and westernized dietary patterns are associated with an increased risk for CRC (Yusof *et al.*, 2012). For instance, westernized diets are higher in dietary trans-fats (Thiebaut *et al.*, 2009; Trattner *et al.*, 2015), and trans-fat intake is positively associated with both colon adenomas (Vinikoor *et al.*, 2008) and CRC (Valenzuela and Morgado, 1999). However, several studies report that low-fat diets do not significantly decrease CRC risk (Williams *et al.*, 2010), including a randomized control intervention study (Beresford *et al.*, 2006). Recently, Habermann *et al.* (2013) reported an interaction between dietary fat intake, CRC risk, and single nucleotide polymorphisms in genes involved in polyunsaturated fatty acid (PUFA) metabolism. Increased intake of certain PUFAs are associated with an increased risk for CRC (Murff *et al.*, 2012), but the results of human studies on PUFA intake and CRC risk are often inconsistent (reviewed in detail by Dommels *et al.*, 2002). Few researchers have investigated associations between specific blood PUFA levels and precancerous lesions such as colon adenomas (Pot *et al.*, 2008; Murff *et al.*, 2012). Instead, most studies investigate associations between PUFAs and CRC related to different fat source intakes, which influence cell growth and survival (Yang *et al.*, 2015). Although the exact mechanism of dietary fat intake and CRC development is not well understood, the metabolites of dietary PUFAs may influence CRC progression (McEntee and Whelan, 2002; Larsson *et al.*, 2004).

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (*www.eurjcancerprev.com*).

DOI: 10.1097/CEJ.0000000000000312

PUFAs are classified as either ω-3 or ω-6. In general, ω-3 PUFAs are considered more 'anti-inflammatory-like', whereas ω-6 PUFAs are considered more 'proinflammatory-like' (Pickens *et al.*, 2015b). Neither ω-3 nor ω-6 essential PUFAs are synthesized by humans; thus, they are only obtained through dietary intake. In clinical trials, ω-3 supplementation reduced CRC proliferation and the risk for colorectal adenomas (CRA) (Peipins and Sandler, 2005). In addition, ω-3 PUFA intake reduces the risk for both CRA and CRC in animal models (Mandir and Goodlad, 2008; Fini *et al.*, 2010). The mechanism through which ω-3 PUFAs decrease CRC risk involves several signaling pathways (reviewed in detail by McEntee and Whelan, 2002). In particular, ω-3 PUFAs can inhibit delta-6-desaturase (D6D) and cyclooxygenase (COX) (McEntee and Whelan, 2002), reduce tissue arachidonic acid (ARA) (Larsson *et al.*, 2004), and reduce ARA eicosanoid biosynthesis (Kinsella *et al.*, 1990).

In contrast to ω-3 PUFAs, ω-6 PUFAs such as linoleic acid (LA) (Whelan and McEntee, 2004), dihomo-γ linolenic acid (DGLA) (Giuliani *et al.*, 2014), and ARA (Murff *et al.*, 2012) may act as tumor promoters. ω-6 PUFAs influence the production of proinflammatory cytokines and prostaglandins (Tokudome *et al.*, 2015). Their biosynthesis begins with the desaturation of LA to form DGLA by D6D, and DGLA is then desaturated by delta-5-desaturase (D5D) to form ARA (Kinsella *et al.*, 1990). ARA is the substrate for enzymatic oxygenation for conversion to prostaglandins (e.g. PGE2). PGE2 in particular may contribute to the growth of intestinal cancer cells (reviewed in detail by Susanna *et al.*, 2004). Inhibiting ARA enzymatic transformation by COX and D6D activity can decrease the risk for CRA and CRC development (reviewed in detail by Whelan, 2002). Although there is inconsistent evidence that diet is associated with CRC risk, the associations between CRA and individual PUFAs are not well studied. The purpose of this study was to determine the association between plasma levels of individual PUFAs, adenomas, and the number of colon polyps present.

Participants and methods

Study population and clinical parameters

The study was approved by the Biomedical and Health Institutional Review Board of Michigan State University (IRB# 08-786). Healthy male participants ($n = 126$, > 96% Caucasian) of 48–65 years of age were enrolled between August 2009 and February 2011, as previously described (Comstock *et al.*, 2014b). In brief, individuals were recruited before undergoing a routine colonoscopy screening. These patients were reported as asymptomatic, and clinical metadata on each individual's comorbidities, family history, and medications were recorded. Individuals were excluded for medical conditions associated with increased CRC risk, as previously described (Comstock *et al.*, 2014b). In brief, the exclusion criteria were as follows: (i) presence of inflammatory bowel diseases, (ii) cancer in the previous 2 years, (iii) familial adenomatous polyposis, (iv) surgery in the previous 2 years, (v) type-1 and type-2 diabetes, and (vi) several autoimmune diseases. Immediately after enrollment, trained staff recorded anthropometric measurements and collected venous blood of study participants, as previously described (Comstock *et al.*, 2014b). In brief, BMI was assessed by recording patient's height using a stadiometer and weight using a digital platform scale. The plasma fraction was separated from whole blood and stored at −80°C. Smoking status was assessed as 'ever smoked' or 'never smoked'. Each individual received a full colonoscopy as previously described (Comstock *et al.*, 2014c). In brief, polyps were identified and removed by a gastroenterologist, and classified by a pathologist.

Plasma phospholipid extraction, isolation, and analysis

Approximately 200 mg plasma per participant was weighed and extracted using a modified Rose and Oaklander extraction method (Rose and Oklander, 1965). PLs were isolated using Isolute-XL SPE aminopropyl columns (500 mg; Bioatage, Charlotte, North Carolina, USA), as described by Agren *et al.* (1992). Fatty acid methyl esters (FAMEs) were prepared as previously described (Burdge *et al.*, 2000; Pickens *et al.*, 2015b). Plasma phospholipid (PPL) FAMEs were analyzed using HS-Omega-3 Index methodology at OmegaQuant Analytics, LLC (Sioux Falls, South Dakota, USA), as previously described (Gurzell *et al.*, 2014).

Statistical analyses

This study and manuscript are a secondary analysis of a previously recruited cross-sectional study. Our study was powered (0.8) to detect differences in several PPL FAs, which include palmitic acid (previously reported by Pickens *et al.*, 2015a), through the package 'pwr' (Champely, 2015) in R v3.3.0 (Team, 2015), using the following functions: pwr.norm.test and pwr.anova.test. We applied an effect size of 0.5 [determined using PPL fatty acids (FA) data in our population] and used the following parameters: pwr.anova.test ($k = 3$, $f = 0.5$, significance level = 0.05, power = 0.8); and pwr.norm.test ($d = 0.5$, significance level = 0.05, power = 0.8, alternative = 'greater').

Frequencies, medians, and SDs were calculated for descriptive analyses (Tables 1 and 2). Each PUFA was expressed as a percentage of total PPL, to allow for cross-study comparison of our results, as a vast majority of epidemiological studies (i.e. outside of Japan) also analyze their data as a percent of total FA (i.e. whole plasma, PPL, plasma cholesterol esters, and red blood cell or whole blood) (Harris *et al.*, 2013; Wu *et al.*, 2013; Newman *et al.*, 2014; Baack *et al.*, 2015; Bigornia *et al.*, 2016). Median and quartile values were obtained for the PPLs (Tables 3 and 4). PPL PUFA enzyme activity estimates (EAE) were calculated as the ratio of product/substrate.

The following variables were calculated and used in our analyses: D5D EAE was calculated as the ratio of ARA/DGLA; D6D EAE was calculated as the ratio of DGLA/LA; eicosapentaenoic acid (EPA) + DHA, herein referred to as EPADHA, was calculated as $\sum$EPA + DHA.

In all statistical tests, PUFAs were analyzed as continuous independent variables, and polyps as categorical dependent variables. Patients were categorized based on polyp type as follows: (i) individuals with no colon polyps, (ii) individuals with at least one hyperplastic polyp and less than one adenoma, and (iii) individuals with at least one adenoma. Patients were categorized based on polyp number as follows: (i) individuals with no colon polyps, (ii) individuals with one colon polyp, (iii) individuals with two colon polyps, and (iv) individuals with at least three colon polyps. Statistical differences in PUFA levels, between polyp type (Table 3) and polyp number (Table 4), were determined using Kruskal–Wallis one-way analysis of variance. Spearman correlations were performed to evaluate the relationship between PUFAS and colon polyps (Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/EJCP/A120*).

Many PUFAs are collinear and cannot be analyzed together as independent variables in regression models; therefore, we used a factor analysis to analyze PUFAs as groups. FA biomarker patterns were generated using factor analysis (Proc Factor, SAS; SAS, Cary, North Carolina, USA), as previously described (Comstock *et al.*, 2014b). In brief, four factors were determined based on the scree plot (Ledesma *et al.*, 2015) and eigenvalues of more than 1 (Kaiser, 1960). The FAME analysis conducted using OmegaQuant Analytics, LLC (Sioux Falls, South Dakota, USA), routinely quantifies 24 FAs that are reported in numerous epidemiological studies and are associated with dietary intake and FA metabolism. Our laboratory has previously reported that saturated and monounsaturated FAs are associated with colon adenomas (Pickens *et al.*, 2015a). In our current study, we report the following PUFAs: LA, linoelaidic, γ-linolenic, DGLA, ARA, DTA, DPA ω-6, ALA, EPA, DPA ω-3, and DHA. However, to reduce bias in the factor analysis, all 24 FAs analyzed were included in factor generation. Next, FAs were removed from the factor analysis only when a FA's correlations were less than 0.3, in the factor-loading matrix, across the four factors (Table 5). Proc Factor assigns each person a factor-loading score for each of the four factors from the data. The Proc Factor option Varimax was specified for orthogonal transformation of factor-loading matrix.

Polytomous logistic regression models for categorical outcome data were used to determine odds ratios (OR) and 95% confidence intervals for polyp type (Tables 6–8) and polyp number (Supplementary Table 2, Supplemental digital content 2, *http://links.lww.com/EJCP/A121*). In all logistic regression models, polyp type (Tables 7 and 8) and polyp number (Supplementary Table 2, Supplemental digital content 2, *http://links.lww.com/EJCP/A121*) were referenced against individuals with no colon polyps. Factor-loading scores (Tables 5 and 6) and PPL PUFAs and EAEs were analyzed as continuous independent variables in logistic regression models. All models were adjusted for age and smoking status except where noted. The logistic regression models were defined as follows: (i) polyp type = age + smoking + FAs, EAEs, or factor-loading scores; or (ii) polyp number = age + smoking + FAs, EAEs, or factor-loading scores. Age was selected as a confounder as age is a risk factor for polyps and colorectal cancer risk (reviewed in detail by Grahn and Varma, 2008), and smoking was selected as a confounder as smoking is associated with colorectal adenomas (Kikendall *et al.*, 1989; Monnet *et al.*, 1991; Zahm *et al.*, 1991; Peipins and Sandler, 1994). BMI, waist circumference (WC), and serum adipokines and cytokines were not included as confounders in our models, because we have previously shown that BMI and WC were highly correlated to several PPL PUFAs (Pickens *et al.*, 2015b), and we have also shown that PPL PUFAs are highly associated with adipokines and cytokines and both BMI and WC (Pickens *et al.*, 2016). Thus, mutual adjustment of collinear factors such as BMI, WC, and adipokines or cytokines could lead to unreliable and unstable estimates of regression coefficients.

There was ~17% missing smoking data due to incomplete data in clinical records, and therefore missing smoking data were imputed in regression analyses. Multiple imputation (Proc MI, seed = 20 121 119, imputations = 7) was used to impute all missing smoking data (Yang, 2011). The multiple imputation procedure applied was the Markov Chain Monte Carlo method using multiple chained equations. Because imputation was used, multiple imputation analysis (Proc MI Analyze) was used to determine the results from analysis of the imputed datasets. In all logistic regression analyses, the odds ratios for linoelaidic, DTA, DPA ω-6, and D6D were calculated on the basis that there is a unit change of 0.1 of the respective β coefficient for each given parameter. *P*-values were considered statistically significant if $P \leq 0.05$ and a statistical trend if $0.05 < P \leq 0.09$. Statistical analyses, excluding power analyses (R, version 3.3.0), were conducted using software SAS, version 9.4 (SAS).

Results

Participant and polyp characteristics

The median age was 58 years and 31% of the participants ever smoked (Tables 1 and 2). As previously reported (Comstock *et al.*, 2014b), 37 (29.4%) participants had adenomas, 20 (15.9%) had hyperplastic polyps, and 69 (54.8%) had no polyps (Table 1). Both BMI and WC increased with polyp type (Table 1) and polyp number

Table 1 Characteristics of individuals by polyp type[a]

Parameters	Overall ($n=126$)	No polyps ($n=69$)	Any polyps ($n=57$)	Hyperplastic ($n=20$)	Adenoma ($n=37$)
Age (years)	58 (53–61)	57 (53–61)	58 (53–60)	58 (53–60)	58 (53–60)
Smoker (% total)	31	15	15	4	12
BMI (kg/m^2)	29 (26–33)	28 (24–31)	31 (28–34)	28 (26–32)	32 (29–36)
WC (in.)	41 (37–46)	40 (36–45)	42 (40–47)	41 (38–45)	43 (40–49)

WC, waist circumference.
[a]Values expressed as median (Q1,Q3).

Table 2 Characteristics of individuals by polyp number categories[a]

Parameters	Overall ($n=126$)	1 Polyp ($n=23$)	2 Polyps ($n=11$)	≥3 Polyps ($n=23$)
Age (years)	58 (53–61)	58 (53–60)	58(52–61)	57 (52–60)
Smoker (% total)	31	4	4	8
BMI (kg/m^2)	29 (26–33)	30 (26–34)	29 (25–32)	32 (28–37)
WC (in.)	41 (37–46)	41 (38–45)	43 (40–48)	45 (40–47)
Hyperplastic (%)	–	43.48	36.36	26.09
Adenoma (%)	–	56.52	63.64	73.91

WC, waist circumference.
[a]Values expressed as median (Q1,Q3).

Table 3 Polyp type is associated with the percentage of n-6 plasma phospholipid polyunsaturated fatty acids[a]

Variables	No Polyp	Hyperplastic	Adenoma	*P*-value
C18:2-c LA	19.83 (17.79–22.79)	18.86 (15.77–21.42)	18.95 (16.76–21.19)	0.111
C18:2-t linoelaidic	0.23 (0.18–0.29)	0.28 (0.23–0.39)	0.30 (0.21–0.40)	*0.064*
C20:3 DGLA	2.56 (1.96–3.21)	2.58 (2.39–3.31)	2.88 (2.16–3.45)	0.484
C20:4 ARA	9.82 (8.64–10.99)	11.60 (9.48–12.72)	10.65 (9.17–12.68)	0.106
C22:4 DTA	0.34 (0.22–0.40)	0.41 (0.35–0.50)	0.37 (0.30–0.50)	**0.031**
C22:5 DPAω-6	0.21 (0.14–0.34)	0.29 (0.19–0.33)	0.26 (0.17–0.32)	0.277
C20:5 EPA	0.63 (0.45–1.07)	0.52 (0.38–0.81)	0.63 (0.45–0.76)	0.228
C22:5 DPAω-3	0.83 (0.65–0.96)	0.84 (0.67–1.08)	0.84 (0.62–1.02)	0.831
C22:6 DHA	2.65 (2.15–3.56)	2.86 (1.96–3.72)	2.72 (2.13–3.40)	0.915
EPADHA[b]	3.32 (2.74–4.65)	3.40 (2.41–4.55)	3.26 (2.63–4.15)	0.740
D5D[c]	3.74 (2.77–4.71)	4.56 (3.14–5.23)	3.41 (2.76–5.16)	0.534
D6D[d]	0.13 (0.10–0.18)	0.15 (0.11–0.19)	0.15 (0.12–0.18)	*0.089*

ANOVA, analysis of variance; ARA, arachidonic acid; D5D, delta-5-desaturase estimated enzyme activity; D6D, delta-6-desaturase estimated enzyme activity; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid.
[a]Fatty acids expressed as percent of total phospholipids. Values expressed as median (Q1,Q3). Kruskal-Wallis one-way nonparametric ANOVA. *P*-values bolded if $P \leq 0.05$ and italicized if $0.05 < P \leq 0.09$.
[b]EPADHA was calculated as $\sum$EPA + DHA.
[c]D5D EAE was calculated as the ratio of ARA/DGLA.
[d]D6D EAE was calculated as the ratio of DGLA/LA.

(Table 2), as previously reported (Comstock *et al.*, 2014b). As the number of colon polyps increased, so did the percentage of participants having at least one adenoma (Table 2).

PPL PUFA proportions and ANOVA results

DTA and linoelaidic were the only ω-6 PUFAs associated with polyp type categories (Table 3). PPL DTA differed significantly across polyp types ($P < 0.05$). There tended to be a difference in PPL linoelaidic ($P = 0.064$) and the D6D EAE ($P = 0.089$) across polyp types. PPL ω-3s were not associated with polyp type or polyp number. Several PPL ω-6s were associated with polyp number (Table 4). There was a significant difference in PPL LA across polyp number categories ($P < 0.05$). PPL DTA tended to differ across polyp number ($P = 0.073$).

Polyps are significantly correlated with PPL PUFAs and EAEs

Polyp number was significantly correlated with PPL linoelaidic, DTA, and D6D EAEs (Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/EJCP/A120*). PPL ARA tended to be correlated with polyp number ($P = 0.079$). Polyp type was correlated to similar PPL PUFAs and EAEs, which were correlated to polyp number. Specifically, polyp type was significantly correlated with PPL linoelaidic, DTA, and the D6D EAE. Polyp type was not associated with ARA. However,

Table 4 Polyp number is associated with the percentage of n-6 plasma phospholipid polyunsaturated fatty acids[a]

Variables	No Polyp	1 Polyp	2 Polyps	≥3 Polyps	*P*-value
C18:2-c LA	19.83 (17.79–22.79)	17.53 (16.07–20.68)	19.16 (17.42–19.64)	20.30 (16.83–23.51)	**0.035**
C18:2-t Linoelaidic	0.23 (0.18–0.29)	0.28 (0.19–0.34)	0.33 (0.23–0.52)	0.30 (0.19–0.45)	*0.099*
C20:3 DGLA	2.56 (1.96–3.21)	2.61 (2.27–3.49)	2.53 (2.16–2.95)	3.06 (2.15–3.53)	0.512
C20:4 ARA	9.82 (8.64–10.99)	10.90 (7.96–13.95)	10.16 (9.03–14.29)	10.35 (9.27–12.51)	0.251
C22:4 DTA	0.34 (0.22–0.40)	0.40 (0.30–0.47)	0.39 (0.34–0.52)	0.38 (0.27–0.50)	*0.073*
C22:5 DPAω-6	0.21 (0.14–0.34)	0.28 (0.24–0.34)	0.25 (0.16–0.31)	0.26 (0.16–0.33)	0.322
C20:5 EPA	0.63 (0.45–1.07)	0.54 (0.43–0.88)	0.63 (0.42–1.01)	0.59 (0.35–0.76)	0.510
C22:5 DPAω-3	0.83 (0.65–0.96)	0.84 (0.62–1.06)	0.83 (0.54–1.12)	0.84 (0.66–1.03)	0.997
C22:6 DHA	2.65 (2.15–3.56)	2.74 (2.07–4.10)	2.75 (2.13–3.24)	2.72 (2.04–3.40)	0.890
EPADHA[b]	3.32 (2.74–4.65)	3.39 (2.53–4.77)	3.43 (3.16–3.96)	3.26 (2.42–4.15)	0.806
D5D[c]	3.74 (2.77–4.71)	3.94 (2.76–5.35)	3.50 (3.40–5.71)	3.17 (2.67–4.93)	0.761
D6D[d]	0.13 (0.10–0.18)	0.15 (0.11–021)	0.13 (0.11–0.18)	0.15 (0.11–0.18)	0.177

ANOVA, analysis of variance; ARA, arachidonic acid; D5D, delta-5-desaturase estimated enzyme activity; D6D, delta-6-desaturase estimated enzyme activity; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid.
[a]Fatty acids expressed as percent of total phospholipids. Values expressed as median (Q1,Q3). Kruskal-Wallis one-way nonparametric ANOVA. *P*-values bolded if $P \leq 0.05$ and italicized if $0.05 < P \leq 0.09$.
[b]EPADHA was calculated as ∑EPA + DHA.
[c]D5D EAE was calculated as the ratio of ARA/DGLA.
[d]D6D EAE was calculated as the ratio of DGLA/LA.

Table 5 Polyunsaturated fatty acids are highly correlated with factor-loading matrix[a]

Fatty acids	ω-3 PUFA	ω-6 PUFA	Trans-FA	VLC SatFA
Adenomas are associated with ω-6 and trans-fatty acid factors				
EPADHA	**0.88**	−0.29	−0.05	−0.04
DHA	**0.70**	−0.24	−0.04	0.01
EPA	**0.61**	−0.52	−0.10	−0.11
Nervonic	**0.66**	−0.08	0.13	0.46
DPA3	**0.48**	0.02	−0.17	−0.05
Docosatetraenoic	−0.14	**0.86**	0.00	0.09
DPA6	−0.12	**0.79**	0.02	−0.03
ARA	0.14	**0.65**	0.01	0.07
DGLA	−0.02	**0.60**	−0.17	−0.08
Linoelaidic	−0.04	−0.10	**0.34**	0.04
Eicosenoic	0.14	−0.15	**0.85**	0.06
Elaidic	−0.11	0.11	**0.81**	−0.05
Behenic	−0.21	−0.07	−0.04	**0.83**
Lignoceric	0.04	0.09	−0.11	**0.78**
Arachidic	0.01	0.05	0.18	**0.78**
Stearic	−0.57	−0.01	0.00	0.18
Palmitic	−0.60	−0.15	−0.20	−0.07
Variance explained by each factor[b]	2.35	2.55	2.45	2.22

ARA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; VLC SatFA, very long chain saturated fatty acid.
[a]Varimax rotated factor-loading matrix generated by (Proc Factor, SAS v9.4). Numbers listed under each factor correspond with each respective FA correlation with each factors.
[b]Total variance explained by each factor, generated from communality estimates (Proc Factor, SAS).

Table 6 ω-6 and trans-fatty acid factors are significantly associated with adenomas[a]

Polyp severity	Factors	*P*-value	OR (95% CI)
Adenomas are associated with ω-6 and trans-fatty acid factors			
Adenomas	ω-3 PUFA	0.882	1.05 (0.63–1.53)
Hyperplastic	ω-3 PUFA	0.234	0.77 (0.48–1.25)
Adenomas	ω-6 PUFA	**0.034**	1.60 (1.04–2.46)
Hyperplastic	ω-6 PUFA	0.090	1.53 (0.93–2.72)
Adenomas	Trans-FA	**0.048**	1.57 (1.00–2.44)
Hyperplastic	Trans-FA	0.800	1.09 (0.56–2.11)

CI, confidence interval; FA, fatty acid; OR, odds ratio; PUFA, polyunsaturated fatty acid.
[a]Model defined as: polyp type = factor loadings.

Table 7 Hyperplastic polyps are associated with ω-6 polyunsaturated fatty acids[a,b]

Variables	Adenoma (OR 95% CI)	*P*-value
C18:2-c LA	0.86 (0.74–0.99)	**0.038**
C18:2-t linoelaidic[c]	1.06 (0.83–1.34)	0.646
C20:3 DGLA	1.08 (0.59–1.96)	0.812
C20:4 ARA	1.17 (0.97–1.41)	0.095
C22:4 DTA[c]	1.43 (1.00–2.06)	*0.052*
C22:5 DPA[c]	1.26 (0.87–1.83)	0.225
C20:5 EPA	0.27 (0.06–1.12)	*0.072*
C22:5 DPA	1.35 (0.40–4.61)	0.628
C22:6 DHA	0.96 (0.64–1.42)	0.821
EPADHA[d]	0.87 (0.64–1.19)	0.385
D5D[e]	1.10 (0.82–1.48)	0.522
D6D[c,f]	1.96 (0.77–5.02)	0.161

ARA, arachidonic acid; CI, confidence interval; D5D, delta-5-desaturase estimated enzyme activity; D6D, delta-6-desaturase estimated enzyme activity; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; OR, odds ratio.
[a]Fatty acids expressed as percent of total phospholipids. *P*-values bolded if $P \leq 0.05$ and italicized if $0.05 < P \leq 0.09$.
[b]Model defined as: polyp type = fatty acid + age + smoking.
[c]Odds ratios have been calculated on the basis that there is a unit change of 0.1 for the respective β coefficient for each given parameter.
[d]EPADHA was calculated as ∑EPA + DHA.
[e]D5D EAE was calculated as the ratio of ARA/DGLA.
[f]D6D EAE was calculated as the ratio of DGLA.

polyp type tended to be inversely correlated with LA ($P = 0.061$).

ω-6 PUFA and trans-FA factors are associated with adenoma presence

A factor analysis was performed to predict how FAs clustered into factors that may be predictive of polyp number or polyp type (Tables 5 and 6). The factor-loading matrix is presented in Table 5. The factor loadings indicate each factor's correlation with each individual FA. Four factors were named as follows: factor 1: ω-3 PUFA; factor 2: ω-6 PUFA; factor 3: trans-FA; and factor 4: very long (VLC) SatFAs. We excluded the logistic regression results from factor 4 loading scores as we have recently reported that saturated FAs are associated with

Table 8 Adenomas are associated with ω-6 polyunsaturated fatty acids[a,b]

Variables	Adenoma (OR 95% CI)	*P*-value
C18:2-c LA	0.91 (0.81–1.02)	0.121
C18:2-t linoelaidic[c]	1.17 (0.98–1.39)	*0.075*
C20:3 DGLA	1.24 (0.77–2.01)	0.376
C20:4 ARA	1.11 (0.95–1.29)	0.190
C22:4 DTA[c]	1.33 (0.99–1.80)	*0.062*
C22:5 DPA[c]	1.20 (0.87–1.64)	0.266
C20:5 EPA	0.70 (0.35–1.39)	0.310
C22:5 DPA	1.48 (0.55–3.99)	0.436
C22:6 DHA	0.97 (0.70–1.33)	0.836
EPADHA[d]	0.94 (0.74–1.19)	0.589
D5D[e]	0.99 (0.77–1.28)	0.945
D6D[c,f]	1.90 (0.88–4.14)	0.104

ARA, arachidonic acid; CI, confidence interval; D5D, delta-5-desaturase estimated enzyme activity; D6D, delta-6-desaturase estimated enzyme activity; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; OR, odds ratio.

[a]Fatty acids expressed as percent of total phospholipids. *P*-values bolded if $P \leq 0.05$ and italicized if $0.05 < P \leq 0.09$.

[b]Model defined as: polyp type = fatty acid + age + smoking.

[c]Odds ratios have been calculated on the basis that there is a unit change of 0.01 for the respective β coefficient for each given parameter.

[d]EPADHA was calculated as $\sum$EPA + DHA.

[e]D5D EAE was calculated as the ratio of ARA/DGLA.

[f]D6D EAE was calculated as the ratio of DGLA/LA.

colon polyps (Pickens *et al.*, 2015a). In our polyp number logistic regressions, polyp number was not significantly associated with ω-3 PUFA, ω-6 PUFA, or trans-FA factors (data not shown). In our polyp type logistic regressions, hyperplastic polyps were not significantly associated with ω-3 PUFA, ω-6 PUFA, or trans-FA loading scores (Table 6). However, ω-6 PUFA and trans-FA factor-loading scores were significantly associated with adenomas, compared with the no-polyps reference group (Table 6). A high ω-6 PUFA score was associated with increased odds of having an adenoma (OR: 1.60, 95% CI: 1.04–2.46) rather than no polyps. A high trans-FA score was also associated with increased odds of having an adenoma (OR: 1.57, 95% CI: 1.00–2.44) compared with the no-polyps reference group.

Polyps are associated with specific PUFAs and EAE in age-adjusted logistic regressions

Colon polyps and several PPL PUFAs and EAEs were correlated with confounding factors such as smoking (data not shown). Polytomous logistic regressions were used to adjust for age, and to determine which PUFAs and EAEs were significantly associated with polyp type (Tables 7 and 8) and polyp number (Supplementary Table 2, Supplemental digital content 2, *http://links.lww.com/EJCP/A121*). In our polyp type models, hyperplastic polyps were significantly associated with ω-6 PUFAs (Table 7). For each unit increase in LA, individuals were 0.86 (0.74–0.99) times as likely to have a hyperplastic polyp compared with the no-polyps reference group. For each unit increase in DTA, individuals were 1.43 (1.00–2.06) times as likely to have a hyperplastic polyp compared with the no-polyps reference group. PPL EPA was the only ω-3 associated with polyps. For each unit increase in EPA, individuals tended to be 0.27 (0.06–1.12) times as likely to have a hyperplastic polyp compared with the no-polyps reference group (Table 7). Adenomas were associated with ω-6 PUFAs in logistic regression analyses (Table 8). For each unit increase in linoelaidic and DTA, individuals tended to be 1.17 (0.98–1.39) and 1.33 (0.99–1.80) times more likely to have an adenoma compared with the no-polyps reference group, respectively.

Associations with polyp number and PPL PUFAs and EAEs are shown in Supplementary Table 2 (Supplemental digital content 2, *http://links.lww.com/EJCP/A121*). LA and DTA were the only PPL ω-6 PUFAs significantly associated with polyp number. For each unit increase in PPL LA, individuals were 0.83 (0.72–0.95) times as likely to have one polyp rather than no polyps. For each unit increase in PPL DTA, individuals were 1.43 (1.02–2.03) times more likely to have one polyp, and tended to be 1.53 (0.95–2.44) times more likely to have two polyps compared with the no-polyps reference group. For each unit increase in PPL DPA ω-6 and linoelaidic, individuals tended to be 1.39 (0.98–1.98) times more likely to have one polyp, and tended to be 1.22 (0.99–1.51) times more likely to have two polyps rather than no polyps, respectively. PPL ω-3s were not associated with polyp numbers. The D6D EAE was significantly associated with having one polyp. For each unit increase in D6D, individuals were 2.45 (1.00–5.96) times more likely to have one polyp compared with the no-polyps reference group.

Discussion

The purpose of this study was to identify specific PPL PUFAs and EAEs associated with colon polyp number and polyp type. We report that PPL ω-6 PUFA and trans-FA factor scores were positively associated with adenoma presence. When analyzed individually, ω-6 PUFA DTA was associated with hyperplasic polyps and adenomas in age-adjusted and smoking-adjusted logistic regressions. Polyp number was not associated with any PPL PUFA. Taken together, these data indicate that certain PPL PUFAs are associated with the presence of hyperplastic polyps and adenomas, but not with the total number of colon polyps. Our results suggest PPL ω-6 PUFAs, in particular DTA, may indicate altered FA metabolism associated with having polyps.

PUFAs are substrates for elongating, desaturating, and oxygenating enzymes. Altered levels of PUFA substrates (i.e. through dietary intake) can alter levels of downstream PUFA metabolites (Keenan *et al.*, 2012), which may promote tumor growth (reviewed in detail by Nakanishi and Rosenberg, 2013). Previous research has shown accumulations of LA and longer chain ω-6s (e.g. DGLA and ARA) in colon adenomas (Fernandez-Banares *et al.*, 1996; Berstad *et al.*, 2012). In addition,

adenocarcinomas have higher concentrations of DTA (Fernandez-Banares *et al.*, 1996). Despite DTA being a downstream metabolite of ARA, few studies investigate DTA effects on colon polyps. Instead, most studies investigating ω-6 PUFA associations with polyps or CRC risk focus on ARA oxygenated metabolites (McEntee and Whelan, 2002; Larsson *et al.*, 2004).

PPL ω-6 PUFAs may be related to an increased risk for CRC (Pot *et al.*, 2008; Azrad *et al.*, 2013), and altered FA metabolism at the tissue level may be detectable in plasma (Warensjo *et al.*, 2009; Bokor *et al.*, 2010; Mathias *et al.*, 2010; Suhre *et al.*, 2011; Hodson and Fielding, 2013). The PPL FA compartment is ideal for biomarker discovery as it contains phospholipids (PL) from sources such as plasma lipoproteins (Khaw *et al.*, 2012) and plasma microvesicle exosomes (Hosseini-Beheshti *et al.*, 2012), which can be secreted from CRC cells (Matsumura *et al.*, 2015). If cellular PUFA metabolism is changed during CRC pathogenesis, then changes in PUFA metabolites could be detectable in the PPLs. What remains unclear is whether differences in ω-6 PUFAs detected in plasma are produced by polyps or other tissues such as the liver. At the time this study was conducted exosome isolation was not possible, but methods and technologies are now available to perform such analyses. Analysis of exosomes could allow researchers to determine whether changes in PPL ω-6 PUFAs originate from colon polyps or other tissues. This distinction may lead to a better understanding of how diet may influence adenoma risk related to ω-6 metabolism and how a diet rich in ω-6s may contribute to CRC risk.

This cross-sectional study was conducted in a population of men ($n = 126$, >96% Caucasian, age: 48–65 years) to identify associations between colon polyps and PPL FAs. We recognize that the generalizability of these observations is limited, and we acknowledge it is possible that some associations reported in this study could be the result of chance due to the number of comparisons relative to our sample size. Therefore, studies need to be conducted in larger, more diverse populations. We also acknowledge that the second category of polyp number was underpowered in this study, but was included in analyses for reporting consistency, as we have several studies associating polyp number with serum and plasma biomarkers in this population (Comstock *et al.*, 2014a, 2014b, 2014c; Pickens *et al.*, 2015a). In addition, using EAEs and ω-6 PUFA factor-loading scores, we report PPL ω-6 PUFA metabolism is altered in individuals with adenomas. These EAEs have yet to be extensively validated and may not fully represent enzyme kinetics. Thus, reported differences in ω-6 PUFAs could be related to other factors (e.g. diet) rather than altered ω-6 metabolism. We did not directly collect or assess dietary and alcohol intake in this study. Our laboratory and others have previously reported that red blood cell PL and PPL PUFAs reflect dietary PUFA intake (Harris *et al.*, 2004; Gurzell *et al.*, 2014; Stark *et al.*, 2016), and our laboratory has also shown PPL PUFA levels are a good proxy of PL PUFA levels in the colon (Gurzell *et al.*, 2014); however, we acknowledge that depending on the lipid compartment (i.e. RBC or plasma) or PUFA there could be variation. We acknowledge that other relevant confounding factors were not included in our statistical analyses (see Statistical Analyses section for detailed description) due to issues of multicollinearity with PPL PUFAs and confounders.

Our research suggests that PPL DTA is associated with both hyperplastic polyps and adenomas. As our study is cross-sectional, our suggestion that elevated PPL DTA is associated with increased colon polyp risk assumes that the observed levels of DTA reflect longer-term DTA levels (i.e. years proceeding). To our knowledge, there is no evidence suggesting long-term exposure to elevated PPL DTA increases the risk for colon polyps. There are strong implications for diet recommendations and cancer prevention strategies: (i) if increased PPL DTA is due to the altered FA metabolism in polyps or organs such as the liver, and if (ii) DTA and DTA metabolites influence polyp formation or progression.

Acknowledgements

The research was supported in part by the National Cancer Institute 1R03CA142000 (J.I. Fenton) and the Clinical and Translational Sciences Institute at MSU.

The authors thank Catherine Belcher for assistance with sample processing, and Sarah Comstock for assistance with PROC FACTOR and output interpretation. They also thank Jason Polreis from OmegaQuant Analytics, LLC for his role in technical and analytical support of PPL PUFA analysis. The authors also thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Conselho Nacional de Desenvolvimento Científico e Tecnológico for enhancing international collaborations.

Conflicts of interest

There are no conflicts of interest.

References

Agren JJ, Julkunen A, Penttila I (1992). Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. *J Lipid Res* **33**:1871–1876.

Azrad M, Turgeon C, Demark-Wahnefried W (2013). Current evidence linking polyunsaturated fatty acids with cancer risk and progression. *Front Oncol* **3**:224.

Baack ML, Puumala SE, Messier SE, Pritchett DK, Harris WS (2015). What is the relationship between gestational age and docosahexaenoic acid (DHA) and arachidonic acid (ARA) levels? *Prostaglandins Leukot Essent Fatty Acids* **100**:5–11.

Beresford SA, Johnson KC, Ritenbaugh C, Lasser NL, Snetselaar LG, Black HR, *et al.* (2006). Low-fat dietary pattern and risk of colorectal cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA* **295**:643–654.

Berstad P, Thiis-Evensen E, Vatn MH, Almendingen K (2012). Fatty acids in habitual diet, plasma phospholipids, and tumour and normal colonic biopsies in young colorectal cancer patients. *J Oncol* **2012**:254801.

Bigornia SJ, Harris WS, Falcon LM, Ordovas JM, Lai CQ, Tucker KL (2016). The omega-3 index is inversely associated with depressive symptoms among individuals with elevated oxidative stress biomarkers. *J Nutr* **146**:758–766.

Bokor S, Dumont J, Spinneker A, Gonzalez-Gross M, Nova E, Widhalm K, *et al.* (2010). Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios. *J Lipid Res* **51**:2325–2333.

Burdge GC, Wright P, Jones AE, Wootton SA (2000). A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid-phase extraction. *Br J Nutr* **84**:781–787.

Champely S (2015). pwr: basic functions for power analysis. R package version 1.1-3. Available at: *http://cran.r-project.org/web/packages/pwr/pwr.pdf.* [Accessed 26 September 2016].

Comstock SS, Lewis MM, Pathak DR, Hortos K, Kovan B, Fenton JI (2014a). Cross-sectional analysis of obesity and serum analytes in males identifies sRAGE as a novel biomarker inversely associated with diverticulosis. *PloS one* **9**:e95232.

Comstock SS, Hortos K, Kovan B, McCaskey S, Pathak DR, Fenton JI (2014b). Adipokines and obesity are associated with colorectal polyps in adult males: a cross-sectional study. *PloS one* **9**:e85939.

Comstock SS, Xu D, Hortos K, Kovan B, McCaskey S, Pathak DR, *et al.* (2014c). Association of insulin-related serum factors with colorectal polyp number and type in adult males. *Cancer Epidemiol Biomarkers Prev* **23**:1843–1851.

Dommels YE, Alink GM, van Bladeren PJ, van Ommen B (2002). Dietary n-6 and n-3 polyunsaturated fatty acids and colorectal carcinogenesis: results from cultured colon cells, animal models and human studies. *Environ Toxicol Pharmacol* **11** (3–4):297–308.

Fernandez-Banares F, Esteve M, Navarro E, Cabre E, Boix J, Abad-Lacruz A, *et al.* (1996). Changes of the mucosal n3 and n6 fatty acid status occur early in the colorectal adenoma–carcinoma sequence. *Gut* **38**:254–259.

Fini L, Piazzi G, Ceccarelli C, Daoud Y, Belluzzi A, Munarini A, *et al.* (2010). Highly purified eicosapentaenoic acid as free fatty acids strongly suppresses polyps in Apc(Min/+) mice. *Clin Cancer Res* **16**:5703–5711.

Giuliani A, Ferrara F, Scimo M, Angelico F, Olivieri L, Basso L (2014). Adipose tissue fatty acid composition and colon cancer: a case-control study. *Eur J Nutr* **53**:1029–1037.

Grahn SW, Varma MG (2008). Factors that increase risk of colon polyps. *Clin Colon Rectal Surg* **21**:247–255.

Gurzell EA, Wiesinger JA, Morkam C, Hemmrich S, Harris WS, Fenton JI (2014). Is the omega-3 index a valid marker of intestinal membrane phospholipid EPA+DHA content? *Prostaglandins Leukot Essent Fatty Acids* **91**:87–96.

Habermann N, Ulrich CM, Lundgreen A, Makar KW, Poole EM, Caan B, *et al.* (2013). PTGS1, PTGS2, ALOX5, ALOX12, ALOX15, and FLAP SNPs: interaction with fatty acids in colon cancer and rectal cancer. *Genes Nutr* **8**:115–126.

Haggar FA, Boushey RP (2009). Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. *Clin Colon Rectal Surg* **22**:191–197.

Harris WS, Sands SA, Windsor SL, Ali HA, Stevens TL, Magalski A, *et al.* (2004). Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. *Circulation* **110**:1645–1649.

Harris WS, Pottala JV, Varvel SA, Borowski JJ, Ward JN, McConnell JP (2013). Erythrocyte omega-3 fatty acids increase and linoleic acid decreases with age: observations from 160,000 patients. *Prostaglandins Leukot Essent Fatty Acids* **88**:257–263.

Hodson L, Fielding BA (2013). Stearoyl-CoA desaturase: rogue or innocent bystander? *Prog Lipid Res* **52**:15–42.

Hosseini-Beheshti E, Pham S, Adomat H, Li N, Tomlinson Guns ES (2012). Exosomes as biomarker enriched microvesicles: characterization of exosomal proteins derived from a panel of prostate cell lines with distinct AR phenotypes. *Mol Cell Proteomics* **11**:863–885.

Kaiser HF (1960). The application of electronic computers to factor analysis. *Educ Psychol Meas* **20**:141–151.

Keenan AH, Pedersen TL, Fillaus K, Larson MK, Shearer GC, Newman JW (2012). Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers. *J Lipid Res* **53**:1662–1669.

Khaw KT, Friesen MD, Riboli E, Luben R, Wareham N (2012). Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study. *PLoS Med* **9**:e1001255.

Kikendall JW, Bowen PE, Burgess MB, Magnetti C, Woodward J, Langenberg P (1989). Cigarettes and alcohol as independent risk factors for colonic adenomas. *Gastroenterology* **97**:660–664.

Kinsella JE, B KS, Whelan JW (1990). Dietary unsaturated fatty acids in nteractions and possible needs in relation to eicosanoid synthesis. *J Nutr Biochem* **1**:123–141.

Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A (2004). Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *Am J Clin Nutr* **79**:935–945.

Ledesma RD, Valero-Mora P, Macbeth G (2015). The scree test and the number of factors: a dynamic graphics approach. *Span J Psychol* **18**:E11.

Mandir N, Goodlad RA (2008). Conjugated linoleic acids differentially alter polyp number and diameter in the Apc(min/+) mouse model of intestinal cancer. *Cell Prolif* **41**:279–291.

Mathias RA, Vergara C, Gao L, Rafaels N, Hand T, Campbell M, *et al.* (2010). FADS genetic variants and omega-6 polyunsaturated fatty acid metabolism in a homogeneous island population. *J Lipid Res* **51**:2766–2774.

Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, *et al.* (2015). Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. *Br J Cancer* **113**:275–281.

Whelan J, McEntee MF (2004). Dietary (n-6) PUFA and intestinal tumorigenesis. *J Nutr* **134** (Suppl):3421S–3426S.

McEntee MF, Whelan J (2002). Dietary polyunsaturated fatty acids and colorectal neoplasia. *Biomed Pharmacother* **56**:380–387.

Methy N, Binquet C, Boutron-Ruault MC, Paillot B, Faivre J, Bonithon-Kopp C (2008). Dietary fatty acids and recurrence of colorectal adenomas in a European intervention trial. *Nutr Cancer* **60**:560–567.

Monnet E, Allemand H, Farina H, Carayon P (1991). Cigarette smoking and the risk of colorectal adenoma in men. *Scand J Gastroenterol* **26**:758–762.

Murff HJ, Shrubsole MJ, Cai Q, Smalley WE, Dai Q, Milne GL, *et al.* (2012). Dietary intake of PUFAs and colorectal polyp risk. *Am J Clin Nutr* **95**:703–712.

Nakanishi M, Rosenberg DW (2013). Multifaceted roles of PGE2 in inflammation and cancer. *Semin Immunopathol* **35**:123–137.

Newman JW, Pedersen TL, Brandenburg VR, Harris WS, Shearer GC (2014). Effect of omega-3 fatty acid ethyl esters on the oxylipin composition of lipoproteins in hypertriglyceridemic, statin-treated subjects. *PloS one* **9**:e111471.

Peipins LA, Sandler RS (1994). Dietary marine n-3 fatty acids in relation to risk of distal colorectal adenoma in women. *Cancer Epidemiol Biomarkers Prev* **14**:835–841.

Peipins LA, Sandler RS (2005). Epidemiology of colorectal adenomas. *Epidemiol Rev* **16**:273–297.

Pickens CA, Sordillo LM, Comstock SS, Harris WS, Hortos K, Kovan B, *et al.* (2014). Plasma phospholipids, non-esterified plasma polyunsaturated fatty acids and oxylipids are associated with BMI. *Prostaglandins Leukot Essent Fatty Acids* **95**:31–40.

Pickens CA, Lane-Elliot A, Comstock SS, Fenton JI (2015a). Altered saturated and monounsaturated plasma phospholipid fatty acid profiles in adult males with colon adenomas. *Cancer Epidemiol Biomarkers Prev* **25**:498–506.

Pickens CA, Sordillo LM, Comstock SS, Harris WS, Hortos K, Kovan B, *et al.* (2015b). Plasma phospholipids, non-esterified plasma polyunsaturated fatty acids and oxylipids are associated with BMI. *Prostaglandins Leukot Essent Fatty Acids* **95**:31–40.

Pickens CA, Matsuo KH, Fenton JI (2016). Relationship between body mass index, C-peptide, and delta-5-desaturase enzyme activity estimates in adult males. *PloS one* **11**:e0149305.

Pot GK, Geelen A, van Heijningen EM, Siezen CL, van Kranen HJ, Kampman E (2008). Opposing associations of serum n-3 and n-6 polyunsaturated fatty acids with colorectal adenoma risk: an endoscopy-based case-control study. *Int J Cancer* **123**:1974–1977.

Rose HG, Oklander M (1965). Improved procedure for the extraction of lipids from human erythrocytes. *J Lipid Res* **6**:428–431.

Shim YJ, Choi KY, Lee WC, Kim MK, Lee SY, Lee-Kim YC (2005). Phospholipid fatty acid patterns in the mucosa of human colorectal adenomas and carcinomas. *Nutr Res* **25**:261–269.

Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem N Jr (2016). Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. *Prog Lipid Res* **63**:132–152.

Suhre K, Shin SY, Petersen AK, Mohney RP, Meredith D, Wagele B, *et al.* (2011). Human metabolic individuality in biomedical and pharmaceutical research. *Nature* **477**:54–60.

Susanna C, Larsson MK, Ingelman-Sundberg M, Wolk A (2004). Dietary long-chain n3 fatty acids for the prevention of cancer mechanisms. *Am J Clin Nutr* **79**:935–945.

Team RC (2015). *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Thiebaut AC, Rotival M, Gauthier E, Lenoir GM, Boutron-Ruault MC, Joulin V, *et al.* (2009). Correlation between serum phospholipid fatty acids and dietary intakes assessed a few years earlier. *Nutr Cancer* **61**:500–509.

Tokudome S, Kuriki K, Yokoyama Y, Sasaki M, Joh T, Kamiya T, *et al.* (2015). Dietary n-3/long-chain n-3 polyunsaturated fatty acids for prevention of

sporadic colorectal tumors: a randomized controlled trial in polypectomized participants. *Prostaglandins Leukot Essent Fatty Acids* **94**:1–11.

Trattner S, Becker W, Wretling S, Ohrvik V, Mattisson I (2015). Fatty acid composition of Swedish bakery products, with emphasis on trans-fatty acids. *Food Chem* **175**:423–430.

Valenzuela A, Morgado N (1999). Trans fatty acid isomers in human health and in the food industry. *Biol Res* **32**:273–287.

Vinikoor LC, Schroeder JC, Millikan RC, Satia JA, Martin CF, Ibrahim J, *et al.* (2008). Consumption of trans-fatty acid and its association with colorectal adenomas. *Am J Epidemiol* **168**:289–297.

Warensjo E, Rosell M, Hellenius ML, Vessby B, De Faire U, Riserus U (2009). Associations between estimated fatty acid desaturase activities in serum lipids and adipose tissue in humans: links to obesity and insulin resistance. *Lipids Health Dis* **8**:37.

Whelan MFMaJ (2002). Dietary polyunsaturated fatty acids and colorectal neoplasia. *Biomed Pharmacother* **56**:380–387.

Williams CD, Satia JA, Adair LS, Stevens J, Galanko J, Keku TO, *et al.* (2010). Associations of red meat, fat, and protein intake with distal colorectal cancer risk. *Nutr Cancer* **62**:701–709.

Wu JH, Marchioli R, Silletta MG, Macchia A, Song X, Siscovick DS, *et al.* (2013). Plasma phospholipid omega-3 fatty acids and incidence of postoperative atrial fibrillation in the OPERA trial. *J Am Heart Assoc* **2**:e000397.

Yang K, Li H, Dong J, Dong Y, Wang CZ (2015). Expression profile of polyunsaturated fatty acids in colorectal cancer. *World J Gastroenterol* **21**: 2405–2412.

Yang Y (2011). Multiple imputation using SAS software. *J Stat Softw* **45**: 1–25.

Yusof AS, Isa ZM, Shah SA (2012). Dietary patterns and risk of colorectal cancer: a systematic review of cohort studies (2000–2011). *Asian Pac J Cancer Prev* **13**:4713–4717.

Zahm SH, Cocco P, Blair A (1991). Tobacco smoking as a risk factor for colon polyps. *Am J Public Health* **81**:846–849.