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Effects of Fish Oil Supplementation on Eicosanoid Production in Subjects at Higher Risk for Colorectal Cancer

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Abstract

Objective—Fish oil supplementation may represent a potential chemopreventive agent for reducing colorectal cancer risk. Fish oil's mechanism of action is unknown but presumed to be related to eicosanoid modification. The purpose of this study was to evaluate the effects of fish oil supplementation on levels of urinary and rectal eicosanoids.

Methods—We conducted a randomized, double-blind, controlled trial of 2.5 grams of fish oil per day compared to olive oil supplementation over a six month period. Study participants had a history of colorectal adenomas. Randomization was stratified based on the gene variant rs174535 in the fatty acid desaturase 1 enzyme (*FADS1*), which affects tissue levels of arachidonic acid.

Results—A total of 141 subjects were randomized. Urinary prostaglandin E₂ metabolite (PGE-M) was measured at baseline, 3 months, and 6 months and rectal prostaglandin E₂ (PGE₂) at baseline and 6 months. Repeated measures linear regression was used to determine the effect of the intervention on each outcome measure. Overall, fish oil supplementation was found to reduce urinary PGE-M production compared to olive oil ($P = 0.03$). Fish oil did not reduce rectal PGE₂

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Conflicts of Interests

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overall, however it did significantly reduce PGE₂ in the subgroup of participants not using aspirin or NSAIDs (P=0.04). *FADS1* genotype did not seem to modify fish oils effects on PGE₂ production.

Conclusions—We conclude that fish oil supplementation has a modest but beneficial effect on eicosanoids associated with colorectal carcinogenesis, particularly in those not taking aspirin or NSAIDs.

Keywords

Colonic neoplasms; fish oil; omega-3 fatty acids; eicosanoids; prostaglandin E₂; controlled clinical trials; randomized

Introduction

An important link between cancer and inflammation involves cyclooxygenase-2, (COX-2) the inducible form of cyclooxygenase, which is overexpressed in multiple malignancies (Wang, Honn et al. 2007). In the COX pathway, the n-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA) is released from cellular membranes and then enzymatically converted into various bioactive lipid molecules including prostaglandin E₂ (PGE₂). PGE₂ promotes carcinogenesis through stimulating cellular proliferation, inhibiting apoptosis, promoting angiogenesis, and/or enhancing invasiveness (Wang and Dubois 2006). Up-regulation of COX-2 occurs in 50% of colon adenomas and 85% of colon cancers and is considered a key and early oncogenic event in colorectal carcinogenesis (Gupta and Dubois 2001).

Eicosapentaenoic acid (EPA), a n-3 long-chain (LC)PUFA found in fish oil, is converted to eicosanoids through the same enzymatic pathways as AA but produces series-3 prostanoids that have less inflammatory actions due to lower receptor affinities when compared with AA-derived series-2 prostanoids (Calder 2002, Wada, DeLong et al. 2007). In addition, EPA reduces tissue levels of AA through directly displacing AA from the sn₂ position (second carbon) in the glycerol backbone in cell phospholipid membranes and inhibits COX enzymes. (Garg, Sebokova et al. 1988, Ferretti, Judd et al. 1991, Blair, Prakash et al. 1993, Smith 2005). Several animal and human studies have demonstrated that increased dietary intake of n-3 LCPUFAs are associated with reduced tissue levels of AA and reduced PGE₂ production (Boudreau, Chanmugam et al. 1991, Ferretti, Judd et al. 1991, Blair, Prakash et al. 1993, Wander, Hall et al. 1997, Broughton and Wade 2002, Trebble, Wootton et al. 2003).

Tissues stores of AA come from both exogenous sources (i.e. diet) as well as endogenous sources (the conversion of linoleic acid to AA). Multiple genome-wide association studies (GWAS) have reported associations between single nucleotide polymorphisms (SNPs) in enzymes responsible for AA biosynthesis pathway and tissue levels of AA. Variants in a single gene, *FADS1* (which encodes Δ 5-desaturase enzyme) has been found to explain up to 28% of the additive variance in tissue levels of AA. Specifically, homozygotes for the T allele in the rs174537 *FADS1* SNP have lower fatty acid desaturase activity and subsequently lower tissues levels of AA (Schaeffer, Gohlke et al. 2006, Rzehak, Heinrich et

al. 2009). A recent GWAS study of loci associated with CRC risk found that SNPs in *FADS1* are associated with colorectal cancer (CRC) risk (Zhang, Jia et al. 2014).

We conducted a randomized, double blind, controlled trial of marine-derived n-3 LCPUFA (fish oil) supplementation compared to olive oil supplementation on markers of colorectal proliferation and apoptosis in subjects with known prior history of colorectal adenomas. Because of the hypothesis that genetically-determined tissue levels of AA might be associated with an increased risk for CRC, participants were stratified based on rs174537 *FADS1* genotype. In this report, we describe the results of the intervention on the secondary outcomes of levels of urinary and rectal eicosanoids.

Material and Methods

Study Participants

Participants were recruited from individuals who were identified as colorectal adenoma cases within the Tennessee Colorectal Polyp Study (TCPS) or were identified through electronic medical record reviews to have undergone a colonoscopy at Vanderbilt University Medical Center and diagnosed with an adenoma. Eligible participants were between the ages of 40 and 80 years, had a past medical history of one or more adenomas, and had a known genotype for the rs174535 SNP of *FADS1*. Participants were excluded if they had a previous resected colorectal cancer; congestive heart failure or coronary artery disease; inflammatory bowel disease; any cancer (except non-melanoma skin cancer); advanced kidney disease; cirrhosis; were pregnant or breast feeding; using fish oil supplements or anti-coagulants; or allergic to fish products.

The Fatty Acid Desaturase Activity, Fish Oil, and Colorectal Cancer Prevention study was approved by the Vanderbilt University Medical Center Institutional Review Board and registered at ClinicalTrials.gov (NCT01661764).

Study Intervention

Participants were randomized to either 3 capsules of the fish oil-sourced supplement Lovaza® each containing 465 mg of EPA and 375 mg DHA; (total daily dose of 1395 mg EPA plus 1125 mg DHA) or 3 capsules of an olive oil supplement with each capsule contained 1,000 mg of olive oil. Olive oil was chosen as the control arm as prior studies have found no effect on colonic eicosanoid production (Hillier, Jewell et al. 1991). Both capsules were similar in size, shape and appearance. The rationale for this dose is that it was similar to us clinical trials that have successfully lowered indexes of rectal mucosal proliferation and is well tolerated without any reported adverse effects. (Bartoli, Palozza et al. 1993, Anti, Armelao et al. 1994) Medications were dispensed by the Vanderbilt University Investigational Drug Service (IDS).

Randomization, Allocation, Adherence and Blinding

Randomization was performed according to a permuted block randomization scheme stratified on *FADS1* rs174537 genotypes (GG, GT, TT). Randomization proceeded within these three strata with a block size of balancing interval, varying randomly according to the

outcome of a computer generated random number. The randomization code was kept by the IDS. Participants and study personnel were unaware of the randomization list or treatment assignment. To assess the study blinding, subjects were asked at the end of the study which treatment they thought they had received. Adherence was assessed by weekly telephone calls, pill counts, and red blood cell n-3 LCPUFA measurements.

Study Procedures

Study participation lasted 24 weeks with three in-person visits: at baseline, 12 weeks, and 24 weeks. At each visit, participants arrived after an overnight fast, and 20 ml of blood and 30 ml of spot urine was collected. Eight rectal epithelial biopsies were collected at baseline and at 24 weeks. Participants did not undergo a bowel preparation and samples were obtained using an anoscope and a 2.4 mm 160 cm Captura® biopsy forceps. Biopsies were taken circumferentially approximately 8 cm from the anal verge using biopsy forceps. Two samples were placed in formalin and six were snap frozen in liquid nitrogen.

Laboratory Measurements

Red Blood Cell Phospholipid Membrane Determination—Total lipids were extracted from 200 µl of double-washed packed red blood cells (RBC) using the method described by Folch et al (Folch, Lees et al. 1957). Phospholipids were isolated using thin layer chromatography on Silica Gel 60 A plates and fatty acids methylated using BF₃ / methanol (Morrison and Smith 1964). The methylated fatty acids were analyzed by gas chromatography using an HP 7890A gas chromatograph equipped with flame ionization detectors and a capillary column (SP2380, 0.25 mm × 30 m, 0.20 µm film, Supelco, Bellefonte, PA). Helium was used as a carrier gas. Fatty acid methyl esters were identified by comparing the retention times to those of known standards. Inclusion of the internal standard, dipentadecanoyl phosphatidylcholine (C15:0), permitted quantitation of the amount of phospholipid in the sample. Fatty acid values are presented as percentage of total RBC membrane phospholipid fatty acid content. The lowest level of detection for individuals' fatty acids is less than 0.5% of the total profile.

Rectal Epithelial Cell Eicosanoid Measurement—Tissue was added to 5mL of ice-cold methanol containing indomethacin to inhibit COX activation and 1.0 ng of the internal standard [²H₄]-PGE₂ (Cayman Chemicals, Ann Arbor, MI). The sample was then extracted and analyzed using GC/NICI-MS as described previously (Liu, Laidlaw et al. 2012, Boutaud, Sosa et al. 2016). The inter-day variability for each assay is < 10%. The precision for each assay is ±5% while the accuracy for each assay is 95%.

Urinary Prostaglandin E₂ Metabolite Determination—To quantify endogenous PGE₂ production, the urinary PGE₂ metabolite PGE-M (11 alpha-hydroxy-9,15-dioxo-2,3,4,5-tetranorpropane-1,20-dioic acid) level was measured using liquid chromatography/tandem mass spectrometric method previously described (Mohebati, Milne et al. 2013). In our laboratory the coefficient of variation for samples analyzed in multiple batches was 7.2%.

Statistical Analysis

The primary outcome of the study was changes in rectal cell markers of proliferation and apoptosis with the secondary outcomes being changes in eicosanoid production. The primary outcomes are completing analyses and pathologic review and this paper reports our secondary outcomes. Sample size estimates were derived using previously described clinical trial information using similar primary outcomes and doses of fish oil supplements. Courtney et al found a change in the mean level of Ki67 labeling of -5.86 ($SD \pm 8.8$) for individuals allocated to fish oil supplements ($n=14$) and 3.62 ($SD \pm 8.0$) for the placebo group ($n=14$) (Cheng, Ogawa et al. 2003, Courtney, Matthews et al. 2007). With a sample size of 22 per group for each genotype would provide at least 90% of power to detect 1 SD (8.4) difference in Ki67 between the two groups within each genotype with type I error rate = 5%. Assuming approximately 10% of drop-out rate, the total of 150 patients (25 patients per group per genotype $\times$ 2 groups $\times$ 3 genotypes = 150) would provide power to detect a clinically significant difference between the intervention and placebo groups.

Baseline characteristics stratified by treatment allocation were compared using Wilcoxon rank order sum test for continuous variables and chi-square for categorical variables. We compared RBC membrane percentages of PUFAs at baseline stratified by genotype using Wilcoxon rank order sum test. Urinary PGE-M and rectal eicosanoids were right skewed and log transformed for analyses. We present medians and interquartile ranges for ease of interpretation. We used repeated measures linear regression to determine the effect of the intervention on urinary PGE-M levels. Models were adjusted for genotype and sample batch. For rectal eicosanoids, we used general linear regression and analysis of covariance to adjust for baseline rectal eicosanoid levels, genotype and batch number. We conducted secondary analysis stratifying the analysis by aspirin/NSAID use (regular use, no regular use), baseline RBC membrane n-3 LCPUFA content (below median, above median), baseline RBC membrane AA content (below median, above median), and *FADS* genotype (G/G, G/T, T/T). We calculated Spearman partial correlation coefficients between urinary PGE-M and rectal PGE₂ adjusting for age, gender, aspirin/NSAID use, genotype and study allocation. All analyses were conducted using SAS v9.4.

Results

Three hundred and seventy-nine potential participants were assessed for eligibility and 141 were randomized to either fish oil ($n = 70$) or olive oil ($n=71$) (Figure 1). Sixty-two participants (89%) allocated to fish oil completed the study and 64 participants (90%) allocated to olive oil completed the study. Reasons for discontinuation are presented in Figure 1.

We were unable to obtain pill/bottle returns for 17 participants (13%). Of participants who did return unused pills or empty bottle, 92% (100/109) reported greater than 80% compliance. In the fish oil arm 33% of participants (20/61) believed they were taking fish oil, 28% (17/61) believed they were taking olive oil, and 39% (24/61) were unsure of their treatment allocation. For the olive oil arm 11% (7/64) reported they were taking fish oil, 33% (21/64) reported they were taking olive oil and 56% (36/64) were unsure of their treatment allocation.

There was no difference in age, gender, or baseline RBC membrane fatty acids between the two groups (Table 1). As expected, RBC membrane AA content was lower in individual who were homozygous for the T allele of the *FADS* gene (Table 2). In addition, RBC membrane content of linoleic acid, a precursor of endogenously synthesized AA, was greater in individuals who had the T allele.

Fish oil supplementation reduced urinary PGE-M in individuals allocated to the fish oil arm compared with the olive oil arm ($P = 0.03$) (Figure 2). The reduction in urinary PGE-M was greater at the 3-month measurement with a waning of the effect at the 6-month measurement period which was no longer statistically significant. We found no evidence of an interaction between fish oil supplementation and urinary PGE-M based on *FADS* genotype ($P_{\text{interaction}} = 0.39$). We also found no evidence of effect modification between n-3 LCPUFA supplementation and urinary PGE-M based on NSAID use ($P_{\text{interaction}} = 0.64$).

Overall, we found no changes in rectal mucosal levels of PGE₂ after 6 months of supplemental fish oil (Figure 3). Rectal PGE₂ did decrease over the course of the study in the fish oil arm compared to the olive oil arm however the difference was not statistically significant. We also compared baseline and 24-month changes in rectal PGE₃, PGD₂ and PGD₃. For each measured rectal eicosanoid there was a decrease from baseline in the fish oil arm but the difference was not statistically significant (Figure 3). Similarly to urinary PGE-M we found no evidence of an interaction between fish oil supplementation and rectal eicosanoids by genotype (data not shown).

We did find a statistically significant interaction between NSAID use and fish oil supplementation ($P_{\text{interaction}} = 0.008$). In participants who did not use regular NSAIDs over the course of the study ($n = 57$, fish oil group = 23, olive oil group = 34), supplemental fish oil reduced rectal PGE₂ levels from 3.10 ± 0.32 (log means $\pm$ S.E.) at baseline to 2.54 ± 0.28 at 6 months (18% reduction) compared with a change of 2.80 ± 0.23 to 3.09 ± 0.19 in participants allocated to placebo (10% increase) ($P = 0.04$). In contrast, participants who did use regular NSAIDs ($n = 84$, fish oil group = 47, olive oil group = 37), supplemental fish oil did not significantly reduce rectal PGE₂ levels compared with placebo; levels went from 2.34 ± 0.22 at baseline to 1.84 ± 0.19 at 6 months, compared with a change of 1.61 ± 0.38 to 1.41 ± 0.36 in participants allocated to placebo ($P = 0.19$). We found no evidence of interaction with other rectal eicosanoids (data not shown).

We found a small but statistically significant positive correlation with an increase in urinary PGE-M being correlated with an increase in rectal PGE₂ levels (adjusted correlation coefficient = 0.25 95% CI 0.06, 0.42, p-value = 0.01).

Over the course of the study RBC membrane percentages of n-3 LCPUFA increased by 76% compared to 6% for individuals allocated to olive oil (Figure 4). Similarly individuals allocated to the fish oil arm had a statistically significant decrease in RBC membrane AA content.

Discussion

We found in a randomized controlled trial that fish oil reduced urinary PGE-M levels compared with olive oil. This effect was strongest during the first three months of the study but we found no effect at 6 months. Changes in rectal mucosa PGE₂ were smaller and significant only in individuals not using NSAIDs. We also found that the *FADS1* polymorphism rs174537 did not appear to interact with fish oil intake even though this variant did influence AA levels. Finally, rectal mucosa PGE₂ and urinary PGE-M had only a negligible correlation.

PGE-M, the major urinary metabolite of PGE₂, is an established marker of systemic PGE₂ (Sterz, Scherer et al. 2012, Wang and DuBois 2013). Several case-control studies have found higher levels of PGE-M in subjects with high-risk adenomas or known CRC than in controls (Cai, Gao et al. 2006, Johnson, Schmidt et al. 2006, Shrubsole, Cai et al. 2012, Bezawada, Song et al. 2014, Davenport, Cai et al. 2016). A recent meta-analysis concluded that PGE-M levels are strongly associated with an increased risk for multiple small tubular adenoma, advanced adenoma, and CRC, and that PGE-M is the most promising urinary biomarker for CRC risk assessment and screening (Altobelli, Angeletti et al. 2016).

PGE-M may also be an important marker to identify subsets of patients who would benefit the most from interventions targeted toward reducing inflammation. A nested case-control study found that regular NSAID use was associated with a reduction in adenoma risk in subjects with high baseline PGE-M but not in those with low PGE-M (Bezawada, Song et al. 2014). This suggests that PGE-M could help with both evaluating risk for CRC and assessing response to interventions – it has the potential to become a “risk-response” biomarker, similar to the role the lipid panel plays in the field of cardiovascular medicine in helping assess risk as well as evaluating response to statin therapy (Colbert Maresso, Vilar et al. 2014). Our finding that fish oil supplementation significantly decreased urinary PGE-M levels in our study population – which was by design a higher risk group in that all subjects had known history of adenomas – further supports the potential use of urinary PGE-M as a response marker. However one concern was the relatively weak correlation between rectal PGE₂ and urinary PGE-M seen in our study. Currently underway is the seAFOod polyp prevention trial, a large randomized controlled trial to examine whether EPA, alone or in combination with aspirin, prevents colorectal adenomas (Hull, Sandell et al. 2013).

Several interventional studies have examined the effect of fish oil on production of eicosanoids and other inflammatory markers, with mixed but overall promising results. In 40 healthy male volunteers, supplementation of 15 grams/day of fish oil for 10 weeks led to a 14% reduction in urinary PGE-M compared with 15 grams/day of placebo oil (Ferretti, Judd et al. 1991). In a crossover feeding trial, 10 healthy male volunteers had a 24% reduction in PGE-M when fed a salmon-rich diet versus a placebo diet with identical macronutrient breakdown (Ferretti 1991). Another crossover trial found that rectal PGE₂ levels were significantly lower after 4 weeks of fish oil supplementation compared with corn oil supplementation (435.5 vs 671.5 pg/mg wet tissue, $p < 0.05$). A third crossover trial found that urinary excretion of thromboxane B₂, a metabolite of platelet activator thromboxane A₂ which is also derived from AA, was significantly reduced with a salmon diet compared with

a reference diet (Prakash, Nelson et al. 1994). A study examining varying doses of fish oil supplementation found that PGE₂ in peripheral mononuclear cells was decreased significantly for all doses of fish oil (0.3 g, 1g, 2g of EPA plus DHA), with a trend toward a dose response (Trebbles, Wootton et al. 2003).

Several meta-analyses have been published on this topic (Jiang, Li et al. 2016). Five of the included studies reported PGE₂ changes in response to fish oil supplementation; only two of these showed reduction in PGE₂ (Andrade, Ribeiro et al. 2007, Tartibian, Maleki et al. 2011). However, of the three studies that did not show a significant reduction in PGE₂, one involved healthy participants (Rees, Miles et al. 2006), and the other two included very limited patient populations, namely patients on hemodialysis (Peck, Monsen et al. 1996) and patients with rheumatoid arthritis (Park, Lee et al. 2013).

In our study, fish oil only statistically significantly lowered rectal mucosa PGE₂ levels in non-users but not in users of aspirin/NSAIDs. Of note the baseline PGE₂ levels were lower in the aspirin/NSAID users than in non-users. After fish oil supplementation for 24 weeks, rectal mucosa PGE₂ levels in the non-users of aspirin/NSAID approached but did not reach the baseline levels in aspirin/NSAID users. This suggests that rectal PGE₂ is reduced with fish oil supplementation, but this effect is not seen with NSAID use. Intriguingly our baseline rectal PGE₂ levels were lower in aspirin/NSAID users who were allocated to olive oil supplementation. This difference was not statistically significant ($P = 0.14$) but may have impacted our stratified results. As this was a secondary analysis and participants were not randomized based on NSAID use, this difference in baseline rectal PGE₂ status may be related to confounding factors. We found no differences in the groups based on age, body mass index, sex, smoking status, or educational level (data not shown) however an unmeasured confounders may have also influenced the results.

Strengths of the study included the randomized, controlled design. We had good compliance with the study medication as determined by pill counts and changes in RBC membrane PUFAs. Our participant demographics are similar to patient populations that would likely benefit most from improved colon cancer prevention strategies – namely middle-aged and older patients who have risk factors for CRC. The study has some weaknesses. Most notably, the results we report are of a secondary outcome of our study, and our study might have been underpowered to detect an overall effect on rectal PGE₂. We did not collect rectal biopsies at 12 weeks and cannot report whether rectal PGE₂ levels changes similarly to urinary PGE-M, with an initial strong decrease followed by a waning effect. Another weakness was our choice of *FADS* polymorphism to stratify the participant. While this polymorphism is associated with endogenous AA production, it has not been associated with CRC in GWAS studies. Our choice of dose for n-3 LCPUFA supplementation could have also impacted the results as limited data exists to determine if a dose-response effect exists between n-3 LCPUFA supplementation and eicosanoid production. Finally, the control arm included olive oil supplementation, which may have had an impact on eicosanoid production.

In conclusion, this study showed that supplemental fish oil reduced urinary PGE-M levels; however this reduction was seen at 3 months and not at 6 months. It would be important to replicate our findings as this could indicate that studies of a shorter duration might suggest a

stronger eicosanoid modifying effect of fish oil than might exist over longer periods of time. Additionally, we found a very weak correlation between urinary PGE-M levels and rectal PGE₂ levels suggesting that generalized PGE₂ production only weakly represents colonic production. Finally, our results suggest that fish oil is more effective in reducing rectal PGE₂ in individuals not using NSAIDs. These results might be better delineated after the results of the seAFOod Polyp Prevention Trial are reported.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Flow diagram of trial recruitment and retention

Figure 2.

Concentrations of Urinary PGEM at baseline, 3-months, and 6-months

^aLinear regression comparing baseline to 3-month values adjusted for baseline value, genotype, and sample batch, ^bLinear regression comparing baseline to 6-month values adjusted for baseline value of urinary PGEM, genotype, and sample batch; ^cRepeated measures analysis of variance adjusted for genotype and sample batch

Figure 3.

Figure 3a–d: Changes in Rectal Eicosanoids after 6-Months of n-3 LCPUFA

Supplementation Linear regression comparing baseline to 6-month values adjusted for

baseline value of rectal eicosanoid, genotype, and sample batch. Panel a: Changes in mean log transformed rectal biopsy PGE₂ concentrations; Panel b: Changes in mean log

transformed rectal biopsy PGE₃ concentrations; Panel c: Changes in mean log transformed rectal biopsy PGD₂ concentrations; and Panel d: Changes in mean log transformed rectal

biopsy PGD₃ concentrations

Figure 4.
Figure 4a–b: Changes in red blood cell phospholipid fatty acid content based on study arm

Table 1

Baseline participant characteristics

Baseline Characteristics	Fish Oil (n = 70)	Placebo (n = 71)	P-value
Age, years, median (IQR)	57 (54, 64)	59 (55, 66)	0.37
Sex, female, n (%)	34 (48.6)	34 (47.9)	0.94
Regular aspirin or NSAID use, n (%)	47 (67.1)	37 (52.1)	0.07
Genotype, n (%)			
G/G	26 (37.1)	25 (35.2)	0.97
G/T	25 (35.7)	26 (36.6)	
T/T	19 (27.1)	20 (28.2)	
Red blood cell phospholipid fatty acids, as % of total			
Linolenic Acid, median (IQR)	11.2 (9.99, 12.6)	11.2 (9.95, 12.6)	0.89
Arachidonic acid, median (IQR)	16.7 (14.7, 18.1)	17.2 (15.2, 18.5)	0.19
Eicosapentaenoic acid, median (IQR)	0 (0, 0.57)	0.35 (0, 0.64)	0.29
Docosahexaenoic acid, median (IQR)	3.48 (2.89, 4.51)	3.55 (2.82, 4.47)	0.93

P values for baseline differences determined using Wilcoxon Rank Order Sum for continuous variables and χ^2 for proportions

Table 2

Baseline Red Blood Cell Phospholipid Membrane PUFAs by Genotype

RBC Membrane %	G/G (n = 51)	G/T (n = 51)	T/T (n = 38)	P-value ^I
Linolenic Acid	10.6 (9.39, 12.09)	11.4 (10.4, 12.6)	11.7 (10.7, 13.5)	0.004
Arachidonic acid	17.7 (16.0, 18.9)	17.2 (14.8, 18.2)	15.4 (12.6, 16.9)	0.0004
Eicosapentaenoic acid	0 (0, 0.58)	0.0 (0, 0.57)	0.47 (0, 0.68)	0.25
Docosahexaenoic acid	3.69 (2.93, 4.54)	3.47 (2.81, 4.39)	3.21 (2.72, 4.60)	0.53

^I P values for baseline differences determined using Wilcoxon Rank Order Sum. Data presented as medians and interquartile ranges