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The effects of aspirin on platelet function and lysophosphatidic acids depend on plasma concentrations of EPA and DHA

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Abstract

Aspirin's prevention of cardiovascular disease (CVD) events in individuals with type 2 diabetes mellitus is controversial. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and aspirin all affect the cyclooxygenase enzyme. The relationship between plasma EPA and DHA and aspirin's effects has not been determined. Thirty adults with type 2 diabetes mellitus ingested aspirin (81 mg/day) for 7 days, then EPA+DHA (2.6 g/day) for 28 days, then both for another 7 days. Lysophosphatidic acid (LPA) species and more classic platelet function outcomes were determined. Plasma concentrations of total EPA+DHA were associated with 7-day aspirin reduction effects on these outcomes in a "V"-shaped manner for all 11 LPA species and ADP-induced platelet aggregation. This EPA+DHA concentration was quite consistent for each of the LPA species and ADP. These results support aspirin effects on lysolipid metabolism and platelet

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aggregation depending on plasma EPA+DHA concentrations in individuals with a disturbed lipid milieu.

Keywords

Lysophosphatidylcholine; lysophosphatidic acid; omega-3 fatty acids; eicosapentaenoic acid; docosahexaenoic acid; aspirin; platelet function

INTRODUCTION

Type 2 diabetes mellitus is a disease of epidemic proportions in the United States and globally[1]. Fatty acid metabolism is disrupted in insulin resistance and plays an important role in the development of obesity-related cardiovascular disease (CVD) and diabetes[2]. This milieu is characterized by an increase in circulating free fatty acids, low blood and tissue levels of omega-3 fatty acids, increased concentrations of highly atherogenic oxidized LDL[2–4], and triglyceride levels higher than in those without diabetes mellitus[5].

It is well-established that low-dose aspirin (81–162 mg/day) has the potential to reduce the rate of recurrent CVD events with a 44% reduction in risk of myocardial infarction[6, 7] and is associated with a much lower risk of gastrointestinal bleeding than higher doses as made clear by the US Preventive Services Task Force[8, 9]. In the primary prevention setting, however, evidence supporting a benefit is much less clear for all individuals[9] and those with diabetes mellitus[10]. Its benefits in preventing CVD events have been most widely ascribed to its antiplatelet effects because it reduces the production of the very potent platelet function agonist thromboxane A₂[8] through the acetylation of cyclooxygenase-1 (COX-1). The term “aspirin resistance” has been used to describe the inability of aspirin to produce a measurable response on tests of platelet function or to protect individual patients from thrombotic complications[11–13]. Those with type 2 diabetes mellitus are particularly predisposed to this issue that has been shown to predict increased risk for CVD events, including death and acute coronary syndromes, as demonstrated by several studies[14–17].

The CVD benefits of omega-3 fatty acid supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were questioned by a meta-analysis of clinical trials [18] suggesting no overall benefit for secondary prevention[19] though the results are controversial[20]. It is important to underline that data analysis in studies where a dietary nutrient is the agent of interest is misleading when blood or other tissue concentrations were not also measured and considered[21, 22]. Our data support a combinatory effect of aspirin and fish oil ingestion on reduction in platelet function[13, 23] and several lysolipids (molecules consisting of a glycerol backbone with 1 attached acyl group)[24], some of which have been shown to enhance platelet function. We thus hypothesized that aspirin's effects are dependent on EPA and DHA plasma concentrations.

PATIENTS AND METHODS

Patients

Thirty adults aged 40 to 80 years with type 2 diabetes mellitus were enrolled in this study. Details of recruitment, eligibility criteria, and data collection have been reported previously[13, 24]. In short, a diagnosis of type 2 diabetes mellitus was based on the Executive Committee of the American Diabetes Association Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus criteria [25]. For the duration of the study, participants were instructed to avoid taking flax seed oil, fish oil other than the study capsules, vitamins, nutritional supplements, herbal preparations, and to limit fish intake to 3 servings/week. The use of diabetic medications was permitted. The list of exclusion criteria included a diagnosis of CVD; recent history of malignancy, peptic ulcer, or bleeding disorder; use of antiplatelet or antithrombotic therapy, oral contraceptives, or NSAIDs; signs of any metabolic disease that would influence lipid metabolism; allergy to aspirin or fish oil; pregnancy; drug or alcohol abuse and tobacco use.

Protocol

This was an 8-week sequential-therapy clinical trial in which each subject served as his or her own control, as described previously[13]. The daily dosing regimens for each study agent were as follows: (1) one over-the-counter non-enteric 81 mg aspirin tablet; and (2) four 1000 mg over-the-counter fish oil capsules (OmegaRx brand; Zone Labs, Marblehead, MA) with each capsule containing 400 mg EPA and 200 mg DHA as per the label. Due to the inconsistent evidence regarding the effect of enteric coating on the bioavailability and biological activity of aspirin [8], non-enteric-coated aspirin was used. The timeline of study activities, all of which took place at the University of Rochester's Clinical Research Center, are summarized in Table 1[13]. Briefly, following screening and recruitment (Day 1), participants underwent a 10-day aspirin-free 'washout' period. The night before each of the 4 study visits, subjects were required to eat a standardized low-fat meal, as directed by a registered dietician, and were instructed not to eat or drink (except water) for 8 hours prior to each visit. On Day 11 (study visit 1), a baseline blood sample was obtained (blood draw 1) before starting the 7-day aspirin regimen. Four hours after the ingestion of the first aspirin, blood draw 2 was obtained. Participants returned on Day 18 (study visit 2), where blood draw 3 was obtained after 7 days of aspirin ingestion. This was followed by the 28-day fish oil regimen, in which some participants (n=8) took aspirin as prescribed by their primary care physician but were instructed to remain aspirin-free for 10 days prior to study visit 3. On Day 46, an initial phlebotomy was done (blood draw 4) reflecting the 28 days of fish oil consumption. Subjects then ingested a single dose (81 mg) of aspirin while continuing the fish oil and had the fifth phlebotomy (blood draw 5) obtained 4 hours later. The combined fish oil plus aspirin regimen continued for 7 days until Day 53 (study visit 4), in which the final phlebotomy (blood draw 6) was obtained and all study regimens were discontinued. Thus, blood draws 2 and 5 represent the acute (4 hours) effects of aspirin ingestion, whereas blood draws 3 and 6 represent its chronic (7 days) effects.

This study was approved by the Research Subject Review Board at the University of Rochester and registered with [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT01181882).

Laboratory Methods

Plasma Total EPA and DHA Assay—EDTA plasma and fish oil capsule samples were assayed for EPA/DHA by a liquid chromatography tandem mass spectrometry (LC-MS/MS) approach using an API 4000 triple-quadrupole mass spectrometer (Applied Biosystem MDS Sciex, Carlsbad, CA). The method used was adopted from a reported GC-MS method, which involved fat hydrolysis, separation, saponification, and ester exchange reaction to generate finally a volatile methyl or ethyl ester [26]. For LC-MS/MS assay, the analytes do not need to be volatile and hence the current method omitted the ester exchange reaction and determined the total EPA/DHA concentration owing to the high sensitivity and specificity of the LC-MS/MS method. This method has been validated for its assay linearity, detection limit (LOD and LOQ), intra- and inter- assay reproducibility and precision. Briefly, plasma (20 μ l) and capsule (20 μ l) samples were treated with 1 M potassium hydroxide in 95% ethanol at 85 $^{\circ}$ C for 90 min. The resulting solution was cooled and then neutralized and diluted with 5% formic acid in methanol and mobile phase. The chromatographic separation of EPA/DHA was carried out on a Symmetry C18 column, 2.1 $\times$ 50 mm, 3.5 μ m (Waters, Milford, MA). The mobile phase consists of 85% methanol and 4 mM ammonium format containing 0.1% formic acid eluting at 0.4 ml/min. The tandem mass spectrometer was operated in negative electrospray mode with the ion spray voltage set at 4.0 KV and temperature to 650 $^{\circ}$ C. EPA/DHA was detected using multiple-reaction monitoring and quantitation achieved through monitoring of mass transition 301.1/257.1 m/z (EPA) and 327.1/283.1 m/z (DHA). All data were acquired and processed with Analyst 1.4.2 software (Applied Biosystem MSD Sciex). The limit of detection was 45 pg for EPA, and 75 pg for DHA.

Lysophosphatidic Acid Species—Eleven different acyl species of plasma lysophosphatidic acid (LPA (in nM)) were measured simultaneously from each blood draw[24]. The acyl chains analyzed were the saturated fatty acids palmitic acid (16:0) and stearic acid (18:0) and the unsaturated fatty acids 16:1, 18:1n9, linoleic acid (18:2n-6), 18:3, arachidonic acid (20:4n-6), EPA (20:5n-3), adrenic acid (22:4n-6), 22:5, and DHA (22:6n-3). As previously specified in detail[24], a modified Bligh and Dyer extract of plasma was directly infused into and analyzed on an ABI QTrap 2000 MS/MS (Life Technologies, Foster City, CA) via an Advion Nanomate robotic device.

Platelet Aggregation Direct Measures—Described in an earlier publication[27], whole blood electrical impedance platelet aggregation was conducted using a Chronolog Whole Blood Lumi-Aggregometer® (Model 560VS) with reagents from Chronolog Corporation (Havertown, PA). The following agonists were tested within 2 hours of the phlebotomy: ADP at 2.5 μ M, 5 μ M, and 10 μ M; collagen at 1 and 2 mg/ml; and arachidonic acid at 0.5 mM.

Thromboxane B2 (TXB2)—A competitive enzyme linked immunosorbent assay (TXB2 EIA Kit, Cayman Chemical Co., Ann Arbor, MI) was used to analyze TXB2 from citrated plasma serially diluted, as necessary, in sample buffer to fall within the standard curve (typically 1:2)[13].

Statistical Methods

As previously reported[24], baseline characteristics of participants were described using relative frequencies for categorical measures, and means and standard deviations (SD) for continuous measures. All EPA+DHA, LPA, ADP, collagen, and thromboxane B2 variables were non-normally distributed, based on a Shapiro-Wilk statistic p -value > 0.05 , and thus were log transformed. Finding a particular plasma EPA+DHA concentration and fitting a V-shaped piecewise linear regression to each outcome of interest was the goal. In order to accomplish this goal, we converted the log-transformed linear combination of EPA and DHA concentrations into actual EPA+DHA plasma concentrations. We first fit the locally weighted scatterplot-smoothing (LOWESS) curve to the scatterplot of the score of the outcome vs. plasma EPA+DHA concentration and determined the location of this particular point by finding the value of the plasma level that corresponds to the lowest point of the LOWESS curve. Each LOWESS curve represents the *change* in each outcome after aspirin ingestion (for example, between blood draw 1 and blood draw 3), not the raw value in a particular blood draw. We then fit a piecewise linear regression consisting of 2 linear functions joined at the particular point. A significant difference between the slopes of the 2 linear functions will support the hypothesis. We also performed sensitivity analyses to ensure that the point selected is the particular point or sufficiently close to it. In particular, we selected other candidates from values of plasma levels near this point and then fit the same V-shaped piecewise linear regression and compared model fit using goodness of fit criteria. If a point existed that led to a better model fit than the point determined earlier, this new point was selected as the particular point.

RESULTS

Thirty adults with type 2 diabetes mellitus completed the study protocol. The baseline characteristics of these study participants are shown in Table 2. The mean concentrations of DHA and EPA in the fish oil capsules were 406 ± 42 mg/ml and 330 ± 46 mg/ml, respectively, and we previously showed that ingestion of fish oil capsules resulted in increases from baseline of 106 ± 48 μ g/ml to 190 ± 65 μ g/ml after 28 days fish oil for plasma DHA and 13 ± 7 μ g/ml to 61 ± 26 μ g/ml EPA in that study[13]. The actual EPA +DHA nM plasma concentrations from the log-transformed linear combination of EPA and DHA concentrations are displayed in Table 3.

In Figure 1 are LOWESS curves that display the relationship between the plasma concentration of EPA+DHA on the x-axis and the LPA *changes* on the y-axis. The lowest level of the curve on the y-axis represents the EPA+DHA concentration for which chronic aspirin ingestion had the greatest effect in reducing the LPA species. Each LPA species' curve is represented individually with them all combined in the same figure to demonstrate the consistency in aspirin's effect associated with EPA+DHA concentration in both Figure 1 A and B. These curves represent EPA+DHA concentrations at the time of blood draw 3 given they reflect concentrations before fish oil ingestion occurred and are the same samples in which the after aspirin ingestion LPA concentrations were measured.

In Figure 2 are LOWESS curves for specific LPA species containing particular acyl groups, because 18:1n9 has been demonstrated to be a potent platelet spreading and aggregation

agonist[28, 29], 20:4n6 is a precursor for thromboxane B2 which is a potent platelet function agonist[12], and the omega-3 fatty acids have known beneficial effects on vascular health[30, 31]. These LPA species have quite consistent particular EPA+DHA concentrations, obtained at blood draw 3, before fish oil ingestion, associated with aspirin's effects after 7 days of ingestion. The curves representing the effects of aspirin 4 hours after ingestion do not, and are much less dynamic. The EPA+DHA concentrations at this time were measured using blood draw 1. We explored the relationship between EPA+DHA concentrations after fish oil ingestion and aspirin effects on each of the LPA outcomes using LOWESS curves and there were no obvious V-shaped curves or such curves were not statistically significant (data not shown). Thus, no level of EPA+DHA measured after fish oil ingestion was correlated with a maximal effect of aspirin in reducing the outcomes.

In Figure 3 are LOWESS curves for combined platelet function measures reflecting changes in each outcome 4 hours after aspirin ingestion (acute) and after daily ingestion for 7 days. The EPA+DHA concentrations were measured using blood draw 1 and 3, respectively. The acute effects of aspirin on thromboxane approaches a "V" shape, whereas this is not true for the other measures. A limitation of these figures is the fairly broad y-axis so that the curve shape dynamics are difficult to demonstrate.

In Figure 4 are LOWESS curves for individual selected platelet function outcomes, each with its own y-axis scale to allow visualization of changes 4 hours (acute) after aspirin ingestion and after its daily ingestion for 7 days (chronic). The acute effects of aspirin are associated with more of a "V" shape than the chronic effects. We explored the relationship between EPA+DHA concentrations after fish oil ingestion and aspirin effects on each of the platelet function outcomes using LOWESS curves and there were no obvious V-shaped curves or such curves were not statistically significant (data not shown). Thus, no level of EPA+DHA measured after fish oil ingestion was correlated with a maximal effect of aspirin in reducing the outcomes.

DISCUSSION AND CONCLUSIONS

Results from this study strongly support the idea that plasma concentrations of total EPA +DHA from adults with type 2 diabetes mellitus enhance the effects of aspirin on lysophosphatidic acid and more classic platelet function measures in a "V"-shaped distribution, one which is not linear. This is because EPA+DHA concentrations were measured in plasma in the same patients and changes were determined longitudinally so that data were matched and major changes in other variables that could affect each individuals' health or medication status are very unlikely. This "V"-shaped relationship was particularly striking for effects on LPAs where significant overlap of curves, particularly at the bottom of the "V" (meaning more effect of aspirin), were present and the EPA+DHA concentrations converge. Similar but less striking "V"-shaped effects on more classic platelet function measures were present. This strongly suggests a combinatory effect of aspirin and EPA +DHA on lysophospholipid metabolism and platelet function exists. As the EPA+DHA concentrations for which the "V"-shaped curves were present were present *de novo* in participating subjects prior to fish oil ingestion, these data suggest that the most robust

correlation between EPA+DHA concentrations and aspirin's effects are for concentrations that exist due to baseline dietary factors, not after fish oil supplementation.

It is important to note that the aspirin effects on the 18:1n9 LPA species, which has been demonstrated to be a strong platelet function agonist[28, 29], are enhanced when the EPA +DHA concentration is at a particular range and that this range is similar to when effects on more traditional and classic platelet function are most prominent. Given the importance of platelet function as a risk factor for cardiovascular disease events[32], this information has public health significance, particularly for those with type 2 diabetes mellitus, who represent an increasing proportion of the global population[1, 33].

The term "aspirin resistance" has been used to describe the inability of aspirin to produce a measurable response on tests of platelet function or to protect individual patients from thrombotic complications[11–13]. Instead of "resistance" we prefer to use the term "nonresponder". Those with type 2 diabetes mellitus are particularly predisposed to this issue which has been shown to predict increased risk for CVD events, including death and acute coronary syndromes, as demonstrated by several studies[14–17]. A meta-analysis concluded that ~28% of individuals are nonresponders. Nonresponders do not benefit from other antiplatelet drugs. The combination of aspirin with other antiplatelet drugs, such as clopidogrel, is associated with a significantly higher risk of major bleeding than with aspirin alone[34]. In contrast, the safety of EPA and DHA (omega-3 fatty acids found in seafood) plus aspirin is supported by the sum of the data available from clinical trials and observational studies[35–39]. Our data support the concept that aspirin nonresponders are those who do not have particular plasma concentrations of EPA and DHA.

Lysophosphatidylcholine (LPC) and LPA are bioactive lipid components of oxidized low-density lipoprotein (LDL) in humans, a robust atherogenic factor[40]. These lysolipids are simple molecules consisting of a glycerol backbone, phosphate or choline groups, and a single fatty acid, whereas triglycerides contain a glycerol backbone with 3 fatty acids. Current thinking is that LPC is converted to LPA by lysophospholipase D (autotaxin)[41–43]. LPA binds to G-protein-coupled receptors, most of which belong to the endothelial differentiation genes family of receptors and mediate LPA pleiotropic and specific actions[44]. LPA stimulates neointima formation (an indicator of endothelial dysfunction and a prelude to atherosclerosis) and its mechanism of action is similar to that for oxidized low-density lipoprotein. LPA is also generated by activated platelets and a very strong platelet function agonist in humans[28, 41]. It has been demonstrated that LPA in oxidized LDL and the highly lipid-rich core of atheromatous plaques directly triggers platelet shape change and aggregation in humans[29, 45]. Serum LPA levels correlate positively with the presence of an acute myocardial infarction (MI)[46], and plasma LPA levels have also been shown to be significantly higher in individuals with cerebrovascular disease than in healthy controls [47]. LPA-induced platelet aggregation is relatively modest, but synergizes strongly with other stimuli (e.g. ADP or epinephrine[45]). The 18:1n9 (oleic acid-18 carbons with one double-bond at the 9th carbon from the methyl group) LPA species has been shown to be the most important atherogenic lipid agonist in the rupture-prone portion of human atherosclerotic plaque[29, 45]. The arachidonic acid (20:4n6; an omega-6 fatty acid, a major platelet function agonist, and source of thromboxane)[48] species of LPA, EPA and DHA

species of LPA [49] are also of potential importance due to the known relationships of their parent fatty acids with CVD.

Our study has strengths and limitations. We had available data from a relatively small number of individuals with type 2 diabetes mellitus. However, we used a sequential clinical trial design, where individuals' baseline values represent their own control, enhancing statistical power and allowing us to analyze multiple outcomes at each time point. Although all subjects were carefully characterized adults with type 2 diabetes mellitus who were seen in a controlled research setting where study protocol adherence was strictly documented, medication adherence between study visits was based on self-report. Although validated measures of lysophospholipids, platelet aggregation and platelet function were used, the local production of lysophospholipids and changes in platelet function in inflamed or injured tissues may not be faithfully reflected in circulation levels. The possibility of measurement error also exists, but efforts were made to minimize this by using reagents from the same batch and the same analytical machine to process specimens. Although we used a validated competitive enzyme linked immunosorbent assay to measure thromboxane B2 in citrated frozen plasma, a limitation is that this value does not represent its direct effects on platelet aggregation. Another limitation is that results from this project were generated using secondary data analyses. Our EPA+DHA assay did not take into account other fatty acids and, thus, did not report their % of total fatty acids. However, since participants in our study had diabetes mellitus and were recruited from Monroe County, NY, which is representative of the general US population, the EPA+DHA concentrations likely are similar to others with this high cardiovascular disease risk condition.

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HIGHLIGHTS

- We modeled the effect of aspirin using LOWESS curves created from scatter-plots
- A consistent plasma concentration of EPA+DHA was associated with aspirin effects
- This concentration was present for effects on lysophosphatidic acid species
- A similar effect was shown for classic platelet function outcomes

Figure 1. Chronic (7-Day) effects of 81 mg aspirin when taking into account EPA+DHA plasma concentrations on all LPA species

All LPA concentrations were measured in nanomolar (nM) units.

The y-axis scale is the log-transformed LPA plasma concentration.

The x-axis scale is the log-transformed EPA+DHA plasma concentration.

Each LOWESS curve represents the effect of chronic aspirin ingestion on the LPA containing a particular fatty acid with the lowest point in the curve representing the particular EPA+DHA concentration at which aspirin has its greatest effect in reducing the LPA.

Figure 2. Acute and chronic effects of 81 mg aspirin when taking into account EPA+DHA plasma concentrations on relevant LPA species

All LPA concentrations were measured in nanomolar (nM) units.

The y-axis scale is the log-transformed LPA plasma concentration.

The x-axis scale is the log-transformed EPA+DHA plasma concentration.

Each LOWESS curve represents the effect of aspirin on the LPA containing a particular fatty acid with the lowest point in the curve representing the particular EPA+DHA concentration at which aspirin has its greatest effect in reducing the LPA.

EPA+DHA cutoffs for LPA 18:1: 4.40 (acute) and 4.30 (chronic).

EPA+DHA cutoffs for LPA 18:3: 4.40 (acute and chronic).
EPA+DHA cutoffs for LPA 20:4: 4.30 (acute) and 4.35 (chronic).
EPA+DHA cutoffs for LPA 20:5n3: 4.40 (acute and chronic).
EPA+DHA cutoffs for LPA 22:6n3: 4.60 (acute) and 4.50 (chronic).

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Figure 3. Acute and chronic effects of 81 mg aspirin when taking into account EPA+DHA plasma concentrations on combined platelet function outcomes

* All platelet function measures are on the y-axis in ohms, with the exception of Thromboxane B2, which is expressed in pg/ml concentration.

The x-axis scale is the log-transformed EPA+DHA plasma concentration.

Each LOWESS curve represents the effect of aspirin on the platelet function measure with the lowest point in the curve representing the particular EPA+DHA concentration at which aspirin has its greatest effect in reducing the measure.

Figure 4. Acute and chronic effects of 81 mg aspirin when taking into account EPA+DHA plasma concentrations on individual platelet function measures

The x-axis scale is the log-transformed EPA+DHA plasma concentration.

Each LOWESS curve represents the effect of aspirin on the platelet function measure with the lowest point in the curve representing the particular EPA+DHA concentration at which aspirin has its greatest effect in reducing the measure

EPA+DHA cutoffs for ADP 2.5: 4.70 (acute) and 4.25 (chronic).

EPA+DHA cutoffs for Thromboxane B2: 4.45 (acute and chronic).

EPA+DHA cutoffs for Arachidonic Acid: 4.40 (acute) and 4.30 (chronic).

TABLE 1

Study timeline of activities

Day	Study Visit	Activity
1		<ul style="list-style-type: none"> Recruitment and screening Collection of baseline demographic and clinical data
1–10		<ul style="list-style-type: none"> Aspirin-free period of 10 days prior to Study Visit 1 Blood draw 1 (before aspirin)
11		<ul style="list-style-type: none"> Ingestion single 81 mg dose aspirin Blood draw 2 (4 hr post aspirin ingestion)
12–17	1	<ul style="list-style-type: none"> Single 81 mg aspirin/day Blood draw 3
18		<ul style="list-style-type: none"> Begin fish oil 4g/day and discontinue aspirin unless subject takes it as prescribed by their doctor
19–45		<ul style="list-style-type: none"> 28 days of fish oil 4g/day
36–45	2	<ul style="list-style-type: none"> Aspirin-free period of 10 days prior to Study Visit 3 Blood draw 4 (before aspirin)
46		<ul style="list-style-type: none"> Continue fish oil and a single dose 81 mg aspirin Blood draw 5 (4 hr post aspirin ingestion)
47–52		<ul style="list-style-type: none"> Continue aspirin and fish oil 4g/day
53	3	<ul style="list-style-type: none"> Blood draw 6 Discontinue fish oil and aspirin

TABLE 2

Baseline participant characteristics (n = 30)

		Minimum	Maximum
Age, mean (SD)	56.6 (8.9)	40.0	74.5
Male, n (%)	15 (50.0)		
Race, n (%)			
African-American	9 (30.0)		
Asian	2 (6.7)		
Caucasian	17 (56.7)		
Other	2 (6.7)		
Smoking status, n (%)			
Former	11 (36.7)		
Never	19 (63.3)		
Fish intake, n (%)			
<once/month	11 (36.6)		
1–3 times/month	10 (33.3)		
once/week	7 (23.3)		
twice/week	2 (6.7)		
Physical activity (hrs/wk), mean (SD)	5.1 (5.2)		
Metformin use, n (%)	25 (83.3)		
Alcohol consumption, n (%)			
None	23 (76.7)		
1–3 days/month	2 (6.7)		
1–4 days/week	4 (13.3)		
5 days/week	1 (3.3)		
Education, n (%)			
<high school	2 (6.7)		
High school or GED	6 (20.0)		
Bachelors or associates degree	12 (40.0)		
Graduate degree	6 (20.0)		
Systolic Blood Pressure, mean (SD)	132.9 (14.0)	98.0	171.0
Diastolic Blood Pressure, mean (SD)	76.0 (9.4)	58.0	94.0
BMI, mean (SD)	34.6 (7.5)	22.1	54.0

TABLE 3

Conversion of log-transformed linear combination of EPA and DHA concentrations into actual EPA+DHA nM plasma concentrations

Log(EPA+DHA)	EPA+DHA (nM)
3.50	46.8
3.75	60.1
4.00	77.2
4.25	99.1
4.50	127.3
4.75	163.5
5.00	209.9
5.25	269.5
5.50	346.0