Differential effects of EPA vs. DHA on postprandial vascular function and
the plasma oxylipin profile in men

Seán McManus¹†, Noemi Tejera¹†, Khader Awwad², David Vauzour¹³, Neil Rigby³, Ingrid Fleming²,
Aedin Cassidy¹, and Anne Marie Minihane¹*¹

¹ Department of Nutrition and Preventive Medicine, Norwich Medical School, University of East Anglia, Norwich, NR4 7UQ, United Kingdom. ² Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe-University, 60590, Frankfurt, Germany. ³ Institute of Food Research, Norwich Research Park, Norwich NR47UA, United Kingdom.

† joint first author

* To whom correspondence should be addressed: Professor Anne Marie Minihane, Norwich Medical School, BCRE, James Watson Rd, Norwich, NR4 7UQ. Telephone: +44 (0)1603 59 2389, e-mail address: a.minihane@uea.ac.uk.

Running title: EPA versus DHA and postprandial vascular function.

Abbreviations used: AIx, Augmentation Index; BP, blood pressure; CI, Confidence interval; CO, control oil; DBP, diastolic blood pressure; DiHDPA, dihydroxy-docosapentaenoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DPA, Docosapentaenoic acid; DRO, DHA-rich oil; EDV, endothelial dependent vasodilation; EpDPE, epoxy-docosapentaenoic acid; EpETE, epoxy-eicosatetraenoic acid; ERO, EPA-rich oil; FMD, flow mediate dilatation; GTN, nitroglycerine; HR, hazard ratio; HDL-C, HDL cholesterol; HEPE, hydroxy -eicosapentaenoic; H₂S, hydrogen sulfide; LC n-3 PUFA, long chain n-3 PUFA; PWV, Pulse Wave Velocity; RCT, randomized controlled trials; RHI, reactive hyperemia index; RR, relative risk; SBP, systolic blood pressure; TC, total cholesterol.
ABSTRACT

Our objective was to investigate the impact of EPA versus DHA, on arterial stiffness and reactivity, and underlying mechanisms (with a focus on plasma oxylipins), in the postprandial state.

In a 3-arm cross-over acute test meal trial men (n=26, 35-55y) at increased CVD risk, received a high fat (42.4g) test meal providing 4.16 g of EPA or DHA or control oil in random order. At 0 h and 4 h, blood samples were collected to quantify plasma fatty acids, LCn-3PUFAs derived oxylipins, nitrite and hydrogen sulfide and serum lipids and glucose. Vascular function was assessed using blood pressure, Reactive Hyperaemia Index (RHI), Pulse Wave Velocity and Augmentation Index (AIx).

The DHA-rich oil significantly reduced AIx by 13% ($P=0.047$) with the decrease following EPA-rich oil intervention not reaching statistical significance. Both interventions increased EPA and DHA derived oxylipins in the acute postprandial state, with an (1.3 fold) increase in 19,20-DiHDPA evident after DHA intervention ($P < 0.001$).

In conclusion, a single dose of DHA significantly improved postprandial arterial stiffness as assessed by AIx, which if sustained would be associated with a significant decrease in CVD risk. The observed increases in oxylipins provide a mechanistic insight for the AIx effect.

**Supplementary keywords**: augmentation index, blood pressure, fish oil, hydrogen sulfide, lipidomics, nitric oxide, nutrition, omega-3 fatty acids, pulse wave velocity.
INTRODUCTION

An impact on arterial stiffness and compliance, associated with reduced blood pressure (1-4), is thought to be a significant contributor to the lower CVD risk associated with increased long chain n-3 PUFA (LCn-3PUFA, EPA plus DHA) intake and status (4-6).

In randomized controlled trials (RCTs) conducted to date, vascular function is typically measured in the fasting non-challenged state. Given that adults following Westernized dietary patterns typically spend 16-18h per day in the postprandial state, and that a postprandial phenotype characterized by an exaggerated lipaemia and glycemia and inflammatory and oxidative stress is associated with vascular dysfunction (7), the postprandial vascular response is likely to be a more significant and discriminating marker of CVD risk. However, the impact of dietary factors and meal composition on acute postprandial vascular function is poorly understood.

Increasing the total fat content of a test meal reduces both cardiac and peripheral postprandial vascular reactivity (7) and a small number of studies providing EPA plus DHA in the test meal have reported a positive effect of fish oil on vascular tone over 2-8h (8-12). Although rarely considered, variability in the EPA and DHA ratio of the n-3 PUFA (fish or fish oil) source used in RCTs is likely to be a major determinant of the apparent inconsistency in findings reported.

A limited number of chronic interventions that measured fasting vascular function, indicate that DHA has a greater beneficial effect on vascular function than EPA (4). However, with the exception of a single trial that examined relative impact on the cardiovascular hemodynamic responses to exercise (11), no study has directly compared the effects of EPA- versus DHA-rich oils on the postprandial response.

Identification of the most bioactive LCn-3PUFA or EPA: DHA ratio on ‘health’ end-points is important in order to refine current dietary recommendations and allow the stratification of intervention to suit an individual’s phenotype. Furthermore, with the emergence of non-fish sources of these fatty acids, such as separate EPA and DHA produced by marine microalga or in transgenic seed oils, and the availability of EPA and DHA concentrates, all with potentially bespoke EPA and DHA content, information on their relative bioactivity is needed in order to inform these production systems (13, 14).
The primary aim of this double-blind, placebo-controlled acute test meal study was to compare the impact of EPA vs. DHA on postprandial (4 h) vascular function as assessed by blood pressure (BP), Pulse Wave Velocity (PWV), Reactive Hyperaemia Index (RHI) and Augmentation Index (AIX). Underlying molecular mechanisms were investigated by quantifying potential circulating mediators of vascular function including nitrite (as a biomarker of nitric oxide (NO), hydrogen sulfide (H_{2}S) and LCn-3PUFA hydroxy, epoxide and thiol metabolites. Although shown to be potent modulators of vascular tone in cell, animal and human ex vivo studies (15, 16), the role of these compounds in modulating EPA- and DHA-induced changes in vascular function in humans is currently unknown.
MATERIALS AND METHODS

This single-center dietary intervention study was conducted at the Norwich and Norfolk University Hospital (NNUH) Clinical Trials and Research Unit, at the University of East Anglia, between September 2012 and September 2013. All participants provided informed consent prior to commencing the study. It was conducted according to the guidelines described in the Declaration of Helsinki, and ethical clearance was provided by the English National Research Ethics Service, East of England Research Ethics Committee (reference 12/EE/0011). This trial was registered at clinicaltrials.gov (NCT01692431).

Subjects

Twenty six males between the ages of 35-55 y were recruited through local and targeted online advertisement. Volunteers were recruited if they possessing one or more of the following CVD risk factors: total cholesterol (TC) \( \geq 6 \text{ mmol/L} \), HDL cholesterol (HDL-C) \( \leq 1.0 \text{ mmol/L} \), systolic BP (SBP) > 140 mmHg, diastolic BP (DBP) > 90 mmHg, or waist circumference > 102 cm, which individually were associated with an aged-adjusted relative risk RR of CVD \( \geq 1.5 \), as previously described by Wilson et al (17). Exclusion criteria included any clinically diagnosed disease, BP \( \geq 160/100 \text{ mmHg} \), abnormal liver function or blood cell count, use of lipid lowering or insulin sensitizing medication, use of steroids or non-steroidal anti-inflammatory drugs including aspirin, antibiotics use or vaccinations within 3 months of enrolment into the study, use of dietary fish-oil or high dose of anti-oxidant vitamin supplements, habitual consumers of more than one portion (140g) of oily fish per week, heavy drinkers (> 30 units (240g) of alcohol per week), smokers or ex-smokers ceasing < 3 months before screening.

Study protocol

On the three separate study days at least 4 weeks apart, EPA-rich oil (ERO), DHA-rich oil (DRO) or control oil (CO) containing meals were consumed in random order. Treatment order for each participant was randomized by an independent researcher, who was not involved in the study design, data collection or data analysis. For three days prior to their clinical visits subjects were asked to follow a restricted diet which included avoidance of n-3 PUFA rich foods and nitrate-, nitrite-, sulphite- and sulphate-rich foods (Supplementary Table S1) in order to standardize plasma n-3
PUFA, NO and H₂S status. Individuals were also asked to avoid alcohol or strenuous exercise in this three day period. The evening before their clinical visits, subjects were requested to consume a standard low fat meal (<10g of fat) which was provided. Adherence to the restrictions was assessed at each visit by questionnaire, and a 24 hour dietary recall.

On the clinical visit, baseline (0 h) vascular and BP measures were taken along with a fasting (> 10 h) blood sample. Following the consumption of the test meal, which participants were requested to consume within a 5 minute period, the vascular and BP measures and blood sampling were repeated at 4 h. The 4 h time point was selected to coincide with the predicted peak plasma lipaemia and EPA and DHA plasma concentrations following lipid consumption (7).

**Meal design**

Test meals were isocaloric (3128 kJ; 9.5% of energy as protein, 51.0% of energy as fat (42.4 g total fat), and 39.5% of energy as carbohydrate) and matched for taste, smell and appearance. They were composed of a high-fat chocolate flavored milkshake containing the test fats and a peppermint extract to mask the fish oils, accompanied by toasted white bread (73g) and a low fruit content jam (30g). In the control meal the milkshake was composed of 40 g of a 4:1 palm oil/soybean oil mixture, skimmed milk (150 g), chocolate flavored powder (15 g), skimmed milk powder (15 g) and 2 g of peppermint oil extract. In the ERO- and DRO- test meals, 6.94 g and 8.33 g of the palm oil/soybean oil mixture was replaced by ERO or DRO (Epax®, FMC Health and Nutrition, Sandvika, Norway, see Supplementary Table S2 for full fatty acid profile) to provide 4.16 g of EPA or DHA, respectively.

**Measures of blood pressure and vascular function**

All vascular measures were taken in a temperature controlled room following a 15 min rest period. BP was taken using an Omron 750 CP-II device (Omron, Milton Keynes, UK) in a supine position.

Arterial stiffness and endothelial dependent reactive hyperemia were assessed using PWV and the Endopat RHI respectively. AIx, a measure of the enhancement of central aortic pressure by a reflected pulse wave, which is dependent on the vascular tone of resistance arteries, was also undertaken.

RHI was measured by arterial tonometry using an Endopat device (Itamar Medical Ltd, Caesarea, Israel) as previously described (18). Measures were taken for 5 minutes at rest, 5 minutes during brachial artery occlusion and another 5 minutes subsequent to occlusion release. RHI was calculated
by expressing the mean amplitude of peripheral arterial tone post occlusion relative to those at baseline. Non-occlusion effects were corrected for by accounting for changes in tone in the control arm probes. The RHI measure was also taken a second time at the postprandial (4h) time point in response to 400mg of sub-lingual nitroglycerine (GTN) in order to assess an impact of treatment on NO independent vasodilation.

A vicorder device (Skidmore Medical, Bristol, UK) was used for the measurement of the PWV and AIx as described previously (19, 20). In brief, measures of carotid-femoral PWV were taken via placement of a BP cuff at the level of the carotid and femoral artery. The distance between the two anatomical sites was measured in each individual in order to allow calculation of pulse wave propagation speed in m/s. For AIx the Vicorder cuff was positioned over the brachial artery and AIx established by analysis of the blood pressure wave form. Specifically, AIx was calculated by expressing augmentation pressure (the difference between blood pressure during occurrence of the anacrotic notch and maximum systolic blood pressure) as a percentage of total pulse pressure.

All BP and vascular measures were taken in triplicate apart from the RHI and RHI*GTN measures, which due to the basis of the methodology and the recovery time needed to return to pre-measurement values, were measured only once.

**Serum/plasma lipids, glucose, nitrite and H\(_2\)S**

Blood samples for lipid and glucose analyses were left to sit at room temperature for 30 min and centrifuged at 2100 RCF at 4°C for 10 min. Serum samples were stored at -80°C until analysis. Serum triacylglycerol (TG), TC, HDL-C, non-esterified fatty acid (NEFA) and glucose were measured on an ILab650 automated analyzer (Instrumentation Laboratory, Warrington, UK) with commercially available kits (Instrumentation Laboratory).

Blood samples for nitrite and H\(_2\)S quantification were drawn into ice cold lithium heparin Vacutainers® and centrifuged immediately at 2100RCF at 4°C for 10 minutes. The obtained plasma samples were snap frozen and immediately stored at -80°C until analysis. Plasma nitrite analysis was conducted by reduction of nitrite to NO with chemiluminescent detection of NO undertaken via reaction with O\(_3\) as previously described (21). H\(_2\)S was measured by reverse-phase HPLC coupled
with fluorescence detection after derivatization with monobromobimane, following methodology
detailed previously by Shen et al (22).

**Plasma fatty acid and oxylipin analysis**

For fatty acid analysis, total lipids (TL) were extracted and analyzed as described previously (23). In
brief, TL were extracted from 500µl of plasma using chloroform/ methanol (2:1, vol/vol). Fatty acid
methyl esters (FAMEs) of the total lipid fraction were obtained by acid-catalyzed transmethylation,
using nonadecanoic acid (19:0) (5% of the total lipid analyzed), as an internal standard. FAMEs were
then purified by thin layer chromatography (TLC) and analyzed by gas-liquid chromatography.
Oxylipin analysis of plasma samples was carried out in the facilities of the Institute for Vascular
Signalling (Frankfurt, Germany). Briefly, samples were spiked with the corresponding deuterated
internal standards (Cayman Europe, Tallinn, Estonia) and lipid epoxide and diol derivatives were
extracted twice into ethyl acetate (0.5 ml) and quantified using a Sciex API4000 mass spectrometer
operating in the multiple reaction monitoring mode as previously described (24, 25).

**Statistical Analysis**

Unless otherwise stated, data are presented as means ± SEM. Sample size calculations were
undertaken with a power of 0.80 to detect clinically relevant changes at the 5% significance level for
Endopat, PWV and AIx. The primary outcome for the trial was the Endopat RHI index, with PWV and
AIx as secondary outcomes. For Endopat, given a SD of 0.4 (26) a clinically relevant difference of
0.25 between treatments would be detectable in 21 subjects per treatment group. For PWV, given a SD
of 1.2m/sec (27), a clinically relevant difference of 1.5m/sec would be detectable with a sample size of
11 participants per treatment group. For AIx, a clinically relevant 4% decrease in AIx would be
detectable in a population of 13 individuals, assuming a population SD of 5% in AIx. We aimed to
recruit 26 participants. This number was chosen to allow for a potential drop-out rate of 15% and to
provide a minimum of 22 participants completing the study.

Statistical analysis was undertaken using the SPSS statistical software (version 22; Chicago, Illinois,
USA) with P < 0.05 taken as being significant. Prior to analysis all datasets were checked for
normality via visual inspection of normal Q-Q Plots. Outliers were identified by analysis of normal Q-
Q Plots and variance in values greater than +/- two standard deviations.
Two-factor repeated measures ANOVA was used to determine the independent and interactive impact of time and treatment on the outcome measures. Assumptions of sphericity were assessed via Mauchly’s Test of Sphericity. In cases where the assumptions of sphericity were not met a Greenhouse-Geisser correction was applied. When significance was reached subsequent post-hoc analysis was undertaken to assess the significance of treatment group effect on the changes from baseline (4h-0h) using repeated measure ANOVA and Bonferroni adjustment. Pearson’s correlation analysis was used to establish the association between AIx responses and oxylipin levels.
RESULTS

Twenty six individuals with a mean age of 45 ± 5 y and BMI of 27.4 ± 3.3 kg/m² completed all three arms of the trial (Table 1). The DHA-rich or EPA-rich interventions had no significant effect on BP, PWV, RHI or RHI in response to sub-lingual GTN administration (400mg) (Table 2). For Alx, there was a statistically significant time (0 h versus 4 h) effect \( (P < 0.010) \) and time*treatment interaction \( (P = 0.005) \) (Figure 1) and post-hoc analysis revealed significant (13.3%, \( P = 0.047 \)) and borderline significant (11.3%, \( P = 0.06 \)) change scores for the DRO- and the ERO- meal compared to control. No time*treatment interactions were evident for TG, NEFA or glucose (\( P = 0.36, 0.90 \) and 0.49 respectively). However time effects were observed with 40% \( (P < 0.001) \) and 15% \( (P = 0.02) \) higher TG and NEFA respectively and 9% lower glucose \( (P < 0.001) \) levels at 4 h for all treatment groups combined (Table 3). No impact of time or treatment was evident for nitrite or H₂S concentrations.

The ERO- and DRO-meals increased plasma EPA and DHA by 158% \( (P = 0.03) \) and 88% \( (P = 0.05) \) respectively. Post-hoc analysis showed no significant change in DPA in response to any of the meals after a Bonferonni correction was applied (Table 4). A total of 7 EPA and DHA hydroxy, epoxide and diols metabolites were quantified (Table 4). The ERO-meal significantly increased plasma concentrations of 15S-HEPE, 14,15-EpETE, 17,18-EpETE, 14,15-DiHETE, and 17,18-DiHETE with the DRO also increasing concentrations of the EPA-derived metabolites 14,15-DiHETE, 17,18-EpETE, 17,18-DiHETE and the DHA-derived metabolite 19,20-DiHDPA. No changes were evident following consumption of the control meal.

14,15-DiHETE, 17, 18-DiHETE and 19, 20-DiHDPA were significantly negatively correlated with Alx \( (r = -0.215, P = 0.007; r = -0.223, P = 0.005 \) and \( r = -0.199, P = 0.013, \) respectively) (Supplementary Table 5S) with the Alx absolute response to DHA correlated with baseline Alx values \( (r = -0.413, P = 0.036) \).
DISCUSSION

Arterial stiffness and vasodilation of the conduit arteries are important determinants of SBP, left ventricular hypertrophy and overall CVD risk and are highly prognostic of future cardiovascular events (28, 29). Specifically AIX, a measure of pulse wave reflections influenced by vascular smooth muscle tone, affects central BP, with a reported HR of 1.68 (95% CI 1.02-2.76) for all-cause mortality and 1.60 (95% CI 1.07-2.39) for combined CVD end-points for men in the highest vs. lowest AIX tertile (30). In line with our recruitment of participants at above average CVD risk, mean baseline AIX values of 24.6% were, approximately 30-50% higher than those observed in RCTs which included healthy males (31, 32) or in the lowest male CVD risk tertile in the Copenhagen City Heart Study (CCHS) (33), and were within the highest risk tertile for CCHS (33). Our main finding is an overall treatment effect on AIX, with the modest differences in responses between DHA and EPA intervention relative to control, although statistically different, unlikely to be of clinical significance. If sustained, the observed DHA mediated 13.3% reduction would equate to a decrease in 10 year CVD risk from 3.3% to 2.8% in this population, using associations generated by the European Society of Cardiology (34). Furthermore EPA and DHA derived epoxides and diols were shown to be highly modifiable in the postprandial state and likely to contribute to the observed improved vascular function.

While to the best of our knowledge no previous RCT has compared the impact of EPA vs. DHA on AIX, a limited number of studies have reported a positive impact of chronic and acute combined EPA+DHA supplementation on this measure of arterial stiffness (9, 10, 35, 36). Using a comparable LCn-3PUFA exposure (4.7g EPA+DHA, EPA:DHA-0.67) Chong et al., reported that the inclusion of fish oil in a test meal reduced AIX in the 1.5-4h postprandial period in healthy adults, with an improved stiffness index only evident in male participants (9). Purcell et al., observed significant reductions in AIX in healthy men after fish oil (5g EPA+DHA, EPA:DHA-1.6) and algal oil (5g DHA only) administration, during a 6 h postprandial assessment which were most evident at 2 h and of comparable size effect to those observed in the current RCT (10). However, they did not include an EPA-only meal which would allow direct comparison of the vasoactivity of EPA vs. DHA on AIX and other measures of vascular function (10).
In the current study no effect of the EPA- or DHA-meals on PWV or RHI was observed. PWV is a proximal measure of gross arterial stiffness determined by the speed at which the pulse wave travels through the arterial tree, with an increased speed indicating increased stiffness and overall vascular dysfunction (28). In a meta-analysis of 17 studies, an increased PWV was associated with an increased risk of total CV events, CV mortality and all-cause mortality (RR (95% CI) of 2.26 (1.89-2.70), 2.02 (1.68-2.42) and 1.90 (1.61-2.70) respectively) (37). The observed lack of impact of treatment on postprandial PWV in the present study corroborates previous observations showing that PWV is not acutely modified in response to altered fatty acid composition (38, 39).

Peripheral arterial tonometry RHI as assessed by the proprietary Endopat device was employed as an observer-independent, high-throughput technique to assess endothelium-dependent vasodilation (40). Since this study was initiated, concerns have been expressed regarding the sensitivity of the Endopat technique, and in particular its ability to assess (subtle changes in) endothelial function in health individuals or at the early stages of vascular dysfunction (41, 42). Future trials should employ more sensitive techniques such as flow-mediated dilatation, in order to assess the impact of EPA vs. DHA on postprandial endothelium-dependent vasodilation. The prognostic value of a compromised flow-induced vasodilation (43), the observation of improved response to flow following chronic EPA+DHA supplementation (3) and of a differential and stronger impact of DHA relative to EPA using a less widely used and indirect measure of endothelium-dependent vasodilation, namely systemic vascular resistance as measured by the Finometer finger arterial BP monitor (11), provides strong justification for such research to be conducted.

Much of the mechanistic focus centered around the modulation of vascular function by EPA and DHA has been on nitric oxide (NO) bioavailability and endothelial NO synthase (eNOS) expression and phosphorylation status (44). There has been a degree of inconsistency with regards to the capacity of LCn-3PUFA to modify NO production postprandially, with some studies indicating an effect (8), whereas others have observed no changes (10). As NO is a labile compound, its status is often estimated via the concentrations of its oxidation products, most notably nitrite and nitrate, with nitrite thought to be the most reflective of changes in NO concentration (45). In agreement with previous
investigations utilizing this method there was no evidence of postprandial EPA or DHA mediated changes in plasma levels of nitrite or total NO metabolites.(10).

H₂S has recently emerged as a novel endothelium derived regulator of vascular function (16, 46). Although, the impact of EPA and DHA on H₂S status in the vasculature is almost completely unknown, a recent study reported an activation of cystathionine-γ-lyase (CSE), the main H₂S biosynthesis enzyme, in response to DHA-rich tuna-oil supplementation in the lung tissue of Sprague-Dawley rats (47). However no EPA or DHA mediated differences were evident in the current RCT and it is unlikely H₂S contributes to reduced arterial stiffness associate with acute DHA consumption. Finally, our study was novel in its investigation of the impact of EPA vs. DHA on the plasma concentration of selected vasoactive cytochrome 450 enzyme-derived EPA and DHA metabolites (oxylipins) and for the first time concurrent assessment of the impact of LCn-3PUFA on the vasoactive oxylinp profile and vascular responses in humans was conducted. Previous in vitro, ex vivo and animal investigations have shown that the LCn-3PUFA epoxides are particularly potent, and that the DHA epoxides account for approximately 75% of DHA’s capacity to illicit vasodilation in in-vitro models (48). Furthermore, the DHA epoxides have emerged as being more vasoactive than their EPA counterparts (15, 48, 49). In 2010, Shearer et al., reported for the first time the presence of EPA- and DHA- derived epoxide and diols in human plasma and their modulation by EPA plus DHA supplementation (50), which has been subsequently corroborated (51, 52). Consistent with the only previous acute assessment (52), which used a supra-physiological DHA-rich fish oil dose (26g EPA+DHA, EPA:DHA- 0.12), it can be confirmed that the hydroxy, epoxide and diol oxylipins are highly modifiable in the postprandial state with several fold higher concentration evident at 4 h post consumption of test meals containing 4.16g of EPA or DHA.

The DHA derived diol 19,20 EpDPE has emerged as a potentially potent vasomodulator with direct effects on vascular smooth muscle tone (48). A limitation of this study is that we were not able to detect the 19,20 EpDPE which fell below the limit of detection. Results from the human serum metabolome project suggest that the molar ratio of 19,20 EpDPE to 19,20 DiHDPA is in the range of 1 in 8 (53), which places plasma concentrations of 19,20 EpDPE in the low picomolar range. However, we did observe a highly significant 1.3 fold increase in the daughter 19,20 DiHDPA diol in response
to DHA consumption, with 19,20 DiHDPA concentration negatively correlated with AIx responses. Given that 19,20 DiHDPA is directly and exclusively derived from 19,20EpDPE via the activity of the soluble epoxide hydrolase, the higher postprandial 19,20 DiHDPA levels are likely reflective of increases in 19,20 EpDPE which is proposed to have contributed to the vascular impact of DHA vs. EPA.

However, we cannot preclude the possibility that EPA-derived oxylipins also contributed to the vascular response following DRO intervention. The strong trend towards an increase in plasma EPA following DRO intervention, was likely due to some EPA being provided as part of the DRO intervention or the known tissue retroconversion of DHA to EPA (54). These sources provided sufficient amounts for the CYP epoxygenases that generally prefer EPA over DHA and arachidonic acid (55) with the DRO meal resulting in an increase in three EPA-derived oxylipin metabolites, with 14,15-DiHETE and 17, 18-DiHETE correlated with AIx. In addition to the CYP derived oxylipins, prostanoids and lipoxygenase derived resolvins may contribute to the vascular response to n-3 PUFA intervention. Although potentially important in the vascular wall, this latter group of compounds have proved difficult to quantify in the human circulation.

Therefore following DHA intervention changes in the tissues status of both DHA and EPA and their associated oxylipins and other metabolites may act in a complementary and additive fashion (56) to mediate the impact on vascular function.

The strengths of the current study were its cross-over design, its inclusion of a range of measures of vascular function, with simultaneous analysis of vascular function and potential lipid and non-lipid derived mediators in the circulation at the clinical assessment time-points. A limitation is the likely lack of sensitivity of the Endopat technique employed to assess the impact of treatment of endothelial dependent vasodilatation.

In summary, the results of this study show that DHA improves postprandial vascular function, with an effect size which would translate into a meaningful reduction in CVD risk, with a strong trend also evident following EPA intervention. The targeted lipidomic profiling indicates that postprandial changes in the LCn-3PUFA epoxides and diol occur and are likely to play a mechanistic role as effector molecules of these changes in vascular tone. Further research is needed to fully establish the
individual impact of EPA vs. DHA on clinical endpoint and disease biomarkers and to gain insight into the bioactive lipids modulating the effects. Ultimately such information would allow, the refinement of current EPA and DHA recommendations, the development of fish oil blends (with defined EPA and DHA content) to suit phenotype, and would inform the design of much needed non-marine sources of these fatty acids.
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AUTHOR CONTRIBUTION

The authors have no conflicts of interest to disclose. SMc, AC and AMM designed the study. SMc conducted the RCT including vascular assessments. IF designed the oxylipin analysis approach. SMc, NT, KA, DV, IF conducted the sample analysis. SMc and NT performed the statistical and data analysis. SMc, NT and AMM wrote the paper. AMM had primary responsibility for final content. All authors read and approved the final manuscript.
REFERENCES


and plasma 8-isoprostane F2alpha concentrations relative to a control high-oleic acid meal: a randomized controlled trial. *The American journal of clinical nutrition* **100**: 1019-1028.


47. Huang, T., M. L. Wahlqvist, and D. Li. 2010. Docosahexaenoic acid decreases plasma homocysteine via regulating enzyme activity and mRNA expression involved in methionine metabolism. *Nutrition (Burbank, Los Angeles County, Calif.)* **26**: 112-119.


FIGURE LEGENDS

Figure 1. Augmentation index (%) at baseline and in response to treatment.

Data are presented as mean ± SEM (n = 26). CO: Control oil; ERO: EPA rich oil; DRO: DHA rich oil. Two-factor repeated measures ANOVA. \( P < 0.010 \), \( P \), time*treatment = 0.005. * indicates significantly different (\( P = 0.047 \)) change scores for DRO when compared to CO.
### Table 1. Study population characteristics (n=26).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>45 ± 5 (36 - 54)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.0 ± 11.5 (73.5-129.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4 ± 3.3 (20.4-39.9)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>95.5 ± 10.4 (76.8-128.0)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.8 ± 0.9 (3.8-7.3)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.4 ± 0.3 (0.86-2.32)</td>
</tr>
<tr>
<td>Systolic Blood pressure (mmHg)</td>
<td>136 ± 10 (101-150)</td>
</tr>
<tr>
<td>Diastolic Blood pressure (mmHg)</td>
<td>86 ± 7 (67.3-104)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM with the range of values in brackets.
Table 2. Vascular measurements at baseline (0h) and in response to treatment (4h).

<table>
<thead>
<tr>
<th></th>
<th>CO meal (0h)</th>
<th>ERO-meal (0h)</th>
<th>DRO-meal (0h)</th>
<th>P, time (^1)</th>
<th>P, time* treatment (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP (mmHg)</td>
<td>77 ± 1</td>
<td>77 ± 2</td>
<td>76 ± 2</td>
<td>75 ± 1</td>
<td>76 ± 1</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125 ± 3</td>
<td>125 ± 2</td>
<td>127 ± 2</td>
<td>125 ± 2</td>
<td>124 ± 1</td>
</tr>
<tr>
<td>RHI</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>RHI*GTN</td>
<td>-</td>
<td>1.7 ± 0.1</td>
<td>-</td>
<td>1.5 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>8.5 ± 0.2</td>
<td>8.3 ± 0.2</td>
<td>8.3 ± 0.1</td>
<td>8.4 ± 0.2</td>
<td>8.2 ± 0.2</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM (n=26). CO: Control oil; ERO: EPA- rich oil; DRO: DHA- rich oil; DBP: diastolic blood pressure; SBP: systolic blood pressure; RHI: reactive hyperaemic index; RHI*GTN: RHI after 400mg of sub-lingual nitroglycerine (GTN); PWV: pulse wave velocity. \(^1\)Two-factor repeated measures ANOVA.
Table 3. Biochemical measures at baseline and in response to treatment.

<table>
<thead>
<tr>
<th></th>
<th>CO meal</th>
<th>ERO-meal</th>
<th>DRO-meal</th>
<th>( P, \text{time}^{1} )</th>
<th>( P, \text{time* treatment}^{1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0h)</td>
<td>(4h)</td>
<td>(0h)</td>
<td>(4h)</td>
<td>(0h)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.7 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>2.3 ± 0.2</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>NEFA (( \mu \text{mol/L} ))</td>
<td>359 ± 24</td>
<td>424 ± 36</td>
<td>335 ± 32</td>
<td>384 ± 35</td>
<td>351 ± 31</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.2 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>Nitrite (mmol/L)</td>
<td>88.7 ± 8.6</td>
<td>80.1 ± 7.2</td>
<td>84.3 ± 6.2</td>
<td>74.3 ± 6.5</td>
<td>78.4 ± 6.5</td>
</tr>
<tr>
<td>( \text{H}_{2}\text{S} ) (mmol/L)</td>
<td>348.3 ± 59.3</td>
<td>386.6 ± 62.0</td>
<td>369.2 ± 59.6</td>
<td>341.7 ± 39.4</td>
<td>377.5 ± 63.6</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM (n=26). CO: Control oil; ERO: EPA- rich oil; DRO: DHA- rich oil; \( \text{H}_{2}\text{S} \): hydrogen sulfide. \(^{1}\)Two-factor repeated measures ANOVA.
Table 4. Plasma long chain n-3 PUFA concentrations along with select hydroxy, epoxide and diol metabolites, measured at baseline and in response to treatment (4h).

<table>
<thead>
<tr>
<th></th>
<th>Control meal</th>
<th>ERO-meal</th>
<th>DRO-meal</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0h)</td>
<td>(4h)</td>
<td>(0h)</td>
<td>(4h)</td>
<td>P, time1</td>
</tr>
<tr>
<td><strong>Fatty acids (mg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>3.0 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>8.5 ± 0.3*</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DPA</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.86</td>
</tr>
<tr>
<td>DHA</td>
<td>6.6 ± 0.2</td>
<td>6.4 ± 0.1</td>
<td>6.7 ± 0.2</td>
<td>7.5 ± 0.2</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Oxylipins (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxy Metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15S-HEPE</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.8 ± 0.5</td>
<td>2.6 ± 0.7*</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>18S-HEPE</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>1.8 ± 1.0</td>
<td>6.5 ± 2.3</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Epoxide Metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14, 15-EpETE</td>
<td>0.6 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.3</td>
<td>2.9 ± 0.8*</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>17, 18-EpETE</td>
<td>0.9 ± 0.5</td>
<td>0.4 ± 0.0</td>
<td>0.6 ± 0.2</td>
<td>9.9 ± 1.8*</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diol Metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14, 15-DiHETE</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.0</td>
<td>1.7 ± 0.1</td>
<td>3.2 ± 0.3*</td>
<td>1.6 ± 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>17, 18-DiHETE</td>
<td>3.0 ± 0.5</td>
<td>2.6 ± 0.2</td>
<td>2.9 ± 0.3</td>
<td>9.7 ± 1.0*</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>19, 20-DiHDPA</td>
<td>0.9 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM (n=26). ERO- EPA rich oil, DRO- DHA rich oil. *Two-factor repeated measures ANOVA. *Indicates a significant difference (P in change from baseline when compared to control.