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Lack of effect of supplementation with EPA or DHA on platelet-monocyte aggregates and vascular function in healthy men☆

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Abstract *Background and Aims:* Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in fish oil are postulated to have favourable effects on platelet, endothelial and vascular function. We investigated whether EPA has differential effects on *in vivo* platelet aggregation and other markers of cardiovascular risk compared to DHA.
Methods and Results: Following a 2 wk run-in taking encapsulated refined olive oil, 48 healthy young men were randomly allocated using a parallel design to receive EPA-rich (3.1 g EPA/d) or DHA-rich (2.9 g DHA/d) triglyceride concentrates or refined olive oil (placebo), for a total supplementary lipid intake of 5 g/d. The specified primary outcome was change in platelet monocyte aggregates (PMA); secondary outcomes were capillary density, augmentation index, digital pulse volume measurements, 24 h ambulatory BP, plasma 8-isoprostanes-$F_{2\alpha}$. Changes in the proportions of DHA and EPA in erythrocytes and non-esterified fatty acid composition indicated compliance to the intervention. There was no significant treatment effect on PMA ($P = 0.382$); mean changes (%) (95% CI) were placebo −0.5 (−2.0, 1.04), EPA 0.4 (−0.8, 1.6), DHA 0.3 (−1.5, 2.0). R-QUICKI, an index of insulin sensitivity, was greater following EPA compared to placebo ($P < 0.05$). No other significant differences were noted.
Conclusion: Neither EPA- nor DHA-rich fish oil supplementation influence platelet-monocyte aggregation or several markers of vascular function after 6 wk in healthy young males. This trial was registered at clinicaltrials.gov as NCT01735357.

Introduction

Epidemiological and observational studies support the theory that fish or fish oil consumption reduces the risk of cardiovascular disease (CVD) [1], while recent meta-analyses found no association between n-3 polyunsaturated fatty acids (n-3 PUFA) supplementation and CVD death, or events [2]. Studies vary in design, populations, duration and doses, as well as the respective ratios of EPA and DHA used, and investigations into their separate effects are limited [3].

Endothelial dysfunction characterizes the initiating stage of atherogenesis, and is closely linked to platelet activation. Nitric oxide produced by the endothelium plays a crucial role in platelet activation, including P-selectin

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expression and platelet-monocyte aggregation [4]. Platelet-monocyte aggregates (PMA) constitute an early marker of CVD risk [5] and are believed to be a more accurate indicator of true *in vivo* platelet function compared to *ex vivo* measures of platelet aggregation, and also to other *in vivo* measures like platelet surface P-selectin [6,7]. On the other hand, platelet activation promotes the homing of endothelial progenitor cells (EPCs) to the sites of endothelial injury and favours their differentiation into endothelial cells [8]. The postulated anti-thrombotic actions of fish oils have been mostly attributed to the ability of EPA to compete with arachidonic acid (AA) in the cyclooxygenase (COX) pathway, leading to the formation of eicosanoids that are less pro-thrombotic than the eicosanoids derived from AA [9]. Both EPA and DHA may also give rise to the production of protectins and resolvins that appear to have potent anti-inflammatory actions [9], and may also directly inhibit platelet aggregation [10], which may account for the anti-thrombotic actions of n-3 PUFAs. On the other hand, the additional double bond in DHA may lead to a greater effect on platelet membrane fluidity compared to EPA [11]. EPA and DHA may also enhance endothelial function [12,13] and improve EPC functionality and numbers in vitro [14] and in healthy subjects at moderate risk of CVD [15], possibly by modulating mobilization, adhesion and angiogenesis mediated by changes in eicosanoid metabolism and/or nitric oxide signalling.

In healthy young men, oily fish consumption (providing ~ 1 g EPA + DHA/d) for 4 wk reduced PMA by 35% [16] and the present study set out to determine whether this effect was primarily influenced by EPA or DHA. It should be noted, however, that the study was designed before the publication of further findings from the same group in 2013, showing that fish oil supplementation (2 g/d, 6wks) did not affect PMA in CHD patients [17], nor in cigarette smokers [18]. The overall aim was to determine whether supplementation with oils enriched with either EPA or DHA, had differential effects on markers of platelet, endothelial and vascular function in healthy young males. Our primary outcomes included PMA and numbers of EPC, as a putative marker of endothelial repair [19]. However, EPCs are very rare events and account for 0.0001–0.01% of all mononuclear cells. Technical difficulties were encountered in the EPC method, which did not allow the recording of sufficient events to provide accurate results. Thus only the PMA data are reported as primary outcomes. Secondary outcomes included finger capillary density (a marker of microvascular function), plasma 8-isoprostane-$F_{2\alpha}$, arterial stiffness (digital and radial pulse wave analysis), ambulatory blood pressure (BP)/heart rate (HR) measurement, and circulating markers of metabolic dysregulation.

Methods

Participants

Ethical approval was granted by the Bromley Research Ethics Committee, London in April 2009 (ref. 08/H0805/2) and informed consent was obtained from all participants. Healthy males (aged 18–45 y) were recruited through internal e-mail circulars and posters among King's College London students and staff, and fitness centre users in the vicinity. Exclusion criteria were history of CVD, diabetes (or fasting glucose ≥ 6.1 mmol/L), cancer, kidney, liver or bowel disease; gastrointestinal disorder or use of drug altering nutrient absorption; smoking, history of substance abuse or alcoholism (>60 units/wk); current alcohol intake > 28 units/wk; BP > 160/90 mmHg, fasting blood cholesterol > 6.5 mmol/L; fasting triacylglycerol concentrations > 2.0 mmol/L, platelet count above or below the normal range or any history indicative of a congenital or acquired platelet or haemostatic defect and recent use of associated medications; allergy or intolerance to study capsules; current consumption of >1 portion oily fish/wk; weight change of >3 kg in preceding 2 months; BMI <18 and >32 kg/m^2. At screening participants filled in a questionnaire on their general health, alcohol and fish consumption; BP, height and weight, body fat percentage, waist and hip circumference were measured, and blood was tested for liver function, fasting glucose, insulin, lipid profile and full blood count.

Intervention

The single-blind, randomized, placebo-controlled, parallel study ran from June 2009 to May 2010. Power calculations were based on 16 subjects per group completing the study to give an 80% power to detect a 10% unit difference in mean PMA at a significance level of 0.05, using standard deviations and expected changes from a previous study [16]. Following a run-in period (5 g refined olive oil/d for 2 wk), participants were randomly assigned to receive either EPA-, DHA-rich oils, or refined olive oil (British Pharmacopoeia specification, maximum unsaponifiable matter 1.5%) in the form of purified triglycerides in soft gel capsules (Incromega™ EPA500TG SR, DHA500TG SR and refined olive oil capsules were provided by Croda Chemicals Europe Ltd, UK). EPA- and DHA-rich oils were blended with refined olive oil and calculations were made to reach a consumption of ~3 g/d of EPA (3.1 g EPA + 0.10 g DPA + 0.71 g DHA) or DHA (2.9 g DHA + 0.17 g DPA + 0.52 g EPA) for a total lipid intake of 5 g/d. During the intervention, participants were asked to avoid medications and consumption of oily fish and dietary supplements. Compliance to treatment was assessed by the incorporation of EPA and DHA into erythrocyte membrane. The composition of NEFA was also assessed as changes in their levels and proportions may impact endothelial and vascular function [20].

Data collection

Participants attended the Metabolic Research Unit at King's College London in the morning at the end of the 2-wk run-in period and of the 6-wk treatment phase. Participants were instructed to consume a low fat evening meal, fast overnight, and avoid drinking alcohol, caffeine