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Fish oil and multivitamin supplementation reduces oxidative stress but not inflammation in healthy older adults: A randomised controlled trial

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ABSTRACT

The effects of daily fish oil supplementation, with and without multivitamins, on biochemical markers of health were examined. Healthy adults (N = 160) were randomised to receive 3 g of salmon oil with a multivitamin, 6 g of salmon oil with a multivitamin, 6 g of salmon oil in isolation or placebo in a double-blind fashion on a daily basis for 16 weeks. Relative to placebo, both 6 g salmon oil groups displayed significantly lower F_2-isoprostane levels at endpoint. Increases in red blood cell polyunsaturated fatty acids correlated with reductions in F_2-isoprostanes. Treatment had no effect on inflammatory cytokines, C-reactive protein, fibrinogen, cholesterol or triacylglycerol.

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Abbreviations: ANZCTR, Australia and New Zealand Clinical Trials Registry; AA, arachidonic acid; CVD, cardiovascular disease; CRP, C reactive protein; DHA, docosahexaenoic acid; EDTA, ethylenediaminetetraacetic acid; EPA, eicosapentaenoic acid; HDL, high density lipoprotein; LC n-3 PUFA, long chain omega-3 polyunsaturated fatty acids; LDL, low density lipoprotein; n-3, omega-3; n-6, omega-6; RBC, red blood cells; RCT, randomised controlled trial; ROS, reactive oxygen species

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1. Introduction

Fish oil supplements containing the long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are one of the most popular dietary supplements in the western world (Morgan et al., 2012). Evidence suggests that higher intake of LC n-3 PUFA is related to better cardiovascular (Kris-Etherton, Harris, & Appel, 2002) and perhaps even neurological outcomes (Fotuhi, Mohassel, & Yaffe, 2009; Tan et al., 2012). Fish oil supplements have been reported to improve the lipid profile, predominantly by lowering triacylglycerol levels (Harris, 1989), exerting vasodilatory effects (Xin, Wei, & Li, 2012) and by lowering blood pressure (Liu, Conklin, Manuck, Yao, & Muldoon, 2014).

LC n-3 PUFAs also play an important role in regulating inflammation (Calder, 2006b). The omega-6 (n-6) arachidonic acid (AA) has pro-inflammatory effects through the production of eicosanoids. Both AA and LC n-3 PUFA compete for space inside cell membrane phospholipids (Simopoulos, 2002). Increasing cellular levels of LC n-3 PUFA decreases the amount of AA in cell membranes thus limiting AA derived eicosanoids (Simopoulos, 2002). The ratio of LC n-3 PUFA to omega-6 fatty acid has thus been put forward as a measure of inflammation (Kalogeropoulos et al., 2010). Observational studies have reported that higher circulating levels of LC n-3 PUFA are associated with lower levels of pro-inflammatory cytokines and lower levels of C-reactive protein (CRP) (Farzaneh-Far, Harris, Garg, Na, & Whooley, 2009; Ferrucci et al., 2006; Kalogeropoulos et al., 2010), which are known to be predictive of cardiovascular disease (CVD) (Ridker, Hennekens, Buring, & Rifai, 2000).

LC n-3 PUFAs may also have antioxidant effects. A study by Mori et al. (1999) reported that a daily fish meal for eight weeks reduced urinary F_2-isoprostane levels in sedentary subjects with non-insulin dependent diabetes. F_2-isoprostanes are lipid peroxidation products derived from the non-enzymatic free radical oxidation of AA in membrane lipids and are considered the most reliable biomarkers of *in vivo* lipid peroxidative damage (Milne, Yin, Hardy, Davies, & Roberts, 2011). Subsequent placebo controlled studies using purified EPA or DHA showed both fatty acids reduced urinary (Mori et al., 2000, 2003) and plasma (Mas et al., 2010) F_2-isoprostanes in type 2 diabetic patients, and in overweight mildly dyslipidaemic men, respectively. A recent double-blind, 4 month randomised, controlled trial (RCT) reported that LC n-3 PUFA supplementation reduced plasma F_2-isoprostane levels by approximately 15%, relative to placebo (Kiecolt-Glaser et al., 2013).

Multivitamin supplements, containing both vitamins and minerals, are commonly used among the general population (Radimer et al., 2004). Evidence suggests that the intake of a range of vitamins and minerals may interact with fatty acid metabolism influencing the overall levels of LC n-3 PUFA measured *in vivo* (Bertrandt, Klos, & Debski, 2004; Durand, Prost, & Blache, 1996; Pipingas et al., 2014; Stangl & Kirchgessner, 1998). There is thus a need to examine the combined effects of multivitamin supplements and LC n-3 PUFAs on all aspects of human health.

The present 16 week RCT was designed to investigate the effect of salmon oil supplementation, with and without the addition of a multivitamin supplement, on cardiovascular and cognitive health in healthy middle-aged and elderly subjects. To the best of our knowledge, this is the first RCT to investigate the combined effects of fish oils and multivitamins on human health. The results of supplementation on the cognitive, cardiovascular and omega-3 uptake into red bloods cells arising from this RCT have been published (Pase et al., 2015; Pipingas et al., 2014). This report examines the secondary outcomes from this study including the effects of supplementation on measures of inflammation, oxidative stress, serum lipids and liver function, in the same subjects. It was hypothesised that treatment with salmon oil would reduce markers of inflammation and oxidative stress as well as triacylglycerol levels. The study also had the novel aim of exploring whether combining salmon oil with a multivitamin would provide added benefits to human health.

2. Materials and methods

2.1. *Participants*

One hundred and sixty healthy, non-smoking, male and female volunteers aged between 50 and 70 years were recruited from the community using newspaper advertisements, posters and community radio announcements. Participants were not currently taking any medication, such as anti-coagulants, anticholinergic, anti-depressant medications and acetylcholinesterase inhibitors, or vitamin/herbal supplements. Subjects were excluded if they fulfilled any of the following; diagnosis of dementia, diabetes, neurological and psychiatric disorders, overt CVD or past or present drug or alcohol abuse. Long term multivitamin and/or omega-3 supplement users were also excluded. The participant flow diagram is shown in Fig. 1.

2.2. *Setting*

The study was conducted at the Centre for Human Psychopharmacology Laboratory at Swinburne University of Technology, Hawthorn, Australia. The study was approved by the Swinburne University Human Research Ethics Committee and all procedures were conducted in accordance with the guidelines of the Australian National Health and Medical Research Council and the Declaration of Helsinki (2008). This trial was registered with the Australian and New Zealand Clinical Trial Registry (ANZCTR no. 12611000094976). Participants gave informed written consent to participate in the study.

2.3. *Interventions, randomisation and blinding*

This study was a 16 week randomised, double-blind, placebo-controlled trial comparing the effects of salmon oil supplements and multivitamins on blood biomarkers. Participants were randomly assigned to one of four treatment groups: multivitamin and salmon oil (3 g); multivitamin and salmon oil (6 g); placebo multivitamin and salmon oil (6g); or placebo multivitamin and placebo salmon oil (Sunola oil).

Participants were instructed to take their assigned treatment daily for 16 weeks. The study medications were provided