Effect of Omega-3 Fatty Acid Supplementation on Arterial Elasticity in Patients with Familial Hypercholesterolaemia on Statin Therapy

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ABSTRACT

Background and aims: Increased arterial stiffness is closely linked with raised blood pressure that contributes substantially to enhanced risk of coronary heart disease in high risk individuals with familial hypercholesterolaemia (FH). Omega-3 fatty acid (ω3-FA) supplementation has been demonstrated to lower blood pressure in subjects with a high cardiovascular disease risk. Whether ω3-FA supplementation improves arterial stiffness in FH subjects, on background statin therapy, has yet to be investigated.

Method and results: We carried out an 8-week randomized, crossover intervention trial to test the effect of 4 g/d ω3-FA supplementation (46% eicosapentaenoic acid and 38% docosahexaenoic acid) on arterial elasticity in 20 adults with FH on optimal cholesterol-lowering therapy. Large and small artery elasticity were measured by pulse contour analysis of the radial artery. ω3-FA supplementation significantly ($P<0.05$ in all) increased large artery elasticity (+9%) and reduced systolic blood pressure (-6%) and diastolic blood pressure (-6%), plasma triglycerides (-20%), apoB concentration (-8%). In contrast, ω3-FAs had no significant effect on small artery elasticity. The change in large artery elasticity was not significantly associated with changes in systolic blood pressure or plasma triglyceride concentration.

Conclusions: ω3-FA supplementation improves large arterial elasticity and arterial blood pressure independent of statin therapy in adults with FH.

Clinical Trial Registration: https://www.clinicaltrials.com/ NCT01577056

Keywords: Atherosclerosis, Genetics, Nutrition, Hypercholesterolaemia

INTRODUCTION

Familial hypercholesterolaemia (FH) is a dominantly inherited disorder principally due to mutations in the LDL-receptor pathway that classically causes markedly elevated plasma LDL-cholesterol concentrations and premature coronary heart disease (CHD) [1, 2]. Despite treatment of FH patients with statins to prevent atherosclerosis, a significant residual cardiovascular risk often remains [3].

Atherosclerosis is a chronic, lipid-driven disease of the arterial wall involving complex and multifactorial processes that leads to arterial wall stiffness [4]. FH patients with untreated hypercholesterolaemia appear accordingly to have impaired arterial elasticity. We have recently demonstrated that hypertension and hypertriglyceridaemia are risk factors which predict coronary artery disease (CAD) in patients with FH [5]. Increased risk of CHD in FH may relate to alterations in the biophysical properties of the arterial wall due to elevated LDL-cholesterol and other co-existent metabolic risk factors, such as hypertriglyceridaemia and inflammation.[4] Increased arterial stiffness, or decreased elasticity, involves endothelial dysfunction and alteration in the collagen matrix in the artery wall. Recent population studies have found that decreased arterial elasticity independently predicts coronary and peripheral diseases [6-9]. Improving arterial elasticity could help to reduce risk of cardiovascular complications in FH subjects.

Compelling evidence suggests that omega-3 fatty acid (ω3-FA) supplementation, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protects against CHD [10, 11]. Clinical evidence indicates that ω3-FA supplementation decreases plasma triglycerides and improves endothelial function, arterial stiffness and blood pressure in patients with obesity and/or type 2 diabetes [12-19]. These

favorable vascular effects of ω3-FAs may contribute to improved cardiovascular outcomes, as demonstrated in large intervention trials [20, 21]. However, the effect of ω3-FA supplementation on arterial stiffness has not been investigated in FH.

In the present study, we tested the hypothesis that supplementation with ω3-FA improves the elasticity of large and small arteries in FH patients receiving standard treatment for lowering LDL-cholesterol.

Methods

Subjects

Twenty-two patients with FH aged 18-70 years [body mass index (BMI) <40 kg/m^2] were recruited from the Lipid Disorders Clinic at Royal Perth Hospital. Diagnosis of FH was defined by the Dutch Lipid Clinic Network criteria (DLCN) score >8 (definite FH) and/or the presence of a pathogenic mutation in the *LDLR* gene. None of the subjects had *APOE2/E2* genotype, proteinuria, creatininaemia (>120 μmol/L), hypothyroidism, intolerance to ω3-FA or abnormal liver enzymes (alanine aminotransferase >120 U/L for men and >90 U/L for women). None reported a cardiovascular event within six months prior to the study, or was taking ω3-FA supplementation or anti-diabetic medication. This study was approved by the Human Research Ethics Committee of the Royal Perth Hospital, and written informed consent was obtained from all subjects.

Clinical Protocol

This study was a randomized, crossover intervention trial. All eligible patients were on statins (or statin plus ezetimibe) at recruitment and throughout the duration of the study. All patients entered a 4-week run-in diet stabilizing period, at the end of which they were randomized to one of the two groups, no treatment or a 8-week treatment period of 4 g/d ω3-FA supplementation (Omacor® 46% EPA and 38% DHA in ethyl ester form, Abbott Products Pty Ltd) with an 8-week washout between each intervention period. This dose is equivalent to an intake of 190-240 g fatty fish per day [22]. Compliance with ω3-FA supplementation was checked by capsule count at

the end of ω3-FA intervention. All subjects were reviewed fortnightly and requested to maintain their diet intake and usual level of physical activity.

Measurements of blood pressure and arterial elasticity

Blood pressure and arterial elasticity were performed at the end of each treatment period. All subjects were admitted to the metabolic ward in the morning after a 14-h fast. Arterial blood pressure was recorded after 3 min in the supine position using a Dinamap1846 SX/P monitor (Critikon Inc, Tampa, FL, USA). Arterial elasticity was measured using pulse contour analysis (Hypertension Diagnostics Inc/PulseWave™ CR-2000, Eagan, Minnesota, USA) with CV of <5%, as described previously [23]. Radial artery waveforms were recorded for 30 s and calibrated by the oscillometric method with a cuff on the opposite arm and an internal calibration. Radial pulse-contour analysis was used to derive large (C1) and small (C2) artery elasticity using a validated modified version of the Windkessel model. C1 and C2 were measured at the end of each intervention period.

Biochemical measurements

Fasting blood samples were collected at the end of each treatment period. Briefly, fasting whole venous blood samples collected in EDTA were immediately centrifuged at 1500 xg for 15 min at 4°C. Plasma was collected and stored at -80°C. Plasma lipid and glucose concentrations were measured using enzymatic methods (Hitachi 917 Biochemical Analyser, Roche Diagnostics Australia Pty Limited, Castle Hill, NSW, Australia). LDL cholesterol was estimated by the Friedewald calculation [24]. Fasting insulin was measured using chemiluminescent immunometric assay (Abbott

Diagnostics, North Ryde, NSW, Australia), and insulin resistance was estimated using homeostasis model assessment (HOMA score) [25].

Statistical Analyses

Data are reported as mean ± SEM unless specified. Significance was defined at the 5% level using a two-tailed test. Groups were compared using independent *t*-tests. Paired *t*-tests were used between the FH patients. All data were analysed using the SPSS 21 (SPSS, Chicago, IL) software. Carryover effect of the cross-over design was estimated using SAS 9.2 (SAS Institute, Cary, North Carolina, USA).

Results

Of the twenty-two eligible subjects, one withdrew consent before completing the first intervention period, and another was withdrawn because of an adverse event related to new onset atrial fibrillation that resolved spontaneously within 24 hrs. A total of 20 patients with FH (10 men and 10 women) completed the study. On average, they were middle-aged (53.3 ± 3.0 years), non-obese (BMI 26.6 ± 5.8 kg/m^2) and normotensive (systolic blood pressure 121 ± 15 mmHg and diastolic blood pressure 69 ± 8 mmHg) at screening. None of the FH patients were current smokers. Using data from our previous studies [17, 26], the FH patients had a comparable large (C1 17.3 ± 3.8 vs. 16.5 ± 4.6 mL/mmHg x10, P>0.05) and small compliance (C2 6.49 ± 3.0 vs. 8.68 ± 3.3 mL/mmHg x100, P>0.05) compared with control subjects (age 44 ± 5 years and BMI 28.2 ± 3.9 kg/m^2). Seventeen patients were genetically diagnosed with FH (i.e. pathogenic *LDLR* mutations) and the other three had a DLCN score >8 (definite phenotypic FH). Nine subjects were on rosuvastatin, eight on atorvastatin and three on simvastatin. Of these, thirteen patients were also on ezetimibe (10 mg/day). Three subjects were on anti-hypertension medication and four reported a history of CAD event.

Table 1 shows the effects of ω3-FA supplementation on clinical and biochemical characteristics in 20 FH patients. Body weight, waist circumference, BMI and pulse pressure did not alter significantly during the intervention (P>0.05 in all). ω3-FA supplementation significantly (P<0.05 in all) lowered systolic blood pressure (-7.7 $\pm$ 2.6 mmHg) and diastolic blood pressure (-4.2 $\pm$ 1.4 mmHg), plasma triglycerides (-0.26 $\pm$ 0.01 mmol/L) and apoB concentration (-0.08 $\pm$ 0.03 g/L). ω3-FA supplementation tended to lower total cholesterol and non-HDL-cholesterol concentrations, but these failed to reach significance (P<0.1 for both). LDL-

cholesterol and HDL-cholesterol concentrations were not significantly altered with ω3-FA supplementation, nor were glucose, insulin concentrations and HOMA score.

Supplementation with ω3-FA significantly increased large artery elasticity by +9% (+1.53 ± 0.45 mL/mmHg x10, $P<0.05$). ω3-FA intervention did not significantly affect small artery elasticity. When dividing the FH patients into two groups (n=10 each) by the median baseline large artery elasticity level (14.5 mL/mmHg x 10), the effect of ω3-FA supplementation on large artery elasticity was chiefly seen in the FH subjects who had lower baseline large artery elasticity levels compared with those had higher baseline level (+14% vs. +4%, $P<0.05$). There was no significant effect on small artery elasticity with ω3-FA supplementation in either group (data not shown). Figure 1 shows the effect of fish oil supplementation on large and small artery elasticity in our 10 FH patients who had baseline arterial elasticity below the median. The increase in large artery elasticity was not significantly associated with changes in systolic blood pressure, diastolic blood pressure, plasma triglycerides or other variables in Table 1.

Discussion

The major finding of this study was that ω3-FA supplementation improved large artery elasticity, and reduced systolic blood pressure, diastolic blood pressure, plasma triglyceride and apoB concentrations. These changes were demonstrated against a background of cholesterol-lowering therapy (statin with and without ezetimibe)

Previous studies have shown conflicting results regarding the effect of ω3-FA supplementation on artery elasticity measured by pulse contour analysis in humans [12-18]. Hill *et al.* and Meyer *et al.* found that dietary supplementation with 4-8 g/day DHA-rich tuna oil (approximately 1.2 to 2.4 g n-3 fatty acids) had no effect on large and small artery elasticity in overweight and statin-treated hypertriglyceridaemic subjects [12, 13]. Wang *et al.* reported that ω3-FA supplementation (3 g/day containing EPA 540 mg and DHA 360 mg) improved large artery elasticity, but had no effect on blood pressure or small artery elasticity in overweight hypertensive subjects [14], consistent with a study by Sjoberg *et al.* in overweight and obese adults [15]. However, McVeigh *et al.* found in non-obese subjects with type 2 diabetes that 6-weeks of dietary supplementation with fish oils (3 g/day) increased large and small artery elasticity [16]. The discrepant findings of the aforementioned studies, particularly regarding large and small artery elasticity, might be accounted for by differences in subject characteristics, experimental protocols and the type (such as ethyl ester versus triglyceride forms of fish oils and ratio of DHA/EPA) and dose of ω3-FA employed. We have previously reported that 12-weeks of high dose ω3-FA supplementation increased large and small artery elasticity in obese

individuals [17]. We also showed that large artery elasticity was improved after 8-weeks of ω3-FA supplementation (4 g/day, 46% EPA and 38% DHA) in patients with chronic kidney disease [18]. We have extended these reports by employing the same dose ω3-FA supplementation and examining FH subjects on a background of statin (with or without ezetimibe).

It is well recognized that elevated LDL-cholesterol and other co-existent metabolic risk factors in FH, such as inflammation, are central to the pathogenesis of atherosclerosis [3]. Hence, it is reasonable to assume that FH patients will have impaired hemodynamic function as reflected by reduced elasticity of large conduit arteries and the microcirculation. However, we found that the large and small artery elasticity were not significantly different between statin-treated patients with FH and control subjects. This observation may relate to the effect of background statin (and or ezetimibe) therapy to restore hemodynamic function in FH.

In this study, we found that ω3-FA supplementation significantly reduced systolic and diastolic blood pressure and plasma triglyceride concentrations. These findings are in agreement with other reports on the favourable effects of ω3-FA supplementation on cardiometabolic risk factors. Moreover, we also demonstrated significant improvements in large artery elasticity [10, 14-18]. It is well established that large artery elasticity is blood pressure dependent [27]. Therefore, it is conceivable that ω3-FA supplementation could improve large arterial elasticity via effects that improve blood pressure. However, that no correlation was observed between the changes in large artery elasticity and systolic blood pressure, plasma triglyceride concentration

or other variables suggests that the improvement in the biophysical properties of these arteries are likely to be due to a direct effect of ω3-FA on the artery wall [28, 29].

Several mechanisms may account for our findings. First, EPA or DHA may directly influence arterial elasticity by enhancing nitric oxide production or release, thereby improving endothelium-dependent arterial relaxation and elasticity [30]. Second, ω3-FA may displace arachidonic acid (AA) in membrane phospholipids, thereby inhibiting AA-mediated inflammatory signalling, cellular adhesion molecules and subendothelial cellular properties [31]. Third, increased production of ω3-FA-derived eicosanoids, such as resolvins, protectins and mono-epoxides, may inhibit inflammation and contribute to a direct vasodilatory effect on arterial smooth muscle cells [32].

While there was an overall improvement in large arterial elasticity with ω3-FA supplementation, its effect was relatively small in those FH subjects who had baseline arterial elasticity above the median. This observation is not unexpected given that arterial function might have been corrected with statin therapy and thus any additional increase in arterial elasticity may not be possible with ω3-FA supplementation. By contrast to our previous study in obese subjects [17], we did not observe an effect of ω3-FA supplementation on small artery elasticity in FH. The precise reason for this is unclear. Whether the effect of ω3-FA supplementation is attenuated by background statin also remains to be elucidated. Nevertheless, the

lack of treatment effect with ω3-FA supplementation is consistent with other reports [12, 13, 14, 17].

Our study has limitations. The sample size was small. Hence, the findings need to be confirmed in a larger population. Compliance with fish oil supplementation was checked only by capsule count and, hence, this needs to be confirmed by analysis of platelet or erythrocyte fatty acid composition. Given the open-label, single-blind design, we cannot exclude the possibility of confounding due to adoption of healthy lifestyle in the active intervention period. Arterial elasticity was also not determined at the end of the weight stabilizing period or after the 8-week wash out period. Given that body weight and BMI remained unaltered during the study and that carry-over effects were not statistically detected, it is unlikely to confound our findings. Nevertheless, our results should be interpreted with caution. We have previously found that 12-weeks of ω3-FA supplementation improved small arterial elasticity in obese subjects [17]. Hence, our results might have been different had we employed a longer treatment period (*e.g.* 12 weeks). We estimated the elasticity of large conduit arteries and the microcirculation using pulse contour analysis derived from a modified Windkessel model. However, measurement of the arterial waveform with PulseWave™ CR-2000 is a convenient, non-invasive and highly reproducible method showing good correlation with other measures of arterial stiffness, such as aortic distensibility measured by magnetic resonance imaging, stroke volume-to-aortic pulse pressure ratio and augmentation index [33-35].

FH is a condition with an extremely high risk of premature CAD. Our data suggest that high dose ω3-FA supplementation, which is equivalent to an intake of 190-240g fatty fish per day, significantly lowers plasma triglyceride and may improve hypertension, as well as large arterial elasticity. Given clinical evidence that arterial elasticity is an independent predictor of cardiovascular mortality and predictor of future coronary artery disease, our new data support the addition of ω3-FA supplementation to cholesterol-lowering therapy to improve arterial elasticity in patients with FH. Future studies should examine the additive effects of fibrates and PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors to statin therapy and their effects on arterial function.

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Disclosures

None to disclose

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FIGURE LEGENDS

Figure 1. Effect of ω3-FA supplementation on large and small arterial elasticity in 10 FH patients who had baseline arterial elasticity below the median

Table 1.

Clinical, biochemical and haemodynamic characteristics with and without ω3-FA supplementation in 20 FH patients

	No Treatment	ω3-FA supplementation	P
Weight (kg)	79.1 ±3.6	79.0 ±3.5	0.801
Waist circumference (cm)	90.5 ±2.9	90.4 ±3.1	0.884
Body mass index (kg/m^2)	27.0 ±1.4	27.0 ±1.3	0.702
Systolic blood pressure (mmHg)	124 ±3.2	117 ±3.4	0.009
Diastolic blood pressure (mmHg)	69.3 ±1.9	65.1 ±1.9	0.006
Pulse Pressure (mmHg)	54.9 ±2.3	51.4 ±2.5	0.121
Total Cholesterol (mmol/L)	4.58 ±0.27	4.20 ±0.16	0.069
Triglycerides (mmol/L)	1.30 ±0.14	1.05 ±0.09	0.011
LDL-cholesterol (mmol/L)	2.81 ±0.29	2.54 ±0.16	0.204
HDL-cholesterol (mmol/L)	1.19 ±0.12	1.12 ±0.05	0.554
Non-HDL cholesterol (mmol/L)	3.39 ±0.27	3.07 ±0.18	0.098
Apolipoprotein B (g/L)	0.83 ±0.06	0.76 ±0.03	0.038
Glucose (mmol/L)	5.19 ±0.10	5.32 ±0.11	0.122
Insulin (mU/L)	7.74 ±0.95	8.77 ±0.89	0.217
HOMA score	1.79 ±0.23	2.08 ±0.21	0.137
CRP (mg/L)	1.90 ±0.54	1.79 ±0.54	0.602
Large artery compliance (mL/mmHg X10)	17.3 ±0.86	18.8 ±0.93	0.006
Small artery compliance (mL/mmHg X100)	6.49 ±0.68	6.47 ±0.78	0.973

Values are Mean ± SEM; the values of clinical, biochemical and hemodynamic characteristics were determined at the end of each 8 week treatment period