Effect of Fish Oil on Arterial Inflammation in Patients with Elevated Lipoprotein (a)

1. Trial Details

Protocol/Clinical Trial Title:	Effect of high-dose fish oil supplementation on arterial inflammation in patients with elevated lipoprotein (a)		
Protocol Number (Version and Date):	Version 1, 23/01/2019		
Amendment (Number and Date):			
Trial Start Date:	January 2019	**Trial Finish Date:**	December 2019
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Elevated lipoprotein (a) [Lp(a)] and low-density lipoprotein cholesterol (LDL-c) are both important risk factors for atherosclerotic cardiovascular disease (ASCVD). Inflammation is a central component in the development of ASCVD and molecular imaging techniques can be used to assess inflammatory processes in the arterial wall and the response to treatments. This open-labelled, pilot study will assess the effect of high-dose fish oil supplementation on arterial inflammation in patients with elevated Lp(a) levels and stable coronary artery disease who are already receiving maximally tolerated doses of lipid-lowering therapy.

2. Rationale / Background

Atherosclerosis is a multi-facetated disease with inflammation playing a central role in both initiation and progression, as well as triggering events such as myocardial infarction and stroke.[1] Elevated low-density lipoprotein (LDL-c) is a well-established risk factor for atherosclerotic cardiovascular disease (ASCVD)[2, 3] and there is widespread consensus on the value of lowering LDL-c to reduce risk.[3] Lipoprotein (a) [Lp(a)] is an LDL like particle in which the apolipoprotein B (apoB) molecule is covalently bound to an apolipoprotein a (apo a) molecule.[4] Circulating levels of Lp(a) are predominately determined genetically, with little influence from diet or environmental factors. Lp(a) has both proinflammatory and atherothrombotic properties,[4, 5] with increased levels associated with an increased risk of coronary artery disease (CAD).[6, 7] This risk is further exacerbated in the presence of high LDL-c levels[8] and both elevated Lp(a) and LDL-c have been demonstrated to be significant and independent predictors of CAD severity in patients with premature CAD.[5]

Fish oils are a rich source of long-chain omega-3 fatty acids, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[9] Compelling evidence suggests that fish oil supplementation protects against coronary heart disease (CHD).[10] The cardioprotective effects of fish oil supplementation are widespread and are mediated by several mechanisms, which include improvements in hypertriglyceridaemia, arterial compliance and endothelial function.[10-12] They also have anti-inflammatory and anti-platelet effects. These favourable vascular effects of fish oils may contribute to improved cardiovascular outcomes, as demonstrated in large intervention trials.[13] Whether fish oil supplementation improves vascular inflammation in patients with elevated Lp(a) concentration remains to be investigated.

Molecular imaging using 18F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) scanning is a widely employed and sensitive technique for directly assessing inflammation in the plaques of the arterial walls.[14] Macrophages in inflammatory plaques have a higher rate of glycolysis than other plaque components and unaffected arterial wall segments. Isotope detection with PET and co-registration of the anatomical site with CT allows for highly reproducible estimates of ^{18}F-FDG uptake by carotid arteries and proximal aorta, allowing sensitive and precise calculation of the maximal target-to-background ratio (TBR_{max}).[15] We have preliminary data demonstrating that elevated Lp(a) is associated with increased arterial inflammation using this method.[15] Therefore, the purpose of this pilot study is to investigate whether high-dose fish oil supplementation can reduce arterial inflammation in patients with elevated Lp(a) levels, stable CAD and already on maximally tolerated doses of statin therapy (with or without ezetimibe).

3. Trial Aims / Objectives / Hypotheses

Aims:
To investigate the effect of high-dose fish oil supplementation on arterial inflammation in participants with stable coronary artery disease receiving maximally tolerated doses of statin with or without ezetimibe to control LDL-c levels to <4 mmol/L.

Hypothesis:
In patients with elevated Lp(a) levels and stable CAD, high-dose fish oil supplementation will significantly reduce arterial inflammation.

Outcomes:
The primary outcome will be reduction in arterial inflammation, as assessed by maximal target to background ratio (TBR_{max}) of the carotid arteries and aorta using ^{18}F-FDG-PET/CT imaging. Secondary outcomes will be changes in lipid profile (Lp(a), total cholesterol, LDL-c, triglycerides, high-density lipoprotein) and markers of inflammation (hs-CRP, IL-6, IL-1β, TNF-α, pentraxin 3).

4. Trial Design

Study Design:
This is a 12 week, open-labelled, uncontrolled, end-point blinded pilot study to assess the effect of high-dose fish oil supplementation on arterial inflammation. All participants and study personnel will be aware of the treatment regimen, with assessment of arterial inflammation measurements and laboratory endpoints performed by someone unaware of the treatment schedule. At baseline (week 0), all participants will undergo an ^{18}F-FDG-PET/CT for assessment of arterial inflammation and provide a fasting blood sample for assessment of lipid profile (Lp(a), total cholesterol, LDL-c, triglycerides, HDL-c) and markers of inflammation (hs-CRP, IL-6, IL-1β, TNF-α, pentraxin 3). Participants will then receive 4g daily of fish oil as capsules containing 45% EPA and 39% DHA as ethyl esters (Blackmores^{TM}) for 12 weeks. All participants will have telephone review every 2 weeks and clinic review at 6 weeks to ensure compliance and to monitor any concerns regarding the treatment. Assessment of ^{18}F-FDG-PET/CT and collection of a fasting blood sample will be repeated on all participants at week 12. For all participants, comparisons between baseline (week 0) and end of treatment (week 12) for arterial inflammation, lipid profile and inflammatory markers will be made, with each person serving as their own control.

Study Treatment:
Fish oil taken as capsules containing 45% EPA and 39% DHA as ethyl esters, taken as 4g per day for 12 weeks.

Outcomes Assessment:
^{18}F-FDG-PET/CT will be performed at the Department of Nuclear Medicine, Sir Charles Gardner Hospital. The procedure will follow imaging protocols and analysis methodology based on published literature. Participants will undergo PET-CT scanning on dedicated PET/CT scanner (Siemens Biograph mCT) at

weeks 0 and 12. Patient preparation includes a 12 hour fast. The patient's blood sugar level should be <7.2mmol/L at the time of administration of ^{18}F-FDG. ^{18}F-FDG will be produced by an onsite cyclotron and radiopharmaceutical labs (RAPID labs) at Sir Charles Gairdner Hospital, using standard methods. ^{18}F-FDG (3 MBq/kg) will be injected intravenously and participants will rest for 2 hours prior to imaging. The imaging protocol includes a low dose CT scan for attenuation correction and anatomic coregistration, followed by static-mode acquisition of images of the neck and thorax. High resolution images (~3 mm slices) will be reconstructed with correction for attenutation, dead time, random coincidences and scatter. PET and CT data will be fused and analysed using MIMvista software. Arterial ^{18}F-FDG uptake in the aorta and carotid arteries will be quantified using regions of interest with SUVmax and target to background ratio (TBR) values obtained. Image analysis will be carried out by the same trained operator, blinded to the treatment schedule.

Fasting blood samples will be collected and stored at -80^{o}C for batch analysis at the completion of the study. Lp(a) will be analysed by immunoassay (Quantia Lp(a) assay), with other lipid profiles analysed by PathWest Laboratories using routine clinical methods. Inflammatory markers will be analysed by commercial ELISA kits.

5. Source and Selection of Participants

Fifteen men and women will be recruited from the Outpatients Clinic, Department of Cardiology at Royal Perth Hospital. Screening and selection of suitable participants will be undertaken by Prof Gerald Watts with all participants undergoing safety biochemistry assessment (fasting glucose, creatinine kinase and liver function) prior to inclusion in the study.

Inclusion criteria include: aged between 45-69 years of age with elevated fasting Lp(a) levels (>0.5g/L), stable coronary artery disease and on maximally tolerated doses of lipid-lowering therapy, including a statin, and achieving a fasting LDL-c of <4.0 mmol/L. Stable coronary artery disease will be defined as previous coronary event (myocardial infarction, stroke, re-vascularisation procedure) >3 months ago or established coronary artery calification (>400) following CT coronary angiogram.

Participants will be excluded from the study if they have unstable coronary artery disease or a cardiovascular event within the last 3 months, fasting blood sugar level >7.2 mmol/L, tachyarrhthmias, women who are pregnant or lactating, a previous diagnosis of a severe co-existing medical condition that would prevent participation (eg: severe dementia or terminal illness), <45 or >70 years of age, or unable to provide a written informed consent.

All participants will provide a written informed consent. Participants will be discontinued from the study if they develop any serious side effects and/or adverse events following commencement of the study treatment. At the completion of the study, all participants will be referred back to the Outpatients Clinic, Department of Cardiology, RPH for ongoing treatment management. Participants may withdraw from the study at any time, without explanation or jeopardising their future medical care. All information collected on participants who withdraw will be retained unless specifically requested otherwise by the participant. Participants who withdraw from the study will be replaced to maintain statistical power via recruitment from the Outpatients Clinic, Department of Cardiology at Royal Perth Hospital.

6. Treatment of Participants

Fish oil capsules containing approximately 90% ω-3-fatty acid ethyl esters (46% EPA and 38% DHA), have been shown to decrease triglycerides, increase HDL cholesterol and improve artery function.[10-12] The dose and treatment period is comparable to that used in previous studies and employed in clinical practice in our clinic at Royal Perth Hospital. Compliance will be monitored via regular telephone contact with participants, accountability logs and tablet count.

7. Assessment of Efficacy

Efficacy will be assessed via changes in the TBR_{max} of the carotid arteries and aorta using ^{18}F-FDG-PET/CT scanning at weeks 0 and 12 of the study.

8. Assessment of Safety

We do not anticipate any significant risks associated with taking part in this study. All participants will be requested to notify the study coordinator of any side effects or adverse events that develop upon commencement of the study treatments. These will be reviewed by the study clinician and the HREC will be notified. All serious and adverse drug reactions will be reported as necessary to the HREC and TGA. Where necessary, the participant will be withdrawn from the study.

There is a minor risk of discomfit and/or bruising associated with providing a blood sample, however this will be minimised by having the procedure performed by a suitably trained and qualified person.

The ^{18}F-FDG-PET/CT scan involves ionising radiation and the insertion of a canula into the arm for several hours while the test is carried out. There is a small risk of brusing and discomfit with cannula insertion, although this will be minimised by having the procedure performed by a suitably trained and qualified person. The amount of radiation exposure during the ^{18}F-FDG-PET/CT scan is small (XX millisieverts). This is slightly higher than the level of radiation exposure one is exposed to through naturally occurring background radiation (~2 millisiverts per year). Although harmful effects of the dose given in the ^{18}F-FDG-PET/CT scan cannot be proven, there is evidence to indicate that such a dose may give a small risk of developing cancer. This risk is believed be decreased for those >50 years of age at the time of exposure.

All medications carry a small risk of side effects. High dose fish oils have been associated with various side effects including eructation, dyspepsia, and taste perversion. Daily consumption of >3 tablets may increase the risk of bleeding in patients already taking blood-thinning medication. Participants will also be provided with a Blackmores™ information sheet (Appendix 2) as well as contact details of the study clinician and nursing support staff.

The potential benefits associated with this research include a reduction in arterial inflammation, which may reduce the risk of a future cardiovascular event.

9. Data Management, Statistical Analysis and Record Keeping

The primary outcome is a reduction in arterial inflammation. This will be assessed using a paired t-test assessing change in maximal target to background ratio of carotid arteries and aorta. All analysis will be performed at the completion of the trial on an intention to treat basis. Participants who withdraw from the study will be replaced.

This is a pilot proof-of-concept study designed to identify if high-dose fish oil supplementation can provide additional benefit to high-risk patients who are already on maximally tolerated lipid-lowering therapy. We anticipate a difference in TBR_{max} of 30%, with 15 participants providing 80% power to test the null hypothesis, with an α-error of 5%.

All hardcopy data will be stored in individual Case Report Forms (CRFs), which will be stored in a locked filing cabinet in the office of the study coordinator at the Medical School, UWA, Level 4 MRF Building, Rear 50 Murray Street, Perth. All electronic data will be re-identified with a unique ID code and stored on a password protected computer that is regularly backed up. Stored biological samples will be re-identified with a unique study ID and stored in -80^{o}C freezer in the Medical School, UWA, Level 4 MRF Building, Rear 50 Murray Street, Perth. All records will be retained for 15 years after the completion of the trial, after which they will be securely destroyed.

10. Monitoring / Audit

Trial-related monitoring, auditing, and regulatory inspections will be granted to HRECs and institutional governance review bodies as requested.

11. Quality Control and Quality Assurance

The trial will be conducted in compliance with the protocol, adhering to the principals of Good Clinical Practice (GCP) and the National Statement on Ethical Conduct in Human Research.

12. Ethics

All participants will provide an informed written consent before inclusion in the trial. All participants will retain a copy of the Participant Information and Consent Forms. Ethics and Governance approval will be obtained from the East Metropolitan Health Services Ethics Committee, with additional Governance approval sought from the North Metropolitan Health Services Ethics Committee, where the ^{18}F-FDG-PET/CT scans will be performed.

13. Budget, Financing, Indemnity and Insurance

The trial is funded by the Royal Perth Hospital Medical Research Foundation and the University of Western Australia. Indemnity and insurance is provided by Crown Indemnity.

14. Publication

The trial will be registered on the Australian and New Zealand Clinical Trials Registry. Results from the study will be published in appropriate peer-reviewed medical and scientific journals. No identifying information will be included.

15. References

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16. Appendices

Appendix 1 – Participant Information and Consent Form
Appendix 2 – Blackmores™ Fish oil Information Brochure