Inflammatory changes following a dietary intervention in the elderly

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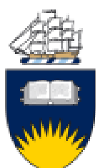

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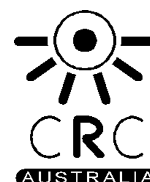

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Non-Technical Summary

2010-738: Inflammatory changes following a dietary intervention in the elderly

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PROJECT OBJECTIVES:
1) To determine, in a group of Australian men and women ≥64 years of age, whether consumption of a higher fish diet (FISH) improves markers of inflammation (i.e. CRP, IL-1, IL-6, TNF-alpha) compared to a usual Australian diet that is low in fish and higher in red meat and lower in fish (CONTROL).

2) To determine whether consumption of a higher fish diet improves fatty acid status in red blood cells and plasma phospholipids compared to CONTROL.

OUTCOMES ACHIEVED

1. A higher fish diet significantly reduced concentrations of C-reactive protein.
2. A higher fist diet significantly increased long chain omega 3 fatty acid levels measured in red blood cells and plasma phospholipids.
3. A higher fish diet produced a modest, yet significant reduction in triglycerides.
4. These outcomes are all beneficial in terms of reducing risk for cardiovascular disease.

LIST OF OUTPUTS PRODUCED

1. A randomized controlled trial (RCT) was undertaken for 8 weeks: 80 older adult participants were randomized to a group to consume fish (~4 servings per week) or meat (~4 servings of meat per week).
2. Of the 80 participants who completed the study: 37 were from the CONTROL group and 43 were from the FISH group.
3. Serum high sensitive C-reactive protein was measured in all 80 participants.
4. Omega 3 and omega 6 fatty acids were measured in both red blood cells and plasma phospholipids in all 80 participants.
5. Serum IL-1β, TNF-α and IL-6 as markers of inflammation were measured in 77-79 participants.

A number of secondary measures were also undertaken:

6. Dietary records, blood levels of HbA1C and iron studies, and depression and memory questionnaires were measured in all participants at baseline and at the end of the study.
7. Body composition (by dual x-ray absorptiometry) was measured in 77 participants.

1.0 Introduction

With ageing, the inflammatory process is aggravated. This may be a result of cachexia (Roubenoff, Roubenoff et al. 1994), neurodegenerative diseases (Holmes, Cunningham et al. 2009; Reale, Iarlori et al. 2009) or depression (Hamer, Molloy et al. 2009). It is becoming increasingly recognized that chronic, low-grade inflammation is associated with increased risk for cardiovascular, and a number of other, chronic diseases (Libby, Ridker et al. 2002; Koenig, Khuseyinova et al. 2008). Pro-inflammatory markers such as interleukin 1 (IL-1) and tumour necrosis factor-alpha (TNF-α) increase interleukin 6 (IL-6) thereby increasing leptin and corticotrophin releasing hormones (Uehara, Sekiya et al. 1989; Oldenburg, Rogy et al. 1993) leading to decreases in appetite (Chapman 2004). These cytokines also are involved in lipolysis and muscle protein breakdown (McLachlan, Serkin et al. 1995; Petersen, Carey et al. 2005); this further contributing to frailty and poor health outcomes in older age. The role of nutrition in the development and resolution of inflammation is under investigation.

In particular, findings from epidemiology studies have generally shown that higher intakes of dietary long chain omega 3 polyunsaturated fatty acids (LC n-3 PUFA) have been associated with lower levels of inflammatory markers including C-reactive protein (CRP), IL-6, and intercellular- and vascular adhesion molecules (Lopez-Garcia, Schulze et al. 2004; Niu, Hozawa et al. 2006; He, Liu et al. 2009). However, results from randomized controlled trials assessing daily fish oil supplementation in doses ranging between 180 mg to 2400 mg for 3 weeks-18 months on *in vivo* and *ex vivo* markers of inflammation have generally been mixed (Thies, Miles et al. 2001; Thies, Nebe-von-Caron et al. 2001; Thies, Nebe-von-Caron et al. 2001; Bechoua, Dubois et al. 2003; Berstad, Seljeflot et al. 2003; Geelen, Brouwer et al. 2004; Wu, Han et al. 2004; Micallef and Garg 2009).

What is less well known is whether consumption of fish in its whole form affects markers of inflammation in healthy older adults. Dietary pattern studies that have included fish were inversely associated with plasma concentrations of CRP (Lopez-Garcia, Schulze et al. 2004; Nettleton, Steffen et al. 2006) and epidemiological studies have reported beneficial associations between regular fish consumption, equivalent to at least 100-120 g per week, with lower levels of inflammation (Lopez-Garcia, Schulze et al. 2004; Niu, Hozawa et al. 2006; Chung, Nettleton et al. 2008; He, Liu et al. 2009; van Bussel, Henry et al. 2011). Comparatively, there have only been four randomized controlled trials in older adults assessing fish consumption on markers of inflammation (Meydani, Lichtenstein et al. 1993; Geelen, Brouwer et al. 2004; Tsitouras, Gucciardo et al. 2008; Pot, Geelen et al. 2009). In these studies, the addition of sardine oil was used to increase LC n-3 PUFA intakes (Tsitouras, Gucciardo et al. 2008), one study had only 22 subjects and all study foods were provided, likely increasing compliance (Meydani, Lichtenstein et al. 1993), and the remaining studies involved patients with either non active/previous cancer (Pot, Geelen et al. 2009) or coronary heart disease (Seierstad, Seljeflot et al. 2005) in which chronic inflammation is inevitable. Currently, no studies have assessed the consumption of fish in a healthy older population who are also likely to be prone to some low-grade systemic inflammation, and none have been conducted in Australia, where consumption of fish is far lower than the current recommendations of 2-3 oily fish servings per week (Howe, Meyer et al. 2006; Flood, Webb et al. 2007; Heart Foundation 2008).

Therefore, the primary aims of the current study were to: 1) to determine, in a group of Australian men and women ≥64 years of age, whether consumption of a higher fish diet (FISH) improves markers of inflammation (i.e. CRP, IL-1β, IL-6, TNF-α) compared to a usual Australian diet that is low in fish and higher in red meat (CONTROL) and 2) determine whether consumption of a FISH diet improves fatty acid status in red blood cells and plasma phospholipids compared to CONTROL. It was hypothesised that a short, 8 week period of higher fish intake, at an amount nearly double the Heart Foundation guidelines of 2-3 oily serves of fish per week (Heart Foundation 2008) would induce an anti-inflammatory effect in healthy older adults.

The secondary aims of the project were to identify whether consumption of a higher fish diet improves depression score and concentrations of lipids, as some studies have identified that higher intakes of LC n-3 PUFA are associated with less likelihood of being depressed (Barberger-Gateau, Jutand et al. 2005; Kamphuis, Geerlings et al. 2006) and a reduction in concentrations of lipids, specifically triglycerides (Brown, Roberts et al. 1990; Milte, Coates et al. 2008).

1.1. Need

1. The older population is growing significantly, and health care costs in this group are highest.
2. The older population often consumes lower intakes of food and may have lower intakes of some key micronutrients.
3. Inflammation, vascular function and oxidative stress all have been implicated in the development of CVD and other chronic disorders (e.g. dementia, depression). As older adults have a compromised immune system, they may have high levels of inflammation.
4. Increasing consumption of fish, in its whole form rather than as a supplement, may improve risk factors for CVD such as inflammation, thereby decreasing risk of developing CVD, depression, and other related cardiovascular diseases.

1.2. Objectives

1) To determine, in a group of Australian men and women ≥64 years of age, whether consumption of a higher fish diet (FISH) improves markers of inflammation (i.e. CRP, IL-1β, IL-6, TNF-α) compared to a usual Australian diet that is low in fish and higher in red meat (CONTROL).

2) To determine whether consumption of a FISH diet improves fatty acid status in red blood cells and plasma phospholipids compared to CONTROL.

2.0 Methods

2.1 Subjects

126 community dwelling men and women ≥64 years of age expressed interest in the study following newspaper advertisements, flyers and verbal invitations. Ninety two were eligible for clinic screening however 8 did not attend. Inclusion criteria were:

BMI ≥18.5 kg/m², consumption of ≤1 serving of fish/seafood per week, willing to consume 8 servings of provided fish or red meat per fortnight, able to provide written informed consent and attend clinic visits at Flinders Medical Centre. Exclusion criteria were: inability to comply with the study protocol, allergies to fish/seafood, vegetarian, intake of lipid-lowering supplements (e.g. psyllium, fish oil capsules, soy lecithin, phytoestrogens) (or to cease 3 weeks prior to study commencement), use of anti-inflammatory medications on a regular basis or if experiencing an acute episode within 1 week of the clinic screening visit, presence of diabetes, liver, kidney, thyroid diseases (unless controlled and stable on replacement medication), presence of other endocrine disorders from self-reported medical history, weight loss or gain of >10% body weight during a period of 6 months before the screening visit, or clinically diagnosed depression or dementia. Of the 84 respondents who attended the clinic visit, all were eligible for the RCT. One participant withdrew before randomization, leaving 83 to be randomized **(Figure 1)**.

2.2 Experimental design

This study was an 8 week randomized controlled, parallel study. Participants were randomized to receive 8 servings of fish per fortnight (FISH group), equivalent to ~800mg EPA+DHA/day, or into a CONTROL group in which participants received 8 serves of meat (beef, pork, lamb, ham) per fortnight **(Appendix 1)**. Participants in both groups were advised to follow their otherwise usual dietary and physical activity patterns throughout the 8 week study. Between study groups, the food provided was less than 10% different for energy (kJ), protein (g), saturated fat (g) and sodium (g), except for total fat which was nearly double in FISH vs. CONTRL (7.0 g/d vs. 4.7 g/day). Over the whole day however, this is only a small difference. Participants collected their study foods from Flinders Medical Centre every 2 weeks, and for those who travelled long distances, at the 4 week time point. At this time, the study researcher questioned each participant about how they were finding the study, compliance of eating the study foods, and ease or otherwise of, including the study foods into their regular diet. For participants who were finding it difficult to consume the study foods, the study researcher provided verbal motivation and listed health benefits for consuming the study foods.

2.3 Measurements

Clinic measurements

All assessments were taken at baseline and at 8 weeks. Body weight was measured to 0.1 kg on a digital scale while wearing light clothing, height was measured to 0.1 cm with a stadiometer without shoes, and blood pressure was measured using a hospital grade non-invasive automated sphygmomanometer (Criticare Systems Inc, USA). Blood pressure was taken three times and the average was used. Whole body scans were performed using dual energy X-ray Absorptiometry (DXA: Lunar Prodigy, GE Healthcare, UK) by a licensed DXA user. The Centre for Epidemiologic Studies-Depression Scale (CES-D) and the Memory Functioning Questionnaire were also completed at both time points by the study participants. Dietary intakes were measuring using 3-day weighed food records and were completed within the first week (week 1) and the last week (week 7) of the study period to assess study compliance. All participants were instructed on how to fill out their food diaries at the baseline visit, and were loaned a set of food scales (Kenwood DS607 Digital Food Scales). All participants were asked to consume at least one of the study foods provided on the weighing days.

2.4 Laboratory measurements

A 10 ml fasting blood sample was taken at baseline and at the end of the 8 week study. After 30 minutes of clotting, whole blood was centrifuged at 3,500 RPM, 24°C for 10 minutes (Beckman GS-6R). Serum was separated, aliquoted and stored at -80°C until the end of the study. Aliquots were then thawed and total cholesterol, HDL-C, triglycerides, glucose and CRP were analysed enzymatically (Siemens Advia 2400 Chemistry System). LDL Cholesterol was calculated using the Friedwald Equation. Inflammatory markers (IL-6, IL-1β and TNF-α) were measured using commercial cytokine enzyme-linked immunosorbent assays (ELISA) (BD Systems, San Diego, CA). The limits of detection for IL-6, IL-1β and TNF-α were all <5 pg/mL. The inter-assay and intra-assay CV for IL-6 was 6-8% and 2-3%, for IL-1β was 7-10% and 2-4% and for TNF-α was 3-5% and 1-3%. Whole blood HbA1C and Hemoglobin were collected in EDTA tubes and measured within 4 hours at baseline and at 8 weeks (Roche Cobas Integra 800).

2.5 Fatty acids

For measurement of fatty acids (DHA, DPA, EPA, total n-3 fatty acids, total n-6 fatty acids and total saturated fatty acids), whole blood was collected into heparinised tubes and within one day of testing, were extracted:

Lipid extraction from plasma

Plasma was separated from erythrocytes by centrifugation (5 min at 1000*g*) and 1ml plasma was added to 0.5 ml saline, 2 ml of methanol and 4 ml of chloroform. The samples were centrifuged and the lower chloroform phase (containing lipids) was transferred to a 20 ml scintillation vial and evaporated to dryness by a vacuum concentrator. 150 μL of chloroform:methanol (9:1) was added to the vial in preparation for thin layer chromatography (TLC).

Lipid extraction from erythrocytes

Erythrocytes were rinsed free of plasma three times in isotonic saline and 1 ml packed cells were mixed with 0.5 ml saline, 2 ml of isopropanol and 4 ml of chloroform. The samples were centrifuged and the lower chloroform phase was transferred to a vial and evaporated to dryness. 150 μL of chloroform:methanol (9:1) was added to the vial in preparation for TLC.

Thin Layer Chromatography (TLC)

The phospholipids from all tissues were isolated using TLC (Silica gel 60 H, Merck, Darmstadt Germany) using a ratio of 3:1 (v/v) of petroleum spirits:acetone. Phospholipid bands were visualised under an ultraviolet lamp and scraped into vials containing 1% H_2SO_4 in methanol for trans-esterification for 3 h at 70°C. After cooling, water and *n*-heptane were added to the mixture and the upper layer containing the resulting fatty acid methyl esters (FAME) was transferred into 2ml gas liquid chromatography (GLC) vials containing anhydrous Na_2SO_4.

Gas chromatographic analysis of FAME

FAMEs were separated and quantified using a Hewlett-Packard 6890 gas chromatograph equipped with a 50m capillary column (0.33 mm ID) coated with BPX-70 (0.25μm film thickness SGE Pty Ltd Victoria Australia). The injector temperature was set at 250°C and the detector (flame ionisation) temperature at 300°C. The initial oven temperature was 140°C and was programmed to rise to 220°C at 5°C per minute. Helium was used as the carrier gas at a velocity of 35 cm per second. Fatty acid methyl esters were identified based on the retention time to authentic lipid standards (GLC-463, Nuchek Prep Inc. Elysian, MN).

2.6 Sample size

The sample size was based on previous studies that showed a 19% (0.4 mg/L) reduction in CRP following 360 mg/day EPA+DHA as fish oil for 3 weeks in patients with hyperlipidaemia (n=15, ~55 years) (Micallef and Garg 2009); a 13% (0.19 mg/L) reduction in CRP following 2 x 150 g of Salmon per week (700 mg EPA, 1400 mg DHA) for 6 months (n=54, ~54 years) (Pot, Geelen et al. 2009); and an 8% (0.13 mg/L) reduction in CRP following 2 x 150 g cod per week (total omega-3, 600 mg) for 6 months (n=51, ~57 years) (Pot, Geelen et al. 2009). The population used in the studies by Pot et al had either non active cancer or were previous cancer patients; however this had no effect on CRP measurements. The calculated sample sizes required from these respective studies was 20, 8 and 17. As the current study population would be older, may have a slightly higher CRP and inflammatory status at baseline, and the total dose of EPA+DHA/day proposed for our study is less, we anticipated a larger sample size to find a similar statistically significant change in CRP. Fish (compared to fish oil supplements) also contain higher amounts of DHA vs. EPA, and the limited conversion from EPA to DHA further supports a larger sample size to detect changes in red blood cell composition of DHA. Therefore the current study aimed to recruit 90 subjects in order to have 40 subjects complete the 8 week study in each group, with an approximate CRP change of 13% (0.24 mg/L).

To control for similar numbers of high and low CRP concentrations at baseline and to identify whether those with higher CRP concentrations respond better to the intervention, the 83 participants were stratified according to their screening CRP concentration. The randomisation schedule was created by an independent statistician using ralloc.ado version 3.6.1 in Stata version 11.1. The randomisation was stratified by CRP at screening (<3 mg/L vs. ≥3 mg/L) and blocks of size 2, 4 and 6 were used. Block sizes were chosen at random and treatment allocations were randomly permuted and balanced within blocks.

2.7 Statistical analyses

Statistical analyses were performed using SPSS 19 for Windows (SPSS, Inc., Chicago, IL, USA). Frequencies and descriptives of the study population are reported as mean (SD), or between groups as mean (SEM). Prior to hypothesis testing, data were examined for normality. Distribution was normal except for CRP, IL-1 and IL-6 which were normalized using natural logarithmic transformation and the exponentiated means [95% confidence intervals] are presented. The primary outcome (CRP) was tested using general linear model: univariate analysis of variance, with CRP at 8 weeks as the dependent variable, group (CONTROL/FISH) as the fixed factor, and adjusting for baseline CRP. When assessing the absolute change in CRP over time, CRP stratification was also included as a fixed factor (group x CRP stratification interaction), with no adjustment for baseline CRP. Significance levels quoted are two-sided. Pearson's correlation coefficients were used to determine the relationships between different variables over time, at 8 weeks, or between the same variable at baseline and at 8 weeks. The alpha level of significance was $P<0.05$.

3.0 Results

3.1 Baseline characteristics

Randomization outcomes in the 80 participants who completed the study are reported in **Table 1**. Mean CRP stratification concentrations in the low and high CRP groups at baseline were 0.8 ± 0.1 mg/L vs. 8.5 ± 1.9 mg/L (P<0.001). Various characteristics of the participants who completed the study are reported in **Table 2**. The mean (SEM) age of the study group was 69.5 (0.6) years (range 64-85 years) and 51% were female.

3.2 3-day weighed food intakes

During the 3-days of food weighing (during week 1 and week 7 of the 8 week study), the majority of participants in the FISH and CONTROL groups consumed 2 portions of the provided study foods. During the first week of the study, there were no significant differences in energy and most nutrients between the CONTROL and FISH groups, except the FISH group had a higher intake of polyunsaturated fatty acids (+3.2 g, P=0.002), and a higher intake of long chain and very long chain PUFA (all P<0.001) **(Table 3)**. At the end of the study, similar results were found (data not shown). In the CONTROL group, there was no statistically significant difference in mean energy and nutrient intakes between the week 1 and week 7 time points. In the FISH group however, a lower intake of long chain fatty acids (-512 mg, P=0.003) specifically, a lower intake of EPA (-147 mg, P=0.031) and DHA (-267 mg, P=0.004) at week 7 compared to week 1 was found.

3.3 Inflammatory markers

CRP-

The mean values of CRP between groups are reported in **Table 2**. There was a strong correlation between baseline and end of study CRP concentrations (n=80, r=.739, P<0.001). In the univariate analysis assessing 8 week CRP concentrations, there was no effect of diet when adjusting for baseline CRP concentrations or CRP stratification group. However, when assessing changes over time and adjusting for CRP stratification, there was a significant diet effect (mean change CONTROL: 1.75 [0.42, 3.09] mg/L vs. mean change FISH: -0.64 [-1.75, 0.47] mg/L, P=0.017) **(Figure 2)**. The magnitude of decrease in CRP in the FISH group was similar between those with low (n=32, Δ=-0.13 ± 0.18 mg/L) or high CRP (n=11, Δ=-1.15 ± 1.5 mg/L, P=0.269); however in the CONTROL group, those with high CRP had a larger increase in CRP over the study (n=7, Δ=+3.37 ± 3.24 mg/L) compared to those with low CRP (n=30, Δ=+0.14 ± 0.23 mg/L, P=0.046).

Cytokines-

All of the TNF-α samples were below the limits of detection in serum (<5 pg/mL) and were therefore unable to be included in the analyses. Mean concentrations of IL-1β and IL-6 that were detectable in serum are reported in **Table 2.** At baseline, there was no significant difference between groups in IL-1β or IL-6.

In the whole group, there was a significant inverse association between the changes in IL-1β and CRP (n= 33, r=-.439, P=0.005) and IL-1β and IL-6 (n=16, r=-.615, P=0.006), but not between the changes in CRP and IL-6 (n=21, r=-.093, P=.344). In the FISH group, there was a strong significant inverse association between the changes in IL-1β and IL-6 (n=8, r=-.756, P=0.015).

3.4 Fatty acids

Mean percentage change in VLCN n-3 PUFA was significantly higher in the FISH vs. CONTROL in both plasma phospholipids (+27% vs. -13%, P<0.001) **(Table 4)** and red blood cells (+14% vs. -12%, P<0.001) **(Table 5).** There were no associations between fatty acid concentrations in plasma phospholipids or red blood cells and CRP at any time point.

3.4.1 Lipids and HDL-C

Baseline and final study concentrations of lipids are reported in **Table 2**. Within the FISH group, there was a small significant decrease in TG over the 8 week study period (mean change compared to baseline: -0.14 ± 0.05 mmol/L, P=0.004, 9%). The change in TG was not significantly different between those who were stratified into the high or low CRP group, respectively (n=32, Δ= -0.18 ± 0.06 mmol/L vs. n=11, Δ= -0.05 ± 0.06 mmol/L, P=0.273).

3.5 Iron studies

There was no significant difference in iron studies at baseline or 8 weeks between groups **(Table 6)**. At baseline, the majority of participants had iron levels in the normal range (10-27 μmol/L; n=72, 90%), 6 (8%) participants had low iron (<10 μmol/L) and 2 participants had higher than normal iron (>27 μmol/L). Frequencies of participants in these categories did not significantly change over the study period. At baseline, the majority of participants had transferrin levels in the normal range (1.5-3.0 g/L; n=72, 90%) and eight (10%) participants had higher than normal transferrin (>3.0 g/L). Frequencies of participants in these categories did not significantly change over the study period.

3.6 Depression

The mean (± SEM) baseline and end of study depression score in the CONTROL and FISH group was 7.3 ± 1.2 vs. 6.0 ± 6.1 and 6.4 ± 0.9 vs. 6.6 ± 1.0, respectively. There was no significant difference in scores between or within groups at 8 weeks or over the study period. The percentage of participants depressed at baseline in the FISH and CONTROL groups was 16% and 7% and at the end of the study was 8% and 9% (not significant between groups or time point).

4.0 Discussion

This project was the first among a group of older adults in Australia to assess whether consumption of 4 servings of fish per week, equivalent to 800 mg EPA+DHA per day, reduces levels of inflammatory markers, compared to a typical Australian diet that is lower in fish and higher in red meat. The main finding of the current study was that CRP concentrations significantly decreased over time following a higher fish diet, compared to the low fish, higher meat diet. Moreover, CRP concentrations significantly decreased in the fish group, regardless of starting CRP concentrations whereas in the control group, those with CRP concentrations >3 mg/L had larger increases in CRP over the study compared to those with CRP concentrations ≤3 mg/L.

Previously, an 8 week single intervention study in 12 adults ~66 years found that consumption of fatty fish (~1300 mg/day) in combination with 15 ml of sardine oil/day (~4-5 g EPA+DHA) decreased CRP concentrations by ~26% compared to 6 weeks consumption of lean fish (Tsitouras, Gucciardo et al. 2008). In 161 patients with non-active/previous cancer, consumption of cod (600 mg EPA+DHA/week) or salmon (2100 mg EPA+DHA/week) over six months reduced serum CRP concentrations by 8% (0.13 mg/L) and 13% (0.19 mg/L), respectively (Pot, Geelen et al. 2009). Several other studies utilising fish oil or omega 3 fortified foods at higher daily LC n-3 PUFA doses (range 1.0 g-6.6 g EPA+DHA per day) than the current study reported mixed effects on CRP (Geelen, Brouwer et al. 2004; Madsen, Christensen et al. 2007; Murphy, Meyer et al. 2007; Micallef and Garg 2009). The limited studies, the different designs and the different dosages and populations used in these studies make it difficult to compare results. However, the current study population was healthy and free of disease, and were slightly older than in those studies and levels of inflammation tend to increase with age (Franceschi, Bonafe et al. 2000; De Martinis, Franceschi et al. 2005; Jones, Stephenson et al. 2009). Yet, mean starting levels of CRP in the current sample was in the normal range (1-3 mg/L); similar to the starting levels in the previous studies. It is therefore difficult to determine why we report a significant reduction in CRP compared to the previous studies which did not. We also report a significant reduction in CRP following the higher fish diet regardless of CRP stratification, yet CRP increased to a greater extent in those with high CRP compared to those with low CRP following the lower fish, higher meat diet. Thus, consumption of a lower fish diet appears unfavourable in the short term in this healthy older group, yet appears more detrimental among those with higher CRP concentrations. Data from several large cohort studies, specifically, the Atherosclerosis Risk in Communities study which included 12,819 apparently healthy middle-aged men, identified that those with CRP >3 mg/L had a significantly increased risk for CHD (Hazard Ratio, 1.72; 95% CI, 1.24 to 2.39) compared to those with CRP 1-3 mg/L (HR: 1.31; 95% CI, 0.96 to 1.80) (Ballantyne, Hoogeveen et al. 2004). Similarly, in the Reykjavik prospective study among 3969 controls without a CHD event, the relative risk (RR) of developing CHD in those with CRP >3 mg/L was 1.45 (95% CI, 1.14 to 1.86) (Danesh, Wheeler et al. 2004). In 3971 men and women ≥65 years of age without prior vascular diseases, after adjustment for age, ethnicity, and sex, the RR of developing CHD for CRP >3 mg/L was 1.82 (95% CI, 1.46 to 2.28) compared with <1 mg/L, while adjusting for conventional risk factors, reduced the RR to 1.45 (95% CI, 1.14 to 1.86) (Cushman, Arnold et al. 2005). These findings suggest that CRP is a useful predictor in risk for CHD development. In our study, we found that regardless of CRP stratification, CRP decreased following the fish diet, whereas in the control group, CRP increased to a greater extent in those with CRP >3 mg/L. Although there was only a small number of participants with high CRP in the control (n=7) and fish groups (n=11), levels of CRP at the end of the dietary intervention still remained >3 mg/L. Both of these sub-groups need to be targeted to reduce CRP to a lower risk range of <3 mg/L. In addition to increasing intake of fish, other beneficial short term dietary strategies to reduce CRP have included consumption of a diet with a low-glycemic load (Neuhouser, Schwarz et al. 2012), and replacing partially-hydrogenated vegetable oils with polyunsaturated fats and oils (Mozaffarian and Clarke 2009); whereas longer term strategies that have been shown to reduce CRP levels to the appropriate range have included consistent physical activity (Plaisance and Grandjean 2006), one year weight loss of 15% among those who are obese (Tchernof, Nolan et al. 2002), two year consumption of a Mediterranean style diet that includes increased intake of whole grains, fruits, vegetables, nuts, and olive oil (Esposito, Marfella et al. 2004), and pharmacologic agents such as aspirin (Ridker, Cushman et al. 1997).

As expected, concentrations of omega 3 fatty acids in phospholipids and red blood cells were significantly increased in the fish group compared to control. However, despite the positive increase in omega 3 fatty acids and the decrease in CRP, there were no associations between fatty acid status and CRP concentrations. Thus, it is unclear what is driving the significant changes. The mechanisms relating long chain fatty acids and inflammation are emerging and relates to the production of eicosanoids. Eicosanoids (e.g. prostaglandins, thromboxanes and leukotrienes) are generated from omega 6 PUFA, particularly arachidonic acid (AA). Arachidonic acid intake is high in most diets (Meyer, Mann et al. 2003; Blasbalg, Hibbeln et al. 2011) therefore, it is the most available substrate for eicosanoid synthesis. Eicosanoids can have different and opposing effects (Calder 2006), however generally inducing higher levels of inflammation (Lewis, Austen et al. 1990; Tilley, Coffman et al. 2001). Comparatively, DHA and to a lesser extent EPA inhibit early atherogenic events by reducing cytokine expression of pro-inflammatory proteins in the endothelium (De Caterina, Cybulsky et al. 1995), partly at the expense of AA. We did not find any associations between the changes in inflammatory markers and changes in omega 3 or omega 6 fatty acid concentrations. In our study population, the effects of a higher fish diet leading to increases in LC n-3 PUFA and a decrease in CRP may be due to independent mechanisms and needs further investigation.

There is consistent evidence showing reductions in triglyceride (TG) concentrations following higher intakes of EPA, DHA or both (Brown, Roberts et al. 1990; Howe, Clifton et al. 1999; Buckley, Shewring et al. 2004; Milte, Coates et al. 2008). In an early Australian study among 12 men aged 18-40 years, 6 weeks of lean fish and 5 g fish oil lowered plasma TG by 12%, whereas a non-significant 7% reduction was observed following consumption of fish only (0.74 g/day LC n-3 PUFA)(Brown, Roberts et al. 1990). Similar to the dose of LC n-3 PUFA in that study, we found a modest 9% reduction in TG in the fish group, however this was not statistically significant compared to that observed at 8 weeks in the control diet. We also found a significant inverse association between TG and omega 3 fatty acids, specifically EPA measured in RBC and phospholipids, but not DHA. Although in a dose response study using DHA oil only, significantly reductions in TG were found, suggestions have been made that the effects of EPA and DHA are equal in their effects to lower TG (Howe, Clifton et al. 1999; Weber 1999). In a meta-analysis of 17 population-based prospective trials including 46,413 men and 10,864 women, a 1.0 mmol/L increase in plasma TG levels significantly increased the RR of CVD by 32% in men and 76% in women (Hokanson and Austin 1996), whereas in patients after acute coronary syndrome, a final TG level <1.69 mmol/L (<150 mg/dL) was associated with a 27% relative reduction in coronary events (Miller, Cannon et al. 2008). The modest 0.14 mmol/L reduction in TG we report is not likely to have a significant effect on cardiovascular disease risk reduction, nor are the final TG concentrations of participants in the control group at significantly elevated risk.

There is growing, albeit inconsistent evidence of a link between various nutritional components, such as n-3 PUFA found in fish, and depression. Increased concentrations of CRP and pro-inflammatory cytokines have been observed in depressed, but otherwise healthy, patients (Miller, Stetler et al. 2002; Tiemeier, Hofman et al. 2003; Schlatter, Ortuno et al. 2004; Thomas, Davis et al. 2005); however not all studies support this (Owen, Eccleston et al. 2001; Schlatter, Ortuno et al. 2004). Likewise, the current study found no effect of fish consumption on depression, and no significant associations with any markers of inflammation. The inconsistent relationships between inflammation and depression suggest mechanisms linking these disorders may be premature; and that these relationships may only occur in some cases/individuals.

The strengths of this study include the randomized controlled design. The intention to supply the fish in an amount nearly twice the current recommendations, and at a dose not too far over the Nutrient Reference Values was to assist in compliance for a relatively short time frame, as consumption of fish in this amount is not usual. In addition, 8 weeks is sufficient to alter the membrane composition and incorporation of omega-3 fatty acids in cells and tissues (Garg, Leitch et al. 2006); and changes in IL-6 and TNF-α, which are mediators of CRP, can be noted within 6 weeks following LC n-3 PUFA in doses slightly higher (Meydani, Lichtenstein et al. 1993) and much higher (Endres, Ghorbani et al. 1989; Gibney and Hunter 1993) than that used in the current study.

In conclusion, 8 week consumption of a higher fish diet significantly reduced CRP concentrations in a group of healthy older Australians. Concomitant to significant increases in long chain omega 3 fatty acids, we also report a modest reduction in triglyceride concentrations. These outcomes are all beneficial in terms of reducing risk for cardiovascular disease. Our study supports that consumption of 4 meals of fish per week, equivalent to 800 mg EPA+DHA/day, significantly reduces CRP, however larger studies are required to confirm our results and to investigate how these changes are sustained in the longer term.

5.0 Benefits and Adoption

This information is important to:
Industry:

- Being aware of the nutritional requirements, specifically intake of long chain omega-3 fatty acids, in the older population, and understanding the relative ease of including a higher fish diet in this population may prompt new fish/seafood products to be formulated that contain higher amounts of EPA+DHA.
- As part of the same 2010/738 project, a survey was conducted in 854 adult's ≥51 years living in Australia. The most frequently reported barrier for consuming lower amounts of *fresh* fish was its cost (37% of the study sample) with 15% reporting this for *canned* fish. The survey also revealed that a number of older adults were aware of the benefits of consuming fish, however multiple barriers, particularly the high cost, restricted higher consumption.
- Conversely, unpublished data has revealed that the cost of consuming fish is a cheaper option compared to red meat to achieve long chain omega 3 fatty acid targets.
- To benefit industry, rather than reducing costs to increase sales, perhaps it is more appropriate to increase awareness of higher values fish (i.e. those high in long chain omega 3 fatty acids, such as salmon or sardines) and couple this with breaking down the barrier of the cost perception indicated by consumers.
- This could be achieved through recipe cards in which preparation of higher omega 3 fatty acid fish meals are included, in combination with the approximate cost of the meal, and importantly, how this translates into potential health benefits.
- Booklets or brochures containing similar information could also be used by GPs and other practitioners to target a broader population of consumers.

The community:

- If new fish products containing high amounts of EPA+DHA, and that also are relatively affordable were to be on the market, this may allow younger and other older populations to attain potential benefits from high fish/seafood consumption.

- The previous survey in Australian adults ≥51 years also found many adults knew the current recommendations for fish and were aware of the health benefits, however this did not translate into sufficient consumption.
- Resource development that includes suitable meal plans containing different types of fish and seafood with higher and lower amounts of omega 3 fatty acids to achieve recommendations and within a suitable budget is likely to be an important strategy to target consumers of many ages to increase consumption.

Older adults and the elderly:

- Low intakes of fish and/or omega-3 fatty acid intake have consistently been related to several diseases that frequently emerge in older adults (i.e. CVD, type 2 diabetes, depression), and these are all linked to a greater inflammatory status.
- The results of this study showing a decrease in an important marker of inflammation may prompt older adults to consume more fish to potentially reduce the risk for disease, or attenuate the disease process in the older population.
- Further to the resource development described above, this strategy will also benefit older adults, particularly when available from GP's and medical practitioners; older adults, particularly those 65 years are the group in which visits to the GP are most frequent and health care expenditure is highest.

6.0 Further Development

This study was a short term trial that found 8 weeks consumption of a higher fish diet significantly reduced CRP and increased long chain omega 3 fatty acid concentrations in the blood. Both of these outcomes have been associated with reduced risk for cardiovascular disease. It is important to replicate these findings on a larger scale. In addition, the inclusion of new outcomes, for example, genetic outcomes involved in cardiovascular disease and inflammatory processes may be another way to identify how valuable the consumption of a higher fish diet is.

7.0 Planned Outcomes

Public Benefit Outcomes

Individual study results and a summary of the overall results will be sent to study participants. They will then have an understanding of the benefits of consuming higher intakes of fish and may choose to continue with the amount provided in the study.

The scientific audience will also gain knowledge on the study findings through presentations at conferences and through published results.

Private Benefit Outcomes

The information that we get from this study will:

- Help us understand how increased consumption of fish (and specifically the fatty acids in fish) may benefit older adults, particularly in relation to inflammation.
- Help us understand whether current recommendations for fish and long chain omega 3 fatty acids for older adults need to be updated.
- Develop further projects utilising fish and other products containing long chain omega 3 fatty acids on larger scale, and test the effects of other cardiovascular outcomes (e.g. genetic assays) in the older population.

Linkages with CRC Milestone Outcomes
At present there are limited data on the opportunity specifically relating to seafood consumption in the older population. The Nutrition, Retail and Foodservice teams at Simplot see an opportunity to use the outcome of this project to develop communication strategies for the increased consumption of fish by older adults. The Simplot team is keen to promote the health benefits of fish products and the scientific evidence supporting the role of fish as a good source of omega-3 will be utilised in promotional activities for foodservice customers.

The published literature shows a clear mechanistic link between the role of n-3 PUFA and inflammation, but there is less evidence in older consumers. Information generated from the RCT will gather further evidence to support fish consumption and increased essential nutrients in the diet, as well as potentially demonstrate the reduction or attenuation of inflammatory disease.

8.0 Conclusion

- Four servings of fish per week, equivalent to 800 mg EPA+DHA per day, significantly reduced CRP concentrations, compared to a diet low in fish and higher in red meat.
- Four servings of fish per week significantly increased long chain omega 3 fatty acids, compared to a diet low in fish and higher in red meat.
- The higher fish diet modestly reduced triglyceride concentrations, but had no effect on other lipids or depression score.
- Consumption of a higher fish diet improved some risk factors for cardiovascular disease (i.e. CRP, triglycerides) at least in the short term.
- Studies of longer duration are required to support these findings.

Tables

Table 1: Randomization in those who completed the study (n=80)

	Low CRP (≤3 mg/L at screening)		High CRP (>3 mg/L at screening)	
	n	CRP (mg/L)	n	CRP (mg/L)
Control	30	0.85 (0.77)	7	6.21 (4.15)
Fish	32	0.85 (0.69)	11	9.07 (8.88)

Mean (SD) CRP concentrations

Table 2: Mean ± SEM baseline and end of study characteristics of the study population

	Baseline			End of study	
	ALL[b]	CONTROL	FISH	CONTROL	FISH
Weight (kg)	74.8 (13.5)	73.8 ± 2.1	75.7 ± 2.2	73.6 ± 2.0	75.8 ± 2.1
BMI (kg/m²)	26.5 (3.8)	26.4 ± 0.6	26.5 ± 0.6	26.3 ± 0.6	26.5 ± 0.6
Systolic blood pressure (mmHg)	126 (15)	126 ± 2	126 ± 2	126 ± 3	124 ± 3
Diastolic blood pressure (mmHg)	68 (9)	67 ± 1	69 ± 1	66 ± 1	68 ± 2
CRP (mg/L)[a]	1.00 [0.75, 1.35]	0.84 [0.54, 1.29]	1.17 [0.78, 1.77]	0.83 [0.51, 1.34]	1.01 [0.68, 1.50]
IL-1β (pg/mL)[a]	8.20 [6.76, 9.95]	6.87 [4.69, 10.06]	8.11 [5.30, 12.42]	7.67 [6.23, 9.44]	7.29 [6.04, 8.80]
IL-6 (pg/mL)[a]	7.76 [6.71, 8.99]	7.87 [5.43, 11.38]	7.21 [5.38, 9.65]	7.52 [5.89, 9.62]	7.73 [5.24, 11.41]
Total cholesterol (mmol/L)	5.49 (0.92)	5.5 ± 0.2	5.5 ± 0.1	5.4 ± 0.2	5.6 ± 0.1
HDL-C (mmol/L)	1.7 (0.4)	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
LDL-C (mmol/L)	3.2 (0.7)	3.3 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.4 ± 0.1
Triglycerides (mmol/L)	1.2 (0.6)	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.0 ± 0.1*

* P=0.004 vs. FISH at baseline

[a] geometric means [95% CI's]

[b] Mean (SD)

Table 3: Mean ± SEM energy and nutrient intakes between the CONTROL and FISH groups after 1 week of study food consumption.

	CONTROL	**FISH**
	Mean ± SEM	Mean ± SEM
Energy and macronutrients		
Energy (including fibre, Kj)	8213 ± 431	8026 ± 326
Protein (g)	89 ± 5	93 ± 4
Total fat (g)	69 ± 5	74 ± 4
Saturated fat (g)	28 ± 2	26 ± 2
Polyunsaturated fat (g)	9 ± 1†	13 ± 1
Monounsaturated fat (g)	26 ± 2	30 ± 2
Cholesterol (mg)	244 ± 21	282 ± 23
Carbohydrate (g)	219 ± 12	199 ± 10
Sugars (g)	105 ± 7	93 ± 6
Dietary fibre (g)	29 ± 2	28 ± 2
Omega 3 fatty acids		
Long chain n-3 PUFA (mg)	1110 ± 12‡	1787 ± 135
Very long chain n-3 PUFA (mg)	27 ± 5‡	1622 ± 129
EPA (mg)	8 ± 2‡	593 ± 48
DPA (mg)	9 ± 2‡	125 ± 16
DHA (mg)	10 ± 2‡	903 ± 77
Linoleic acid (g)	7.8 ± 0.7	7.6 ± 0.6
Alpha linolenic acid (g)	1.2 ± 0.1	1.0 ± 0.1
Vitamins and minerals		
Thiamin (mg)	1.9 ± 0.1	1.7 ± 0.1
Riboflavin (mg)	2.3 ± 0.2	2.1 ± 0.1
Niacin equivalents (mg)	45 ± 2	49 ± 2
Vitamin C (mg)	125 ± 9	162 ± 33
Vitamin D (μg)	2.7 ± 0.3‡	8.0 ± 0.6
Vitamin E (mg)	8.0 ± 0.8	9.0 ± 0.6
Dietary folate equivalents (μg)	450 ± 26	411 ± 29
Vitamin A RE (μg)	985 ± 83	1079 ± 94
Sodium (mg)	2017 ± 95	1966 ± 99
Potassium (mg)	3423 ± 146	3539 ± 164
Magnesium (mg)	376 ± 22	366 ± 19
Calcium (mg)	858 ± 49	816 ± 51
Phosphorus (mg)	1505 ± 66	1517 ± 61
Iron (mg)	12.4 ± 0.6	12.6 ± 0.8
Zinc (mg)	12.5 ± 0.9	11.4 ± 0.7

Iodine (µg)	105 ± 7	122 ± 7
Caffeine (mg)	210 ± 35	203 ± 28

* P<0.05, † P<0.01 ‡P<0.001 between groups

Table 4: Levels of fatty acids measured in phospholipids before and after 8 weeks consumption of either a CONTROL (n=37) or FISH diet (n=43)

	Diet	Baseline	Week 8	Change	Mean difference [95% CI]
Total saturated fatty acids (%)	CONTROL	45.6 ± 0.2	44.6 ± 0.6	-2.0 ± 2.0	-1.26 [-2.72, 0.20]
	FISH	45.4 ± 0.2	45.7 ± 0.3	0.7 ± 0.7	
Total n-6 fatty acids (%)	CONTROL	32.5 ± 0.4	32.0 ± 0.6*	-1.0 ± 2.0	2.04 [0.60, 3.47]
	FISH	32.9 ± 0.4	30.3 ± 0.4 [c]	-7.0 ± 1.0*	
Total n-3 fatty acids (%)	CONTROL	7.1 ± 0.3	6.1 ± 0.3‡ [c]	-12.0 ± 4.0	-2.48 [-3.23, -1.73]
	FISH	6.9 ± 0.3	8.5 ± 0.3 [c]	26 ± 4‡	
Very long chain n-3 (%)	CONTROL	6.7 ± 0.3	5.6 ± 0.3‡ [c]	-13 ± 3	-2.49 [-3.25, -1.74]
	FISH	6.6 ± 0.3	8.2 ± 0.3 [c]	27 ± 4‡	
EPA (%)	CONTROL	1.5 ± 0.1	1.3 ± 0.1‡ [a]	-12 ± 5	-0.82 [-1.19, -0.46]
	FISH	1.4 ± 0.1	2.0 ± 0.1 [c]	55 ± 10‡	
DPA (%)	CONTROL	1.2 ± 0.0	1.1 ± 0.04 [b]	-7 ± 2	-0.07 [-0.15, 0.006]
	FISH	1.1 ± 0.03	1.1 ± 0.03	-0.4 ± 2*	
DHA (%)	CONTROL	4.0 ± 0.2	3.4 ± 0.2‡ [c]	-15 ± 3	-1.60 [-2.03, -1.17]
	FISH	4.1 ± 0.2	5.0 ± 0.2 [c]	29 ± 5‡	

* P<0.05, †P<0.01, ‡ P<0.001 between groups

[a] P<0.05, [b] P<0.01, [c] P<0.001 within groups over time

Table 5: Levels of fatty acids measured in red blood cells before and after 8 weeks consumption of either a CONTROL (n=37) or FISH diet (n=43)

	Diet	Baseline	Week 8	Change	Mean difference [95% CI]
Total saturated fatty acids (%)	CONTROL	44.2 ± 0.2	44.1 ± 0.3	-0.2 ± 0.4	-0.9 [-1.8, -0.1]
	FISH	44.1 ± 0.2	44.8 ± 0.2	0.7 ± 0.2	
Total n-6 fatty acids (%)	CONTROL	27.0 ± 0.4	26.6 ± 0.4	-0.4 ± 0.4	1.3 [0.2, 2.4]
	FISH	27.2 ± 0.4	25.5 ± 0.3	-1.7 ± 0.3	
Total n-3 fatty acids (%)	CONTROL	9.7 ± 0.4	8.4 ± 0.3	-1.3 ± 0.2	-2.3 [-2.9, -1.8]
	FISH	9.3 ± 0.3	10.4 ± 0.2	1.3 ± 0.2	
Very long chain n-3 (%)	CONTROL	9.5 ± 0.4	8.2 ± 0.3	-1.3 ± 0.2	-2.3 [-2.9, -1.8]
	FISH	9.2 ± 0.3	10.2 ± 0.2	1.0 ± 0.2	
EPA (%)	CONTROL	1.5 ± 0.1	1.1 ± 0.1	-0.4 ± 0.1	-0.7 [-1.0, -0.5]
	FISH	1.3 ± 0.1	1.7 ± 0.1	0.3 ± 0.1	
DPA (%)	CONTROL	3.0 ± 0.1	2.8 ± 0.1	-0.2 ± 0.04	-2.1 [-0.3, -0.1]
	FISH	2.8 ± 0.1	2.8 ± 0.1	-0.03 ± 0.04	
DHA (%)	CONTROL	5.0 ± 0.2	4.4 ± 0.2	-0.6 ± 0.1	-2.3 [-2.9, -1.8]
	FISH	5.0 ± 0.2	5.7 ± 0.1	0.7 ± 0.1	

* P<0.05, †P<0.01, ‡ P<0.001 between groups

[a] P<0.05, [b] P<0.01, [c] P<0.001 within groups over time

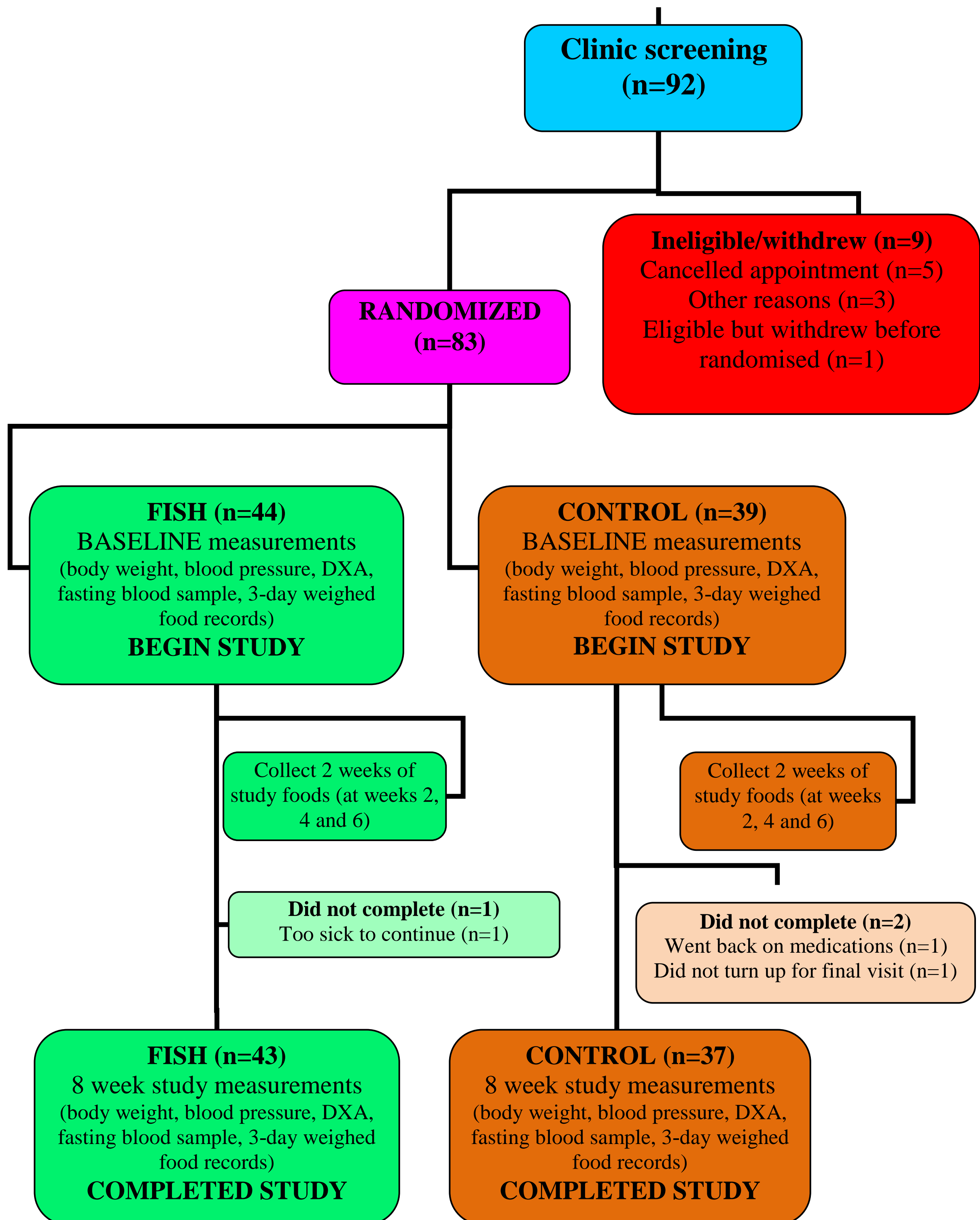
Table 6: Mean ± SEM levels of iron study measurements before and after 8 weeks consumption of either a CONTROL (n=37) or FISH diet (n=43)

		Baseline		End of study	
	ALL[a]	CONTROL	FISH	CONTROL	FISH
Iron (µmol/L)	17.4 (5.6)	17.9 ± 0.9	16.9 ± 0.9	16.6 ± 0.8	17.3 ± 1.1
Transferrin (g/L)	2.6 (0.3)	2.6 ± 0.07	2.6 ± 0.04	2.6 ± 0.06	2.6 ± 0.05
saturation (%)	27.3 (9.3)	28.0 ± 1.6	26.7 ± 1.4	26.1 ± 1.5	27.2 ± 1.7
Ferritin (µg/L)	143.6 (109.4)	144.6 ± 19.3	142.7 ± 15.8	143.2 ± 22.1	130.4 ± 15.3

[a] Mean (SD)

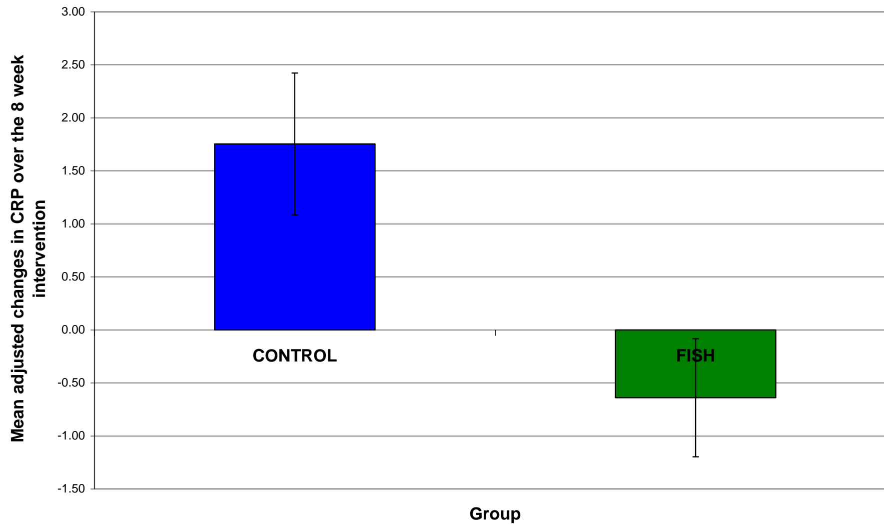
Figures

Figure 1: Recruitment and study flow-chart



Clinic screening
(n=92)
Ineligible/withdrew (n=9)
Cancelled appointment (n=5)
Other reasons (n=3)
Eligible but withdrew before
randomised (n=1)
RANDOMIZED
(n=83)
FISH (n=44)
BASELINE measurements
(body weight, blood pressure, DXA,
fasting blood sample, 3-day weighed
food records)
BEGIN STUDY
CONTROL (n=39)
BASELINE measurements
(body weight, blood pressure, DXA,
fasting blood sample, 3-day weighed
food records)
BEGIN STUDY
Collect 2 weeks of
study foods (at weeks 2,
4 and 6)
Collect 2 weeks of
study foods (at weeks
2, 4 and 6)
Did not complete (n=1)
Too sick to continue (n=1)
Did not complete (n=2)
Went back on medications (n=1)
Did not turn up for final visit (n=1)
FISH (n=43)
8 week study measurements
(body weight, blood pressure, DXA,
fasting blood sample, 3-day weighed
food records)
COMPLETED STUDY
CONTROL (n=37)
8 week study measurements
(body weight, blood pressure, DXA,
fasting blood sample, 3-day weighed
food records)
COMPLETED STUDY

Figure 2: Mean adjusted changes in CRP over the 8 week study period between groups



Univariate analysis: P=0.007 between groups, adjusting for CRP stratification

APPENDIX

Appendix 1: Study foods provided: FISH

Week 1 and 2	**Energy (kJ)**	**Protein (g)**	**Total fat (g)**	**Saturated fat (g)**	**Sodium (mg)**	**PUFA (g)**	**EPA (mg)**	**DHA (mg)**	**EPA+DHA (mg)**
JW Atlantic Salmon (Skin Off) 145g	1056	32.0	13.6	3.2	59	3	945.0	1035	1980
JW Atlantic Salmon (Skin Off) 145g	1056	32.0	13.6	3.2	59	3	945.0	1035	1980
JW Ocean Trout-skin on (150g serving)	1630	28.2	30.3	7.8	41	7	1210.0	2200	3430
JW Ocean Trout-skin on (150g serving)	1630	28.2	30.3	7.8	41	7	1210.0	2200	3430
JW Salmon Tempters Onion & Tomato 95g pack	559	13.3	6.8	1.0	271	0.7	114	233	347
JW Tuna Tempters Lemon & Cracked Pepper 95g pack (87g drained)	537	17.3	6.3	0.8	326	0.2	17	148	164
JW Sardines in spring water, 110g	710	13.7	12.5	3.4	180	3.3	750.0	1120	1880
JW Pink Salmon 105g pack (79g drained)	484	18.6	4.9	1.2	280	1.7	502	683	1185.0
Week 3 and 4									
JW: Atlantic Salmon with Asian marinade (145g)	1380	27.1	23.5	5.4	312	9.4	1300	1530	2830
JW: Atlantic Salmon with Asian marinade (145g)	1380	27.1	23.5	5.4	312	9.4	1300	1530	2830
JW Red Salmon 105g pack (79g drained)	557	18.4	6.9	1.5	324	2.3	494	762	1256
JW Salmon Tempters Onion & Tomato 95g pack	559	13.3	6.8	1.0	271	0.7	114	233	347
JW Tuna slices in springwater (82g drained weight)	397	21.7	0.7	0.2	185	0.3	29	215	244
BE Lightly Seasoned Fish Fillets (Hoki) Lemon & Cracked Pepper 200g	1340	29.2	14.8	2.0	260	3.8	200	500	700
JW light tuna tempters: spring water	227.0	12.1	0.5	0.3	139.0	167.0	0.2	20.0	147.0
Total all 4 weeks	13502	332	195	44	3060	219	9129	13444	22750
Average per day	**482**	**12**	**7.0**	**1.6**	**109**	**7.8**	**326**	**480**	**813**

Study food: MEAT

Week 1 and 2	Energy (kJ)	Protein (g)	Total fat (g)	Sat Fat (g)	Sodium (mg)	EPA (mg)	DHA (mg)	DPA (mg)
Beef, fillet, scotch, lean, raw 125g	758.75	29	9.375	2.875	68.75	30	5	33.75
Beef, fillet, scotch, lean, raw 125g	758.75	29	9.375	2.875	68.75	30	5	33.75
Coles veal Cordon blue with cheese and ham, 140g serve (4 in a pack)	1250	16.9	13.4	3.1	910			
Lamb rump, lean, raw, 125g	677.5	28.125	5.375	1.75	81.25	23.8	13	46.3
Beef, fillet, scotch, lean, raw 125g	758.75	29	9.375	2.875	68.75	30	5	33.75
Hans, Luncheon Meats: Leg Ham, Champagne, 97% Fat-Free, Fine Sliced, 50g	184	7.9	1.5	0.3	293			
Hans, Luncheon Meats: Leg Ham, Champagne, 97% Fat-Free, Fine Sliced, 50g	184	7.9	1.5	0.3	293			
Week 3 and 4								
Beef mince, regular fat, 10% fat, 150g	1112	30.15	16.2	5.85	96	141	31.5	217.5
Beef mince, regular fat, 10% fat, 150g	1112	30.15	16.2	5.85	96	141	31.5	217.5
Lamb rump, lean, raw, 125g	677.5	28.125	5.375	1.75	81.25	23.8	13	46.3
Beef, fillet, scotch, lean, raw 125g	758.75	29	9.375	2.875	68.75	30	5	33.75
Pork loin chop, separable lean (62% pork or raw chop) (38% inedible). ~138g chop to get 100g actual meat, but nutrients based on 100g edible portion	440	22.2	1.7	0.6	66	0	6	9
Coles frozen meals, Beef satay, 375g	2378	25.9	20.6	8.6	675			
Beef, fillet, scotch, lean, raw 125g	758.75	29	9.375	2.875	68.75	30	5	33.75
Pork loin chop, separable lean (62% pork or raw chop) (38% inedible). ~138g chop to get 100g actual meat, but nutrients based on 100g edible portion	440	22.2	1.7	0.6	66	0	6	9
Total all 4 weeks	12249	365	130	43	3001	480	125	714
Average per day	**437**	**13**	**4.7**	**1.5**	**107**	17	4.5	25.5
% difference between fish and meat (over 4 weeks)	-10%	9%	-49%	-2%	-2%	-	-	-

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