Improving erythrocyte omega-3 fatty acid profiles and health status in adults through increased consumption of canned tuna

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Non-technical summary

Consumer awareness of omega-3 health benefits is increasing continually, which is reflected in increasing sale figures of omega-3 supplements in Australia between 2004 and 2006. The success of omega-3 supplements might have resulted from the intense clinical research with fish oil supplements. In contrast, very little research has been conducted in the area of processed seafood. Therefore, the fishery and fish processing industry should take the opportunity to support and initiate research which is aiming to highlight the advantages of seafood over supplements.

The aim of this project, a follow-up of the "*Shape Up For Life*" study, was to demonstrate that regular consumption of John West canned fish can lead to increased omega-3 levels in red blood cells, and how the increase is associated with a range of cardio metabolic health benefits, such as lower blood triglycerides, blood pressure and heart rate. The Seafood CRC and Simplot funded the follow-up study to obtain reliable research data on processed fish products. A positive outcome could help to increase the awareness of health benefits of processed fish in general, and would allow Simplot to increase the attractiveness of their John West canned fish products to consumers. Overall, the promotion of the study targets to increase the demand for canned seafood and seafood in general.

The follow-up study utilized food frequency questionnaires and blood samples from a large-scale community based trial, the "*Shape-up for Life"* Whyalla Nutrition and Exercise Study, undertaken by the Nutritional Physiology Research Centre (NPRC) and the Australian Technology Network (ATN) Centre for Metabolic Fitness. Participants were split into a control group and an intervention group. The intervention group received a 16-week structured but non-prescriptive lifestyle education program based on the Australian national diet and physical activity guidelines. Food samples were provided to participants in the intervention group as examples of healthy food products, including canned tuna and salmon provided by John West. During the 16-week intervention, participants were offered several cans per week of individual portions of fish. The control group did not receive free samples or the education lifestyle program. After 4 months, the intervention group was split into an active follow up group, who was contacted on a monthly basis, or an inactive follow up group, who received no further contact. Fish samples were not provided to either group during the 8 months of follow-up. All volunteers were reassessed at the end of the 12 months from baseline.

Outcomes: During the study the consumption of canned fish was increased. The provision of a lifestyle education program and canned fish samples resulted in an 8% increase in the number of people eating tinned fish at the end of 4 months. At this time, the quantity of canned fish consumed increased almost 3 fold. At the 12 month assessment, canned fish consumption was still increased, but this was no longer statistically significant. The additional fish consumption was not reflected in significant elevated DHA, EPA or total omega-3 levels in red blood cells at 4 and 12 months, except for DHA, which was significantly increased after 4 months in the intervention group. This small effect is consistent with the relatively low intake of EPA+DHA from canned fish (estimated to average 60mg /day). The increased fish intake, along with other diet and lifestyle changes, appears to have contributed to the change in waist circumference after 12 months.

Conclusion: This study has demonstrated that regular consumption of canned fish can modestly elevate erythrocyte DHA (with the potential to improve the health status of individuals with Metabolic Syndrome).

Acknowledgement

We wish to acknowledge and thank the Seafood CRC and Simplot Australia for their financial support of this project; the Uni of South Australia for conducting the clinical study and providing their scientific expertise, and to Port Lincoln Tuna Processors for providing John West Tuna products. Last but not least we thank all the volunteers who participated in the study.

Background

This project addressed the Seafood CRC Output 1.6 *"Removal or reduction of barriers to seafood consumption"*, Milestone 1.6.1 *"Barriers to and drivers of seafood consumption identified in domestic markets"*, Milestone 1.6.2 *"Individually tailored approaches to overcoming barriers trialled and evaluated"*, Output 2.5 *"Communication of consumer health benefits and risks"* and Milestone 2.5.3 *"Health benefit and risk communication strategies trialled for four sectors, consumer responses analysed and guidelines refined"*.

The aim of this project was to demonstrate that regular consumption of John West canned fish can lead to long-term increases in incorporation of docosahexanoic acid (DHA) and eicosapentenoic acid (EPA) into red blood cells (erythrocytes) which are consistent with improved health status. Simplot had the intention to use the data to educate consumers about omega-3 health benefits and to promote John West canned fish products.

The project utilised data and blood samples obtained in a large-scale community based trial, the "*Shape Up For Life"* Whyalla Nutrition and Exercise Study undertaken by the Nutritional Physiology Research Centre (NPRC) and the Australian Technology Network (ATN) Centre for Metabolic Fitness. The study aimed to investigate the efficacy and sustainability of combined lifestyle changes (dietary modification without energy restriction and increased physical activity) in improving metabolic fitness in a community setting. At the end of the study, blood samples and data, which includes food frequency questionnaires and consumption rates of fish (split into fresh, fried and canned fish), were available for analysis from 120 adults at 3 time points (0, 4 and 12 months). This was a substantial data set representing a valuable source of information on the fatty acid status of an at-risk population of people with metabolic syndrome. It also offered an important insight into the longer term impact of fish consumption on omega-3 levels[Ω].

Simplot, market leader in the canned seafood category (John West), was interested in using the outcome of the follow-up study to develop strategies to increase the demand for canned seafood and seafood in general. This is in accordance with one of John West's key strategies to demonstrate expert knowledge and educate consumers on health benefits of seafood. A collaboration with a credible research institute, such as the NPRC, is a opportunity for John West to demonstrate leadership, and to gain trust and a reputation as an omega-3 expert in Australia.

Scientific Background

Fish and seafood consumption is associated with a range of cardio metabolic health benefits, including lower blood triglycerides, blood pressure and heart rate[1]. Regular fish consumption has been strongly associated with a reduced risk of cardio vascular disease, including stroke, and sudden cardiac death[2,3]. Despite the clear benefits associated with fish consumption, in many countries dietary intake levels are low[4]. Many of the health benefits of seafood consumption have been attributed to omega-3s, both DHA and EPA[5]. The importance of omega-3 in our diet has been recognised by authorities in Australia such that the NHMRC. Nutrient Reference Values state that the suggested dietary intake target is 610mg/day for men and 430mg/day for women[6]. These levels are 3-fold higher than the intakes that were last reported in the 1995 National Nutrition Survey but are in line with anticipated recommendations from the National Heart Foundation and the current American Heart Association guidelines[7]. The importance of omega-3 for maintaining cardiovascular health has also been recognized through the establishment of the Omega 3 Index, which is based on the content of EPA and DHA in red blood cell membranes[8]. This concept suggests that a high level of red blood cell EPA+DHA is associated with reduced risk of mortality

[Ω] The focus of the study was on DHA and EPA, which represent the two most common long chain poly unsaturated omega-3 fatty acids. In this report DHA + EPA will be referred as "omega-3".

from coronary heart disease (CHD), with a level >8% associated with the greatest protection and <4% associated with the least protection. Previous studies have demonstrated increases in red blood cell membrane omega-3 following fish consumption[16,17] indicating that fish consumption can provide protection against CHD. Recent studies have also examined the benefits of regularly consuming salmon in non obese populations and found improvements in traditional risk factors for cardiovascular disease, suggesting that fish consumption may decrease the risk of metabolic syndrome[9]. The NPRC reported in two separate studies a beneficial effect of omega-3 intake through fish oil supplementation on body composition and components of the metabolic syndrome[10, 11].

The purpose of the *"Shape Up For Life"* follow-up study was to assess the impact of regular canned fish consumption on incorporation of omega-3 in red blood cells and determine whether any changes achieved were related to improvements in markers of cardio metabolic health.

Need

The prime objective for John West is to maintain continued growth by keeping key existing categories relevant and attractive to customers and consumers.

Health is one of the key drivers of consumer demand for fish products. This is reflected in a growing omega-3 supplements market in Australia. A reason for the success of the supplement market might be that almost all studies indicating and promoting omega-3 health benefits are related to fish oil supplements, but very little research has been done in the area of processed seafood. Therefore, intervention studies which demonstrate the omega-3 bioavailability of fish or canned fish might have the potential to promote processed fish as an alternative to fish oil supplements.

Simplot supports this study to assist in providing relevant health messages to consumers and to promote the importance of processed fish in the diet. Building and increasing consumers' awareness of fish and processed fish as a good source of omega-3 is an opportunity to boost the sales in the canned fish category and other categories.

Objective

The objective of this study was to demonstrate that regular consumption of canned fish leads to long-term increases of omega-3 in red blood cells and to determine that the changes have a positive influence on cardio metabolic health markers. The data would be used by Simplot to educate consumers and promote John West canned fish products.

Methods

Intervention trial

An intensive 16 week diet and exercise intervention study (*Shape Up For Life*) with 8 months follow-up was conducted in Whyalla. The study used a randomised controlled parallel group design. Participants who met the International Diabetes Federation criteria for metabolic syndrome were randomly allocated to the intervention or control group. Pregnant/lactating women, individuals taking medications for weight loss or to lower blood lipids or blood pressure, and individuals with established cardiovascular disease or diabetes were excluded from the study.

The intervention group received a 16-week structured but non-prescriptive lifestyle education program based on the Australian national diet and physical activity guidelines. Dietary information provided was based upon the dietary guidelines for Australian adults[12], with a focus on improving diet quality rather than achieving energy restriction. Participants were not provided with specific diet plans, energy or macronutrient targets. Instead, dietary education sessions focused on providing information that could be used to improve the quality and variety of diet, including information on balancing energy needs with energy expenditure, glycemic index, types of dietary fats, increasing fibre intake, reducing salt, and limiting take-away food. Practical information was also provided on reading and understanding food labels during supermarket tours, and practical cooking sessions were used to provide examples of methods of preparing healthy meals. Food samples were provided to participants in the intervention group as examples of healthy food products, which included tinned tuna and salmon (Port Lincoln Tuna Processors, Port Lincoln, Australia/Simplot Australia, Mentone, Australia). During the 16-week intervention, participants were offered several cans per week of individual portions of fish.

After 4 months, the intervention group was then split into an active follow up group, who was contacted on a monthly basis, or an inactive follow up group, who received no further contact until the end of the study. Samples of the Tuna and Salmon Tempters were not provided to either group during the 8 months of follow up. All volunteers were reassessed at the end of the follow up (12 months from baseline).

Outcome measures

Cardio metabolic markers

Fasting blood samples were collected at the beginning and end of the 16 week intervention and at the end of the 8 months follow-up. Red blood cell membrane fatty acid content was determined and a range of cardio metabolic biomarkers were measured such as systolic and diastolic blood pressure, triglycerides, and waist circumference.

Dietary analysis

Dietary intakes were assessed using the Victorian Cancer Council Food Frequency Questionnaire (FFQ) which has been validated for use in intervention studies[13, 14]. The FFQ indicated how often fish was consumed in each of three categories:

1) Steamed, grilled or baked fish.
2) Fried fish (including take-away).
3) Tinned fish (salmon, tuna, sardines etc).

Options of frequency included:

- Never,
- Less than once per month,
- 1-3 times per month,
- 1, 2, 3 times per week,
- 4, 5, 6 times per week,
- 1, 2, 3 or more times per day.

Participants were also asked to indicate portion sizes that they typically eat for a variety of foods. Based on this information each answer in the FFQ was converted into a gram quantity.

Red blood cell fatty acid analysis

Following a 10 hour fast, blood was collected from an antecubital vein of the forearm into vaccutainer tubes containing ethylene diamine tetra acetate (EDTA). EDTA blood was centrifuged (4,000 rpm, 10 min, 4 ºC) and the supernatant discarded. Red blood cells were washed with 0.9% isotonic saline and isolated from solution by a second centrifugation. Packed red blood cells were frozen (24 h, -20 ºC), followed by storage at -80 ºC until later analysis. Samples were thawed and mixed with 10mL Tris:EDTA (10:1, v/v). Ultracentrifugation (48,000 rpm, 30 min, 4ºC) in a Beckman Optima LE-80K Preparative Ultracentrifuge (Beckman Instruments, Fullerton, CA) formed a red blood cell pellet, which was dissolved in 300 μL water and 2mL methanol:toluene (4:1, v/v). The fatty acids were transesterified according to the method of Lepage and Roy[15]. The upper toluene phase containing the fatty acid methyl esters was removed and analysed by flam-ionization gas-chromatography (model GC-20A, Shimadzu, Kyoto, Japan). Individual fatty acids were identified by comparison with known standards (NuChek Prep Inc., Elysian, MN, USA).

Results

Shape Up for Life Intervention subject retention

153 participants were enrolled in the study and blood samples were collected from 146 at baseline 129 after 4 months and 115 after 12 months.

Dietary Analysis- seafood intake

FFQ data was available from 143 participants (47 in the control group (CON) and 96 in the intervention group (INT) at baseline and 123 participants after the 4 month intervention (39 CON, 84 INT. At 12 months 101 participants completed the FFQ (32 CON, 40 INTA and 29 INTB).

The majority of participants reported eating some fish at baseline (Table 1). During the intervention, canned fish was provided to participants and this increased the number of people reporting eating canned fish by 8%. Of the 10 participants who did not eat canned fish at baseline, half of these increased their canned fish consumption to over 30g per day by the end of the intervention. Estimated fish consumption in each category ranged from 8 to 30g per day at baseline. There was a significant increase in total fish consumption in the INT group after the 4 month intervention (Table 2). This was attributable to an increase of 33 ± 5/day ($p < 0.001$) in canned fish consumption; changes in other types of fish consumed were minor.

Table 1: Numbers of participants in each group (% of the group) who ate the different types of fish at baseline and after both 4 months and 12 months.

	CON 0 mth	**CON 4 mth**	**CON 12 mth**	**INT 0 mth**	**INT 4 mth**	**INTA 12 mth**	**INTB 12 mth**
FFQ completed	**47**	**39**	**32**	**96**	**84**	**40**	**29**
Fresh (Steamed, grilled, baked)	37 (79%)	30 (77%)	25 (78%)	78 (81%)	72 (86%)	36 (90%)	27 (93%)
Fried (include take-away)	30 (64%)	26 (67%)	19 (59%)	76 (79%)	52 (62%)	21 (53%)	20 (69%)
Canned (salmon, tuna, sardines etc.)	38 (81%)	36 (92%)	29 (91%)	86 (90%)	82 (98%)	38 (95%)	26 (90%)

Table 2: Amounts of fish (g/day) consumed in each category. Data shown as Mean ± SEM. * denotes increases in consumption of canned fish and total fish in the INT group (P <0.001).

	CON 0 mth	**CON 4 mth**	**CON 12 mth**	**INT 0 mth**	**INT 4 mth**	**INT A 12 mth**	**INT B 12 mth**
Total number of FFQ	**47**	**39**	**32**	**96**	**84**	**40**	**29**
Fresh (Steamed, grilled, baked)	17 ± 3	18 ± 3	21 ± 4	16 ± 3	18 ± 2	24 ± 6	21 ± 7
Fried (incl take-away)	8 ± 2	7 ± 1	11 ± 3	9 ± 2	6 ± 1	5 ± 2	5 ± 1
Canned (salmon, tuna, sardines etc.)	20 ± 4	26 ± 4	29 ± 6	17 ± 2	**49 ± 5 ***	31 ± 5	27 ± 5
Total fish consumption	45 ± 6	51 ± 6	60 ± 10	41 ± 5	**73 ± 6***	60 ± 10	54 ± 10

During the initial 4 months, there was an increase of at least 15g/day (equating to at least 1 can of fish per week) in 28% of participants in CON and 59% of subjects in INT group. After 12 months the proportions who had increased their fish consumption at least 15g/day above baseline levels had declined to 19% of CON and 28% of INT.

Omega 3 levels in Canned Fish

During the intervention several flavours of canned tuna (Tuna Tempters) and 1 style of canned salmon (Salmon Tempters) were offered free to participants in INT. Data on the omega-3 content of canned fish is shown in Table 3. When all tuna and salmon were considered together, each 100g can provided an average EPA + DHA of 184 mg (EPA = 34mg, and DHA = 150mg). Tuna had a lower average EPA and DHA level (19 mg, 116 mg respectively) compared with salmon (93mg, 287 mg respectively). Thus the increase in canned fish consumption of 33g/day equates to an increased average intake of 60 mg EPA +DHA per day.

Table 3: Analysis of LCn3PUFA (mg/100g) in canned fish varieties provided to participants in INT during the first 4 months.

	EPA (mg/100g)	**DHA** (mg/100g)	**EPA + DHA** (mg/100g)
Salmon Tempters (chili)	93	287	380
Tuna Tempters (Tomato Salsa)	16	115	131
Tuna Tempters (Chili)	17	110	127
Tuna Tempters (onion and tomato savory sauce)	28	129	156
Tuna Tempters (olive oil)	16	109	125
Average of above varieties	34	150	184

Omega-3 Incorporation into erythrocyte membranes

Table 4 shows the EPA and DHA contents of erythrocyte membranes. Data are expressed as percent total fatty acids. Table 5 shows the changes to 4 months and 12 months in the both CON and INT groups. DHA incorporation after 4 months was significantly increased in the INT group compared with CON (p=0.05). After 12 months a 1-way ANOVA revealed a significant difference in changes in EPA+DHA levels although post-hoc tests were unable to detect significant differences between means.

Table 4: EPA and DHA levels (% total fatty acids) in erythrocytes at baseline and after 4 months and 12 months in the control (CON) and intervention (INT) groups. Data are Mean ± SEM.

	CON			INT		INT A	INT B
	Baseline	**4 mth**	**12 mth**	**Baseline**	**4 mth**	**12 mth**	**12 mth**
	48	**38**	**34**	**98**	**91**	**40**	**29**
EPA (%)	0.8 ± 0.1	0.9 ± 0.1	0.9 ±0.1	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.0
DHA (%)	4.1 ± 0.2	4.0 ± 0.2	4.3 ± 0.2	3.8 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.1
EPA + DHA (%)	4.9 ± 0.2	4.8 ± 0.2	4.7 ± 0.4	4.8 ± 0.2	4.9 ± 0.1	5.0 ± 0.2	4.4 ± 0.3

Table 5: Change in EPA and DHA levels (% total fatty acids) in erythrocytes after 4 and 12 months in control (CON) and intervention (INT) participants. Data are mean ± SEM, *significantly different from the corresponding change in CON (p<0.05).

	Change after 4 months		Change after 12 months		
	CON	**INT**	**CON**	**INT A**	**INT B**
Change in EPA (%)	0.02 ± 0.1	0.01 ± 0.03	0.02 ± 0.1	0.03 ± 0.2	-0.1 ± 0.04
Change in DHA (%)	-0.1 ± 0.2	**0.2± 0.1***	0.1 ± 0.1	0.2 ± 0.7	0.1 ± 0.1
Change in EPA + DHA (%)	-0.1 ± 0.2	0.2 ± 0.1	-0.4 ± 0.3	0.3 ± 0.9	-0.4 ± 0.3

Correlations between Fish Intake and erythrocyte fatty acids content

As seen in Fig 1, total consumption of fish at baseline correlated significantly with the erythrocyte content of DHA and EPA+DHA but not EPA alone. Table 6 shows that this correlation was due to significant relationships between DHA or EPA+DHA and intakes of fresh fish or canned fish (P <0.001) but not fried/take away fish.

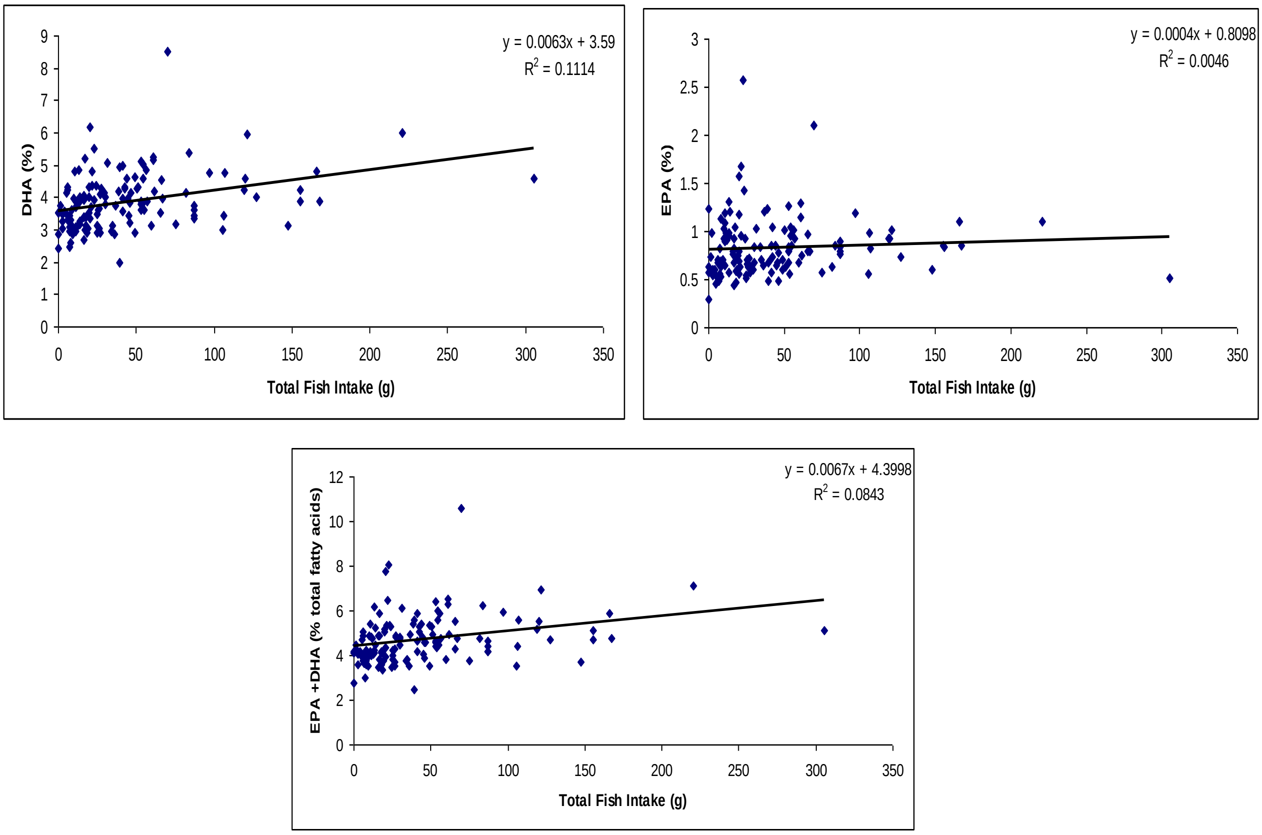

Figure 1: Associations between total fish intake and erythrocyte n-3 PUFA at baseline.

Table 6: Associations between type of fish intake and erythrocyte fatty acid content at baseline (n=143). Correlation coefficients (R value) and level of significance (P values) shown.

Type of Fish	EPA	DHA	EPA+DHA
Fresh (Steamed, grilled, baked)	R= 0.05	**R=0.31**	**R=0.26**
	P=0.54	**P<0.001**	**P=0.002**
Fried (include take-away)	R=-0.08	R=0.03	R=0.004
	P=0.33	P=0.69	P=0.96
Canned (salmon, tuna, sardines etc.)	R=0.13	**R=0.29**	**R=0.27**
	P=0.13	**P<0.001**	**P=0.001**

Changes after 4 months and 12 months in fish intake and erythrocyte fatty acid content

There were no significant correlations between the changes in fish intake for fresh, fried, canned or total fish intake and the changes in EPA, DHA or EPA +DHA in erythrocytes after 4 months of intervention (Table 7). However, after 12 months there was a positive relationship between the amount of DHA incorporated and the amount of fresh fish and total fish consumed while a marginally significant negative association was found with DHA incorporation and fried fish intake. Interestingly the change in the combined level of EPA+DHA was significantly associated with increased intakes of fresh, canned and total fish.

Table 7: Associations between change in fish intake (g) and change in erythrocyte fatty acids (%) after 4 months and 12 months. Correlation coefficients (R value) and level of significance (P values) shown. * denotes P <0.05 and significant correlations are highlighted.

	Change after 4 months (n=123)			**Change after 12 months (n=101)**		
(Type of Fish)	**EPA**	**DHA**	**EPA+DHA**	**EPA**	**DHA**	**EPA+DHA**
Fresh (Steamed, grilled, baked)	R=-0.07 P=0.48	R=0.11 P0.29	R=0.07 P=0.52	R =0.04 P=0.70	**R=0.28** **P=0.01***	**R=0.24** **P=0.02***
Fried (include take-away)	R=0.03 P=0.78	R=-0.15 P=0.88	R<-0.001 P=0.97	R=-0.01 P=0.94	**R=-0.23** **P=0.03***	R=-0.19 P=0.73
Canned (salmon, tuna, sardines etc.)	R=0.03 P=0.75	R=0.07 P=0.5	R=0.06 P=0.53	**R=0.26** **P=0.01**	R=0.18 P=0.10	**R=0.23** **P=0.03**
Total Fish Intake	R=-0.01 P=0.95	R=0.10 P=0.31	R=0.08 P=0.42	R=0.15 P=0.17	**R=0.23** **P=0.03***	**R=0.23** **P=0.03***

Correlations between fish Intake, erythrocyte fatty acids and cardiometabolic biomarkers

Baseline Associations

At baseline there were positive relationships between intakes of fresh fish and total fish and blood pressure, both systolic (SBP) and diastolic (DBP). However no other significant associations were found between fish intake and cardiometabolic biomarkers (Table 8). There were significant inverse relationships between erythrocyte DHA content and waist circumference and EPA+DHA erythrocyte levels and both plasma TG and waist circumference (WC) (Table 9).

Table 8: Associations between fish intake (g) and cardiometabolic parameters at baseline (n=146). Correlation coefficients (R value) and level of significance (P values) shown. * denotes P <0.05

Type of Fish	**TG (**mmol/L)	**SBP** (mmHg)	**DBP** (mmHg)	**WC** (cm)
Fresh (Steamed, grilled, baked)	R=-0.09 P=0.30	**R=0.19** **P=0.02***	**R=0.19** **P=0.03***	R=-0.01 P=0.94
Fried (include take-away)	R=-0.02 P=0.86	R=0.04 P=0.66	R=0.14 P=0.10	R=0.07 P=0.40
Canned (salmon, tuna, sardines etc.)	R=-0.08 P=0.37	R=0.06 P=0.49	R=0.05 P=0.55	R=-0.09 P=0.32
Total Fish Intake	R= - 0.08 P=0.32	R=0.15 P=0.07	**R=0.18** **P=0.03***	R= - 0.02 P=0.80

Table 9: Associations between erythrocyte fatty acids and cardiometabolic parameters at baseline (n=146). Correlation coefficients (R value) and level of significance (P values) shown.

Erythrocyte fatty acid contents	**TG** (mmol/L)	**SBP** (mmHg)	**DBP** (mmHg)	**WC** (cm)
EPA	R=-0.15 P=0.07	R=0.12 P=0.17	R=0.02 P=0.85	R=-0.12 P=0.14
DHA	**R=-0.17** **P=0.040**	R=0.12 P=0.16	R=-0.01 P=0.87	**R=- 0.25** **P<0.001**
EPA +DHA	**R=- 0.18** **P=0.03***	R=0.13 P=0.13	R=-0.01 P=0.94	**R= - 0.24** **P<0.001**

Associations between changes after 4 months and 12 months

There were several significant associations between the changes in cardiometabolic biomarkers and changes in fish intake (Table 10) and fatty acid incorporation (Table 11). The changes in plasma TG and fried fish consumption after 4 months were inversely related. After 12 months, the change in waist circumference was inversely associated with changes in fresh fish, canned fish and therefore total fish consumption. After 4 months, the only significant association between change in erythrocyte fatty acid composition and change in the measured biomarkers was an inverse association between DHA content and waist circumference. No significant associations were seen after 12 months between changes in erythrocyte fatty acid content and any biomarkers.

Table10: Associations between changes in fish intake and changes in cardiometabolic parameters

	Change after 4 months (n=143)				**Change after 12 months (n=101)**			
Type of Fish	**TG**	**SBP**	**DBP**	**WC**	**TG**	**SBP**	**DBP**	**WC**
Fresh (Steamed, grilled, baked)	R<0.001 P=0.10	R=0.10 P=0.30	R=0.04 P=0.65	R=-0.14 P=0.13	R=-0.02 P=0.82	R=0.10 P=0.33	R=0.01 P=0.90	**R=-0.37** **P<0.001**
Fried (include take-away)	**R=-0.19** **P=0.04**	R=-0.07 P=0.44	R=-0.07 P=0.43	R=0.03 P=0.79	R=-0.00 P=0.98	R=-0.01 P=0.90	R=-0.07 P=0.50	R=-0.11 P=0.92
Canned (salmon, tuna, sardines etc)	R=-0.03 P=0.76	R=-0.02 P=0.87	R=-0.01 P=0.92	R=-0.10 P=0.29	R=-0.00 P=0.98	R=0.03 P=0.80	R=-0.09 P=0.38	**R=-0.25** **P=0.02**
Total Fish Intake	R=-0.06 P=0.51	R=0.02 P=0.83	R=-0.002 P=0.99	R=-0.14 P=0.13	R=-0.02 P=0.86	R=0.08 P=0.44	R=-0.05 P=0.63	**R=-0.37** **P<0.001**

Table 11: Associations between changes in erythrocyte fatty acids and cardiometabolic parameters

	Change after 4 months (n=129)				Change after 12 months (n=115)			
	TG	**SBP**	**DBP**	**WC**	**TG**	**SBP**	**DBP**	**WC**
Change in EPA	R=0.004 P=0.96	R=0.06 P=0.54	R=0.04 P=0.65	R=0.10 P=0.28	R=-0.04 P=0.68	R=0.17 P=0.08	R=0.10 P=0.33	R=0.06 P=0.55
Change in DHA	R=-0.17 P=0.06	R=-0.03 P=0.79	R=-0.03 P=0.72	**R=-0.23** **P=0.01**	R=-0.07 P=0.47	R=0.13 P=0.19	R=0.10 P=0.30	R=-0.13 P=0.17
Change in EPA+DHA	R=-0.13 P=0.16	R<0.001 P=1.00	R=-0.01 P=0.90	R=-0.15 P=0.11	R=-0.07 P=0.48	R=0.03 P=0.77	R=0.11 P=0.25	R=-0.09 P=0.36

Discussion

This study examined relationships between habitual consumption of fish and n-3 PUFA levels in erythrocytes. It further tested whether increases in canned fish consumption were associated with increased incorporation of EPA and/or DHA into erythrocytes and whether this was related to changes in cardiometabolic biomarkers.

Data on fish consumption obtained from FFQs are limited in that they do not differentiate between types of fish, particularly fatty versus lean fish, and variations in cooking and preserving methods. Nevertheless, they provided useful estimates of average fish consumption by intervention and control groups in this study. Although the proportion of participants consuming fish increased by a small margin in both groups, the provision of free canned fish in the form of John West Tuna and Salmon Tempters® to participants in the INT group resulted in a twofold increase in the quantity of canned fish consumed after 4 months, with no significant change in the control group. At the 12 month assessment, however, 8 months after cessation of the complimentary canned fish, canned fish consumption was no longer significantly elevated. Erythrocyte DHA increased significantly by 4 months in the INT group but the change was small (< 10%) and was no longer significant after 12 months. This small effect is consistent with the relatively low intake of EPA+DHA from canned fish (estimated to average 60mg /day).

Despite the small magnitude of change, cross-sectional analysis revealed significant associations between fish consumption, erythrocyte n-3 PUFA and waist circumference. At baseline, the DHA content of erythrocytes was related to the level of consumption of both canned and fresh fish.. After the first 4 months of the intervention, there were no significant associations between changes in fish consumption and changes in erythrocyte fatty acid levels. However, after 12 months, the increase in EPA +DHA content of erythrocytes correlated with higher fish consumption (all except fried fish). Although fish consumption may have declined after the initial 4 months, fatty acids continue to accumulate in erythrocytes and this is reflected in the changes to 12 months.
These results should be interpreted with caution as 1) the analysis of change data is less reliable than the baseline data which reflects habitual consumption patterns and steady state erythrocyte fatty acid levels; 2) the likelihood of type 2 error increases with multiple correlations.

The provision of canned fish to participants in the intervention trial proved to be an effective strategy for increasing the relatively low level of habitual fish consumption in a typical Australian community. A previous report to Simplot indicates that the canned fish was popular with participants. However, its n-3 PUFA content was lower than expected, thus limiting the likelihood of detecting associations between fish intake and improvements in cardiometabolic variables. Nevertheless, the increased fish intake, along with other diet and lifestyle changes, appears to have contributed to the change in waist circumference. The results from this study may inform future studies designed to investigate associations between increased n-3 PUFA in the diet, their incorporation into erythrocytes and their potential impact on health status.

Benefits and adoption

The aim of the study was to prove the omega-3 bioavailability of canned fish products and the impact on cardio metabolic markers. The study did not indicate any relationship between fish consumption and increased omega-3 levels in red blood cells or associated cardio metabolic markers. Therefore, there are no benefits identified in this study and an adoption to other sectors of the industry is not possible.

Further development

No further development is planned, as the omega-3 bioavailability of canned fish could not be shown.

Planned outcomes

Outcome: Reliable scientific data on the omega-3 status of John West canned seafood products
Output: Simplot regularly tests the omega-3 content of canned fish. This does not take into account bioavailability. Recent development of Nutrient Reference Values – Adequate Intakes and Suggested Dietary Targets for omega-3 acids allows comparisons of omega-3 content to dietary recommendations, beyond the permitted Food Standard Code source and good source of omega-3 claims, allowing consumers to assess their intake.

Achievement: Additional content data was gathered on the John West seafood products, however the omega-3 bioavailability of John West canned fish products could not be proven. The omega-3 content per can is above 60mg, which allows Simplot to use the “Good Source” claim. John West’s marketing strategy already addresses this in their promotional activities; further recommendations beyond this can not be developed from the present study.

Outcome: Build further brand equity
Output: There are two different target audiences for omega-3 communications; the nutrition health professionals and consumers. The information provided to health professionals will be the intrinsic health benefits of processed fish. The message to consumers will build the current awareness of omega-3 benefits, using scientific data to explain practically and simply how that translates to the consumer’s diet. By promoting the omega-3 health benefits of fish the focus will be on fish in general and on John West canned in particular. Simplot wants to generate a connection between omega-3, health and John West. By increasing on-pack claims it aims to attract consumers’ attention at the point of purchase and to drive sales ahead of competitors.

Achievement: Communication about the bioavailability of omega-3 in processed fish in order to build further brand equity is not possible based on the results of this study.

Conclusion

This study has demonstrated that regular consumption of canned fish can modestly elevate erythrocyte DHA (with the potential to improve the health status of individuals with MetS).

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