British Journal of Nutrition (2011), **106**, 1231–1239
doi:10.1017/S000711451100153X

Consumption of salmon *v.* salmon oil capsules: effects on *n*-3 PUFA and selenium status

Welma Stonehouse[1]*, Melanie R. Pauga[1], Rozanne Kruger[1], Christine D. Thomson[2], Marie Wong[1] and Marlena C. Kruger[3]

[1]*Institute of Food, Nutrition and Human Health, Massey University, Private Bag 102 904, North Shore Mail Centre, 0745 Auckland, New Zealand*
[2]*Department of Human Nutrition, University of Otago, Dunedin, New Zealand*
[3]*Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand*

(Received 29 July 2010 – Revised 13 January 2011 – Accepted 25 February 2011 – First published online 18 May 2011)

Abstract

Salmon provides long-chain (LC) *n*-3 PUFA and Se, which are well recognised for their health benefits. The *n*-3 and Se status of the New Zealand population is marginal. The objective of the present study was to compare the effects of consuming salmon *v.* supplementation with salmon oil on LC *n*-3 and Se status. Healthy volunteers (*n* 44) were randomly assigned to one of four groups consuming 2 × 120 g servings of salmon/week or 2, 4 or 6 salmon oil capsules/d for 8 weeks. Linear regression analysis predictive models were fitted to the capsule data to predict changes in erythrocyte LC *n*-3 levels with intakes of LC *n*-3 from capsules in amounts equivalent to that consumed from salmon. Changes in Se status (plasma Se and whole-blood glutathione peroxidase) were compared between the groups consuming salmon and capsules (three groups combined). Salmon, 2, 4 and 6 capsules provided 0·82, 0·24, 0·47 and 0·69 g/d of LC *n*-3 fatty acids. Salmon provided 7 μg/d and capsules < 0·02 μg/d of Se. The predictive model (r^2 0·31, P = 0·001) showed that increases in erythrocyte LC *n*-3 levels were similar when intakes of 0·82 g/d LC *n*-3 from salmon or capsules (1·92 (95 % CI 1·35, 2·49) *v.* 2·32 (95 % 1·76, 2·88) %) were consumed. Plasma Se increased significantly more with salmon than with capsules (12·2 (95 % CI 6·18, 18·12) *v.* 1·57 (95 % CI −2·32, 5·45) μg/l, P = 0·01). LC *n*-3 status was similarly improved with consumption of salmon and capsules, while consuming salmon had the added benefit of increasing Se status. This is of particular relevance to the New Zealand population that has marginal LC *n*-3 and Se status.

Key words: Salmon: Fish oil: Long-chain *n*-3 fatty acids: Selenium

Salmon is a good source of both long-chain (LC) *n*-3 PUFA and Se. The LC *n*-3 fatty acids, EPA and DHA, are well recognized for their protective effects against CVD[1]. Possible mechanisms include reductions in TAG, blood pressure, cardiac arrhythmias, platelet aggregation, the inflammatory response, growth of atherosclerotic plaque and improved endothelial function[2]. In addition, these fatty acids may play important roles in inflammatory disease[3] and in brain and retinal development[4]. It may also be of benefit for cognitive decline during ageing[4], bone health[5] and mood disorders[6], but more research is still needed to elucidate the effects of LC *n*-3 fatty acids on these conditions. The 'omega-3 index', calculated as the percentage EPA and DHA of total erythrocyte fatty acids, has been introduced as a novel, physiologically relevant, modifiable and independent marker of risk for sudden cardiac death[7]. Although very few data are available on the intakes of LC *n*-3 fatty acids by the New Zealand population, food disappearance data indicate that intakes are low, as in most other Western countries[8]. Recommendations for intake of LC *n*-3 fatty acids for the prevention of CVD include the consumption of 500 mg LC *n*-3 fatty acids/d or one to two servings/week of fatty fish[9]. This recommendation assumes equivalence between fatty fish intake and fish oil supplements with regard to bioavailability. However, the bioavailability of the fatty acids might be affected by the different matrices of fish and fish oil capsules but there are not many studies that have investigated the effects of fish *v.* fish oil on LC *n*-3 status. These studies had different study designs ranging in quality[10–13]. Visioli *et al.*[13] and Elvevoll *et al.*[11] demonstrated that *n*-3 fatty acids from fish were more effectively incorporated into plasma/serum lipids than when administered as capsules. However, two recent studies could not confirm this and concluded that *n*-3 fatty acids from fish or

Abbreviations: DPA, docosapentanoic acid; GPx, glutathione peroxidase; LC, long chain.

* **Corresponding author:** Dr W. Stonehouse, fax +64 9 4439640, email w.stonehouse@massey.ac.nz

capsules were equally effective in enriching blood lipids with *n*-3 fatty acids[10,12].

Consuming fish might be a more beneficial way of improving the LC *n*-3 status than consuming capsules because of its other nutritional properties; for example, it provides protein to the diet and it is also a good source of Se, a critical component of numerous selenoproteins in humans[14]. Increased intakes of Se-rich foods are of particular importance to the New Zealand population, which has been shown to have a marginal Se status due to low concentrations of Se in the soil[15]. Marginal Se status has been associated with a number of chronic diseases such as CVD[16], some types of cancer (e.g. lung, prostate and colorectal and liver cancer)[17], altered immune function, viral infections, miscarriage, reduced sperm motility, mood, hypothyroidism, inflammatory conditions, asthma and others[18].

The objective of the present study was to compare the effects of consuming two servings/week of farmed New Zealand King Salmon with daily intakes of salmon oil capsules for 8 weeks in healthy men and women on erythrocyte levels of LC *n*-3 fatty acids, the 'omega-3 index' and Se status.

Experimental methods

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Massey University Human Ethics Committee (Southern A, Reference no. 07/72). Written informed consent was obtained from all subjects. The study was conducted between April and June 2008.

Subjects and study design

A total of forty-four healthy volunteers aged between 21 and 40 years, from the Auckland region in New Zealand, participated in the study. It was calculated that a sample size of ten per group would provide 80 % power to detect a significant difference (α 0·05, two-tailed) between groups of one unit in omega-3 index and in total erythrocyte *n*-3 fatty acid levels. Subjects (eleven per group) were recruited to allow for a 10 % dropout in each group. Subjects were eligible to participate if they had a low habitual intake of fatty fish (less than two servings/month), had not taken fish oil or Se supplements over the past 6 months, did not smoke, did not have allergies to seafood and did not have any known condition or disease or taking medication for any condition or disease.

A randomised parallel study design was used. Subjects were matched for age and sex and then randomly assigned to one of four groups receiving one of the following treatments for a duration of 8 weeks: 2 × 120 g servings/week of farmed New Zealand King salmon fillets (*Oncorhynchus tshawytscha*) (The New Zealand King Salmon Company Limited, Nelson, New Zealand); 2, 4 or 6 capsules of salmon oil/d (Healtheries of New Zealand Limited, Auckland, New Zealand). Since the LC *n*-3 fatty acid content (specifically EPA and DHA) of salmon and salmon oil capsules is not equal and the quantities of EPA and DHA in different batches of salmon and with cooking may vary, the following method was used to compare intakes of LC *n*-3 fatty acids from salmon and capsules on *n*-3 fatty acid levels in erythrocytes. Data obtained from different dosages of capsules were used to create a linear predictive model from which changes in *n*-3 fatty acid levels in erythrocytes with intakes of LC *n*-3 fatty acids from capsules in amounts equivalent to that consumed from salmon could be predicted (this is explained in more detail in the Statistics section). To ensure that the most accurate value for LC *n*-3 fatty acids consumed from salmon could be determined, each subject received an additional portion of salmon for each meal, which they were requested to prepare in the same manner. For each salmon meal, one portion was served and eaten and the duplicate portion was stored in a coded plastic bag in a freezer. The duplicate cooked salmon portions were collected from subjects on a weekly basis and stored at −80°C until the end of the study when the fatty acid and Se content of the portions were analysed. Subjects were also asked to return any unconsumed salmon in a separate coded plastic bag. The amount of unconsumed salmon was subtracted and the amount of LC *n*-3 fatty acids and Se consumed was calculated. The fatty acid and Se content of the salmon oil capsules were also analysed and the amounts consumed were calculated based on percentage compliance.

Demographic information including medical history, medication and supplement use and alcohol consumption habits were collected at recruitment using questionnaires. Fasting blood samples and anthropometric measurements (height, weight and BMI) were obtained at baseline and after 8 weeks, as any change may indicate a change in lifestyle. Subjects were requested not to consume any other fatty fish or supplements and to maintain their normal daily routines (e.g. eating patterns, physical activity, alcohol consumption, etc.) for the duration of the study. Food consumption pre- and post-intervention was assessed using a validated FFQ[19]. Subjects completed a weekly compliance diary, including consumption of salmon and capsules, maintenance of normal daily routines, occurrence of any illnesses and any medication used during the study, in order to ascertain compliance to the study protocol.

Subjects received guidelines for the consumption and storage of salmon oil capsules and preparation of salmon. Guidelines for preparation of salmon were based on cooking methods shown to achieve optimal sensory qualities and preservation of *n*-3 fatty acid levels such as oven baking, pan frying and steaming[20]. Subjects were advised to store the capsules in the refrigerator in opaque containers and to consume the capsules every day with a meal. Subjects were requested to return any left-over salmon and capsules, which was weighed and counted to determine the compliance. The oxidation levels (anisidine and peroxide values) of the salmon oil capsules were analysed. Fish oil, due to its high degree of unsaturation, is susceptible to oxidation, which results in decreased *n*-3 fatty acid levels and is associated with chronic inflammation and oxidative stress[21].

At the end of the study, each subject completed a computer-based tolerance questionnaire. The first question was an open-ended question that asked about any side effects experienced during the study without any probing to ensure

British Journal of Nutrition

responses were not led by suggestion. Subjects were not able to go back to this question to change the answer. The subsequent questions, adapted from Freeman & Sinha[22], asked about specific side effects, including dizziness, diarrhoea, nausea, bloating, burping, heart burn/reflux, difficulty in swallowing the treatment, unpleasant breath and tiredness.

Blood samples and biochemical analysis

Registered phlebotomists collected fasting venous blood samples from subjects between 07.00 and 09.30 hours after fasting overnight (at least 8 h with no food or beverage, except water). An EDTA blood sample was collected for the analysis of erythrocyte fatty acids, plasma Se and whole-blood glutathione peroxidase (GPx) activity. Blood was centrifuged for 10 min at 2000 ***g*** at 4°C within 2 h after blood collection, plasma was removed and the erythrocytes were then washed three times with saline (0·9 % NaCl). Aliquots of whole blood, plasma and erythrocyte were stored at −80°C until analysis was carried out in one batch at the end of the study.

Erythrocyte fatty acids were analysed using a Shimadzu GC 2010 ported to a GCMS-QT2010 Mass Spectrometer (Shimadzu Corporation, Kyoto, Japan) as described previously[23]. The CV for EPA and DHA assays were 2·7 and 3·6 %, respectively. Erythrocyte fatty acids are expressed as weight percentage of total fatty acids. The omega-3 index was calculated as follows[7]:

$$\text{Omega-3 index}(\%) = \frac{\text{erythrocyte EPA} + \text{erythrocyte DHA}}{\text{erythrocyte total fatty acids}} \times 100.$$

Plasma Se concentrations were analysed by graphite furnace atomic absorption spectroscopy with Zeeman background correction (Perkin-Elmer Model 3100; Perkin-Elmer, Norwalk, CT, USA), using a modified version of the method of Jacobson & Lockitch[24]. Accuracy was assessed by analysing certified reference material with each batch (Seronorm Reference Serum, batch no. JL4409; Laboratories of SERO AS, Billingstad, Norway), with a certified Se concentration of 0·92 (95 % CI 0·84, 1·00) μmol Se/l, which gave a mean concentration of 0·88 (SD 0·04) μmol/l (CV 4·9 %, *n* 45). Whole-blood GPx activity was determined using a modification of the coupled enzyme procedure by Paglia and Valentine[25] on the Cobas Fara autoanalyser (Hoffman-La Roche, Basle, Switzerland). Whole-blood GPx was assayed as a measure of erythrocyte GPx, which has been shown previously by us to constitute 95 % of total whole-blood activity using this assay method[26]. Because no external controls were available at the time, pooled samples of whole blood were analysed with each batch, which gave a mean activity of 45·5 (SD 2·8) U/g Hb (CV 6·2 %, *n* 45).

Analysis of salmon and salmon oil capsules

The salmon samples from each subject (*n* 16) were thawed, pooled and homogenised (resulting in one sample per subject). In total, nine salmon oil capsules, previously stored at −80°C, were thawed at room temperature in the dark and analysed.

The procedure for the analysis of fatty acids in salmon and salmon oil capsules was described by Larsen *et al.*[20]. The CV′ values for EPA and DHA concentrations in oil were 2·52, 2·62 % and 1·74, 1·94 % for the salmon and capsules, respectively.

The Se content of the salmon and salmon oil capsules was analysed by inductively coupled plasma MS using a Perkin-Elmer Sciex Elan 6000 (Perkin-Elmer, Inc., Waltham, MA, USA). Approximately 0·5 g sample was weighed into 68 ml polypropylene tubes (Environmental Express, Mt Pleasant, SC, USA) with calibrated volume markings. Tetramethylammonium hydroxide solution (12·5 %; 5 ml) was added and digested on a hot block set (Environmental Express) to achieve a digest temperature of 90°C for 1 h. The digest was made to 50 ml in digest tubes, shaken and filtered through a 0·45 μm cellulose acetate syringe filter (Sartorius Minisart; Sartorius Stedim Biotech S.A., Aubagne Cedex, France). The isotope ^{82}Se was used to monitor Se.

Oxidation values of capsules

At 0, 4 and 8 weeks, samples of salmon oil capsules (nine in total) were collected randomly during the study and stored at −80°C until the end of the study for analysis of oxidation values (anisidine and peroxide values). Peroxide values were analysed using the AOCS Official Method Cd8-53 with modifications and anisidine was analysed using the AOCS Official Method Cd18-90[27].

Statistical analysis

Data were analysed using the SPSS package version 16 (SPSS, Inc., Chicago, IL, USA). Normally distributed variables are presented as means and standard deviations and non-normally distributed variables as medians (25–75th percentiles). A P value of $<0{\cdot}05$ was considered to be statistically significant. The variables were tested for a normal distribution using Kolmogorov–Smirnov and Shapiro–Wilk tests, together with examination of normal Q–Q plots, box and stem and leaf plots. Differences within groups between baseline and end values were analysed using the paired-samples *t* test for parametric variables and the Wilcoxon ranked-sum test for non-parametric variables. One-way ANOVA with *post hoc* tests (Tukey's honest significance difference test) were used to determine the differences between groups for parametric variables. Differences between groups for non-parametric variables were analysed using the Kruskal–Wallis test with *post hoc* analysis and Bonferroni adjustments. Differences in plasma Se concentrations between the salmon and the capsule groups (three groups combined) were analysed while controlling for baseline Se using the ANCOVA test, while differences in whole-blood GPx were analysed using the Mann–Whitney *U* test. All statistical tests were two-sided. Linear regression analysis predictive models were fitted to the capsule data. All assumptions for linear regression analyses were met; residuals were normally distributed and independent (Durbin–Watson test) and variance was constant (as assessed by plotting standardised residuals against the predicted

Table 1. Baseline characteristics of the study subjects
(Mean values and standard deviations)

	Salmon group (*n* 11)		Two-capsule group (*n* 11)		Four-capsule group (*n* 9)		Six-capsule group (*n* 10)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P**
Sex (*n*)									
Male	5		5		5		4		
Female	6		6		4		6		
Age (years)	30·3	5·53	30·6	6·19	30·8	5·76	29·6	5·74	0·97
BMI (kg/m^2)	24·7	3·14	24·5	2·10	26·0	4·91	23·0	2·12	0·26

* Differences between the groups were determined by one-way ANOVA (two-sided).

values). Linear regression analysis was carried out with a dummy variable to calculate the predicted change and 95 % CI in erythrocyte *n*-3 fatty acid levels to the intake of LC *n*-3 fatty acids from capsules in amounts equivalent to that consumed from salmon.

Results

Baseline characteristics and compliance

Of the forty-four subjects recruited, three subjects withdrew from the study, one due to illness (six-capsule group) and two for personal reasons (four-capsule group). Subject characteristics are described in Table 1. There were no differences between the intervention groups at baseline. Compliance to the interventions was 98 (SD 3·3) % and did not differ between the four intervention groups. Anthropometric measurements remained constant throughout the study.

Interventions

Cooked salmon contained on average 1·01 g EPA, 0·33 g docosapentanoic acid (DPA), 1·11 g DHA and 0·02 mg Se/100 g salmon. Each gram of salmon oil capsule contained on average 45 mg EPA, 14 mg DPA and 60 mg DHA. Se was not detected in salmon oil capsules. Mean peroxide values (3·30 (SD 0·06) meq/kg oil) and anisidine values (8·36 (SD 0·67)) of salmon oil capsules were below the maximum permitted levels during the study (5 meq/kg for the peroxide value and 10 for the anisidine value[28]).

The total daily average LC *n*-3 fatty acids and Se consumed from the different treatments by the subjects per day are summarised in Table 2. Analysis showed that the salmon oil capsules contained less LC *n*-3 fatty acids than claimed by the company supplying the capsules (0·045 g EPA and 0·06 g DHA/capsule *v.* 0·06 g EPA and 0·10 g DHA/capsule as claimed by the company). This resulted in the higher level of *n*-3 fatty acids supplied by six capsules being less than the *n*-3 fatty acids supplied by salmon, which could possibly affect the linear regression analysis. However, it can be argued that the linear regression analysis was still valid to use, based on evidence in the literature showing a linear relationship between dosage of *n*-3 fatty acids even higher than that used in the present study and erythrocyte *n*-3 fatty acid levels[29,30]. In the present study, the relationship between intake of LC *n*-3 fatty acids from capsules and erythrocyte *n*-3 fatty acid levels was indeed linear and is described below.

Fatty acid status

Baseline erythrocyte fatty acid levels did not differ between the intervention groups (Table 3). As expected, erythrocyte EPA, DHA and omega-3 index levels increased and arachidonic acid levels decreased significantly from baseline to end with all treatments. Erythrocyte DPA levels increased significantly only in the salmon and six-capsule groups. The increase in erythrocyte EPA and omega-3 index were significantly higher in the salmon and six-capsule groups compared with the two-capsule group. This trend was also seen for erythrocyte DHA, but the differences were not significant. Although some small

Table 2. Daily consumption of long-chain (LC) *n*-3 fatty acids and selenium during the 8-week intervention with salmon and salmon oil capsules
(Mean values and standard deviations)

	Salmon group (*n* 11)		Two-capsule group (*n* 11)		Four-capsule group (*n* 9)		Six-capsule group (*n* 10)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EPA (g/d)	0·34	0·02	0·09	0·001	0·17	0·001	0·25	0·01
DPA (g/d)	0·11	0·01	0·03	<0·001	0·06	<0·001	0·09	0·003
DHA (g/d)	0·37	0·02	0·12	0·002	0·24	0·002	0·35	0·01
Total LC *n*-3 (g/d)	0·82	0·06	0·24	0·004	0·47	0·004	0·69	0·02
Se (μg/d)	6·70	0·65	ND		ND		ND	

DPA, docosapentanoic acid; ND, not detected.

British Journal of Nutrition

Table 3. Erythrocyte fatty acid levels (% of total fatty acids) during the 8-week intervention with salmon and salmon oil capsules (Mean values and standard deviations)

		Baseline		End		Change			
Fatty acids	Treatment*	Mean	SD	Mean	SD	Mean	SD	*P*†	*P*‡
Myristic acid (14 : 0)	Salmon	0·54	0·16	0·57	0·11	0·03	0·11	0·47	0·75
	Two capsules	0·57	0·09	0·62	0·10	0·05	0·07	0·04	
	Four capsules	0·61	0·26	0·79	0·70	0·18	0·72	0·47	
	Six capsules	0·57	0·14	0·62	0·12	0·04	0·14	0·33	
Palmitic acid (16 : 0)	Salmon	19·4	0·74	19·2	0·90	−0·23	0·39	0·08	0·83
	Two capsules	19·5	0·90	19·1	0·78	−0·36	0·81	0·17	
	Four capsules	19·6	0·77	19·5	0·93	−0·13	0·47	0·44	
	Six capsules	20·0	0·85	19·7	0·73	−0·37	0·85	0·20	
Stearic acid (18 : 0)	Salmon	15·3	0·94	14·7	0·34	−0·58	0·84	0·05	0·63
	Two capsules	14·7	0·41	14·5	0·63	−0·22	0·46	0·14	
	Four capsules	14·9	0·90	14·7	0·71	−0·22	0·83	0·44	
	Six capsules	14·8	0·64	14·5	0·69	−0·33	0·70	0·17	
Oleic acid (18 : 1*n*-9)	Salmon	11·3	0·83	11·4	0·63	0·07	0·65	0·73	0·74
	Two capsules	11·5	0·59	11·3	0·53	−0·18	0·47	0·24	
	Four capsules	11·3	0·75	11·5	0·94	0·18	0·78	0·50	
	Six capsules	10·8	0·94	10·8	0·67	−0·03	1·05	0·92	
Linoleic acid (18 : 2*n*-6)	Salmon	7·33	0·80	7·84	0·93	0·51	0·72	0·04	0·74
	Two capsules	8·05	1·10	8·35	0·99	0·31	0·87	0·27	
	Four capsules	7·90	1·43	7·87	0·56	−0·03	1·23	0·95	
	Six capsules	7·62	1·31	7·93	0·98	0·31	1·42	0·51	
α-Linolenic acid (18 : 3*n*-3)	Salmon	0·11	0·04	0·14	0·04	0·02	0·07	0·24	0·30
	Two capsules	0·13	0·02	0·16	0·05	0·03	0·04	0·05	
	Four capsules	0·14	0·06	0·16	0·06	0·02	0·05	0·26	
	Six capsules	0·16	0·03	0·15	0·03	−0·01	0·05	0·42	
Arachidonic acid (20 : 4*n*-6)	Salmon	11·4	0·98	10·9	0·57	−0·47	0·61	0·03	0·22
	Two capsules	11·1	0·88	10·8	0·71	−0·28	0·37	0·03	
	Four capsules	11·0	0·74	10·5	0·97	−0·50	0·63	0·04	
	Six capsules	10·9	0·59	10·1	0·56	−0·83	0·74	0·01	
EPA (20 : 5*n*-3)	Salmon	0·78	0·20	1·58	0·24	0·80[a]	0·33	<0·001	0·002
	Two capsules	0·90	0·24	1·26	0·32	0·36[b]	0·23	<0·001	
	Four capsules	0·86	0·22	1·38	0·21	0·51	0·24	<0·001	
	Six capsules	0·78	0·31	1·62	0·53	0·84[a]	0·40	<0·001	
DPA (22 : 5*n*-3)	Salmon	1·64	0·32	1·83	0·26	0·18	0·12	<0·001	0·03
	Two capsules	1·61	0·17	1·70	0·16	0·09	0·13	0·05	
	Four capsules	1·69	0·31	1·76	0·31	0·07	0·14	0·17	
	Six capsules	1·56	0·26	1·81	0·16	0·25	0·21	0·004	
DHA (22 : 6*n*-3)	Salmon	5·06	0·70	5·99	0·52	0·93	0·53	<0·001	0·10
	Two capsules	5·21	0·49	5·68	0·62	0·47	0·34	0·001	
	Four capsules	5·08	0·47	5·97	0·53	0·89	0·58	0·002	
	Six capsules	5·42	0·79	6·34	0·78	0·92	0·50	<0·001	
Omega-3 index (%)	Salmon	5·84	0·72	7·58	0·68	1·74[a]	0·78	<0·001	0·007
	Two capsules	6·10	0·63	6·94	0·79	0·84[b]	0·38	<0·001	
	Four capsules	5·91	0·56	7·35	0·61	1·44	0·75	<0·001	
	Six capsules	6·20	1·01	8·0	1·10	1·76[a]	0·69	<0·001	

DPA, docosapentanoic acid.
[a,b] Mean values with unlike superscript letters were significantly different for *post hoc* tests ($P<0·05$).
* Salmon group, *n* 11; two-capsule group, *n* 11; four-capsule group, *n* 9; six-capsule group, *n* 10.
† Differences within groups (from baseline to end) were analysed using the paired-samples *t* test (two-sided).
‡ Differences between changes from baseline to end in groups were determined by one-way ANOVA and *post hoc* tests using Tukey's honest significance difference test (two-sided).

significant differences were seen within-group changes for some of the other fatty acids, there was no clear trend in changes in these fatty acids with the interventions (Table 3).

Increased intakes of LC *n*-3 fatty acids from capsules were linearly related and significantly predicted changes in total erythrocyte *n*-3 fatty acid, erythrocyte EPA, erythrocyte DHA and omega-3 index levels, but did not significantly predict erythrocyte DPA levels, which are as follows:

- Change in total erythrocyte *n*-3 fatty acids (%) = 0·36 + 2·39 × total LC *n*-3 fatty acid intake (g/d), *n* 30, $F(1,29) = 12·7$, $P=0·001$, r^2 0·31, $\beta = 2·39$ (95 % CI 1·02, 3·76).
- Change in erythrocyte EPA (%) = 0·09 + 1·04 × total LC *n*-3 fatty acid intake (g/d), *n* 30, $F(1,29) = 13·0$, $P=0·001$, r^2 0·32, $\beta = 1·04$ (95 % CI 0·45, 1·64).
- Change in erythrocyte DHA (%) = 0·28 + 1·02 × total LC *n*-3 fatty acid intake (g/d), *n* 30, $F(1,29) = 5·10$, $P=0·032$, r^2 0·15, $\beta = 1·02$ (95 % CI 0·10, 1·95).
- Change in omega-3 index (%) = 0·38 + 2·07 × total LC *n*-3 fatty acid intake (g/d), *n* 30, $F(1,29) = 12·71$, $P=0·001$, r^2 0·31, $\beta = 2·07$ (95 % CI 0·88, 3·26).
- Change in erythrocyte DPA levels: *n* 30, $F(1,29) = 4·03$, $P=0·05$, r^2 0·13.

British Journal of Nutrition

Table 4. Changes and predicted changes in erythrocyte long-chain (LC) *n*-3 fatty acids after an 8-week consumption of 0·82 g/d LC *n*-3 fatty acids from salmon or salmon oil capsules

(Mean values and 95 % confidence intervals)

	Predicted values*			
	Salmon intake		Salmon oil capsule intake	
	Mean	95 % CI	Mean	95 % CI
Total erythrocyte *n*-3 fatty acids (%)	1·92	1·35, 2·49	2·32	1·76, 2·88
Erythrocyte EPA (%)	0·80	0·58, 1·02	0·95	0·70, 1·19
Erythrocyte DHA (%)	0·93	0·58, 1·29	1·12	0·74, 1·50
Omega-3 index (%)	1·73	1·21, 2·26	2·08	1·59, 2·66

* Changes predicted from linear regression models.

The changes and predicted changes in erythrocyte LC *n*-3 fatty acid levels and omega-3 index with intake of 0·82 g/d of LC *n*-3 fatty acids from salmon and salmon oil capsules are summarised in Table 4. Based on the large overlap in 95 % CI levels between salmon and salmon oil capsules, it can be concluded that the increase in erythrocyte total *n*-3 fatty acid levels, EPA, DHA and omega-3 index were similar to the intake of salmon and salmon oil capsules.

Selenium status

Changes in plasma Se concentrations and whole-blood GPx activities are summarised in Table 5. Plasma Se increased significantly with intake of salmon compared with salmon oil capsule intake (mean difference between the change in plasma Se with salmon and salmon oil capsules, 10·6 (95 % CI 3·44, 17·8) μg/l, $F(1,36) = 9{\cdot}04$, $P = 0{\cdot}005$). Whole-blood GPx did not change significantly in either the salmon or the salmon oil capsule groups.

Food consumption

Food intake from the major food groups (including milk, bread, butter/margarine/spreads, breakfast cereal, starchy foods, meat, poultry, fish/seafood, eggs, legumes, cooking fat/oil, condiments, vegetables, fruit, beverages and takeaways) did not change during the study in any of the intervention groups and no differences were observed between the groups (data not shown). As expected, fatty fish intake increased significantly in the salmon group (from a median intake of 0·5 (25–75th percentile 0·3–0·8) to 3·0 (25–75th percentile 1·0–3·5) servings/week)) compared with the capsule groups (from a median intake of 0·6 (25–75th percentile 0·4–1·0) to 1·0 (0·5–1·3) servings/week) ($P = 0{\cdot}002$; Mann–Whitney *U* test). Fatty fish intake did not differ between the three capsule groups. Since total fish and seafood intake did not change, we suspect that subjects in the salmon group exchanged their habitual intake of fish and seafood for salmon.

Tolerance of treatments

Unprompted, only six subjects reported side effects, all of them from the salmon oil capsule groups and included complaints about bloating (1 ×), nausea (2 ×), burping (2 ×), indigestion (1 ×) and stomach pain (1 ×). With regard to specific side effects, only one person in the salmon group reported one side effect (bloating), whereas more complaints were reported by subjects in the capsule groups (two-capsule group: nausea (2 ×), bloating (2 ×), burping (4 ×), unpleasant breath (1 ×) and tiredness (1 ×); four-capsule group: diarrhoea (2 ×), nausea (1 ×), burping (1 ×), heart burn/reflux (1 ×), difficulty in swallowing the treatment (2 ×), unpleasant breath (2 ×) and tiredness (4 ×); six-capsule group: nausea (2 ×), bloating (1 ×), burping (3 ×) and difficulty in swallowing the treatment (3 ×).

The number of capsules consumed did not seem to affect the number of side effects reported. Most of these side effects were experienced by the subjects as mild/insignificant.

Discussion

The present study showed that consumption of similar amounts of LC *n*-3 fatty acids either from two weekly servings of salmon or daily dosages of salmon oil capsules was equally effective in increasing LC *n*-3 status in healthy volunteers.

Table 5. Plasma selenium concentrations and whole-blood glutathione peroxidase (GPx) activities during the 8-week consumption of salmon or salmon oil capsules

(Mean values and 95 % confidence intervals or medians and 25–75th percentiles)

	Salmon group (*n* 11)		Salmon oil capsule group (*n* 26)		
	Mean	95 % CI	Mean	95 % CI	*P*
Plasma Se (μg/l)					
Baseline	124	109, 139	118	110, 126	0·42
End	135	121, 149	120	115, 126	0·02
Change*	12·2	6·18, 18·2	1·57	−2·32, 5·45	0·01
	Median	25–75th Percentile	Median	25–75th Percentile	
Whole-blood GPx (Ug Hb)					
Baseline	46·9	39·8–53·1	44·6	37·5–55·3	0·57
End	44·8	38·8–53·1	43·9	37·9–57·4	0·94
Change	0·26	−2·44–4·24	−0·58	−1·57–2·77	0·57

* Adjusted mean and 95 % CI (adjusted for baseline plasma Se). Differences in plasma Se between the groups were analysed while controlling for baseline Se concentrations using ANCOVA (two-sided); differences in whole-blood GPx between the groups were analysed using the Mann–Whitney *U* test.

British Journal of Nutrition

However, consuming salmon had the added benefit of increasing plasma Se concentrations and was better tolerated than salmon oil capsules.

In two studies, it has been shown that fish consumption was more effective at increasing plasma/serum EPA and DHA levels than capsules[11,13], whereas two other studies have shown no difference between fish *v.* capsules[10,12]. Visioli *et al.*[13] also used a dose–response model to compare the effects of daily portions of salmon with different daily dosages of fish oil capsules for 6 weeks. They added data from another study with a similar design to complete the dose–response curve, resulting in a less than optimal study design. They showed that, to obtain the same increment in plasma EPA induced by 0·38 g/d of EPA from salmon, a dose of 0·80 g/d (more than twofold) was needed when using capsules. For DHA, a dose almost ninefold was required to obtain the same increment in plasma DHA as with salmon intake. Furthermore, subjects had to consume 100 g of salmon every day, which might not be feasible in real life. Elvevoll *et al.*[11] also showed that fish (salmon or cod) consumption was more effective in increasing serum EPA and DHA over a period of 8 weeks than supplementing the diet with cod-liver oil. In the present study, however, no attempt was made to match the EPA and DHA content of the fish and fish oil capsules or the type of fish and fish oil. Harris *et al.*[12] demonstrated that consumption of equal amounts of EPA and DHA from oily fish (salmon and albacore tuna) on a weekly basis or from fish-oil capsules on a daily basis by premenopausal women for 16 weeks was equally effective in enriching erythrocyte and plasma phospholipids with *n*-3 fatty acids. Arterburn *et al.*[10] compared the availability of 600 mg DHA/d from algal oil capsules with equal amounts from cooked salmon. In this 2-week study, changes in DHA levels in plasma phospholipids and erythrocyte were similar between consumption of salmon and algal oil capsules. The authors from the first two studies[11,13] speculated that the greater bioavailability of *n*-3 fatty acids from fish than from capsules could be due to capsules providing a small lipidic bolus following ingestion resulting in the processes of lipid absorption not being adequately activated. Fat from fish, on the other hand, is in a diluted emulsion after eating, which may result in higher surface interaction between food and the intestinal wall and more favourable secretion of drivers that aid digestion and absorption at the intestinal level[11,13]. In the present study, subjects were requested to consume the capsules with food, which could partly explain why salmon was not more effective at increasing LC *n*-3 status than the capsules. In addition, in the present study, the lipids in both salmon and salmon oil capsules were in the form of TAG, whereas in the study of Visioli *et al.*[13], EPA and DHA were provided as ethyl esters. On the other hand, Harris *et al.*[12] also used *n*-3 fatty acids in the ethyl ester form. The strengths of the present study are that erythrocyte EPA and DHA levels were used as biomarkers, and subjects with high habitual intakes of fatty fish and *n*-3 fatty acid supplements were excluded and *n*-3 fatty acid intakes from fish and capsules were well matched, similar to the studies by Harris *et al.*[12] and Arterburn *et al.*[10]. In the present study, the *n*-3 fatty acid levels in salmon were analysed after cooking. Since cooking methods can result in varying amounts of *n*-3 fatty acid content[20], this would have resulted in more accurate assessment of actual intakes of *n*-3 fatty acids by each subject, whereas in other studies, the effect of cooking on *n*-3 fatty acid levels was not taken into account[11–13].

It could be argued that 8 weeks were not a sufficient length of supplementation since erythrocyte *n*-3 fatty acids are considered a long-term marker of *n*-3 intakes and do not achieve a plateau until about 6 months[30]. However, 8 weeks were long enough to result in a significant increase in erythrocyte *n*-3 fatty acid levels and it was noted in previous studies that the greatest increases were achieved within the first 4–8 weeks of supplementation[30,31]. The study was powered to detect relatively large differences (13–17 %) in LC *n*-3 status and may not have been adequately powered to detect modest differences between salmon and salmon oil capsule intake. However, the large overlap between 95 % CI levels indicates that it is unlikely that LC *n*-3 fatty acid levels differed significantly between intakes of salmon and salmon oil capsules.

The consumption of two portions/week of salmon or daily dosage of six capsules of salmon oil (providing 0·82 g and 0·69 g/d, respectively, of LC *n*-3 fatty acids) almost achieved omega-3 index levels of 8 %, which is considered to be cardioprotective[7]. A longer duration would have resulted in an even greater increase. Cao *et al.*[31] showed that supplementation with approximately 2 g/d of LC *n*-3 fatty acids, a dosage much higher than in the present study, for 8 weeks nearly reached the optimal value of 8 %. The magnitude of increase is probably largely dependent on baseline levels, which in the present study ranged from 5·84 to 6·20 % compared with 4·3 % in the study of Cao *et al.*[31]. Thus, the lower the baseline levels, the higher the dosage needed over 8 weeks to achieve an optimal omega-3 index level of 8 %. Furthermore, the predictive equation from the capsule data indicates that the omega-3 index will increase with 2·07 % (β-coefficient) with every 1 g/d increase in the intake of LC *n*-3 fatty acids. Taken together (from the present study and the study by Cao *et al.*[31]), every approximately 1 g/d intake of LC *n*-3 fatty acids may result in an approximately 2 % increase in omega-3 index over 8 weeks.

Salmon was consumed twice weekly without any tolerance problems. However, similar to other studies, the most frequent side effect reported for capsule intake (burping and unpleasant breath) was related to eructation[12,22].

Mean plasma Se concentration at baseline (120 (SD 20·4) μg/l) was higher than that previously reported for New Zealand residents (ranging from 66 to 88 μg/l)[15]. These higher concentrations might be due to greater importation of Australian wheat and other cereal products especially in the North Island of New Zealand, where subjects in the present study resided, as well as due to increased Se concentrations in animal foods[32]. Despite the relatively high baseline Se concentrations, the small amount of Se provided by consuming two portions of salmon/week (6·7 μg Se/d) resulted in a significant increase of almost 10 % in plasma Se after 8 weeks compared with capsule intake. A study by Thorngen & Akesson[33] has shown a similar increase in plasma Se (13 %)

with much greater amounts of Se from fish (herring, salmon or mackerel providing 40–50 μg Se/d for 6 weeks).

Whole-blood GPx, a functional measure of Se status, did not change during the study. This could be explained by plasma Se concentrations at baseline being higher than the levels needed for the full expression of GPx[34].

In conclusion, consumption of similar amounts of LC *n*-3 fatty acids either from salmon or salmon oil was equally effective in increasing erythrocyte LC *n*-3 status. The *n*-3 status can be improved by either method according to consumer preference. However, consuming salmon had the added benefit of increasing Se status and was better tolerated than salmon oil capsule intake.

The model used in the present study could be used in future studies to compare different food matrices, e.g. fortified food *v.* natural sources, and different lipid forms, e.g. phospholipids *v.* TAG *v.* ethyl esters. These studies should include a larger sample size and be of longer duration (4–6 months), but good compliance should be ensured over this period.

Acknowledgements

We thank all the subjects who participated in the present study. We gratefully acknowledge Yan Wang for setting up and helping to analyse the fatty acid content of the salmon, salmon oil capsules and erythrocytes; Dr Karl Bailey and Ms Jody Miller, Department of Human Nutrition, University of Otago for Se and GPx analyses. The present study was funded by the Institute of Food, Nutrition and Human Health, Massey University, the Foundation for Research Science and Technology and the New Zealand King Salmon Company Limited, who supplied the salmon. None of the authors has any conflict of interest. W. S. is the main author of the paper and the project leader, involved in all aspects of the study, including conceptualising and design of the research project, the acquisition of the data and statistical analysis. M. R. P. is an MSc student in the project, involved in planning and conducting of the research project, the analysis and statistical analysis of the data and approving the manuscript. R. K. was involved in the planning of the study, the acquisition of the data, analysis of the data and revising and approving the manuscript. C. D. T. helped in the approval of the study design, analysis and interpretation of the results related to Se, revising and approving the manuscript. M. W. also helped in the approval of the study design, overseeing set-up and analysis of fatty acids in salmon, salmon oil capsules and erythrocytes, revising and approving of the manuscript. M. C. K. was involved in the conceptualisation and design of the research project, revising and approving the manuscript.

References

1. Mozaffarian D & Rimm EB (2006) Fish intake, contaminants, and human health – evaluating the risks and the benefits. *JAMA* **296**, 1885–1899.
2. Kris-Etherton PM, Harris WS, Appel LJ, *et al.* (2003) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arterioscler Thromb Vasc Biol* **23**, E20–E31.
3. Goldberg RJ & Katz J (2007) A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. *Pain* **129**, 210–223.
4. Uauy R & Dangour AD (2006) Nutrition in brain development and aging: role of essential fatty acids. *Nutr Rev* **64**, S24–S33.
5. Poulsen RC, Firth EC, Rogers CW, *et al.* (2007) Specific effects of gamma-linolenic, eicosapentaenoic, and docosahexaenoic ethyl esters on bone post-ovariectomy in rats. *Calcif Tissue Int* **81**, 459–471.
6. Parker G, Gibson NA, Brotchie H, *et al.* (2006) Omega-3 fatty acids and mood disorders. *Am J Psychiatry* **163**, 969–978.
7. Harris WS & von Schacky C (2004) The omega-3 index: a new risk factor for death from coronary heart disease? *Prev Med* **39**, 212–220.
8. Hibbeln JR, Nieminen LRG, Blasbalg TL, *et al.* (2006) Healthy intakes of *n*-3 and *n*-6 fatty acids: estimations considering worldwide diversity. *Am J Clin Nutr* **83**, 1483S–1493S.
9. Gebauer SK, Psota TL, Harris WS, *et al.* (2006) *n*-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am J Clin Nutr* **83**, 1526S–1535S.
10. Arterburn LM, Oken HA, Hall EB, *et al.* (2008) Algal-oil capsules and cooked salmon: nutritionally equivalent sources of docosahexaenoic acid. *J Am Diet Assoc* **108**, 1204–1209.
11. Elvevoll EO, Barstad H, Breimo ES, *et al.* (2006) Enhanced incorporation of *n*-3 fatty acids from fish compared with fish oils. *Lipids* **41**, 1109–1114.
12. Harris WS, Pottala JV, Sands SA, *et al.* (2007) Comparison of the effects of fish and fish-oil capsules on the *n*-3 fatty acid content of blood cells and plasma phospholipids. *Am J Clin Nutr* **86**, 1621–1625.
13. Visioli F, Rise P, Barassi MC, *et al.* (2003) Dietary intake of fish vs. formulations leads to higher plasma concentrations of *n*-3 fatty acids. *Lipids* **38**, 415–418.
14. Mozaffarian D (2009) Fish, mercury, selenium and cardiovascular risk: current evidence and unanswered questions. *Int J Environ Res Public Health* **6**, 1894–1916.
15. Thomson CD (2004) Selenium and iodine intakes and status in New Zealand and Australia. *Br J Nutr* **91**, 661–672.
16. Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, *et al.* (2006) Selenium and coronary heart disease: a meta-analysis. *Am J Clin Nutr* **84**, 762–773.
17. Rayman MP (2005) Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proc Nutr Soc* **64**, 527–542.
18. Rayman MP (2000) The importance of selenium to human health. *Lancet* **356**, 233–241.
19. Russell D, Parnell WR & Wilson N (1999) *NZ Food: NZ People Key Results of the 1997 National Nutrition Survey*. Wellington: Ministry of Health.
20. Larsen D, Quek SY & Eyres L (2010) Effect of cooking method on the fatty acid profile of New Zealand King Salmon (*Oncorhynchus tshawytscha*). *Food Chem* **119**, 785–790.
21. Kolanowski W, Jaworska D & Weissbrodt J (2007) Importance of instrumental and sensory analysis in the assessment of oxidative deterioration of omega-3 long-chain polyunsaturated fatty acid-rich foods. *J Sci Food Agric* **87**, 181–191.
22. Freeman MP & Sinha P (2007) Tolerability of omega-3 fatty acid supplements in perinatal women. *Prostaglandins Leukot Essent Fatty Acids* **77**, 203–208.
23. Stonehouse W, Kruger A, Smuts CM, *et al.* (2010) Plasma polyunsaturated fatty acids and liver enzymes in HIV-infected subjects: the Prospective Urban and Rural Epidemiology (PURE) Study. *Am J Clin Nutr* **91**, 729–735.

British Journal of Nutrition

24. Jacobson BE & Lockitch G (1988) Direct determination of selenium in serum by graphite-furnace atomic absorption spectrometry with deuterium background correction and a reduced palladium modifier: age-specific reference ranges. *Clin Chem* **34**, 709–714.
25. Thomson CD, Robinson MF, Campbell DR, *et al.* (1982) Effect of prolonged supplementation with daily supplements of selenomethionine and sodium selenite on glutathione peroxidase activity in blood of New Zealand residents. *Am J Clin Nutr* **36**, 24–31.
26. Thomson CD, Robinson MF, Butler JA, *et al.* (1993) Long-term supplementation with selenate and selenomethionine: selenium and glutathione peroxidase (*EC* 1.11.1.9) in blood components of New Zealand women. *Br J Nutr* **69**, 577–588.
27. Society AOCs (1993) *Official Methods and Recommended Practice of the American Oil Chemists' Society*, 5th ed. Champaign, IL: AOCS Press.
28. Ministry of Economic Development (2009) *Consolidated List of Approvals Relating to Part II of the Tariff of New Zealand*. Wellington: Ministry of Economic Development.
29. Brown AJ, Pang E & Roberts DC (1991) Persistent changes in the fatty acid composition of erythrocyte membranes after moderate intake of *n*-3 polyunsaturated fatty acids: study design implications. *Am J Clin Nutr* **54**, 668–673.
30. Katan MB, Deslypere JP, van Birgelen A, *et al.* (1997) Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res* **38**, 2012–2022.
31. Cao J, Schwichtenberg KA, Hanson NQ, *et al.* (2006) Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin Chem* **52**, 2265–2272.
32. Thomson CD, McLachlan SK, Parnell WR, *et al.* (2007) Serum selenium concentrations and dietary selenium intake of New Zealand children aged 5–14 years. *Br J Nutr* **97**, 357–364.
33. Thorngren M & Akesson B (1987) Effect of dietary fish on plasma selenium and its relation to haemostatic changes in healthy adults. *Int J Vitam Nutr Res* **57**, 429–435.
34. Thomson CD (2004) Assessment of requirements for selenium and adequacy of selenium status: a review. *Eur J Clin Nutr* **58**, 391–402.

British Journal of Nutrition