

## Bioequivalence of *n*-3 fatty acids from microencapsulated fish oil formulations in human subjects

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### Abstract

Fish oil *n*-3 fatty acids (FA) have known health benefits. Microencapsulation stabilises and protects fish oil from oxidation, enabling its incorporation into foods. The aim of the present study was to compare the bioavailability of *n*-3 FA delivered as two microencapsulated fish oil-formulated powders or fish oil gel capsules (FOGC) taken with a flavoured milk in healthy participants. Formulation 1 (F1) composed of a heated mixture of milk protein–sugar as an encapsulant, and formulation 2 (F2) comprised a heated mixture of milk protein–sugar–resistant starch as an encapsulant. Participants consumed 4 g fish oil (approximately 1.0 g EPA and DHA equivalent per dose). Bioavailability was assessed acutely after ingestion of a single dose by measuring total plasma FA composition over a period of 48 h (*n* 14) using a randomised cross-over design, and over the short term for a period of 4 weeks using an unblinded parallel design (after daily supplementation) by measuring total plasma and erythrocyte FA composition at baseline and at 2 and 4 weeks (*n* 47). In the acute study, F1 greatly increased (% Δ) plasma EPA and total *n*-3 FA levels at 2 and 4 h and DHA levels at 4 h compared with FOGC. The time to reach maximal plasma values ( $T_{\max}$ ) was shorter for F1 than for FOGC or F2. In the short-term study, increases in plasma and erythrocyte *n*-3 FA values were similar for all treatments and achieved an omega-3 index in the range of 5.8–6.3% after 4 weeks. Overall, the results demonstrated human bioequivalence for microencapsulated fish oil powder compared with FOGC.

**Key words:** Bioequivalence; *n*-3 Fatty acids; Fish oil; Microencapsulation

There is growing evidence from epidemiological studies, animal studies and randomised controlled clinical trials in human subjects that long-chain (LC) *n*-3 fatty acids (FA), mainly EPA and DHA, found in oily fish have multiple health benefits<sup>(1)</sup>. They are best known for positive outcomes of cardiovascular health<sup>(2–6)</sup>. There is also accumulating evidence for the role of *n*-3 FA in the prevention or treatment of colorectal cancer<sup>(7)</sup> and inflammatory conditions<sup>(8–10)</sup>, including inflammatory bowel disease<sup>(11–13)</sup>, and associated evidence for positive neurological effects<sup>(14–17)</sup>. Since there is a relatively low intake of *n*-3 FA in the Australian population<sup>(18)</sup>, there have been recommendations to increase the intake of *n*-3 FA through increased consumption of fish or supplements, or functional foods enriched with LC *n*-3 FA, especially to reduce the risk of CVD<sup>(19)</sup>.

Fish oil is unstable and highly susceptible to oxidation<sup>(20)</sup>, leading to the development of undesirable flavours. Therefore, despite the health benefits associated with LC *n*-3 FA, eating fish or taking fish oil capsules is not a desirable

option for everyone. This is possibly due to food preferences of some consumers and undesirable effects of taking fish oil supplements such as fishy aftertaste and gastrointestinal (GI) upset<sup>(21)</sup>. To mask the fishy taste and protect unsaturated FA from oxidation, fish oil may be microencapsulated before addition to food products. This approach has enabled successful incorporation of oils containing unsaturated FA into a range of dairy products<sup>(20)</sup>, yogurt<sup>(22)</sup>, luncheon meat<sup>(23)</sup> and bread<sup>(24)</sup>. Microencapsulated fish oils may have different profiles of lipolysis, release and uptake of FA along the GI tract, and thus influence the bioavailability of *n*-3 FA<sup>(25–28)</sup>. Dietary lipids are normally digested and absorbed rapidly after a meal. The LC FA and monoacylglycerols are packed into chylomicrons, which transport the absorbed TAG and other dietary lipids via the lymphatic system into the blood with peak absorption occurring in the first 2–4 h after ingestion<sup>(24)</sup>.

Previously, we have demonstrated in a rat model that microencapsulated fish oil formulations stabilised by heated protein–carbohydrate encapsulants can deliver fish oil to the

**Abbreviations:** CSIRO, Commonwealth Scientific and Industrial Research Organisation; F1, formulation 1; F2, formulation 2; FA, fatty acids; FAME, fatty acid methyl ester; FOGC, fish oil gel capsule; GI, gastrointestinal; LC, long chain.

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GI tract<sup>(27)</sup>, with *n*-3 FA being bioavailable alone<sup>(29)</sup> or in combination with other bioactive compounds<sup>(30)</sup>. Some human studies have examined the bioequivalence of fish oil, and different emulsion and powdered formulations acutely over a period of 48 h<sup>(28)</sup> and over the short term for a period of 3 weeks<sup>(25)</sup>. Recently, bioavailability of microencapsulated fish oil incorporated into different food matrices such as cereal bar, orange juice or yogurt was assessed in ileostomy patients, revealing that over 99% of *n*-3 FA were absorbed into the GI tract<sup>(31)</sup>. From this study, it has been suggested that bioavailability, absorption and accumulation of *n*-3 FA in the blood needs to be confirmed in healthy subjects<sup>(31)</sup>.

It is important to test the bioavailability of different microencapsulated formulations in the intended food product as both the microencapsulated formulation and the food vehicle may influence bioavailability. We have previously developed fish oil powders, which are shelf stable, by formulating fish oil emulsions stabilised by heated mixtures of proteins and carbohydrates and converting them into powders by spray-drying<sup>(32)</sup>. The aim of the present study was to compare the bioavailability of *n*-3 FA delivered as microencapsulated fish oil powders or fish oil gel capsules (FOGC) taken with a flavoured milk in healthy participants. The microencapsulated fish oil powders were formulated either with heated mixtures of milk protein–sugar (formulation 1 (F1)) or milk protein–sugar–resistant starch (formulation 2 (F2)). We hypothesised that microencapsulated fish oil formulations would be bioequivalent to a FOGC preparation, with regard to the uptake of EPA and DHA into the plasma and erythrocyte membranes. To do this, we investigated the indices of LC *n*-3 FA bioavailability (1) in an acute study for 48 h (with a single dose approximately 1.0 g DHA + EPA) by measuring the accumulation of LC *n*-3 FA in plasma, and (2) in a short-term study (with daily supplementation of approximately 1.0 g DHA + EPA over a period of 4 weeks) by measuring the accumulation of LC *n*-3 FA in plasma and erythrocytes, including the omega-3 index over a period of 4 weeks. Plasma lipid profiles were also assessed.

## Participants and methods

### Participants

According to the power calculation, about twelve participants (per group) were required to have an 80% chance of detecting a 30 and 45% increase in DHA compared with the FOGC (control) ( $\alpha = 0.05$ ) in the acute and short-term studies, respectively. An extra five participants per treatment group (i.e. *n* 17) were included to safeguard against participant non-compliance and other contingencies. Standard deviations for the above variables were sourced from the literature<sup>(26,28)</sup>.

Participants were recruited from an existing database of volunteers in Adelaide, South Australia, who have previously given permission to be contacted again to receive information with regard to other studies. Each participant was screened by responses given to a standard Commonwealth Scientific and Industrial Research Organisation (CSIRO) health questionnaire using inclusion and exclusion criteria. Participants

were recruited from the same pool for the 'acute' and 'short-term' studies.

Inclusion criteria for apparently healthy participants (with no reported current or previous metabolic diseases, GI disorders, renal or CVD) were either male or female between the age of 18 and 65 years with normal dietary habits (with no medically prescribed diet, no slimming diet, and no vegan or macrobiotic diet) and BMI between 18 and 30 kg/m<sup>2</sup>. Exclusion criteria included regular use of supplements containing *n*-3 FA, regular consumption of foods supplemented with *n*-3 FA such as some breads and milk products, consumption of fish as part of regular dietary patterns, high alcohol consumption (>21 standard drinks/week), reported participation in another biomedical trial 3 months before the start of the study or during the study, food allergies or intolerances (such as dairy products, lactose, peanuts and shellfish), and recent history (within 12 months) of substance abuse including alcohol. Age and anthropometric data including height and weight were collected in duplicate by a trained researcher following the International Society for the Advancement of Kinanthropometry protocol and standards, using A & D Weighing HW-PW200 digital electronic scales and a Seca stadiometer. All the participants gave written and informed consent. The Human Research Ethics Committee of CSIRO Food and Nutrition Flagship approved the study (no. 10/20), which included a power calculation and was registered as ACTRN12611000046909.

### Acute study

A randomised, cross-over, non-blinded study design was used for the acute study. A total of fourteen participants were included for this study. There was a 4-week run-in period before the start of taking the treatment dose, followed by a 6-week washout period between consumption of each of the three treatments. Group A consumed four FOGC containing approximately 1049 mg EPA + DHA in total (647 mg EPA and 402 mg DHA) taken with 250 ml flavoured milk. Group B consumed 8 g F1 containing approximately 1013 mg EPA + DHA in total (618 mg EPA and 395 mg DHA) that was supplied as a sachet and mixed in 250 ml flavoured milk by the participants. Group C consumed 8 g F2 containing approximately 1026 mg EPA + DHA in total (629 mg EPA and 397 mg DHA) that was supplied in a sachet and mixed in 250 ml flavoured milk by the participants. The treatment dose was consumed immediately after taking a fasted blood sample. Participants were randomised into the following treatment orders: ABC; BCA; CAB. Blood samples were taken at 0, 2, 4, 6, 24 and 48 h for each of the three treatment groups. Participants were advised to avoid consumption of fish or fish oil products for the entire study duration of 16 weeks, and recorded any deviations from this protocol. A list of foods and supplements to be avoided was also provided to all the participants.

### Short-term study

A randomised, parallel, non-blinded study design was used for the short-term study. A total of forty-seven participants

were randomised to one of the three treatment groups. Participants followed a diet that excluded fish or fish oil-supplemented foods for 8 weeks (i.e. during the 4-week run-in period and 4-week supplementation period), as indicated in the Acute study section. The baseline characteristics of the participants in the short-term study are summarised in Table 2. The three treatment groups were the same as those in the acute study except that a daily dose was taken for 4 weeks. Participants took four FOGC (1049 mg EPA + DHA in total) with 250 ml flavoured milk, and mixed 8 g F1 powder (1013 mg EPA + DHA in total) or F2 powder (1026 mg EPA + DHA) into 250 ml of flavoured milk before consumption. The inclusion/exclusion criteria were the same as those for the acute study, and participants were advised to avoid consumption of fish or fish oil products for the entire study duration of 8 weeks and recorded any deviations. A compliance checklist was developed to monitor the regular consumption and occasions where foods high in *n*-3 FA had been inadvertently consumed.

#### *Production of microencapsulated fish oil powders*

Fish oil with an 18:12 ratio of EPA/DHA was obtained from Catalent Pharma Solutions. Microencapsulated fish oil powders (containing 50% (w/w) fish oil) were manufactured following the procedures described previously<sup>(27)</sup>. Briefly, aqueous mixtures of sodium caseinate–glucose–dried glucose syrup (ratio 1:1:1, F1) or sodium caseinate–glucose–processed resistant starch (ratio 1:1:1, F2) adjusted to pH 7.5 were heated in a retort (100°C, 40 min) and then cooled down to 60°C. Fish oil (preheated to 60°C) was dispersed into the heated protein–carbohydrate encapsulants and homogenised at 35 MPa and 10 MPa in two stages using a homogeniser (Rannie, The Frain Group). The homogenised emulsion was then spray-dried into powders and packed into aluminium foil sachets (8 g powder per sachet) and supplied to all the participants. The same fish oil was used in the manufacture of the microencapsulated fish oil powders and the FOGC.

#### *Measurement of plasma and erythrocyte fatty acids by GC*

Blood was collected into 9 ml Vacutainer tubes containing EDTA, cooled to 4°C and separated into plasma and erythrocytes by centrifugation at 1500 *g* for 10 min. Erythrocytes were washed twice with isotonic saline and reconstituted to the original volume in water to lyse the cells. Aliquots of plasma and lysed erythrocytes were stored at –80°C until analysis.

For the measurement of total plasma FA composition, 50  $\mu$ l 17:0 FA as the internal standard (1.0 mg/ml) in chloroform (as triheptadecanoin, Sigma T-2151), 100  $\mu$ l plasma and 1 ml methanol were added to culture tubes (16 mm  $\times$  125 mm). The tubes were mixed briefly by shaking; 2 ml chloroform (0.005% butylated hydroxytoluene) was added followed by vigorous vortex mixing for 30 s. Then, 0.5 ml of 0.1 M-HCl were added to the tube, vortexed again for 30 s and centrifuged for 10 min at 1000 *g*. The aqueous (top) layer

was removed by aspiration, and the chloroform layer was transferred carefully to a culture tube (20 mm  $\times$  150 mm) and evaporated to dryness with a stream of dry N<sub>2</sub> at 35–40°C. The residue was methylated with 1.5 ml of 1% H<sub>2</sub>SO<sub>4</sub> in dry methanol in sealed tubes at 50°C for 18 h. After cooling to room temperature, 3 ml of pure water and 5 ml petroleum spirit (boiling point 40–60°C) were added and mixed vigorously for 30 s. The top organic phase was transferred to a glass tube (16 mm  $\times$  125 mm). The aqueous phase was extracted again with 5 ml petroleum spirit; the organic phase was combined with the first extract and evaporated to dryness with a stream of dry N<sub>2</sub>. FA methyl esters (FAME) were dissolved in 1.5 ml hexane and applied to solid-phase extraction columns made from glass Pasteur pipettes blocked with glass wool and packed to a height of 20 mm with Florisil (F-7752; Sigma-Aldrich) to remove contaminants. The columns were washed with an additional 1.0 ml hexane and FAME eluted with 2 ml of 10% diethyl ether in hexane into chromatography vials. Solvents were evaporated using a stream of dry N<sub>2</sub>. FAME were dissolved in 50  $\mu$ l isoctane for GC analysis.

Frozen lysed erythrocyte samples were thawed on ice and prepared for analysis by a method based on a published report<sup>(33)</sup>, which uses a one-step extraction and transesterification procedure. Briefly, 0.5 ml of lysed erythrocytes were added to 9.0 ml of cold Tris–EDTA buffer (10 mM-Tris, 1 mM-EDTA, pH 7.4) in a 10 ml polycarbonate ultracentrifuge bottle (Beckman 355603), mixed by inverting the tube gently four times and followed by centrifugation at 50 000 *g* at 5°C for 30 min. The supernatant was carefully removed by aspiration, and the erythrocyte membrane pellet was transferred quantitatively in a total volume of 200  $\mu$ l of water to a screw cap glass tube containing the internal standard (50  $\mu$ g 17:0 FA as triheptadecanoin; Sigma T-2151). Then, 2 ml of methanol–toluene (4:1, v/v) were added, briefly vortex mixed and followed by careful dropwise addition of 200  $\mu$ l of acetyl chloride while vortex mixing the tube to minimise vigorous reaction. The tubes were capped firmly and placed in a heating block at 100°C for 1 h. After cooling, 3 ml of 10% K<sub>2</sub>CO<sub>3</sub> were added and mixed. Toluene (200  $\mu$ l) was added, tubes were capped and shaken vigorously for 60 s. After centrifugation for 5 min at 1000 *g*, the upper toluene phase was transferred to a glass vial and evaporated to dryness under a stream of N<sub>2</sub>. FAME were redissolved in 1 ml hexane and applied to Florisil solid-phase extraction columns as described for the measurements of plasma FA composition before GC analysis.

Plasma and erythrocyte FAME were quantified by GC. A 0.2  $\mu$ l sample was injected onto a bonded-phase vitreous-silica BPX70 column (30 m  $\times$  0.53 mm  $\times$  0.5  $\mu$ m; SGE) in an Agilent 6890N gas chromatograph equipped with a cool on-column injector. The oven temperature programme was initially set at 120–180°C (6°C/min) and increased to 230°C (3°C/min), and the flame ionisation detector was set at 300°C. Peak identification was based on a comparison of retention times with Supelco 37-Component FAME Mix 47885-U (Sigma-Aldrich Pty Limited). Individual FA were calculated as a percentage of the total FA. The omega-3 index is the content of EPA plus DHA, expressed as a percentage of total FA<sup>(34)</sup>.

**Table 1.** Baseline characteristics of the study participants in the acute (0–48 h) study (Mean values with their standard errors; *n* 14)

	Group	
	Mean	SE
Sex ( <i>n</i> )		
Male	6	
Female	8	
Age (years)	58.2	2.5
BMI (kg/m <sup>2</sup> )	27.9	0.9

### Measurement of plasma lipids

Cholesterol, HDL-cholesterol and TAG concentrations in the plasma were measured on a Boehringer Mannheim/Hitachi 902 clinical analyser (Hitachi Science Systems Limited) using appropriate diagnostic kits from Roche Diagnostics Australia. LDL-cholesterol was calculated using the Friedewald equation<sup>(35)</sup>.

### Statistical analyses

Data are presented as means with their standard errors. All statistical analyses were performed using GraphPad Instat (GraphPad Software). The graphs were plotted using GraphPad Prism version 5.04 for Windows (GraphPad Software) and used to calculate the AUC, the time to maximal value ( $T_{max}$ ) and the maximal value ( $C_{max}$ ). The FA data of plasma and erythrocytes and blood lipid parameters of cholesterol, HDL-cholesterol and TAG were found to have no significant differences between standard deviations, as determined by the method of Bartlett, and the data were sampled from populations that followed Gaussian distributions using the Kolmogorov–Smirnov method. The effects of the dietary treatments on FA and lipid parameters were determined using two-way ANOVA. When significance was attained ( $P < 0.05$ ), selected pairwise multi-comparison procedures were adopted using the Bonferroni post-test.

## Results

### Participant compliance

There were no withdrawals or lack of compliance in the acute study. Of the fifty-one participants who commenced the short-term study, four participants withdrew. None of the participants withdrew from the FOGC group, three withdrew from the F1 group due to health problems with only one reporting an aversion to taste and odour of the test products, and one withdrew from the F2 group due to family health issues. The baseline characteristics of the participants in the acute study are summarised in Table 1. There were no significant differences between age and BMI of the treatment groups (Table 2).

### Acute study

**Fatty acid composition of plasma.** In all the treatment groups, the percentage of EPA, DHA and total *n*-3 FA in the plasma rose steadily from 0 to 6 h, with a further increase in

the percentage of EPA up to 24 h. Although the percentage values were marginally decreasing after 24 h, they remained elevated at 48 h with no significant differences between the groups at individual time points (Fig. 1). The difference ( $\Delta$ ) from  $t = 0$  h and the individual time points was also calculated (as a percentage of total FA) for EPA, DHA and total *n*-3 FA for each treatment group (Fig. 2). The percentage of  $\Delta$  values for EPA in the F1 group was found to be significantly higher than that of the FOGC group at 2 h ( $P < 0.001$ ) and 4 h ( $P < 0.01$ ) (Fig. 2(A)). Similarly, for DHA, the percentage of  $\Delta$  values was significantly higher in the F1 group than in the FOGC group at 4 h ( $P < 0.05$ ; Fig. 2(B)). Also, for total *n*-3 FA, the F1 group had higher percentage of  $\Delta$  values at 2 and 4 h ( $P < 0.05$ ) than the FOGC group (Fig. 2(C)). Furthermore, the time to reach maximum value ( $T_{max}$ ) for the total *n*-3 FA was shorter in the F1 group than in the F2 and FOGC groups (Table 3).

### Short-term feeding study

**Fatty acid composition of plasma.** There were no significant changes in the values of individual SFA, individual MUFA, or total SFA and total MUFA across the treatment groups or within the treatment groups across the study duration of 4 weeks (Table 4). There were no significant changes in the values of 18:2*n*-6 (linoleic acid), 20:4*n*-6 (arachidonic acid), total *n*-6 FA or 18:3*n*-3 ( $\alpha$ -linolenic acid) from 0 to 4 weeks for the FOGC, F1 and F2 groups (Table 4). No differences were observed between the treatment groups in relation to the changes in the values of *n*-6 FA. There were no significant differences between the three groups with respect to the increases in the values of EPA, DPA, DHA, total *n*-3 FA and omega-3 index from 0 to 4 weeks ( $P < 0.01$ ).

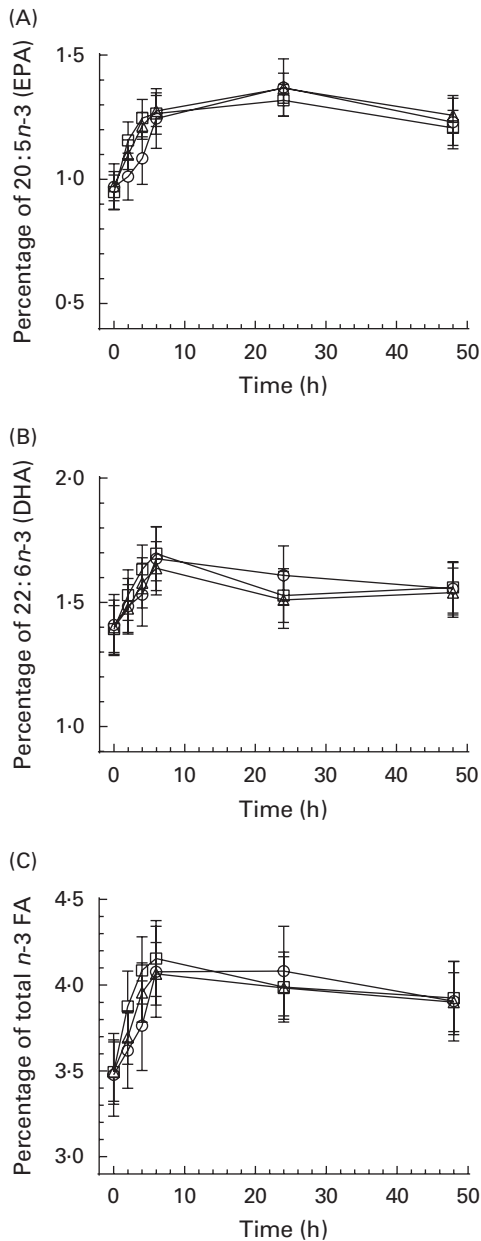
**Fatty acid composition of erythrocytes.** There were no significant changes in the values of individual SFA, individual MUFA or total SFA and total MUFA across the treatment groups or within the treatment groups across the study duration of 4 weeks (results not shown). At 2 and 4 weeks, there were no significant changes in individual *n*-6 FA values in erythrocytes compared with those at 0 weeks, but total *n*-6 FA values had significantly decreased at 4 weeks for all the treatment groups. For EPA, the values increased significantly at 2 and 4 weeks compared at 0 weeks ( $P < 0.05$ ) in all the three

**Table 2.** Baseline characteristics of the study participants in the short-term (4 weeks) study (Mean values with their standard errors)

	FOGC group ( <i>n</i> 17)		F1 group ( <i>n</i> 14)		F2 group ( <i>n</i> 16)		<i>P</i> *
	Mean	SE	Mean	SE	Mean	SE	
Sex ( <i>n</i> )							
Male	5		5		6		
Female	12		9		10		
Age (years)	56.2	2.7	58.3	3.1	56.2	2.1	0.82
BMI (kg/m <sup>2</sup> )	29.0	0.8	28.4	1.0	27.1	1.1	0.36

FOGC, fish oil gel capsule; F1, formulation 1; F2, formulation 2.

\* *P* values were obtained from one-way ANOVA (two-sided) for the determination of differences between the treatment groups.

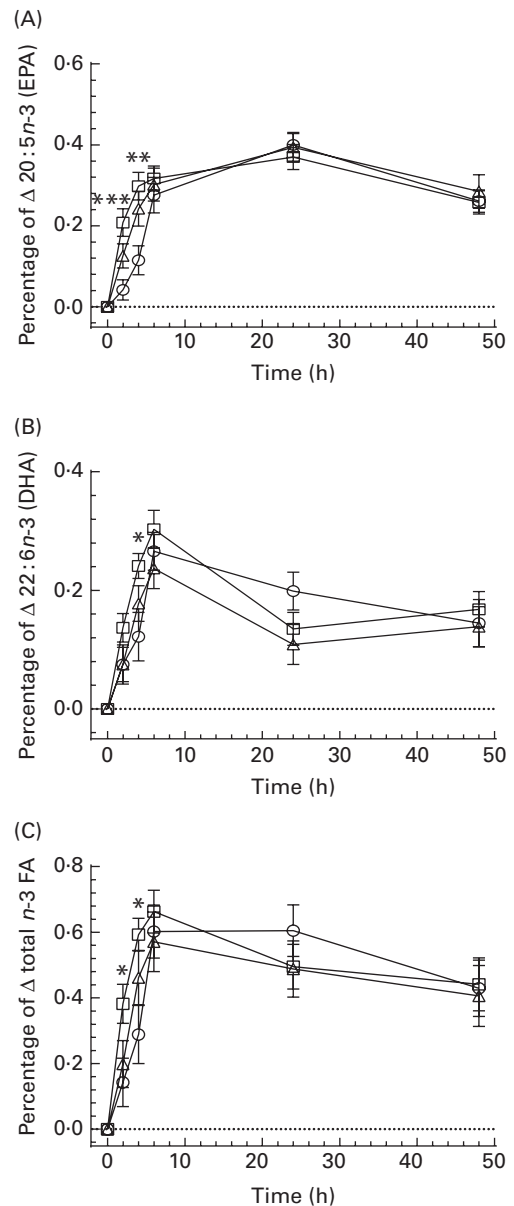


**Fig. 1.** Acute time course from 0 to 48 h of the composition of fatty acids (FA) as a percentage of the total FA pool in the plasma of the study participants ( $n$  15) given at  $t=0$  h a single dose of fish oil gel capsules taken with a flavoured milk ( $\circ$ ), or a flavoured milk containing formulation 1 ( $\square$ ) or formulation 2 ( $\Delta$ ). The FA shown are EPA (A), DHA (B) and total  $n$ -3 FA (C), which includes  $\alpha$ -linolenic acid, EPA, docosapentaenoic acid and DHA (note: the ordinate axis does not start at 0%). Values are means, with their standard errors represented by vertical bars.

groups (Table 5). Total  $n$ -3 FA values significantly increased in the FOGC groups at 2 and 4 weeks, but increased significantly in the F1 and F2 groups at 4 weeks (Table 5). The omega-3 index value increased in the FOGC and F1 groups at 2 and 4 weeks, but for the F2 group, the increase was significant only at 4 weeks (Table 5). When these results were expressed as the change (percentage of  $\Delta$ ) in total  $n$ -3 or  $n$ -6 from baseline for all the treatment groups at 2 and 4 weeks, the  $n$ -3 FA values were increased and  $n$ -6 FA values were decreased

( $P<0.05$ ; Fig. 3(A)). The  $n$ -3: $n$ -6 FA ratio was increased only at 4 weeks ( $P<0.05$ ; Table 5 and Fig. 3(B)).

**Plasma lipids.** Total cholesterol, HDL-cholesterol and LDL-cholesterol levels did not change significantly over the 4 weeks of supplementation for any of the treatment groups (Table 6). However, for the FOGC group, the changes ( $\Delta$ ) in HDL-cholesterol levels were significantly increased compared with the F1 and F2 groups ( $P<0.05$ ) at 4 weeks. There were no significant changes in plasma TAG concentrations between



**Fig. 2.** Acute time course showing the change ( $\Delta$ ) from baseline  $t=0$  h to  $t=2, 4, 6, 24$  and  $48$  h in the composition of fatty acids (FA) as the percentage of the total FA pool in the plasma. Participants ( $n$  15) were given at  $t=0$  h a single dose of fish oil gel capsules taken with a flavoured milk ( $\circ$ ), or a flavoured milk containing formulation 1 ( $\square$ ) or formulation 2 ( $\Delta$ ). The FA shown are EPA (A), DHA (B) and total  $n$ -3 FA (C), which include  $\alpha$ -linolenic acid, EPA, docosapentaenoic acid and DHA. Values are means, with their standard errors represented by vertical bars. Mean value for the formulation 1-treated group was significantly different from that of the fish oil gel capsule-treated group: \*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$  (ANOVA with Bonferroni post-test).



**Table 3.** Net changes in acute (0–48 h) plasma fatty acid (FA) composition as AUC (arbitrary units), time (h) to maximal value ( $T_{max}$ ) and maximal value (%) ( $C_{max}$ ) in the study participants ( $n$  14) administered a single dose of fish oil gel capsules (FOGC) taken with a flavoured milk or a flavoured milk containing formulation 1 (F1) or formulation 2 (F2)

(Mean values with their standard errors)

FA	Value	FOGC		F1		F2		$P^*$
		Mean	SE	Mean	SE	Mean	SE	
EPA (20:5 $n$ -3)	AUC	14.6	1.4	15.0	1.1	15.5	1.5	0.89
	$T_{max}$	22.8	1.2	21.9	2.7	24.7	3.0	0.71
	$C_{max}$	0.40	0.03	0.42	0.03	0.44	0.04	0.70
DHA (22:6 $n$ -3)	AUC	8.98	1.34	8.65	1.08	6.85	1.24	0.42
	$T_{max}$	17.6	3.9	9.7	3.0	10.9	3.9	0.26
	$C_{max}$	0.29	0.03	0.32	0.03	0.26	0.03	0.38
Total $n$ -3 FA†	AUC	24.8	3.3	24.2	2.4	22.2	3.5	0.83
	$T_{max}$	21.6 <sup>a</sup>	4.1	8.5 <sup>b</sup>	2.1	18.8 <sup>a</sup>	4.4	0.04
	$C_{max}$	0.71	0.08	0.71	0.06	0.69	0.08	0.98

<sup>a,b</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\*  $P$  values were obtained from ANOVA with Bonferroni post-test on selected groups.

† Total  $n$ -3 FA includes  $\alpha$ -linolenic acid, EPA, docosapentaenoic acid and DHA.

the treatment groups. The TAG:HDL-cholesterol ratio did not change significantly at 2 or 4 weeks (Table 6).

### Discussion

The recent challenge to the food industry has been to introduce LC  $n$ -3 FA into functional foods where oxidation and rancidity is avoided and fishy taste and aroma are masked<sup>(36)</sup>. Microencapsulation technology using heated protein and carbohydrates was developed in our laboratory to stabilise oxygen-sensitive oils and mask the undesirable taste of fish oil and other bioactive compounds<sup>(27,29,30)</sup>. Microencapsulated fish oil formulations were used in the present study to

facilitate the delivery of  $n$ -3 oils into food and beverage products. The present study measured the acute absorption kinetics and short-term bioavailability (steady state) of two microencapsulated fish oil formulations in healthy human subjects. In the acute study, the F2 group was expected to be better protected from gastric and intestinal fluid and digestive enzymes due to the presence of high-amylose resistant starch in the encapsulant, and hence delayed release as observed in a previous animal study<sup>(27)</sup>. However, the delayed release of  $n$ -3 FA from the F2 powder (formulated with pre-processed resistant starch) was not observed in the acute study, which was similar to the results obtained in an earlier animal study<sup>(29)</sup>. Resistant starch has been found to delay

**Table 4.** Plasma fatty acid (FA) composition of the study participants administered daily with fish oil gel capsules (FOGC) taken with a flavoured milk, or formulation 1 (F1) or formulation 2 (F2) supplemented with a flavoured milk in the short-term study for 4 weeks

(Mean values with their standard errors)

	Time (weeks)												$P^*$
	0						4						
	FOGC		F1		F2		FOGC		F1		F2		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Total SFA†	30.22	0.35	30.31	0.49	30.31	0.58	30.71	0.39	30.44	0.68	29.85	0.27	0.99
Total MUFA†	27.63	0.74	28.56	0.70	27.17	0.76	26.78	0.55	26.66	0.81	26.69	0.59	0.39
18:2 $n$ -6 LA†	27.50	0.78	26.82	0.93	27.76	0.79	25.72	0.77	26.55	1.13	26.45	0.66	0.53
20:3 $n$ -6	1.84 <sup>a</sup>	0.11	1.87	0.08	1.92 <sup>a</sup>	0.08	1.53 <sup>b</sup>	0.08	1.55	0.10	1.57 <sup>b</sup>	0.07	0.0017
20:4 $n$ -6 AA	7.40	0.39	6.95	0.43	7.22	0.30	6.68	0.36	6.33	0.32	7.11	0.27	0.31
Total $n$ -6	37.36	0.95	36.25	1.00	37.51	0.83	34.50	0.80	34.91	1.13	35.61	0.59	0.08
$\Delta$ † $n$ -6	–	–	–	–	–	–	–2.86	0.81	–1.34	0.58	–1.90	0.73	0.34
18:3 $n$ -3 ALA	0.63	0.04	0.69	0.06	0.62	0.06	0.61	0.03	0.73	0.03	0.57	0.04	0.17
20:5 $n$ -3 EPA	1.27 <sup>a</sup>	0.09	1.26 <sup>a</sup>	0.05	1.22 <sup>a</sup>	0.08	3.20 <sup>b</sup>	0.15	3.17 <sup>b</sup>	0.19	3.00 <sup>b</sup>	0.14	0.0001
22:5 $n$ -3 DPA	0.64 <sup>a</sup>	0.02	0.73 <sup>a</sup>	0.04	0.62 <sup>a</sup>	0.02	0.91 <sup>b</sup>	0.03	0.91 <sup>b</sup>	0.04	0.82 <sup>b</sup>	0.02	0.0001
22:6 $n$ -3 DHA	2.26 <sup>a</sup>	0.08	2.21 <sup>a</sup>	0.11	2.55 <sup>a</sup>	0.12	3.28 <sup>b</sup>	0.09	3.19 <sup>b</sup>	0.11	3.57 <sup>b</sup>	0.13	0.0001
Total $n$ -3	4.80 <sup>a</sup>	0.16	4.88 <sup>a</sup>	0.12	5.01 <sup>a</sup>	0.18	8.01 <sup>b</sup>	0.24	7.99 <sup>b</sup>	0.29	7.95 <sup>b</sup>	0.28	0.0001
$\Delta$ † $n$ -3	–	–	–	–	–	–	3.21	0.19	3.11	0.26	2.94	0.21	0.67
$n$ -3: $n$ -6 FA ratio	0.13 <sup>a</sup>	0.01	0.14 <sup>a</sup>	0.01	0.13 <sup>a</sup>	0.01	0.23 <sup>b</sup>	0.01	0.23 <sup>b</sup>	0.01	0.22 <sup>b</sup>	0.01	0.0001

LA, linoleic acid; AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DPA, docosapentaenoic acid.

<sup>a,b</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\*  $P$  values were obtained from ANOVA with Bonferroni post-test on selected groups.

† FA are expressed as a percentage of the total FA pool, for fourteen to seventeen individuals per group (see Table 2).

‡  $\Delta$  values represent the change from  $t=0$  weeks to  $t=4$  weeks. The  $n$ -3: $n$ -6 FA ratio is the total percentage of  $n$ -3 FA divided by the total percentage of  $n$ -6 FA.

**Table 5.** Erythrocyte membrane fatty acid (FA) composition of the study participants administered daily with fish oil gel capsules (FOGC) taken with a flavoured milk, or formulation 1 (F1) or formulation 2 (F2) in the short-term study for 2 and 4 weeks (Mean values with their standard errors)

	Time (weeks)																		<i>P</i> *
	0						2						4						
	FOGC		F1		F2		FOGC		F1		F2		FOGC		F1		F2		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
18:2 <i>n</i> -6 LA†	8.07	0.22	8.23	0.24	8.02	0.17	7.53	0.18	7.67	0.24	7.58	0.18	7.54	0.20	7.78	0.20	7.47	0.18	0.0686
20:3 <i>n</i> -6	1.59	0.09	1.65	0.07	1.66	0.11	1.49	0.09	1.52	0.07	1.51	0.10	1.41	0.08	1.49	0.07	1.51	0.09	0.5516
20:4 <i>n</i> -6 AA	14.73	0.26	14.19	0.29	14.32	0.25	14.48	0.25	14.06	0.24	14.10	0.30	13.90	0.20	13.54	0.24	13.90	0.25	0.0644
Total <i>n</i> -6	24.38 <sup>a</sup>	0.25	24.08 <sup>a</sup>	0.34	24.00 <sup>a</sup>	0.20	23.49	0.23	23.25	0.23	23.2	0.28	22.85 <sup>b</sup>	0.24	22.80 <sup>b</sup>	0.21	22.88 <sup>b</sup>	0.28	0.0001
Δ <i>n</i> -6‡							-0.89	0.14	-0.82	0.15	-0.77	0.29	-1.53	0.15	-1.27	0.18	-1.12	0.15	0.0283
18:3 <i>n</i> -3 ALA	0.03	0.01	0.05	0.02	0.05	0.01	0.03	0.01	0.05	0.02	0.04	0.01	0.03	0.01	0.04	0.01	0.05	0.02	0.0877
20:5 <i>n</i> -3 EPA	0.71 <sup>a</sup>	0.04	0.84 <sup>a</sup>	0.07	0.81 <sup>a</sup>	0.03	1.32 <sup>b</sup>	0.05	1.40 <sup>b</sup>	0.07	1.29 <sup>b</sup>	0.04	1.55 <sup>b</sup>	0.06	1.58 <sup>b</sup>	0.06	1.51 <sup>b</sup>	0.04	0.0001
22:5 <i>n</i> -3 DPA	1.98	0.09	2.18	0.08	2.04	0.07	2.16	0.10	2.30	0.09	2.11	0.09	2.21	0.10	2.33	0.09	2.25	0.05	0.0866
22:6 <i>n</i> -3 DHA	3.81 <sup>a</sup>	0.20	3.83 <sup>a</sup>	0.26	4.33	0.14	4.19	0.14	4.22	0.33	4.52	0.17	4.29	0.16	4.25	0.26	4.83 <sup>b</sup>	0.14	0.0163
Total <i>n</i> -3 FA	6.54 <sup>a</sup>	0.20	6.90 <sup>a</sup>	0.34	7.23 <sup>a</sup>	0.20	7.70 <sup>b</sup>	0.24	7.97 <sup>a,b</sup>	0.44	7.96 <sup>a,b</sup>	0.26	8.07 <sup>b</sup>	0.28	8.19 <sup>b,c</sup>	0.35	8.63 <sup>b,c</sup>	0.20	0.0001
Δ <i>n</i> -3‡							1.16	0.15	1.06	0.19	0.76	0.13	1.53	0.18	1.29	0.19	1.40	0.18	0.0281
Omega-3 index§	4.52 <sup>a</sup>	0.13	4.67 <sup>a</sup>	0.31	5.14 <sup>a</sup>	0.15	5.50 <sup>b</sup>	0.16	5.62 <sup>b</sup>	0.39	5.81 <sup>a,b</sup>	0.19	5.84 <sup>b</sup>	0.19	5.82 <sup>b</sup>	0.30	6.34 <sup>b</sup>	0.16	0.0001
<i>n</i> -3: <i>n</i> -6 FA ratio	0.19 <sup>a</sup>	0.01	0.20 <sup>a</sup>	0.02	0.21 <sup>a</sup>	0.01	0.23 <sup>a,b</sup>	0.01	0.24 <sup>a,b</sup>	0.02	0.25 <sup>a,b</sup>	0.01	0.26 <sup>b</sup>	0.01	0.26 <sup>b</sup>	0.01	0.28 <sup>b</sup>	0.01	0.0001

LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; DPA, docosapentaenoic acid.  
<sup>a,b</sup> Mean values within a row with unlike superscript letters were significantly different (*P*<0.05).  
 \* *P* values were obtained from ANOVA with Bonferroni post-test on selected groups.  
 † FA are expressed as a percentage of the total FA pool, for fourteen to seventeen individuals per group (see Table 2).  
 ‡ Δ values represent the change from *t*=0 weeks to *t*=2 and 4 weeks.  
 § Omega-3 index is EPA + DHA, expressed as a percentage of total FA.





and the calculated omega-3 index ranged from 4.5 to 5.1% at baseline and increased to 5.8–6.3% after 4 weeks. In the present study, daily supplementation with fish oil powder containing at least 1.0 g EPA + DHA did not raise the omega-3 index to 8%, as recommended for potential cardiovascular protection<sup>(34,39)</sup>, but was in the intermediate range between 4 and 8%<sup>(39)</sup>. In a recent trial, participants were supplemented with 1.7 g EPA + DHA/d as an emulsified preparation (Coromega, Inc.) with the erythrocyte omega-3 index value increasing from 3.2 to 5.2% within a period of 4 weeks<sup>(40)</sup>. In another earlier trial, the omega-3 index value increased from 4.3 to 7.8% within a period of 8 weeks in participants who were administered soft gel fish oil capsules containing a total of 2.16 g EPA + DHA/d<sup>(41)</sup>. These results, and studies by others<sup>(36)</sup>, have suggested that to achieve optimal erythrocyte omega-3 index values of about 8%, supplementation above 2 g EPA + DHA/d for at least 8 weeks is required in one form or the other. It was concluded that approximately 1 g/d intake of LC *n*-3 FA may result in an approximate increase of 2% in the omega-3 index value over an 8-week period<sup>(36)</sup>. This is the dose amount also proposed to elicit anti-inflammatory actions, but few dose-finding studies in human subjects have been performed<sup>(8)</sup>.

At a dose of at least 1.0 g EPA + DHA/d in the present study, there was a trend towards lower TAG levels over a period of 4 weeks for all the three treatment groups. This is not surprising as recent evidence suggests that the TAG-lowering effect of LC *n*-3 FA is best seen at a concentration above the normal range of 1.7 mmol/l<sup>(42)</sup>. However, the  $\Delta$  HDL-cholesterol values decreased significantly for the F2 group compared with the FOGC group over a period of 4 weeks. The calculated TAG:HDL-cholesterol ratio, which is also used as a risk factor for CVD<sup>(43)</sup>, with an increased ratio recognised as increased risk, did not change. In a human study where 1.7 g EPA + DHA/d was ingested for 4 weeks, there was no reported change in TAG levels<sup>(40)</sup>. In an earlier study, a cohort of human subjects who ingested 5.9 g micro-encapsulated (multi-core microcapsules as a gelatine and polyphosphate by complex coacervation) fish oil powder containing 2.0 g EPA + DHA, plasma TAG levels declined significantly from 2.68 to 1.86 mmol/l with a concomitant drop in the TAG:HDL-cholesterol ratio<sup>(25)</sup>. Again, this suggests that the amount of *n*-3 FA used in the present study may not have been at the threshold required to significantly lower plasma TAG levels in healthy volunteers. However, for participants with hyperlipidaemia, a meta-analysis showed that fish oil supplementation produced a clinically significant dose-dependent reduction in fasting blood TAG levels, but not total, HDL- or LDL-cholesterol levels<sup>(43)</sup>.

In agreement with our hypothesis, we conclude that F1 and F2 demonstrated equivalence with regard to bioavailability and uptake of LC *n*-3 FA into plasma for 48 h (acute study) and plasma and erythrocyte membranes over a period of 4 weeks (short-term study) compared with FOGC in human subjects, and raised the omega-3 index value into the intermediate range for all the treatments.

The model used in the present study could be used to compare different food matrices with those presented here

using different types and forms of lipid. It is suggested that a higher dose of EPA + DHA (>2 g/d) could be used over longer durations of up to 16 weeks with good compliance encouraged over this period.

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