European Journal of Clinical Nutrition (2014), 1–5

www.nature.com/ejcn

ORIGINAL ARTICLE

Bioavailability of long-chain n-3 fatty acids from enriched meals and from microencapsulated powder

HH Hinriksdottir[1], VL Jonsdottir[2], K Sveinsdottir[2], E Martinsdottir[2] and A Ramel[1]

BACKGROUND: Despite the potential benefits of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs), intake is often low because of low consumption of oily seafood. Microencapsulated fish oil powder can improve tolerance and acceptance of LC n-3 PUFAs. Bioavailability is important to achieve efficacy. We investigated the bioavailability of LC n-3 PUFAs from microencapsulated powder in comparison with meals enriched with liquid fish oil.

METHODS: Participants ($N = 99$, age $\geqslant 50$ years) of this 4-week double-blinded dietary intervention were randomized into three groups. Group 1 ($n = 38$) received 1.5 g/d eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as ready-to-eat meals enriched with liquid fish oil; group 2 ($n = 30$) received the same amount of these LC n-3 PUFAs as microencapsulated fish oil powder and regular meals; and group 3 ($n = 31$) was the control group, which received placebo powder and regular meals. Blood samples were taken from fingertips at baseline and at the end point.

RESULTS: Seventy-seven subjects (77.8%) completed the study. The amount of EPA in blood doubled in both groups that received LC n-3 PUFAs ($P < 0.05$), but it did not change in the control group. The changes in DHA were less but still significant in both intervention groups. According to multivariate analysis, both intervention groups had higher end-point LC n-3 PUFA concentrations compared with placebo, but differences between intervention groups were not significant.

CONCLUSION: Bioavailability of LC n-3 PUFAs in encapsulated powder is very similar to the bioavailability of LC n-3 PUFAs in ready-to-eat meals enriched with liquid fish oil. Thus, encapsulated powder can be considered useful to increase LC n-3 PUFA concentrations in blood.

European Journal of Clinical Nutrition advance online publication, 19 November 2014; doi:10.1038/ejcn.2014.250

INTRODUCTION

Studies have shown potential benefits of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs) against various diseases,[1] for example, cardiovascular disease, in which eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can reduce the cardiovascular risk in persons with high-risk factors.[1] The therapeutic value of LC n-3 PUFAs in the treatment of inflammatory diseases has also been noticed with a particular focus on rheumatoid arthritis.[2,3] Studies have also suggested that supplementation of LC n-3 PUFAs in parenteral nutrition is safe and may, for instance, improve immune and hyper-inflammatory responses in surgical intensive care unit patients after surgery.[4]

Oily seafood is the main dietary source of LC n-3 PUFAs. The Nordic Nutrition Recommendations recommend that LC n-3 PUFAs should at least contribute 1% of total energy intake (E%) for adults and children from 2 years of age.[5] Despite recommendations and awareness of the benefits from seafood consumption, food habits have changed over the years and few people meet the recommendations of consumption, which is two fish meals per week with a focus on oily fish.[6–8] It should be mentioned here that α-linolenic acid frequently found in seeds and plant oils is another potential source of EPA and DHA; however, the conversion rate (especially to DHA) is considered to be very low.[9]

Considering the low consumption of seafood products in large parts of the population, attempts have been made to fortify food accordingly or to produce dietary supplements with LC n-3 PUFAs from marine sources. It has been shown that enriched foods with LC n-3 PUFAs increase plasma phospholipid DHA and EPA.[10,11] However, it can be problematic to fortify foods with LC n-3 PUFAs from marine sources, because they have a strong odor and taste that can be hard to hide. Therefore, it is a challenge to find the right amount of LC n-3 PUFAs for fortification to get both the health benefits and still a satisfactory flavor.

As an alternative, flavor-neutral microencapsulated oil rich in marine LC n-3 PUFAs in powder form has been suggested for fortification of foods. Microencapsulation of a liquid is a process in which small droplets are coated to give small units with useful properties. In this context, it is important to consider bioavailability, because bioavailability of LC n-3 PUFAs has been reported to depend on various factors,[12] and because greater bioavailability may enhance the efficacy (for example, reduction of circulating triglycerides) particularly at lower dosages.[13] Microencapsulation has the potential to increase bioavailability; because of the small droplet size, digestion and absorption might be increased. Most likely owing to the uncommon use of fish oil in Western countries, only few studies have been conducted in this area. Some of them indicated comparable bioavailability of, for example, microencapsulated fish oil and fish oil gelatin.[14,15] However, to the best of our knowledge, there have not been any human studies comparing the bioavailability of LC n-3 PUFAs in powder form (microencapsulated fish oil) with meals enriched with liquid fish oil.

Given these considerations, the current randomized, double-blinded, dietary intervention study investigated the bioavailability

[1]Unit for Nutrition Research, National University Hospital & Faculty of Food Science and Nutrition, University of Iceland, Reykjavik, Iceland and [2]The Icelandic Food and Biotech R&D Institute, Reykjavik, Iceland. Correspondence: Dr A Ramel, Landspitali-University Hospital & Faculty of Food Science and Nutrition, University of Iceland, Unit for Nutrition Research, Eiriksgata 29, Reykjavik, 101, Iceland.
E-mail: alfonsra@hi.is
Received 4 April 2014; revised 10 September 2014; accepted 12 October 2014

of LC n-3 PUFAs either consumed as microencapsulated fish oil powder or consumed in meals enriched with liquid fish oil over a 4-week period.

MATERIALS AND METHODS

Subjects

All participants ($N = 99$) from the capital area of Iceland were recruited through advertisements on the Internet, through e-mail lists at the University of Iceland and through advertisements published in regional health-care facilities. The study was conducted from May until October 2013. Inclusion criteria were as follows: age 50 years or more and regular consumption of fish or fish meals (defined as at least once a week according to the information given by the participants). The only exclusion criterion was a previous record of digestive disease, which could interfere with the digestion or absorption of dietary fat. The study was approved by the National Bioethics Committee (VSNb201302008/03.07) and was notified by the Data Protection Authority (S6241/2013). All persons gave their informed consent before their inclusion in the study.

Study design

This was a 4-week randomized, placebo-controlled, double-blinded dietary intervention study. Bioavailability was investigated by the increase of LC n-3 PUFAs in whole blood. Subjects were randomized into three groups: group 1 ($n = 38$) received 6 meals/week fortified with a liquid oil blend (see below) providing 1.75 g of EPA and DHA daily and 6 sachets of placebo powder; group 2 ($n = 30$) received 6 conventional meals/week and 6 sachets of microencapsulated powder (22.7 g) providing 1.75 g of EPA and DHA daily (see below); and group 3 ($n = 31$) was the control group, which received conventional meals and placebo powder. Calculated on a weekly basis, the amount of EPA+DHA provided was 1.5 g/day ($1.75 \times 6 / 7$).

In the current study, we used fortified meals instead of liquid fish oil. The reason therefore was twofold: this made blinding easier and we hoped for better tolerance. The meals were fortified with fish oil and olive oil blend provided by BioActive Foods AS, Trondheim, Norway (www.1life63.com). The microencapsulated LC n-3 powder (particle size 30–50 μm) was also from BioActive Foods in Norway and is based on the same oil blend (see Table 1). The participants received 6 powder sachets each week and were given written instructions on how to use the powder. The meals were produced by Grimur Kokkur ehf, Vestmanneyjar, Iceland (www.grimurkokkur.is/en). All the dishes were kept frozen until cooking or heating. The nutrient profile of each meal is given in Table 2. The fat content of the meals was between 5.3 and 11.1% of total weight (including water). Enriched meals always contained more fat than conventional meals, with a mean difference of 3.3% of total weight. LC n-3 PUFAs partly replaced other fat normally used in the recipes and/or were added. The conventional meals did not provide any noteworthy amount of LC n-3 PUFAs (0.12 g/day). Protein powder with light vanilla flavor was used as placebo powder in groups 1 and 3; unfortified meals were used in groups 2 and 3. Subjects were told to exclude all LC n-3 PUFAs from their diet at least for 2 weeks before the intervention and also while the intervention lasted. Compliance was assessed by a questionnaire each week when the participants received the meals and powder for the following week.

All measurements were conducted at baseline and at the end point of the study.

Anthropometric measurements

Body weight was measured in light clothing on a calibrated scale (model no. 708, Seca, Hamburg, Germany). Height was measured and body mass index was calculated from the recorded height and weight (kg/m^2). For the measurement of waist circumference, a flexible tape was applied horizontally midway between the lowest rib margin and the iliac crest. Body fat percentage was estimated using a hand-held bioimpedance measurement device (Body Fat Monitor BF 306, Omron Healthcare UK Ltd, Milton Keynes, United Kingdom).

Blood parameters

Fasting blood samples from the fingertips were collected using a home test kit and sent to Norway for analysis of fatty acids (FAs), based on the methods published by Saga *et al.*[16] The contents of the home test kit were as follows: user manual (Grako AS, Oslo, Norway), an absorption paper for the blood spot samples (Grako AS), a disposable automatic lancing device (Med-Kjemi AS, Asker, Norway), aluminum bag (Whatman, Roskilde, Denmark) for preservation and storage of the test paper and an enclosed envelope (Grako AS) addressed to St Olav's Hospital, Trondheim University Hospital, Norway, where the analyses were conducted.

Table 1. Composition of the microencapsulated LC n-3 PUFA powder and of the liquid oil

	Content in 100 g powder	*Content in 100 g oil*
Energy (kcal)	630	
Protein (g)	10	
Carbohydrates (g)	34	
Sugars (g)	15	
Ash (g)	2.5	
Moisture (g)	2.5	
Fat (g)	51	
SFA (g)	15.4	30.2
MUFA (g)	22.9	44.9
OA (g)	18.3	35.9
PUFAs (g)	12.1	23.7
n-6 Fatty acids(g)	1.70	3.33
LA (g)	1.34	2.62
GLA (g)	0.07	0.13
AA (g)	0.32	0.63
n-3 Fatty acids (g)	9.40	18.43
ALA (g)	0.34	0.66
ETA(g)	0.21	0.41
EPA (g)	5.30	10.39
DPA (g)	0.56	1.10
DHA (g)	2.40	4.71

Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ETA, eicosatetraenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; LA, linoleic acid; OA, oleic acid. LC n-3 powder group consumed 22.7 g of powder six times a week corresponding to 1.5 EPA and DHA a day. Enriched meal group consumed 11.5 g of oil six times a week corresponding to 1.5 EPA and DHA a day.

The blood spot samples were stored at 4 °C after arrival at the hospital laboratory, and they were then prepared and analyzed within a week. The transesterification and extraction procedure was based on the method published by Marangoni *et al.*[17] with slight modifications. Briefly, the pieces of absorbent paper containing the blood samples were transferred to screw-capped glass vials and treated with 1ml of 0.5 M HCl in MeOH. Samples were then stored at 70 °C in a dry bath for 1 h to achieve transesterification of FAs to FA methyl esters. After cooling, 1 ml of H2O and 1ml of saturated KCl were added, before FAs were extracted using 2 ml of hexane. The solvent was then evaporated by N_2 and the samples were re-dissolved in 50 ml of hexane. Resultant FA methyl esters were analyzed on a gas chromatography mass spectrometry system (HP Agilent 6890GC unit; HP Agilent 5973MS detector; Agilent Technologies, Santa Clara, CA, USA). The GC unit was equipped with a 15-m capillary column (Supelco Omegawax 100; Sigma-Aldrich, St Louis, MO, USA). Samples were injected with a split ratio of 100:1, and the column was heated from 150 to 260 °C with a rate of 40 °C/min, followed by 15 min of isothermal conditions at 260 °C. Pure reference compounds were used as standards, and all chemicals and gases were of analytical grade. Heptadecanoic acid and tricosanoic acid were used as internal standards.[16]

Statistical analyses

The data were entered into the SPSS statistical package, version 21.0 (SPSS, Chicago, IL, USA), and checked for normality using the Kolmogorov–Smirnov test. Data are presented as means ± s.d.. The paired-samples *T*-test (normally distributed) and Wilcoxon Signed Rank test (not normally distributed) were used to assess whether the content of FAs measured in fingertip blood changed during the course of the intervention. Differences between groups in end-point FA concentrations were calculated using a general linear model including 'group' as factor and the baseline concentration of the relevant FA as covariate. The significance level was set at $P \leqslant 0.05$.

Table 2. Nutrient profile of the test meals

Sample	*Protein (g)*	*Fat (g)*	*EPA+DHA (% of total fat)*	*EPA+DHA (g)*	*CHO (g)* [a]	*Energy (kcal)* [b]
Fish in white sauce (conventional)	13.0	12.4	2.5	0.3	22.4	253.2
Fish in white sauce (enriched)	12.4	20.4	10.4	2.1	18.8	308.4
Gratinated haddock with broccoli (conventional)	12.6	8.6	1.0	0.1	20	207.8
Gratinated haddock with broccoli (enriched)	11.8	10.6	8.5	0.9	22	230.6
Haddock in lobster sauce (conventional)	21.4	10.6	1.9	0.2	14	237.0
Haddock in lobster sauce (enriched)	21.8	18.4	8.9	1.6	11.2	297.6
Haddock in curry sauce (conventional)	23.4	14.6	1.5	0.2	14.2	281.8
Haddock in curry sauce (enriched)	19.8	22.2	9.1	2.0	14	335.0
Fish cakes (conventional)	21.8	9.6	1.3	0.1	30	293.6
Fish cakes (enriched)	19.4	22.0	10.9	2.4	27.2	384.4
Vegetable cakes (conventional)	7.0	17.0	0.0	0.0	48.4	374.6
Vegetable cakes (enriched)	6.6	18.4	7.5	1.4	49.2	388.8

Abbreviations: CHO, carbohydrates; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. [a]Calculated as 100 - water - protein - fat - ash. [b]Calculated as fat × 9 + protein × 4 + carbohydrates × 4.

Table 3. Baseline characteristics of the participants

	Enriched meal group (n = 38)	*LC n-3 powder group (n = 30)*	*Control group (n = 31)*
Gender female/male	17/10	16/9	19/6
Age (years)	57 ± 6	56 ± 6	55 ± 4
Weight (kg)	82.3 ± 16.3	84.8 ± 16.3	78.6 ± 21.3
BMI (kg/m^2)	28.5 ± 5.2	29.6 ± 6.0	27.0 ± 5.8
Waist circumf. (cm)	98.1 ± 14.1	103.0 ± 16.3	94.5 ± 18.2
Body fat (%)	33.7 ± 8.3	34.3 ± 8.6	33.0 ± 7.3
Blood glucose (mmol/l)	6.0 ± 1.7	5.9 ± 1.5	5.7 ± 0.9

Data are shown as means ± s.d. Abbreviations: BMI, body mass index; LC n-3, long-chain n-3.

RESULTS

Seventy-seven subjects completed the study (77.8%). Dropout rates were 28.9%, 16.7% and 19.4% for group 1, group 2 and group 3, respectively (not significantly different). The most common reason for dropout was lack of time or lack of interest.

Baseline characteristics of the participants are shown in Table 3. More than two-thirds of the participants were women. Results from the fingertip blood mesurements are shown in Table 4.

General linear models showed end-point differences between both intervention groups compared with placebo as follows: eicosatetraenoic acid (enriched meal group: − 0.098%, $P = 0.048$; LC n-3 powder group: − 0.213%, $P < 0.001$); EPA (enriched meal group: 0.758%; LC n-3 powder group: 0.937%; both $P < 0.001$); docosapentaenoic acid (enriched meal group: 0.166%, $P = 0.016$; LC n-3 powder group: 0.260%, $P < 0.001$); and DHA (enriched meal group: 0.393%, $P = 0.015$; LC n-3 powder group: 0.424%, $P = 0.009$). Differences in other FAs or differences between intervention groups were not significant.

The significant changes in both LC n-3 groups and no changes in the control group indicate good compliance to the study protocol. No significant difference was observed between women and men (not shown in table). According to the questionnaires, more than 97% of the meals were eaten during the intervention.

DISCUSSION

The present study investigated the bioavailability of LC n-3 PUFAs in microencapsulated fish oil powder and in ready-to-eat meals enriched with liquid fish oil. The results show that after 4 weeks of

Table 4. Whole-blood fatty acid measurements (% of total fatty acids) from fingertip test (completers only)

	Enriched meal group Mean ± s.d.	*LC n-3 powder group Mean ± s.d.*	*Control group Mean ± s.d.*
LA			
t0	19.21 ± 2.94	20.24 ± 2.05	20.03 ± 3.36
t1	19.50 ± 2.95	20.24 ± 2.13	20.88 ± 3.08
GLA			
t0	0.23 ± 0.11	0.22 ± 0.10	0.23 ± 0.09
t1	0.28 ± 0.18	0.26 ± 0.22	0.26 ± 0.18
AA			
t0	6.65 ± 1.62	7.79 ± 1.16	7.25 ± 0.99
t1	7.36 ± 1.35[a]	8.11 ± 1.15[a]	7.86 ± 1.12[a]
ALA			
t0	0.46 ± 0.20	0.49 ± 0.23	0.54 ± 0.14
t1	0.44 ± 0.21	0.46 ± 0.24	0.51 ± 0.11
ETA			
t0	1.41 ± 0.35	1.43 ± 0.28	1.44 ± 0.25
t1	1.35 ± 0.26	1.25 ± 0.28[a]	1.47 ± 0.24
EPA			
t0	1.15 ± 1.15	0.83 ± 0.26	1.06 ± 0.39
t1	1.91 ± 0.69[a]	2.02 ± 0.70[a]	1.13 ± 0.36
DPA			
t0	1.40 ± 0.41	1.50 ± 0.22	1.49 ± 0.25
t1	1.56 ± 0.32[a]	1.69 ± 0.27[a]	1.43 ± 0.20
DHA			
t0	3.04 ± 1.03	3.16 ± 0.69	3.40 ± 0.78
t1	3.62 ± 0.79[a]	3.72 ± 0.72[a]	3.42 ± 0.65
n6/n3 ratio			
t0	8.52 ± 3.81	10.29 ± 3.79	7.64 ± 2.63
t1	4.23 ± 1.36[a]	4.78 ± 3.07[a]	7.58 ± 2.34

Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; GLA, gamma-linolenic acid; LA, linoleic acid; LC n-3, long-chain n-3. t0, baseline; t1, end point. [a]Significant difference between baseline and end point according to paired-samples *T*-test. Distribution of EPA was not normal, and thus Wilcoxon Signed Rank test was used.

regular consumption, the amount of EPA in blood approximately doubled in both groups that received LC n-3 PUFAs, but it did not change in the control group. The changes in DHA were less pronounced but still significant and in the same direction as changes in EPA. The n-6/n-3 ratio decreased in the two LC n-3 PUFA groups, but it did not change in the control group. According to multivariate statistics, end-point concentrations in LC n-3 PUFAs in both intervention groups were significantly higher compared with placebo, but they were not different from each other. Thus, the study shows that the bioavailability of LC n-3 PUFAs in encapsulated powder is very similar to the bioavailability of LC n-3 PUFAs in ready-to-eat meals enriched with liquid fish oil. Therefore, it can be assumed that both ways can be equally used if increased intake of LC n-3 PUFAs is indicated.

Bioavailability of LC n-3 PUFAs is considered important, because greater bioavailability may enhance the efficacy (for example, reduction of circulating triglycerides) particularly at lower dosages.[13] Bioavailability of LC n-3 PUFAs depends on several factors. α-Linolenic acid, frequently found in seeds and plant oils, is not considered a good source of EPA and DHA. Although part of α-linolenic acid is theoretically converted to EPA and DHA, recent research has shown that conversion is often low and this is in particular true for DHA.[9] Bioavailability of FAs can vary significantly depending on their various chemical forms, for example, natural triglycerides, re-esterified triglycerides or ethyl esters.[18] Not only the type of chemical bonds but also the concomitant intake of food affect the uptake of LC n-3 PUFAs. For example, calcium ions can reduce the availability of LC n-3 PUFAs by forming a complex with free FAs.[12] Microencapsulation, as used in the present study, can possibly affect bioavailability by interacting with fat dispersion in the stomach owing to the mechanical influence of stomach peristalsis. Pharmaceutical preparations and dietary supplements often use coatings that are resistant to gastric acid intended to reduce the gastrointestinal side effects such as reflux or heartburn, which occur in some people.[12] Wallace *et al.*[14] compared the bioavailability of LC n-3 PUFAs from microencapsulated fish-oil-enriched foods to the bioavailability of LC n-3 PUFAs from fish oil gelatin capsules. Their findings indicate that foods enriched with microencapsulated fish oil have the same bioavailability as LC n-3 PUFA fish oil capsules. This is in good agreement with our findings in which LC n-3 PUFAs from foods enriched with fish oil and LC n-3 PUFAs from microencapsulated fish oil powder had comparable bioavailability. A recently published study found a greater bioavailability of microencapsulated n-3 FA compared with liquid fish oil in capsule form. However, the investigators used exines, that is, emptied pollen, to form hollow shells, to encapsulate fish oil. Thus, the results can not be necessarily extrapolated to other encapsulated preparations.[19]

The observed changes in LC n-3 PUFAs in the present study were smaller than those previously reported by Metherel *et al.*[20] who measured also changes in LC n-3 PUFAs in fingertip blood after 4 weeks of intervention. This difference can be explained by the dose used by Metherel *et al.*,[20] which was more than three times higher than in the present study (EPA+DHA = 4.8 vs 1.5 g/day).

The omega-3 index has been defined as the amount of EPA and DHA in red blood cell membranes (expressed as a percentage of total FAs). It is a good measure of long-term incorporation of FAs in tissue and has also been used to estimate heart disease risk.[21] Several studies have shown that higher omega-3 index is associated with a reduction of heart disease.[22–24] In our study, we measured FAs in fasting whole blood, which represents a combination of two different pools of LC n-3 PUFAs, that is, FAs in plasma and in red blood cell. It is not known whether measurements from fingertip blood have the same predictive value as EPA and DHA in red blood cell membranes; however, it has been shown that the EPA+DHA content of erythrocyte membrane is highly correlated with other plasma-based measures of EPA+DHA content.[22]

Dropout and adherence

In this study, the dropout rate was 22.2%. In group 2, which received the newly developed LC n-3 PUFA powder, dropout was not higher than in the other two groups, indicating that the participants tolerated the powder. Dropout was mostly related to lack of time or lack of interest.

Adherence to the study protocol seemed to be excellent. According to the questionnaires, more than 97% of the meals were eaten during the intervention. Additionally, the changes in LC n-3 PUFAs in blood were in good accordance with the different LC n-3 PUFA consumption in all of the three groups.

Strengths and limitations

The strength of the present study is that it is both double-blinded and randomized. It is, however, a limitation that the background diet was not controlled during the intervention period.

CONCLUSION

The present study investigated the bioavailability of LC n-3 PUFAs in microencapsulated fish oil powder and in ready-to-eat meals enriched with liquid fish oil. Bioavailability of LC n-3 PUFAs in encapsulated powder is very similar to bioavailability of LC n-3 PUFAs in ready-to-eat meals enriched with liquid fish oil. Thus, encapsulated powder can be considered useful to increase LC n-3 PUFA concentrations in blood.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This project was funded by Nordic Innovation no 11057: Enriched Convenience Seafood Products. The test meals were provided by Grimur Kokkur ehf, Vestmanneyjar, Iceland; the microencapsulated powder was provided by BioActive Foods AS, Trondheim, Norway. None of these three entities participated in data collection, data interpretation or publication.

REFERENCES

1 Delgado-Lista J, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. *Br J Nutr* 2012; **107**: S201–S213.
2 Calder PC. Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids* 2006; **75**: 197–202.
3 Calder PC. Omega-3 fatty acids and inflammatory processes. *Nutrients* 2010; **2**: 355–374.
4 Han YY, Lai SL, Ko WJ, Chou CH, Lai HS. Effects of fish oil on inflammatory modulation in surgical intensive care unit patients. *Nutr Clin Pract* 2012; **27**: 91–98.
5 Nordic Councils of Ministers. *Nordic Nutrition Recommendations 2012: Integrating nutrition and physical activity*. 5th ed Norden: Copenhagen, 2012.
6 Nordic Councils of Ministers. *Nordic Nutrition Recommendations 2004: Integrating nutrition and physical activity*. 4th ed Norden: Copenhagen, 2004.
7 Thorgeirsdottir H, Valgersdottir H, Gunnarsdottir I, Gisladottir I, Gunnarsdottir BE, Thorsdottir I *et al.* The Diet of Icelanders. Dietary Survey of The Icelandic Directorate of Health 2010-2011. Main findings 2011.
8 Steingrimsdottir L, Thorgeirsdottir H, Olafsdottir AS. The Diet of Icelanders. Dietary Survey of The Icelandic Nutrition Council 2002. Main findings 2002.
9 Lane K, Derbyshire E, Li W, Brennan C. Bioavailability and potential uses of vegetarian sources of omega-3 fatty acids: a review of the literature. *Crit Rev Food Sci Nutr.* 2014; **54**: 572–579.
10 Metcalf RG, James MJ, Mantzioris E, Cleland LG. A practical approach to increasing intakes of n-3 polyunsaturated fatty acids: use of novel foods enriched with n-3 fats. *Eur J Clin Nutr* 2003; **57**: 1605–1612.

11 McCowen KC, Ling PR, Decker E, Djordjevic D, Roberts RF, Coupland JN *et al.* A simple method of supplementation of omega-3 polyunsaturated fatty acids: use of fortified yogurt in healthy volunteers. *Nutr Clin Pract* 2010; **25**: 641–645.
12 Schuchardt JP, Hahn A. Bioavailability of long-chain omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2013; **89**: 1–8.
13 Kastelein JJ, Maki KC, Susekov A, Ezhov M, Nordestgaard BG, Machielse BN, Kling D, Davidson MH. Omega-3 free fatty acids for the treatment of severe hypertriglyceridemia: the EpanoVa fOr Lowering Very high triglyceridEs (EVOLVE) trial. *J Clin Lipidol* 2014; **8**: 94–106.
14 Wallace JM, McCabe AJ, Robson PJ, Keogh MK, Murray CA, Kelly PM *et al.* Bioavailability of n-3 polyunsaturated fatty acids (PUFA) in foods enriched with microencapsulated fish oil. *Ann Nutr Metab* 2000; **44**: 157–162.
15 Higgins S, Carroll YL, O'Brien NM, Morrissey PA. Use of microencapsulated fish oil as a means of increasing n-3 polyunsaturated fatty acid intake. *J Hum Nutr Diet* 1999; **12**: 265–271.
16 Saga LC, Liland KH, Leistad RB, Reimers A, Rukke EO. Relating fatty acid composition in human fingertip blood to age, gender, nationality and n-3 supplementation in the Scandinavian population. *Int J Food Sci Nutr* 2012; **63**: 790–795.
17 Marangoni F, Colombo C, Galli C. A method for the direct evaluation of the fatty acid status in a drop of blood from a fingertip in humans: applicability to nutritional and epidemiological studies. *Anal Biochem* 2004; **326**: 267–272.
18 Dyerberg J, Madsen P, Møller JM, Aardestrup I, Schmidt EB. Bioavailability of marine n-3 fatty acid formulations. *Prostaglandins Leukot Essent Fatty Acids* 2010; **83**: 137–141.
19 Wakil A, Mackenzie G, Diego-Taboada A, Bell JG, Atkin SL. Enhanced bioavailability of eicosapentaenoic acid from fish oil after encapsulation within plant spore exines as microcapsules. *Lipids* 2010; **45**: 645–649.
20 Metherel AH, Armstrong JM, Patterson AC, Stark KD. Assessment of blood measures of n-3 polyunsaturated fatty acids with acute fish oil supplementation and washout in men and women. *Prostaglandins Leukot Essent Fatty Acids* 2009; **81**: 23–29.
21 Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med* 2004; **39**: 212–220.
22 Harris WS. The omega-3 index as a risk factor for coronary heart disease. *Am J Clin Nutr* 2008; **87**: 1997–2002.
23 Siscovick DS, Raghunathan TE, King I, Weinmann S, Wicklund KG, Albright J *et al.* Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA* 1995; **274**: 1363–1367.
24 Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis* 2007; **193**: 1–10.