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Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study

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Abstract. Tissue levels of n–3 fatty acids reflect dietary intake, but quantitative data about rate of incorporation and levels as a function of intake are scarce. We fed 58 men 0, 3, 6, or 9 g/d of fish oil for 12 months and monitored fatty acids in serum cholesteryl esters, erythrocytes, and subcutaneous fat during and after supplementation. Eicosapentaenoic acid (EPA) in cholesteryl esters plateaued after 4–8 weeks; the incorporation half-life was 4.8 days. Steady-state levels increased by 3.9 ± 0.3 mass % points (± SE) for each extra gram of EPA eaten per day. Incorporation of docosahexaenoic acid (DHA) was erratic; plateau values were 1.1 ± 0.1 mass % higher for every g/d ingested. Incorporation of EPA into erythrocyte membranes showed a half-life of 28 days; a steady state was reached after 180 days. Each g/d increased levels by 2.1 ± 0.1 mass %. C22:5n–3 levels increased markedly. Changes in DHA were erratic and smaller. EPA levels in adipose tissue rose also; the change after 6 months was 67% of that after 12 months in gluteal and 75% in abdominal fat. After 12 months each gram per day caused an 0.11 ± 0.01 mass % rise in gluteal fat for EPA, 0.53 ± 0.07 for C22:5n–3, and 0.14 ± 0.03 for DHA. Thus, different (n–3) fatty acids were incorporated with different efficiencies, possibly because of interconversions or different affinities of the enzymatic pathways involved. EPA levels in cholesteryl esters reflect intake over the past week or two, erythrocytes over the past month or two, and adipose tissue over a period of years. These findings may help in assessing the intake of (n–3) fatty acids in epidemiological studies.—**Katan, M. B., J. P. Deslypere, A. P. J. M. van Birgelen, M. Penders, and M. Zegwaard.** Kinetics of the incorporation of dietary n–3 fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J. Lipid Res.* 1997. **38:** 2012–2022.

Supplementary key words n–3 polyunsaturated fatty acids • diet • fish oil • cholesteryl esters • erythrocytes • adipose tissue • biomarkers of intake

The human body has an absolute requirement for polyunsaturated fatty acids of both the n–6 (omega 6) and the n–3 series. These essential fatty acids occur in the body in a variety of forms, which include phospholipids in membranes, fatty acid esters of cholesterol and other lipids circulating in the blood or stored in cells, and triglycerides stored in adipose tissue. Although the fatty acid composition of membrane phospholipids and cholesteryl esters is under homeostatic control it also reflects dietary intake (1–12). This is even more so for storage of triglycerides (13). The proportion of linoleic acid, and increasingly also that of eicosapentaenoic acid (C20:5n–3, EPA) in plasma or adipose tissue are therefore used as a marker of intake (10, 14). A number of studies have indeed shown significant correlations between intake and plasma or adipose tissue levels of eicosapentaenoic and/or docosahexaenoic acid (C22:6n–3, DHA) (15–21). It is also clear that different plasma lipid classes reflect changes in intake at different rates, and that adipose tissue is slowest of all in reaching a new steady state. However, rational use of tissue or plasma fatty acids as indicators of intake requires two types of quantitative data: the time frame of intake which a specific marker represents, and the quantitative change in the tissue or plasma fatty acid per g of fatty acid consumed. Little such information is presently available.

The present study aimed to provide such data. We report herein the rate and extent to which EPA, docosa-

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; BHT, butylated hydroxytoluene (2,6-di-tert-butyl-p-cresol); EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EDTA, ethylenediamine tetraacetate; LDL, low density lipoprotein.

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pentaenoic acid (C22:5n–3), and DHA were incorporated into cholesteryl esters, erythrocyte membranes, and adipose tissue in volunteers as a function of dosage and duration in an 18-month controlled trial.

SUBJECTS, MATERIALS AND METHODS

Subjects

Fifty-eight monks from one Trappist and three Benedictine monasteries participated in the study. Their mean age (±SD) was 56.2 ± 16.5 years. We excluded subjects who used drugs that influence lipid metabolism or the cyclo-oxygenase enzyme (i.e., non-steroidal anti-inflammatory drugs), and subjects with serious illnesses (diabetes, cancer, or coronary heart disease), a body mass index greater than 30 kg/m^2, a systolic blood pressure above 160 mmHg or a diastolic blood pressure above 95 mmHg, total cholesterol > 7 mmol/l or triglycerides > 3 mmol/l, and age younger than 21 or older than 90 years. Ten participants ate lacto-ovovegetarian diets exclusively.

The protocol of the study was approved by the Human Ethics Committee of the Department of Human Nutrition, and all volunteers gave their written consent after having been thoroughly informed about the nature of the study.

Design and supplements

The study consisted of a 2-week base-line period, followed by a 1-year treatment with fish oil and a 6-month wash-out period. Within each monastery subjects were randomly divided into four groups, which received each day during the 12-month treatment period either 0 (n = 14), 3 (n = 15), 6 (n = 15), or 9 (n = 14) capsules, each containing 1 gram of fish oil. The oil consisted of triglycerides, not synthetic esters. In each group the number of capsules was made up to 9 by adding 9, 6, 3, or 0 placebo capsules, respectively. Thus every subject received 9 g of oil per day on top of his normal intake. Three capsules were taken at each meal. We performed a capsule count and delivered new capsules every 2 months.

All capsules were provided by S.A. Sanofi Labaz Inc. (Brussels, Belgium). The placebo capsules contained olive oil (Puget, Marseille, France) plus palm oil (Loders Croklaan, Wormerveer, The Netherlands) to which cholesterol (Merck, Darmstadt, Germany) and vitamin E (Organon, Oss, The Netherlands) were added. The placebo oil was composed in such a way that the content of saturated fatty acids, vitamin E, and cholesterol was equal and that of linoleic acid nearly equal to that in the fish oil (**Table 1**). The study thus comprised a comparison of monounsaturated fatty acids, which can be made in the body, with essential n–3 polyunsaturates, at a constant total intake of fat. The low fish oil group received 0.81 g EPA, 0.16 g DHA, and 0.09 g C 22:5n–3/d, the middle group 1.62, 0.33, and 0.18 g/d, and the high fish oil group 2.43, 0.49, and 0.27 g/d, respectively.

TABLE 1. Composition of the fish oil and placebo capsules

Fatty Acid	Placebo Oil	Fish Oil
	g/100 g fatty acids	
14:0	0.3	9.0
16:0	16.6	10.8
16:1	0.7	10.6
18:0	3.4	2.2
18:1n–9	67.5	12.6
18:2n–6	9.4	0.7
18:3n–3	0.5	0.4
18:4n–3	0.0	1.5
20:0	0.4	0.7
20:1n–9	0.2	1.2
20:5n–3	0.0	27.5
22:1n–9	0.0	0.8
22:5n–3	0.0	3.1
22:6n–3	0.0	5.7
Sum	99.0	86.7[a]
Cholesterol (mg/g)	1.1	1.2
Vitamin E (I.U./g)	0.6	0.6

Each volunteer took 9 gram per day of fish oil, placebo oil, or a mixture of the two.

[a]Other peaks were either less than 0.5% or not identifiable.

Blood sampling and a clinical examination were done 12 times during the trial, on days −14, 0 (start of supplementation), 3, 7, 14, 28, 56, 182, 363 (end of supplementation), 396, 424, and 536 (end of washout period). Subjects kept records of everything they ate and drank for 3 days on days −3 to −1, 179 to 181, and 360 to 362. These records were each time supplemented by a dietary history interview of every subject by a trained dietician who weighed portion sizes and foods. Nutrient intakes were calculated using the Netherlands Nutrient Data Bank supplemented with figures for EPA and DHA contents of foods.

All subjects recorded any illnesses, use of drugs, alcohol intake, omission of capsule intake, and other relevant facts in a diary.

Sampling and storage

Venous blood was drawn after an overnight fast into one 10-ml Vacutainer tube containing EDTA and another one without anticoagulant. Serum was stored at −70°C and used for the analysis of cholesteryl esters. The EDTA tubes were centrifuged on the spot at low speed and 4°C to isolate red blood cells. The cells were washed twice with ice-cold isotonic saline, transferred

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into stoppered plastic tubes, and hemolyzed by freezing at −80°C for at least 2 h. The hemolysates were stored at −20°C for a maximum of 7 days before further processing.

Both gluteal (buttock) and abdominal subcutaneous fat aspirates were taken at days 0, 182, and 363 by the method of Beynen and Katan (22) using evacuated 10-ml tubes instead of a syringe. Abdominal subcutaneous fat microbiopsies were taken 2–3 cm to the left of the umbilicus. A final gluteal sample was taken at day 536; the abdominal sampling was omitted because of possible discomfort to subjects. The amount of fat obtained was 20 to 100 mg. Samples were stored at −80°C.

Chemical analyses

Serum cholesteryl ester fatty acids were analyzed largely as previously described (11). All glassware was washed twice with distilled water and once with hexane. Antioxidant BHT (2,6-di-tert-butyl-*p*-cresol, BDH Biochemicals, Poole, UK) was added to the isopropanol and n-octane to a final concentration of 0.005%. The fatty acids were analyzed with a gas chromatograph (Packard 438 AS United Technologies, Delft, The Netherlands), using a 50-m fused silica column (inside diameter 0.25 mm) coated with 0.2 μm CP Sil 88 (Chrompack, Middelburg, The Netherlands). The injection volume was 1 μl and the split ratio was 30:1. Nitrogen was used as carrier gas at a flow of 20 ml/min and a pressure of 120 kPa. The oven temperature was programmed to rise after 3 min of steady state from 160°C to 215°C at a rate of 3°C/min during the first 2 min followed by a rate of 15°C/min, and then kept constant at 215°C. The temperature of the injector was 225°C and that of the hydrogen flame ionization detector was 250°C. Peak areas were measured with an electronic integrator (SP 4100, Spectra Physics, Santa Clara, CA). Fatty acids were identified by comparison with known standards (Chrompack, Middelburg, the Netherlands). The sum of all peak areas of the fatty acids identified was taken as 100%. A quality control serum pool was analyzed in duplicate in each run. Over a 4-month period the combined within and between run coefficient of variation was 4–12% for EPA and 3–8% for DHA.

For analysis of erythrocyte fatty acids, 0.5 g of hemolysate was mixed with 4.5 mL isopropanol, 6 mL hexane containing 50 mg of BHT per L was added, the tubes were shaken for 45 min, and the clear supernatant was poured off and centrifuged for 5 min at 1200 *g* at 4°C. Four mL of the upper layer was dried down at 60°C under a stream of nitrogen and phospholipids were transmethylated by mixing with 4 mL of acid methanol (40 mL H_2SO_4/L methanol) and incubating at 60°C for 18 h as previously described (11). The methyl esters were extracted from the mixture with hexane and analyzed by gas–liquid chromatography as described above, except that the temperature program involved an initial 3 min at 145°C, an increase of 3°C/min for 2 min, and a further increase of 25°C/min to a final temperature of 215°C. Injector and detector temperatures were 250°C. A quality control pool of fatty acids from hemolyzed erythrocytes was analyzed in duplicate in each run; the combined within and between run coefficients of variation over a 4-month period was 6–12% for EPA, 6–8% for C22:5n–3, and 3–5% for DHA.

Analysis and quality control procedures for fat tissue aspirates have been previously described (23).

Mathematical modeling and statistical analysis

The proportion of EPA or DHA in cholesteryl esters or erythrocyte membranes as a function of time was modelled with the equation:

$$C(t) = C(\infty) - [C(\infty) - C(0)]\ e^{-kt}$$

where C(t) is the proportion of the fatty acid of interest (mass% fatty acids) after t days of fish oil consumption, C(∞) is the final steady-state proportion, C(0) is the initial proportion, and k is the rate constant. Essentially the same equation was used by Dayton et al. (24) to model changes in adipose tissue linoleic acid in the Veterans Administration trial. Each subject's data were fitted to this equation using SAS procedure NLIN. This yielded estimates of the parameters C(0), C(∞), and k which were then averaged per fatty acid per dosage group. The incorporation half-life $t_{1/2}$ was then calculated as $t_{1/2} = \ln(2)/k$.

Statistical analysis was performed using *t*-tests or the two-tailed Wilcoxon rank sum test. Changes in fatty acids concentration were calculated as the value at a given point of time minus the baseline value, i.e., the mean of the values at day −14 and day 0. We calculated slopes and intercepts of the mean changes of n–3 fatty acids levels per dosage group over 12 months as a function of the amount of n–3 fatty acids supplied per day. For example, the relation between EPA in cholesteryl esters at 363 days and EPA supplied was calculated using the model $\Delta Y(g/100\ g) = a + b \times X\ (g/d) + \text{error}$, where ΔY is the mean change in cholesteryl ester EPA between baseline and day 363, and X is the amount of extra EPA supplemented (0, 0.81, 1.62 or 2.43 g/d; cf Methods).

RESULTS

Compliance and food intake

Throughout the study period the subjects showed an excellent compliance as judged from the capsule count and the regular visits to the monasteries.

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TABLE 2. Intake of very-long-chain n–3 fatty acids of the volunteers at the start of the study and during supplementation

	Baseline (t = 0)	Supplementation Group (means of t = 6 and 12 months)			
		Zero	Low	Mid	High
n	57	14	15	15	14
Fish oil (g/d)	0	0	3	6	9
Placebo oil (g/d)	0	9	6	3	0
EPA (g/d)	0.04	0.19	1.01	1.79	2.58
C22:5n–3 (g/d)	0.00	0.00	0.09	0.17	0.26
DHA (g/d)	0.06	0.18	0.41	0.57	0.73

Intakes of EPA and DHA include those from foods as calculated from 3-day records. Figures for C22:5n–3 refer exclusively to intake from capsules, as no data for foods were available.

Nutrient intake on the baseline diet was highly similar for the four dosage groups and it showed little change over the course of the trial. At weeks 6 and 12 combined, mean energy intake of the 58 men, including the contribution from oil supplements, was 9.7 MJ/d (2326 kcal/d), with protein providing 12%, carbohydrates 46%, alcohol 1%, total fat 41%, saturated fatty acids 16%, monounsaturated fatty acids 14–15%, and linoleic acid 4–5%; mean cholesterol intake was 373 mg/d. The intake of n–3 polyunsaturates from foods unfortunately showed some variation with time: EPA and DHA intakes from foods were 40 and 60 mg/d, respectively, at baseline, 368 and 352 mg/d at 6 months, and 112 and 117 mg/d, respectively, at 12 months. The rise at 6 months was due to meat being replaced by fish during the pre-Christmas Advent period in the non-vegetarian Benedictine monasteries. The averages of intakes at 6 and 12 months are shown in **Table 2.** The extra intake of n–3 fatty acids from foods was reflected in serum cholesteryl esters and erythrocytes. Brothers from abbey No 4, which reported the highest fish and n–3 fatty acid intake at 6 months (day 182), indeed showed the most marked elevation in erythrocyte and cholesteryl ester n–3 fatty acids at that time point. However, it occurred to about the same extent in the supplemented groups as in the concurrent placebo group, and as a result the effect was eliminated when all changes were calculated relative to changes in the placebo group.

Cholesteryl esters

The proportion of EPA in the cholesteryl esters was increased in all three fish oil groups at the first blood sampling, after 3 days of fish oil consumption (**Fig. 1, top**). The rise in EPA was compensated for largely by decreases in linoleic acid, with little change in arachidonic acid (**Table 3**). Crude EPA levels were slightly elevated at 6 mo, reflecting the unscheduled intake of fish around this period (see above); thus levels in the control group were 0.9 g/100 g at 0 and 12 mo, and 1.3 at 6 mo. This effect disappeared when all changes were corrected for changes in the control group (Fig. 1, top).

Using a first-order exponential model (see Methods) we calculated that half-maximal levels were reached after an average 4.8 days of supplementation (**Table 4**). The increase reached a plateau at 56 days (Fig. 1, top). After 1 year EPA concentrations in cholesteryl esters had changed by −5% in the placebo group, +367% in

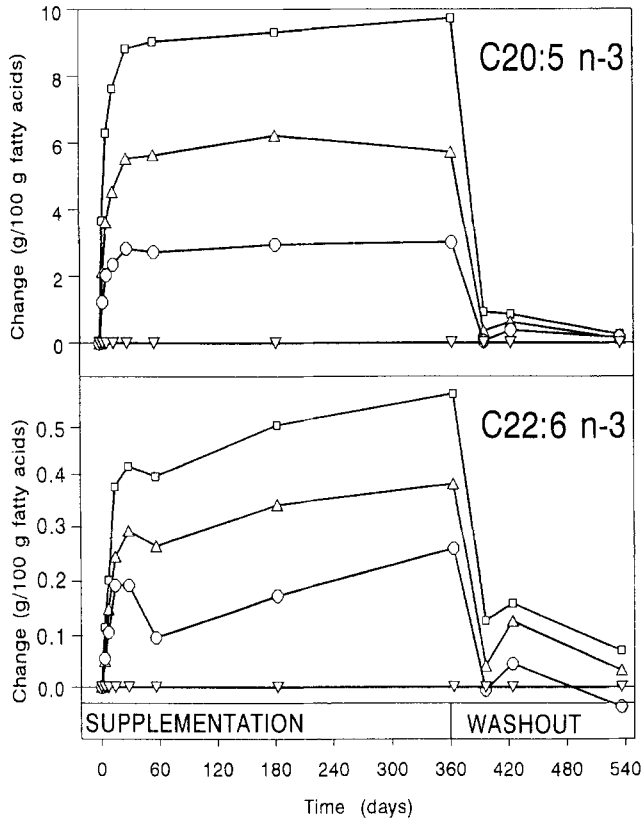


Fig. 1. Serum cholesteryl esters: changes in the levels of very-long-chain n–3 polyunsaturated fatty acids in volunteers supplemented for 12 months with 0 g/d (▽, n = 14), 3 g/d (○, n = 15), 6 g/d (△, n = 15), or 9 g/d (□, n = 14) of fish oil rich in EPA. Values represent changes from baseline corrected for changes in the placebo (0 g/d) group. Top: Eicosapentaenoic acid (EPA), C20:5n–3. Bottom, docosahexaenoic acid (DHA), C22:6n–3. Docosapentaenoic acid (C22:5n–3) was not detectable.

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TABLE 3. Proportions of key essential fatty acids in cholesteryl esters and erythrocyte membranes at the start and after 12 months of fish oil supplementation, as a function of the dose of fish oil consumed

Dose and Fatty Acid	Cholesteryl Esters		Erythrocyte Membranes	
	t = 0	t = 12 mo	t = 0	t = 12 mo
	proportion (g/100 g fatty acids)			
0 gram/day				
18:2n–6	54.2 ± 3.2	57.2 ± 4.6	10.2 ± 1.4	10.1 ± 1.3
20:4n–6	5.4 ± 0.7	5.7 ± 1.2	12.0 ± 1.3	11.6 ± 1.3
20:5n–3	0.9 ± 0.4	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.3
22:5n–3	[a]	[a]	2.4 ± 0.2	2.2 ± 0.2
22:6n–3	0.6 ± 0.2	0.7 ± 0.2	4.6 ± 0.6	4.4 ± 0.8
3 gram/day				
18:2n–6	55.5 ± 3.9	55.2 ± 6.2	10.4 ± 1.6	9.0 ± 1.4
20:4n–6	5.3 ± 1.1	5.2 ± 0.8	12.7 ± 1.6	9.6 ± 0.7
20:5n–3	0.8 ± 0.3	3.8 ± 1.0	0.8 ± 0.2	2.8 ± 0.3
22:5n–3	[a]	[a]	2.5 ± 0.3	3.9 ± 0.3
22:6n–3	0.6 ± 0.2	0.9 ± 0.2	4.6 ± 0.6	4.6 ± 0.5
6 gram/day				
18:2n–6	56.2 ± 5.0	53.7 ± 5.6	10.3 ± 1.6	8.7 ± 1.2
20:4n–6	5.1 ± 0.7	4.9 ± 1.0	11.9 ± 1.1	8.2 ± 0.9
20:5n–3	0.9 ± 0.4	6.6 ± 2.6	0.7 ± 0.3	4.3 ± 0.9
22:5n–3	n.d.	n.d.	2.3 ± 0.2	4.5 ± 0.5
22:6n–3	0.6 ± 0.2	1.1 ± 0.3	4.7 ± 0.6	4.8 ± 0.5
9 gram/day				
18:2n–6	56.3 ± 3.2	49.1 ± 6.9	10.3 ± 1.5	7.6 ± 1.1
20:4n–6	4.9 ± 0.8	5.0 ± 0.8	12.2 ± 1.5	7.3 ± 0.8
20:5n–3	0.7 ± 0.3	10.4 ± 2.8	0.7 ± 0.2	5.9 ± 1.2
22:5n–3	n.d.	n.d.	2.3 ± 0.3	5.0 ± 0.4
22:6n–3	0.5 ± 0.2	1.2 ± 0.2	4.4 ± 0.4	4.8 ± 0.5

[a] Levels of C22:5n–3 in cholesteryl esters were too low for reliable quantitation.

TABLE 4. Kinetic parameters of the incorporation of dietary very-long-chain n–3 fatty acids into plasma cholesteryl esters and erythrocytes[a]

	Supplementation Group (g fish oil per day)			
	3	6	9	Mean
Cholesteryl esters				
EPA				
n	15	15	14	45
k (d^{-1})	0.151	0.127	0.155	0.145
Half life $t_{1/2}$ (d)	4.6	5.4	4.5	4.8
DHA				
n	14	14[b]	14[b]	42
k (d^{-1})	0.061	0.067	0.076	0.068
Half life $t_{1/2}$ (d)	11.4	10.3	9.1	10.3
Erythrocytes				
EPA				
n	15	15	14	45
k (d^{-1})	0.025	0.024	0.025	0.025
Half life $t_{1/2}$ (d)	27.7	28.9	27.8	28.1
C22:5n–3				
n	15	15	14	44
k (d^{-1})	0.019	0.017	0.018	0.018
Half life $t_{1/2}$ (d)	36.7	40.1	38.8	38.5

[a] C22:5n–3 was not detectable in cholesteryl esters. The time course of DHA in erythrocytes could not be properly fitted with the model.

[b] n = 14 instead of 15 because data of one subject could not be fitted with the model.

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TABLE 5. Average change (± SEM) in the proportion of various n–3 fatty acids in tissues after 12 months of supplementation, per gram supplemented with diet

	Increase per g supplemented per day		
	EPA	C22:5n–3	DHA
	g/100 g fatty acids		
Cholesteryl esters	3.94 ± 0.27	ND	1.12 ± 0.13
Erythrocytes	2.07 ± 0.11	9.99 ± 1.93	1.09 ± 0.21
Gluteal fat tissue	0.11 ± 0.01	0.53 ± 0.07	0.14 ± 0.03
Abdominal fat tissue	0.12 ± 0.00	0.83 ± 0.07	0.24 ± 0.03

The highest dose group received 2.4 g/day EPA, 0.3 g C22:5n–3, and 0.5 g DHA. ND, not detected.

the 3 g/d group, +600% in the 6 g/d group and +1426% in the 9 g/d group relative to baseline. The change was linear with dosage.

The concentration of DHA increased also (Fig. 1, bottom), but changes were much smaller and more erratic than for EPA. The calculated half-life for reaching a steady state level was 10.3 days, significantly longer than the 4.8 days for EPA. The change in DHA was again roughly linear with dosage (cf. Fig. 1, bottom), but each g of DHA supplemented per day produced an increase of DHA in cholesteryl esters of only 1.1 g/100 g fatty acids, as opposed to 3.9 g/100 g for EPA (**Table 5**).

Levels of C22:5n–3 in cholesteryl esters were too low for reliable quantitation.

Red blood cell membranes

The proportion of EPA in erythrocytes was already increased after 3 days of supplementation (**Fig. 2, top**). Crude values were again elevated in all groups at 6 mo; thus EPA levels in the placebo control group were 0.8 throughout the trial but 1.1 at 6 mo. Again, correction of all changes for changes in the control group removed this effect. The mathematical analysis showed that half-maximal concentrations were reached in 28 days (Table 4). The plateau values (Fig. 2, top) represented changes over baseline of +5%, +261%, +482% and +737%, for the placebo group, and the 3 g/d, 6 g/d, and 9 g/d groups, respectively. The change was linear with dosage, with one g of EPA/d, corresponding with about 4 g of fish oil, producing an increase in red blood cell membrane concentrations of 2 g/100 g fatty acids (Table 5). Levels of C22:5n–3 were somewhat slower to rise than those of EPA, the half-life being 39 instead of 28 days Table 4 and Fig. 2, middle). The rise in C22:5n–3 in erythrocytes was much higher than would be expected based on the amount of C22:5n–3 supplied; the mean increase was 10 g/100 g fatty acids for each gram supplied per day (Table 5). In contrast, DHA in erythrocytes rose by only 1 g fatty acid per gram ingested from fish oil (Table 5).

While EPA and C22:5n–3 rose, levels of linoleic and

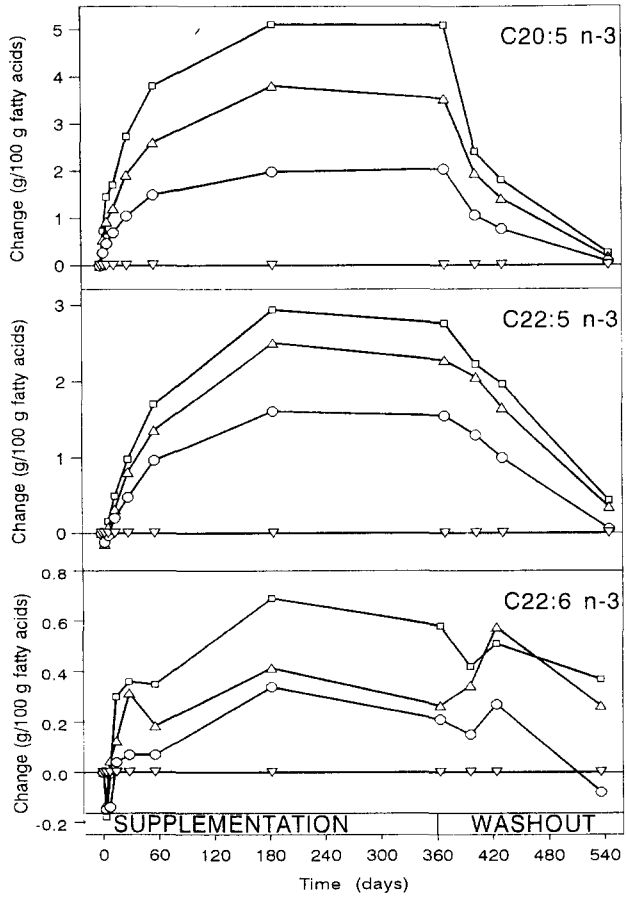


Fig. 2. Red blood cells: changes in the levels of EPA (C20:5n–3), DPA (C22:5n–3), and DHA (C22:6n–3) as a function of intake at various times after the start of supplementation with fish oil in amounts of 3 g/d (○, n = 15), 6 g/d (△, n = 15), or 9 g/d (□, n = 14). Values were corrected for changes in the concurrent placebo control group (▽, n = 14). Top: Eicosapentaenoic acid (EPA), C20:5n–3; middle: docosapentaenoic acid (DPA), C22:5n–3; bottom, docosahexaenoic acid (DHA), C22:6n–3.

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arachidonic acid declined (Table 3). Reductions in oleic acid were less than 1 g/100 g in all groups.

When supplementation was suspended, levels of EPA and C22:5n–3 came down at approximately the same rate at which they had risen (Fig. 2). Elevations of EPA fell to 51% of peak values after 1 month, 37% after 2 months, and 4% after 6 months (means of the three fish oil groups, corrected for placebo). C22:5n–3 fell more slowly.

Adipose tissue

The proportion of EPA in gluteal fat aspirates rose throughout the 12-month supplementation period, without a plateau becoming evident (**Fig. 3, top**). As in the erythrocyte membranes, the relative amount of C22:5n–3 incorporated was again much higher than that of EPA or DHA (Table 5). Six months after cessation of fish oil intake, the elevation of EPA in gluteal fat had fallen to 50% and that of DHA to 45% of peak levels (Fig. 3). The number of data points was insufficient for calculation of half-lives.

There was no clear-cut compensatory decline in other fatty acids, possibly because the absolute amounts of n–3 fatty acids entering the fat tissue pool were so small.

After 12 months of supplementation, the increase in EPA in abdominal subcutaneous fat tissue was similar to that in gluteal fat tissue, but C22:5n–3 and DHA were both incorporated more extensively into abdominal than into gluteal fat tissue (Table 5).

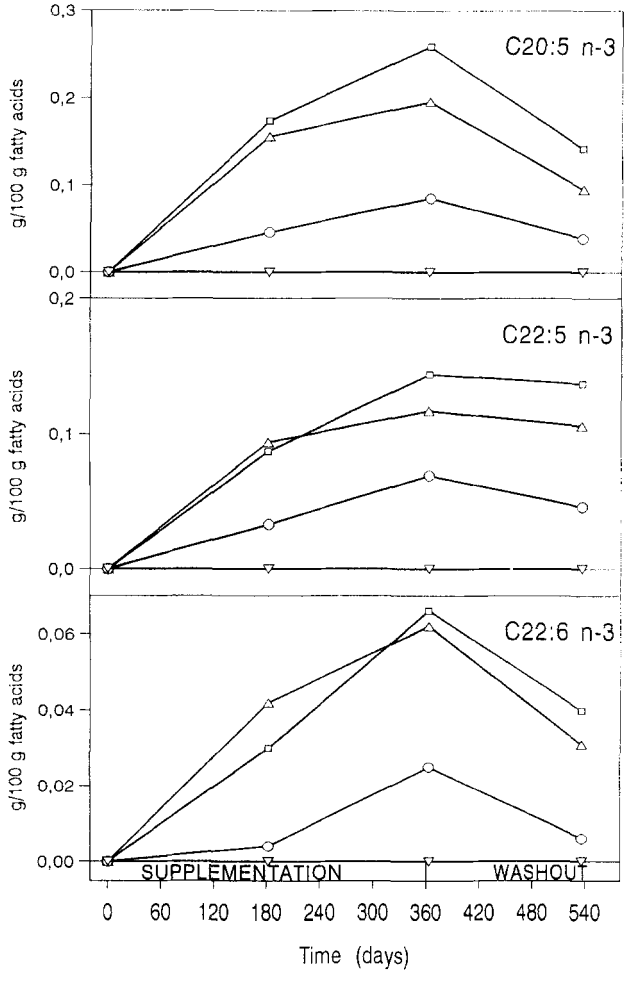


Fig. 3. Changes in the levels of very-long-chain in n–3 polyunsaturated fatty acids in gluteal (buttock) fat tissue aspirates in volunteers supplemented for 12 months with 0 g/d (▽), 3 g/d (○), 6 g/d (△), or 9 g/d (□) of fish oil rich in EPA. Values represent changes from baseline corrected for changes in the placebo (0 g/d) group. *Top*: Eicosapentaenoic acid (EPA), C20:5n–3; middle: docosapentaenoic acid (DPA), C22:5n–3; bottom, docosahexaenoic acid (DHA), C22:6n–3.

DISCUSSION

We have found that the incorporation half lives of EPA into the human body are about 5 days for serum cholesteryl esters, almost a month for erythrocytes, and longer than a year for subcutaneous fat tissue. The extent of incorporation was in general linear with the amount consumed up to 9 grams of fish oil per day. These data should provide a firm footing for the use of these fatty acids as markers for dietary intake.

Cholesteryl esters

The spectrum of fatty acids found in cholesteryl esters is largely determined by the specificity of the enzyme lecithin:cholesterol acyl transferase (LCAT). LCAT has a preference for linoleic acid (C18:2n–6), which is the predominant fatty acid in cholesteryl esters even when linoleic acid intakes are low. However, the composition of cholesteryl esters is influenced by diet; even saturated and *trans*-monounsaturated fatty acids are incorporated in a diet-dependent fashion (25). After 1 year of supplementation with 9 g of fish oil per day, the ratio of linoleic acid over EPA was 4.5 in the diet of our subjects, including the capsules, and 4.7 in their cholesteryl esters. Excess linoleic acid was probably also still being supplied from fat stores, in which the ratio of linoleic acid to EPA was 38. This suggests that the enzymatic pathway leading to cholesteryl ester formation has as high an affinity for EPA as for linoleic acid. This contrasts with in vitro studies where LCAT showed a marked preference for linoleic acid over (n–3) polyunsaturated fatty acids (26, 27). However, Parks, Bullock, and Rude (26) already pointed out that many factors influence the LCAT reaction including phospholipid species, lipid environment, and the size and shape of the macromolecular substrate particles.

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The rate of incorporation of EPA into cholesteryl esters could depend on the turnover rate of the main carrier of cholesteryl esters in serum, LDL. The half-life of LDL in middle-aged men is about 3 days, which is of the same order of magnitude as the half-life for the incorporation of EPA into cholesteryl esters of 5 days observed here. This should make the level of EPA in cholesteryl esters a suitable indicator of the intake in the preceding 1 to 2 weeks.

The extent of incorporation of EPA as a function of diet found by us was in reasonable agreement with values reported previously. Our data predict an increase of 3.9 g/100 g fatty acids for every extra gram of EPA eaten per day (Table 5). From the data of Bronsgeest-Schoute et al. (28) one can calculate a ratio of about 2.3, but this was after only 1 week of supplementation; our data show that steady-state levels are not reached within that time.

The increase of DHA in the cholesteryl esters was quite small relative to the amount supplied. This is in agreement with the data of Bronsgeest-Schoute et al. (28) and Von Lossonczy et al. (29) who found no effect on cholesteryl ester DHA concentration after supplementation with high doses of DHA (4.2 g/d) during 4 weeks. These observations could point to a retroconversion of DHA into EPA (30) but may also be due to DHA being a poor substrate for LCAT (26, 27, 31) or to its being less efficiently incorporated into the *sn*-2 position of phosphatidylcholine, which is the substrate for the LCAT reaction.

Erythrocyte membranes

Mean initial levels in erythrocyte fatty acids were 0.8% for EPA, 2.4% for C22:5n−3, and 4.7% for DHA. These data are in agreement with published values. Previous studies have shown significant increases of erythrocyte EPA and DHA levels during supplementation (32, 33). In our subjects the rise in erythrocyte EPA was much higher than that in DHA, but on a gram per gram basis EPA and DHA were incorporated about equally effectively. In contrast, levels of C22:5n−3 rose markedly even though very little was supplemented. This may point to formation of C22:5n−3 from EPA, DHA, or from both (34).

Brown, Pang, and Roberts (33) concluded that each extra gram of dietary EPA raised erythrocyte EPA by 1.2 g/100 g fatty acids. This is less than our figure of 2.2. However, the median duration of the studies summarized by Brown et al. was only 9 weeks; according to our data that is insufficient to reach a steady state (Fig. 2). Brown et al. (33) also suggested that turnover of DHA in erythrocyte membranes is slower than that of EPA, possibly because DHA is located in the inner leaflet of the phospholipid bilayer and does not exchange with plasma phospholipids. In our study the time course of incorporation of DHA was more erratic than that of EPA, which precludes firm conclusions as to its half-life.

Animal studies (35) have suggested that the incorporation of essential (n−3) fatty acids into tissues may level off when dietary requirements are exceeded. This was evidently not the case in these grown men; levels went up with intake and did not plateau even at doses that clearly exceeded requirements. Such plateauing is thus not a valid way to determine requirements in man.

The lifetime of a red blood cell is about 120 days, but we saw a doubling of EPA concentration in erythrocyte membranes already at 3 days after the start of the supplementation. Evidently the red blood cells exchanged phospholipids or fatty acids with their environment rather easily; possibly the phospholipid transfer protein plays a role here. The decrease in (n−3) fatty acids after cessation of fish oil supplementation was equally rapid; about half of the EPA gained during supplementation was lost in the first 4 weeks of the wash-out period. Our data on this do not agree with those of Sanders, Vickers, and Haines (36) who reported that n−3 composition of erythrocyte membranes was unaltered 5 weeks after withdrawal. The reason for this discrepancy is unclear.

Fat tissue

Levels of linoleic acid in human or animal fat tissue closely resemble those in the fat eaten when both are expressed as gram per 100 gram of fatty acids. One might expect the same relation for the very-long-chain n−3 fatty acids. Indeed, basal values for EPA in fat tissue in our subjects were similar to those in the diet, at 0.07 and 0.04 gram per 100 g fatty acids, respectively. Comparable levels have been published by others (10, 18). However, after 1 year of fish oil consumption, the proportion of EPA in abdominal or gluteal fat was only about one-sixth and that of DHA about one-third of that in the dietary fat. The values for DHA could be accounted for if the half-life for incorporation of DHA into gluteal fat tissue were assumed to be 2 years; the model described in Methods would then yield values in fat tissue at 363 days close to those observed, and at $t = \infty$ values identical to those in the diet. The half-life for the incorporation of linoleic acid into fat tissue is 680 days (24); 2 years for DHA is thus a plausible value.

On the other hand, the values observed for EPA could be explained only if the half-life for incorporation of EPA were assumed to be 6 years. Such a long half-life is implausible in view of the linoleic acid data, and it is also incompatible with the fairly rapid rise in EPA content of gluteal fat over the first 180 days and the equally rapid fall during the wash-out period, between days 363 and 540 (Fig. 3, top). One explanation

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could be preferential mobilization of EPA from fat tissue, as observed by Connor et al. in rabbits (37). Another possibility is that part of the dietary EPA is not available for incorporation into fat tissue, and that the stationary-state levels in body fat will be lower than those in the dietary fat. This agrees with some preliminary results of Popp-Snijders (38) who suggested that adipose tissue triglycerides have a poor affinity for omega–3 polyunsaturated fatty acids.

The opposite phenomenon was seen with C22:5n–3, where the rise in gluteal fat tissue was 4- to 5-fold higher than that of the other two very-long-chain n–3 fatty acids relative to the amounts fed (Table 5). A possible explanation is again production of C22:5n–3 from EPA or DHA in tissues.

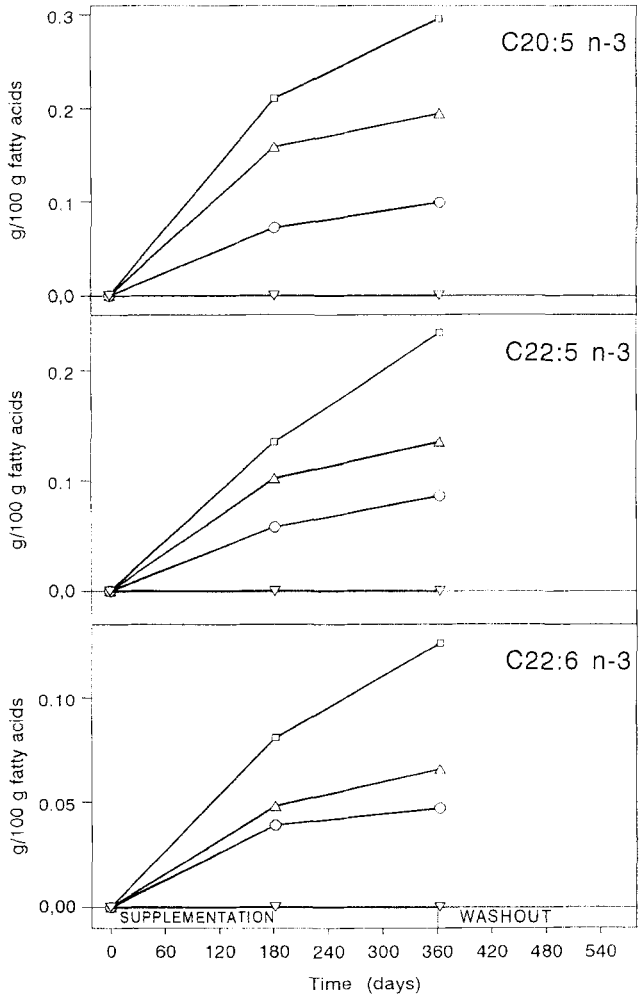


Fig. 4. Changes in the levels of very-long-chain n–3 polyunsaturated fatty acids in abdominal fat tissue aspirates in volunteers supplemented for 12 months with 0 g/d (▽), 3 g/d (○), 6 g/d (△), or 9 g/d (□) of fish oil rich in EPA. Values represent changes from baseline corrected for changes in the placebo (0 g/d) group. No abdominal samples were taken at the end of the washout period. Top: Eicosapentaenoic acid (EPA), C20:5n–3; middle: docosapentaenoic acid (DPA), C22:5n–3; bottom, docosahexaenoic acid (DHA), C22:6n–3.

Data from other studies on the level of n–3 fatty acids in body fat as a function of long-term intake are scarce. Leaf and coworkers (39) studied seven patients who had received 10–15 g of fish oil per day for periods of 12–27 months for the treatment of dyslipidemic disorders; five patients not on fish oil treatment plus three volunteers with a low fish intake served as controls. Levels of n–3 fatty acids were markedly elevated in adipose tissue as well as in all plasma lipid classes studied, but quantitative data on incorporation half-life and steady-state levels as a function of intake were not available.

There are a number of indications that fatty acids in abdominal fat tissue turn over more rapidly than those in gluteal fat tissue. Kather et al. (40) showed that the maximal effect of adrenaline on adenylate cyclase activity was larger in abdominal than in gluteal subcutaneous fat tissue. Field and Clandinin (41) studied the turnover of labeled fatty acids in human adipose tissue and found a half-life of 363 days for gluteal and of 134 days for abdominal fat. In our subjects levels of DHA and C22:5n–3 indeed rose more rapidly in abdominal than in gluteal fat (Figs. 3 and 4), but at day 363 they were still far below dietary levels. Either turnover times in adipose tissue were longer under our conditions, or the levels in abdominal adipose tissue never reach those in the diet.

Thus our fat tissue data do not allow estimation of absolute long-term intakes of (n–3) fatty acids, but they do show that subjects can be ranked by intake, which is all that is required in most epidemiological studies. Our data on cholesteryl esters and erythrocytes suggest that such measurements provide valid estimates of differences in intakes over the past weeks and months, respectively, and should thus be helpful in ranking subjects by intake of n–3 fatty acids in free-living populations.

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