Dietary Intake of Fish vs. Formulations Leads to Higher Plasma Concentrations of n-3 Fatty Acids

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ABSTRACT: The n-3 fatty acids from fish appear to be more efficacious, in terms of cardioprotection, than equivalent amounts provided as capsules. Volunteers were given, for 6 wk, either 100 g/d of salmon, providing 383 mg of EPA and 544 mg of DHA, esterified in glycerol lipids, or 1 or 3 capsules of fish oil/d, providing 150 mg of EPA and 106 mg of DHA or 450 mg of EPA and 318 mg of DHA, as ethyl esters. Further, we reevaluated data from a previous study carried out with the same design, i.e., with 3 and 6 capsules/d of fish oil, providing 1290 and 2580 mg/d EPA and 960 and 1920 mg/d DHA. Marked increments in plasma EPA and DHA concentrations (μg/mg total lipid) and percentages of total fatty acids were recorded at the end of treatment with either n-3 capsules or salmon. Net increments of EPA and DHA in plasma lipids were linearly and significantly correlated with the dose after capsule administration. Further, increments in plasma EPA and DHA concentration after salmon intake were significantly higher than after administration of capsules. The same increments would be obtained with at least two- and ninefold higher doses of EPA and DHA, respectively, if administered with capsules rather than salmon. We provide experimental evidence that n-3 fatty acids from fish are more effectively incorporated into plasma lipids than when administered as capsules and that increments in plasma concentrations of EPA and DHA given as capsules are linearly correlated with their intakes.

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Several "minor components" of the diet are indispensable for a number of vital processes. The essentiality of various nutrients and recognition of their healthful effects have, occasionally, promoted their utilization as formulated preparations for clinical studies. However, clinical trials have often proved such preparations to be less effective than what was anticipated by epidemiologic data based on the dietary intakes of the same bioactive compounds (1,2).

The n-3 fatty acids (FA) are essential components of functionally important cell membranes, e.g., in the cardiovascular and nervous systems, and they must be derived from the diet. The average Western diet provides <200–300 mg/d n-3 FA, mostly from fish, out of a total fat intake of >100 g/d. Several epidemiologic and clinical studies demonstrated the cardioprotective activities of these minor components (3,4). Controlled studies have also shown that fish rich in n-3 FA is cardioprotective (5) even when consumed in small amounts, i.e., a few grams/d (6). These data suggest that fish consumption is more efficacious in terms of biological effects than administration of formulated preparations, e.g., capsules, that provide comparable amounts of n-3 FA (6–8). Despite this apparent contradiction, the comparative effects on plasma n-3 status of the administration of n-3 when taken with food or as capsules have not been addressed specifically to date. Yet given the popularity of n-3 prescription in secondary prevention, this comparison may bear important therapeutic consequences.

We investigated the relationships between consumption of given amounts of salmon or administration of capsules containing EPA and DHA acids ethyl esters, i.e., the most widely used pharmaceutical form of n-3 administration, and n-3 plasma concentrations in healthy subjects. These compounds are derived exclusively from the diet, and to assess their status in the plasma compartment, conventional measurements of their plasma levels as percentages of total FA may not be completely adequate. In fact, this value provides information on relative changes, i.e., increments in certain FA are associated with reductions in others. Also, their measurements in selected lipid classes, e.g., in phospholipids (PL) as conventionally performed, may provide incomplete information because although DHA is preferentially associated with PL, EPA is appreciably associated also with other lipid classes such as cholesterol esters. Therefore, we included measurements of their concentrations in plasma lipids [μg/mg total lipids (TL)], in turn establishing the magnitude of the circulating n-3 FA pool and its increase after administration.

For comparative purposes, we also reanalyzed and included the results of a previous study, carried out by following exactly the same protocol, in which different preparations of EPA and DHA were administered to healthy individuals (9).

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Abbreviations: cps, capsule; FA, fatty acids; PL, phospholipids; TL, total lipids.

STUDY DESIGN

This study conforms with the ethical standards on human experimentation of the University of Milan and is in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Normolipidemic, healthy volunteers (ages, 26–38 yr; mean body mass index, 21.6 kg/m^2; mean total cholesterol concentration, 190.5 mg/dL; mean triglyceride concentration, 95.5 mg/dL) were recruited from within our Department, gave informed consent to the study, and were instructed to abstain from fish consumption during the 2 wk (T_{-2}) preceding the treatment, which was carried out for 6 wk. We did not control for alcohol intake, although subjects were instructed not to drink more than one alcoholic beverage per day.

Eight subjects (4 men and 4 women) were given 100 g/d of smoked salmon (Chinook, Tourin, Italy), which provided 383 mg of EPA and 544 mg of DHA (EPA/DHA 0.70). Two groups of four subjects each (6 men and 2 women) took 1 or 3 capsules of fish oil per day (Now Foods, Bloomingdale, IL), providing 150 mg of EPA and 106 mg of DHA or 450 mg of EPA and 318 mg of DHA as ethyl esters, respectively (EPA/DHA 1.41). These subjects were instructed to take the capsules with the main meal. No placebo oil was used because the aim of the study was to compare the effects of n-3 FA intake through two different sources. Also, n-3 FA are exclusively derived from the diet and therefore, unless ingested, no change will ever occur in their plasma lipids levels.

Blood was drawn by venipuncture from fasting subjects at –2, 0, and 6 wk (T_{-2}, T_0, and T_6) using heparin as anticoagulant; plasma was separated by centrifugation. In the previous study (9), performed by following the same protocol, three (1290 mg EPA and 960 mg DHA) or six (2580 mg EPA and 1920 mg DHA) 1-g capsules/d (Pharmacia, Upjohn, Milan, Italy) were given to two groups of eight healthy subjects each. The EPA/DHA ratio was 1.34. In the previous paper, however, relationships between intakes and plasma concentrations were not reported (9).

Quantification of administered n-3 FA. To evaluate the amount of n-3 FA in the different sources, several 50-g samples of salmon were ground, and five aliquots of the final mixture were extracted according to the method of Folch *et al.* (10). The profile of fatty acid methyl esters was subsequently analyzed and quantified by gas–liquid chromatography, as fully described by Marangoni *et al.* (11). Conditions were as follows: column Omegawax 320 (Supelco, Bellefonte, PA), 30 m, i.d. 0.32 mm, film thickness 0.25 μm. Gas chromatographic run programming: 1.2 min at 125°C, to 205°C at 2.5°C/min, 205 min for 20 min, to 220°C at 5°C/min, at 220°C for 30 min with programmable temperature vaporizing injector and flame ionization detector. Calibration standards were purchased from Nu-Chek-Prep (Elysian, MN). The n-3 FA contents in the capsules were also controlled by quantitative gas–liquid chromatography after derivatization to methyl esters (11).

Analysis of plasma FA. Lipids were extracted from plasma according to the method of Folch *et al.* (10) and quantified by microgravimetry. FA methyl esters were prepared from TL extracts, and plasma concentrations were determined by gas chromatography made quantitative by the use of 19:0 and 21:0 as internal standards (11). We report the values both as percentage of total plasma FA and as mg/mg total plasma lipids (TL).

Statistical analysis. Statistical analysis was carried out using a nonparametric method (Wilcoxon's matched pairs signed rank test) to allow for the limited number of participants (12). A value of $P < 0.05$ was considered significant. Statistics were performed by use of SPSS 11.0 for Windows (Chicago, IL).

RESULTS

The FA composition and contents of salmon and capsule lipids and their individual lipid classes are reported in Table 1. The major FA were 16:0 and 18:1, whereas n-3 FA (EPA, docosapentaenoic acid, and DHA) represented about one-third of total FA. The n-6 FA were found in small amounts, whereas among the monounsaturates, 20:1 and 22:1 were present in appreciable concentrations.

The total amounts of the n-3 FA, in the form of ethyl esters, in a 1-g capsule, were: Now Foods, USA EPA, 150 mg/capsule and DHA 106 mg/capsule; Pharmacia-Upjohn EPA 430 mg/capsule and DHA 320 mg/capsule.

In plasma, there was no appreciable change in triglyceride and cholesterol levels (not reported) after treatment, an expected finding because the subjects were normolipidemic.

In plasma lipids, there was no appreciable change in EPA and DHA percentage values and concentrations between T_{-2} and T_0; therefore, we report only differences between T_0 and

TABLE 1
Lipid Content and Fatty Acid Composition of Salmon and Capsules Administered to Volunteers[a]

Fatty acid	Salmon (%)	Capsules (%)
14:0	4.7	5.1
16:0	15.9	16.8
18:0	3.5	3.7
16:1	6.9	9.2
18:1	16.3	12.6
20:1	6.2	1.4
22:1	5.8	1.3
18:2	3.2	1.8
20:4	0.8	1.3
18:3	0.9	1.2
20:5	11.4	22.7
22:5	4.9	2.5
22:6	16.1	16.1
n-6	4.4	3.4
n-3	35.5	46.1
n-3/n-6	8.0	13.5
Unsaturation index	236	274
Total n-3 in salmon (mg/100 g)		Total n-3 in capsules (mg/capsules)
EPA	383	150
DHA	544	106

[a]The unsaturation index is Σ (% of each fatty acid × number of double bonds).

T_6 for all five treatments. Basal EPA ranged between 0.44 and 0.67% of total plasma FA in the different groups of the five studies, whereas DHA ranged between 1.38 and 1.97%. After 6 wk, the percentage values of EPA increased from 0.97 to 4.03 percentage points in the different experimental groups, whereas DHA increased from 0.91 to 3.07 percentage points.

Measurements of plasma FA concentrations allow the evaluation of the net changes associated with treatments. The initial plasma concentrations in the different groups ranged between 0.97 and 3.2 μg/mg TL for EPA and between 6.03 and 6.63 μg/mg TL for DHA (Table 2, which also reports FA concentrations at the end of treatments). DHA concentrations were substantially higher and more uniform throughout the various groups than those of EPA. The mg/mL plasma concentrations (not reported) were somewhat more variable than the values expressed as mg/mg TL. However the basal (T_0) values for DHA were rather uniform, ranging from 26.9 ± 7.2 (SD) in the salmon group to 35.9 ± 4.5 in the group taking 6 capsules from the previous study (9). Despite the different initial EPA and DHA plasma concentrations in the different groups, increments (μg/mg plasma TL) of both FA were linearly correlated with the doses after 6 wk of capsule administration ($R^2 = 0.98$ and 0.92, respectively (Figs. 1 and 2, respectively). Such increments were much smaller for DHA (Fig. 2) than for EPA (Fig. 1) (slopes: 1.34×10^{-3} and 9.74×10^{-3}, respectively). It can be extrapolated from the equations that increasing the intake by 100 mg/d for a 6-wk period results in a 0.97 mg/mg TL increment for EPA and in a 0.151 mg/mg increment for DHA. The increments of plasma EPA and DHA observed 6 wk after the consumption of salmon were substantially greater than those that can be extrapolated from the curves for equivalent intakes of these FA as capsules. In fact, to obtain the same increment in plasma EPA induced by 383 mg/d for 6 wk in salmon by using capsules, a dose of 800 mg, i.e., more than twofold, would be required; as for DHA, a dose of 4858 mg, i.e., almost ninefold compared with 544 mg given with salmon, would be required by using capsules.

DISCUSSION

The initial values of EPA and DHA expressed as mg/mg TL and especially as mg/mL plasma allow the assessment of the basal n-3 FA status. The values were rather uniform in the case of DHA, which can be synthesized from the precursor EPA through a rate-limiting and tightly controlled process involving peroxisomal reactions; this is more relevant in terms of both the quantitative aspects and the physiologic roles. Based on evaluations of plasma volume/body weight (i.e., 60 mL/kg body mass) and mg TL/mL plasma values, it can be calculated that the basal amounts of circulating DHA are ~1.6–2.1 mg/kg body weight.

This study, comparing different n-3 FA intakes, provides two new sets of information. First, by increasing and maintaining the intakes of EPA and DHA for a 6-wk period, in groups of normolipidemic subjects and using the same type of formulation, i.e., ethyl esters, there was a progressive increment of plasma levels that has a linear relationship with dose. It is of interest that the increments were related exclusively to the doses, and were independent of the initial concentration value. The situation in essence resembles what happens when a certain volume of fluid is added to a container in which some fluid is already present: The added volume simply adds up to the previous one and the increment is independent of the initial value. The data on the effects of salmon consumption were obtained with a single level of intake. The possibility that a similar relationship occurs with increasing intakes of salmon warrants further investigation.

Second, net increments of n-3 FA, notably those of DHA, after 6 wk of salmon consumption were much higher than after capsule administration. Plasma EPA increments after a fish meal providing a dose of 383 mg/d correspond to those obtained with a more than double dose of EPA administered as capsules, and the difference is even greater in the case of DHA (an almost ninefold greater dose would be required using capsules rather than fish to obtain the same increment in plasma).

The greater "bioavailability" of n-3 FA from fish than from capsules could be attributed to the association of n-3 with a larger amount of fat, as part of 100 g of ingested food, ensuring administration in a diluted form. In addition, EPA and DHA in fish are esterified mainly in the *sn*-2 position of triacylglycerols and glycerophospholipids, which represent a significant proportion of fish lipids. The glycerol *sn*-2 position is to a large extent preserved from hydrolysis during digestion and intestinal absorption of exogenous fat (13).

Conversely, capsules provide a small lipidic bolus and, in the eventual absence of a concomitant intake of other fats, although the subjects were instructed to do so, the processes of lipid absorption may not be adequately activated. The results

TABLE 2
Plasma Concentrations of EPA and DHA Before (T_0) and After (T_6) 6 Wk of Administration of Either Salmon or Fish Oil Capsules[a,b]

		EPA T_0	EPA T_6	DHA T_0	DHA T_6
		(mg fatty acid/total lipids)			
Fish	($n = 8$)	1.68 ± 0.66	10.58 ± 3.14[c]	6.41 ± 1.15	15.11 ± 2.25[c,d]
1 capsule current study	($n = 4$)	1.58 ± 0.39	4.21 ± 1.62[c]	6.69 ± 1.47	8.77 ± 1.71
3 capsules current study	($n = 4$)	0.97 ± 0.42	6.28 ± 2.01[c]	6.03 ± 1.48	9.08 ± 2.24
3 capsules previous study (9)	($n = 8$)	2.87 ± 0.37	16.59 ± 0.28[c]	6.16 ± 0.32	9.54 ± 0.69[c]
6 capsules previous study (9)	($n = 8$)	3.28 ± 0.34	29.57 ± 3.01[c]	6.51 ± 0.37	11.41 ± 0.4[c]

[a]See study design section for experimental details.
[b]Data are means ± SD.
[c]Different from T_0, $P < 0.05$.
[d]Different from 3 capsules in current and previous studies $P < 0.05$.

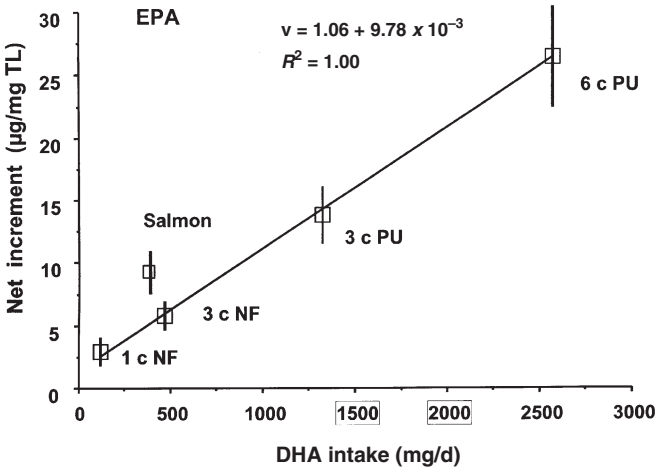

FIG. 1. Plasma net increments of EPA after supplementation of healthy volunteers with either fish oil capsules or salmon. Data are means ± SD. NF refers to the capsule data from the current study (1 and 3 capsules of fish oil per day from Now Foods, USA, Bloomingdale, IL), whereas PU refers to capsule data reported in Tremoli *et al.* (9) (3 and 6 capsules of fish oil per day from Pharmacia Upjohn, Milan, Italy). TL, total lipids; c, capsules.

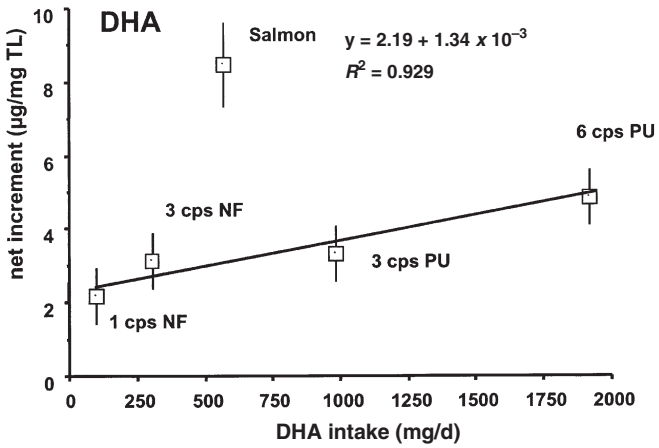

FIG. 2. Plasma net increments of DHA after supplementation of healthy volunteers with either fish oil capsules or salmon. Data are means ± SD. NF refer to the capsule data from the current study (1 and 3 capsules of fish oil/d from Now Foods, USA), whereas PU refers to capsules data reported in Tremoli *et al.* (9) (3 and 6 capsules of fish oil per day from Pharmacia Upjohn). TL, total lipids; cps, capsules. For manufacturers

clearly indicate that fish is more efficient than capsules in providing n-3 FA. In turn, these data might explain why fish consumption, even with low doses and infrequent consumption, is highly protective toward cardiovascular disease. From our data, it could be postulated that 10–20 g salmon/d would effectively raise plasma DHA levels.

It should be considered, however, that treatments were carried out only for 6 wk and that n-3 FA tend to be maintained in plasma and cell lipids for long time periods after their intake is interrupted (11); therefore, long-term and regular intakes of n-3 FA with capsules might attain plasma levels comparable to those obtained with fish intake. A "pharmacological" treatment may be recommended when fish consumption is not accepted or is not feasible.

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