Enteral Absorption in Man of Eicosapentaenoic Acid in Different Chemical Forms

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After administering the equivalent of 1 g of eicosapentaenoic acid (EPA) in four different chemical forms, the kinetics of EPA incorporation into plasma triglycerides (TG) were compared by gas liquid chromatography on a capillary column following separation of the lipid fraction by thin layer chromatography.

EPA incorporation into plasma TG was markedly smaller and later when EPA was administered as an ethyl ester rather than as EPA free fatty acid, EPA arginine salt or 1,3-dioctanoyl-2-eicosapentaenoyl glycerol (2-EPA). Our results and the data in the literature are compatible with the hypothesis that 2-EPA is absorbed with minimum hydrolysis and escapes random distribution between the other positions of the glycerol molecule during the absorption process.

Lipids 22, 711-714 (1987).

Dietary fat modification is now considered to be an effective tool for modifying the phospholipid fatty acid composition of cell membranes, especially in blood platelets. The effect of dietary manipulation and the metabolism of polyunsaturated fatty acids (PUFA) have been extensively studied in the aim of influencing the physiological responses mediated by the oxygenated metabolites of the eicosapolyenoic acid cascade.

Several studies have been devoted to the action and metabolism of eicosapentaenoic acid (EPA) in animals or in man, especially its antithrombotic effect (1-7). Owing to the greater stability of esterified PUFA vs free fatty acids, and to the potential toxicity of methanol released by methyl ester hydrolysis, many of the studies concerning dietary manipulation with EPA have used the ethyl ester of this acid, as in a recent study by Tamura et al. (8). However, some data in the literature raise questions about the relative difficulty of its hydrolysis by pancreatic lipase (9-11); this difficulty could result in some impairment of its subsequent intestinal absorption.

For this reason, we compared in this study the kinetics of EPA incorporation into plasma triglycerides (TG) in man after ingestion of four chemical forms of EPA: ethyl ester, free fatty acid, arginine salt and triglyceride (1,3-dioctanoyl-2-eicosapentaenoyl glycerol [2-EPA]). The kinetics of this incorporation after EPA ethyl ester ingestion differed strikingly from the kinetics following ingestion of the three other forms.

MATERIALS AND METHODS

Reagents. Free fatty acid, arginine salt, ethyl ester of EPA and 2-EPA of pure analytical grade were generously provided by Roussel-Uclaf Laboratories (Romainville, France).

The purity of the chemical forms was checked by gas chromatography-mass spectrometry; when useful, this was completed by NMR spectroscopy.

Organic solvents, hexane (Uvasol), chloroform, methanol, inorganic compounds and 2′,7′-dichlorofluorescein were from E. Merck (Darmstadt, FRG). The lipid and fatty acid standards were from Fluka (Buchs, Switzerland) or Sigma Chemical Co. (St. Louis, MO).

Experimental protocol. Eight normal volunteers, 25-29 years of age, were given the equivalent of 1 g of EPA per os at 8 a.m. after an overnight fast; immediately afterward they ate a light lipid-free breakfast. Under the same conditions, four of the subjects received the four chemical forms of EPA, each form taken at subsequent 1-wk intervals. The other four received only two forms, the free fatty acid and 2-EPA.

Blood samples (7 ml on ethylene diamine tetraacetate) were taken 0, 1, 2, 3, 4, 5, 6, 9, 12 and 24 hr after EPA ingestion. At 6 and 12 hr the samples were taken just before meals of low lipid content, and at 0 and 24 hr after an overnight fast.

Analytical methods. Plasma lipids were extracted by chloroform/methanol according to the method of Folch et al. (12). The lipid classes were separated by thin layer chromatography on Kieselgel 60 F 254 (Merck) using hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as the developer. The lipid fractions were detected under UV light after spraying the plates with 2′,7′-dichlorofluorescein. The TG fraction was scraped from the plates and transesterification was performed for 30 min at 80 C in methanol/sulfuric acid (19:1, v/v) without extraction from the gel. The methyl esters extracted with hexane were analyzed by gas liquid chromatography using a Fractovap 2900 (Erba Science) chromatograph, FFAP capillary columns (25 m × 0.32 mm) and a flame ionization detector at 250 C. The injection temperature was 230 C, and the oven

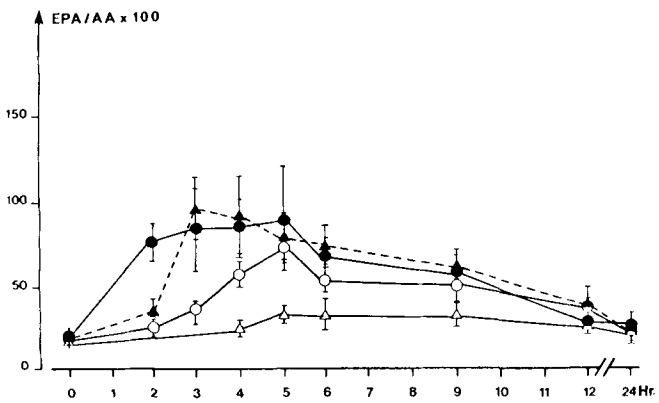

Fig. 1. EPA/AA × 100 ratio variation in plasma TG; mean value (± SEM) after ingestion of 1 g of EPA in the form of 2-EPA (○) free EPA (▲), arginine EP (•) and ethyl EP (△).

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Abbreviations: PUFA, polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; 2-EPA, 1,3-dioctanoyl-2-eicosapentaenoyl glycerol; EP, eicosapentaenoate; TG, triglyceride.

was programmed to increase the temperature 10 C/min from 50 C to 150 C, and then 2 C/min from 150 C to 200 C. The esters were identified by their retention times with respect to pure fatty acid standards. The relative proportions of EPA and arachidonic acid (AA) were evaluated using a digital integrator (I.C.A.P-5 or Spectra-physics).

RESULTS

After 1 g EPA was ingested, its absorption was followed by substantial incorporation into plasma TG characterized by a rise in the ratio of EPA to AA in the plasma lipid fraction. The different absorption rates of EPA as given in the four different chemical forms were estimated from the corresponding variations of the ratio of EPA to AA in plasma TG.

Figure 1 compares the variation of the mean values of the ratio of EPA to AA in plasma TG after ingestion of the four different chemical forms. There is a striking difference among the kinetics observed after administration of EPA as ethyl ester and the three other chemical forms. When administered as ethyl ester, the rise of EPA in plasma TG was about three times less and occurred at least 3 hr later than with the three other chemical forms. The difference between EPA ethyl ester and the other forms is statistically significant ($p < 0.05$ at 5 and 6 hr), as is the increase in EPA/AA after ethyl ester ingestion ($p < 0.05$ at 5, 6 and 9 hr. vs control at 0 hr).

Tables 1-4 detail the individual variations of the ratio of EPA to AA in plasma TG after ingestion of the four different chemical forms of EPA.

After ingestion of an amount of the TG 2-EPA equivalent to 1 g of EPA, the ratio of EPA to AA increased in all eight subjects (Table 1). The maximum (3 to 10 times the basal value) was observed 4 or 5 hr after ingestion of TG. The maximum of the mean values (four to five times the mean basal value) was observed between 4 and 5 hr.

When the subjects were given 1 g of free EPA (Table 2), the increase in the ratio of EPA to AA was rapid; the maximum occurred at 3 hr and reached 5 to 12 times the initial value (mean value 5.5).

The variation of the ratio of EPA to AA after ingestion of an equivalent of 1 g of EPA as arginine salt was similar to that of 2-EPA; the maximum of the mean value is five times the basal value and occurred at 5 hr.

After ingestion of the ethyl ester of EPA, the ratio of EPA to AA in plasma TG rose slowly, and the maximum of the mean value was only two times higher than the basal value and was delayed until 9 hr.

TABLE 1

EPA/AA × 100 Ratio Variation in Plasma TG After Ingestion of 1 g of EPA in the Form of 2-EPA ($\bar{X}$ ± SEM)

Time (hr)	Subjects								Mean ± SEM		
	1	2	3	4	5	6	7	8	$\bar{X}$		
0	15	22	20	15	15	10	10	12	15	±	1,5
2			37		15	15	12	25	21	±	4,6
3				42	50	15	10	25	28,5	±	7,7
4	50	48	150	27	65	50	25	25	55	±	14,5
5	100	140	72	60	50	65	30	30	68	±	13
6	60	85	56	32	40	70	30	35	51	±	7
9		90	55	45	50	40	25	15	46	±	9
12	45	70	60	30	20	20	20	15	35	±	7,4
24	30	22	15	35	10	25	15	12	21	±	3

TABLE 2

EPA/AA × 100 Ratio Variation in Plasma TG After Ingestion of 1 g of free EPA ($\bar{X}$ ± SEM)

Time (hr)	Subjects								Mean ± SEM		
	1	2	3	4	5	6	7	8	$\bar{X}$		
0	22	22	20	22	20	22	12	9	17,4	±	2
2			20	41	45	25	17		30	±	5,6
3		172	140	131	100	65	25	30	95	±	21
4	52	250	62	68	65	145	35	37	89	±	26
5	57	170	56	77	65	95	46	46	76	±	15
6	107	110	36	47	60	85	55	70	71	±	9,6
9	83	75	46	50	45	95	50	20	58	±	8,6
12	55	62	46	50	20	30	20	15	37	±	6
24	32	52	22	15	10	25	15	12	22	±	3

DISCUSSION

The percentage of AA in plasma TG can be considered constant over the 24-hr period studied here, since this fatty acid was not supplied by food during this period. Under these conditions, the kinetics of EPA absorption can be estimated from the variation of the ratio of EPA to AA in plasma TG. This method has been used successfully by others (13). Our results show significant modifications of the ratio of EPA to AA in plasma TG after ingestion of the equivalent of 1 g of EPA in different chemical forms.

In spite of several individual variations, common characteristics clearly appeared, e.g., there was a 5- to 12-fold rise in the ratio of EPA to AA in 19 of the 20 experiments in which EPA was ingested as TG (2-EPA), free fatty acid and arginine salt. However, after ingestion of EPA ethyl ester, the ratio of EPA to AA increased only 1.5- to 3-fold over the basal value. This indicates that EPA was less incorporated into plasma TG after ingestion of the ethyl ester than after ingestion of the three other forms, but it does not imply that EPA ethyl ester is an ineffective therapeutic agent. The rise in the EPA/AA ratio is statistically significant at 5, 6 and 9 hr vs control at 0 hr. EPA ethyl ester has, in fact, been shown to be able to modify platelet function. The results obtained here with EPA ethyl ester are in agreement with data in the literature (14), e.g., a twofold rise above the basal level was obtained by Nagakawa et al. (5) even after repeated administration and by Tamura et al. (14) even after administration of a higher dose (3.5 g/day).

EPA was less readily incorporated into plasma TG after ingestion of ethyl ester than after ingestion of the TG (2 EPA), free fatty acid or arginine salt. The difference could result from impairments at the EPA ethyl ester hydrolysis step, at the EPA absorption step or from interference with some unknown process involved in the PUFA absorption mechanism. EPA is well absorbed when given as free fatty acid or arginine salt. The difference is possibly the result of poor hydrolysis of EPA ethyl ester by pancreatic lipase and a subsequent decrease in the incorporation of EPA ethyl ester into the mixed micelle. This resistance could result from both the EPA and the ethyl components of the molecule. An unusual resistance of the ethyl esters was in fact reported in 1958, i.e., the hydrolysis of ethyl oleate was found to be less than the hydrolysis of triolein (9), and the hydrolysis of ethyl esters was generally less than the hydrolysis of the homologous methyl esters (10,11). EPA structure is also involved in the relatively low susceptibility of EPA esters to hydrolysis by pancreatic lipase. Hydrolysis of eicosapentaenoyl or docosahexaenoyl glycerides is less than hydrolysis of oleyl glycerides (15,16) even when these fatty acids are situated at positions 1 and 3 on the triglyceride molecule, which are known to be the preferential sites of pancreatic lipase action (17). This resistance has been related to the proximity of the first double bond to the carboxyl group (17,18). Although the EPA glyceryl or docosahexaenoyl glycerol esters are resistant to hydrolysis by pancreatic lipase even at positions 1 and 3, the n-3 docosapentaenoyl glycerol is not (19). In the last compound, five methylene groups separate the ester function from the nearest double bond vs only two or three in the other molecules. Thus, $\Delta 4$ and $\Delta 5$ polyunsaturated fatty acids such as docosahexaenoic, eicosapentaenoic (and probably arachidonic) acids are scarcely released from their glyceride combinations by pancreatic lipase. On the other hand, $\Delta 7$ fatty acids such as n-3 docosapentaenoic acid are readily released by this enzyme and more easily available for further metabolism. A steric hindrance by the ω methyl group has also been suggested (16,19) to be the cause for the resistance of esters of the other fatty acids.

If such a resistance to the digestive enzymatic process impairs EPA ethyl ester hydrolysis, the same must hold for 2-EPA, especially when the ester bond is at position 2, which is resistant to pancreatic lipase. However, in all of the subjects studied, EPA incorporation into plasma TG was much greater after ingestion of 2-EPA than of ethyl ester. It is possible that the absorption of the ethyl ester takes place without prior enzymatic hydrolysis. This hypothesis is now under investigation.

TABLE 3

EPA/AA × 100 Ratio Variation in Plasma TG After Ingestion of 1 g of EPA in the Form of Arginine EP ($\bar{X}$ ± SEM)

Time (hr)	Subjects				Mean ± SEM		
	1	2	3	4	$\bar{X}$		
0	22	17	13	15	17	±	2
2			60	82	71	±	11
3		70	40	132	81	±	27
4	60	130	70	76	82,5	±	16
5	160	90	35	70	89	±	26
6	85	32	60	75	63	±	11
9	75	32	45	70	56	±	10
12	40	20		20	27	±	6,7
24	21	32	20	23	24	±	2,7

TABLE 4

EPA/AA × 100 Ratio Variation in Plasma TG After Ingestion of 1 g of EPA in the Form of Ethyl Ester ($\bar{X}$ ± SEM)

Time (hr)	Subjects				Mean ± SEM		
	1	2	3	4	$\bar{X}$		
0	16	16	12	12	14	±	1,2
2	—	—	—	—		—	
3	—	—	—	—		—	
4	22	21	15	22	20	±	2
5	35	32	14	36	28	±	4,7
6	35	30	20	26	28	±	3,1
9	38	32	17	38	31	±	5
12	30	25		20	25	±	2,9
24	30	20	18	20	22	±	2,7

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[Received July 22, 1986]