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NORMALIZATION BY DIETARY COD-LIVER OIL OF REDUCED THROMBOGENESIS IN ESSENTIAL FATTY ACID DEFICIENT RATS

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ABSTRACT

Rats, deficient in essential fatty acids (EFA), were given diets containing 5 energy% sunflowerseed oil (SO, rich in linoleic acid), cod-liver oil (CLO, rich in timnodonic acid and cervonic acid), or hydrogenated coconut oil (HCO), containing no EFAs at all. SO and CLO feeding resulted in normalization of the reduced arterial thrombus formation in EFA-deficient animals. SO feeding was associated with the normalization of the arachidonic acid content of platelet phospholipids. CLO feeding did not have this effect but greatly increased the availability of timnodonic acid (EPA) and cervonic acid (DHA). Further reseach is required to investigate whether these changes in fatty acid composition can be hold responsible for the normalizing effect of dietary CLO on the disturbed arterial thrombosis tendency in EFA deficient rats, possibly via the formation of eicosanoids.

INTRODUCTION

Essential fatty acids (EFAs) are important structural elements of cellular membranes because they are involved in the homeostasis of membrane fluidity and, thereby, in the regulation of cellular function. Certain EFAs, moreover, are the precursor fatty acids of prostanoids, leukotrienes and hydroxy fatty acids, many of which are important physiological regulators in their own right.

The importance of EFA for normal physiological functions in clearly illustrated in EFA-deficiency, which has been observed in a large number of animal species as well as in man, and which causes a great variety of deficiency symptoms (1-3).

Key words: Arterial thrombosis, essential fatty acid deficiency, rat, fatty acid, phospholipid, blood platelet

In 1974 we demonstrated that in EFA deficient rats certain functions of blood platelets are fundamentally disturbed, leading to a highly reduced arterial thrombosis tendency (4). Oils rich in linoleic acid, 18:2(n-6), were able to restore platelet function and arterial thrombus formation, demonstrating that linoleic acid and/or its longer-chain, more unsaturated derivatives are essential for normal thrombotic functions (5), possibly by increasing the formation of prothrombotic thromboxane A_2 (TxA_2), which is reduced in EFA-deficiency (6). However, the effect of linoleic acid may have been accomplished also by normalization of the fatty acid composition of the plasma membranes and, thereby, by restoration of normal membrane function.

Columbinic acid, 18:3(n-6c,9c,13t), is able to take over various structural functions of linoleic acid (7,8), but it is not elongated and, consequently, it is not a prostanoid precursor (7,9). Feeding columbinic acid to EFA deficient rats did not normalize the disturbed arterial thrombosis tendency in these animals (10), suggesting that normalization of the structural membrane defects in EFA-deficiency per sé is insufficient to regain normal platelet function and arterial thrombus formation. Consequently, the normalization of disturbed thrombus formation in EFA-deficient rats, as achieved by feeding linoleic acid, mainly requires restoration of prostanoid production.

The same suggestion follows from the work by MacDonald and all (11), who studied cats which are unable to synthesize significant amounts of arachidonic acid from linoleic acid. In animals fed an EFA-deficient diet, linoleic acid supplementation did not normalize the reduced platelet aggregation in response to collagen.

Cod-liver oil, containing timnodonic acid (20:5(n-3), eicosapentaenoic acid, EPA) and cervonic acid (22:6(n-3), docosahexaenoic acid, DHA), has been shown to have an antithrombotic effect in rats, most probably because it lowers the arachidonic acid (20:4(n-6), AA) content of membrane phospholipids and, thereby, the formation of prothrombotic TxA_2 (12). Since prostanoids of the 3-series are hardly formed (13), it can be expected that feeding polyunsaturated fatty acids (PUFAs) of the (n-3) family to EFA-deficient animals, just like columbinic acid, does not normalize the disturbed thrombotic functions in EFA-deficient animals. We now demonstrate, however, that the administration of cod-liver oil, rich in (n-3) PUFAs, to EFA-deficient rats increases the thrombosis tendency of these animals to normal values. The possible implications of this finding for the regulation of arterial thrombus formation will be discussed.

ANIMALS, MATERIALS AND METHODS

Newly weaned male Wistar rats, specific pathogen free, were housed at a temperature of 23°C and a relative humidity of 55% and were given a fat-free diet (Table 1) for 13 weeks. During this period they develop an EFA-deficiency, as is indicated by an increased trans-epidermal water loss (14) and by typical changes in the fatty acid profile of serum, liver and platelet lipids (15, data not shown). Subsequently, the animals were stratified over 4 groups of 16 animals each, so that the average body weight per group was about equal. One group continued to consume the fat-free diet. The other groups were given diets (Table 1) containing 5 en% sunflowerseed oil (SO, rich in linoleic acid), cod-liver oil (CLO, rich in (n-3) PUFAs, or hydrogenated coconut oil (HCO, containing no PUFAs at all). The fatty acid composition of the dietary oils is given in Table 2. The diets were freshly prepared 3 times a week and

TABLE 1

Composition of Experimental Diets (g/1000 kCal).

Ingredients	Fat free (FF)	Fat containing (FC)	en % FF	en % FC
Casein	62.0	62.0	23	23
Minerals[1)]	5.4	5.4		
Vitamin mix[1)]	1.0	1.0		
Cellulose	15.0	15.0		
Sucrose	187.8	175.6	77	72
Experimental oils	0.0	5.4		5
Total	271.2	264.4	100	100

1) For detailed composition: see ref. 16

kept in daily portions at 4 oC under nitrogen to reduce oxidation.
After 8 weeks of feeding, arterial thrombosis tendency of the animals was determined by measuring the time (Obstruction time, OT) between insertion and complete obstruction of a loop-shaped polyethylene canula, inserted into the abdominal aorta (17). At least 3 weeks after loop obstruction, some animals

TABLE 2

Fatty Acid Composition of Experimental Oils
(% of Total Fatty Acids)

Fatty acid	HCO	SO	CLO
8:0	4.0		
10:0	9.5		
12:0	43.2		
14:0	19.4		4.1
16:0	10.4	6.4	10.0
16:1(n-7)		0.3	9.2
17:0			1.0
18:0	13.0	4.6	2.1
18:1(n-9)		15.5	24.3
18:2(n-6)		70.8	2.1
18:3(n-3)		0.4	0.6
18:4(n-3)			3.4
20:0		0.4	
20:1(n-9/11)		0.3	12.0
20:4(n-3)			0.7
20:5(n-3)			15.2
22:0		0.1	
22:1(n-9/11)		0.8	3.0
22:5(n-3)			0.5
22:6(n-3)			10.4
24:0		0.2	
24:1(n-9)			1.0

were bled under ether anaesthesia. Blood was collected in EDTA and platelets were isolated and washed free from plasma as described before (18). The platelets were pooled per 4 animals and used to measure the fatty acid composition of the platelet phospholipids as described elsewhere (18).

RESULTS

Arterial thrombosis tendency

In Table 3 the average obstruction times per group are given. As demonstrated before (4,5), EFA-deficiency is associated with a long OT, which reflects a reduced arterial thrombosis tendency. This condition is normalized by feeding a diet containing 5en% SO (ca. 3en% linoleic acid). The same amount of HCO, containing no essential fatty acids, does not affect the disturbed thrombosis tendency of the EFA-deficient animals in a significant way. 5 en% cod-liver oil in the diet, however, has a normalizing effect on the arterial thrombosis tendency of EFA-deficient rats: OTs of CLO-fed animals do not differ significantly from those after feeding the SO-containing diet but are significantly ($P_2<0.01$) shorter than those of the EFA+HCO group (Bonferroni inequality test).

TABLE 3

Effect of Dietary Sunflowerseed Oil (SO), Hydrogenated Coconut Oil (HCO) and Cod-Liver Oil (CLO) on the Arterial Thrombosis Tendency of Essential Fatty Acid (EFA) Deficient Rats. Obstruction Time (OT, hours)

Group	n	log OT ± sem	OT (h)
EFA-deficient	16	2.30 ± 0.064	201
EFA + HCO	16	2.43 ± 0.076	269
EFA + SO	16	2.06 ± 0.039	115
EFA + CLO	15	2.12 ± 0.048	132

Fatty acid composition of platelet phospholipids

The fatty acid composition of platelet phospholipids is given in Table 4. EFA-deficiency is associated with low amounts of (n-6) and (n-3) PUFAs, whereas appreciable amounts of mead acid, 20:3(n-9), are found, as well as of its elongation product dihomo-mead acid, 22:3(n-9). Feeding with SO, which is rich in linoleic acid, greatly increases the presence of (n-6) PUFAs, and of arachidonic acid in particular, whereas the CLO diet, low in linoleic acid, but containing large amounts of the (n-3) PUFAs, does not increase the (n-6)PUFA content but the amounts of the (n-3)PUFAs instead. Both PUFA-rich oils greatly lower the amounts of mead acid and dihomo-mead acid.

DISCUSSION

In essential fatty acid deficient rats, arterial thrombus formation is greatly disturbed (4,5). This indicates that EFAs have a major role in normal thrombogenesis, either because they are important structural membrane components, and/or because they serve as precursor fatty acids for the formation of prostanoids, prothrombotic thromboxane A_2 (TxA_2) in particular.

An important role of TxA_2 in our model of arterial thrombogenesis is supported by the negative relationship between arterial thrombus formation and platelet TxA_2 production, which was observed after feeding various dietary fats (10). Studies with columbinic acid, which has similar structural functions as linoleic acid (7,8) but is not a prostanoid precursor (9), demonstrated that restoration of 'membrane structure' alone is insufficient to normalize arterial thrombus formation. This indicates that prostanoid formation is instrumental in normal thrombogenesis (10).

TABLE 4

Fatty Acid Composition of Platelet Phosholipids
(% of Total Fatty Acids)

Fatty acid	EFA-def	HCO	SO	CLO
12:0	-	0.1	-	0.1
14:0	0.8	1.0	0.7	1.0
16:0 dma	1.2	1.6	1.8	2.0
16:0	27.3	26.5	28.6	29.8
16:1(n-7)	3.0	3.4	2.1	4.1
17:0	0.6	-	0.6	1.1
18:0 dma	0.7	1.7	1.8	2.1
18:0	11.7	12.4	13.4	11.5
18:1(n-9)	20.1	16.7	10.1	15.2
18:2(n-6)	1.6	0.7	3.4	0.9
18:3(n-6)	0.2	0.3	-	-
18:3(n-3)+20:0	0.6	0.3	0.4	0.3
20:1(n-9)	1.4	1.3	0.8	1.3
20:3(n-9)	15.5	17.7	0.4	1.3
20:3(n-6)	-	-	0.5	0.4
20:4(n-6)+22.0	5.6	6.1	25.2	6.1
20:5(n-3)	0.2	0.2	tr(1)	12.7
21:0	0.5	0.4	-	-
22:1(n-9)	0.7	0.7	0.8	1.2
22:3(n-9)	3.6	3.9	0.4	0.2
22:4(n-6)	0.7	0.7	5.5	0.8
22:5(n-3)	0.2	-	0.4	3.0
22:6(n-3)	-	-	-	2.8
24:1(n-9)	1.9	2.2	2.2	1.7
(n-3)	1.0	0.5	0.8	18.8
(n-6)(2)	8.1	7.8	34.6	8.2
(n-9)	43.2	42.5	14.7	20.9
Unidentified	1.9	2.1	0.9	0.4

(1) trace
(2) minor amounts of 20:0 and 22:0 included

To further support this view, EFA-deficient rats were fed with (n-3) PUFAs which, at least partly, also restore normal membrane function in EFA deficiency (19), but which are poor substrates for prostanoid formation in rats (20-24). Moreover, the thrombogenicity of TxA_3 is thought to be much smaller than that of TxA_2 (22,23). Therefore, if prostanoids are instrumental in normal thrombogenesis, CLO feeding of EFA-deficient animals can be expected to

have no major influence on the disturbed arterial thrombogenesis in EFA-deficient animals. However, this did not appear to be true: a diet containing 5 en% CLO and fed for 8 weeks to EFA-deficient animals normalized the disturbed arterial thrombogenesis almost as efficiently as did SO, which is rich in linoleic acid (Table 3). Unfortunately, the design of the present study did not allow us to compare SO with CLO for their effectiveness in this respect, because the feeding time was rather long, which may have obscured a more rapid action of either of the two oils.

The normalization of the arterial thrombosis tendency by SO is associated with a considerable increase in the amount of arachidonic acid in platelet phospholipids (Table 4). Since AA availability is a major determinant of the stimulated prostanoid formation in vitro (18), SO feeding will have increased the potency of activated platelets to produce prothrombotic thromboxane A_2, as has been demonstrated before (10,18). It is likely that the normalization of arterial thrombogenesis upon SO feeding to EFA deficient animals results from this improved TxA_2 production capacity (10).

CLO feeding to EFA deficient animals does not increase the AA content of their platelet phospholipids (Table 4). Consequently, the potential of these platelets to produce TxA_2 will not have increased. Actually, a further decrease may be expected, since timnodonic acid, present in large amounts upon CLO feeding, may successfully compete with the low amount of AA for the cyclooxygenase (20, 23-26). This TA may also provide the potential for the formation of TxA_3 by activated blood platelets. Although in earlier studies with fish oil-fed animals the formation of TxA_3 could not be detected (13), the very high TA/AA ratio in the platelet phospholipids of the present CLO group may enable the formation of TxA_3 after all. Preliminary studies indicated that CLO feeding further reduced the formation of platelets TxA_2 indeed, whereas some TxA_3 was formed instead (Data not shown).

Studies with human platelets indicated that TxA_3 is less thrombotic than TxA_2 (22,23), but is should be realized that this is due to the concomittant production of PGD_3, which has stronger anti-platelet properties than PGD_2 (26). In the rat, however, PGD_2 hardly inhibits platelet aggregation (27), and if this holds for PGD_3 as well, TxA_3 might, after all, be responsible for the normalization by CLO of the arterial thrombosis tendency in EFA deficient animals. This has to be investigated in further studies.

TA is a good substrate for the lipoxygenase of rat, rabbit and human platelets an can be easily converted into (5Z, 8Z,10E, 14Z, 17Z)-12-L-hydroxyeicosapentaenoic acid, HEPE (13, 28, 29). The endogenously formed lipoxygenase product of arachidonic acid, HETE, has been demonstrated to promote platelet aggregation under certain conditions (30,31). Further research is required to investigate the role of HEPE in this respect, and of the lipoxygenase products of clupanodonic acid (22:5(n-3), see ref. 32) and cervonic acid (33), the availability of which also increases upon feeding CLO (Table 4, see also refs. 12 and 18).

The amount of mead acid is high in the platelet phospholipids of both EFA deficient groups and low in both PUFA-fed groups. This could imply that the presence of mead acid may result in an antithrombotic condition. However, the lipoxygenase product of mead acid has been shown to stimulate thrombin-induced platelet aggregation (34), which is not consistent with an antithrombotic effect of mead acid. Moreover, feeding columbinic acid to EFA deficient rats lowers the amount of mead acid in their platelet phospholipids (35) but does not normalize the disturbed thrombogenesis in EFA-deficient rats (Table 3). Therefore, mead acid is unlikely to be of major importance in thromboregulation.

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