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THE PROTECTIVE EFFECTS OF DIETARY FISH OIL ON FOCAL CEREBRAL INFARCTION

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ABSTRACT

The protective effect of the (n-3) fatty acids in menhaden fish oil on acute cerebral ischemia was investigated in cats. Cerebral ischemia was produced by ligation of the left middle cerebral artery of cats fed either a basal diet of feline cat chow or the basal diet supplemented with 8% of the calories as menhaden oil for 18-24 days. Fatty acid esters of 20:5(n-3) were increased and the 18:2(n-6) decreased in the heart and liver of cats fed supplemental fish oil, but the brain lipid showed no effect of the diet. We found that the neurological deficit and the volume of brain infarction in the group treated with fish oil was less than that of the control group. The present findings suggest that moderate dietary supplements of fish oil may be beneficial in the prophylactic treatment of ischemic cerebral vascular disease

INTRODUCTION

A variety of oxidized products of arachidonic acid (prostaglandins, thromboxanes, SRSA) are important in regulating the homeostatic integrity of the vascular system. The synthesis of these materials can occur only from substrates derived from dietary polyunsaturated fatty acids (PUFA) and therefore the abundance of ingested PUFA precursors can influence the onset of thrombosis and vasospasm. There are two main classes of dietary polyunsaturated acids, (n-6) and (n-3), and the (n-6) class of PUFA contains the precursors of the principal type of prostaglandins (the dienoic series; e.g. PGE_2, $PGF_{2\alpha}$,PGI_2,TXA_2) The (n-3) class of PUFA contains inhibitors (1) of the synthesis of the dienoic (n-6) prostaglandins and thromboxanes. Also, one member of the (n-3) class, eicosapentaenoate (20:5(n-3)), is the precursor of the trienoic series of prostaglandins which is proposed to include an anti-aggregating agent, PGI_3 (2).

Considerable attention has been given in the past to the beneficial effects of polyenoic acids in dietary fats, but much less attention has been given to specific differences among the types of polyenoic acids. This situation may change with the recent increased interest in identifying interventions that will reduce infarct size following coronary and/or cerebral artery occlusion. Reports of the low incidence of acute myocardial infarction in Eskimos in Greenland (3,4) have refocused attention upon the role of diet in regulating the abundance of prostaglandins in human tissues. The high concentration of the (n-3) class of fatty acids (especially 20:5 and 22:6) in oils from fish and marine animals contrasts markedly with the composition of PUFA in grain oils and meats that are rich in the (n-6) type of PUFA. The high content of (n-3) fatty acids in fish may be one of the factors causing the low incidence of infarction in Eskimos.

Our current knowledge of prostaglandin metabolism should help shift future dietary considerations away from the total amount of PUFA in the diet to the nutritional balance among specific types ((n-3) vs. (n-6)) of polyunsaturated acids. To help determine the degree to which dietary PUFA can influence cerebrovascular integrity, we studied the effect of ligating the middle cerebral artery in cats upon the subsequent infarction and impaired neurological function. We compared cats on a normal diet to those on a diet supplemented with fish oil.

The present report provides qualitative and quantitative information on the fatty acid composition of the heart, liver and brain, in association with a smaller infarct size and better neurological status following fish oil supplementation.

METHODS

Animals and diets. Ten male adult cats weighing between 3 and 4 kg were used in these experiments. All animals were allowed to eat no more than 95 Cal/kg/day for 18 to 24 days prior to middle cerebral artery occlusion (MCA). Five cats in the control group were fed a basal diet of c/d Feline cat chow (Ravena Foods, Topeka, Kansas) and the experimental group (5 cats) was fed the basal diet supplemented with 8% of the calories as menhaden oil (Zapata Haynie, Reedville, Virginia). The composition of the menhaden oil is presented in the last column of Table I.

Surgical procedure. Middle cerebral artery occlusion was performed following a modification of the technique of Sundt and Waltz (5). Cats were paralyzed with succinylcholine (30 mg/kg i.p.) and ventilated via a Harvard Apparatus Respirator (Model 607). Cannulae were placed in the right femoral artery and vein under sterile conditions using lidocaine (15 mg subcutaneously) anesthesia. Femoral artery pressure was recorded continuously on an Electronics for Medicine machine connected to the arterial cannula via a Gould Statham P 23 pressure transducer. Lead 1 of the EKG was monitored throughout the experiment, and arterial pH, P_aO_2, P_aCO_2, and HCO_3 were maintained within the following ranges: P_aO_2 104 ± 20 mmHg, P_aCO_2 26 ± 8 mmHg, and pH 7.4 ± 0.07. Rectal temperatures were maintained in the range of 97.6 to 101.3°F. Two Kleinert-Kutz angled microvessel clips (blades 5 mm x 1 mm) were applied to the left MCA immediately lateral to the optic nerve to occlude the cerebral artery. The animals received 2 mg/kg i.v. ketamine hydrochloride every 10 minutes for surgical anesthesia beginning approximately 1 hour prior to ligation of the left MCA.

Tissue analysis. Samples of heart, liver and brain were removed and homogenized with 6 ml of chloroform:methanol (2:1) using a Brinkman Polytron Model PT 10-35 tissue homogenizer. Pentadecanoic acid (15:0) was added as an internal standard for calculation of recovery and quantitation of individual fatty acids. The homogenate was heated at 70°C for 10 minutes. Two milliliters of water was added and evaporated to dryness under nitrogen. The lipids were then converted to methyl esters by heating for 45 minutes at 70°C with 8% H_2SO_4 in methanol. The composition of total fatty acids methyl esters was measured by gas liquid chromatography using a flame ionization detector. Each methyl ester was identified by its derived equivalent chain length calculated relative to the retention time of standard acids, and quantitated relative to the 15:0 internal standard.

Volume of infarction. The location and extent of brain lesions caused by the occlusion of the left MCA were determined primarily by histological study of brain sections stained with hematoxylin and eosin as described earlier (6). After spontaneous or induced death, the chest was opened, and the descending aorta ligated. A catheter was inserted into the aorta, and the head perfused with normal saline, followed by 10% formalin-saline. The brain was then removed, and examined to rule out the presence of intracranial hemorrhage and sliced in the frontal plane at intervals of 4 mm. These brain slices were also examined to determine if any hemorrhage was present. Representative sections of each brain slice were mounted and stained, and the margin between infarcted and healthy tissue was verified by light microscopy.

Estimation of the volume of brain infarction was achieved by the following procedures:

1) The total volume of each slice was measured directly by the displacement of water in a graduated cylinder.

2) Slices with infarcted areas were analyzed by polar planimetry of photographs of histologic sections from these slices made to the same scale. The areas of the infarcted tissue determined on the two faces of the slice were added, divided by 2, and multiplied by the thickness of the slice.

3) Division of the calculated volume of infarcted brain by the total original brain volume yielded the fraction of the brain that was infarcted.

TABLE I. TOTAL FATTY ACID COMPOSITION

(Mole % values reported as mean ± S.D.)

	HEART		LIVER		BRAIN		
FATTY ACID	+ OIL (n=3)	CONTROL (n=1)	+ OIL (n=3)	CONTROL (n=1)	+ OIL (n=3)	CONTROL (N=1)	FISH OIL
12:0	0.1±0.1	0.1	0	0.1	0	0.3	0
14:0	2 ±0.8	2	0.3±0.1	0.2	0.9±0.1	1	9
15:1	4 ±0.8	5	0	0	0	0	0
16:0	19 ±2	17	20 ±2	20	21 ±2	21	21
16:1	3 ±0.9	2	1 ±0.1	0.9	1 ±0.2	1	11
17:1	2 ±1	2	0.5±0.1	0.4	0.5±0.1	0.4	0
18:0	14 ±2	14	24 ±1	24	15 ±2	12	3
18:1(n-9)	28 ±3	25	15 ±2	14	36 ±4	34	12
18:2(n-6)	12 ±1	21	12 ±3	24	1 ±0.4	1	1
20:1(n-9)	1 ±0.2	0.1	0	0.1	7 ±6	13	2
20:2(n-9)	0.1±0	0	0.4±0.6	0.3	0	0.1	0
20:3(n-9)	0	0	0	0	0.1±0	0.1	0
20:3(n-9)	0.5±0.2	0.4	1 ±1	0.6	0.7±0.2	0.9	0
20:4(n-6)	7 ±3	8	14 ±2	9	4 ±0.6	3	2
22:1(n-6)	0.9±0.8	0.1	0	0.1	0	0	0
20:5(n-3)	3 ±0.6	0.7	2 ±0.5	0.3	0.4±0	0.2	14
22:5(n-6)	0.3±0.1	0.3	0.2±0.1	0.2	3 ±0.7	5	0
22:5(n-3)	1 ±1	1	0.5±0.1	0.1	0.2±0.1	0	2
22:6(n-3)	2 ±0.8	2	6 ±1	5	4 ±1	2	10

Because ischemic changes in brain tissue cannot be demonstrated with certainty by hematoxylin and eosin staining prior to 24 hours after the onset of ischemia, the described analysis of brains was carried out only with animals which lived 24 hours or longer after the ligation.

Neurological competence. Three days after occlusion of the MCA, animals were examined for alterations of consciousness, forced motor behavior (such as circling), gait disturbances and the ability to right. The gait was graded on a scale of 0 to 4 as follows:

0- No neurological deficit

1- Mild right hemiparesis, normal gait

2- Moderate right hemiparesis, limping gait

3- Severe right hemiparesis, can stand without support

4- Right hemiplegia, can stand with support.

To determine the righting reflex score, the cats were held upside down 3 feet above a cushion. The animals were released upside down above the cushion and their ability to turn upright (righting reflex) was scored on a scale of 0 to 2:

0- Normal

1- Slightly impaired, attempts to right

2- Severely impaired, no attempt to right.

RESULTS

One of the 5 cats which was given menhaden oil as 8% of its caloric intake died in hypotensive shock 10 hours after ligation of the left MCA. The remaining 4 animals were active and alert for 3 days after ligation of the middle cerebral artery until they were killed with an overdose (80 mg/kg, i.v.) of pentobarbital. The 5 cats on the control diet survived the 3 day period, but only one was used for lipid analysis. Tissues removed at the termination of the experiment showed little difference in the composition of total fatty acid esters between the two experimental conditions (Table 1). The composition of brain lipids was particularly interesting in showing no effect of the diet for the period of time studied. The primary significant differences were increased amounts of 20:5(n-3) and decreased amounts of 18:2(n-6) in the heart and liver of cats fed supplemental fish oil. Also there was a somewhat higher amount of 20:4(n-6) in the liver of the oil-supplemented animals. In addition to the results in Table 1, we determined the composition of the non-esterified fatty acids (FFA) in the plasma of cats fed the supplemental fish oil. The average mole% of 16:0 (26), 16:1 (4), 18:0 (14), 18:1 (32) and 18:2 (11) were similar to those noted for the total heart lipids in Table 1. In contrast, the average content of 20:4(n-6), the precursor of dienoic prostaglandins was 1.3%, whereas that for 20:5(n-3), the inhibitor of the dienoic prostaglandins formation was 2.0%.

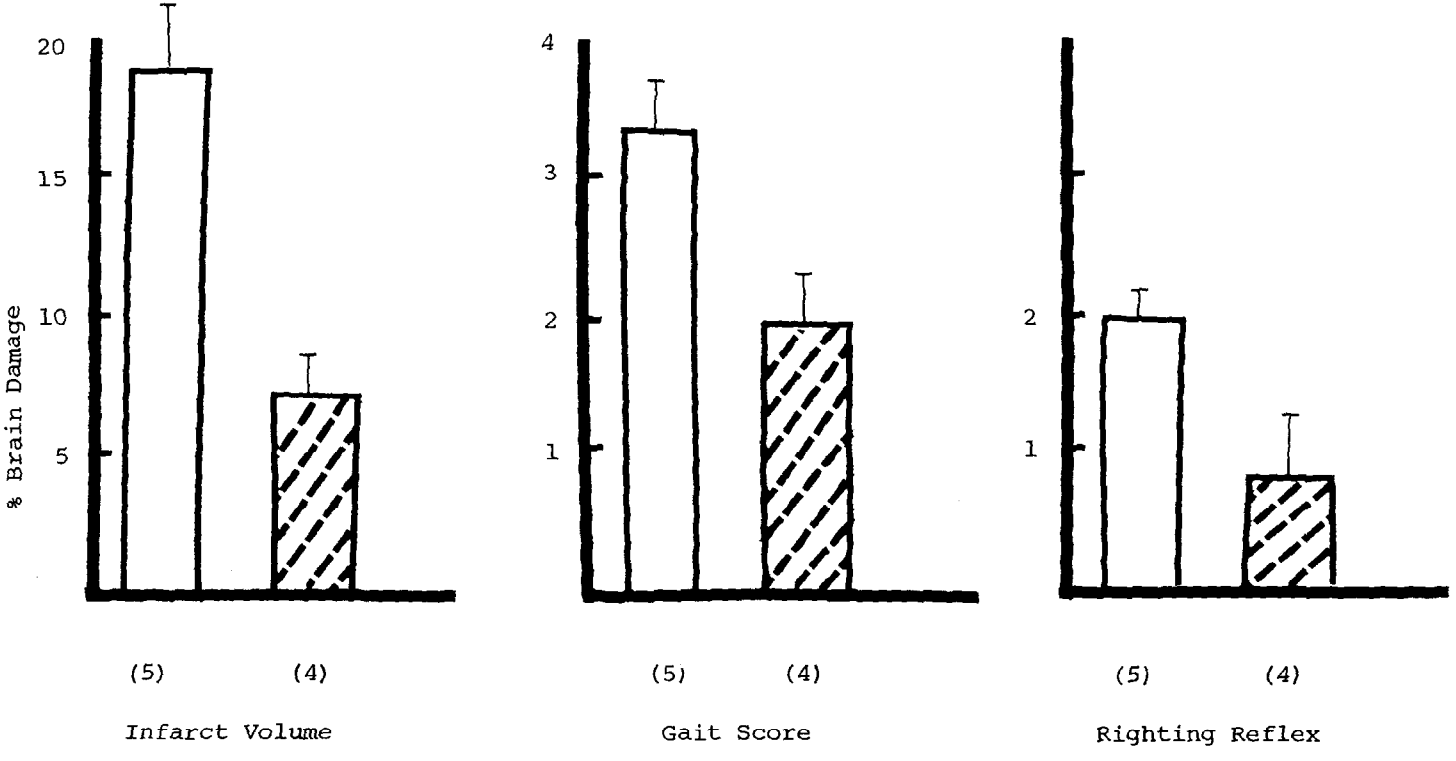

FIGURE 1. Lower Infarct Volume and Neurologic Impairment Following Supplemental Fish Oil.

Values expressed as mean ± SEM for the number of animals shown in parentheses. Results with control animals are indicated by open bars and those for supplemental cats are hatched.

The mean volume of infarcted brain in the group treated with menhaden oil was 7 ± 1% of the original brain volume. In contrast, the 5 cats which received normal diets without menhaden oil (control group) had a volume of necrosis of 19 ± 2% of the original brain volume. The volume of brain necrosis was significantly less in the group treated with menhaden oil compared to the control group. These relationships are demonstrated graphically in Figure 1. We also observed that the mean gait score was less affected in the menhaden oil treated animals (1.8 ± 0.5) compared to that for the control animals (3.4 ± 0.3). Also, the animals treated with menhaden oil scored significantly better (lower) on the righting reflex (0.8 ± 0.5 vs. 2.0 ± 0. for the controls.

DISCUSSION

The markedly different pattern of fatty acid composition of the plasma lipids of Eskimos living in Greenland (7) relative to those living in Denmark strongly indicated an influence of the (n-3) type of polyunsaturated acid derived from linolenate that is so abundant in the Eskimo maritime food (8). A special metabolic effect of the (n-3) type of fatty acids in reducing coronary occlusion was envisioned earlier by Owren (9). The higher tendency to thrombosis regularly found in atherosclerotic disease was reported to be reduced by administering linseed oil (which contains appreciable 18:3(n-3)). Reduced platelet adhesiveness following three days of linseed oil intake (20 ml/day) was reported for other dietary oils in proportion to their content of the (n-3) polyunsaturated acid (9). Unfortunately subsequent experiments did not confirm an antithrombotic effect of linolenate (10), and further research is necessary to establish the utility and mechanism of the proposed use of linseed oil.

Dietary linolenate is rapidly desaturated and elongated in animal tissues to 20:5, 22:5, and 22:6 which are then accumulated in cellular glycerolipids. Thus, some of the earlier antithrombotic findings with linolenate (11) may reflect effects of the longer C-20 and C-22 polyunsaturated derivatives in the tissue rather than the nutrient linolenate *per se*. Fish oil in contrast to that from plants, provides large amounts of 20:5(n-3) (10 to 13% in menhaden and 19% in anchovy), 22:5(n-3) (2% in menhaden and tuna) and 22:6(n-3) (9 to 13% in menhaden and 23% in tuna) (12-14).

Polyunsaturated fatty acids of the (n-3) type have little stimulatory effect upon platelet aggregation (15) in contrast to the dramatic effect of arachidonate (20:4(n-6)) in producing stroke in rats (16) and sudden death in rabbits (17). The action of arachidonate seems clearly due to its ability to form thromboxane A_2 (18) which mediates thrombogenesis. The important additional action of thromboxane A_2 in causing arterial vasospasm (19,20,21,22) gives further impetus to understanding how the C-20 and C-22 polyunsaturated acids can influence these fatal vascular events.

Dyerberg *et al.* (3,4) indicated that the antiaggregatory action of 20:5 (n-3) might be due to the competitive inhibition of TXA_2 synthesis. This is in accord with our earlier report (1) that fatty acids of the (n-3) type are not effective substrates for cyclooxygenase from sheep vesicular gland. Rather, these acids are bound to the enzyme site with affinities (1.7 to 15 μM) equal to or greater than the Km value for arachidonate, and they are thus

effective competitive inhibitors (1). Moncado and Vane (2), agreeing with this and citing the evidence adumbrated by Raz (23), have concluded that the use of eicosapentaenoate could afford a "dietary protection against thrombosis" (2). In 1959, Ahrens et al. (24) reported that feeding a diet supplemented with menhaden oil to a hyperlipidemic and a hypercholesterolemic patient lowered the serum lipids. Total cholesterol was lowered 68% in the hyperlipidemic patient and 39% in the hypercholesterolemic. The cholesterol lowering effect did not depend on essential fatty acids. Recently, normal subjects were fed mackerel and Gouda cheese in a study conducted by von Lossonczy et al. (25). They reported that subjects on the fish diet for three weeks showed 7.5% lower cholesterol and 35% less serum triglycerides whereas the high density lipoprotein cholesterol increased an average of 3%. Despite an equal amount of (n-6) acids in the diet there was a replacement of the (n-6) in the serum lipid by the (n-3) fatty acids.

A diet supplemented with tuna, salmon, and variety of other fish has been used in therapy of 80 patients with coronary artery disease (26). Patients on the diet survived a mean of 109 months compared to 58 months for 126 control patients. Salmon (with 16% 22:6) lowered serum cholesterol by 42% in one patient. Dietary prostaglandin precursors and their high ratio to inhibitory fatty acids are likely factors causing vasospasm, infarction and sudden death. The relative composition of fatty acids in the heart lipids reflect that of the plasma FFA which contains a ratio of 20:4/20:5 which is nearly midway between that observed for the dietary oil and the liver which secretes serum lipoproteins.

The fatty acid composition of the major phospholipids from platelets and platelet function was studied in 12 healthy male volunteers on a saturated dietary regimen followed by a diet rich in (n-6) polyunsaturated fats (27). There was a significant ($p < 0.001$) fall in serum cholesterol on the PUFA diet (27). The phosphatidylcholine class of phospholipids showed the greatest change. Linoleic acid (18:2) increased from 9.7 to 16.6% ($p < 0.001$). Our results also show linoleate as the acid most responsive to the diet; in this case, decreasing with a diet poor in (n-6) fatty acids.

One primary concern in the management of stroke is to minimize the extent of damage incurred as a result of the original ischemic insult. Many interventions tested (carbon dioxide inhalation, hyperventilation, induced hypertension, vasodilating agents and stellate ganglion blockade) now have little current use. Barbiturates in very high doses, however, have a protective effect on cerebral ischemia (28,29), and clinical trials report favorable results (30). Experiments in which pentobarbital was administered to cats within 2 hours of cerebral artery occlusion showed less infarct size and neurological deficit at the high level used (50 mg/kg) (6). The drug greatly reduced the metabolic rate and increased the mortality, apparently by creating a secondary adrenal insufficiency. Thus a further search for better intervention is needed, and more studies of the blockade of prostaglandin synthesis may help.

Although some impaired neurological functions may improve within hours or days of the focal cerebral ischemia (31), the present study was designed to identify differences in persistent neurological deficits. Hemiparesis (impairment in gait) appears to involve the internal capsule (31). This structure is usually affected during unprotected occlusion of the MCA.

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Maintenance of collateral microvascular capillary channels may be the key event in limiting infarct size following focal cerebral ischemia (32,33). The collateral microvascular channels may continue to supply ischemic neurons and glial cells with limited circulation for 30 minutes after occlusion (34-36). However, there is a subsequent progressive narrowing of these collateral channels (34), and occlusion of the microcirculation is complete by 3 hours. Perivascular glial swelling and brain edema was associated with the capillary narrowing. It has been demonstrated that reducing brain edema and perivascular swelling with osmotic agents or barbiturates (37) can maintain the patency of collateral microchannels after cerebral ischemia (32,33) and reduce the volume of infarction.

Narrowing of vascular channels after cerebral ischemia may also reflect a disturbance in the normal balance among different prostaglandins which constrict and dilate the vascular bed in a manner that gives a net vasospasm of the cerebral vessels. Both prostaglandin E_2 and prostaglandin $F_{2\alpha}$ are potent constrictors of cerebral vessels, and they produce prolonged vasospasm when given intracisternally (38,39). Thromboxane A_2, another product of arachidonic acid metabolism, has been shown to be a potent spasmogenic agent when applied topically to cerebral vessels (20). Thus all three products of cyclooxygenase action on arachidonic acid may provide the exaggerated response to occlusion in normal animals. The protective effect of fish oil on cerebral infarction may result from the increased amounts of (n-3) fatty acids that inhibit cyclooxygenase action and reduce the synthesis of the (n-6) dienoic prostaglandins. The relatively small amounts of the (n-3) acids needed to provide beneficial effects suggest that a balance between the two classes of PUFA is needed rather than a replacement of one by the other.

The conversion of dietary linoleate to arachidonate (and subsequently into prostaglandin derivatives) appears to occur to a lesser extent in cats (40) than in rats or other mammals. Cats are reported to have an absence (41) or, at least, an impairment (42) of the desaturase reaction which forms γ-linolenic acid (18:3(n-6)) from linoleic acid (18:2(n-6)). This relative inability of cats to desaturate fatty acids at the 6-position may make the composition of their tissue polyunsaturated fatty acids much more sensitive to altered amounts of dietary fatty acids than the tissue compositions acquired by other animals. We can expect, therefore, that similar beneficial effects of dietary acids may occur with different animal species, although relatively higher amounts of dietary fish oil may be required to secure the same degree of protection.

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