Plasma opioid peptides and ACTH responses to fish oil and vitamin E supplementation in male subjects

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Fish oils, high in omega-3 fatty acids, affect lipid and carbohydrate metabolism partly through their effects on the levels of hormones involved in their metabolism. Recently, a role for opiates in glucose homeostasis and lipid metabolism has been reported. It is possible that some of the effects of fish oils on carbohydrate and lipid metabolism may be, in part, mediated through changes in opiates. We therefore studied the effects of fish oil and fish oil plus vitamin E, a potent antioxidant, on plasma opiates and adrenocorticotrophic hormone (ACTH) in normal subjects. Forty healthy men consumed diets providing 40% of energy from fat and a minimum of 25 mg vitamin E for 28 weeks. During the first 10 weeks, diets were supplemented with placebo, 15 g mixed fat/day. During the second 10 weeks, placebo was replaced by 15 g/day of fish oil concentrate. During the last 8 weeks, 200 U/day of vitamin E was added to fish oil. Plasma opioid peptides were measured by radioimmunoassay after eluting from C_{18} Sep-Pak cartridges. Fish oil feeding significantly decreased plasma β-endorphin compared with placebo, but had no significant effects on plasma ACTH, met-, and leu-enkephalins. Fish oil plus vitamin E appeared to further decrease β-endorphin and significantly increased both enkephalins. Thus, it is possible that in addition to hormonal changes, alterations in opiate tone may also partly explain the effect of fish oil and vitamin E on plasma glucose, triglyceride, and other metabolic and physiologic processes.

Keywords: fish oil feeding; omega-3 fatty acids; vitamin E; β-endorphin; enkephalins; ACTH

Introduction

Omega-3 fatty acids from fish oils are active in metabolic and biological processes. They affect both lipid and carbohydrate metabolism, and their use as diet supplements has been proposed to possibly prevent diseases associated with defects in the metabolism of these nutrients. Fish oil supplementation has been shown to have a protective effect against atherosclerotic changes by lowering serum triglyceride and very low density lipoprotein levels,[1-4] in addition to platelet function and thrombogenicity. In noninsulin-dependent diabetic subjects, fish oil supplements led to an increase in fasting plasma glucose levels, although insulin sensitivity was also increased.[5-6] Alterations in the levels of hormones that are counterregulatory to insulin such as glucagon, epinephrine, growth hormone,[7] and dehydroepiandrosterone-sulfate (DHEA-S) also have been reported.[8] Because glucose homeostasis is a complex phenomenon influenced by other factors such as prostaglandins,[9] opiates, and neuropeptides,[10-13] it is conceivable that omega-3 fatty acids influence these factors as well.

Opiates and neuropeptides are also involved in lipid metabolism.[14,15] Many of the opiates also have cardiotropic effects[16-18] and play a role in hypertension.[19,20] It is possible that some of the beneficial effects of fish oil with respect to lipid metabolism and in the prevention of cardiovascular disorders may be via changes in opiate tone, however no data on the effect of fish oil

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supplementation on plasma opioid peptide levels in humans or animals are available. We therefore investigated the influence of dietary omega-3 fatty acids on plasma opioids and adrenocorticotrophic hormone (ACTH). Supplementation with fish oil induces a high level of polyunsaturated fatty acids to the diet thus causing concern for the potential damaging effects of lipid peroxidation. Vitamin E, an antioxidant, may reduce the lipid peroxidation caused by fish oils. We therefore examined the effects of omega-3 fatty acids with and without additional vitamin E supplementation. The results reported here indicate the existence of opiate-specific responses attributable to omega-3 fatty acids.

Materials and Methods

The protocol of the study was approved by the Human Studies committees of the Georgetown University School of Medicine and the National Cancer Institute. The details of the study design have been described elsewhere.[8] Briefly, 40 healthy male subjects consumed controlled diets that provided approximately 40% of energy from fat when fed in conjunction with a daily 15 g fat supplement. No alcoholic beverages, vitamin, mineral, or other dietary supplements, other than those provided, were consumed by the subjects during the study. Periodic caloric adjustments were made to maintain initial body weight. Subjects were initially assigned to a caloric level considered appropriate for weight maintenance. When necessary, adjustments were made in 1.67-MJ (400-kcal) increments. All meals were prepared in the Human Study Facility of the Beltsville Human Nutrition Research Center (BHNRC). Breakfast and evening meals on weekdays were eaten in the BHNRC dining facility and carry-out meals were provided for weekday lunches and all weekend meals. A 14-day menu cycle formulated from commonly available foods was used to ensure variety and acceptibility. The nutrient composition of basal and experimental diet is given in *Table 1*. *Table 2* gives the fatty acid composition of the placebo and fish oil supplements.

The study was divided into three periods of 10, 10, and 8 weeks according to the supplement given. During period 1 (placebo period), all subjects consumed basal diet and were given supplementary capsules each day containing 15 g of mixed fat (48% stripped lard, 40% beef tallow, and 12% corn oil) and 33 IU of vitamin E as dl-α-tocopherol (22 mg α-tocopherol/day). This diet provided approximately 40% of energy from fat. During period 2 (fish oil period), the mixed fat capsules were replaced with capsules containing 15 g concentrate of fish oil (ROPUFA 50%, a triacylglycerol containing 50% concentrate of refined anchovy oil, Hoffmann-La Roche Pharmaceutical, Nutley, NJ USA) and 15 IU of vitamin E. During period 3, in addition to 15 g of fish oil, subjects received 200 IU of vitamin E as dl α-tocopherol daily. Both fat supplements were provided as soft capsules containing 1 g of the fat plus 1 IU of vitamin E per capsule. The placebo capsules were manufactured to resemble the fish oil capsules in external appearance. The subjects received seven capsules (7 g) at breakfast and eight capsules (8 g) at dinner. Vitamin E was provided during period 3 as 200 IU dl-α-tocopherol (Hoffman-La Roche) in a single capsule at breakfast. The nutrient compositions of the basal diet and of the supplements were reported earlier.[8] The fatty acid composition of the fat supplements is given in *Table 2*.

Table 1 Estimated daily intake on controlled diets

Nutrient	Supplement	
	Placebo	Fish oil
Fat (percent of energy)	40	40
Carbohydrate (percent of energy)	46	46
Protein (percent of energy)	16	16
Cholesterol (mg/day at 2800 Kcal)	360	360
Vitamin E (IU/day, minimum)	33	33
Total tocopherol (mg/day, minimum)	41	41

Table 2 Fatty acid composition of the placebo and fish oil supplements

Fatty acid	Placebo	Fish oil
(g/100 g Supplement)		
12:0	0.16	0.13
14:0	2.08	4.87
16:0	21.81	9.29
16:1 (N-7)	2.43	6.48
18:0	13.28	1.37
18:1 (N-9)	36.39	5.43
18:2 (N-6)	13.73	1.86
18:3 (N-3)	0.44	1.04
18:4 (N-3)	ND	4.62
20:1 (N-9)	0.44	0.38
20:4 (N-6)	0.15	1.41
20:4 (N-3)	ND	1.11
20:5 (N-3)	ND	30.18
22:5 (N-3)	ND	2.51
22:6 (N-3)	ND	13.06
Saturates	38.42	16.47
Monounsaturates	42.77	15.68
Polyunsaturates	14.38	63.60
Omega-6 fatty acids	13.94	3.71
Omega-3 fatty acids	0.44	52.52
Ratio omega-3/omega-6	0.03	14.16

ND, None detected.

At the end of each dietary period, after an overnight fast, venous blood samples were collected in tubes containing EDTA (1.4 mg/mL), Trasylol (100 U/mL; FBA Pharmaceuticals, New York NY USA), bestatin (88 μmol/L), and citrate (23 mmol/L). This combination of protease inhibitors prevents the degradation of opioid peptides by plasma proteases.[21,22] Plasma was aliquoted and stored at −70° C until analyzed.

Plasma opioid peptides were measured by radioimmunoassay after eluting from Sep-Pak cartridges as described previously.[22] Briefly, 2 mL of plasma was acidified with 400 μL of 1N HCl and applied slowly onto a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA USA), which was activated with 10 mL of methanol and washed with 20 mL of distilled deionized water. The cartridges were washed with 20 mL of 4% acetic acid and the adsorbed opiates were eluted with 5 mL of glacial acetic acid and 90% ethanol (1:24) mixture. The eluates were aliquoted and lyophilized on a Speed-Vac concentrator (Savant Instruments, Farmingdale, NY USA). The lyophilized aliquots representing 0.5 mL of plasma each were dissolved in 1% BSA-borate buffer for β-endorphin, in BSA-phosphate buffer for met-enkephalin, and in peptone-borate buffer for leu-enkephalin. The opiates were then measured by radioimmunoassay using kits

from INCSTAR (Stillwater, MN USA; Cat # 16065, 18100, 19100). Plasma ACTH was measured by radioimmunoassay using a kit from INCSTAR (Cat. No. 24130). The data were analyzed statistically by paired *t* test between the dietary periods.[23] All *P* values less than 0.05 are considered statistically significant. The data were also analyzed by analysis of variance (ANOVA), and essentially the same statistical significances were observed.

Results

The mean age of the study population was 37.9 ± 5.9 years and body mass index was 25.7 ± 1.9 kg/m^2. The average plasma glucose, triglyceride, and cholesterol levels were 4.6 ± 0.06 mmol/L, 1.62 ± 0.11 mmol/L, and 4.60 ± 0.14 mmol/L, respectively.[8] *Table 3* shows the effect of fish oil and fish oil with vitamin E supplementation of plasma β-endorphin, ACTH, and met- and leu-enkephalin levels. Fish oil feeding significantly decreased plasma β-endorphin levels but had no effect on ACTH. Supplementation of fish oil-fed subjects with vitamin E (200 IU) had no significant effect on plasma β-endorphin or ACTH. Plasma met- and leu-enkephalins showed an opposite trend to β-endorphin. Thus, levels of both enkephalins were not significantly altered after fish oil supplementation compared with the placebo dietary period. However, the supplementation with vitamin E (200 IU) in addition to fish oil significantly elevated the plasma levels of both enkephalins.

Discussion

In the present study we observed significant effects of fish oil supplementation on plasma β-endorphin and possibly of vitamin E supplementation on enkephalins in humans. It is not clear whether greater changes in both enkephalins and β-endorphin observed after vitamin E supplementation of fish oil are due to an independent effect of vitamin E or a continuing and magnified effect of prolonged fish oil supplementation.

Omega-6 fatty acids have been shown to affect both β-endorphin and ACTH secretion from pituitary.[24,25] Pagano et al.[24] injected arachidonic acid in the right ventricle and showed a three to four fold increase in pituitary β-endorphin, possibly due to increased prostaglandin E_2, which also increases β-endorphin in pituitary when injected in the cerebral ventricle.[26] Similarly, arachidonic acid and its lipoxygenase products also stimulate ACTH release from pituitary cells.[25] It is possible that omega-3 fatty acids may also have an effect on pituitary β-endorphin and ACTH and therefore possibly on plasma levels. Omega-3 fatty acids form prostaglandins of the 3-series as opposed to those of 2-series produced by arachidonic acid (omega-6). The prostaglandins and other eicosanoids formed from omega-3 and omega-6 fatty acids compete with each other and have opposite effects. This may partly help to explain decreased β-endorphin levels in plasma of subjects during fish oil feeding compared with the placebo period. Plasma ACTH levels were not affected by fish oil feeding with or without vitamin E supplementation.

In humans and animals, supplementation with fish oils or purified omega-3 fatty acids has been reported to affect several metabolic and physiologic processes. Fish oils lower plasma triglycerides, cholesterol, and very low density lipoproteins,[1-4] elevate blood glucose levels,[5,8,24] increase hepatic glucose output,[27] and decrease plasma insulin, glucagon, growth hormone, and somatomedin-C levels.[8]

It is important to note that opiates that affect glucose homeostasis and lipid metabolism are colocalized, often in the same cells or granules, in the pancreas, and in many instances cosecreted with hormones that are known to control these processes. β-endorphin is colocalized and cosecreted with glucagon,[28] and enkephalin with insulin.[29] It remains to be established whether the effects of omega-3 fatty acids on plasma opiate levels observed in this study are direct or secondary to alterations in pancreatic hormones insulin, glucagon, and somatostatin. Opiates and ACTH are also present in central nervous system and other extrapancreatic organs such as pituitary, hypothalamus, stomach, and adrenals, and the plasma levels depend on secretion from all these sources besides the pancreas. The levels of opiates are differentially altered in different tissues in many metabolic conditions such as obesity, diabetes, and hypo- and hyperinsulinemic states.[28,30]

In conclusion, we have reported that fish oil, and possibly vitamin E supplements, affect plasma opioid peptide levels in humans. The changes in plasma opioid peptide levels brought about by fish oil does not appear to be beneficial, especially in those subjects with hyperglycemia or glucose intolerance. However, to what extent these small but significant changes in opioid peptides account for the changes observed in biochemical, metabolic, hormonal, and physiologic processes

Table 3 The effect of supplement of fish oil concentrate (ROPUEA 50%) and vitamin E on plasma levels of immunoreactive β-endorphin, ACTH, and met- and leu-enkephalins in healthy men*

Dietary period	β-endorphin pmol/L	ACTH ng/L	Met-enkephalin pmol/L	Leu-enkephalin pmol/L
Placebo fat	5.41 ± 0.51^a	28.5 ± 2.1^a	67.0 ± 8.1^a	1012.7 ± 118.6^a
Fish oil	3.91 ± 0.48^b	29.7 ± 3.5^a	79.7 ± 7.7^a	1121.0 ± 106.2^a
Fish oil and vitamin E	3.11 ± 0.33^b	24.2 ± 1.9^a	148.6 ± 6.0^b	1593.7 ± 125.1^b

*Mean ± standard error of mean. Means not sharing the same letter within a column are significantly different according to a paired *t* test.

after fish oil supplementation remains to be determined. Similarly, how vitamin E affects changes in plasma opioid peptides also remains unexplained. The present study suggests some interesting possibilities. Clearly more definitive work needs to be done to elucidate the mechanism and significance of our observations.

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