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Effects of a new fluid fish oil concentrate, ESKIMO-3, on triglycerides, cholesterol, fibrinogen and blood pressure

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Eskimos have a very low incidence of cardiovascular disease, at least in part due to a high intake of n-3 fatty acids. ESKIMO-3 is a new stabilized (insensitive to oxidation) fluid fish oil concentrate, 30 ml of which contains an amount of eicosapentaenoic acid and total n-3 fatty acids equivalent to the daily intake among Eskimos.

Thirty-three volunteers, healthy or with coronary artery disease, were given ESKIMO-3, at a dose of 15 or 30 ml d^{-1}, corresponding to 2.7 or 5.4 g of eicosapentaenoic acid d^{-1}, or placebo oil, for a period of up to 6 months.

ESKIMO-3 had a pronounced dose-dependent effect on several risk factors for coronary artery disease. Intake of one tablespoon (15 ml) daily for 6 months significantly reduced levels of triglycerides (−64%), total cholesterol (−8%), plasma fibrinogen (−23%) and diastolic blood pressure (−9%). Bleeding time was unchanged. Intake of two tablespoons daily for 4 weeks increased plasma eicosapentaenoic acid levels by 490%, and decreased arachidonic acid by 20%. The HDL concentration increased by 21%. No change in the above mentioned variables was observed after intake of placebo oil.

Keywords: blood pressure, cholesterol, fibrinogen, n-3 fatty acids, triglycerides.

Introduction

Eskimos have a low incidence of atherosclerosis and coronary artery disease, which is believed to be due to a high intake of omega-3 (n-3) fatty acids, about 10 g d^{-1} [1].

ESKIMO-3 is a new stabilized (insensitive to oxidation) fluid fish oil concentrate, 30 ml of which corresponds to the daily intake of n-3 fatty acids and eicosapentaenoic acid (EPA) among Eskimos.

We have studied the effects of this fish oil preparation on triglycerides, cholesterol, fibrinogen and blood pressure, all of which are known risk factors for the development of atherosclerosis and coronary artery disease.

Abbreviations: DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, HDL = high density lipoprotein, LDL = low density lipoprotein, PgI = prostaglandin I, PgI2 = prostacyclin, TxA = thromboxane A, VLDL = very low density lipoprotein.

Study population

Thirty-three volunteers (23 men and 10 women), healthy or with coronary artery disease, of mean age 48 years (range 22–63 years), were studied in two different trials.

In one study, seven subjects were given placebo (soybean oil) and 13 individuals were given ESKIMO-3 for 4 weeks, at a dose of 15 or 30 ml d^{-1} in a cross-over study with an interval of 2 weeks between the two treatment periods. Seven subjects started with the 15-ml regime, and the other six were given 30-ml doses. Blood samples were taken and other tests performed before and after the various treatment periods.

In another study, 13 subjects were given 15 ml of ESKIMO-3 daily for 6 months. Tests were performed before the study, and after 1, 3 and 6 months.

The diets remained unchanged during the experimental period. Before blood sampling, the subjects fasted overnight. They were also instructed to

abstain from alcohol consumption for 2 days beforehand, vigorous physical activity for 1 day beforehand and use of acetylsalicylic acid or similar medication for at least 1 week before sampling. Blood collection was always performed at about 08.00 hours. All individuals gave their informed consent, and the study was approved by the local Ethics Committee.

Methods

Fish oil preparation. ESKIMO-3 (Cardinova, Sweden) consists of 35% n-3 fatty acids as triglycerides, 18% EPA and 12% docosahexaenoic acid (DHA). It has been stabilized against oxidation by natural antioxidants [2]. In the 4-week trial 0.3 IU and in the 6-month trial 1.5 IU of α-tocopherol g^{-1} was added. This highly purified preparation is almost odourless and has a very low peroxide value, which increases only slightly during the consumption period.

Blood sampling. Venous blood samples were taken after resting for at least 15 min without stasis. The subject lay in the supine position.

Fatty acids in plasma phospholipids. Plasma lipids were extracted with chloroform to which 0.005% butylated hydroxytoluene had been added as an antioxidant. The lipid esters were separated by thin layer chromatography. After transmethylation the fatty acid methyl esters were separated by gas liquid chromatography [3].

Measurements of serum lipids. Cholesterol and triglyceride concentrations were determined in serum and in the isolated HDL fraction by enzymatic methods, using Boehringer-Mannheim kits 126012 and 124087 (Munich, W. Germany) modified for use in a Multistat III F/LS apparatus (Instrumentation Laboratories, Lexington, MA, USA) [3]. Serum HDL levels were determined in the supernatant after selective precipitation with sodium phosphotungstate and magnesium chloride [4]. The cholesterol ratio, which is also referred to as the atherogenic index, was calculated as follows: (total cholesterol −HDL cholesterol)/HDL cholesterol.

Fibrinogen. Fibrinogen in plasma was assayed as clottable fibrinogen by the method of Nilsson and Olow [5].

Blood pressure. Blood pressure was measured in the supine position by a single observer after resting for at least 20 min. A conventional sphygmomanometer was used. Systolic blood pressure was recorded at the point of appearance of Korotkoff sounds, and diastolic blood pressure at the point of disappearance of the sounds.

Bleeding time. Bleeding time was measured by the same observer in all cases, using a Simplate-II device (General Diagnostics, Morris Plains, NJ, USA). Two standard incisions were made longitudinally on the volar surface of the forearm, during application of a constant pressure of 40 mmHg on the upper arm with a standard sphygmomanometer cuff. The bleeding time was calculated as the time that elapsed from incision until blood ceased to appear on a filter paper applied to the edge of the incision every 30 s.

Table 1. Effects of administration of the fluid fish oil concentrate ESKIMO-3 (15 or 30 ml d^{-1} for 4 weeks, $n = 13$), and of placebo oil ($n = 7$), on levels of fatty acids in plasma

	Linoleic acid (18:2, n-6)	Arachidonic acid (20:4, n-6)	Eicosapentaenoic acid (20:5, n-3)	Docosahexaenoic acid (22:6, n-3)
Before treatment	23.4±2.6†	7.9±1.0	1.5±0.5	4.7±0.9
Placebo	+13%***	−2%*	−9%	−2%
ESKIMO-3 15 ml	−20%***	−13%***	+300%***	+35%***
ESKIMO-3 30 ml	−28%***	−20%***	+490%***	+47%***

*$P < 0.005$. **$P < 0.01$. ***$P < 0.001$.
†Mean values±SD are shown.

Statistics

Student's *t*-test for paired observations was used to compare values in the same subjects before and after the intervention period.

Results

ESKIMO-3 was tolerated by all subjects without any adverse effects.

Fatty acid composition of plasma phospholipids

During treatment with ESKIMO-3, there was a significant increase in long-chain polyunsaturated fatty acids of the n-3 series (20:5, EPA and 22:6, DHA). There was a concomitant decrease in n-6 fatty acids (18:2, linoleic acid and 20:4, arachidonic acid). EPA increased by 490% and 300% and DHA increased by 47% and 35% after administration of 30 and 15 ml, respectively, of ESKIMO-3 (Table 1). The EPA/arachidonic acid ratio increased by 637% and 363%, respectively. After a 2-week wash-out period, there was still a slight but significant increase in both EPA and DHA, and a fairly large decline in arachidonic acid (Fig. 1).

Administration of soybean oil as a placebo resulted in no significant changes in n-3 fatty acids, a slight increase in the n-6 fatty acid 18:2 and a slight decrease in the n-6 fatty acid 20:4 (Table 1).

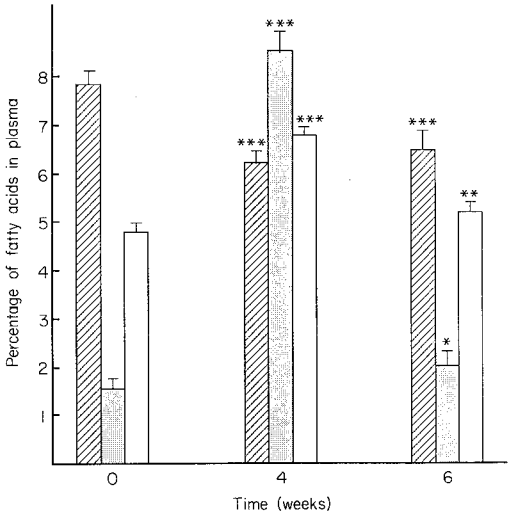

Fig. 1. Relative proportions of different fatty acids in plasma before (0) and after daily intake of 30 ml of fish oil for 4 weeks (4), and after a wash-out period of 2 weeks (6) ($n = 13$). ▨ = 20:4, ▩ = 20:5, □ = 22:6.

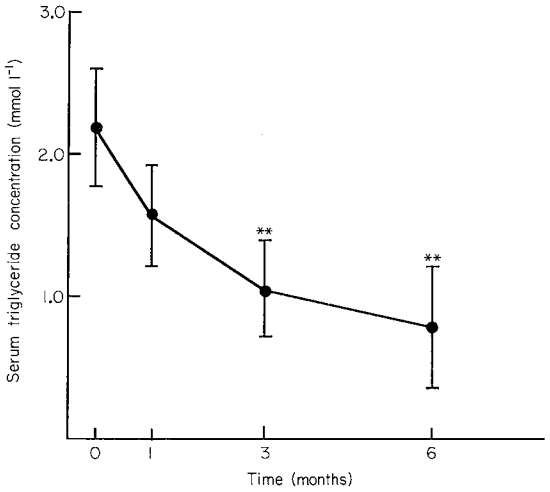

Fig. 2. Serum triglyceride concentration at various times during intake of 15 ml of fish oil daily ($n = 13$).

Table 2. Effects of administration of the fluid fish oil concentrate ESKIMO-3 (15 ml or 30 ml d^{-1} for 4 weeks, $n = 13$), and of placebo oil ($n = 7$), on levels of plasma lipids and lipoproteins

	Serum triglycerides (mmol l^{-1})	Total cholesterol (mmol l^{-1})	HDL cholesterol (mmol l^{-1})	Cholesterol ratio†
Before treatment	1.9±1.7‡	5.1±1.0	1.2±0.4	4.0±2.4
Placebo	+3%	+1%	+3%	−3%
ESKIMO-3 15 ml	−32%	−6%	+16%	−18%
ESKIMO-3 30 ml	−43%*	−10%*	+21%**	−22%**

*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.
†Calculated as (total cholesterol − HDL cholesterol)/HDL cholesterol.
‡Mean values ± SD are shown.

Serum lipids and lipoproteins

When 30 ml of fish oil concentrate d^{-1} was given for 4 weeks, there was a significant decrease in serum triglycerides, cholesterol and cholesterol ratio (atherogenic index). HDL cholesterol levels were significantly increased (Table 2). After treatment with 15 ml of fish oil concentrate d^{-1} for 4 weeks a similar trend was observed, but no significant changes were evident at the end of that period. There were no significant changes in these variables after administration of placebo oil.

There was a significant correlation between the basal serum level of triglycerides and the decrease due to the fish oil concentrate ($r = 0.99$), the subjects with the highest initial levels showing the greatest decrease.

When ESKIMO-3 was administered at a dose of 15 ml d^{-1} for 6 months there was a continuous decrease in both triglycerides and cholesterol. At the end of the 6-month period, the triglyceride (Fig. 2) and total cholesterol (Fig. 3) levels had decreased by 64% and 8%, respectively.

Plasma fibrinogen

Administration of fluid fish oil at a dose of 30 ml d^{-1} for 4 weeks resulted in a significant decrease in plasma fibrinogen, whereas a dose of 15 ml d^{-1} had no effect on this variable (Table 3).

When ESKIMO-3 was supplied at a dose of 15 ml d^{-1} for 6 months, there was a further continuous decrease in plasma fibrinogen, which reached 23% of original levels (Fig. 4).

Blood pressure

There was a significant, 5% decrease in systolic blood pressure after intake of 15 and 30 ml of ESKIMO-3 for 4 weeks (Table 3). The diastolic blood pressure

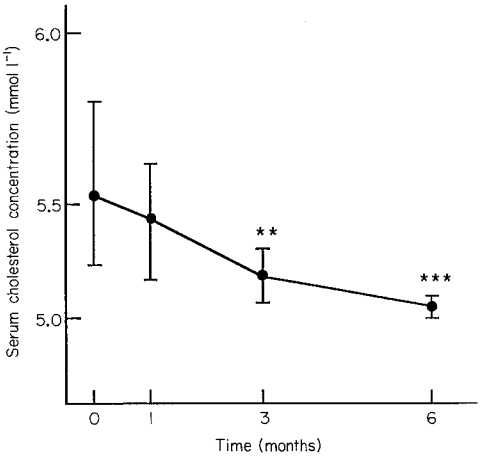

Fig 3. Total serum cholesterol concentration at various times during intake of 15 ml of fish oil daily ($n = 13$).

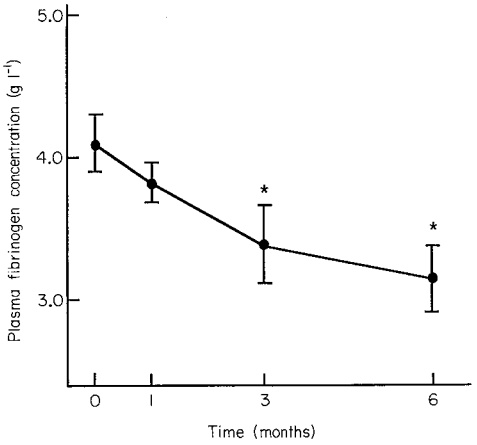

Fig. 4. Plasma fibrinogen concentration at various times during intake of 15 ml of fish oil daily ($n = 13$).

Table 3. Effects of administration of the fluid fish oil concentrate ESKIMO-3 (15 or 30 ml d^{-1} for 4 weeks, $n = 13$), and of placebo oil ($n = 7$), on plasma fibrinogen, bleeding time, and systolic and diastolic blood pressure

	Plasma fibrinogen ($g\ l^{-1}$)	Bleeding time (min)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Before treatment	3.7±0.9†	4.8±1.4	126±10	80±13
Placebo	−5%	+38%	−1%	−2%
ESKIMO-3 15 ml	+1%	+8%	−5%*	−5%
ESKIMO-3 30 ml	−9%**	+10%	−5%*	−1%

*$P < 0.05$. **$P < 0.01$.

†Mean values±SD are shown.

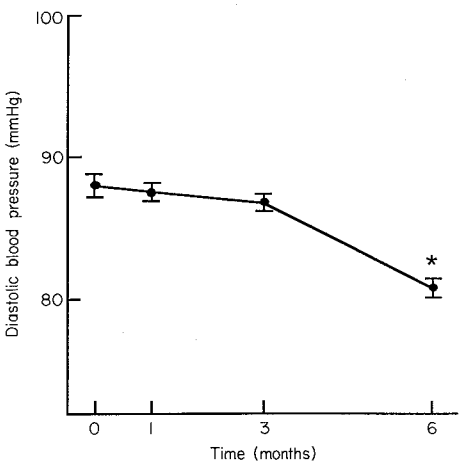

Fig. 5. Diastolic blood pressure at various times during intake of 15 ml of fish oil daily ($n = 13$).

was not significantly altered after that period, but showed a progressive decrease in the long-term experiment, and had declined by 9% after 6 months (Fig. 5). After intake of placebo oil there was no change in blood pressure.

Bleeding time

There was no significant change in bleeding time after intake of either 15 or 30 ml of ESKIMO-3 for 4 weeks (Table 3), or after administration of this concentrate for 6 months.

Discussion

This investigation has shown that the fluid fish oil concentrate has a pronounced effect on plasma fatty acid composition, and on triglycerides, cholesterol, fibrinogen and blood pressure, all of which are known risk factors for atherosclerosis and coronary artery disease. The effect was dependent on both the dose and on the duration of the administration period.

Fatty acids in plasma

The EPA/arachidonic acid ratio increased by 363% and 637% after administration of 15 ml and 30 ml, respectively, of ESKIMO-3 daily for 4 weeks (2.7 or 5.4 g EPA). It has been reported that administration of 3.6 g of EPA as ethyl ester for the same period of time increased this ratio by only 173%, leaving the arachidonic acid unchanged. This discrepancy might be explained by reduced bioavailability of ethyl esters [6].

Triglycerides

Our results confirm the well-established effect of fish oil concentrates in decreasing triglycerides [7], an independent risk factor for ischaemic heart disease [8], particularly among women. Triglycerides appear to be very sensitive to fish oil treatment, although fairly high doses of fish oil concentrate are needed even for a decrease in triglycerides. Thus administration of 15 ml d^{-1} for 4 weeks did not result in a significant decrease. This is in agreement with the earlier finding that, after intake of 5 g of MaxEPA (Seven Seas Health Care Ltd, UK) capsules per day for 4 weeks, there was no significant decrease in triglycerides [9]. With regard to the mechanism of fish oil action on triglycerides, *in vitro* studies have shown that EPA and DHA have a reversible inhibitory effect on hepatic VLDL triglyceride synthesis [10].

Cholesterol

The reported effects of fish oil on cholesterol are conflicting, with results ranging from an increase to a decrease. These discrepancies probably reflect differences in the quality and dose of the fish oil preparation used, and in the duration of the treatment period. Decreased intake of saturated fat during consumption of a fish oil diet may also play a role. When fish oil is administered, larger doses and more prolonged therapy are required to obtain a decrease in cholesterol levels than to obtain a decline in triglycerides. Low doses of fish oil, e.g. 5 g of MaxEPA capsules per day, have been found to result in an increase in total cholesterol and LDL levels [9]. The present findings have shown that a decrease in total cholesterol and an increase in HDL cholesterol can be achieved by intake of fluid fish oil, although the decrease in total cholesterol was moderate. However, the fact that there was an 8% reduction in total cholesterol after a daily intake of 15 ml of ESKIMO-3 for 6 months is promising and, in combination with the very pronounced decrease in triglycerides (64%), makes this treatment of particular interest in mixed hyperlipidaemia.

In one study, apoprotein-B synthesis was reduced but the fractioned catabolic rate was unchanged after administration of fish oil [11]. The reduction in LDL apoprotein-B synthesis may be secondary to a

decrease in hepatic synthesis of VLDL. Both VLDL and LDL have been found to be smaller and more dense after intake of fish oil, and are considered to reduce atherogenicity [12].

Fibrinogen

The present results confirm the finding [13] that daily administration of 14 g of fish oil containing 50% EPA+DHA for 3 weeks resulted in a 13% decrease in plasma fibrinogen. The effect is probably due to a decrease in the production of fibrinogen in the hepatocytes. With a lower dose of n-3 fatty acids (20 ml cod liver oil daily), no effect on fibrinogen was observed [14].

It is of interest that there appears to be a positive correlation between the plasma fibrinogen level and the risk of cardiovascular disease [15].

Blood pressure

The reduction in systolic and diastolic blood pressures in response to consumption of fish oil in the present study confirms several earlier observations [16]. Some investigators found no significant decrease in blood pressure, a result which may have reflected the use of smaller doses or different fish oil preparations. A decrease in whole blood viscosity that was observed in the present study (to be reported elsewhere) may be partially responsible for the effect on blood pressure. The effect of fish oil on blood pressure may also be partly attributable to its influence on eicosanoid formation, with a decrease in the formation of TxA2 and an increase in that of PgI3. EPA and DHA displace arachidonic acid from membrane phospholipids and decrease the conversion of linoleic acid to arachidonic acid; they also act as competitive inhibitors of cyclo-oxygenase and lipoxygenase [17]. Some eicosanoids formed from EPA, such as TxA3, are less active than those formed from arachidonic acid, e.g. TxA2, whereas PgI3 is as active as PgI2. It is not known whether fish oil acts synergistically with other anti-hypertensive drugs. The heart rate is decreased after intake of fish oil, and the reduction in the heart rate—systolic blood pressure product is beneficial because it causes a reduction in the myocardial oxygen demand. [18].

Bleeding time

Administration of the fluid fish oil did not result in an increased bleeding time. Such an increase was previously noted when larger doses were used [19], but no clinical signs of bleeding have been found to be associated with fish oil intake.

An increased bleeding time has been reported in Eskimos, but it is possible that other factors in their food may have contributed to this finding. Selenium, for example, an element which is present in high concentrations in fish, can cause an increase in bleeding time when taken as a dietary supplement [20].

Mode of administration and dose of fish oil

Until now, concentrated fish oil has generally been available only in the form of capsules, which are an expensive and in many ways inconvenient form of medication. One tablespoon (15 ml) of the fluid fish oil concentrate is equivalent to 14 1-g capsules.

The reason why fluid fish oil has been used less frequently than capsules may be that previously available preparations had an unpleasant taste and odour, and were very sensitive to oxidation. In the case of the fish oil concentrate used in the present study, these disadvantages appear to have been overcome.

This preparation is also apparently as or more potent (per g n-3 fatty acids) than previously used preparations in decreasing triglycerides, cholesterol, fibrinogen and blood pressure. Administration of an ethyl ester preparation that provided 7.8 g of n-3 acids daily for 6 weeks did not result in any change in total cholesterol or HDL-cholesterol, and decreased triglycerides by only 38% [7]. Ethyl esters and triglycerides must be enzymatically converted into free fatty acids and monoglycerides if they are to be absorbed. The enzyme responsible, pancreatic lipase, has a much higher specificity for triglycerides than for esters, which might explain the discrepancy, at least in part. The effect of the fluid fish oil that we used was dose-dependent, a 30-ml dose being more potent than 15 ml administered daily over a 4-week period. As there was a further continuous decrease in all four variables mentioned above when 15 ml was supplied daily for 6 months, it would be of interest to study an even lower dose over a longer time period.

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