The Long-Term Effect of Dietary Supplementation with Fish Lipid Concentrate on Serum Lipids, Bleeding Time, Platelets and Angina

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Summary

The effect of a fish lipid concentrate rich in eicosapentaenoic acid was studied in 107 subjects for periods up to 2 years. Ninety-two had heart disease or hyperlipidaemia and 15 had no history of heart disease.

Serum triglyceride levels were rapidly reduced, cholesterol fell more slowly but high density lipoprotein (HDL) cholesterol was increased. Bleeding time increased significantly and the consumption of glyceryl trinitrate (GTN) tablets decreased together with a reduction in anginal attacks. The data are consistent with a reduction in hepatic triglyceride synthesis rather than an increased rate of triglyceride clearance.

Total and HDL cholesterol changes are suggestive of an enhanced removal of cholesterol from the tissues.

Increased bleeding time and changes in GTN consumption are consistent with decreased platelet aggregation.

Key words: Angina – Atherosclerosis – Bleeding time – Eicosapentaenoic acid – Glycerol trinitrate (GTN) – Platelets – Serum lipids

Introduction

Much interest has recently been focused on the low incidence of ischaemic heart disease in Eskimos living in the Umanak district of Greenland. The Eskimo diet of fish and other marine animals is rich in long-chain polyunsaturated fatty acids [1–3]

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mainly of the linolenic class (ω-3). A low incidence of ischaemic heart disease has also been reported in coastal-dwelling Turks [4] consuming large quantities of fish compared with town dwellers on a mixed diet. Eicosapentaenoic acid (EPA 20:5, ω-3) is present in large quantity in the diet of fish-eating populations but present in only small amounts in the Westernised diet. Eskimos have lower serum triglyceride, cholesterol and increased high density lipoprotein cholesterol (HDL cholesterol) levels than Danes taking a Western type diet. They also have a longer bleeding time [2]. A number of studies [5–8] have shown that the incorporation of fish oils into the diet lowers serum lipid levels in human subjects and animals.

When EPA partially replaces arachidonic acid within the platelet membrane, changes occur in platelet behaviour [9–11]. Platelet survival increases and platelet count falls [12]. The enzyme cyclo-oxygenase converts arachidonic acid to cyclic endoperoxides in platelets. Cyclic endoperoxides are rapidly converted by thromboxane synthetase to thromboxane A2 (TXA2). However, when EPA is introduced into the platelet membrane it is metabolised to thromboxane A3 which unlike TXA2, is a much less effective pro-aggregator of platelets. The major effect of EPA is to counteract TXA2 action by competitive inhibition of cyclo-oxygenase [13].

The purpose of the present work was to assess the effect of a marine lipid concentrate on the serum lipids, bleeding time and glycerol trinitrate consumption of patients with angina, hyperlipidaemia or those having suffered a myocardial infarction.

Materials and Methods

Subjects

Ninety-two patients and 15 normal volunteers participated in the study. Of these 88 were males and 19 females. Myocardial infarction was diagnosed in 46 patients, 31 had angina, 1 xanthelasma, 1 diabetes and 2 had peripheral vascular disease, 11 patients had undergone coronary artery bypass surgery, and coronary dilatation procedure had been successfully performed on 3 of the patients with angina. Ages ranged between 31 and 75 years on joining the study. Fredrickson types are shown in Table 1.

TABLE 1
SUBJECTS CLASSIFIED BY DIAGNOSIS AND FREDRICKSON TYPE

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Fredrickson type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>10</td>
</tr>
<tr>
<td>Angina</td>
<td>8</td>
</tr>
<tr>
<td>Xanthelasma</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>0</td>
</tr>
<tr>
<td>Coronary artery by-pass surgery</td>
<td>1</td>
</tr>
<tr>
<td>Symptomless volunteers</td>
<td>8</td>
</tr>
</tbody>
</table>
Subjects joined the study continuously over the 2-year period and therefore the group at pre- and 1 month included all 107 subjects. At 3 months there were pairs of laboratory values i.e. pre-oil compared with 3 months on oil, from 91 subjects, at 6 months 72 pairs, 9 months 51 pairs, 12 months 42 pairs and at 24 months 16 pairs.

Maxepa
This material is a selected fish lipid concentrate containing 19% EPA and was kindly supplied by Seven Seas Health Care Ltd., Hull, U.K.

Blood sampling and clinical procedures
Serum for lipid estimation was separated by centrifugation at $800 \times g$ for 10 min from blood withdrawn after a 12-14-h fast. Two blood samples were taken at 7-day intervals before supplementation of the diet with Maxepa and further samples at 1, 3, 6, 12 and 24 months after commencing therapy. All blood samples were taken between 09.00 and 10.00 a.m. to avoid diurnal lipid changes. Each participant was asked to take 10 ml of Maxepa twice daily with food. No further modification of diet was attempted.

Fat absorption test
The serum triglyceride response to fat loading was measured in 7 subjects. After withdrawing a fasting blood sample the subjects were given a breakfast containing 75 g of fat in the form of fried bacon, cream with cornflakes and butter. Further blood samples were taken at hourly intervals for 6 h and serum triglyceride, cholesterol and HDL cholesterol measured. The patients were then instructed to take 10 ml Maxepa twice daily and the above procedure repeated 1 month later.

Laboratory procedures
The serum concentration of triglyceride and total cholesterol were measured using the procedures Boehringer Mannheim (Catalogue Nos. 125032 and 290319). High density lipoprotein cholesterol was measured by the method above after isolation of the HDL by polyanion precipitation of other lipoprotein fractions with heparin/MnCl$_2$ [14].

The very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) were separated from the serum by electrophoresis on an agar medium [15] known commercially as Lipidophor (Immuno Ltd). This technique uses polyanion precipitation of the lipoprotein bands prior to densitometric measurement on the Liposcript.

Bleeding times were measured by the modified ‘Simplate’ method and platelets were counted on a Coulter counter.

Twelve patients were taking glyceryl trinitrate (GTN) tablets as required for the relief of anginal pain. The number of tablets taken weekly was recorded before starting treatment and again after 9 months.

Statistical analysis
Statistical analyses were paired $t$-test or by Wilcoxon 2-sample test and values were taken as significant at a $P$-value of 0.05 or less.
<table>
<thead>
<tr>
<th></th>
<th>Triglyceride (mmol/l)</th>
<th></th>
<th>Cholesterol (mmol/l)</th>
<th></th>
<th>HDL cholesterol (mmol/l)</th>
<th></th>
<th>Platelet count (×10^5/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Pre-oil</td>
<td>2.68</td>
<td>0.153</td>
<td>6.85</td>
<td>0.132</td>
<td>1.19</td>
<td>0.027</td>
<td>255.4</td>
</tr>
<tr>
<td>1 month</td>
<td>1.68</td>
<td>0.072</td>
<td>&lt; 0.001</td>
<td>6.75</td>
<td>0.128</td>
<td>NS</td>
<td>1.31</td>
</tr>
<tr>
<td>3 months</td>
<td>1.71</td>
<td>0.109</td>
<td>&lt; 0.001</td>
<td>6.74</td>
<td>0.136</td>
<td>&lt; 0.05</td>
<td>1.27</td>
</tr>
<tr>
<td>6 months</td>
<td>1.57</td>
<td>0.067</td>
<td>&lt; 0.001</td>
<td>6.59</td>
<td>0.137</td>
<td>&lt; 0.05</td>
<td>1.31</td>
</tr>
<tr>
<td>9 months</td>
<td>1.65</td>
<td>0.108</td>
<td>&lt; 0.001</td>
<td>6.64</td>
<td>0.175</td>
<td>NS</td>
<td>1.23</td>
</tr>
<tr>
<td>12 months</td>
<td>1.69</td>
<td>0.108</td>
<td>&lt; 0.001</td>
<td>6.62</td>
<td>0.171</td>
<td>NS</td>
<td>1.29</td>
</tr>
<tr>
<td>24 months</td>
<td>1.58</td>
<td>0.179</td>
<td>&lt; 0.001</td>
<td>6.51</td>
<td>0.234</td>
<td>&lt; 0.001</td>
<td>1.36</td>
</tr>
</tbody>
</table>

TABLE 2
MEANS, SEM AND SIGNIFICANCE OF SERUM TRIGLYCERIDE, CHOLESTEROL, HDL CHOLESTEROL AND PLATELET COUNTS
Results

In Table 2 are set out mean concentration, standard error of mean (SEM) of serum triglyceride, total cholesterol, HDL cholesterol and platelet counts. The significance of these results was obtained by paired $t$-test on 1, 3, 6, 9, 12 and 24 months individually compared with the pre-Maxepa results.

The triglyceride concentration was substantially reduced with a mean change of 37% at one month. This change was constant throughout the period of observation and a mean fall of 41% was observed at 24 months. When the values for subjects with initially high triglyceride level were assessed separately the 1-month mean change was 42% (range 19–74%) and 47% at 24 months with a range of 30–71%. Total cholesterol, however, was reduced more slowly over a longer period and did not reach significance until 24 months. The HDL cholesterol increased significantly after 1 month on Maxepa but at 9 and 12 months there was a slight fall although this did not reach the pre-treatment level. The rise in HDL cholesterol was again significant at the 24-month point. Platelet counts fell at 1. 3 and 6 months but then increased to the pre-Maxepa level at 12 and 24 months.

A group of 8 subjects was asked to take 10 ml Maxepa (1.8 g EPA) daily. The bleeding time of this group was compared to that of 11 subjects taking 20 ml
Fig. 3. VLDL, LDL and HDL measured densitometrically after electrophoresis on agarose medium, before and after 6 months treatment with Maxepa (10 ml b.d.).

Fig. 4. Pre- and post-prandial measurement of serum triglyceride before and after taking Maxepa for 1 month. •—•, before Maxepa; •—•—•, after Maxepa.
Maxepa (3.6 g EPA) daily. Measurements in both groups were made before commencing treatment and 12 months later. The results shown in Fig. 1 demonstrate a highly significant increase in bleeding time in people taking the high dose of oil. Figure 2 indicates the change in pattern of GTN consumption.

Densitometric scans of VLDL, LDL and HDL were evaluated before Maxepa and after 6 months. The results in Fig. 3 show a significant depression of VLDL accompanied by a somewhat smaller depression of LDL. A significant increase in the HDL fraction was observed.

Figure 4 demonstrates the changing pattern in serum triglyceride after a fat loading in subjects before treatment and at 1 month on Maxepa. Not only was the fasting triglyceride level reduced at 1 month but the overall response to fat loading was modified.

Discussion

The results presented here on the effect of Maxepa on serum triglycerides show a rapid and highly significant depression of this particular lipid fraction. This reduction in triglyceride and VLDL concentration could result from a reduction in synthesis of triglycerides by the liver or an enhanced clearance of VLDL and chylomicrons from the serum. A reduction in fatty acid availability for the synthesis of triglyceride could result from the inhibitory effect of ω-3 fatty acids on hepatic lipogenesis [16] where EPA (20:5, ω-3) has been shown to reduce acetyl CoA carboxylase activity.

The overall reduction in serum triglyceride levels demonstrated by the fat absorption test after 1 month on fish oil is consistent with a reduction in hepatic triglyceride synthesis rather than an increased rate of triglyceride clearance. Indeed, previous work [17] has shown that the lipoprotein lipase activity was not significantly different in individuals fed ω-3 or saturated fat diets.

The precursor–product relationship of VLDL and LDL is well known and this study demonstrates a fall in LDL which may result from the reduced synthesis of VLDL. This decrease in LDL concentration probably accounts for the fall in total cholesterol concentration. The increased HDL is suggestive of an enhanced removal of cholesterol and that a shift in body cholesterol from the serum to tissue pool is not the mechanism by which the total cholesterol is reduced.

Although the changes occurring in platelet count are transient and demonstrate a fall which is significant only during the first 6 months, the modifications to platelet membrane lipids are important [18]. The partial replacement of arachidonic acid in the membrane by EPA would appear to weaken the platelets' ability to form aggregates due to the release of thromboxane A3 at the expense of the A2 form [9–11]. Some evidence to support this is suggested by the increased bleeding time and decreased GTN consumption. The latter suggests a reduction in anginal attacks and, in fact, the patients reported fewer episodes and an increasing exercise tolerance. This agrees with the suggestion that one of the mechanisms involved in angina is the hyper-activity of platelets in forming aggregates which temporarily or permanently occlude arteries already affected by atheromatous deposits [19].
This present work suggests that the incidence of arterial thrombosis may be reduced and the development of atheroma delayed in subjects on a mixed diet when taking a supplement of fish oil rich in \( \omega-3 \) fatty acids.

**Acknowledgement**

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**References**