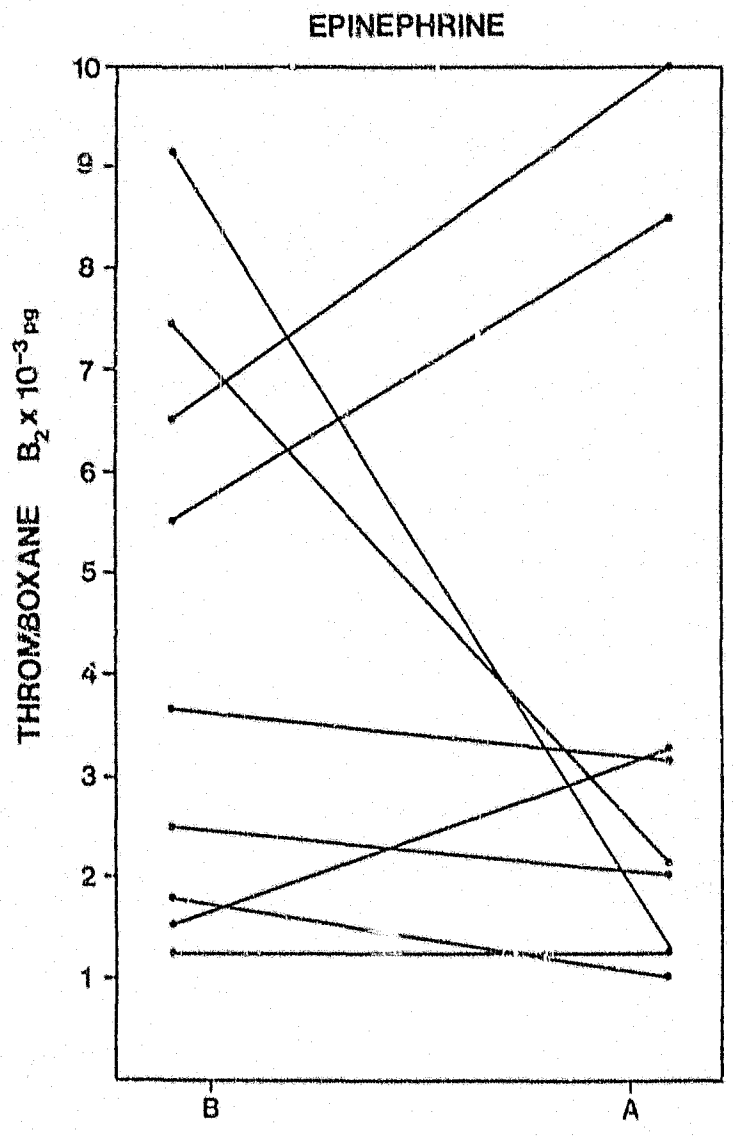


Fig 7.8 Thromboxane $B_2$ production by 9 subjects in response to epinephrine before (B) and after (A) a vegetarian diet.

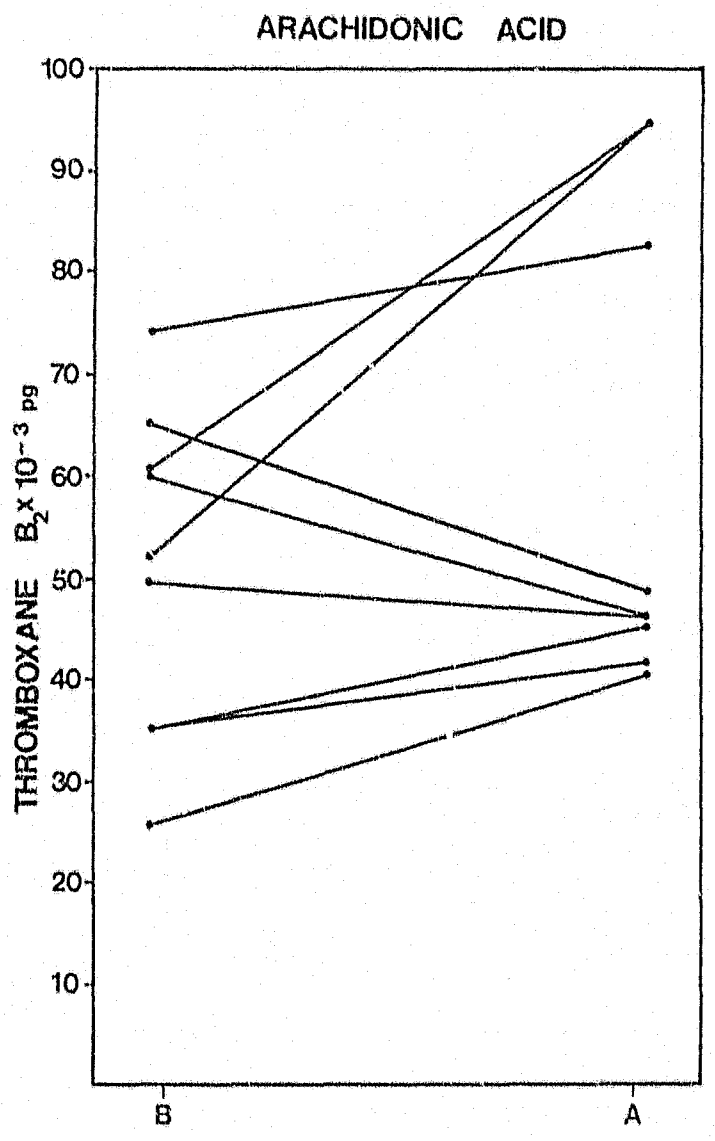


Fig 7.9 Thromboxane $B_2$ production by 9 subjects in response to Arachidonic Acid stimulation before (B) and after (A) a vegetarian diet.

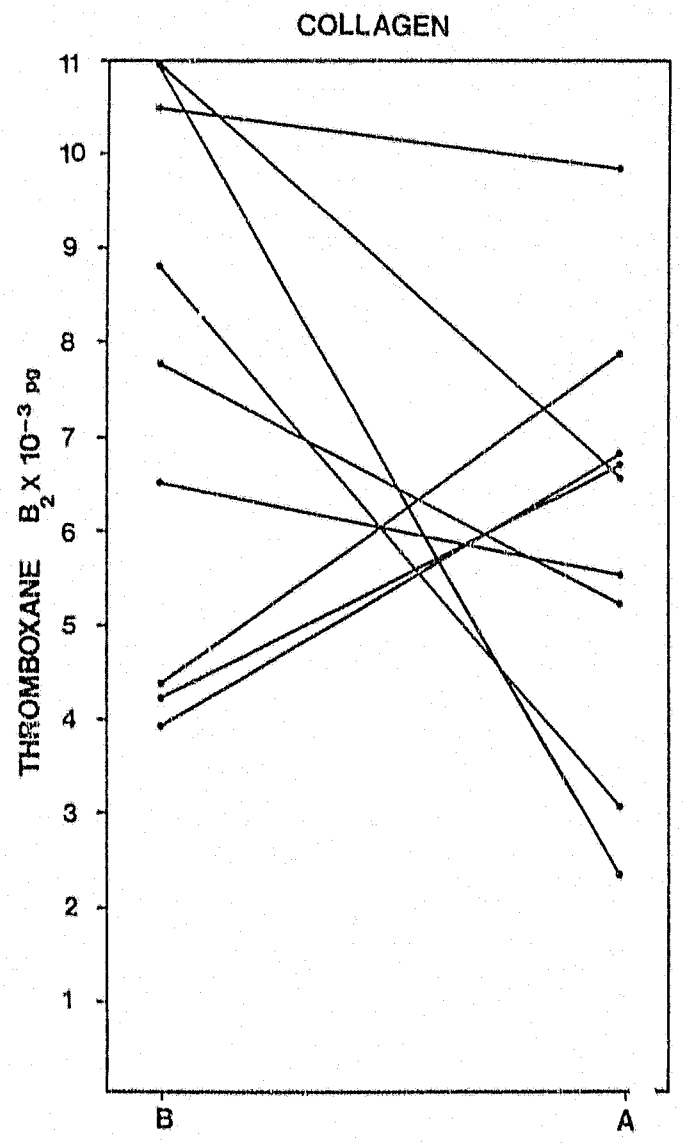


Fig. 7.10 Thromboxane $B_2$ production by 9 subjects in response to collagen stimulation before (B) and after (A) a vegetarian diet.

Table 7.4 $TXB_2$ production by platelets before and after a vegetarian diet.

| | ADP | EPI | COLL | AA |
|---|---|---|---|---|
| Before | 3929±2711 | 4393±2902 | 7608±3027 | 50750±16053 |
| After | 3552±2826 | 3723±3416 | 5969±2351 | 60055±23477 |
| p value | NS | NS | NS | NS |

Values shown are the mean ± SD in pg/ml of PPP

### 7.3.3. Platelet Indices

The platelet count (Table 7,5), Plateletcrit (Table 7.6), Mean platelet volume (Table 7,7) and platelet distribution width (Table 7.8) did not change significantly after the vegetarian diet.

Table 7.5 Platelet count of 9 subjects before and after vegetarian diet. Results expressed as counts/ul.

| Subject | Before | After |
|---|---|---|
| 1 | 192000 | 216000 |
| 2 | 338000 | 319000 |
| 3 | 368000 | 401000 |
| 4 | 270000 | 266000 |
| 5 | 295000 | 344000 |
| 6 | 187000 | 198000 |
| 7 | 368000 | 348000 |
| 8 | 236000 | 247000 |
| 9 | 390000 | 395000 |
| Mean | 282000 | 292000 |
| SD | 73000 | 71000 |
| P | NS | |

Table 7.6 Plateletcrit (%) of nine subjects before and after a vegetarian diet.

| Subject | Before | After |
|---|---|---|
| 1 | 0,155 | 0,181 |
| 2 | 0,182 | 0,187 |
| 3 | 0,263 | 0,263 |
| 4 | 0,295 | 0,321 |
| 5 | 0,266 | 0,264 |
| 6 | 0,258 | 0,296 |
| 7 | 0,330 | 0,305 |
| 8 | 0,192 | 0,194 |
| 9 | 0,343 | 0,340 |
| Mean | 0,253 | 0,261 |
| SD | 0,06 | 0,05 |
| P | >0.5 | |

Table 7.7 Mean platelet volume (fℓ) of 9 subjects before and after a vegetarian diet

| Subjects | Before | After |
|---|---|---|
| 1 | 8.0 | 8.4 |
| 2 | 9.7 | 9.4 |
| 3 | 7.7 | 8.2 |
| 4 | 8.0 | 8.0 |
| 5 | 9.8 | 9.9 |
| 6 | 8.7 | 8.6 |
| 7 | 8.9 | 8.7 |
| 8 | 8.1 | 7.8 |
| 9 | 8.8 | 8.6 |
| Mean | 8.6 | 8.6 |
| SD | 0.7 | 0.6 |
| P | >0.5 | |

Table 7.8 Platelet distribution width of 9 subjects before and after a vegetarian diet.

| Subjects | Before | After |
|---|---|---|
| 1 | 15.9 | 15.8 |
| 2 | 17.2 | 16.4 |
| 3 | 16.0 | 15.3 |
| 4 | 15.2 | 15.1 |
| 5 | 16.8 | 16.2 |
| 6 | 16.2 | 15.9 |
| 7 | 16.3 | 16.4 |
| 8 | 16.5 | 16.7 |
| 9 | 15.6 | 15.5 |
| Mean | 16.2 | 15.9 |
| S.D. | 0.6 | 0.5 |
| P | >0.5 | |

7.3.4. Platelet Fatty Acid

Platelet fatty acid composition (Table 7.10) did not change significantly except for an increase in the percentage of arachidonic acid. This can probably be explained by the increase in the linoleic (C18:2) and linolenic (18:3) acid intake during the vegetarian diet period (Gurr and James, 1980). Both these fatty acids can be metabolised to Arachidonic acid (Gurr and James, 1980).

Table 7.9 Fatty acid composition of platelets before and after a a vegetarian diet.

| Fatty acid | Before | After | p Value |
|---|---|---|---|
| C16* | 23.08 ± 1.65 | 21.34 ± 4.04 | NS |
| C18 | 30.04 ± 5.64 | 26.69 ± 4.19 | NS |
| C18:1 | 15.98 ± 5.93 | 15.27 ± 4.58 | NS |
| C18:2 | 7.98 ± 1.37 | 8.33 ± 3.94 | NS |
| C20:4 | 6.79 ± 1.88 | 12.17 ± 4.43 | P <0.01 |
| C20:5 | ND | ND | |
| Others | 16.13 | 16.20 | NS |

Values shown are the percentage of total fatty acids
* = Including di-methyl-acetals ND = Not detected

7.3.5. Serum Lipids

Serum lipids before and after the experimental diet is shown in figures 7,11 - 7,14 and summarized in table 7.10. Serum triglycerides and LDL cholesterol did not change but there were significant reductions in total and HDL cholesterol levels.

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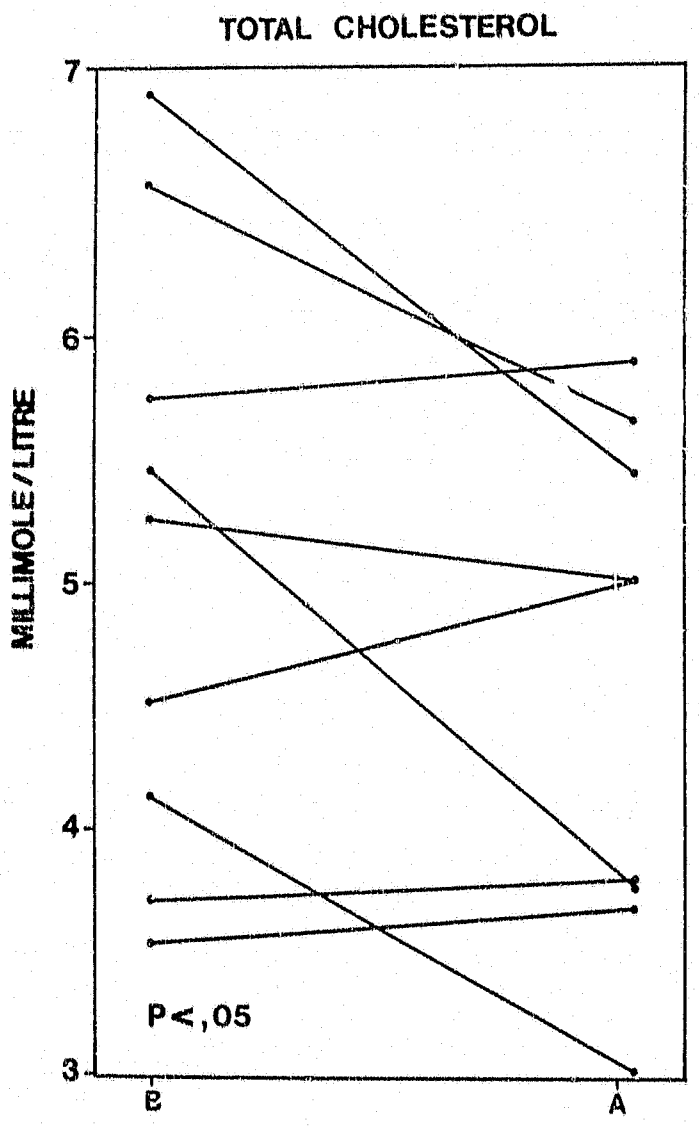


Fig 7,11 Total cholesterol of 9 subjects before (B) and after (A) the vegetarian diet

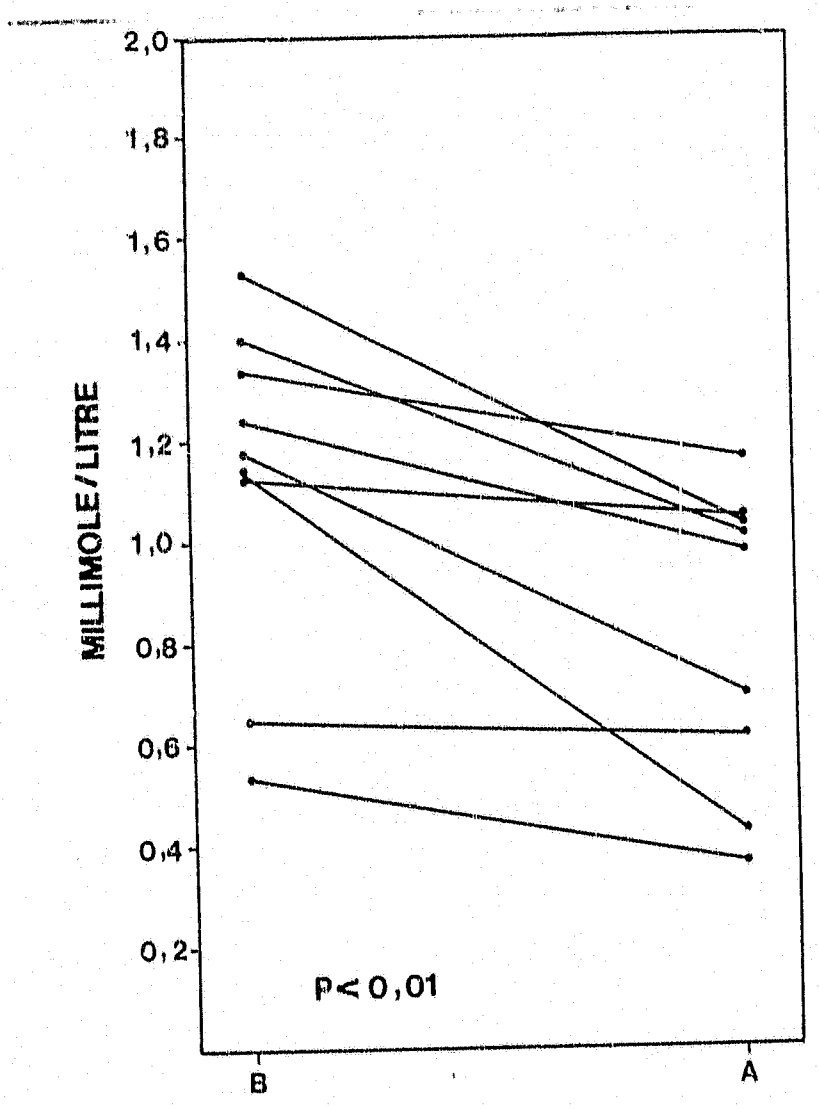


Fig 7,12 HDL cholesterol of 9 subjects before (B) and after (A) the vegetarian diet.

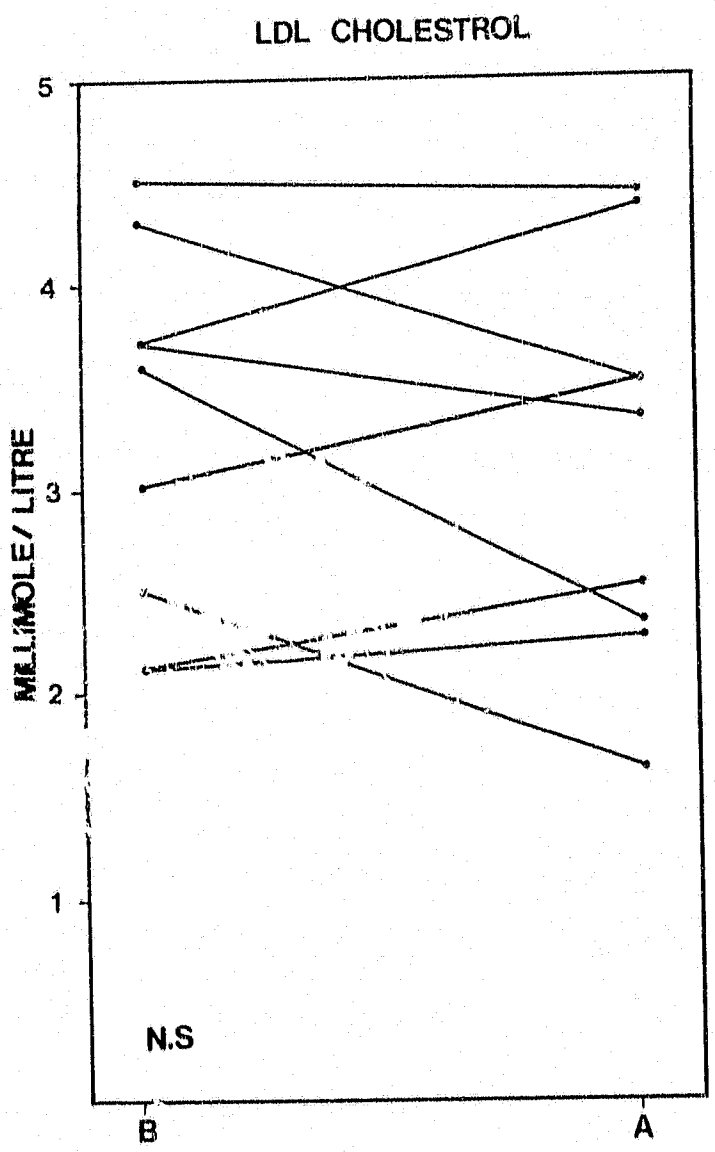


Fig 7,13 LDL cholesterol of 9 subjects before (B) and after (A) a vegetarian diet.

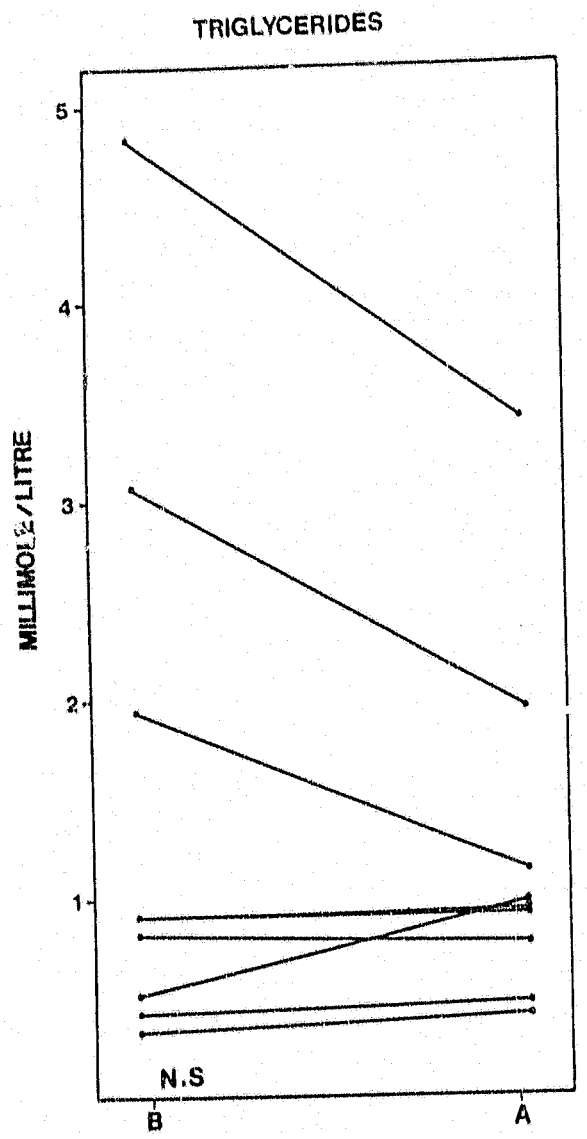


Fig 7.14 Trigiycerides of 9 subjects before (B) and after (A) a vegetarian diet.

Table 7.10. Serum lipids before and after a vegetarian diet.

| | CHOLESTEROL | | | |
|---|---|---|---|---|
| | TOTAL | HDL | LDL | TRIGLYCERIDES |
| Before | 5.11 ± 1.23 | 1.13 ± 0.33 | 3.28 ± 0.9 | 1.53 ± 1.52 |
| After | 4.6 ± 1.03 | 0.82 ± 0.31 | 3.10 ± 0.97 | 1.45 ± 1.08 |
| Value | <0.05 | <0.01 | NS | NS |

Values shown are in mmoles per litre

Table 7.10. Serum lipids before and after a vegetarian diet.

| | CHOLESTEROL | | | |
|---|---|---|---|---|
| | TOTAL | HDL | LDL | TRIGLYCERIDES |
| Before | 5.11 ± 1.23 | 1.13 ± 0.33 | 3.28 ± 0.9 | 1.53 ± 1.52 |
| After | 4.6 ± 1.03 | 0.82 ± 0.31 | 3.10 ± 0.97 | 1.45 ± 1.08 |
| Value | <0.05 | <0.01 | NS | NS |

Values shown are in mmoles per litre

## DISCUSSION

Unlike the effect of the fish diet (chapter 6) the vegetarian diet did not cause any reduction in platelet aggregability and Thromboxane $B_2$ production after 4 weeks of the diet. On the contrary there was a small but statistically significant increase in the height of the aggregation responses to epinephrine and arachidonic acid and an increase in the rate of reaction and a decrease in lag phase in response to the latter. The biological significance of this change is uncertain. Some of the studies on the effect of EPA on platelet function have used diets poor in arachidonic acid (Siess et al., 1980; Goodnight et al., 1981) although similar inhibitory effects were observed without reducing dietary AA (Thorngren and Gustafson, 1981; Hay et al., 1982). The present study demonstrates that a reduction in dietary AA did not decrease platelet function. The slight increase in aggregability to AA and epinephrine suggest that a low intake of AA may even oppose the inhibition by dietary EPA. In order to achieve inhibition of platelet function by dietary means it may be sufficient to increase EPA without any reduction in AA consumption.

A definite increase in the AA content of the platelets was observed during the vegetarian diet despite the reduction in dietary intake of AA. This can probably be explained by an increase in the linoleic (C18:2)and linolenic (18:3) acid intake during the diet since both these fatty acids can be metabolised to arachidonic acid (Gurr and James, 1980). The diet in this study contained many foods rich in linoleic acid including cereals (40-60% 18:2; 1-9% 18:3), soya beans (52% 18:2;) beans (28% 18:2;) cucumber (29% 18:2) Potatoes (56% 18:2) spinach (12% 18:2) mushrooms (13% 18:2) Bananas (16% 18:2) Apples (64% 18:2) Turnips (16% 18:2) (Paul and Southgate, 1978) as well as other vegetables, sunflower seed oil and nuts. In general, polyunsaturation in animals is accomplished by three separate desaturases designated as $\Delta 4$, $\Delta 5$ and $\Delta 6$ because they introduce double bonds between carbon atoms 4-5, 5-6 and 6-7 respectively (fig 7.15) (Gurr and James, 1980). The most important substrates for the first 'polydesaturation' are oleic acid (either synthesised or dietary), linoleic and linolenic acids (coming only from the diet). The structural relationships between the 'families' of fatty acids that arise from these three precursors are most easily recognised by using the system that numbers the double bonds from the methyl end of the chain. Hence, oleic acid gives rise to a series of omega 9 fatty acids, the

linoleic family to omega 6 fatty acids and the ∝-linolenic family to omega 3. The first desaturation is at C6 and all three precursors compete for desaturation by the same enzyme as illustrated in Fig. 7.15. The affinity of the substrates for the enzyme is in the order 18:3 > 18:2 > 18:1. Under conditions in which there is ample linoleic acid in the diet (linolenic acid is a minor dietary component unless the diet is rich in fish) linoleic acid competes successfully with oleic acid for the 6 desaturase with the result that the major bio-synthetic pathway is generally the one that gives rise to the omega 6 family of polyunsaturates, (Gurr and James, 1980). This excess of linoleic acid in the vegetarian diet could account for the increase in platelet arachidonic acid content, despite a reduction in dietary AA.

The plasma total cholesterol and HDL cholesterol levels in this study fell significantly but the LDL cholesterol and triglycerides did not change. These results were similar to those obtained after a fish diet (see previous section) and was presumably related to the high intake of polyunsaturated fatty acids (Rosenstock et al., 1982).

Platelet counts did not fall in this study in contrast to the fall observed after diets rich in

eicosapentaenoic acid in some studies (Goodnight et al.,1981; Hay et al., 1982). On the contrary the mean platelet count increased from 282,000 to 292,000 per ul after this study and the significance of this inconsistent effect would seem to be minor.

A comparison of the effects of the diet rich in EPA and poor in AA to a diet poor in AA is shown in table 7,11.

These findings support the view that the reduction in platelet functions observed after a diet rich in EPA but poor in AA can be attributed to the increased EPA content of the platelets consequent upon the increased dietary intake and not to a reduction in dietary AA. If this is true it should be possible to design an EPA rich diet that could inhibit platelet function and would be acceptable to many populations. It would only be necessary to increase the consumption of fish to perhaps 200-400g per day or to administer an oil containing 1-4g EPA per day. AA containing foods such as meat, poultry and eggs could be permitted.

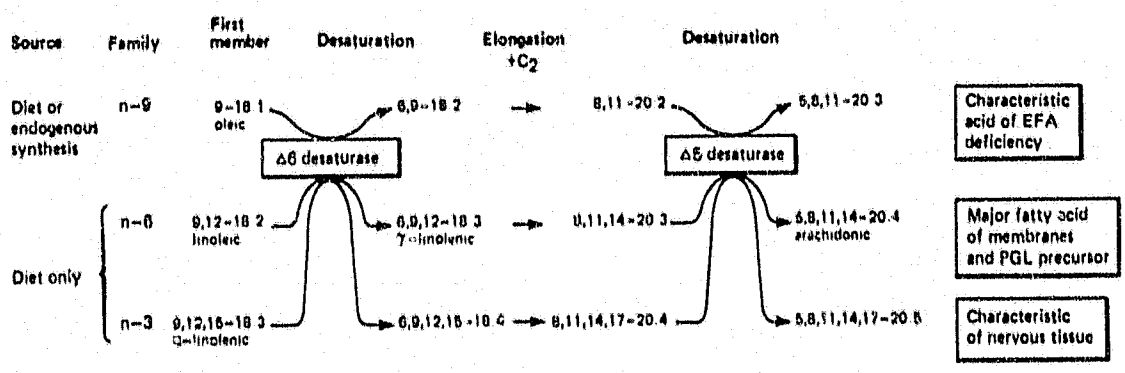


Fig. 7.15 Metabolic transformations of three major unsaturated fatty acid families by desaturation and elongation (Adapted from Gurr and James, 1981).

Table 7.11 Summary of the effects of the two diets – rich in EPA (Fish) and poor in Arachidonic Acid (Vegetarian).

| | FISH DIET | VEGETARIAN DIET |
|---|---|---|
| PLATELET FUNCTION | | |
| Platelet aggregation | ↓ | N.C. |
| $TXB_2$ production | ↓ | N.C. |
| Platelet counts | N.C. | N.C. |
| SERUM LIPIDS | | |
| Total cholesterol | ↓ | ↓ |
| HDL cholesterol | ↓ | ↓ |
| LDL cholesterol | N.C. | N.C. |
| Triglycerides | N.C. | N.C. |
| PLATELET FATTY ACIDS | | |
| EPA/AA | ↑ | N.C |
| AA content | ↓ | ↑ |

Increases ↑
Decreases ↓
N.C. No change

Table 7.11 Summary of the effects of the two diets - rich in EPA (Fish) and poor in Arachidonic Acid (Vegetarian).

| | FISH DIET | VEGETARIAN DIET |
|---|---|---|
| PLATELET FUNCTION | | |
| Platelet aggregation | ↓ | N.C. |
| $TXB_2$ production | ↓ | N.C. |
| Platelet counts | N.C. | N.C. |
| SERUM LIPIDS | | |
| Total cholesterol | ↓ | ↓ |
| HDL cholesterol | ↓ | ↓ |
| LDL cholesterol | N.C. | N.C. |
| Triglycerides | N.C. | N.C. |
| PLATELET FATTY ACIDS | | |
| EPA/AA | ↑ | N.C |
| AA content | ↓ | ↑ |

Increases ↑
Decreases ↓
N.C. No change

SUMMARY

Nine healthy subjects taking an average mixed "Western" diet were placed on a vegetarian diet poor in arachidonic acid for four weeks. All animal and marine foods except for cows milk and milk products were excluded. Platelet aggregation responses to arachidonic acid and epinephrine increased slightly whereas responses to ADP and collagen, were unchanged. Platelet thromboxane production, platelet counts, serum LDL cholesterol and triglycerides did not change but total and HDL serum cholesterol levels fell significantly. There was a significant rise in platelet arachidonic acid content but other platelet fatty acids did not change significantly. A reduction in dietary arachidonic acid did not inhibit platelet aggregation or thromboxane production.

# CHAPTER 8

## 8. The effect of a dietary supplementation with an extract of fish oil (MAXEPA) on platelet aggregation, platelet fatty acids and serum lipids.

### 8.1 Introduction

The findings in chapter 7 support the view that increasing the consumption of fish to 300-400g per day, or to administer an oil containing 1-4g EPA per day, would be sufficient to inhibit platelet function. This would be achieved without any further modifications in the diet and AA containing foods such as meat, poultry and eggs could be permitted.

Fish oils contain substantial amounts of C20-C22 n3 polyunsaturated fatty acids, notably EPA and docosahexaenoic acid (C22:6 n3) (DHA) (Stansby, 1969). Vegetable oils which are usually rich in linoleic acid (18:2 n6), are devoid of EPA and DHA and fats from land animals only contain trace amounts (Sanders and Roshanai, 1983).

Experiments with diet supplemented by fish oils have been performed in man. Sanders et al., (1981), showed that a daily supplement of 20 ml Cod liver oil taken for 6 weeks by male volunteers prolonged template bleeding time and led to a marked fall in plasma

triglyceride concentrations and to a slight increase in the HDL cholesterol concentrations. Moreover EPA and DHA displaced AA and docosatetraenoic acid (22:4 n6), from erythrocyte and platelet lipids.

In another study (Sanders and Roshanai, 1983), five healthy male volunteers received 5,10 and 20g MaxEPA (British Cod Liver Oils Ltd, Hull, U.K.) per day for three week periods in random order with a six week wash out period between experiments. At all levels of intake the concentration of EPA in platelets was increased mainly at the expense of AA. Platelet aggregation in vitro stimulated by 3um compound U46619 (a stable prostaglandin endoperoxide analogue) or by collagen at 10, 2 and 1mg/ml were unaffected. Bleeding time, however, tended to be prolonged with the supplement but did not follow any clear dose-dependent trend.

In a further study (Sanders, 1983) examined the influence of 10g MaxEPA per day for 14 weeks in 12 healthy males: 12 healthy controls who received no treatment were studied simultaneously. There was no effect on platelet aggregation measured in vitro. However, template bleeding times were prolonged in the MaxEPA treated group by one minute. The level of EPA in the erythrocyte lipids rose throughout the

experimental period in the MaxEPA treated group. Greaves et al., 1983) studied the effects of MaxEPA (Seven Seas Health Care Limited, Hull, U.K.) in a dosage of 1.8g EPA daily for 4 weeks in 6 normal subjects. They found that the EPA is readily incorporated into platelet membrane phospholipids when administered in the above dosage. The incorporation of EPA was seen to be selective for phosphatidyl choline and phosphatidyl ethanolamine and there appears to be little incorporation of EPA into phosphatidyl serine or phosphatidyl inositol. They found no effect on platelet aggregation in response to collagen and arachidonic acid and $TXB_2$ production. Despite the above findings, they found that the skin bleeding times were significantly prolonged.

In a very recent study, Galloway et al., (1985) studied the effects of dietary supplementation with MaxEPA (Seven Seas Health Care Limited, Hull U.K.) in six normal volunteers, They administered the equivalent of 1.8g EPA for 4 weeks. Throughout this study there were no significant changes in platelet aggregation responses or in platelet thromboxane production. There was a marked increased in the relative EPA content of phosphatidyl choline and phosphatidyl ethanolamine but not phosphatidyl inositol and phosphatidyl serine.

However, Ahmed and Holub (1984) found a significant reduction in thrombin induced aggregation after 30mls of cod liver oil in 7 volunteers for 2 weeks. Hay et al., 1982, gave an oil rich in EPA to 12 patients with ischaemic heart disease. The fish oil provided 3.5g EPA a day. After 5 weeks the fish oil caused a 10% lengthening in platelet survival times, a 15% fall in platelet count; a 75% fall in plasma levels of platelet factor 4, and a 30% fall in plasma B-TG, indicating reduced platelet activity

Brox et al., (1981) reported that platelet aggregation induced by 0,9mg of collagen/ml of PRP was inhibited after 4 weeks in subjects who had taken 25ml cod liver oil daily, which provided 1.4g EPA per day, for 4 weeks.

Studies on Greenland Eskimos (Dyerberg and Bang, 1979a,b) and the results of a previous study (chapter 6), have suggested a relationship between a lipid-rich diet, containing a high proportion of fatty acids of the n-3 family, and a bleeding tendency with prolonged skin bleeding time. Subsequent evidence suggests that dietary supplementation with n-3 fatty acids, particulaly EPA, has a platelet anti-aggregatory effect (Siess et al., 1980; Sanders et al., 1981; Thorngren and Gustafson, 1981; Goodnight et al., 1981; Hirai et al., 1982).

However, the mechanism underlying the anti-aggregatory effect of EPA is unknown although several hypothesis have been proposed including the competitive inhibition of cyclo-oxygenase by EPA (Needleman et al., 1979) and the increased synthesis of thromboxane $A_3$ and prostaglandin $I_3$ from EPA, resulting in reduced platelet aggregability (Needleman et al., 1979; Gryglewski et al., 1979; Whitaker et al., 1979; Fisher and Weber, 1984).

The effects of supplementing diets with fish oils have revealed conflicting results. In chapter 6, it was found that a diet rich in marine fish inhibited platelet aggregation and thromboxane production. This study was undertaken in order to determine the effect of a supplement of fish oil (MaxEPA - Seven Seas Health Care Ltd, Hull U.K.) on normal healthy volunteers without any further dietary modifications.

## 8.2 Materials and Methods

### 8.2.1 Subjects

Eight volunteers aged 19 to 37 years (mean 28 years, 3 males and 5 females) participated in the study. All gave informed consent and the study was approved by the ethics committee of the University of Witwatersrand Medical School. The subjects agreed not to take any

medication including aspirin for two weeks prior to and during the study.

8.2.2 Experimental Design

Each subject was venesected on two separate occasions before the experimental period, for baselines values. During the experimental period of 4 weeks, the subjects ingested 10 capsules (10g) of fish oil concentrate (Max EPA Seven Seas Health Care, Hill, U.K.) per day. The daily intake of EPA was 1.8 g. During the experiment, the subjects ate their normal regular diet, with no restrictions. The diets of the subjects contained only small amounts of fish. The subjects were venesected at 1 week, 2 weeks, 3 weeks and at 4 weeks after the beginning of the experiment.

8.2.3 Methods

Platelet aggregation, platelet indices, platelet fatty acids and serum lipids were determined at each venesection. Bleeding times were measured before and after the experimental period. Fatty acid composition of the capsules were determined by G.L.C. methods as described earlier.

## 8.3 Results

### 8.3.1 Fatty acid composition of Max EPA

The fatty acid composition of the Max EPA used is shown in Table 8.1. These figures compare favourably with that obtained by Sanders and Roshanai (1983) as well as the specifications as supplied by Seven Seas Health Care Limited. (Appendix 1).

Table 8.1 Fatty acid composition of the Max EPA fed to the subjects.

| Fatty Acid | %of Total Fatty Acids |
|---|---|
| C16:0 | 16.89 |
| C16:1 | 7.00 |
| C18:0 | 4.90 |
| C18:1 | 13.30 |
| C18:2 W6 | 1.45 |
| C18:3 W3 | 0.30 |
| C20:5 W3 | 17:32 |
| C22:5 | 2:90 |
| C22:6 W3 | 11:08 |

8.3.2. Platelet aggregation

Platelet aggregation responses after stimulation with ADP, Epinephrine, Collagen and Arachidonic Acid is shown in tables 8,2 - 8,5. Aggregation responses after Max EPA supplementation did not change significantly, although there was a tendency for the height of aggregation to decrease after 4 weeks. Height of aggregations in response to, ADP decreased from 6,7 ± 1,0 cm to 5.7 ± 1,6 cm, in Epinephrine from 5.2 ± 1.7 cm to 4.9 ± 1.2 cm, in collagen from 7.9 ± 0.9 cm to 6.5 ± 0,8 cm and in AA from 7.9 to ± 0.5 cm to 7.3 ± 0.9 cm. These changes were negligible.(Wilcoxon signed rank test).

8.3.3. Bleeding times

Bleeding times of 8 subjects are shown in table 8.6. The mean bleeding time tended to be prolonged by the MaxEPA supplement (P`0,05)

Table 8.2 Platelet aggregation in response to ADP stimulation after 4 weeks of Max EPA ingestion. Values shown are the height of the aggregation response in cms.

| Subject | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 6.4 | 7.0 | 4.2 | 3.2 | 4.8 |
| 2 | 7.2 | 6.2 | 7.4 | 6.8 | 6.8 |
| 3 | 5.1 | 5.0 | 2.8 | 2.6 | 5.1 |
| 4 | 6.2 | 3.0 | 5.7 | 4.3 | 3.0 |
| 5 | 7.8 | 8.3 | 8.0 | 7.9 | 6.3 |
| 6 | 8.2 | 7.6 | 6.7 | 7.3 | 9.0 |
| 7 | 6.8 | 7.8 | 5.0 | 6.2 | 5.3 |
| 8 | 5.7 | 8.5 | 5.0 | 6.2 | 5.3 |
| Mean | 6.7 | 6.7 | 5.8 | 5.6 | 5.7 |
| ±SD | 1.0 | 1.8 | 1.6 | 1.9 | 1.6 |

Table 8.3. Platelet aggregation in response to epinephrine stimulation after 4 weeks of Max EPA ingestion. Values shown are the heights of the aggregation responses in cms

| Subject | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 6 | 7.6 | 5.9 | 6.0 | 5.6 |
| 2 | 4.5 | 3.2 | 6.0 | 2.7 | 3.0 |
| 3 | 4.3 | 4.5 | 3.4 | 2.1 | 5.6 |
| 4 | 7.3 | 7.5 | 7.2 | 7.5 | 4.0 |
| 5 | 7.8 | 7.7 | 7.5 | 7.3 | 7.3 |
| 6 | 3.6 | 4.3 | 2.2 | 3.2 | 5.2 |
| 7 | 5.7 | 7.4 | 6.8 | 6.8 | 5.0 |
| 8 | 2.6 | 5.5 | 3.5 | 5.2 | 3.8 |
| Mean | 5.2 | 5.9 | 5.3 | 5.1 | 4.9 |
| ±SD | 1.7 | 1.7 | 1.9 | 2.0 | 1.2 |

Table 8.4. Platelet aggregation in response to collagen stimulation after 4 weeks of MaxEPA ingestion. Values shown are the height of the aggregation response in cms.

| Subject | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 8.1 | 7.7 | 4.0 | 5.1 | 5.6 |
| 2 | 6.7 | 7.0 | 3.5 | 5.4 | 5.0 |
| 3 | 6.9 | 6.3 | 7.2 | 6.6 | 6.0 |
| 4 | 8.5 | 6.2 | 6.3 | 6.5 | 6.8 |
| 5 | 7.5 | 6.8 | 7.8 | 7.0 | 6.7 |
| 6 | 7.5 | 7.5 | 7.6 | 7.4 | 7.1 |
| 7 | 9.5 | 7.8 | 7.4 | 7.0 | 7.1 |
| 8 | 7.5 | 7.3 | 5.0 | 5.3 | 7.3 |
| Mean | 7.9 | 7.1 | 6.1 | 6.2 | 6.5 |
| ± | 0.9 | 0.6 | 1.6 | 0.8 | 0.8 |

Table 8.5. Platelet aggregation in response to Arachidonic acid stimulation after 4 weeks of MaxEPA ingestion. Values shown are the height of the aggregation response in cms.

| Subject | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 8.1 | 8.7 | 8.5 | 8.5 | 5.6 |
| 2. | 7.2 | 7.5 | 8.5 | 8.0 | 8.0 |
| 3 | 7.0 | 7.4 | 7.3 | 7.2 | 6.5 |
| 4. | 8.0 | 7.7 | 7.5 | 8.0 | 8.0 |
| 5. | 8.5 | 7.0 | 7.7 | 7.0 | 6.5 |
| 6 | 8.5 | 8.5 | 8.5 | 7.5 | 8.5 |
| 7 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 |
| 8 | 8.1 | 8.0 | 7.4 | 6.5 | 8.0 |
| Mean | 7.9 | 7.8 | 7.9 | 7.5 | 7.3 |
| ± | 0.5 | 0.5 | 0.5 | 0.6 | 0.9 |

Table 8.6. Bleeding times in 8 subjects before and after 4 weeks MaxEPA ingestion. Results in minutes.

| Subjects | Baseline | 4 weeks |
|---|---|---|
| 1 | 5.2 | 5.6 |
| 2 | 5.4 | 6.0 |
| 3 | 6.0 | 6.4 |
| 4 | 6.8 | 7.2 |
| 5 | 6.6 | 7.0 |
| 6 | 5,0 | 5.8 |
| 7 | 7.0 | 7.4 |
| 8 | 5.8 | 6.4 |
| Mean | 6.0 | 6.5 |
| ± SD | 0.75 | 0.67 |
| P | <0,05 | |

8.3.4 Platelet Indices

Platelet counts, plateletcrit, mean platelet volume and the platelet distribution widths are shown in tables 8.7 - 8.10. There were no significant changes in any of the indices, after and during the experimental period.

8.3.5 Serum Lipids

The serum cholesterol, triglycerides and LDL cholesterol (Tables 8.11 - 8.13) were not significantly affected by Max EPA after 4 weeks of ingestion. However there was a tendency for the triglycerides to decrease after four weeks from 0,87 $\pm$ 0.62 to 0,69 $\pm$ 0,81, but this decrease was not significant (P= 0.12350 (Wilcoxon signed rank test). The HDL cholesterol (Table 8.14) increased significantly after 1 week of Max EPA (P=0,0117) and remained high for the duration of the experimental period.

Table 8.7 Platelet counts before, during and after 4 weeks of Max EPA ingestion. Results in counts/uℓ.

| Subject | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 282000 | 382000 | 357000 | 354000 | 339000 |
| 2 | 383000 | 384000 | 374000 | 326000 | 331000 |
| 3 | 249000 | 256000 | 312000 | 278000 | 280000 |
| 4 | 265000 | 276000 | 231000 | 297000 | 261000 |
| 5 | 231000 | 274000 | 306000 | 303000 | 287000 |
| 6 | 284000 | 317000 | 329000 | 313000 | 287000 |
| 7 | 245000 | 184000 | 278000 | 259000 | 205000 |
| 8 | 231000 | 249000 | 236000 | 222000 | 203000 |
| Mean | 271250 | 290250 | 290375 | 294000 | 274125 |
| ± SD | 46408 | 63790 | 46024 | 38308 | 47194 |

Table 8.8 Plateletcrit before, during and after 4 weeks of Max EPA ingestion. Results in %.

| Subject | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 0.233 | 0.315 | 0.289 | 0.286 | 0.285 |
| 2 | 0.352 | 0.349 | 0.331 | 0.308 | 0.350 |
| 3 | 0.215 | 0.234 | 0.244 | 0.255 | 0.224 |
| 4 | 0.243 | 0.252 | 0.220 | 0.267 | 0.254 |
| 5 | 0.234 | 0.277 | 0.282 | 0.284 | 0.285 |
| 6 | 0.256 | 0.309 | 0.322 | 0.325 | 0.291 |
| 7 | 0.329 | 0.232 | 0.284 | 0.334 | 0.222 |
| 8 | 0.202 | 0.222 | 0.219 | 0.195 | 0.174 |
| Mean | 0.258 | 0.274 | 0.274 | 0.278 | 0.260 |
| ±SD | 0.050 | 0.043 | 0.040 | 0.045 | 0.050 |

Table 8.9 Mean Platelet Volume before, during and after 4 weeks of Max EPA ingestion. Results in fℓ.

| Subject | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 8.2 | 8.2 | 8.0 | 8.0 | 8.4 |
| 2 | 9.1 | 9.0 | 9.2 | 9.4 | 9.2 |
| 3 | 8.6 | 8.4 | 7.8 | 8.1 | 7.9 |
| 4 | 9.1 | 9.1 | 9.5 | 8.9 | 9.7 |
| 5 | 10.1 | 10.0 | 9.2 | 9.7 | 9.9 |
| 6 | 9.0 | 9.7 | 9.7 | 10.3 | 10.1 |
| 7 | 13.3 | 12.6 | 10.2 | 12.8 | 10.7 |
| 8 | 8.7 | 8.9 | 9.2 | 8.7 | 8.5 |
| Mean | 9.5 | 9.5 | 9.1 | 9.5 | 9.3 |
| ±SD | 1.5 | 1.3 | 0.8 | 1.5 | 0.9 |

Table 8.10. Platelet distribution width before, during and after 4 weeks of Max EPA ingestion.

| Subject | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 9.9 | 9.9 | 10.0 | 10.0 | 9.8 |
| 2 | 8.5 | 9.4 | 9.6 | 9.5 | 9.6 |
| 3 | 9.9 | 10.0 | 10.0 | 10.0 | 10.0 |
| 4 | 9.8 | 9.9 | 9.9 | 9.7 | 9.6 |
| 5 | 9.6 | 9.6 | 9.8 | 9.6 | 9.5 |
| 6 | 10.2 | 9.8 | 9.7 | 9.8 | 9.8 |
| 7 | 11.7 | 10.7 | 10.3 | 10.5 | 10.2 |
| 8 | 10.0 | 9.9 | 10.1 | 9.9 | 9.9 |
| Mean | 10.8 | 9.90 | 9.93 | 9.88 | 9.80 |
| ±SD | 0.65 | 0.35 | 0.21 | 0.29 | 0.22 |

Table 8.11 Total serum cholesterol before, during and after 4 weeks of Max EPA ingestion. Values in mmoles/liter.

| Subjects | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 4,19 | 5,05 | 4,58 | 4,66 | 3,91 |
| 2 | 4,35 | 4,93 | 3,94 | 3,71 | 4,43 |
| 3 | 4,67 | 4,77 | 4,47 | 4,61 | 4,69 |
| 4 | 4,50 | 4,55 | 4,28 | 5,06 | 4,74 |
| 5 | 3,61 | 4,02 | 3,97 | 4,56 | 4,92 |
| 6 | 6,67 | 7,08 | 7,28 | 7,44 | 7,52 |
| 7 | 5,02 | 5,58 | 5,93 | 5,71 | 4,44 |
| 8 | 3,74 | 4,10 | 3,97 | 4,35 | 3,81 |
| Mean | 4,59 | 5,01 | 4,80 | 5,01 | 4,81 |
| ±SD | 0,89 | 0,91 | 1,12 | 1,06 | 1,08 |

Table 8.12. Serum triglycerides before, during and after 4 weeks of Max EPA ingestion. Values in mmoles/liter.

| Subjects | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 0,83 | 0,36 | 0,37 | 0,34 | 0,31 |
| 2 | 1,27 | 0,66 | 0,52 | 0,48 | 0,43 |
| 3 | 0,6 | 0,41 | 0,42 | 0,35 | 0,49 |
| 4 | 0,35 | 0,23 | 0,14 | 0,31 | 0,22 |
| 5 | 0,29 | 0,18 | 0,27 | 0,34 | 0,33 |
| 6 | 2,33 | 3,03 | 2,84 | 3,61 | 2,82 |
| 7 | 0,5 | 0,33 | 0,33 | 0,28 | 0,32 |
| 8 | 0,81 | 0,46 | 0,85 | 1,05 | 0,57 |
| Mean | 0,87 | 0,70 | 0,72 | 0,85 | 0,69 |
| ±SD | 0,62 | 0,88 | 0,83 | 1,07 | 0,81 |

Table 8.13 Serum LDL cholesterol before, during and after 4 weeks of Max EPA ingestion. Values in mmoles/liter.

| Subjects | Baseline | 1st week | 2nd week | 3rdweek | 4th week |
|---|---|---|---|---|---|
| 1 | 2.75 | 3.30 | 3.14 | 2.78 | 2.29 |
| 2 | 2.48 | 2.68 | 2.15 | 1.98 | 2.78 |
| 3 | 2.83 | 2.60 | 2.59 | 2.72 | 2.59 |
| 4 | 3.23 | 2.91 | 2.71 | 3.33 | 2.77 |
| 5 | 2.37 | 2.47 | 2.36 | 2.64 | 2.49 |
| 6 | 5.08 | 5.13 | 5.39 | 5.22 | 5.59 |
| 7 | 3.86 | 4.11 | 4.19 | 4.32 | 3.14 |
| 8 | 2.46 | 2.85 | 2.53 | 2.75 | 2.53 |
| Mean | 3.13 | 3.26 | 3.13 | 3.21 | 3.02 |
| ±SD | 0,87 | 0,86 | 1,04 | 0,98 | 0,99 |

Table 8.14. Serum HDL cholesterol before, during and after 4 weeks of MaxEPA ingestion. Values in mmoles/liter.

| Subjects | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|
| 1 | 1,05 | 1,58 | 1,27 | 1,72 | 1,47 |
| 2 | 1,28 | 1,94 | 1,55 | 1,50 | 1,45 |
| 3 | 1,56 | 1,60 | 1,68 | 1,72 | 1,87 |
| 4 | 1,10 | 1,53 | 1,48 | 1,58 | 1,59 |
| 5 | 1,10 | 1,46 | 1,48 | 1,76 | 1,74 |
| 6 | 0,52 | 0,56 | 0,58 | 0,56 | 0,63 |
| 7 | 0,93 | 1,31 | 1,26 | 1,58 | 1,15 |
| 8 | 0,91 | 1,03 | 1,05 | 1,11 | 1,01 |
| Mean | 1,06 | 1,38 | 1,29 | 1,44 | 1,36 |
| ±SD | 0,28 | 0,39 | 0,32 | 0,38 | 0,38 |

8.3.6. Platelet fatty acids

The fatty acid composition of the platelets before and after the diet is shown in Table 8.15. The C20:4 and C20:5 values for each week is shown in Table 8.16. There was a significant decrease in C16:0 and a significant increase in C20:5 and C22:6. There were no significant changes in the other fatty acids.

Table 8.15 Platelet fatty acid composition before and after MaxEPA ingestion

| Fatty Acid | Before | After | p Value |
|---|---|---|---|
| C16:0 | 18.78+4/31 | 13.20+4,07 | <0.01 |
| C18:0 | 18.99±5.33 | 17,63±2.27 | NS |
| C18:1 | 5.79±3.33 | 7.92±2.09 | NS |
| C20:4 | 13.36±1.24 | 14,16±1.43 | NS |
| C20:5 | 0.25±0.15 | 0.54±0.31 | <0.05 |
| C22:5 | 1.80±0.17 | 3.20±0.20 | <0.05 |
| C22:6 | 1.32±0.83 | 3.68±1.48 | <0.05 |
| Others | 28.39 | 32.4 | |

Table 8.16. Platelet Arachidonic Acid (C20:4 and Eicosapentaenoic Acid (C20:5) composition in 8 subjects before, during and after 4 weeks MaxEPA ingestion.

| Subject | Fatty Acid | Baseline | 1st week | 2nd week | 3rd week | 4th week |
|---|---|---|---|---|---|---|
| 1 | C20:4 | 13,00 | 13,76 | 18,10 | 16,41 | 14,4 |
| | C20:5 | 0,07 | 0,77 | 0,77 | 0,71 | 0,30 |
| 2 | C20:4 | 13,00 | 17,02 | 15,90 | 14,99 | 11,70 |
| | C20:5 | 0,47 | 1,38 | 1,23 | 1,4 | 0,90 |
| 3 | C20:4 | 14,20 | 15,90 | 17,90 | 16,60 | 16,60 |
| | C20:5 | 0,20 | 0,40 | 0,69 | 1,2 | 0,97 |
| 4 | C20:4 | 11,3 | 16,59 | 10,05 | 12,57 | 13,7 |
| | C20:5 | 0,45 | 1,96 | 2,07 | 0,44 | 0,56 |
| 5 | C20:4 | 15,1 | 14,2 | 10,42 | 11,90 | 12,90 |
| | C20:5 | 0,04 | 0,50 | 1,02 | 0,60 | 0,12 |
| 6 | C20:4 | 14,9 | 13,9 | 17,9 | 14,68 | 15,7 |
| | C20:5 | 0,25 | 0,66 | 0,80 | 0,826 | 0,88 |
| 7 | C20:4 | 13,30 | 12,00 | 17,30 | 16,80 | 13,80 |
| | C20:5 | 0,30 | 1,52 | 1,43 | 0,60 | 0,30 |
| 8 | C20:4 | 12,07 | 16,70 | 10,66 | 15,15 | 14,50 |
| | C20:5 | 0,20 | 0,44 | 0,74 | 0,50 | 0,30 |

## DISCUSSION

Before discussing the significance of the results obtained in this study the diet warrants consideration. The MaxEPa capsules were not well tolerated and subjects complained of an after taste hours after the ingestion of the capsules.

The MaxEPA which provided 1,8g EPA, led to a considerable increase in the proportion of EPA in platelet lipids. However this increase did not compare to that obtained by Sanders and Roshanai (1983). The increase in the proportion of C22:5 n3 was large considering the small amount of this fatty acid provided by the MaxEPA supplement. This would suggest that a portion of the dietary EPA is chain elongated to 22:5 n3 (Sanders and Roshanai, 1983). The proportion of DHA was considerably increased by the MaxEPA supplement.

Despite these changes in the fatty acid composition platelet aggregations were not altered significantly. Several studies have reported reductions in platelet aggregation after oils rich in EPA ingestion (Brox et al.,1981; Hay et al., 1982; Ahmed and Holub, 1984). However, the results of other studies using diets rich in EPA (Sanders et al., 1981; Sanders and

Roshanai, 1983; Greaves et al.,1983), do not indicate any consistent change in platelet aggregation measured in vitro.

Several studies have shown that bleeding time is prolonged by a diet rich in fish oil (Sanders et al., 1981; Goodnight et al., 1981; Thorngren and Gustafson, 1981; Sinclair, 1982; Sanders and Roshanai, 1983; Greaves et al.,1982 ; Woodcock et al 1984). In the present study the bleeding times increased ($P<0,05$) with the MaxEPA supplement in the absence of altered platelet reactivity. Relatively small amounts of EPA are claimed to lower whole blood viscosity and to increase erythrocyte deformability (Terano et al., 1983). This may well explain the prolonged bleeding times observed with modest intakes of fish oils (Sanders, 1983). However substantial reductions in plasma triglyceride concentrations also occur and this in itself may influence whole blood viscosity (Sanders, 1983).

The ability of n3 fatty acids to lower plasma total cholesterol concentration is well documented (Stansby, 1969) and the potency increases with the degree of unsaturation. This study showed that the plasma triglyceride concentrations were decreased by 10g

(10 tablets) of MaxEPA per day, which provided about 1,8g of C20-22 n3 fatty acids. Similar findings were reported by Sanders and Roshanai (1983). Bronsgeest-Shoute et al., (1981) found that 7g of C20-22 n3 fatty acids per day but not lower doses decreased plasma triglyceride concentrations. These workers were using a cod liver oil concentrate which was a mixture of non-esterified fatty acids and methyl esters and was prepared by removing the more saturated fatty acids as urea adducts. It has been known (Stansby, 1969; Kinsell and Sinclair 1957; Kingsburg et al., 1961) that fatty acids of fish oils prepared in this way often yield different results from those expected. Firstly the absorption of the fatty acids may be poor compared with that of the triglyceride and secondly peroxidized fatty acids and their breakdown products such as aldehydes, hydroxy acids and ketones are concentrated in the non- urea adductable fraction (Andrews et al., 1960)

Our results suggest that the HDL cholesterol concentrations may be slightly increased by 10g MaxEPA per day. Other studies using diets rich in fish oils have noted a similar trend (Sanders et al.,1981; Saynor and Verel 1980; Von Lossonczy et al., 1978; Sanders and Roshanai 1983). Ten grams MaxEPA did not decrease total cholesterol levels, on the contrary

serum cholesterol levels increased after 4 weeks. Although further work is necessary to determine whether MaxEPA raises HDL cholesterol, MaxEPA certainly does not reduce the HDL cholesterol concentration as do some drugs used to lower plasma triglyceride concentrations such as clofibrate (Sanders and Roshanai 1983). The use of MaxEPA as a dietary means of treating hypertriglyceridaemia deserves a controlled trial as preliminary studies (Saynor and Verel 1981; Phillipson et al., 1985) on patients with hypertrigyceridaemia are encouraging.

In conclusion these results suggest that modest amounts of fish oil significantly influence blood lipids and haemostatic function such as bleeding times without alteration in platelet aggregability.

SUMMARY

The diets of 8 normal healthy subjects were supplemented with 10g of fish oil concentrate for 28 days (MaxEPA, Seven Seas Health Care, Hull UK). The daily intake of EPA was 1.8g. During the experiment, the subjects ate their normal regular diet, with no restrictions. Dietary supplementation with 1,8g MaxEPA showed no reduction in platelet aggregation response despite an increase in platelet EPA. Bleeding times were prolonged in the absence of any change in platelet function. Serum HDL cholesterol was increased and triglycerides reduced. These findings suggest that the mechanism by which EPA-rich fish oil influences primary haemostasis requires re-evaluation. This study remains compatible with the proposition that dietary fish or fish oil delays primary haemostasis and influences serum lipids, and may decrease the incidence of arterial thrombosis in the human population. The mechanism of this effect has not yet been determined.

# CHAPTER 9

## 9. General Discussion and conclusion

### 9.1. Aspirin

It is still not possible to provide accurate guidelines for the use of platelet suppressive agents in individual patients with thromboembolic disease. However, a picture is gradually emerging of the therapeutic benefit of platelet suppressive agents, for well-defined groups of patients. These include unstable angina and coronary artery by-pass surgery, where aspirin alone and aspirin and dipyridamole respectively have shown to be of value. Results of studies of anti-platelet drugs in patients with amaurosis fugax and thromboembolism associated with prosthetic heart valves are also encouraging. The more important question of secondary prevention of myocardial infarction remains unresolved. Here, problems relating to study design cannot be over-emphasized. Although the currently available results do not allow us to draw firm conclusions, the trend is nevertheless sufficiently encouraging to suggest that further studies would be worthwhile.

The present studies suggest a dosage frequency of at least once a day at a dose of 150-300 mg/day since longer intervals would permit recovery of platelet function in some cases. If *in vivo* aggregation is likely to occur in response to a combination of aggregating agents, it seems likely that the need for daily administration of aspirin

would be even greater than our data suggest in order to achieve constant suppression of platelet function.

The concept of a low dose of aspirin or "therapeutic window" that would discriminate between the inhibitory effects on platelets and on the endothelium, although attractive, may well be illusory. Even if such a dose exists, its clinical value has yet to be demonstrated.

## 9.2. Dietary fish oils

Perhaps the most convincing evidence for the influence of fish oils on platelet vessel wall interactions is their protective effect on experimental cerebral infarction in cats (Black et al., 1979) and experimental myocardial infarction in dogs (Culp et al., 1980). In both these studies infarct size was reduced and there was evidence of improved collateral circulation. In the latter study platelet fatty acids were modified considerably by the diet but platelet aggregation measured in vitro was not affected.

Many studies using fish oils or fatty fish have only considered the influence of EPA and not the other n-3 fatty aids. Docosahexaenoic acid (DHA) (22:6) is usually a major component of fish oils and is the major polyunsaturated fatty acid found in human brain and retina. DHA is thought to play an important role in

brain and retinal function (Tinoco et al., 1979). Sanders and Bolster (1983) found that the potassium salts of DHA added to PRP inhibited platelet aggregation induced by low doses of collagen at similar concentrations to EPA. The role DHA may play in haemostasis deserves more attention.

### 9.2.1. Side Effects

If fish oil concentrates are to be used for prolonged periods as prophylactic agents any potentially undesirable side effects must be considered. The thrombocytopenia observed after the sudden introduction of very large quantities of salmon into the diet is an observation that cannot be ignored (Goodnight et al., 1981). Platelet counts in Eskimos living on a high fish diet were also lower than in Danish control subjects (Dyerberg and Bang, 1979a,b). Furthermore Hay et al., (1982) found a slight lowering of the platelet count using MaxEPA for 5 weeks although Saynor et al., (1984) reported that this effect was transient and disappeared after several months. Sinclair (1982 and 1984) documented a significant fall in his own platelet count on a diet of high EPA content. Sinclair (1984) attributed this to the gadoleic (20:1 n-11) and cetoleic (22:1 n-11) acids in the marine foods. Although there is some support for this view, the fact that a mild fall in platelet count can occur after administration of MaxEPA

is not explained since this preparation is almost free of long chain mono-enoic acids. The possibility that other compounds in marine foods may lower platelet counts cannot be excluded altogether.

Toxic effects on the heart and skeletal muscles have been attributed to erucic acid in rats (Christopherson et al., 1982; FAO/WHO, 1977), and cynomolgus monkeys (Schiefer, 1982). The pathological lesion is a transient myocardial lipidosis and fibrosis. In an attempt to assess the significance of this lesion Ruiter et al., (1978) fed 100 g of mackerel oil to young piglets. These animals are known to be sensitive to the effects of n-3 fatty acids in their diets. Despite the development of "yellow fat disease", and an increase in the triglyceride content of the heart muscle, the animals had normal rates of growth, calculated metabolizable energy, liver weight, and reached the same final body weight as the controls. The authors indicate that " no detrimental effects of long chain mono-enoic acids were observed". The pathological changes were not accompanied by clinical symptoms.

It may be that the absence of myocardial damage attributable to long chain mono-enoic fatty acids in human populations consuming marine based diets is due to a metabolic adaptation similar to that in the rat (Bradlow, 1985).

The "yellow fat disease" of pigs (Ruiter et al., 1978) is thought to be due to Vitamin E deficiency resulting from increased requirements due to the highly unsaturated nature of the n-3 fatty acids. This condition has not been reported in humans or in other species (Goodnight et al., 1982) and even in piglets there were no clinical effects and growth was normal.(Ruiter et al., 1978) Nevertheless the addition of Vitamin E to a diet rich in n-3 fatty acids would seen prudent. Available concentrates do contain added Vitamin E (Appendix 1).

An increased intake of fish oils could pose the hazard of hypervitaminosis A and D. Cod liver oil in amounts of 20 ml per day would provide four times the daily allowance for each vitamin (Goodnight et al., 1982). This could cause toxic consequences and it is therefore necessary to eliminate or at least reduce the concentrations of these vitamins in fish oil preparations especially if they are to be used for prolonged periods.

In recommending an increased intake of the appropriate fish species including salmon, mackerel, herring, sardines, etc it is also necessary to consider the salt

intake from such a diet. These fish are frequently salted or pickled and if eaten daily might cause a salt intake great enough to produce or aggrevate hypertension. Consequently any benefits due to the effects on lipids and platelets could be completely negated. An ideal concentrate would therefore contain high levels of EPA and DHA, but little or no cholesterol, long chain mono-enoic fatty acids (erucic, cetoleic, gadoleic), Vitamin A and D and salt. Vitamin E should be added (Bradlow, 1985).

9.2.2. Conclusion

Available evidence provides a convincing case for further research into the potential prophylactic and therapeutic value of the n-3 fatty acids.

At the experimental level more information is required about the mechanism and nature of the effects on lipid metabolism, platelet and leucocyte functions and endothelial cell biochemistry. Studies on the effects of these substances on cell-cell interaction particularly those involving endothelial cells may yield significant data.

Clinical studies on patients with a variety of cardio-vascular disorders are also necessary in order to assess potential benefits. Angina pectoris, peripheral

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Clinical studies on patients with a variety of cardio-vascular disorders are also necessary in order to assess potential benefits. Angina pectoris, peripheral

vascular disease and transient ischemic cerebral attacks are some of the patient groups worthy of study.

The prophylactic value needs to be tested in a large group of healthy subjects in a prospective double blind controlled trial. Studies of selected groups of patients at high risk of thromboembolic disease would also be valuable. Examples include post myocardial infarction, post coronary by-pass, diabetics and familial hyperlipidemias.

The role of n-3 fatty acids in the prevention of atherosclerosis and its thrombotic complications is a topic that warrants a major research effort. At the present time the epidemiological evidence (Kromhout et al., 1985; Glomset, 1985; Bradlow, 1985) favours their value as prophylactic agents but the case is as yet unproven.

# CHAPTER 10

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