

https://dspace.org/

Drug and dietary modification of platelet function

Chetty, Nanthakumarn

2014-12-15
http://hdl.handle.net/10539/15991

Downloaded from a DSpace repository. https://dspace.org/

5.2.2 Experimental design

The study consisted of three phases:

(i) The acute effect of a single dose of 150 mg aspirin (Bayer) on platelet function was studied 2 hours after administration;

(ii) the time course and pattern of recovery from this single dose was studied over the subsequent 7-10 days; and

(iii) the effect on platelet function of the daily administration of 162mg aspirin (Angier) was studied after 7 and after 28 days.

All blood samples for determination of platelet activity were drawn by venepuncture after an overnight fast. All patients and control subjects were instructed not to smoke on the morning of blood sampling. All patients had abstained from using aspirin or other analgesic drugs known to affect platelet function for at least 2 weeks before admission to the study. None was taking any other medications known to affect platelet function or count. All were given a supply of paracetamol (acetaminophen) to use as necessary in the event of occasional headache or other pain.

5.2.3. Methods

5.2.3.1. Platelet Aggregation

Platelet aggregation was measured as described in section 3.3 with the following modifications:
On the first day of the study blood was collected before and again 2 hours after the administration of 150 mg aspirin. PRP was prepared from both samples immediately after collection. The PRP from the first sample was kept at room temperature for 2 hours, by which time PRP from the second specimen had been prepared. Aggregation studies were then carried out on both preparations and on mixtures of the two. The pre-aspirin PRP was tested for sensitivity to each of the aggregating agents to be tested, and the lowest concentration that gave maximum aggregation in each case was used for all subsequent aggregation studies on the pre- and post-aspirin PRP mixtures.

The concentration of agonists used and, the proportions of the mixtures of aspirinized and non-aspirinized platelets were as described in section 4.2.2.(1). Full response was defined as more than 75% and partial response as more than 25% of the height in millimeters of the maximum response recorded for the platelets prior to aspirin ingestion.

Aggregation responses were then tested daily, using the concentrations of agonists determined as above. The day on which the aggregation response had fully recovered was determined for each subject. Full aggregation response was defined as above.
After full recovery of the response the subjects were given 162 mg aspirin daily for 4 weeks. Aggregation responses were determined after 7 and 28 days using the concentrations of agonists determined on the first day (see above).

For each agonist the height of the maximum aggregation response above the baseline at the chosen concentration was measured in millimetres before and at intervals after giving the dose. Each post-aspirin observation was expressed as a percentage of the pre-aspirin response. In the case of ADP only the height of the secondary wave was measured. Many of the post-aspirin responses to ADP showed a primary wave followed by disaggregation. When this occurred the response was recorded as zero. For the other three agonists the maximum response, whether a single wave or two waves of aggregation, was measured.

5.2.3.2 Beta-Thromboglobulin (B-TG) and Circulating Platelet Aggregates (CPA)

B-TG and CPA was measured in both groups before ingestion of Aspirin, after full recovery of a single dose of 150mg aspirin and again 4 weeks after the daily administration of 162 mg Aspirin (see methods).

5.2.3.3. Thromboxane B_2

In some subjects thromboxane B_2 (TXB_2) was also measured. After aggregation had occurred the specimens were centrifuged for 30 minutes at 1800g to produce PPP. TXB_2 in the PPP was measured by Radioimmuno assay (See section 3.5).

5.3. Results

5.3.1. Platelet aggregation

The proportion of normal platelets required to restore partial and full aggregation responses to mixtures of normal and aspirin-inhibited platelets in the normal subjects and in the F.H. patients is shown in Figs 5.1 and 5.2. There was no significant difference between the normal subjects and the patients.

Figure 5.3. shows the day on which full recovery of platelet function occurred after a single dose of 150 mg aspirin. The differences between the normal subjects and

5.2.3.2 Beta-Thromboglobulin (B-TG) and Circulating Platelet Aggregates (CPA)

B-TG and CPA was measured in both groups before ingestion of Aspirin, after full recovery of a single dose of 150mg aspirin and again 4 weeks after the daily administration of 162 mg Aspirin (see methods).

5.2.3.3. Thromboxane B_2

In some subjects thromboxane B_2 (TXB_2) was also measured. After aggregation had occurred the specimens were centrifuged for 30 minutes at 1800g to produce PPP. TXB_2 in the PPP was measured by Radioimmuno assay (See section 3.5).

5.3. Results

5.3.1. Platelet aggregation

The proportion of normal platelets required to restore partial and full aggregation responses to mixtures of normal and aspirin-inhibited platelets in the normal subjects and in the F.H. patients is shown in Figs 5.1 and 5.2. There was no significant difference between the normal subjects and the patients.

Figure 5.3. shows the day on which full recovery of platelet function occurred after a single dose of 150 mg aspirin. The differences between the normal subjects and

the patients were not significant. Figure 5.4. shows the percentage of the initial aggregation for each agonist at the end of 7 days of daily ingestion of 162 mg aspirin and Figure 5.5. the percentage of initial response at the end of 28 days. A statistical analysis comparing the patients with the normal subjects is shown in table 5.2. None of the comparisons differ significantly.

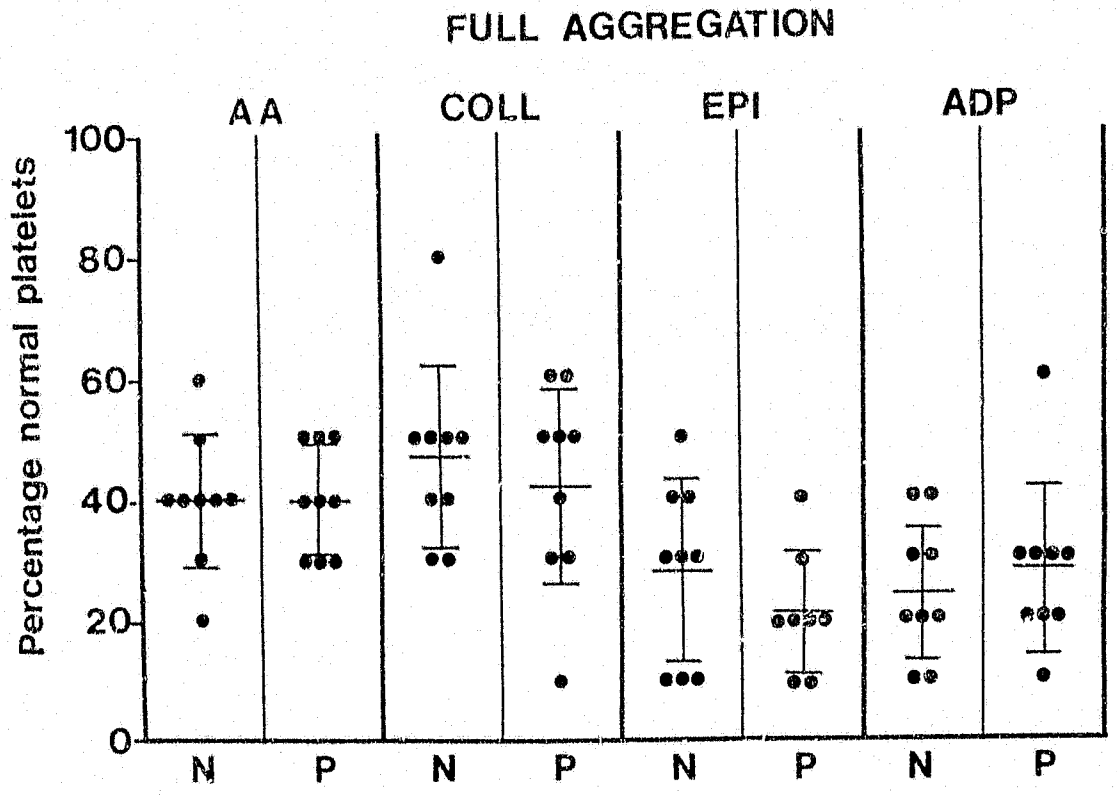


Fig. 5.1. Percentage of pre-aspirin platelets necessary to restore full aggregation to mixtures of pre-aspirin and post-aspirin platelets in normal subjects (N) and F.H. patients (P) (normal platelets = pre-aspirin platelets; COLL = collagen; EPI = epinephrine. Full aggregation was defined as more than 75% of the pre-aspirin response (height of the curve in mm).

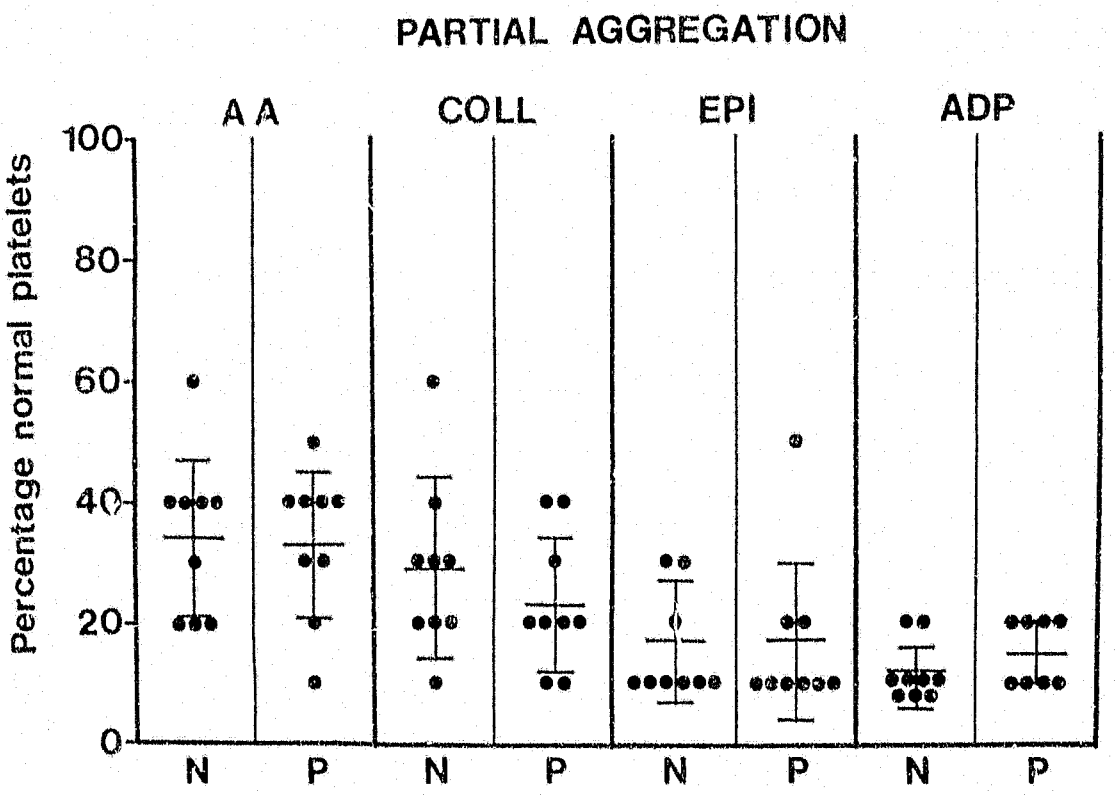


Fig 5.2. Percentage of pre-aspirin platelets necessary to restore partial aggregation to mixtures of pre-aspirin and post-aspirin platelets in normal subjects (N) and F.H. patients (P) (abbreviations as in Fig. 5.1.). Partial aggregation was defined as more than 25% of the pre-aspirin response (height of the curve in mm).

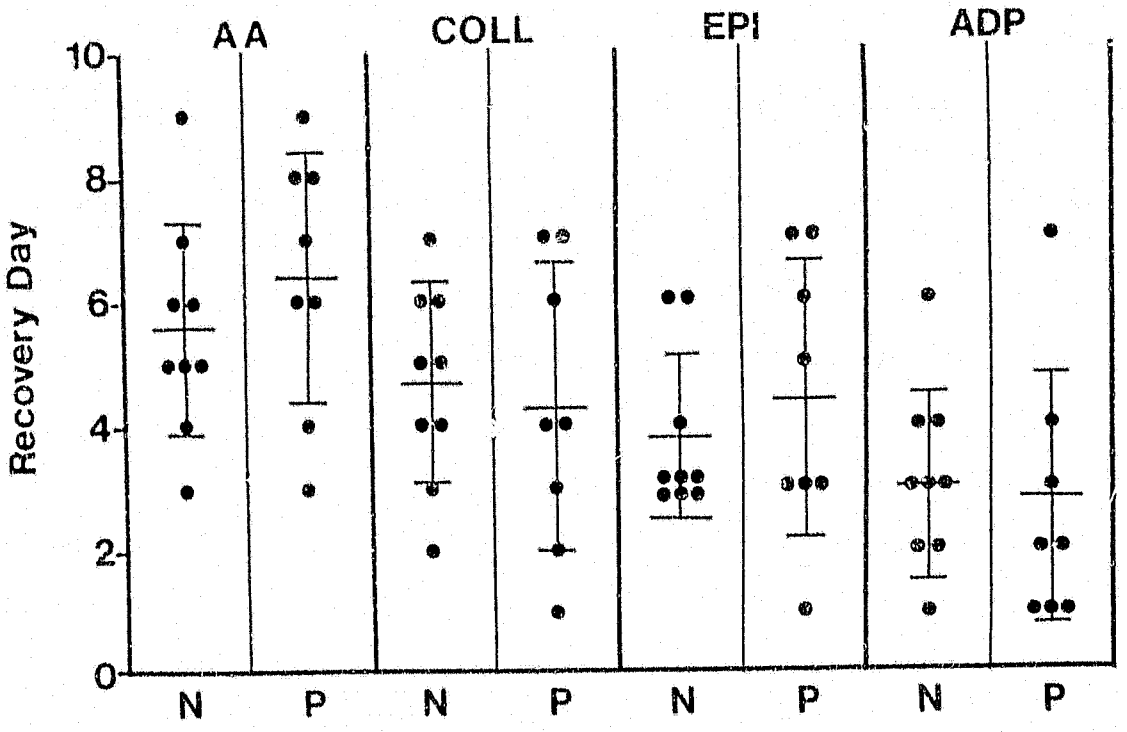


Fig.5.3 Number of days after administration of a single 150 mg dose of aspirin before full aggregation (75%) was restored in normal subjects (N) and FH patients (P) (abbreviations as in fig 5.1).

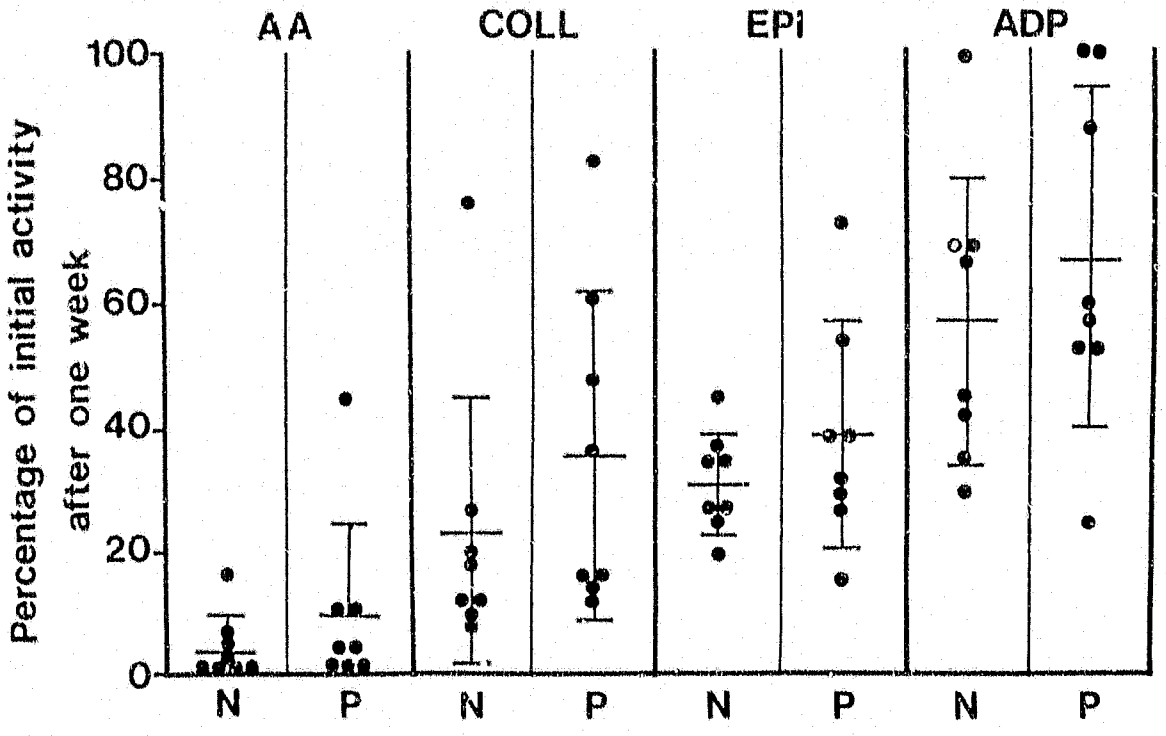


Fig 5.4. Percentage of the initial aggregation for each agonist at the end of 7 days of daily ingestion of 162 mg aspirin (Abbreviations as in Fig 5.1).

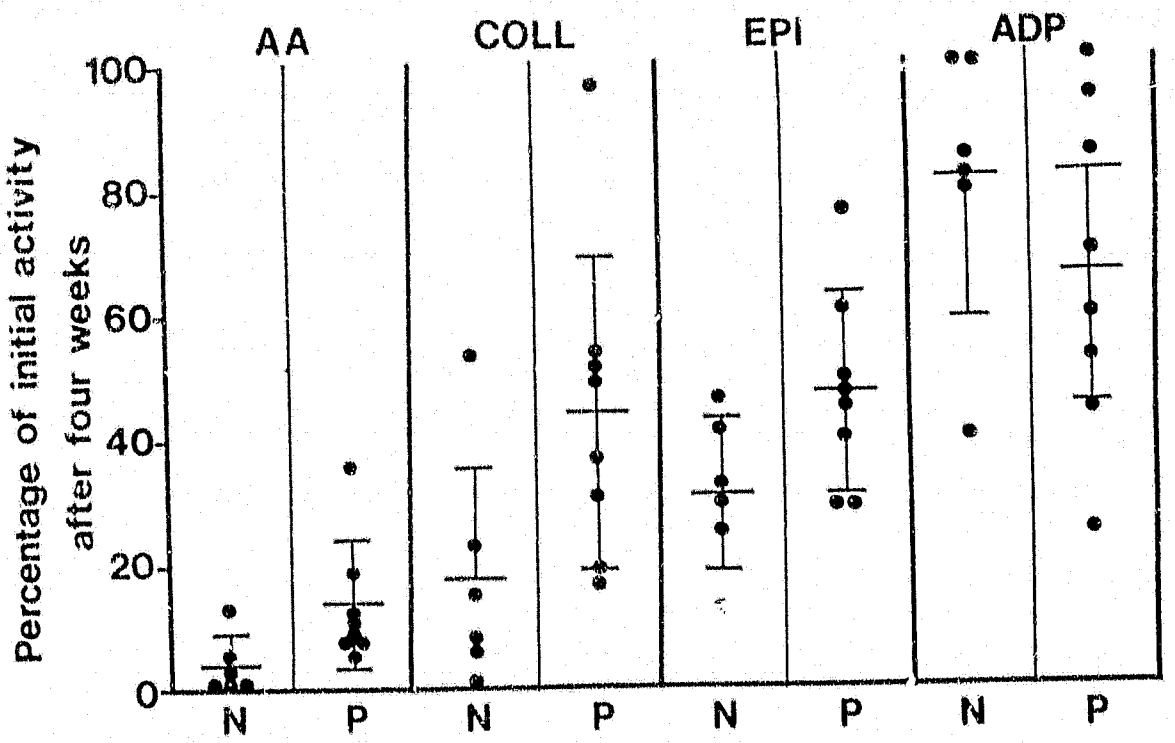


Fig.5.5.Percentage of the initial aggregation for each agonist at the end of 28 days of daily ingestion of 162 mg aspirin (abbreviations as in Fig 5.1).

Table 5.2 Platelet Aggregation Responses After Aspirin 162mg/d for 1 and 4 weeks in FH Patients and Control Subjects

		Controls			Patients			
		Mean	SD	N	Mean	SD	N	Significance
1 Week	AA	3,75	5,52	8	9,00	14,74	8	P=0,36
	COLL	22,88	22,18	8	35,00	26,04	8	P=0,33
	EPI	31,00	7,82	8	37,75	17,66	8	P=0,34
	ADP	57,12	26,36	8	65,62	27,13	8	P=0,51
4 Weeks	AA	3,83	4,88	6	12,63	9,87	8	P=0,07
	COLL	17,67	18,99	6	43,25	25,20	8	P=0,06
	EPI	31,00	12,03	6	45,75	15,64	8	P=0,08
	ADP	81,00	22,00	6	65,12	26,16	8	P=0,25

One subject from each group did not complete this part of the study, and by week 4 a further 2 controls had been lost to follow-up. The mean values shown represent the percentage of the initial (pre-aspirin) aggregation response in millimetres (See methods) P values of 0,05 or more indicate that the patients' responses were not significantly different from those of the control subjects.
Coll=collagen; EPI=epinephrine (adrenaline).

Table 5.2 Platelet Aggregation Responses After Aspirin 162mg/d for 1 and 4 weeks in FH Patients and Control Subjects

		Controls			Patients			
		Mean	SD	N	Mean	SD	N	Significance
1 Week	AA	3,75	5,52	8	9,00	14,74	8	P=0,36
	COLL	22,88	22,18	8	35,00	26,04	8	P=0,33
	EPI	31,00	7,82	8	37,75	17,66	8	P=0,34
	ADP	57,12	26,36	8	65,62	27,13	8	P=0,51
4 Weeks	AA	3,83	4,88	6	12,63	9,87	8	P=0,07
	COLL	17,67	18,99	6	43,25	25,20	8	P=0,06
	EPI	31,00	12,03	6	45,75	15,64	8	P=0,08
	ADP	81,00	22,00	6	65,12	26,16	8	P=0,25

One subject from each group did not complete this part of the study, and by week 4 a further 2 controls had been lost to follow-up. The mean values shown represent the percentage of the initial (pre-aspirin) aggregation response in millimetres (See methods) P values of 0,05 or more indicate that the patients' responses were not significantly different from those of the control subjects.
Coll=collagen; EPI=epinephrine (adrenaline).

5.3.2. Circulating platelet aggregates

Circulating platelet aggregate ratios are shown in table 5.3. All patients had normal ratics before and after aspirin ingestion. Wilcoxons sign ranked test did not reveal any significant differences between the groups.

5.3.3. Beta-Thromboglobulin (B-TG)

B-TG values are shown in table 5.4. The normal subjects (28±7) are much lower than the F.H. patients (51±18) indicating platelet hyperreactivity of F.H. patients. Aspirin therapy had no influence on B-TG values in both groups.

5.3.4. Thromboxane B_2 (TXB_2)

Thromboxane B_2 production after stimulation with each of four agonists was measured in the normal subjects (5.5) as well as the F.H. patients (Table 5.6), before aspirin ingestion, after aspirin, and 7 and 28 days after continuous aspirin therapy. The degree of inhibition of TXB_2 production was the same in both groups.

Table 5.3. Circulating platelet aggregate ratios before and after aspirin.

	Normal Subjects			F.H. Patients		
	Before Therapy	After Acute Phase	After 4* weeks	Before Therapy	After Acute phase	After 4* weeks
Mean	0,98	0,97	0,95	0,94	0,90	0,93
SD	0,04	0,05	0,07	0,07	0,09	0,05
N	9	8	6	9	8	8

*After 28 days continuous ingestion of 162 mg Aspirin

Table 5.4. Beta thromboglobulin (ng/mℓ) before and after aspirin therapy.

	Normal subjects			F.H. Patients		
	Before therapy	After Acute phase	*After 4 weeks	Before therapy	After acute phase	After 4 weeks
Mean	28	30	25	51	54	53
SD	7	9	11	18	27	23
N	9	8	6	9	8	8

*After 28 days continuous ingestion of 163 mg Aspirin.

Table 5.4. Beta thromboglobulin (ng/mℓ) before and after aspirin therapy.

	Normal subjects			F.H. Patients		
	Before therapy	After Acute phase	*After 4 weeks	Before therapy	After acute phase	After 4 weeks
Mean	28	30	25	51	54	53
SD	7	9	11	18	27	23
N	9	8	6	9	8	8

*After 28 days continuous ingestion of 163 mg Aspirin.

able 5.5 Thromboxane B_2 production of normal subjects before and after aspirin (ASA) Therapy.

ggregating Agent	Thromboxane B_2 (pg/ml)			
	Normal (pre-aspirin)	2 hrs post Aspirin	7days Continuous ASA	28 days Continuous ASA
rachidonic Acid	75500±23641	620±169	555±180	195±132
ollagen	10500±3416	41±30	43±11	18±8
pinephrine	5625±2910	55±29	79±19	15±4
DP	4150±1981	285±68	65±13	30±7

ean ± S.D. of 3 control subjects.

Table 5.5 Thromboxane B_2 production of normal subjects before and after aspirin (ASA) Therapy.

Aggregating Agent	Thromboxane B_2 (pg/ml)			
	Normal (pre-aspirin)	2 hrs post Aspirin	7days Continuous ASA	28 days Continuous ASA
Arachidonic Acid	75500±23641	620±169	555±180	195±132
Collagen	10500±3416	41±30	43±11	18±8
Epinephrine	5625±2910	55±29	79±19	15±4
ADP	4150±1981	285±68	65±13	30±7

Mean ± S.D. of 3 control subjects.

Table 5.6 Thromboxane B_2 production of F.H. patients before and after aspirin (ASA) therapy.

Aggregating Agents	Thromboxane B_2 (pg/mℓ)			
	Normal (pre-aspirin)	2 hrs post aspirin	7 days continuous ASA	28 days continuous ASA
Arachidonic Acid	77500±26900	197±80	237±427	533±231
Collagen	8400±3908	425±260	160±121	385±162
Epinephrine	2300±1500	58±31	51±17	12±3
ADP	1250±799	56±29	55±14	60±19

Mean ± S.D. of 3 F.H. Pateints.

DISCUSSION:

The beneficial effect of aspirin in preventing arterial thrombosis is recognized in a number of conditions (Salzman, 1982). There are differences in the literature regarding optimal dosage of aspirin which will achieve the desired combination of inhibiting platelet thromboxane A_2 production while leaving endothelial prostacyclin secretion intact (Burch et al., 1978; Masotti et al., 1979; Patrignani et al., 1982; Marcus, 1983). The dosage required to inhibit platelet thromboxane A_2 synthesis has generally been reported to be small and very much less than that necessary to block vascular endothelial prostacyclin production, although one recent report suggests that a mere doubling of the daily dose from 40 mg to 80 mg may be all that is required for this differential effect to disappear (Hanley et al., 1981).

In view of these considerations, the efficacy of daily low-dose aspirin in patients with FH was investigated, since this treatment might provide some protection against the sequelae of coronary atherosclerosis. Furthermore these patients' untreated platelets exhibit increased aggregation in response to various stimuli (Carvalho et al., 1974; Shattil et al., 1975; Shattil et al., 1977; Joist et al., 1979; Bradlow et al.,

1982), and platelet survival is reduced (Corash, 1976). In addition, the enrichment of platelets *in vitro* with cholesterol enhances production of thromboxane B_2 (Stuart et al., 1980).

The major point of interest in this study is that despite the previously demonstrated hyperaggregability of the platelets in FH, aggregation and thromboxane B_2 production can be inhibited by a small dose (150 mg) of aspirin to the same extent as occurs in normal platelets. This was true for all four of the agonists tested. There was also no significant difference between the results after 1 and 4 weeks of daily ingestion of 162 mg aspirin, although statistical analysis (Table 5.2) showed that after 4 weeks of aspirin therapy the platelets from the FH patients did have greater aggregation responses to some stimuli. However, the probability that this difference was significant was only between 90% and 95%. Nevertheless, significant inhibition of platelet aggregation did persist throughout the 4 week study period. It must be pointed out that platelet aggregation in two patients with FH was only very slightly inhibited by the aspirin after 4 weeks, indicating that while FH patients as a group are sensitive to the antiplatelet action of aspirin a minority may be somewhat resistant. The B-TG levels of the FH patients were significantly higher than

in the normals. This indicated that the platelets were hypereactive *in vivo*. This supports the findings of Zahavi *et al*., (1979) and Simons *et al*.,(1981) who also reported that the B-TG levels were elevated in FH. However, low dose aspirin therapy had no effect on circulating B-TG. The concept that platelet aggregates may develop in the circulation and interfere with the vascular supply in the microcirculation of vital organs is obviously of interest. However, we detected no increase in circulating platelet aggregates in the FH patients whereas, Wu *et al*., (1975) and Lowe *et al*., (1979), reported increased circulating platelet aggregates in FH.

Whether the method of Wu and Hoak (1974) of detecting circulating platelet aggregates in blood that has just been removed from a vein actually detects platelet aggregates that are circulating *in vivo* or whether the aggregates form after the blood is removed, has not been resolved. But it seems that if platelet hypersensitivity *in vivo* does result in the formation of circulating platelet aggregates, it is desirable to have tests that would detect them.

A putative role for platelets in the process of atherogenesis itself, owing to the stimulation of myo-intimal proliferation by platelet-derived growth

factor, has also been suggested (Ross and Glomset, 1976). If aspirin or other antiplatelet drugs affect this process too, an additional benefit to patients with FH and other 'prothrombotic states' would follow.

In conclusion, low dosage aspirin (150-162 mg) inhibits the aggregation of platelets of patients with FH as effectively as those of normal subjects. Considerable individual variation in response to aspirin exists, so that in some persons this dose may be inadequate. Whether the long term addition of a small daily dose of aspirin to the conventional treatment of patients with FH will prove beneficial in reducing the incidence of myocardial infarction needs to be investigated.

SUMMARY

Since the platelets of patients with familial hypercholesterolaemia (FH) are hyperaggregable, the inhibitory effect of low-dose aspirin on platelet agregation in response to adenosine diphosphate, collagen, adrenaline and arachidonic acid was tested in 9 young adult patients with FH and compared with that in 9 normal subjects. After a single oral dose (150mg) of aspirin the degree of inhibition of platelet aggregation was the same in the two groups, as was the recovery time (in days) for full restoration of platelet aggregation. In a second experiment in which 162mg aspirin was administered daily for 28 days, inhibition of platelet aggregation was sustainable in both the patient and the control groups. These results suggest that despite the recognised hyperaggregability of platelets from patients with FH, daily low-dose aspirin ingestion effectively inhibits their in vitro aggregation and may therefore prove beneficial as supplemental therapy to reduce the rate of myocardial infarction in this high-risk group.

CHAPTER 6

6. THE EFFECTS OF A MIXED FISH DIET ON PLATELET FUNCTION, PLATELET FATTY ACIDS AND SERUM LIPIDS

6.1 Introduction

Dietary supplementation with fatty acids that replace arachidonic acid or inhibit arachidonic acid metabolism in platelets and thus manipulate the Thromboxane and prostacyclin balance play a role in the prevention of coronary and cerebral thrombosis. A prime condidate for dietary manipulation in this regard is 5,8,11,14,17 - eicosapentaenoic acid (EPA n-3), the precursor of the triene PG's. Comparative studies of the actions of metabolites of arachidonate and eicosapentaenoate on platelets in platelet-rich-plasma (PRP) produced the following results (Raz et al ., 1977; Needleman et al., 1979). Arachidonate, and it's metabolites, PGH_2 and thromboxane A_2, were found to be potent aggregators of human platelets; in sharp contrast, neither eicosapentaenoic acid nor exogenous addition of its purified metabolites, PGH_3 or thromboxane A_3, caused platelet aggregation with other agonists.

When EPA is substituted for AA in the platelet membrane the two fatty acids compete for cyclo-oxygenase (Needleman, 1982). Though EPA and AA have

approximately equal affinities for cyclo-oxygenase, EPA is transformed much more slowly than AA to cyclic endoperoxides. (Needleman et al., 1979). At least part of the action of EPA on platelet function is thus mediated by competitive inhibition of cyclo-oxygenase (Jakubowski and Ardlie, 1980; Needleman et al., 1979). Some EPA is probably metabolised to thromboxane A_3 (TXA_3), which is not only much less active than TXA_2 but also may inhibit the action of TXA_2 by competitive inhibition (Thorngren and Gustafson, 1981).

The effect of EPA on platelet function was first observed by Dyerberg and colleagues (Dyerberg and Bang, 1979 (a); Dyerberg and Bang, 1979 (b); Dyerberg and Bang, 1978; Dyerberg et al., 1978). They found that, in platelets stimulated with ADP, aggregation was enhanced by preincubation with arachidonic acid but diminished with EPA (Dyerberg and Bang, 1978). They also observed that in Eskimos in whom no secondary-phase platelet aggregation could be induced even after stimulation with collagen, incubation with arachidonic acid induced normal aggregation so that there was normal ability to synthesise prostaglandins, bleeding times were prolonged, platelet counts low, and serum fibrinogen high, while platelet adhesiveness and prothrombin time were normal. In platelets n-6 fatty acids were partly replaced by n-3 fatty acids. On the

basis of these findings they suggested that EPA might be responsible for the above changes and thus prevent thrombosis and atherosclerosis (Dyerberg et al., 1978). Sinclair (1982) likewise attributed the absence of ischaemic heart disease in Eskimos on their traditional diet to their very low arachidonic acid and high EPA, intake which gives rise to a thromboxane-TXA_3-that does not aggregate platelets (Raz et al., 1977). On a diet strictly limited to marine animal food (seal, fish, and crustaceans) with water for 100 days (Sinclair, 1982), the linoleic acid in Sinclair's serum fell dramatically in all three lipid fractions (Cholesterol esters, phospholipids, triglycerides) of all four lipoproteins (VLDL, IDL, LDL, HDL), in erythrocyte phospholipids, in platelets, in adipose tissue, and in skeletal muscle; simultaneously EPA rose. VLDL decreased, LDL rose slightly, HDL increased so that the ratio of HDL to LDL rose from 0.76 to 0.96, both values being compared with a normal of 0.55. TXA_2 and PGI_2 decreased in plasma. Prostaglandins decreased significantly in seminal fluid and small amounts of the 3-series appeared. The platelet count fell dramatically and giant forms appeared. Platelet function was studied in great detail: bleeding time increased significantly (over 50 minutes later returning to 15 minutes) with spontaneous haemorrhages; aggregation induced by ADP, thrombin,

ristocetin, or adrenalin was unaffected but aggregation by collagen was reduced. Subsequently, experiments with diets enriched with EPA have been done in man (Von Lossonczy et al., 1978; Siess et al., 1980; Bronsgeest-Schoute et al., 1981; Goodnight et al., 1981; Thorngren and Gustafson, 1981; Sanders et al., 1981; Hay et al., 1982), Sanders and Roshanai, 1983; Mortenson et al.,1983; Galloway et al.,1985), pigs (Ruiter et al., 1978), cats (Black et al., 1979) dogs (Culp et al., 1979; Culp et al., 1980), rabbits (Vas Dias et al., 1982) and rats (Hornstra et al., 1981).

Von Lossonczy et al., (1978), found in 42 volunteers that mackerel providing about 8g of n-3 fatty acids daily caused a considerable fall in serum triglycerides and VLDL, a slight fall in LDL, and a slight rise in HDL. In a second trial (Bronsgeest-Schoute et al., 1981) similar results were obtained with volunteers over 4 weeks. Siess et al., (1980), with seven volunteers for one week used a mackerel diet that provided between 7 and 11g of EPA daily. Platelet aggregation and thromboxane synthesis induced by low dose collagen stimulation were significantly reduced after the mackerel diet. Sanders et al., (1981), gave 12 males a supplement of cod-liver oil for 6 weeks. The HDL, bleeding time, and platelet aggregation with

ADP were increased, while plasma triglycerides and antithrombin III were decreased. In a subsequent experiment, Sanders and Roshanai, (1983), gave supplements rich in long-chain n-3 fatty acids to 5 subjects for 2 or 3 weeks and similar results were found. Thorngren and Gustafson (1981) enriched the diet of 10 male volunteers with mackerel and salmon for 11 weeks. This diet provided between 2-3 g EPA per day. Within 3 weeks the fish diet produced prolonged bleeding times and reduced platelet aggregability to collagen and ADP. The changes in platelet phospholipid fatty acid composition consisted of increases in the n-3 series (C 20:5 and C22:6) and decreases in the n-6 series (C18:2 and C20:3). Goodnight et al., (1981), fed normal subjects large amounts of salmon (about 500g/day) and salmon oil (60-90 ml / day) which corresponded to approximately 3.5g EPA daily. This diet led to the incorporation of EPA into platelets with a reduction in AA. Bleeding times were prolonged and platelet retention on glass beads was mildly reduced. Platelet aggregation in response to dilute concentrations of ADP was inhibited. Hay et al., (1982) gave an oil rich in EPA to 13 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 time, a 15% fall in platelet count, a 75% fall in plasma levels of platelet

factor 4, and a 30% fall in plasma B-thromboglobulin B-TG. Extensive work has been done by a group of Japanese workers comparing a fishing with a farming village (Hirai, et al., 1980; Kobayashi et al., 1981) and giving a supplement of purified EPA to volunteers (Hirai et al.,1982). Platelet aggregation, blood viscosity, and ischaemic heart disease were lower in the fishing village, and the supplement of 1,4g EPA daily to each of 8 volunteers for 4 weeks decreased platelet aggregation by collagen and ADP and collagen induced TXB_2 production. In rats Hornstra (1981) found that the occlusion time in vascular prostheses inserted into the aorta was prolonged by feeding of cod liver oil.

Saynor et al.,(1984) supplemented the diet of 107 volunteers with 20 mls MaxEPA for 2 years. They found a significant decrease in triglycerides and total cholesterol and a significant increase in HDL.

Ahmed and Holub (1984) fed 30 ml of cod liver oil to 7 volunteers for 2 weeks. The bleeding times increased by 81% and there was a significant reduction in thrombin induced aggregation. EPA rose significantly from 0,3% to 2.9% phosphatidylcholine.

Kromhout et al., (1985), reported an inverse relation between fish consumption and 20 year mortality from coronary heart disease. This relation persisted after multiple logistic - regression analysis. Mortality from CHD was found to be more than 50% lower among those who consumed at least 30 mg of fish per day than among those who did not eat fish. Phillipson et al., (1985), reported a reduction of plasma lipids, lipoproteins, and apoproteins following supplements of fish oil (Salmon oil - 30g of n3 fatty acids) in 20 patients with hypertriglyceridaemia. They found that fish oils, compared with vegetable oils (each for 4 weeks), resulted in major falls in plasma cholesterol, triglycerides and VLDL

In conclusion it seems that fatty fish, such as mackerel or salmon, can greatly lower plasma triglycerides and VLDL, lower LDL, usually raise HDL, and decrease platelet aggregation probably by altering the fatty acids in platelet membranes and by decreasing platelet production of thromboxanes.

The present study was undertaken in order to assess the effects of a mixture of fish rich in EPA, other than mackerel or salmon, on platelet and plasma fatty acid composition, platelet aggregation, thromboxane B_2 production, platelet count, plateletcrit , mean platelet

volume, platelet distribution width, serum triglycerides, serum cholesterol and serum LDL and HDL cholesterols. The amount of fish in this diet was kept at a low level that could be maintained for prolonged periods if desired.

Before conducting the study, the fatty acid composition of fish available in South Africa was determined. While data for fish overseas is available (Paul and Southgate, 1978) such information was not available for fish obtained locally. Moreover, the fatty acid composition of a fish may vary depending on its diet, sea water temperature and other factors. Only fish with a high 20:5/20:4 ratio were selected for the diet.

6.2 Materials and Methods

6.2.1.Fatty acid composition of Fish

A random selection of fresh and canned fish available inland (Johannesburg) was analysed. Maputo prawns were included for comparison. A portion of the fish (1g) was extracted with chloroform-methanol (2:1 v/v) by the method of Folch et al., (1957). Fatty acid methyl esters were prepared by the method of Morrison and Smith (1964). Esters were separated in a Packard Becker 417 Chromatograph (see methods, section 3.9.2.)

6.2.2. Subjects

Eight volunteers aged 21 to 37 years (mean 29 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 the Witwatersrand Medical School. The subjects agreed not to take any medication including aspirin for two weeks prior to and during the study. Each subject was venesected on two separate occasions while still taking the diet normally consumed prior to commencing the fish diet. They all ate a mixed diet containing meat, poultry, dairy products, eggs, fruit, vegetables and cereals. At the commencement of the test period the experimental diet was taken and maintained for periods of 8 - 21 days (mean = 16 days). At the end of the test period a further blood sample was taken.

6.2.3. The Experimental Diet

The diet was designed to increase the EPA and reduce the AA content of the food. All animal products including meats, poultry, dairy foods and eggs were eliminated. Unrestricted quantities of soft margarine containing at least 50% polyunsaturated fats and a dried polyunsaturated 'filled' milk containing 65% linoleic, 23% oleic, 7% palmitic and 5% stearic acids (Mendelsohn, 1982-personal communication) (manufactured by Continental Ethicals, Johannesburg, S.A) were permitted in place of butter and milk respectively. Fruit, vegetables and cereals including bread were not restricted. All foods containing dairy products or eggs were excluded from the diet.

In addition, the subjects were asked to eat at least one helping of 300 - 400g of one or more of the selected fish species every day. The fish selected had an EPA content of at least 7% of total fatty acids and an EPA:AA ratio of at least 7 (see table 6.1). Sardines, pilchards, herrings and kabeljou were used and were prepared according to the individual taste and skill of the subjects. The amount of EPA in the diet was estimated to be 1-4 g per day.

6.2.4 Platelet aggregation

Platelet aggregations were performed as described in section 3.3. Platelet aggregation was tested twice prior to commencing and again at the end of the experimental period. On the first occasion the lowest concentration of each of the agonists that produced a maximal aggregation response was determined. This concentration of agonist was used for all subsequent aggregation studies. The concentrations used were : ADP from 2,1 to 10,6 uM, EPI from 1.3 to 55 uM, collagen from 1 to 4 ug per ml, AA from 0.4 to 0.8 mM. Aggregation responses were quantitated by measuring the maximum height in cms of the aggregation response above the base line of the aggregation recordings. When a primary and secondary wave occured the height of the latter was measured.

The mean value of the two observations prior to the experimental period was recorded.

6.2.5. Thromboxane B_2 Assay

After aggregations had occurred the specimens were centrifuged for 30 minutes at 1800g to produce platelet poor plasma (PPP). Thromboxane B_2 in the PPP was measured as described in section 3.5.

6.2.6 Platelet Indices

Blood samples for platelet indices were collected in 1.5 ug potassium EDTA per ml blood. All samples were run on a Coulter S. Plus (Coulter Electronics)

6.2.7. Serum Lipids

Total and low density lipoprotein (LDL) cholesterol and triglyceride levels (Lipid Research Clinics, 1974) and high density lipoprotein (HDL) cholesterol (Burstein et al., 1970) were measured in serum after an overnight fast. Serum lipids were measured twice prior to commencing and again at the end of the experimental period.

6.2.8. Fatty acid analysis

Lipids were extracted from platelets and plasma (Folch et al., 1957) in solvents containing the antioxidant 2,6 - di-tert- butyl-4-methylphenol (BHT, 0,005%, W/V) Fatty acid methyl esters of the lipid extracts were prepared (Morrison and Smith, 1964) using 14% (W/V) boron trifluoride-methanol reagent. By continuing the methanolysis for 75 mins, fatty acids derived from triglycerides, phospholipids as well as sphingolipids were obtained. The methyl esters were separated by gas-liquid chromatography, (Section 3.9.2.).
The dimethylacetals (DMA) obtained from fatty aldehydes were identified after GLC and excluded from the

calculations. The DMA were identified by comparing retention times with those of known standards.

6.2.9. Statistical analysis

The Wilcoxon sign ranked matched paired test was used for comparison of platelet aggregation, thromboxane production and serum lipids before and after the diet. Students "t" test was used for comparison of the fatty acid results and the platelet indices.

6.3 Results

6.3.1.Fish Analyses

The fish were listed according to the ratio of 20:5 to 20:4 (Tables 6.1 and 6.2.). Smoked salmon had the highest ratio, followed by pickled herring, canned sardines and pilchards. Canned pilchards had the highest percentage of 20.5 (22.3%). In general, all the fish studied had high percentages of 20:5 and low percentages of 20:4. The data clearly illustrate the suitability of marine foods as a source rich in eicosapentaenoic acid.

Table 6.1. Fatty Acid composition of Fish with high 20:5/20:4 ratios.

Fatty acid	Smoked Salmon	Pickled Herring	Canned Sardines	Canned Pilchards	Raw Kablj.	Fried Kablj.	Fried Sole
16:0	29.37	15.37	15.39	21.06	16.26	15.50	7.78
16:1	2.09	10.02	12.21	10.08	6.42	8.01	0.91
18:0	8.69	1.85	2.18	6.38	4.71	5.82	4.55
18:1	6.05	13.19	21.69	15.23	17.01	13.83	23.00
18:2 n6	4.84	2.62	2.40	3.70	1.61	17.16	57.20
18:3 n3	0.44	0.55	1.20	0.56	0.75	0.21	0.00
20:1	2.20	18.42	12.64	1.12	8.13	1.46	0.30
20:4 n6	0.22	0.44	0.65	1.57	0.86	1.87	0.20
20:5	7.04	7.85	9.59	22.29	12.41	14.25	1.62
22:1	4.84	20.82	8.07	1.23	7.06	0.83	0.81
22:5 n3	2.31	1.96	1.85	3.02	3.75	2.50	1.41
22:6 n3	11.44	6.76	9.92	12.10	20.01	17.16	2.22
20:5 / 20:4	32.00	17:80	14.75	14.20	14.40	7.60	8.10

Table 6.2 Fatty acid composition of fish with low 20:5/20:4 ratios

Fatty acid	Raw haddock	Raw Hake	Raw Kingklip	Raw Sole	Raw Prawns	Canned Tuna
16.0	20.5	18.62	16.52	17.6	18.69	11.2
16.1	3.4	4.89	3.40	1.5	7.98	0
18.0	7.52	6.0	4.80	7.25	12.10	3.2
18.1	12.88	20.7	15.90	12.6	14.70	24.4
18.2 n6	1.96	1.46	0.82	13.02	2.94	50.4
18.3 n3	0.82	0.52	0.31	0.63	0.53	7.5
20.1	2.16	3.85	2.24	2.94	2.94	0
20.4 n6	2.06	2.18	3.06	3.05	8.70	0.2
20.5 n3	9.27	6.14	7.14	6.4	12.70	0.2
22.0,1	2.06	2.81	0.31	1.26	1.70	0.2
22.5 n3	2.99	4.26	3.98	7.35	1.70	0.4
22.6 n3	33.40	26.62	35.40	25.31	12.10	1.7
20.5/20.4	4.50	2.80	2.33	2.10	1.46	1.2

6.3.2 Platelet Aggregation

Figures 6.1 - 6.4 show the aggregation patterns of one of the subjects before and after 14 days on the fish diet. There was a marked reduction in the height of the aggregation responses to ADP, EPI and collagen but a marked increase in the response to AA. The results for the group of 8 subjects are shown in figs 6.5 - 6,8 and summarized in table 6.3. The responses to ADP, EPI and collagen were significantly reduced after the diet but there was no significant change in the groups response to AA. Were it not for one subject who showed a very marked and unexplained decrease in her response the group response to AA would have been significantly increased after the diet.

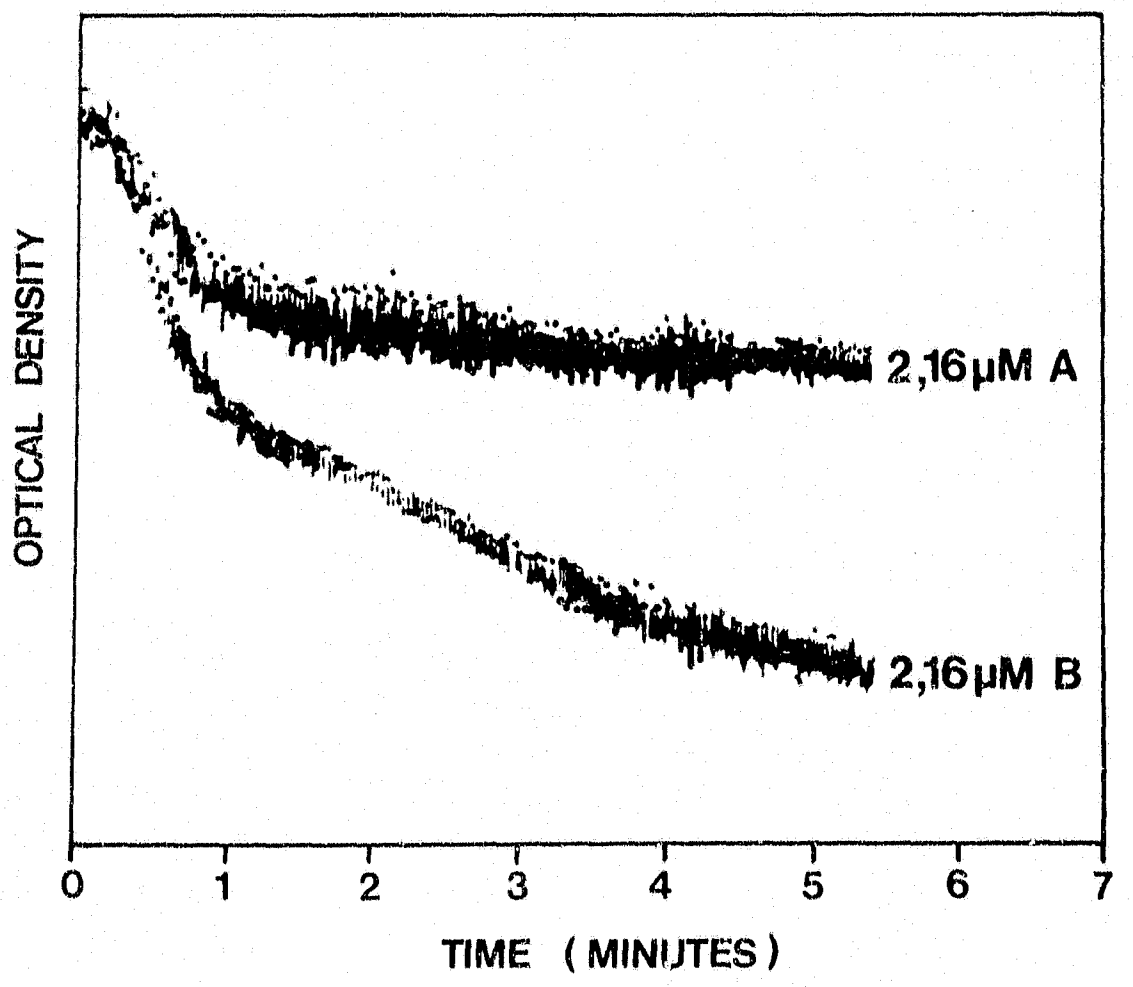


Fig 6.1. A representative experiment showing platelet aggregation of one subject in response to ADP before (B) and after (A) the fish diet.

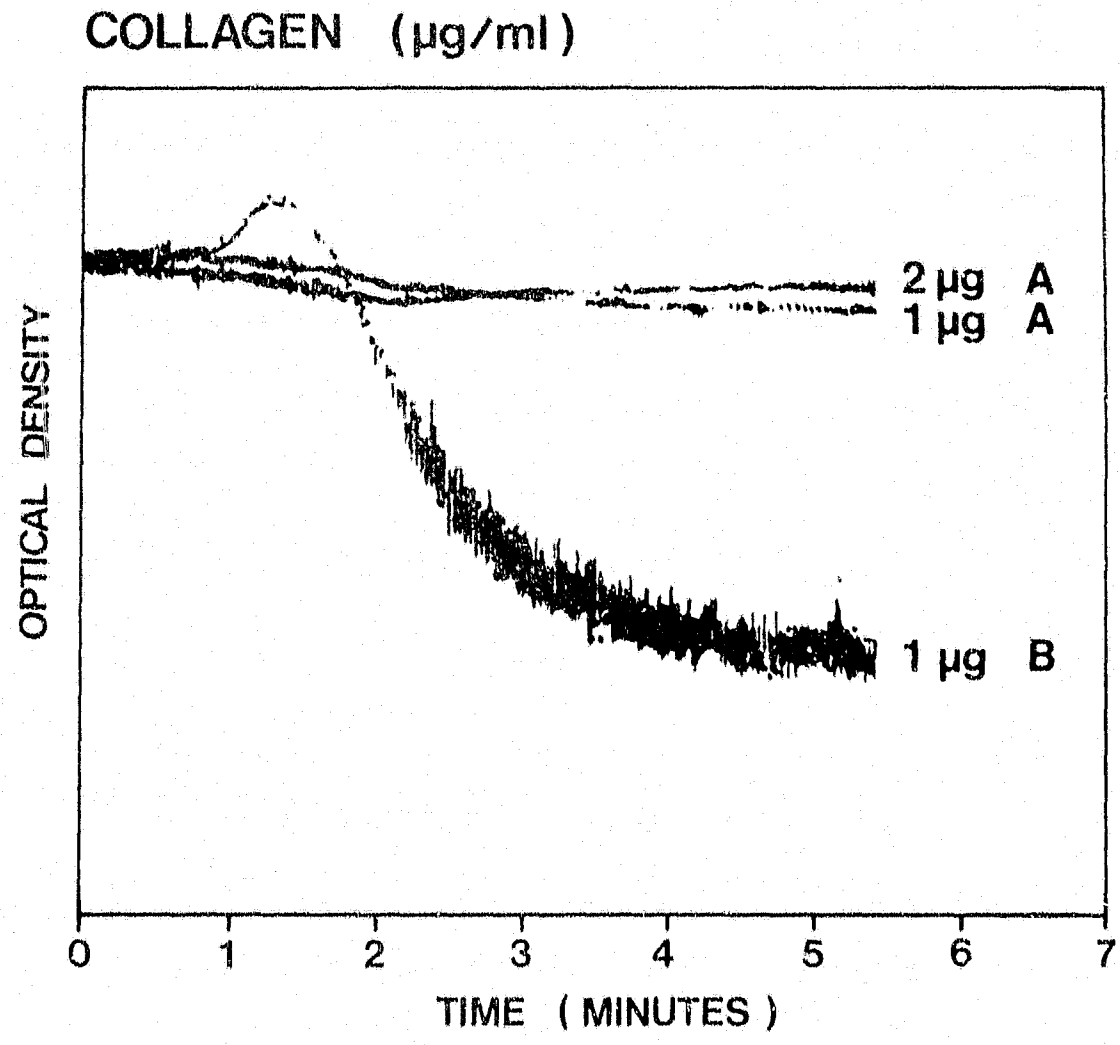


Fig 6.2. A representative experiment showing platelet aggregation of one subject in response to collagen before (B) and after (A) the fish diet.

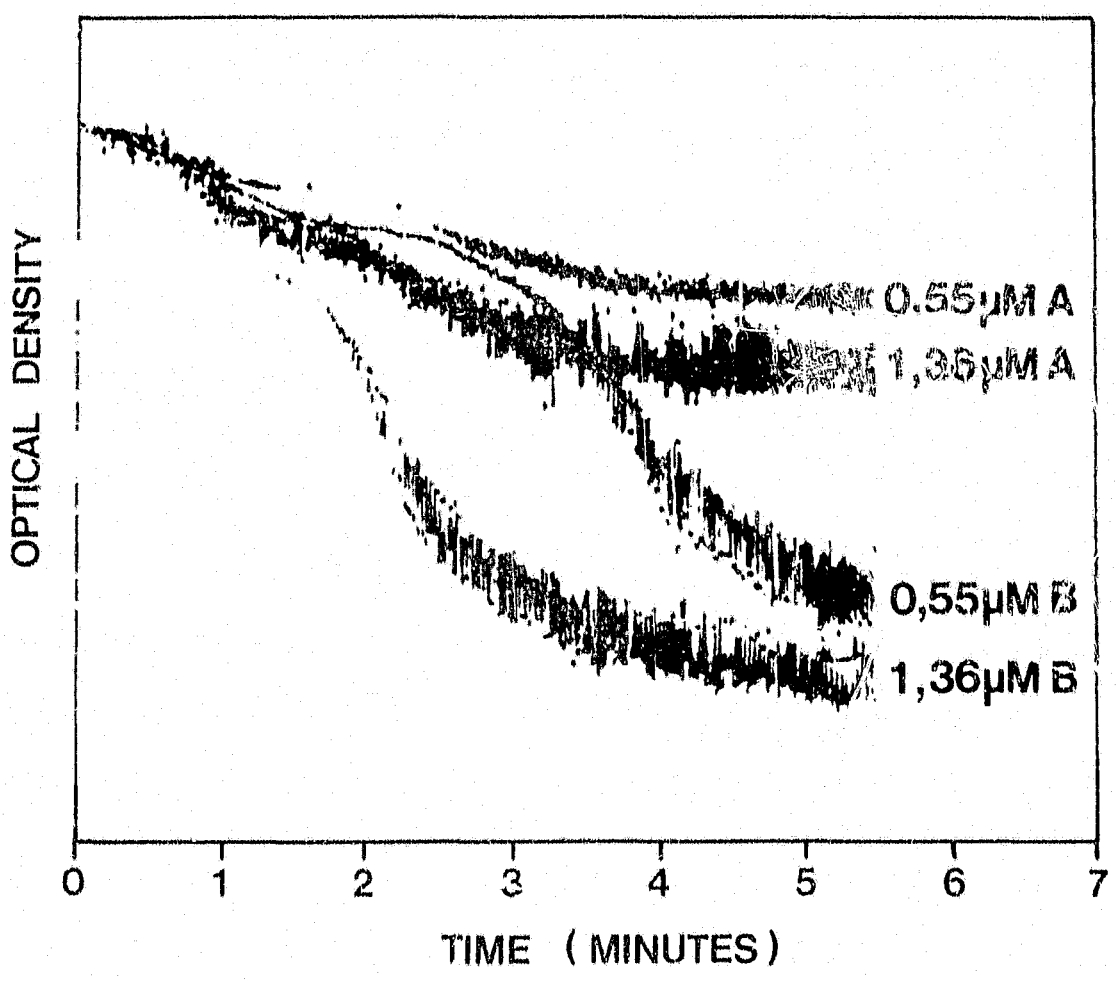


Fig 6.3. A representative experiment showing platelet aggregation of one subject in response to epinephrine before (B) and after (A) the fish diet.

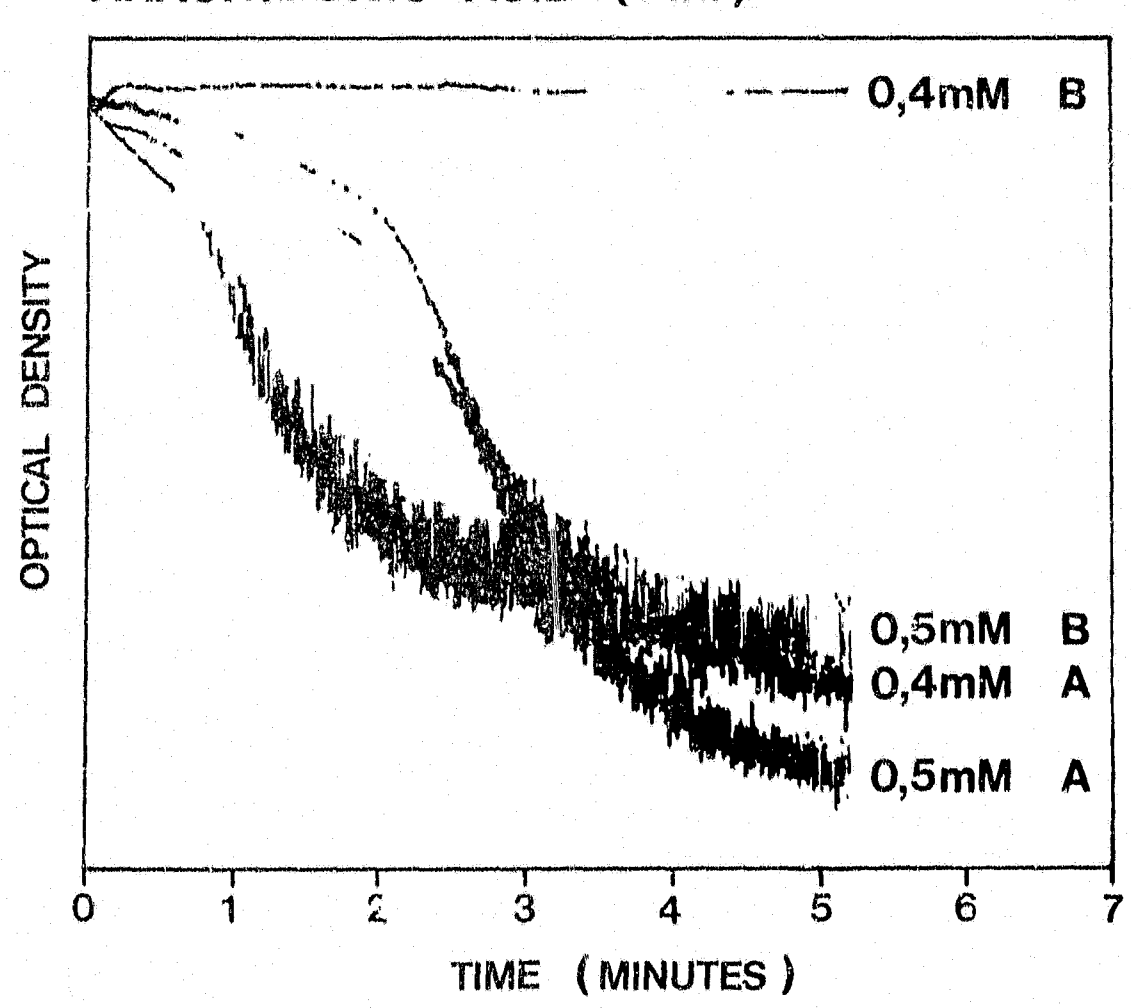


Fig 6.4. A representative experiment showing platelet aggregation of one subject in response to Arachidonic Acid before (B) and after (A) the fish diet.

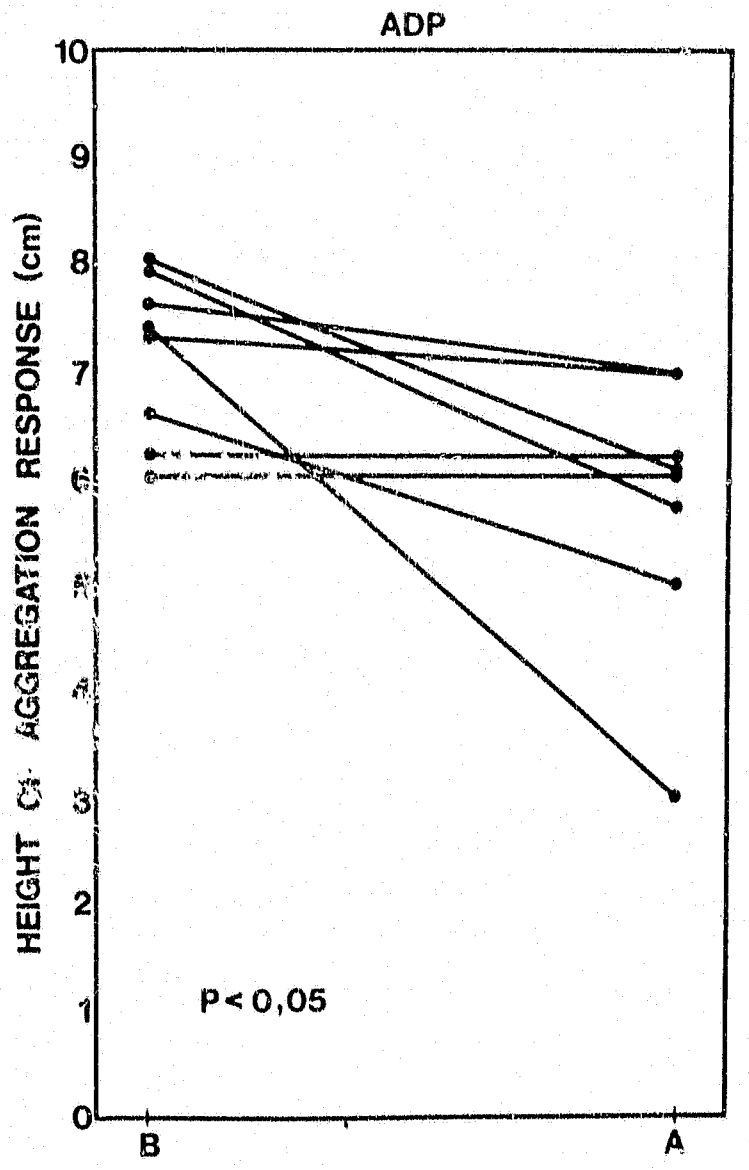


Fig 6.5. Aggregation responses of all 8 subjects in response to ADP before (B) and after (A) the fish diet.

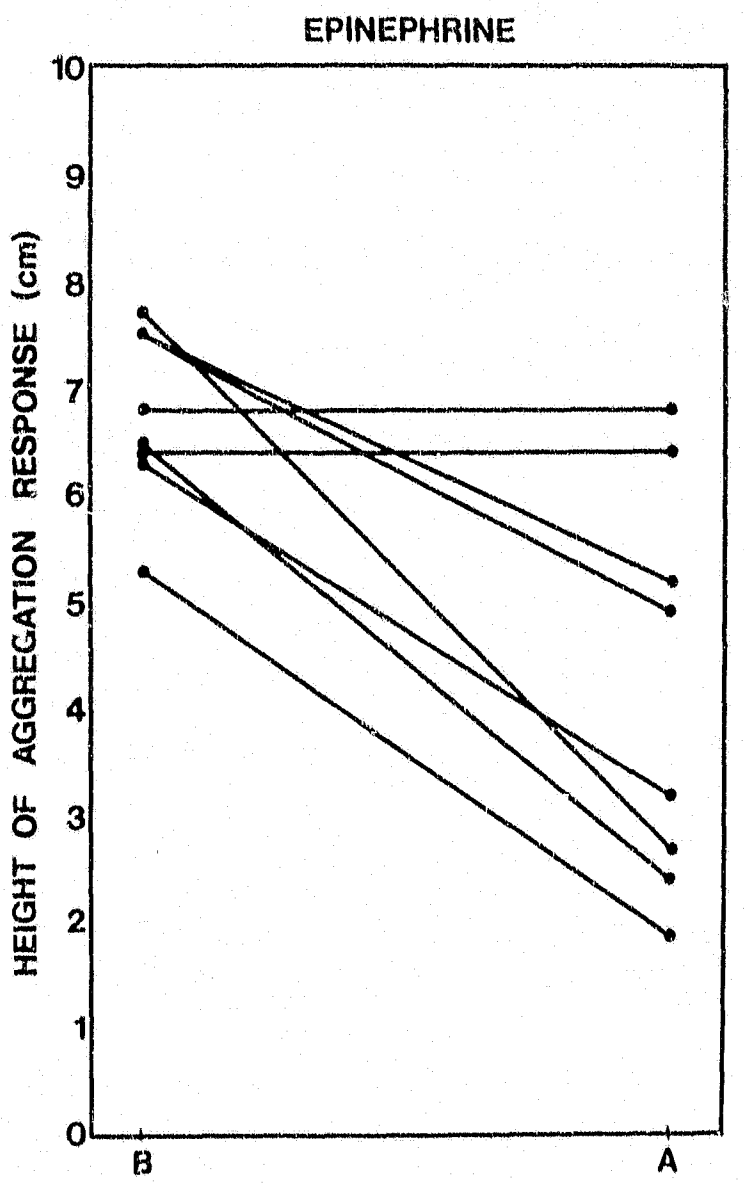


Fig 6.6 Aggregation responses of all 8 subjects in response to Epinephrine before (B) and after (A) the fish diet.

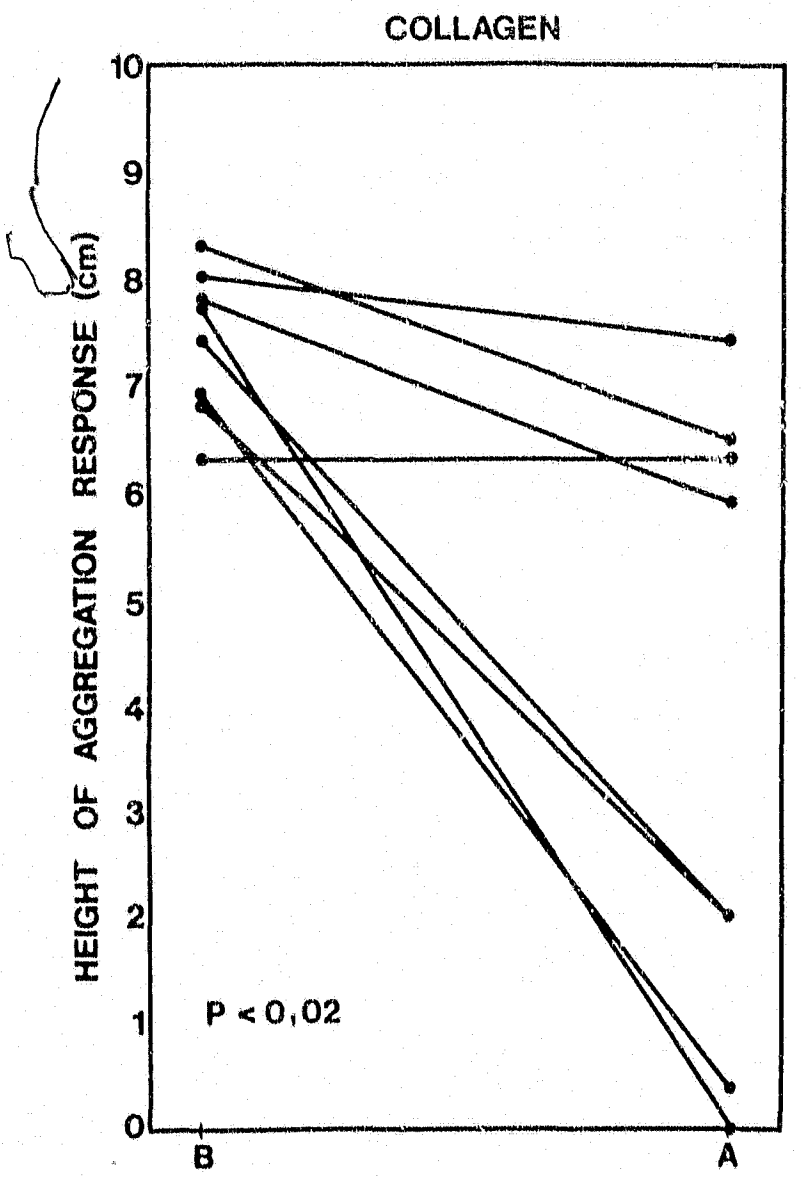


Fig 6.7. Aggregation responses of all 8 subjects in response to collagen before (B) and after (A) the fish diet.

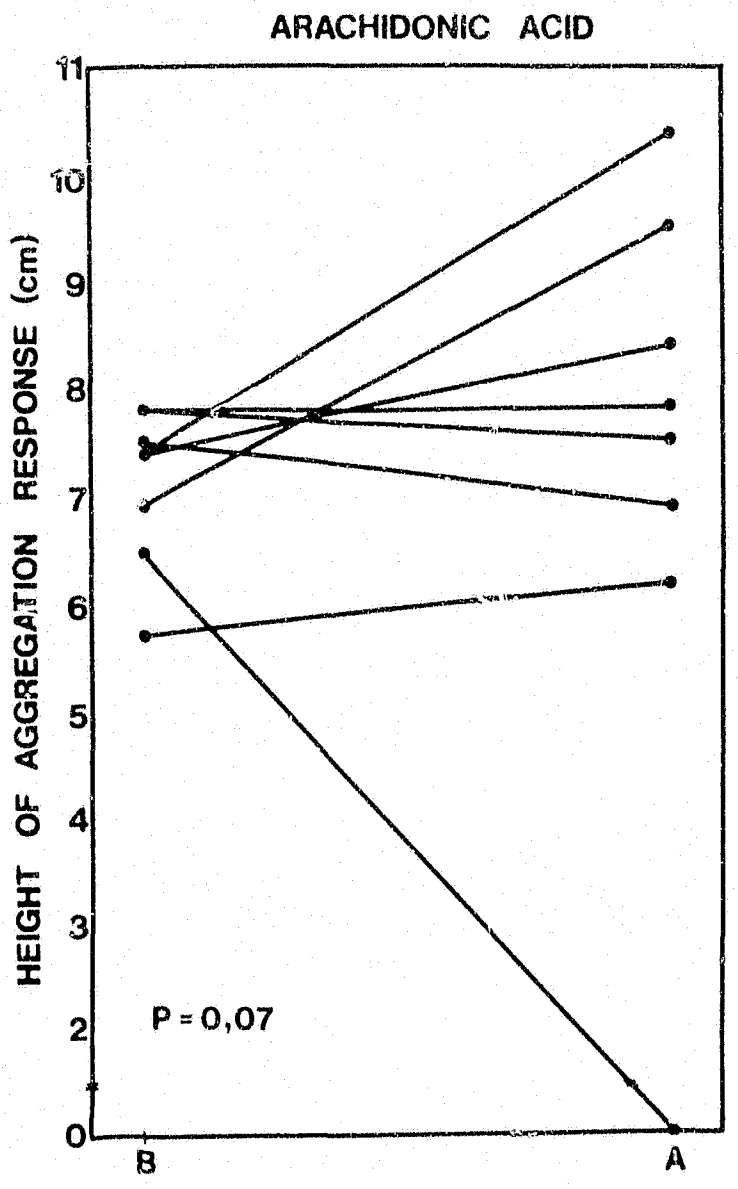


Fig 6.8. Aggregation responses of all 8 subjects in response to arachidonic acid before (B)and after (A) the fish diet.

Table 6.3 Platelet Aggregation before and after a "fish diet".

	ADP	EPI	COLLAGEN	AA
Before	7.13±0.76	6.75±0.8	7.4 ±0.68	7.13±0.74
After	5.75±1.29	4.19±1.88	3.81±3.01	7.11±3.18
P	`0.05	`0.05	`0.02	=0.07

Values shown are the mean ± SD of the height of the aggregation response in cms.

6.3.3. Thromboxane B_2 Production

Production of Thromboxane B_2 (TXB_2) in all 8 subjects in response to the different agonists is shown in figures 6,9 - 6,13. There was a significant decrease in the mean TXB_2 production after the diet in response to stimulation by EPI, collagen and AA but in response to ADP the mean change was not significant. Six of the eight cases did show a fall in TXB_2 production after ADP stimulation but two showed a marked increase. The responses to the other three agonists were reduced after the diet in every subject except for one who showed a slight increase in response to EPI.

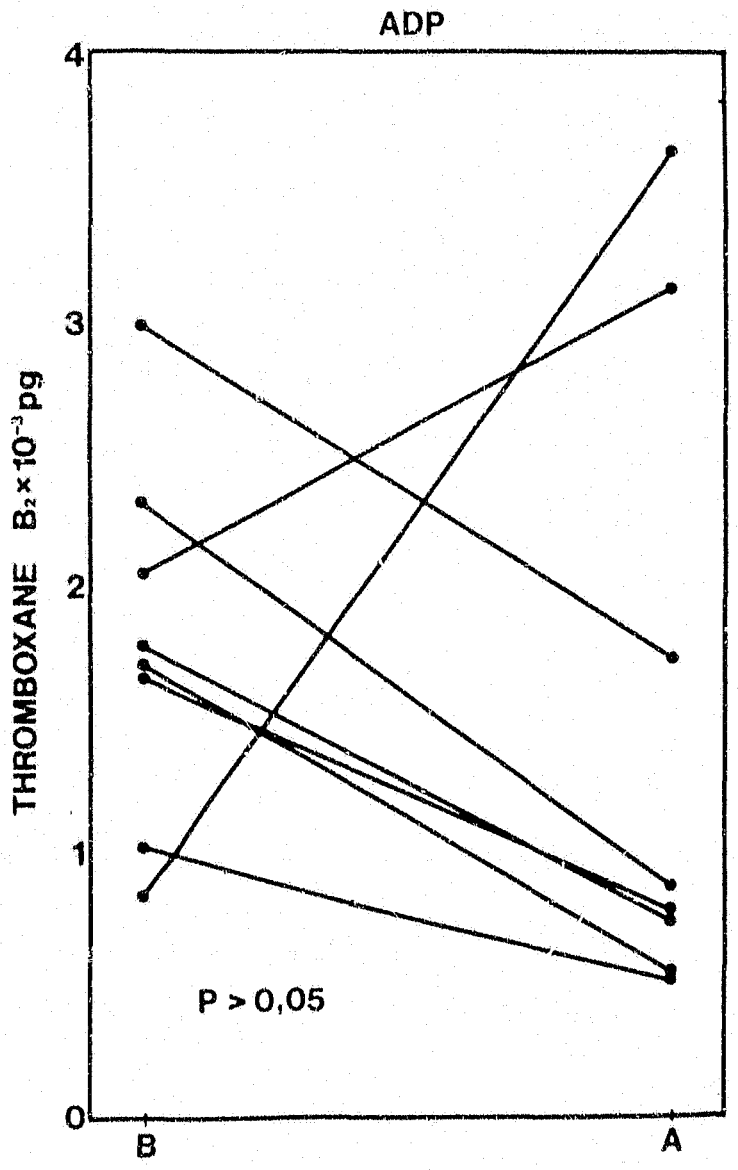


Fig 6.9. Thromboxane B_2 production in all 8 subjects in response to ADP stimulation before (B) and after (A) the fish diet.

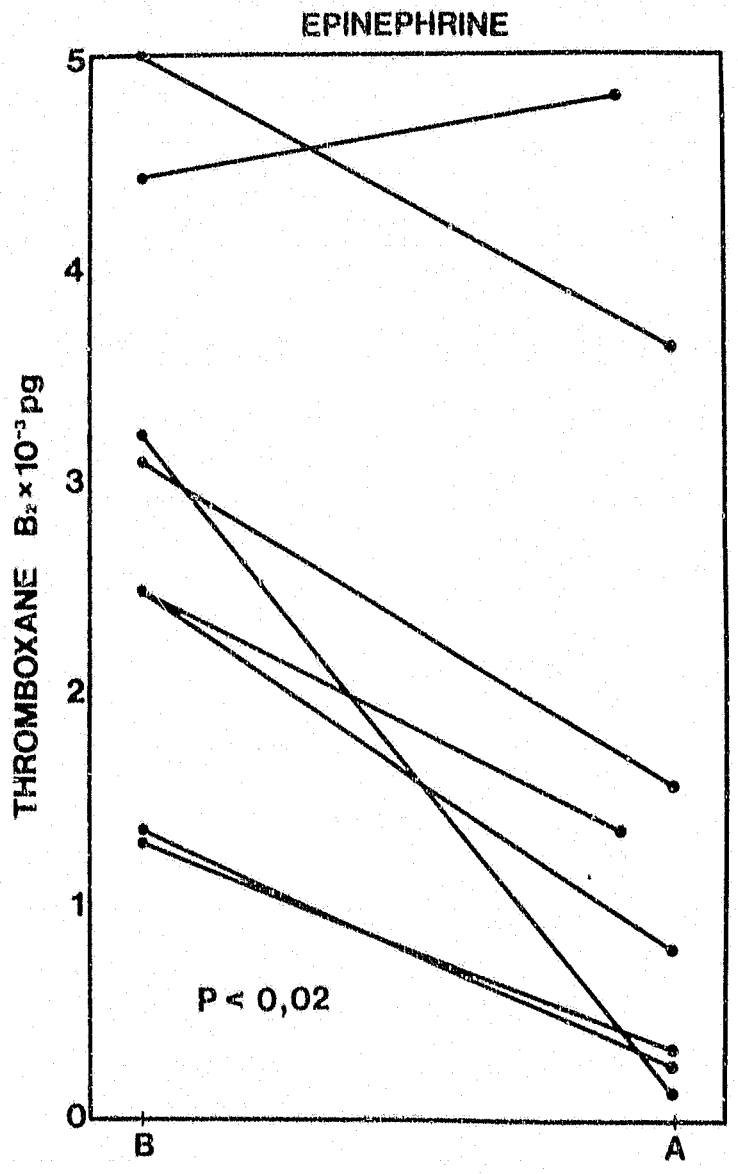


Fig. 6.10. Thromboxane B_2 production in all 8 subjects in response to epinephrine stimulation before (B) and after (A) the fish diet.

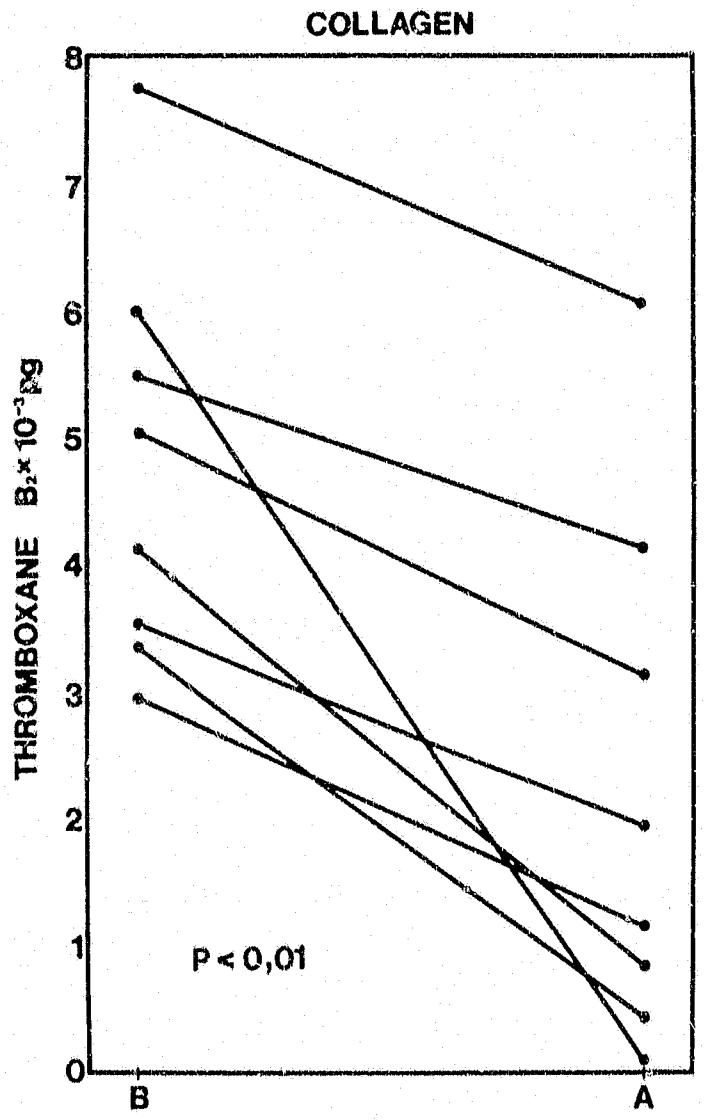


Fig 6.11. Thromboxane B_2 production in all 8 subjects in response to collagen stimulation before (B) and after (A) the fish diet.

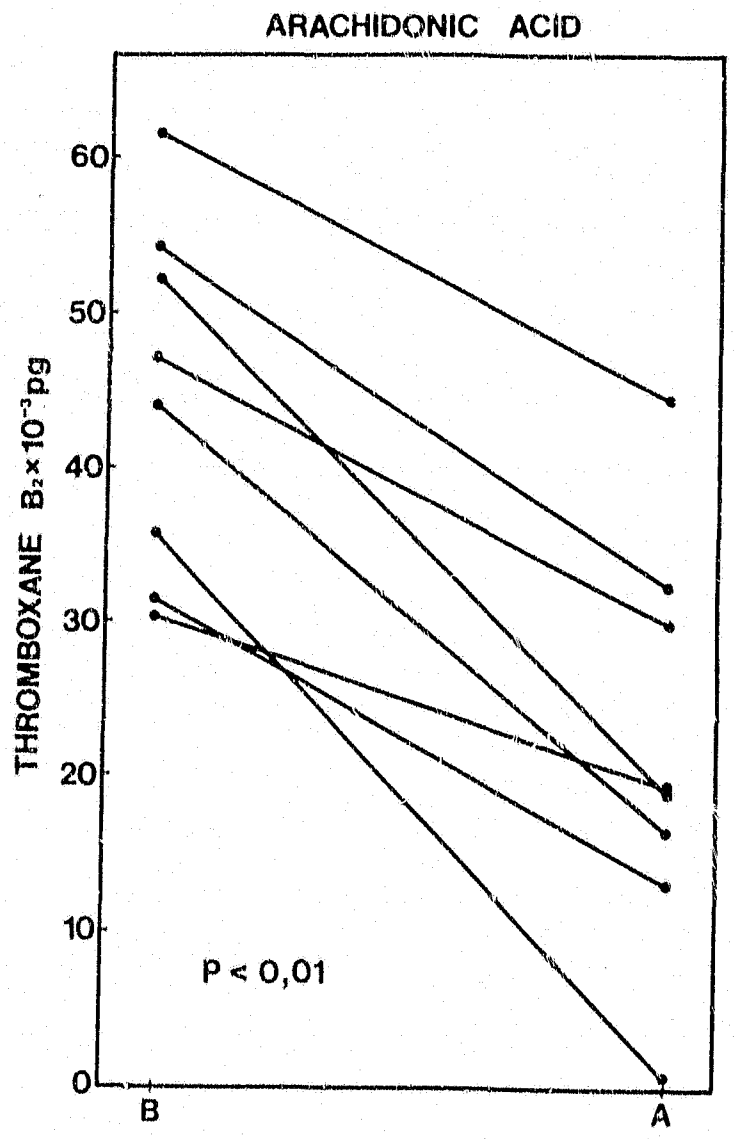


Fig 6.12 Thromboxane B_2 production in all 8 subjects in response to Arachidonic Acid stimulation before (B) and after (A) the fish diet.

6.3.4. Platelet Indices

The platelet counts before and after the fish diet are summarized in table 6.4. The difference was not significant (P>0,05). In one case the platelet count rose by 21% but in all the others there was a decrease ranging from 1 to 29% (mean = 14%) of the initial count.

The plateletcrit before and after the diet is summarized in table 6.5. The mean platelet volumes are shown in table 6.6. and the platelet distribution width of all 8 subjects are summarized in table 6.7. There were no significant changes in any of the results.

Table 6.4. Platelet counts before (B) and after (A) a "Fish Diet". Results expressed as counts/uℓ.

	B	A
Mean	253,500	231,500
SD	38,890	64,360
P	>0,05	

Table 6. 5 Plateletcrit Before (B) and After (A) a "Fish Diet" .

	B	A
Mean	0.219%	0.203%
S.D.	0.03	0.03
P	>0.05	

Table 6.6 Mean Platelet volume Before (B) and After (A) a "Fish Diet"

	B	A
Mean	8.15 fℓ	8.72 fℓ
S.D.	0.6	1.07
P	>0,05	

Table 6.7. Platelet distribution width Before (B) and after (A)"Fish Diet".(A)

	B	A
Mean	9.8	9.97
S.D	0.15	0.20
P	>0,05	

6.3.5. Serum lipids

Table 6.8 shows the cholesterol and triglyceride values before and after the diet. Figures 6.13 - 6.16 show the results of the 8 subjects before and after the diet. There were significant decreases in the mean total and HDL cholesterol values after the diet but the small decreases in the mean LDL cholesterol and triglyceride values were not significant. In the case of the total and HDL cholesterol values every one of the eight subjects showed a fall after the fish diet.

Table 6.8 Serum lipids before and after a fish diet.

	Cholesterol			Triglycerides
	Total	HDL	LDL	
Before	5.4±0.7	1.2±0.8	3.6±0.6	1.2±1.6
After	4.6±0.9	0.5±0.3	3.4±0.8	0.8±0.7
P	<0,01	<0,01	N.S.	N.S.

Values shown are in mmoles/liter.

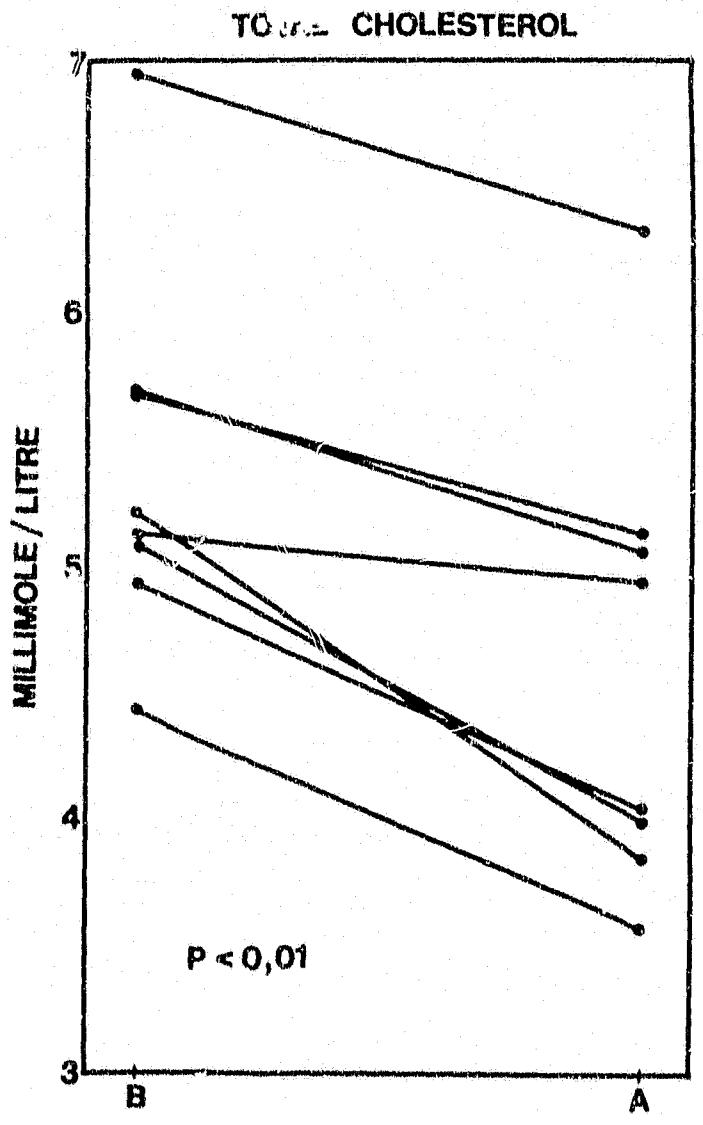


Fig 6.13. Total cholesterol of all 8 subjects before (B) and after (A) the fish diet.

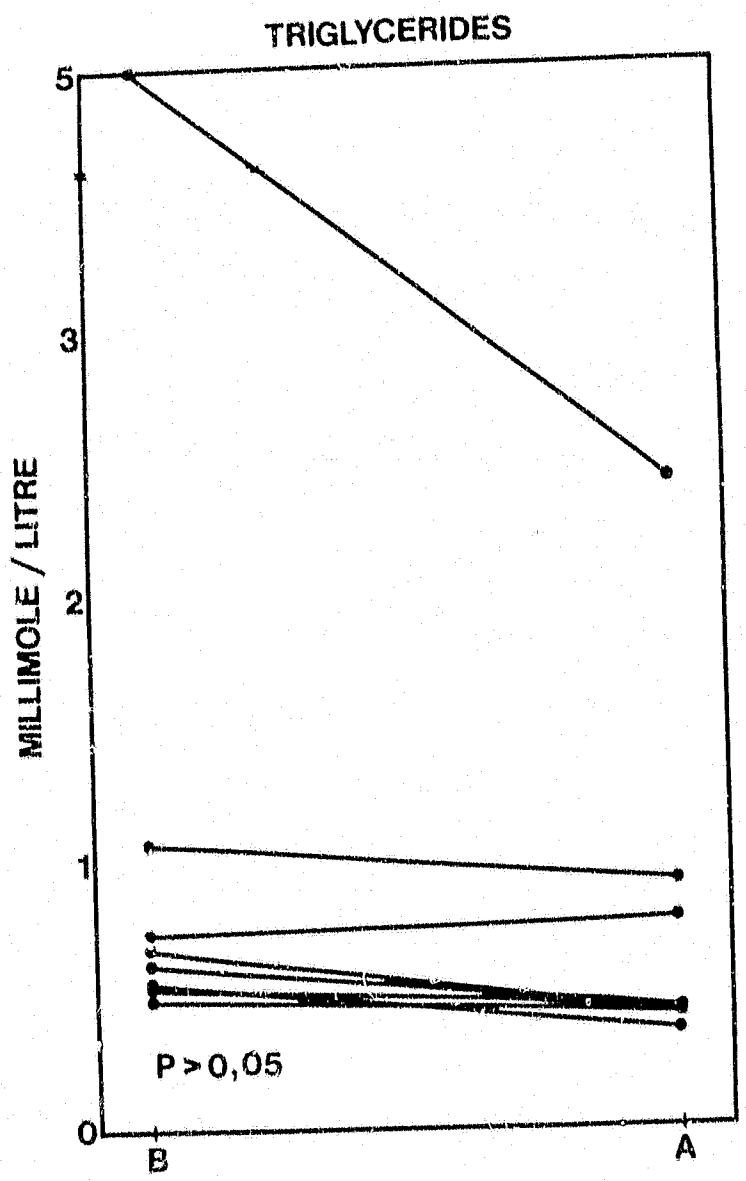


Fig 6.14 Triglycerides of all 8 patients before (B) and after (A) the fish diet.

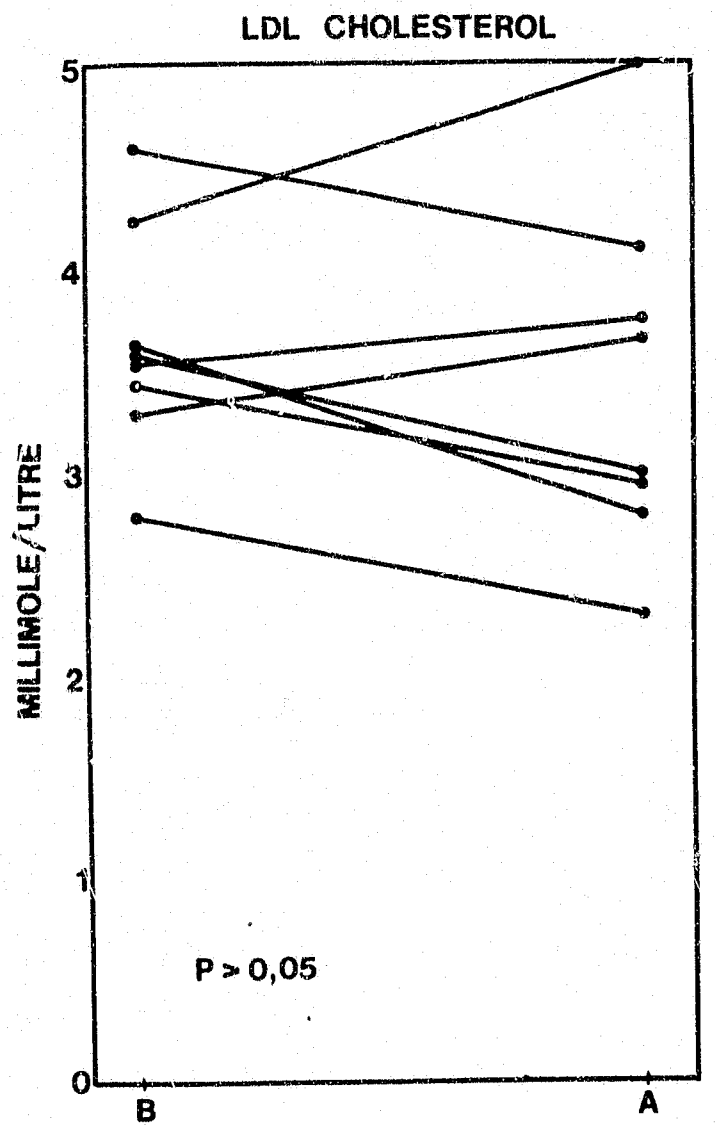


Fig 6.15 LDL cholesterol of all 8 subjects before and after the fish diet.

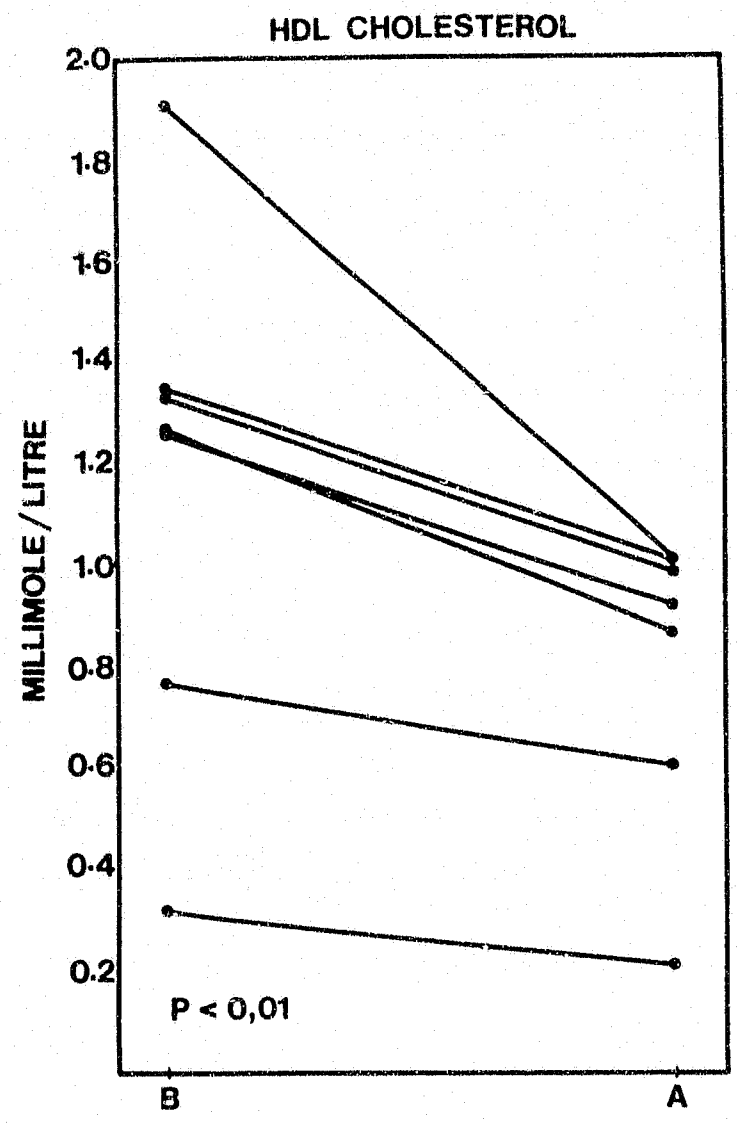


Fig 6.16. HDL cholesterol of all 8 subjects before and after the fish diet.

6.3.6. Fatty Acids

The fatty acid composition of platelets before and after the diet is shown in table 6.9. There were statistically significant increases in C18:3, and C20:5 and a significant decrease in C20:3. The C20:4 decreased after the diet and were it not for one subject (Fig 6.19) who showed an unexplained increase in C20:4 in both platelets and plasma, the decrease in C20:4 would have been significant. The ratio of C20:5 to C20:4 also increased significantly. The plasma fatty acids showed essentially similar changes. The platelet and plasma linoleic (18:2), linolenic (18:3), Arachidonic (20:4) and eicosapentaenoic acids of the 8 subjects before and after the diet is shown in figures 6.17 - 6.20. Fig 6.21 shows the ratio of 20:5 to 20:4 in the platelets and plasma.

Table 6.9. Fatty acid composition of platelets before (B) and after ingestion of a fish diet.

	% of Total Fatty Acids		
Fatty acids	Before	After	F
16:0	18.83±2.54	19.13±3.45	N.S.
16:1	1.33±1.19	0.95±0.66	N.S.
18:0	16.19±3.59	12.85±2.60	N.S.
18:1	17:53±2.69	15.34±2.88	N.S.
18:2	10.50±5.28	13.89±11.20	N.S.
18:3	1.02±0.44	2.84±1.52	<0.005
20:0	0.92±0.49	0.82±0.26	N.S.
20:1	0.90±0.41	1.02±0.82	N.S.
20:2	0.46±0.18	1.48±2.63	N.S.
20:3	0.89±0.36	0.56±0.29	<0.005
20:4	14.88±3.29	11.60±5.29	N.S.
22:0:1	1.25±0.35	1.63±0.41	N.S.
20:5	0.43±0.38	1.12±0.85	<0.05
22:2	0.0	0.0	-
22:3	0.14+0.23	0.19+0.36	N.S.
22:4	1.54±0.57	1.68±0.65	N.S.
22:5	0.26±0.74	0.84±0.94	N.S.
24:1	3.25±1.24	2.16±1.53	N.S.
22:6	3.98±2.28	4.04±2.17	N.S.
26:0	4.72±6.26	8.43±11.01	N.S.
$\frac{20:5}{20:4}$	0.029±0.02	0.090±0.06	p`0.005

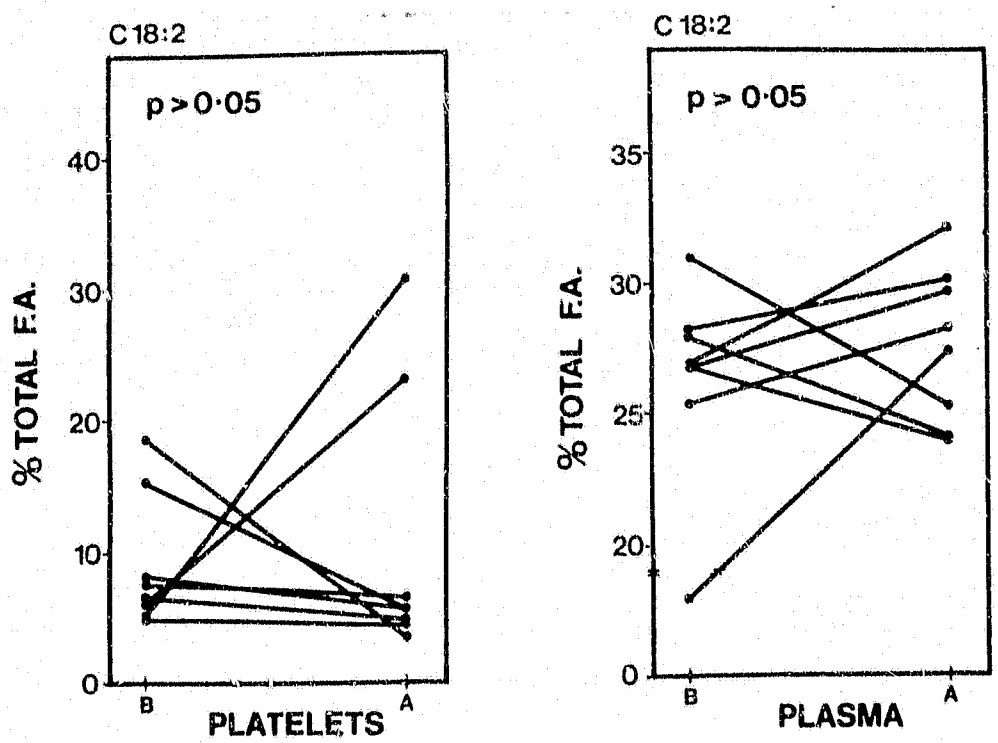


Fig 6.17 Platelet and Plasma linoleic acid in all 8 subjects before (B) and after (A) the fish diet.

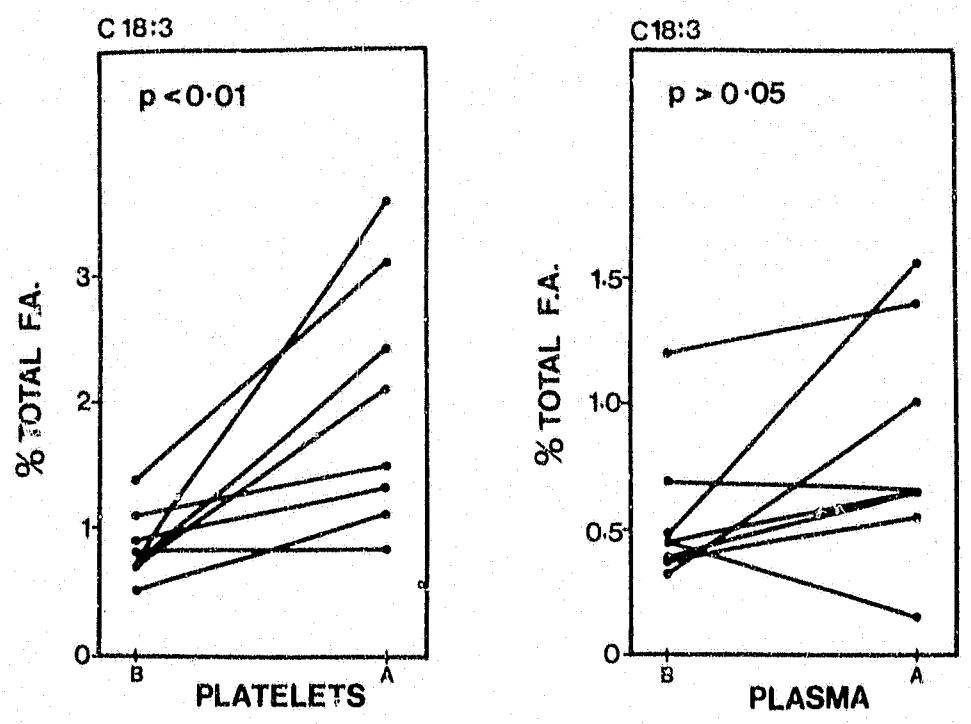


Fig 6.18 Platelet and Plasma linolenic acid in all 8 subjects before (B) and after (A) the fish diet.

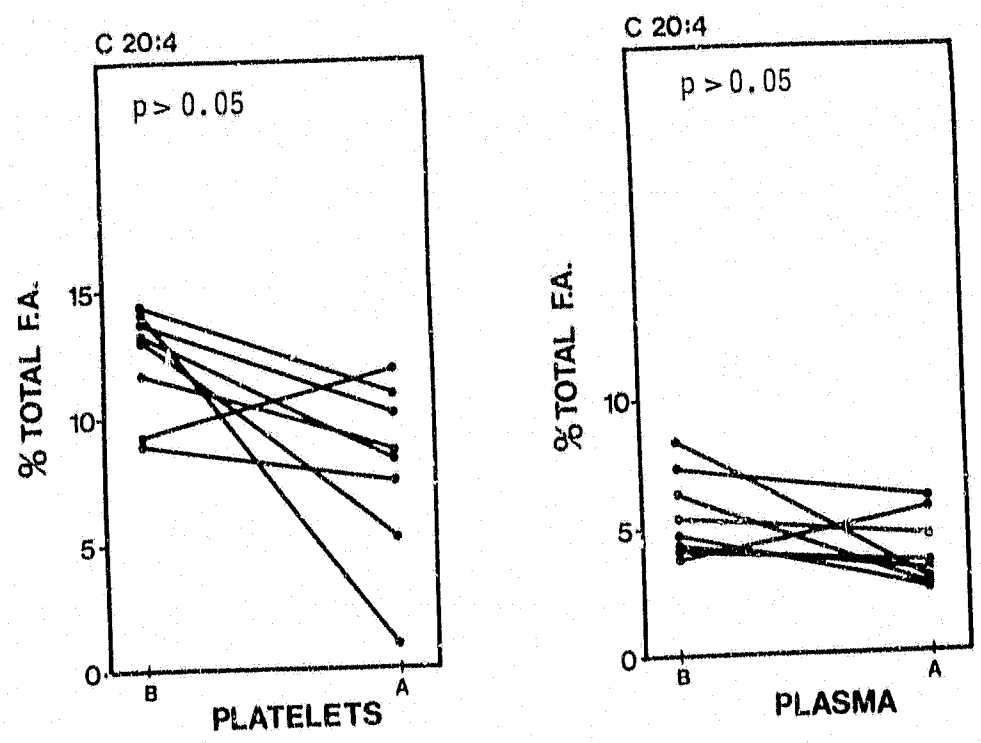


Fig 6.19. Platelet and Plasma arachidonic acid in all 8 subjects before (B) and after (A) the fish diet.

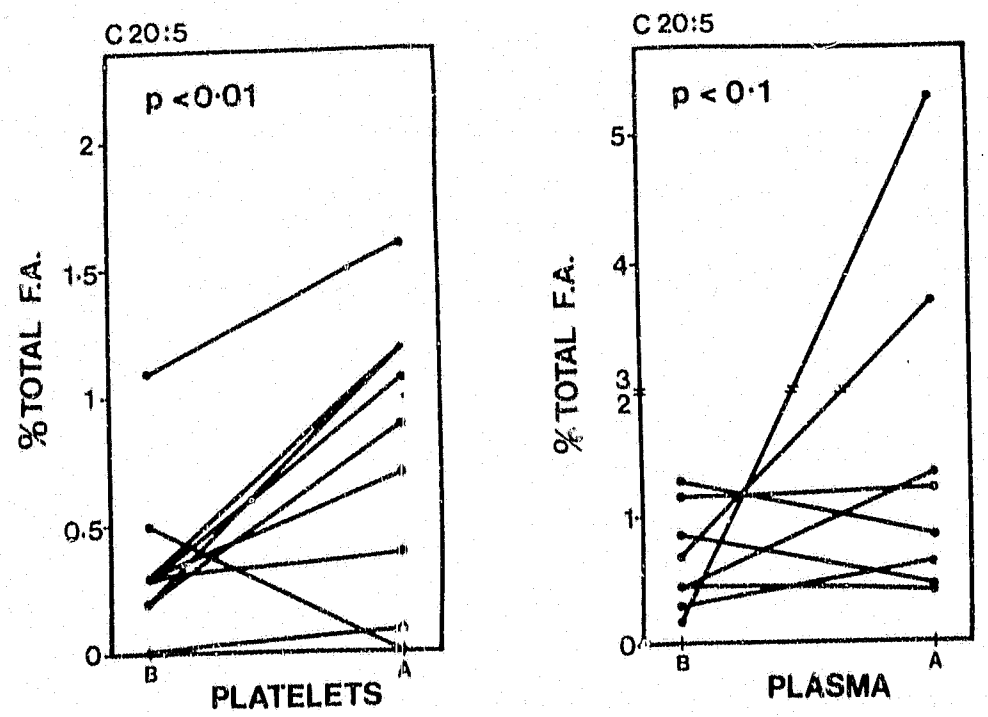


Fig 6.20. Platelet and plasma eicosapentaenoic acid in all 8 subjects before (B) and after (A) the fish diet.

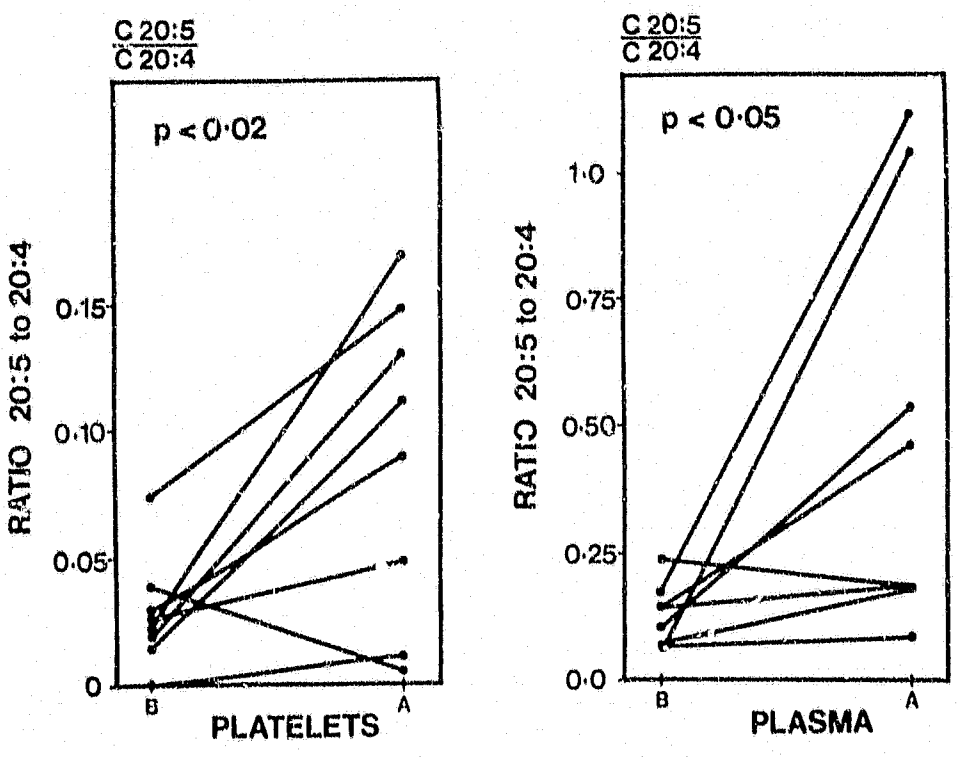


Fig 6.21 Ratio of 20:5/20:4 in platelets and plasma of all 8 subjects before (B) and after (A) the fish diet.

DISCUSSION

This study indicates that in a short term experiment a mixed fish diet is associated with significant changes in platelet aggregation, TXB_2 production, platelet fatty acid composition and serum lipids. The diet was characterised by a high content of long chain polyunsaturated fatty acids of the n-3 series particularly EPA but differered from that used in earlier studies (Siess et al., 1980; Goodnight et al., 1981., Thorngren and Gustafson, 1981; Hay et al., 1982) in that the chief source of EPA was sardines, pilchards, herring and kabeljou. The amount of EPA in this diet was estimated to be between 1 and 4 g per day. This amount was less than the 7-11g used by Siess et al., (1980) and Goodnight et al., but was similar to that used by Thorngren and Gustafson (1981). The amount of fish in this diet was close to a quantity that might be acceptable to many. The amounts of fish used by Siess et al.,(1980) and Goodnight et al.,(1981), were larger and probably too much to eat for long periods. (Their study continued for 4 weeks)

Reduced aggregation responses to ADP, epinephrine and collagen were observed during the diet but there was no reduction in the aggregation response to Arachidonic acid. In fact were it not for one subject the responses to arachidonic acid would have been

significantly increased. This fact suggests that the diminished responses were not due to inhibition of cyclo-oxygenase. A more likely explanation for the diminished responses would seem to be the increased ratio of EPA to AA in the platelet membrane. EPA is readily acylated into platelet phospholipids (Needleman et al., 1979; Whitaker et al., 1979) and no distinction exists between EPA and AA release from tissue lipids (Needleman et al., 1982). Since EPA acts as a competitive antagonist for AA, the simultaneous release of these fatty acids should effectively suppress AA conversion to its aggregatory metabolites. The lowering of endogenous AA and increase in EPA which must result from a diet of this type and that which the Greenland Eskimos ingest would be anticipated to reduce the generation of PGH_2 and thromboxane A_2 which cause aggregation (Needleman, 1982). Furthermore, some EPA is probably metabolised to thromboxane A_3 which is much less active than TXA_2, and it may also inhibit the action of TXA_2 by competitive inhibition (Needleman 1982).

In the present study the increase in EPA in the whole platelet extracts was significnt although the ratio of EPA to AA only rose from 0.03 to 0.09. This increase was similar to that found by Thornren and Gustafson (1981) which was 0.08, but much lower than that found

by Goodnight et al., 1981) and Siess et al., 1980) which was 0.3. In Eskimos ratios of above 0.9 have been reported (Dyerberg and Bang, 1979a). In favour of this view is the observed reduction in TXB_2 production after aggregation by all four agonists. The effect of EPA on endothelial cell production of prostacyclin was not assessed in this study. Since PGI_3 synthesised from EPA acts similarly to PGI_2 from AA (Needleman et al., 1979) the combined effect of increasing EPA in platelets and endothelial cells is likely to shift the balance of effects of the products of prostaglandin synthesis against platelet aggregation and platelet thrombus formation on endothelial cells.

Apart from the changes in EPA and AA in the platelets there was also a significant fall in 20:3 (n-6) and a rise in 18:3 fatty acids during the fish diet. The latter was probably due to increased consumption of vegetables and the decrease in 20:3 due to a reduction in consumption of n-6 fatty acids. A similar reduction in 20:3 was reported by Thorngren & Gustafson (1981). Neither of these changes in platelet fatty acid content have been implicated as causes of diminished platelet aggregation (Harlan and Harker, 1981). None of the other platelet fatty acids showed significant changes.

The whole blood platelet count was not altered significantly by the diet used in the present study. Thorngren and Gustafson (1981) also reported that the platelet count did not change but Goodnight et al's salmon diet (1981) and Hay et al's fish oil (1982) caused a significant fall. Lower counts were also reported in Eskimos than in Danish controls (Dyerberg and Bang, 1979a). The low platelet counts are unlikely to be due to increased destruction since Hay et al., (1982) found prolonged mean platelet survival time during the experimental period. Whatever the explanation, platelet counts never fell to haemostatically dangerous levels and the significance of this inconsistent effect is uncertain.

The plasma total cholesterol and HDL cholesterol levels in this study fell significantly but LDL cholesterol and triglycerides did not change. Plasma cholesterol and plasma triglycerides also fell after a mackerel diet (Siess et al., 1980) and a salmon oil diet in hypertriglyceridaemia patients (Phillipson et al., 1985) Hay et al., (1982) found that total cholesterol did not change but HDL cholesterol levels did rise. Lower levels of total and LDL cholesterol (Bang et al., 1971; Bang and Dyerberg, 1972) and higher levels of HDL cholesterol (Bang et al., 1971) were found in eskimos than in Danish controls. The fall in HDL

cholesterol observed in the present study differed from the latter findings but it is conceivable that the immediate short term effect of the fish diet may differ in its effect on HDL cholesterol levels from the effect of the diet over a longer period. A fall in HDL cholesterol levels could be harmful (Gordon et al., 1977; Miller and Miller, 1975) but the findings in eskimos suggest that the fall is unlikely to be sustained.

A second potential effect of EPA on atherogenesis is suggested by the observation of Lee and his colleagues (1985) that a diet containing fish-oil polyunsaturated fatty acids alters the function of monocytes. Monocytes adhere to the arterial endothelium and migrate into the intima at early stages of hypercholesterolaemia - induced atherosclerosis (Faggiotto, et al., 1984). They are then transformed into macrophages, which not only act as scavengers of plasma lipoproteins and cholesterol but also probably release growth factors that stimulate the proliferation of arterial smooth muscle cells (Glomset, 1985). Leukotriene B_4 influences the adherence of neutrophils to the arterial endothelium (Hoover et al., 1984). If a similar phenomenon occurs with monocytes, then monocytes containing increased amounts of EPA, and which show decreased production of

leukotriene B_4, may not adhere. Since the leukotriene isomer derived from EPA is LTB_5 which is approximately one tenth as potent as LTB_4 (Goldman et al., 1983; Lee et al., 1984; Lewis and Austen, 1984) migration and activity of granulocytes is reduced.

This study supports previous evidence that an increase in platelet EPA inhibits platelet aggregation. The combined effects on platelets and endothelial cells would seem to be potentially anti-atherogenic. As yet a pharmacological agent with similar effects has not been found. The amounts of fish used in the present study (300-400 g per day) would seem to be within the range that might be acceptable to many populations. It is possible that even smaller amounts may be effective if taken over long periods. This has been shown by Kromhout et al., 1985. These workers showed a negative correlation between fish ingestion and CHD over a 20 year period. The diet used in the present study also excluded sources of AA. To what extent this was necessary is uncertain. It may be that a compromise diet involving a reduction in foods rich in AA including meat and poultry together with an increase in fish rich in EPA may be equally effective.

These considerations suggest that it may be possible to design a sufficiently palatable and widely acceptable

diet that would alter platelet function and achieve an antithrombotic and antiatherogenic effect. The prophylactic value of such a diet could then be tested in a long term prospective controlled trial.

SUMMARY

Eight healthy subjects were fed a diet containing 1-4 g eicosapentaenoic acid (EPA) daily for 8-21 days. The EPA was derived from 300-400 g per day of sardines, pilchards, herring and/or kabeljou. Sources of arachidonic acid (AA) in the diet were reduced. At the end of the experimental period there was an increase in the ratio of EPA to AA in the platelets and a decrease in platelet aggregation to ADP, epinephrine and collagen. Aggregation to AA was not reduced. Thromboxane production in response to all four agonists was reduced. Serum total and HDL cholesterol levels fell significantly but platelet counts, LDL cholesterol and triglyceride values did not change. In conclusion a relatively modest intake of EPA derived from a mixed fish diet together with a reduction of AA intake can alter *in vitro* platelet function and serum lipids significantly. A long term controlled trial of a palatable mixed fish diet to assess possible antithrombotic and antiatherogenic effects is justifiable.

CHAPTER 7

7. The Effects of a Vegetarian Diet on Platelet Function, Fatty Acids and Serum Lipids

7.1 Introduction

In the previous chapter (6), it was shown that a diet poor in arachidonic acid (AA) and rich in eicosapentaenoic acid (EPA) was found to inhibit platelet aggregation in response to collagen, ADP and epinephrine and suppress thromboxane B_2 (TXB_2) production. Changes in platelet fatty acid composition were also observed. The EPA rich diet also decreased serum cholesterol values. These effects on platelet function have been observed by others (Siess *et al.*, 1980; Goodnight *et al.*,1981 Thorngren and Gustafson, 1981; Hay et al., 1982; Saynor *et al.*, 1984; Ahmed and Holub, 1984) and attributed to the increase in platelet EPA consequent upon the increased dietary intake of this fatty acid. The possible effects of the concomitant reduction in dietary AA on platelet function have not been established and could possibly have influenced the changes observed in previous studies. To prove that these haemostatic changes were due to increased dietary intake of EPA and not a reduction in dietary AA, 9 healthy subjects were placed on a vegetarian diet free of arachidonic acid for four weeks, to assess the effects of eliminating AA from the

diet on platelet aggregation, TXB_2 production, fatty acid composition and serum lipids.

7.2. Materials and Methods

7.2.1 Volunteer subjects

Nine healthy normal subjects (8 males, 1 female, ages 19-34 years, Mean = 25.1 years) were studied. They were all Indian subjects and followers of the Hindu religion. During a four week period known as Perattasi Hindu persons are forbidden to eat any animal food (including marine foods) except for milk and dairy products. Cakes, ice cream or any food containing eggs are not permitted. The chief foods consumed during this period are: milk, cheese, margarine, cereals, including bread, lentils, rice, all vegetables, many in the form of salads, salad oil (sunflower seed oil) mushrooms and nuts. At all times other than the period of Perattasi the subjects ate a mixed diet including meat (mutton, beef and pork), poultry, eggs and fish. Each subject was venesected twice prior to Perattasi while on a mixed diet and again at the end of the four week period while still on the vegetarian diet. Materials and Methods used for platelet aggregation, serum lipids, TXB_2 production and fatty acids are discussed in chapter 6. In addition aggregation responses to AA were quantitated by measuring the lag

phase and the rate of reaction. The lag phase was defined as the interval in seconds between the addition of the agonist and the point where the line delineating the slope of reaction intercepted the base line. The rate of reaction was defined as the slope of the aggregation curve during the exponential phase of the reaction (the steepest portion of the curve) in cms per sec.

7.3 Results

7.3.1. Platelet Aggregation

The platelet aggregatory responses of the 9 subjects in response to stimulation by ADP, EPI, collagen and arachidonic are shown in figures 7.1 - 7.4 and summarized in table 7.1. There was an increase in the height of the aggregation response to AA and EPI. The responses to collagen and ADP did not change. In the case of AA the mean concentration required for maximum aggregation fell from 0.8 $\pm$ 0,17 to 0,4 $\pm$ 0,18mm ($P < 0,005$) (Table 7.2) and the rate of reaction increased from 0,8 $\pm$ 0,2 to 1,2 $\pm$ 0,4 cms per sec ($p < 0,01$) and the lag time decreased from 73.9 $\pm$ 33.3 to 35.5 $\pm$ 15.2 ($p<0,05$) (Table 7.3), indicating a definite increase in platelet aggregability. The concentration of EPI required for maximum aggregation did not change significantly. Figs 7.5 and 7.6 show a representative experiment of the aggregation tracings in response to

EPI and arachidonic acid respectively, before and after the vegetarian diet. The increase in the height of aggregation (Fig 7.5 and 7.6) and the increase in the rate of reaction and decrease in lag phase is clearly illustrated.

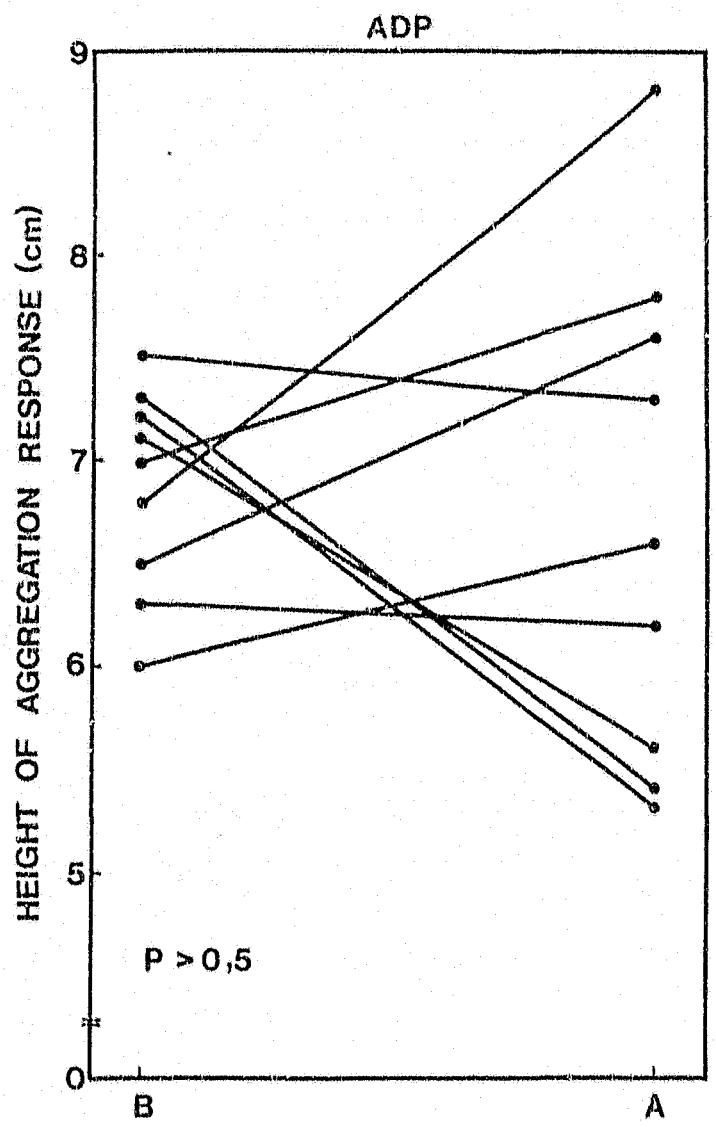


Fig 7.1 Aggregation responses of all 9 subjects in response to ADP before (B) and after (A) a vegetarian diet.

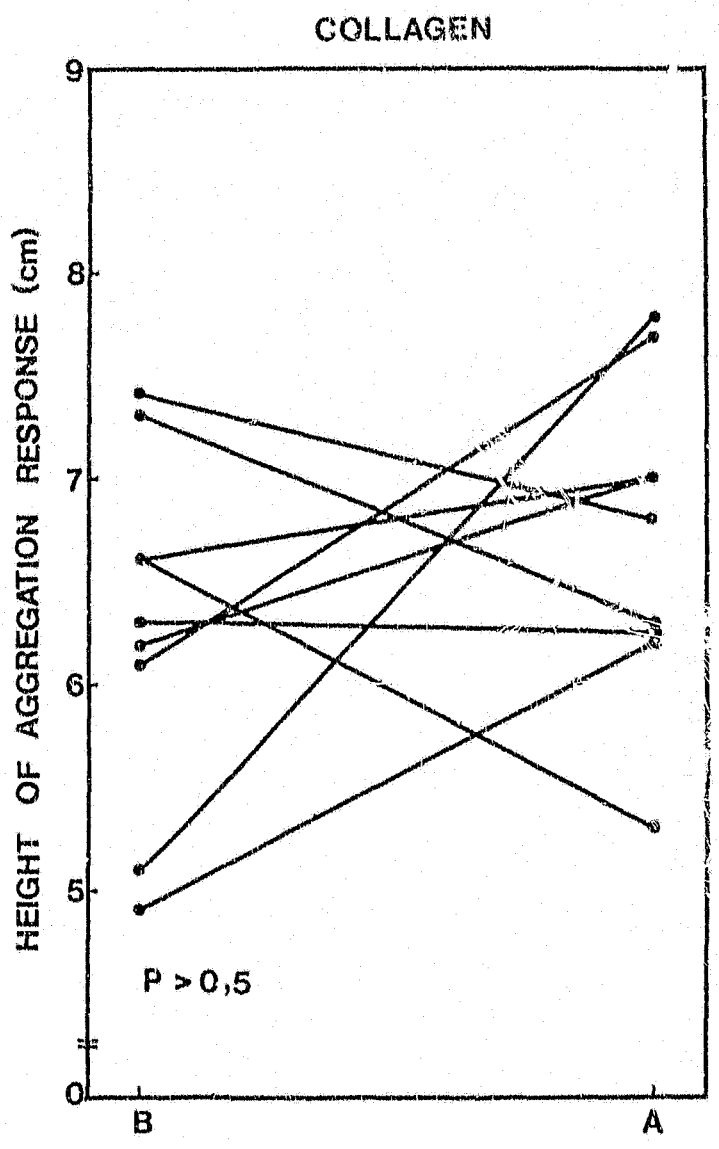


Fig 7.2 Aggregation responses of 9 subjects in response to collagen before (B) and after (A) a vegetarian diet.

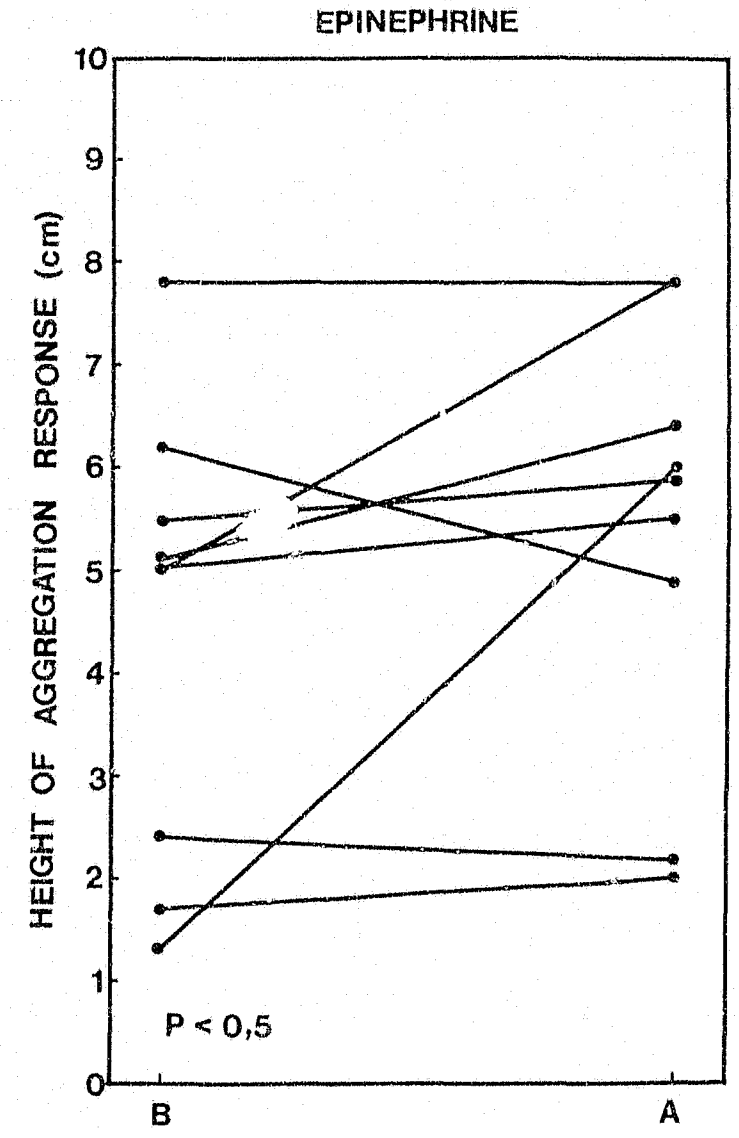


Fig 7.3 Aggregation responses of 9 subject in response to epinephrine before (B) and after (A) a vegetarian diet.

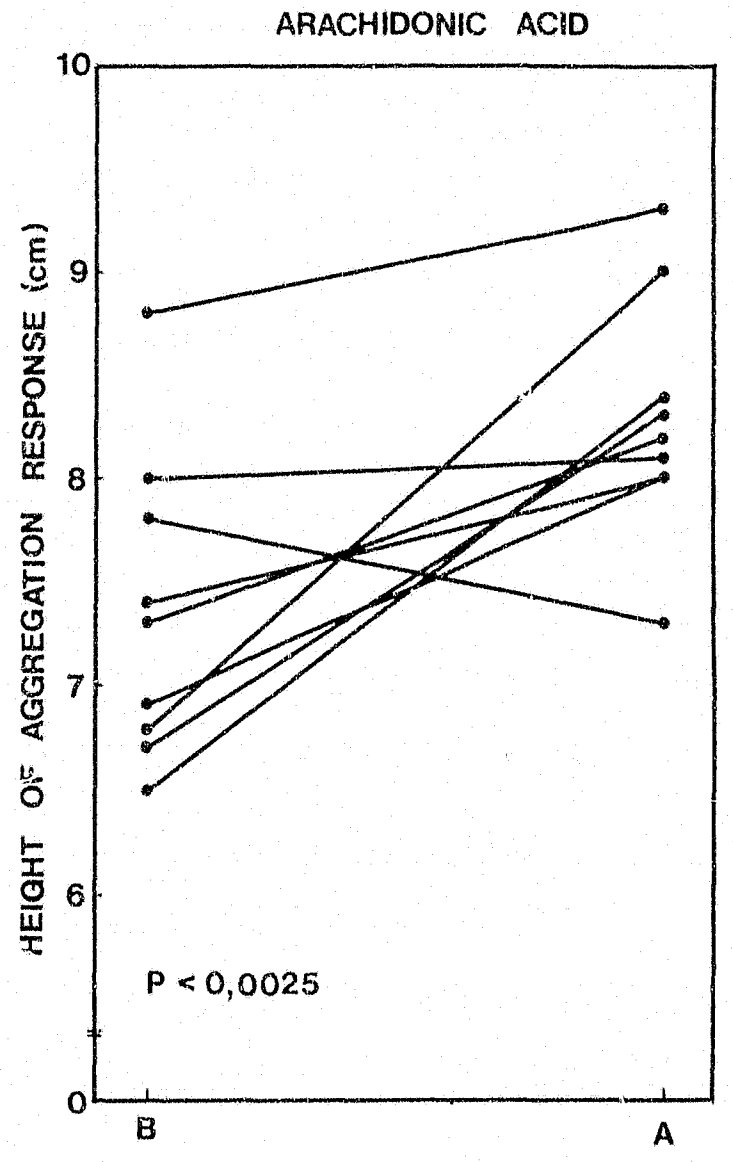


Fig 7.4. Aggregation responses of 9 subjects in response to arachidonic acid before (B) and after (A) a vegetarian diet.

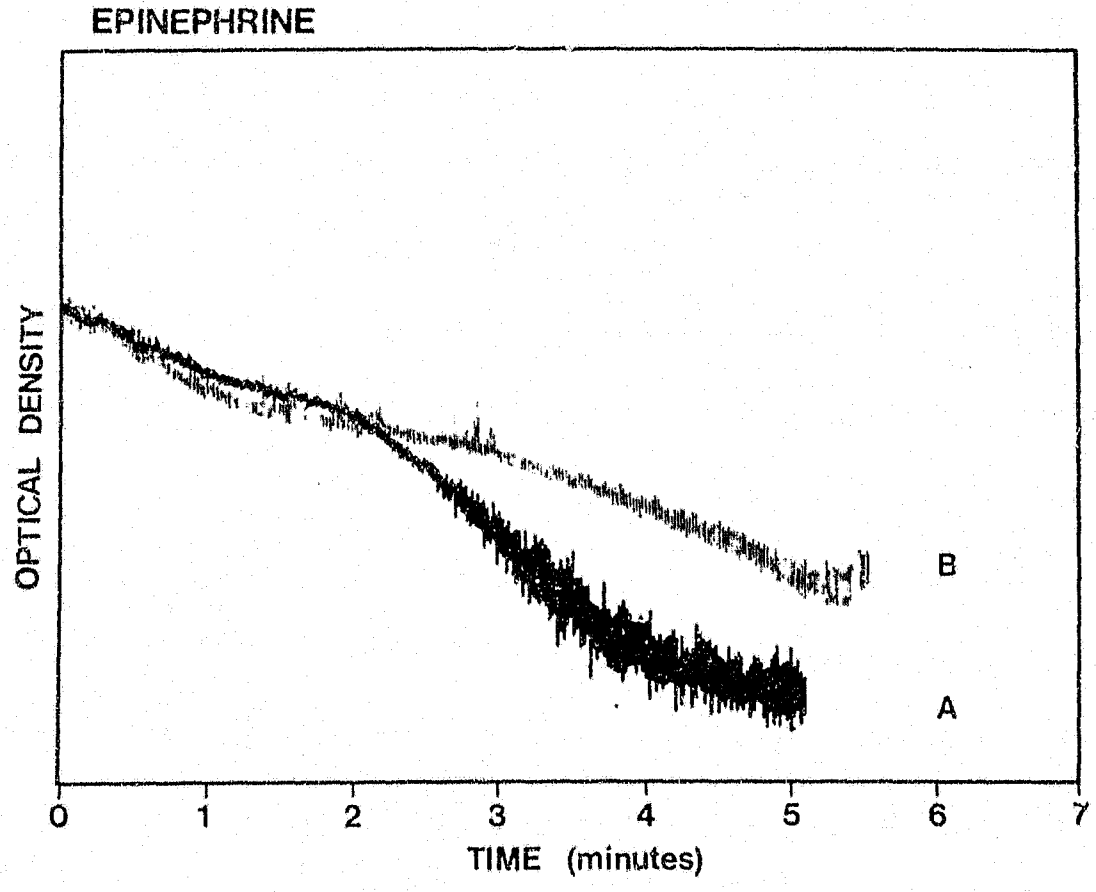


Fig. 7.5. A representative experiment showing an increase in the height of aggregation in response to epinephrine after a vegetarian diet.

B = Before Diet

A = After Diet

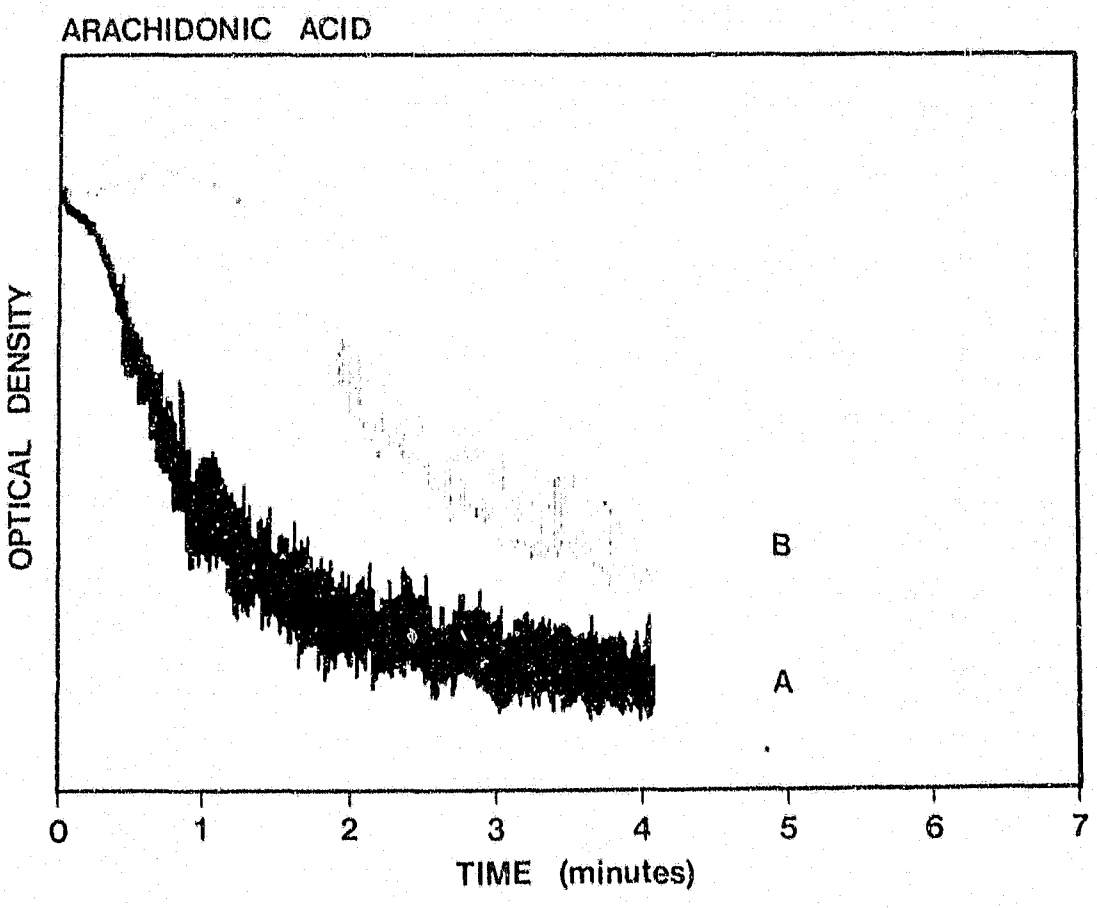


Fig 7.6 A representative experiment showing an increase in the height of aggregation, increase in the reaction rate and a decrease in the lag phase in response to Arachidonic Acid after a vegetarian diet.



Table 7.1. Platelet aggregation before and after a vegetarian diet.

	ADP	EPI	COLL	AA
Before	6.86 ± 0.5	4.44 ± 2.18	6.28 ± 0.85	7.36 ± 0.74
After	6.62 ± 1.04	5.39 ± 2.10	6.62 ± 0.69	8.30 ± 0.56
p value	NS	<0.05	NS	<0.0025

Values shown are the mean ± SD of the height of the aggregation response in cms (see methods) NS = Not significant.

Table 7.2 The minimal concentration of Arachidonic Acid (mM) required to produce maximal aggregation response before and after a vegetarian diet.

Subject	Before	After
1	0,6	0,3
2	0,8	0,2
3	0,9	0,4
4	0,7	0,5
5	1,0	0,8
6	0,8	0,3
7	1,0	0,4
8	0,5	0,3
9	0,9	0,3
Mean	0,8	0,4
S.D.	± 0,17	± 0,18
P	<0,005	

Table 7.3. Reaction rate (cms per sec.) and lag phase (s) in response to Arachidonic Acid. Before and after a vegetarian diet.

	Reaction rate		Lag Phase	
Subject	Before	After	Before	After
1	2.55	3.67	120	21.6
2	2.67	3.10	31.2	19.2
3	2.5	3.64	64.8	21.6
4	1.82	1.93	91.2	55.2
5	2.0	1.75	124.8	60
6	1.77	3.5	81.6	24
7	1.83	4.0	60	43.20
8	2.64	3.09	38.4	38.4
9	1.32	1.78	52.8	36
Mean	0.83	1.2	73.9	35.5
S.D.	$\pm$0.2	$\pm$0.37	$\pm$33.3	$\pm$15.2
P	<0.01		<0.005	

7.3.2 Thromboxane B_2

Thromboxane B_2 production in response to stimulation by ADP, EPI, collagen and arachidonic acid is shown in figures 7.7 - 7.10 and the mean change summarized in table 7.4. Thromboxane B_2 production did not change significantly in response to any of the agonists.

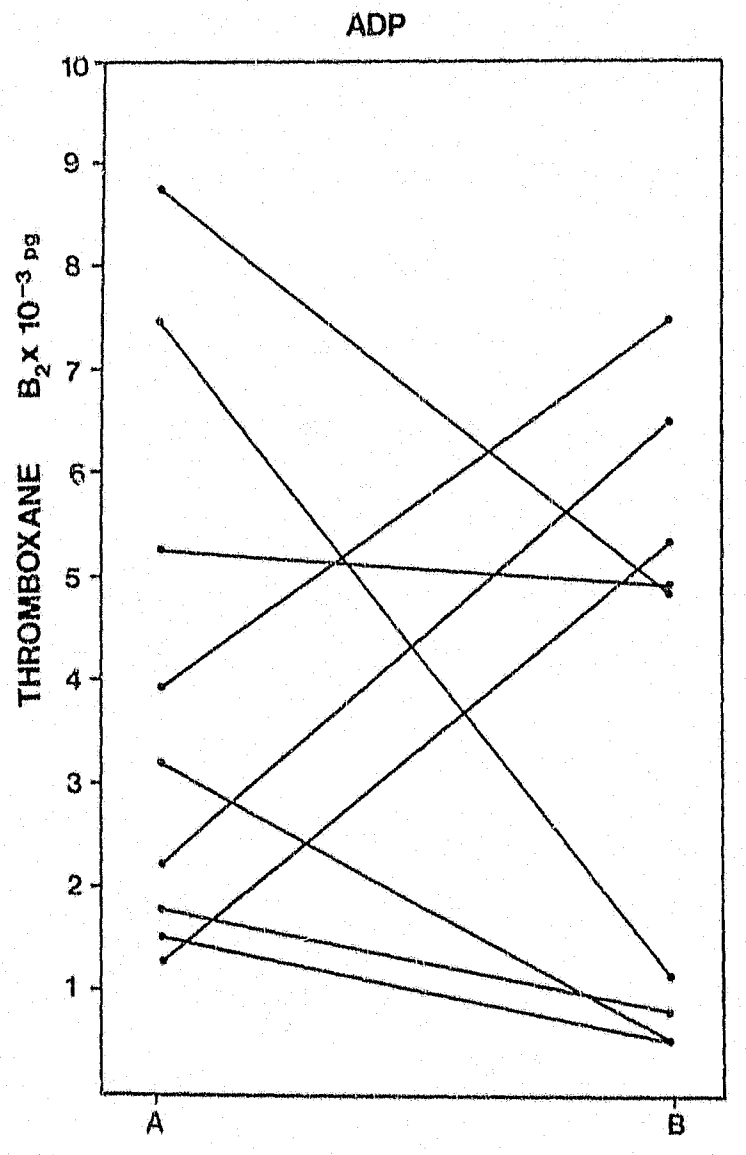


Fig 7.7. Thromboxane B_2 production by 9 subjects in response to ADP stimulation before (B) and after (A) a vegetarian diet.

Author Chetty N
Name of thesis Drug and dietary modification of platelet function 1985

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.