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Platelet - Vessel Wall Interaction: Influence of Diet [and Discussion]

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Platelet – vessel wall interaction: influence of diet

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The interaction of platelets with the vessel wall is influenced by the diet. Of major importance is the dietary polyunsaturated fatty acids (PUFA). Epidemiological evidence from Greenland Eskimos with low incidences of acute myocardial infarction has drawn attention to the role of the $n-3$ PUFA family.

Experiments *in vitro* have demonstrated an anti-aggregatory effect of eicosapentaenoic acid (EPA). However, EPA does not inhibit vascular prostacyclin production. Antiaggregatory substances generated from EPA have not yet been demonstrated *in vivo*.

Studies *in vivo* in both animals and humans have demonstrated an antithrombotic effect of EPA. In a study where volunteers were given 6 g EPA per day for 3 weeks, moderate decreases in collagen-induced and ADP-induced platelet aggregation, lower thromboxane B_2 (TXB_2) synthesis and prolongation of bleeding time were found.

These observations indicate that dietary factors modulate the interaction of platelets and the vessel wall. Dietary advice aiming at lowering the incidence of ischaemic diseases must include this aspect. This necessitates a re-evaluation of advice hitherto given to the population in general.

Introduction

Attempts have been made for two or three decades to influence the alarmingly high incidence of mortality from ischaemic heart disease (i.h.d.) in western societies by dietary advice. The so-called lipid hypothesis, based on epidemiological evidence of a causal role of high blood cholesterol and, in later years, low levels of high-density lipoproteins in the pathogenesis of ischaemic heart disease has, however, proved unprofitable in spite of the effort made at its implementation. One of the main points in lipid lowering régimes has been the recommendation of a high intake of polyunsaturated fatty acids, which has been synonymous with a high intake of linoleic acid, the principal member of the $n-6$ family. The inconsistency of the scientific data supporting this recommendation has led the Food and Nutrition Board (1980) under The National Academy of Science in U.S.A. in its report *Toward healthful diets* to state that 'the benefit of altering the diet (towards a higher P:S ratio) has not been established'. In his comment on this report, Olson (1980) states, 'the Lipid Hypothesis is not proved and has failed as a strategy for reduction of IHD. New excitement has been generated about the "Platelet Hypothesis". Feeding aspirin and marine oils may provide a new strategy for prevention of coronary disease.'

Great differences in the incidence of ischaemic heart disease exist, however, between different societies, and many observations point to environmental causes for this difference. Thus, the study of different populations and their way of life may still prove useful in identifying the factors leading to ischaemic heart disease.

Some observations indicate that this difference in morbidity pattern may be associated with differences in platelet – vessel wall interaction, and that differences in this balance may be attributed to differences in dietary habits.

I shall illustrate this by referring to studies on Greenland Eskimos and to the experimental evidence that we have, elucidating the mechanisms involved in the Eskimo pattern of morbidity. Finally I shall discuss the results of an experiment designed to see if the platelet – vessel wall interaction can be changed by dietary alterations.

The Greenland Eskimos: studies on the mechanism of their low incidence of i.h.d.

It has been known (but not documented) for decades that myocardial infarction in Greenland Eskimos is very rare. Recently this common knowledge has gained support from medical statistics as reported in *The state of health in Greenland*, an annual report from the chief medical officer in Greenland (1978), from which it can be calculated that the overall mortality from ischaemic heart diseases in the years 1973–6 averaged 3.5%. This is not due to short life expectancy, for the average life span of Greenlanders is now about 60 years as reported by the Ministry of Greenland (1979). We have had the opportunity of investigating this problem during four expeditions to northwestern Greenland in the years 1970–8. First we examined the plasma lipid and lipoprotein concentrations (Bang & Dyerberg 1972; Dyerberg *et al.* 1977) and table 1 shows the average differences in plasma lipid and lipoprotein concentrations between 130 Greenlanders and 470 Danish controls, matched for age and sex.

The Eskimos had lower cholesterol and triglyceride concentrations owing to lower LDL and VLDL levels and also higher HDL concentrations. Such differences are documented by several epidemiological studies as associated with a lower risk of developing i.h.d. The differences were, however, not large enough to explain satisfactorily the difference in mortality due to i.h.d., which is about 50% in Denmark.

In investigating the fatty acid pattern of the plasma lipids (Dyerberg *et al.* 1975), we found that the distribution of polyunsaturated fatty acids was dominated by those belonging to the linolenic class or the $n-3$ class in contrast to the $n-6$ class, which is the prevailing type in Danes. This is illustrated in table 2 in which the fatty acid distribution in the plasma phospholipids is given.

During two expeditions to the same area in Greenland in 1972 and 1976 we examined the diet of Greenlanders (Bang *et al.* 1980). The total consumption of fat was no greater than in Danes, whereas the protein content due to the higher meat consumption was higher and the carbohydrate content correspondingly lower. Of the fat in the diet (table 3), the $n-3$ class was the prevailing type of polyunsaturated fat, in contrast with the $n-6$ class in Danes.

This dominance was due to a high content of eicosapentaenoic acid (EPA), docosapentaenoic acid and docosahexaenoic acid (table 4), and we calculated that approximately 5–6 g of EPA was eaten per day.

At the time of our work it was found by Samuelsson's group in Sweden (Hamberg *et al.* 1975) and Vane's group in Beckenham (Moncada *et al.* 1976) that unstable metabolites of arachidonic acid were generated by platelets and the vessel wall respectively and that these metabolites had opposite effects on platelet – vessel wall interactions. It was further postulated that a balanced effect between these substances regulated haemostasis (Moncada & Vane 1978). By combining these findings with our observations of a shift from arachidonic acid into

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Table 1. Average differences in plasma lipid and lipoprotein concentrations between Caucasian Danes and Greenland Eskimos

cholesterol	1.15 mmol/l
triglycerides	0.66 mmol/l
LDL	0.76 g/l
VLDL	0.86 g/l
HDL	−0.66 g/l

Table 2. Fatty acid composition of plasma phospholipids in Greenland Eskimos (G.E., $N = 16$) and Danish controls (D., $N = 20$)

(Values are means as a percentage of total.)

	G.E.	D.		G.E.	D.
8:0–15:0	4.2	0.3	20:4	3.8	7.4
16:0	32.2	30.0	20:5	7.4	1.8
16:1	4.3	0.8	22:0–22:5	2.2	2.2
16:2–17:1	—	0.7	22:6	3.6	2.2
18:0	17.0	14.6	24:0–24:1	2.5	4.2
18:1	14.9	12.4	saturated	54.5	47.3
18:2	5.4	22.3	monoenes	24.4	15.3
18:3	0.1	0.2	polyenes	21.1	34.2
18:4–20:3	3.3	0.7	20:5/20:4	1.95	0.24

Table 3. Dietary fat types in Eskimo and Danish food

	Eskimos	Danish
saturated (percentage of total fatty acids)	22.8	52.7
monounsaturated (percentage of total fatty acids)	57.3	34.6
polyunsaturated (percentage of total fatty acids)	19.2	12.7
P:S ratio	0.84	0.24
linoleic class $(n-6)$/(g/day/3000 kcal)	5.4	10.0
linolenic class $(n-3)$/(g/day/3000 kcal)	13.7	2.8
monoenes, except 16:1 and 18:1/(g/day/3000 kcal)	29.6	2.1

Table 4. Fatty acid composition of food lipids in Greenland Eskimos, 1976 (G.E., $N = 178$) and in Danish food, 1972 (D.)

(Values are means as a percentage of total.)

	G.E.	D.		G.E.	D.
12:0	4.8	13.4	20:1	14.7	0.4
16:0	13.6	25.5	20:4	0.4	—
16:1	9.8	3.8	20:5	4.6	0.5
16:2–17:1	0.4	—	22:1	8.0	1.2
18:0	4.0	9.5	22:5–22:6	8.5	0.3
18:1	24.6	29.2	saturated	22.8	52.7
18:2	5.0	10.0	monoenes	57.3	34.6
18:3	0.6	2.0	polyenes	19.2	12.7
20:0	0.1	4.3	20:5/18:2	0.92	0.05

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eicosapentaenoic acid, the precursor of the prostaglandin-3 family in Eskimos, we hypothesized that such a shift may alter haemostasis and thrombosis tendency towards an antithrombotic direction and thereby give an additional explanation to the low morbidity of acute myocardial infarction in Eskimos. An indication of such a shift was found in nosographic reports from Greenland, in which throughout the centuries we find descriptions of an enhanced bleeding tendency in Eskimos ascribed by many to the heavy intake of blubber (Bang & Dyerberg

Table 5. Fatty acid composition of platelet lipids in Greenland Eskimos, 1978 (G.E., $N = 24$) and Danish controls (D., $N = 20$)

(Values are means as a percentage of total.)

	G.E.	D.		G.E.	D.
12:0–14:0	0.2	0.1	22:0–24:1	8.8	5.9
16:0–16:1	23.8	20.4	22:5	3.4	1.0
18:0	12.0	17.2	22:6	6.1	1.5
18:1	18.2	17.2	(unidentified	1.0	3.6)
18:2	4.0	8.2	saturated	35.6	41.3
20:0–20:1	5.6	2.3	monoenes	32.8	21.8
20:4	8.9	22.1	polyenes	30.3	33.0
20:5	8.3	0.5	20:5/20:4	0.93	0.02

1980). We hypothesized that this was due to the influence of EPA on the balance between pro- and antiaggregatory conditions in the platelet and vessel wall, and we demonstrated that EPA inhibits platelet aggregation (Dyerberg & Bang 1978). Furthermore, we suggested that EPA in the vessel wall could be converted into an antiaggregatory substance (Dyerberg *et al.* 1978). Consequently the haemostasis in Greenlanders was examined (Dyerberg & Bang 1979). We found that the bleeding time in Eskimos averaged 8.05 min, which was significantly longer than in Danes (4.76 min). This difference was due to a decreased platelet aggregability, as measured by ADP and collagen induced aggregation. It was paralleled by a difference in fatty acid composition of the platelet lipids (table 5), the Eskimos being dominated by the $n-3$ class, especially 20:5, $n-3$ (EPA) to which we in our hypothesis ascribed the platelet hypoaggregability.

We concluded from our studies that the low incidence of ischaemic heart disease in Eskimos was associated with platelet hypoaggregability and high content of EPA in structural and transport lipids, coming from a dietary intake of 5–6 g of this substance per day. Furthermore, the Eskimos showed no sign whatsoever of deficiency of essential fatty acids. I mention this because Gudbjarnason & Oskarsdottir (1975) found that rats fed with a marine oil diet showed a higher mortality after isoproterenol injection than control animals. The diet was, however, also rich in vitamin D, which is known to increase the sensitivity of these animals to isoproterenol. It was also rich in long-chain monoenoic acids, which in rats (in contrast to several other species) produce cardiac muscle degeneration. In investigating human hearts the same authors actually found a higher content of arachidonic acid in persons dying a sudden cardiac death than in controls (Gudbjarnason & Hallgrimsson 1975).

Another epidemiological observation should be mentioned in this context. During the World War II a distinct decline in mortality from i.h.d. was observed in Oslo, Norway (Strøm & Jensen 1951) followed by a marked rise after the war. In this period the consumption of food containing fish increased very substantially owing to a shortage of dairy products. We have calculated (Bang & Dyerberg 1981) that this resulted in an average daily EPA intake comparable with that of the Eskimos. No conclusion can, of course, be drawn from such figures.

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The mechanism of action of EPA on platelet–vessel wall interaction

The mechanism by which EPA may influence platelet–vessel wall interaction is only partly clarified. EPA inhibits platelet aggregation (Dyerberg & Bang 1978; Silver *et al.* 1973). This inhibition may be due to a competitive inhibition of the conversion of arachidonic acid into thromboxane A_2 (TXA_2) (Lands *et al.* 1973; Culp *et al.* 1979). This mechanism, however, is not the only one. Gryglewski *et al.* (1979) have found that EPA very effectively blocks TXA_2 receptors in the platelet membrane, and we and others have demonstrated that it blocks

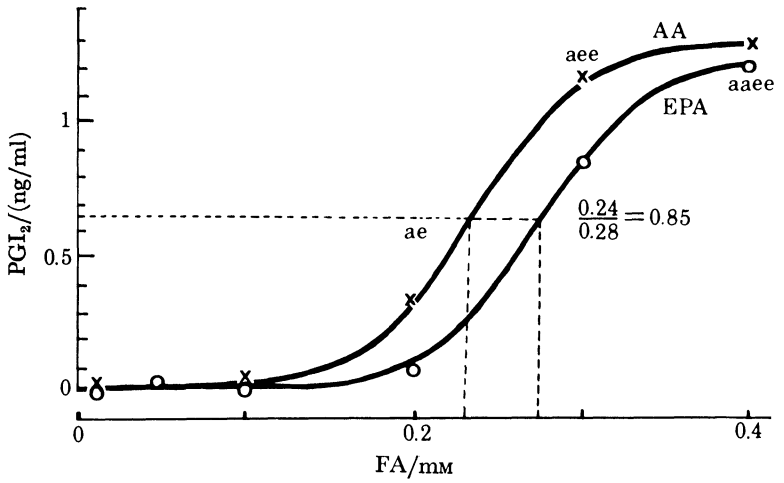

Figure 1. Production of PGI-like material in human umbilical vascular tissue measured by bioassay. The production of PGI-like material in mixed incubates is indicated by the letters a and e; ae means 0.1 mM AA + 0.1 mM EPA, aee means 0.1 mM AA + 0.2 mM EPA in the incubation mixture, and so on. AA and EPA are 20:4, $n-6$ and 20:5, $n-3$, respectively (Dyerberg & Jørgensen 1980).

prostaglandin-independent thrombin receptors too (Jakubowski & Ardlie 1979). The conversion of EPA into prostaglandins of the 3-series seem to be low and thromboxane A_3 (TXA_3) has low proaggregatory properties (Needleman *et al.* 1979; Whitaker *et al.* 1979). It is not known whether EPA is converted by human vascular tissue into PGI_3 or PGD_3, which are both powerful antiaggregatory substances. We have data suggesting such a conversion, and from the same set of experiments there is a clear indication that EPA does not inhibit the conversion of arachidonic acid into PGI_2 (Dyerberg & Jørgenson 1980). These data are given in figure 1. Powdered washed human vascular tissue was incubated with either arachidonic acid (AA) or EPA, or the two in combination. The prostacyclin produced was then measured by bioassay. We could not demonstrate any inhibition by EPA of the production of antiaggregatory material from AA. This situation contrasts with that of the platelets where the conversion of AA is markedly depressed.

The present knowledge of the influence of EPA on haemostatic functions is summarized in table 6.

EPA inhibits TXA_2 formation in platelets, whereas it does not inhibit PGI_2 synthesis in human vascular tissue *in vitro*. EPA blocks prostaglandin-dependent and prostaglandin-independent platelet membrane receptors. If any metabolic conversion of EPA takes place, TXA_3 is a very weak proaggregatory substance whereas PGD_3 and PGI_3 are powerfully

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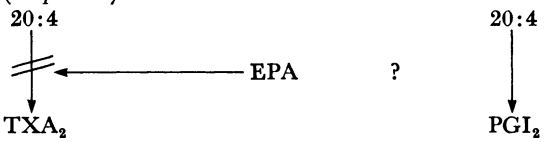
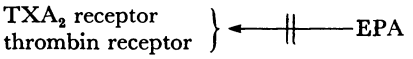
antiaggregatory. Thus, the effect of incorporating EPA into structural lipids would appear as a shift in the haemostatic balance in an antithrombotic direction, which was exactly what we found in Eskimos. We thus felt encouraged to look further into the question of whether a supplement of EPA to the food would influence thrombogenesis.

TABLE 6. EFFECTS OF EPA ON HAEMOSTASIS

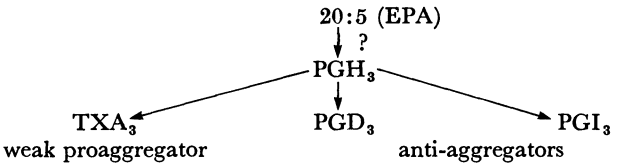
1. *blocks prostaglandin synthesis (competition)*



2. *receptor blocking*



3. *metabolic conversion*



FEEDING EXPERIMENTS INTENDED TO ALTER PLATELET–VESSEL WALL INTERACTION

Di-homo-γ-linolenic acid (20:3, $n-6$) has been suggested as a dietary antiaggregatory substance (Kernoff *et al.* 1977; Willis *et al.* 1974). Reports on its effect have, however, been inconclusive (Oelz *et al.* 1976; O'Brien 1980).

Prostaglandin synthesis from 20:3, $n-6$ results in eicosanoids of the 1-family. TXA_1 is without proaggregatory properties and no PGI compound can be synthesized owing to the lack of a double bond in the 5-position. Another aspect that should, however, be kept in mind is that 20:3, $n-6$ is readily metabolized to AA (20:4, $n-6$) in man.

The Western diet contains very little AA, whereas it is rich in linoleic acid (18:2, $n-6$), which is converted into AA with di-homo-γ-linolenic acid as an intermediate. The almost indetectable amount of 20:3, $n-6$ in human body lipids indicates the facility of this conversion.

Feeding experiments with the $n-3$ family are very scarce and in this context I must again refer to the lifelong experiment that the Greenland Eskimos represent. Another fact to be remembered is that in short-term experiments, thrombotic tendency is often measured very indirectly by haemostatic variables. In an interesting experiment by Black *et al.* (1979) a group of cats were given 8% of their dietary calories as fish oil for 3 weeks, after which cerebral infarction was induced by ligation of the left middle cerebral artery. Both the neurological deficit and the volume of brain infarction in the group of cats given fish oil was significantly less than that of the control group.

Seiss *et al.* (1980) gave 5–800 g of mackerel daily to seven volunteers for a week. A shift in 20:5/20:4 ratio in platelet lipids was paralleled by a decrease in platelet aggregability and TXB_2 formation. Sanders *et al.* (1980) have reported on the effect on bleeding time of giving

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Table 7. Composition of EPA ethyl ester concentrate

(Values are percentage of total.)

18:1	4.2	22:1	5.1
18:2	0.7	22:5	0.2
18:3	0.7	22:6	5.4
20:1	9.3	saturated	0
20:2	2.3	monoenes	18.6
20:4	4.0	polyenes	80.1
20:5	66.6	20:5/20:4	16.7

Table 8. Changes in plasma lipids and lipoproteins during 3 weeks of intake of 10 ml per day of a 60% EPA ethyl ester concentrate, $N = 20$

	before		during		significance
	$\bar{X}$	s.e.m.	$\bar{X}$	s.e.m.	of difference
total lipids/(g/l)	5.64	0.18	5.12	0.17	$p < 0.01$
cholesterol/(mmol/l)	4.87	0.17	4.67	0.16	$p < 0.05$
triglycerides/(mmol/l)	1.02	0.10	0.69	0.06	$p < 0.01$
LDL/(g/l)	3.85	0.17	3.73	0.17	n.s.
VLDL/(g/l)	1.10	0.14	0.78	0.08	$p < 0.01$
HDL/(g/l)	2.95	0.12	2.71	0.11	n.s.

Table 9. Platelet total fatty acid distribution during 3 weeks of daily intake of 10 ml of a 60% EPA ethyl ester concentrate, compared with baseline values, $N = 20$

	before	during	significance of difference
12:0–14:0	0.11	0.08	n.s.
16:0–16:1	19.8	19.5	n.s.
18:0	16.9	15.6	$p < 0.01$
18:1	17.7	16.4	$p < 0.01$
18:2	10.4	8.1	$p < 0.01$
18:3	0.01	0.31	$p < 0.01$
20:0–20:2	1.7	2.4	$p < 0.01$
20:4	21.1	16.3	$p < 0.01$
20:5	0.01	4.5	$p < 0.01$
22:0–24:1	6.5	9.2	$p < 0.01$
22:5	1.4	4.3	$p < 0.01$
22:6	1.5	1.4	n.s.
saturated	42.5	42.0	n.s.
monoenes	20.4	20.9	n.s.
polyenes	34.3	34.4	n.s.
20:5/20:4	0	0.28	$p < 0.01$

Table 10. Changes in haemostatic variables during 3 weeks of daily intake of 10 ml of a 60% EPA ethyl ester concentrate, compared with baseline values, $N = 20$

platelet aggregation:		
ΔO.D.$_{max}$ ADP, rel.	−13.1%	$p < 0.05$
ΔO.D.$_{max}$ collagen, rel.	−11.1%	$p < 0.01$
Δt O.D.$_{50\%}$ collagen, rel.	+13.0%	$p < 0.01$
change in TXB_2, collagen	−15.5 ng/ml	$p < 0.05$
change in bleeding time, Ivy, rel.	+11.1%	$p < 0.05$

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volunteers 5 g of cod-liver oil four times daily for 6 weeks. A significant increase in bleeding time of 1.8 min was observed.

In collaboration with the Wellcome Research Laboratories, we have recently performed a study on 20 volunteers, giving them a daily dose for 3 weeks of 10 ml of an EPA-ethyl ester concentrate made from cod-liver oil. This concentrate was properly tested for toxicity on monkeys and rats given a tenfold dose per kilogram body mass. The composition of the oil is shown in table 7.

The volunteers were examined once a week for 2 weeks before the study, during the study, and for 2 weeks after the cessation of oil supplement. During this period they were asked to refrain from any intake of alcohol or medicine as this may affect platelet function.

Modest but significant decreases in plasma cholesterol, triglyceride and VLDL concentrations were found (table 8).

The oil intake led to an increase in EPA content of plasma lipids and of the fatty acids of the platelet lipids, increasing from almost undetectable amounts to an average of 4.5% (table 9). This increase was seen during the first week of treatment and did not increase further during the feeding period.

Haemostasis was measured by platelet aggregation, bleeding time determinations (by the technique of Ivy) and by TXB_2 formation in platelet-rich plasma during collagen-induced aggregation. The results are given in table 10.

A significant fall in ADP- and collagen-induced aggregation was found, measured both as the $O.D._{max}$ obtained and as the time elapsed for reaching 50% of maximal aggregation response. TXB_2 formation was also decreased. The bleeding time was prolonged by a factor comparable with the alteration in platelet aggregability. This could be interpreted as indicating unaltered vessel wall function. The modest alterations do not, however, warrant far-reaching conclusions.

Does diet influence platelet – vessel wall interaction? The data summarized in this survey and the experimental results presented indicate that it does. As it is generally accepted that the haemostatic process influences thrombogenesis and atherogenesis, this means that dietary advice given to the public aiming at lowering the incidence of ischaemic heart disease must include this aspect. It does not indicate the sort of advice that should be given, but it opens up new possibilities in an area rich in disappointments.

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Discussion

J. McMichael, F.R.S. (2 *North Square*, *London*, *U.K.*). Comparisons between countries can be very insecure. Africans die from their infestations and few reach the coronary age. When medical care is substandard, the accuracy of diagnosis is poor. Of the coronary deaths in this country, 85% are over the age of 65 years, and less than 10% below 60 years. Low wartime recording of coronary deaths in Norway could be ascribed to an absence of doctors.

J. Dyerberg. I would agree. However, Eskimos do live to the coronary age.

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