Prevention of coronary heart disease: the role of essential fatty acids

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Summary

There are 2 classes of essential fatty acids (EFA), the linoleic (n-6) and linolenic (n-3). They are required for the glycerophosphatides (phospholipids) of cellular membranes; the transport and oxidation of cholesterol; the formation of prostaglandins.

In deficiency of EFA, cellular membranes are imperfectly formed which causes increased susceptibility to various insults and increased permeability. Low-density lipoproteins (LDL) transport cholesterol mainly as cholesteryl linoleate and supply EFA to tissue. A relative deficiency of EFA (i.e. a high ratio in the body of non-EFA such as long-chain saturated fatty acids to EFA) causes an increase in plasma cholesterol. EFAs cause decreased aggregation of platelets.

Atherosclerosis is not caused by increased aggregation of platelets, and can be prevalent in a population in which coronary thrombosis is rare.

Introduction

Coronary thrombosis or myocardial infarction is not directly caused by increased aggregation of platelets through ingestion of certain non-EFA: coronary thrombosis is not related in time to the consumption of meals. But free fatty acids (mainly oleic and palmitic) are present in atheroma and, when a plaque ruptures, the free long-chain fatty acids will come in contact with platelets and cause instant thrombosis. In addition, the platelets will have increased tendency to aggregate from the relative deficiency of EFA and decreased production of prostacyclin by endothelial cells at sites of atheroma. The free fatty acids in atheroma are in equilibrium with fatty acids in plasma and so can be changed by diet.

Eskimos have a diet very high in fat but relatively very rich in EFA; they do not get ischaemic heart disease. A study on the effect of the Eskimo diet on the author showed that platelet aggregation was

greatly decreased, very interesting changes in body lipids occurred, and there was probably toxicity of cetoleic acid (C22 : 1n-11).

It is suggested that the British diet should contain less saturated fat and more fatty acids of both classes of EFA, i.e. by eating less fat of ruminants (dairy produce, beef and mutton fat), less fat of animals fed diets low in EFA (pigs, poultry), and less of certain hardened vegetable fats (cooking fat, chocolate, ice-cream and certain margarines). The n-6 EFAs are obtained from unhydrogenated vegetable seed oils (corn oil, sunflower seed oil), from certain soft margarines, and from offal (liver, kidney and brain) and meat particularly of animals fed diets high in EFA. The n-3 EFAs are obtained from fish and crustaceans, and from vegetables; the dietary increase of fatty fish (mackerel, herring) is important, but more research is needed on the possible toxicity of cetoleic acid.

Essential and certain non-essential fatty acids

We are concerned with broad classes of fatty acids: the essential fatty acids (EFAs) and certain non-essential fatty acids (non-EFAs) that are antagonistic to EFA and include long-chain saturated fatty acids and isomers of EFA (such as *trans*-linoleic acid). A third group, the monoenoic fatty acid classes (oleic and palmitoleic), are neutral for the purposes of this discussion but can give rise in the body to polyunsaturated fatty acids that are not EFAs. We should therefore contrast EFAs with certain non-EFAs, and not contrast polyunsaturated fatty acids (which include *trans*-linoleic acid and C20 : 3n-9 from oleic) with saturated (which would not include *trans*-linoleic acid which behaves biologically as saturated).

Two classes of EFA occur naturally, the linoleic (C18 : 2n-6) and the linolenic (C18 : 3n-3); each can be desaturated and elongated. Certain fatty acids relevant to this discussion can be summarized thus:

There are 3 functions for which EFAs are required. First and most important, they are part of the glycerophosphatides of all animal cellular membranes, and most of the signs of deficiency of EFAs arise from this structural requirement. A low ratio of EFAs to non-EFAs in the region where cellular membranes are being formed causes non-EFAs to be incorporated in the membranes in place of EFA, altering the fluidity and the shape of the glycerophosphatides (since EFAs are kinked at each double-bond whereas *trans*-isomers and saturated fatty acids are straight and have higher melting-points). The second function is for the normal transport and oxidation of cholesterol. Cholesterol forms about 44% of low-density lipoproteins (LDL, Sf0-12) and about 22% of high-density lipoproteins (HDL); in each about 80% of the cholesterol is esterified mainly (55%) with linoleic acid. Cholesteryl linoleate is more easily mobilized and transported than is cholesteryl oleate, and the approximate melting-points of the esters are different: saturated, 70 to 85°C; oleate, 50°C; linoleate, 42°C; linolenate, 36°C. All cells require free cholesterol for their membranes and this is probably fitted into the curved EFA of the glycerophosphatides. Although cells can synthesize their own cholesterol, they are supplied with it by LDL which attach to receptors on cells, become engulfed by endocytosis, fuse with lysosomes, and then the protein of the LDL is degraded while an acid hydrolase forms free cholesterol which inhibits the synthesis within the cell and also the regeneration of new receptors on the cell. The details of these important processes have been elucidated by the excellent work of Goldstein and Brown (1977). Free cholesterol in the plasma membrane of the cell is taken up by HDL and with the aid of lecithin-cholesterol acyltransferase is converted into cholesteryl linoleate. HDL pass to the liver where they are degraded and part of the cholesterol is oxidized to form bile acids and excreted.

The third function of EFA is for the formation of prostaglandins (Bergström, Danielsson and Samuelsson, 1964; van Dorp *et al.*, 1964). Dihomo- γ -linolenic acid (C20 : 3n-6) forms the first series, arachidonic acid (C20 : 4n-6) the second, and timnodonic acid (C20 : 5n-3) the third. In any series, a cyclo-oxygenase forms hydroperoxides, e.g. PGG₂ and PGH₂, which can then form either prostaglandins (e.g. PGD₂, PGE₂, PGF_{2 α}) or a thromboxane (e.g. TXA₂) (Hamberg *et al.*, 1974) or a prostacyclin (e.g. PGI₂) (Moncada *et al.*, 1976). Different fatty acids have different effects on the clotting of blood and thrombosis. Connor and Pool (1961) showed that long-chain saturated fatty acids (C16 upwards) were strongly active in producing thrombi, C12 : 0 and C14 : 0 were slightly active, mono-unsaturated fatty acids had little effect and arachidonic acid (C20 : 4) was slightly less active than saline. Kloeze (1969) found that whereas PGE₁ de-aggregated platelets PGE₂ aggregated them and PGE₃ was 10 times less active than PGE₂; PGI₂ is about 30 times as active in de-aggregating as is PGE₁ (Moncada *et al.*, 1976). Some prostaglandins of the 3-series are also strongly de-aggregating (Gryglewski *et al.*, 1979): PGD₃ is more active than PGD₂ because PGE₂ inhibits the effect of PGD₂ whereas PGE₃ does not inhibit that of PGD₃. Needleman, Minkes and Raz (1976) showed that TXA₃ was unlike TXA₂ in not aggregating platelets.

Atherosclerosis

This chronic process is different from that which produces coronary thrombosis. In countries such as Jamaica (Robertson, 1959) where coconut oil is eaten atherosclerosis may be severe because of the presence in this food of C12 : 0 and C14 : 0 which are strongly atherogenic in lower animals but have little effect on thrombosis. In European countries during World War II deaths attributed to ischaemic heart disease fell immediately the war started and the pre-war rise was resumed after hostilities ended

except in Britain where Lease-Lend in 1942 caused a dietary change with marked increase in non-EFA from hydrogenated margarine, fat bacon and Spam; the pre-war rise was resumed in 1943. The suddenness of these changes in national mortality figures, which were paralleled by those for deaths attributed to pulmonary embolism and infarction, could not be caused by a change in so chronic a process as atherosclerosis but could indicate an altered thrombotic tendency in blood. Indeed, in Norway there was a parallelism between deaths attributed to circulatory diseases and thrombo-embolic phenomena in surgical wards in Oslo (Dedichen *et al.*, 1951).

The so-called 'lipid hypothesis' as usually enunciated is: High dietary saturated fat → high plasma cholesterol → atheroma → coronary thrombosis. For a variety of reasons the author has never subscribed to this (Sinclair 1956/57, 1961, 1968). Firstly, as just mentioned, gross atheroma can occur in coconut-eating people without causing coronary thrombosis. Secondly, atheroma and coronary thrombosis can occur without elevated plasma cholesterol. Thirdly, national changes in diet associated immediately with changes in deaths attributed to ischaemic heart disease must be affecting the thrombotic tendency of blood and not the degree of atheroma. Further, the author's own studies of deposition of cholesterol in the early 1950s showed that this could occur with abnormally low plasma cholesterol. When cholesterol accumulates, for instance in the diabetic or in homozygous Type II hyperlipidaemia, it does so in avascular tissues, which are tissues not supplied by capillaries nor drained by lymphatics: intima, epidermis, cornea (both endothelium and epithelium), lens, tendon, cartilage (not bone, which is vascular), the granulosa cells of the ovary and the cells of the seminiferous tubules in the male. The enamel of teeth is also avascular, and the only other tissues in which cholesterol accumulates are liver and adrenal cortex. Studies on the epidermis of the rat (Basnayake and Sinclair, 1956) showed that rats with pure deficiency of EFAs (on a fat-free diet) had abnormally low plasma cholesterol but accumulated cholesteryl oleate in the epidermis which, it was concluded, was probably locally synthesized since the amount in the dermis was not increased; adding non-EFA to the diet, and thereby producing a relative deficiency of EFA, caused abnormally high plasma cholesterol and increased the deposition in the epidermis. It was believed, therefore, that a relative deficiency of EFA in tissues that have a precarious supply being without capillaries and dependent on diffusion of nutrients from a distance would cause accumulation of locally synthesized cholesterol particularly where cells were rapidly dividing and needing EFA for cellular membranes.

In the relative absence of EFA the cholesterol would be esterified with oleate and this ester is less easily removed than is the usual cholesteryl linoleate. Atheroma occurs predominantly where branches come off arteries and at these sites there is increased cell division (Wright, 1968); in fatty streaks the main cholesteryl ester is oleate (Böttcher, 1964); the lipid is within smooth-muscle cells and relatively deep in the intima. But one of the most marked effects of deficiency of EFA is increased permeability (of capillaries, epidermis, plasma membrane of cells, etc.) and so the local deficiency will facilitate LDL entering the intima particularly if the plasma concentration is high and if there is hypertension. In advanced lesions LDL is found, the main ester is cholesteryl linoleate as in LDL, and the lipid is extracellular (Smith, 1974).

Coronary thrombosis

Dietary fats affect the thrombotic tendency of blood, long-chain fatty acids increasing this and EFA of either class decreasing it. But dietary fat does not immediately cause coronary thrombosis since this is not related in time to the eating of a meal (e.g. 4 hr afterwards). The atheromatous plaque contains some free fatty acids which are usually oleic and palmitic acids, and these are in equilibrium with the albumin-bound fatty acids in plasma (Zilvermit *et al.*, 1961). According to Constantinides (1966) coronary thrombosis always results from a ruptured plaque, and when this rupture occurs platelets will come in contact with the FFAs in the plaque and will instantly be aggregated by long-chain saturated ones. Aggregation will be facilitated if the platelets are unusually 'sticky', which is so in coronary thrombosis (McDonald and Edgill, 1957). Thrombosis could be averted by altering the composition of the fatty acids in the plaque, by making platelets less 'sticky' by drugs such as aspirin or sulphinpyrazone, or better by diet discussed in conclusion.

Quality of fat and coronary thrombosis

The author concluded in 1956 (Sinclair, 1956) that atheroma and coronary thrombosis were related to a relative deficiency of EFA and not to the total fat in the diet (which at that time was regarded as the relevant factor by Keys (1953) and by Brock and Bronte-Stewart (1955)). On the one hand, Eskimoes on their traditional diet have the highest dietary fat in the world (Sinclair, 1953) but very rich in EFAs of the linolenic class, whereas the Japanese have very low dietary fat but this is also relatively rich in EFAs from fish (linolenic class) and soy-bean oil (51% linoleic acid and 7% linolenic).

In 1944, the author studied the cornea and lens of Eskimoes, using a slit-lamp microscope with

crossed polaroids, to detect early cholesterol deposition: even in elderly Eskimoes there was none. In 1976, the author joined another expedition to the long-lived community of Eskimoes in Igdlorssuit in north-western Greenland (Dyerberg and Bang, 1979) who are still subsisting mainly on seal and fish although 'Western' food is also now used, having been available for several years (Bang, Dyerberg and Sinclair, 1980).

Since travel by dog-sledge precludes many sophisticated investigations, the author decided to go himself for 100 days on to a diet rigidly limited to seal and other marine animal food (mainly fish) with only water to drink. The diet was started in March 1979. This diet is extremely high in fat and protein with no carbohydrate, high in EFA of the linolenic class (mainly C20:5 and C22:6) and very low in EFA of the linoleic class, high in cetoleic

acid, high in vitamins A and D and in cholesterol, very low in ascorbic acid, with no fibre. Muscle biopsies were done to monitor toxicity of cetoleic acid, to which Eskimoes presumably become adapted as do lower animals so that myocardial fibrosis does not occur. But cetoleic acid appeared in plasma membranes of the author's cells and in adipose tissue, and the glycerophosphatides and triglycerides in these respectively altered in composition, n-6 fatty acids being largely replaced by n-3 except that arachidonic acid was remarkably conserved. Estimations were carried out of prostaglandins in semen and of stable metabolites in plasma, and these with the extensive estimations that are still being done will be published in due course.* An expected finding was that bleeding time, which started at 3 to 4 min,

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FIG. 1. Essential fatty acids, prostaglandins and platelet aggregation.

FIG. 2. Diagram of the suggested mechanisms of atherosclerosis and of coronary thrombosis, EFA = essential fatty acids, HDL = high density lipoproteins, SFA = long-chain saturated fatty acids.

rose in the middle of the experiment to > 50 min. This is probably mainly the result of substituting 3-series prostaglandins (from C20 : 5n-3) for the usual 1- and 2-series (the latter from C20 : 4n-6), as discussed above and summarized in Fig. 1.

Obviously the next stage of this work is to see how small an amount of EFA of the linolenic class, for instance from occasional mackerel or other fatty fish, is sufficient to make platelets 'unsticky'. But the experiment has also indicated that cetoleic acid is toxic and that the linoleic class of EFA are definitely required; the Eskimos have no doubt adapted to the toxicity of the former, and as in the author's experiment appear to conserve their limited amounts

of arachidonic acid. Since C20 : 5n-3 inhibits the desaturation and elongation of linoleic to arachidonic acid and since our usual diets contain little of the latter, too much of the linolenic class may be undesirable. A balance is needed between the EFA of the linoleic and linolenic classes.

A tentative diagram of the mechanisms of atherosclerosis and of coronary thrombosis is given in Fig. 2.

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References

- BANG, H.O., DYERBERG, J. & SINCLAIR, H.M. (1980) An examination of food consumed by Greenland Eskimos. *American Journal of Clinical Nutrition* (in press).
- BASNAYAKE, V. & SINCLAIR, H.M. (1956) The effect of deficiency of essential fatty acids upon the skin. In: *Biochemical Problems of Lipids*, p. 476. Butterworth, London.
- BERGSTRÖM, S., DANIELSSON, H. & SAMUELSSON, B. (1964) The enzymatic formation of prostaglandin E_2 from arachidonic acid. *Biochimica et biophysica acta*, **90**, 207.
- BÖTTCHER, C.J.F. (1964) Chemical constituents of human atherosclerotic lesions. *Proceedings of the Royal Society of Medicine*, **57**, 792.
- BROCK, J.F. & BRONTE-STEWART, B. (1955) Arteriosclerosis in African populations. *Minnesota Medicine*, **38**, 852.
- CONNOR, W.E. & POOLE, J.C.F. (1961) The effect of fatty acids on the formation of thrombi. *Quarterly Journal of Experimental Physiology*, **46**, 1.
- CONSTANTINIDES, P. (1966) Plaque fissures in coronary thrombosis. *Journal of Atherosclerosis Research*, **6**, 1.
- DEDICHEN, J., STRÖM, A., ADELSTEN-JENSEN, R. & CLOSS, K. (1951) Incidence of atherosclerotic disease during war years. *Transactions of the 5th Conference of the Josiah Macy Junior Fund of New York*, 117.
- DYERBERG, J. & BANG, H.O. (1979) Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *Lancet*, **ii**, 433.
- GOLDSTEIN, J.L. & BROWN, M.S. (1977) The low-density lipoprotein pathway and its relation to atherosclerosis. *Annual Review of Biochemistry*, **46**, 897.
- GRYGLEWSKI, R., SALMON, J., UBATUBA, F., WEATHERBY, B., MONCADA, S. & VANE, J. (1979) Effects of all *cis*-5,8,11,14,17 eicosapentaenoic acid and PGH_3 on platelet aggregation. *Prostaglandins*, **18**, 453.
- KEYS, A. (1953) Atherosclerosis: a problem in newer public health. *Journal of the Mount Sinai Hospital*, **20**, 118.
- KLOEZE, J. (1969) Relationship between chemical structure and platelet-aggregation activity of prostaglandins. *Biochimica et biophysica acta*, **187**, 285.
- HAMBERG, M., SVENSSON, J., WAKABAYASHI, T. & SAMUELSSON, B. (1974) Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proceedings of the National Academy of Sciences of the United States of America*, **71**, 345.
- MCDONALD, L. & EDGILL, M. (1957) Coagulability of the blood in ischaemic heart-disease. *Lancet*, **ii**, 457.
- MONCADA, S., GRYGLEWSKI, R., BUNTING, S. & VANE, J.R. (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*. London, **263**, 663.
- NEEDLEMAN, P., MINKES, M. & RAZ, A. (1976) Thromboxanes: selective biosynthesis and distinct biological properties. *Science*, **193**, 163.
- ROBERTSON, W.B. (1959) Atherosclerosis and ischaemic heart disease: observations in Jamaica. *Lancet*, **i**, 444.
- SINCLAIR, H.M. (1953) The diet of Canadian Indians and Eskimos. *Proceedings of the Nutrition Society*, **12**, 69.
- SINCLAIR, H.M. (1956) Deficiency of essential fatty acids and atherosclerosis etcetera. *Lancet*, **i**, 381.
- SINCLAIR, H.M. (1956/57) Essential fatty acids and their relation to other vitamins. *Lectures on the Scientific Basis of Medicine*, **6**, 260.
- SINCLAIR, H.M. (1961) Animal and vegetable fats as human foods. *Proceedings of the Nutrition Society*, **20**, 149.
- SINCLAIR, H.M. (1968) Nutrition and atherosclerosis. *Symposium of the Zoological Society of London*, **21**, 275.
- SMITH, E.B. (1974) The relationship between plasma and tissue lipids in human atherosclerosis. *Advances in Lipid Research*, **12**, 1.
- VAN DORP, D.A., BERTHUIS, R.K., NUGTEREN, D.H. & VONKEMAN, H. (1964) Enzymatic conversion of all-*cis* polyunsaturated fatty acid into prostaglandins. *Nature*. London, **203**, 839.
- WRIGHT, H. & PAYLING (1968) Endothelial mitosis around aortic branches in normal guinea-pigs. *Nature*. London, **220**, 78.
- ZILVERSMIT, D.B., MCCANDLESS, E.L., JORDAN, P.H., HENLY, W.S. & ACKERMAN, R.F. (1961) The synthesis of phospholipids in human atheromatous lesions. *Circulation*, **23**, 370.