SESSION I

BIOCHEMISTRY AND PATHOLOGY OF THE ARTERIAL WALL

Chairman: DR K. P. BALL

Essential fatty acids and the vulnerability of the artery during growth

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Summary

Essential fatty acids not only control blood lipid levels, but are the precursors of prostaglandins responsible for regulation of platelet aggregation. Dietary deficiency of essential fatty acids may play an important role in the development of coronary heart disease, particularly during the early growth period.

Introduction

The discussion on the role of diet in the aetiology of coronary heart disease has tended to focus on the negative: that is, on the allegedly harmful aspects of the diet such as saturated fats, cholesterol, sucrose or purified carbohydrates. The underlying atherosclerosis can be considered as a degenerative disease, strongly linked with environmental factors, which ends in coronary occlusion in adulthood. Yet little attention has been given to the positive nutrients on which the healthy growth of the artery must depend.

Sinclair, as early as 1968, suggested that an essential fatty acid deficiency was responsible for atherosclerosis, yet few of the more recent expert committee reports on the prevention of coronary heart disease (CHD) mention the function of the essential fatty acids (EFA) in the context of arterial growth and health.

In practice, the growth of every cell membrane in the body needs EFA which cannot be synthesized

in the body and must be supplied in the diet in the same way as essential amino acids.

In addition to their structural importance, EFA have a wide range of physiological functions. They control blood lipid levels and are the precursors of the prostaglandins responsible for the regulation of platelet aggregation.

In the experimental approach to atherogenesis, the dietary fats have been supplied as saturated fats or cholesterol, but these diets have also been low in EFA. This approach to atherogenesis has neglected the simultaneous manipulation of dietary essential fats which are needed for proper growth and tissue repair.

First to be discussed will be essential fatty acids which could be important factors in the growth of the artery and in thrombus formation. It will be suggested that, as with other nutritional problems, the period when the artery is most sensitive to dietary distortion is during its period of growth.

Function of essential fatty acids and their metabolism

The EFAs are important for growth and development because in combination with protein they are used in cell membranes and cell structures in every tissue in the body. The EFAs are the essential components of structural lipid, the invisible fats described by the early histologists. After protein, structural

FIG. 1. The metabolism of essential fatty acids to the longer-chain polyunsaturated acids and prostaglandin derivatives

lipid is the most important component of all soft tissues, except in the nervous system where there is more lipid than protein (Crawford and Sinclair, 1972).

Structural lipids contain phospholipid and cholesterol. Triglycerides are mainly employed as energy stores. The fatty acid composition of the phospholipid varies in different tissues (Carney and Walker, 1971; Sun, Go and Sun, 1974; Crawford *et al.*, 1976b). The fatty acid composition of triglyceride esters and of phospholipids can be modified by dietary manipulation. However, the fact that the phospholipids change more slowly suggests some homeostatic control.

There are two positions for fatty acids in the phospholipid molecules. The first is usually occupied by a saturated and the second by a polyunsaturated fatty acid (PUFA). If an excess of saturated fatty acids is contained in the diet they can compete with the PUFA and force their way into the second position, and so alter the physical properties of the phospholipid and hence of the membrane itself (Van Golde, Pieterse and van Deenan, 1968). An increase in dietary saturated fat increases the basic requirement for essential polyunsaturated fat. (Dhopeswarkar and Mead, 1961).

Cholesterol has only one position available for esterification and up to 70% of the fatty acids used for making its esters may be polyunsaturated; in man, linoleic acid is the major fatty acid found. During the early development of atheromatous

lesions cholesterol esters have a low linoleic acid content (Bottcher, 1964; Smith, 1965).

The triglycerides have three positions available for esterification. When triglycerides contain a PUFA it is usually in the middle position while the other two contain a saturated fatty acid. The fatty acid composition of triglyceride varies with the diet but seldom has a ratio of more than one PUFA to two saturated or non-essential fatty acids. Triglycerides have a greater affinity for non-essential, and phospholipids for essential fatty acids (Sinclair, 1975); EFAs are taken up preferentially by the structural lipids.

In addition to their role in cell structures, EFAs also give rise to prostaglandins, substances with a wide range of physiological action at low concentrations (van Dorp, 1975). That is, EFA have both a structural and a physiological function.

The structural function of the EFA is largely dependent on their chain lengths, degree of unsaturation and position of the double bonds. The higher the degree of unsaturation, the lower will be the melting point of the fat. By contrast, saturated fatty acids give rise to hard or rigid fats with high melting points (Chapman, 1972). Consequently membrane or tissue fats can be either rigid, as in the myelin sheath (Baumann *et al.*, 1972); or elastic, as in the arterial wall, and these are associated with either a low or high content of PUFA.

Linoleic and α-linolenic acids are the two parent EFA and are metabolized by desaturation and chain

elongation to their long-chain derivatives which include arachidonic and docosahexaenoic acids (Fig. 1). Linoleic and α -linolenic acids have a chain length of eighteen carbons with two or three double bonds. Their long-chain derivatives have carbon chain lengths of 20–22 carbons with four, five and six double bonds. These long-chain derivatives are important because they are the principal fatty acid constituents of membrane systems such as those of the nervous system, the vascular endothelium and platelets, and also because they are the direct precursors of prostaglandins (van Dorp *et al.*, 1964).

The significance of rate limitations

Both *in vivo* and *in vitro* studies have shown that desaturation reactions impose limitations on the rate of conversion of the parent EFA to their higher homologues (Hassam, Sinclair and Crawford, 1975; Marcel, Christiansen and Holman, 1968). The rate limitations are so profound that in the rat *in vivo*, specific activity studies have shown that only one-thirtieth of radioactively-labelled linoleate appears as arachidonate (Table 1) in 24 hr (Hassam and Crawford, 1976).

TABLE 1. Recovery of orally administered isotope in the arachidonic acid fraction of developing rat brain phosphoglyceride (Hassam and Crawford, 1976)

Isotope administered	Radioactivity recovered from brain phosphoglyceride arachidonate fraction (as percentage administered dose)
[1- ¹⁴ C]-18 : 2 ω 6	0.06 $\pm$ 0.004
[1- ¹⁴ C]-18 : 3 ω 6	0.16 $\pm$ 0.004
[1- ¹⁴ C]-20 : 3 ω 6	0.40 $\pm$ 0.03
[1- ¹⁴ C]-20 : 4 ω 6	2.00 $\pm$ 0.03

It is likely that these rate limitations control the synthesis of arachidonic and docosahexaenoic acids for cell structures and for prostaglandin synthesis. There is evidence that the desaturation is regulated by hormones such as insulin and thyroxine (Brenner, 1974).

These findings are not only of relevance to dietary considerations in relation to thrombosis but also provide added strength to the view that the rate limitations in the synthesis of the long-chain polyunsaturated fatty acids may provide a regulatory mechanism in man for platelet aggregation.

Vascular endothelium and prostaglandin production

In addition to the role of prostaglandins in platelet aggregation, Moncada *et al.* (1976) reported that the vascular endothelium synthesises a different prostaglandin (PGI₂) which prevents platelets from sticking to the walls of the artery and releases those

which have become adherent. If the arterial endothelium is damaged, local synthesis of PGs cannot occur and a mural thrombus is likely.

Hence, the specific prostaglandin functions in relation to the vascular system are relevant to homeostasis and repair and probably to the events in coronary thrombosis.

TABLE 2. Long-chain polyunsaturated fatty acid content of the ethanolamine phosphoglyceride fraction in the human aorta endothelium and platelets

Fatty acid	Ethanolamine phosphoglyceride fraction	
	Aorta endothelium (g/100 g fatty acid)	Platelet methyl esters
20 : 3 ω 6	0.9	1.0
20 : 4 ω 6	26.0	33.3
22 : 4 + 22 : 5 ω 6	9.9	3.6
20 : 5 ω 3	2.6	1.8
22 : 5 ω 3	5.7	4.2
22 : 6 ω 3	11.0	5.6

The long-chain PUFA in arterial endothelium and platelet membranes

The arterial endothelium contains high concentrations of long-chain PUFA in the phospholipids (Table 2). Comparative studies have shown that the arterial phospholipids have higher concentrations of arachidonic acid and lower concentrations of linoleic acid than those in muscle or liver (Crawford and Woodford, 1971). Similarly, the amounts of arachidonic acid in platelets are impressive. Damage to the membrane causes release of large amounts of arachidonic acid, which results in platelet aggregation. This mechanism is physiologically important both in haemorrhage and in thrombosis.

The sensitivity of the vascular system during early development

Experimental atherogenic diets in which the fat is provided as cholesterol or saturated fat are deficient in EFA. Excess saturated fat competes with EFA thus exacerbating the effect of EFA deficiency. These points were made by Sinclair (1968). During early development of the child, substitution of cow's milk for human milk results in a diet low in EFA, which is associated with changes in blood chemistry. Cow's milk has more protein and minerals to meet the need for body and skeletal growth in the calf; human milk has more EFA (Crawford *et al.*, 1976b).

Babies fed on cow's milk do not maintain their EFA balance in the same way as do breast-fed babies (Pikaar and Fernandes, 1966; Sanders and Naismith, 1976; Crawford, Hassam and Hall, 1977).

TABLE 3. Triglyceride levels of $\omega 6$ and $\omega 3$ long-chain polyunsaturated fatty acids (LCP) in breast- and bottle-fed infants (Crawford, Hassam and Hall, 1977)

	Milk	Week 1	g/100 fatty acid methyl esters		Adult
			Week 6	Week 18	
$\omega 6$ LCP	Human ($n=10$)	4.5 ± 0.31	2.2 ± 0.11	1.7 ± 0.08	0.52 ± 0.04
	Cow ($n=12$)	4.3 ± 0.24	1.0 ± 0.08	0.20 ± 0.01	
$\omega 3$ LCP	Human ($n=10$)	3.8 ± 0.17	2.5 ± 0.16	1.0 ± 0.09	0.44 ± 0.02
	Cow ($n=12$)	3.4 ± 0.15	0.91 ± 0.11	0.13 ± 0.06	

The human fetus accumulates high concentrations of arachidonic and docosahexaenoic acids which, after birth, soon fall to adult levels. However, in cow's-milk-fed infants the levels of these fatty acids fell well below those of breast-fed infants or those expected in adults (Table 3). Although there was no clinical evidence of EFA deficiency in these infants, in view of the concern that arterial changes may occur in infancy, the lipid status of the artificially-fed infant should be examined by more detailed research.

Blood cholesterol and triglycerides

In communities with a low incidence of CHD, low levels of blood lipids are found (Shaper and Jones, 1962). Louw, du Plessis and Van den Berg, 1969, studied blood cholesterol levels in Bantu children and found them to be lower than those in South African children of European descent. The present authors found no difference in blood cholesterol levels in East African infants compared with European infants, but they could be clearly distinguished by the age of 7-8 years (Table 4).

TABLE 4. Blood cholesterol levels of European and East African males at different age groups

Age group (years)	European (mg/100 ml blood)	East African (n)
0-1	116 ± 14	120 ± 10 ($n=45$)
6-8	165 ± 11	109 ± 7 ($n=37$)
12-16	196 ± 16	128 ± 13 ($n=26$)
25-45	210 ± 18	141 ± 11 ($n=43$)

These findings suggest that the risk factors associated with heart disease are not confined to adults but are already apparent in young children. The absence of a difference in infants suggests that variation in susceptibility in racial groups is an environmental, rather than a genetic, phenomenon.

In experimental studies, the most pronounced effects of nutritional changes are seen during early growth. McCay (1952) showed that underfeeding growing rats increased their longevity. In their own experiments on dietary cardiomyopathy in guinea-pigs (McKinney and Crawford, 1965) the authors

found that it was only possible to produce an effect if weanling animals were used. Even in experimental atherosclerosis it is customary to use young growing animals because this is the period in which results are best obtained. If this evidence can be translated to the human, it is most likely that the human artery is most sensitive to dietary influence during the most rapid period of body growth.

During early growth the appropriate fatty acids are important for arterial membranes. It is likely that if the vascular system is predisposed to atherosclerosis during its growth, then subsequently dietary fatty acids could be important either in repair or in exacerbation of disease. They might also be important as precursors of prostaglandins, which could be relevant to thrombus formation.

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