The effect of dihomo-γ-linolenic acid (20:3,*n*-6) on the composition of phospholipid fatty acids in the liver of rats deficient in essential fatty acids

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1. Rats were fed on either a diet deficient in essential fatty acid (EFA) or one supplemented with dihomo-γ-linolenic acid (20:3,*n*-6) at levels that represented 0·25, 0·5, 1·0 and 2·0% of the dietary energy.

2. Supplementation of the diet of EFA-deficient animals with 20:3,*n*-6 reversed most of the fatty acid changes induced in the liver phospholipid fraction.

3. The EFA potency of 20:3,*n*-6 was found to be similar to that of γ-linolenic acid (18:3,*n*-6) which has been shown to be higher than that of linoleic acid (18:2,*n*-6).

Experimentally-induced deficiency in essential fatty acids (EFA) in laboaratory rats has been reversed by supplementing the EFA-deficient diet with either linoleic (18:2,*n*-6), γ-linolenic (18:3,*n*-6) or arachidonic (20:4,*n*-6) acids (Mohrhauer & Holman, 1963; Hassam, Rivers & Crawford, 1977). Arachidonic acid supplementation was found to be more effective than linoleic acid supplementation in improving weight gain and dermal score, and in reducing the ratio of eicosatrienoic (20:3,*n*-9) to arachidonic (20:4,*n*-6) acids, i.e. the ratio, triene:tetraene of the EFA-deficient animals (Mohrhauer & Holman, 1963). However, in terms of efficiency of supplementation, γ-linolenic acid (18:3,*n*-6) was intermediate between linoleic and arachidonic acids (Hassam *et al.* 1977). The lower EFA potency of linoleic acid is due to the rate-limitation in the conversion of linoleic to arachidonic acid at the Δ6 desaturation. The metabolic pathway is as follows:

$$\underset{\text{linoleic acid}}{18:2,n\text{-}6} \xrightarrow[\text{desaturation}]{\Delta 6} \underset{\gamma\text{-linolenic acid}}{18:3,n\text{-}6} \xrightarrow[\text{elongation}]{\text{chain}} \underset{\text{dihomo-}\gamma\text{-linolenic acid}}{20:3,n\text{-}6} \xrightarrow[\text{desaturation}]{\Delta 5} \underset{\text{arachidonic acid}}{20:4,n\text{-}6}$$

Dihomo-γ-linolenic acid (20:3,*n*-6) is the chain elongation product of γ-linolenic acid. It is desaturated by the Δ5 desaturase to arachidonic acid, and is also the precursor of the prostaglandins of the I series which have antiaggregatory properties in the platelets (Willis, Comai, Kuhn & Paulsrud, 1974). In this paper we describe the potency of dihomo-γ-linolenic acid in reversing the biochemical signs of EFA deficiency in the laboratory rat.

MATERIALS AND METHODS

Weanling female albino rats (Wistar strain) were maintained on a low-fat diet (Hassam *et al.* 1977) for 3 months. At the end of that period, three randomly-selected rats showed liver lipid patterns characteristic of EFA deficiency, i.e. lower levels of *n*-6 fatty acids, higher levels of oleic (18:1,*n*-9) and eicosatrienoic (20:3,*n*-9) acids and a high ratio triene: tetraene. The animals were then housed in groups of four. The low-fat diet was supplemented with dihomo-γ-linolenic acid (as all *cis* methyl-dihomo-γ-linolenate, purity > 99%; Bio-Oil Research Ltd, Nantwich, Cheshire) to supply 0, 0·25, 0·5, 1 and 2% of the

Table 1. *Fatty acid composition of liver phospholipid fraction of rats receiving different amounts of dihomo-γ-linolenic acid* (20:3,*n*-6) *as a supplement to an EFA-deficient diet*

(Results expressed as weight per cent and given as mean with standard error of 4 rats)

	20:3,*n*-6 supplement to the EFA-deficient diet (% total energy)					
Fatty acids	0	0·25	0·5	1·0	2·0	Controls on stock diet*
16:0	10·6±0·50	10·7±0·87	10·2±0·23	11·8±1·48	12·0†	15·5±0·1
18:0	22·3±0·30	25·4±0·38	26·7±0·50	24·5±3·6	30·1	27·0±0·05
16:1,*n*-7	8·1±0·66	4·0±0·54	3·6±0·23	2·2±0·6	1·6	1·3±0·05
18:1,*n*-9	24·0±1·19	17·5±0·5	15·5±0·58	9·2±0·8	7·2	2·9±0·15
18:2,*n*-9	0·95±0·32	1·1±0·32	0·83±0·07	0·10±0·06	0·06	ND
20:3,*n*-9	19·4±0·95	14·5±0·32	10·7±0·35	2·7±0·03	0·40	ND
18:2,*n*-6	ND	ND	ND	ND	ND	8·9±0·15
20:3,*n*-6	ND	3·2±0·23	4·2±0·23	4·5±0·41	3·1	1·3±0·15
20:4,*n*-6	3·9±0·23	13·3±0·37	18·2±0·52	26·7±0·75	29·6	20·4±0·10
22:4,*n*-6	0·43±0·13	0·38±0·03	0·43±0·03	1·9±0·7	1·7	0·4±0·01
22:5,*n*-6	1·4±0·15	1·7±0·37	3·0±0·13	5·9±0·56	10·5	1·3±0·05
20:5,*n*-3	0·30±0·06	0·18±0·05	0·10±0	0·04±0·03	ND	1·9±0·05
22:5,*n*-3	0·65±0·05	0·85±0·38	0·30±0·15	0·87±0·48	0·1	1·8±0·01
22:6,*n*-3	4·1±0·17	4·1±0·24	3·9±0·06	3·5±0·09	2·6	15·7±0·15
Triene:tetraene						
20:3,*n*-9/	4·98	1·09	0·59	0·10	0·01	—
20:4,*n*-6	±0·34	±0·05	±0·04	±0·01	—	

ND, not detected.
* Animals given the laboratory rat diet.
† Mean of 2 animals.

dietary energy. Stearic acid (purity > 99%; BDH Ltd, Poole) was added to provide a total lipid content of 6% dietary energy. The control group was maintained on the standard laboratory rat diet (Diet 86, Scientific Products Farm, Ash, Canterbury). After 7 d on the diets the animals were killed and their livers were removed, washed in ice-cold saline (9 g sodium chloride/l) and weighed. They were homogenized in chloroform–methanol (2:1, v/v, containing antioxidant) and lipids were extracted according to the procedure of Folch, Lees & Sloane-Stanley (1957). The liver lipids were fractionated by thin-layer chromatography and the phosphoglyceride fraction scraped off the plate and methylated with 5% concentrated sulphuric acid in methanol. The methyl esters were analysed by means of a gas-liquid chromatograph (Pye Research, Series 104). Glass columns packed with 10% ethylene glycol succinate silicone (EGSS-X) and 10% polyethylene glycol adipate (PEG-A) were used. The fatty acid esters were identified by use of pure known standards. The peak areas were quantitated by an automatic digital integrator. The details of these methods have already been published (Hassam *et al.* 1977). The results are expressed as mean weight per cent ± SEM.

RESULTS AND DISCUSSION

The fatty acid compositions of the liver phospholipid fraction from the experimental and control animals are presented in Table 1.

The EFA-deficient group not supplemented with 20:3,*n*-6 showed the characteristic fatty acid pattern: there were increased levels of palmitoleic (16:1,*n*-7) and oleic (18:1,*n*-9) acids, detectable amounts of 18:2,*n*-9, an increased content of 20:3,*n*-9 and decreased levels of linoleic acid (18:2,*n*-6) and its metabolites, notably arachidonic acid (20:4,*n*-6). The

α-linolenic acid (18:3,*n*-3) and its metabolites were also reduced. The ratio triene:tetraene ([20:3,*n*-9]/[20:4,*n*-6]) (a biochemical index to measure the EFA status) in the deficient group was 4·9, whereas in rats on normal diets the ratio is usually < 0·02.

Supplementation with dihomo-γ-linolenic acid (20:3,*n*-6) to the EFA-deficient diet reversed most of the changes in the liver fatty acids induced by EFA deficiency. There were reduced levels of 16:1,*n*-7, 18:1,*n*-9, 18:2,*n*-9 and 20:3,*n*-9 which were lowered further with increasing levels of supplementation. The supplementation led to a rise in the metabolites of 20:3,*n*-6, especially arachidonic acid (20:4,*n*-6). The ratio triene:tetraene also declined with increasing levels of dihomo-γ-linolenic acid (20:3,*n*-6).

There were no changes in the contents of α-linolenic acid (18:3,*n*-3) or its metabolites in the supplemented group as compared with the deficient group. As the supplemented groups had no 18:3,*n*-3 or its metabolites in the diet, the levels of these fatty acids remained low. To compensate for their low levels, there was a striking increase in the metabolites of 20:3, *n*-6, especially of 22:5,*n*-6. In the controls, no such increase was seen.

Supplementation of the EFA-deficient diet with dihomo-γ-linolenic acid reversed the biochemical signs of EFA deficiency. From the results of earlier studies (Mohrhauer & Holman, 1963; Hassam *et al.* 1977) it would seem that dihomo-γ-linolenic acid has an ability, similar to that of γ-linolenic acid, to reverse the biochemical signs of EFA deficiency in the rat; the potency of γ-linolenic acid in this respect is intermediate between that of linoleic acid (18:2,*n*-6) and arachidonic acid (20:4,*n*-6). The potency of 20:3,*n*-6 to reduce the ratio triene:tetraene is greater than that of its precursor 18:2,*n*-6, but less than that of arachidonic acid (20:4,*n*-6). Thus, both dietary γ-linolenic acid (18:3,*n*-6) and dihomo-γ-linolenic acid 20:3,*n*-6) appear to have similar essential fatty acid activity.

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