# The influence of α-linolenic acid (18: 3ω3) on the metabolism of γ-linolenic acid (18: 3ω6) in the rat

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1. Essential fatty acid-deficient rats were fed γ-linolenic acid (18:3ω6) at 2% dietary energy and α-linolenic acid (18:3ω3) at 0, 1·6, 2·8 and 4·0% of the dietary energy.
2. 18:3ω3 at 1·6% apparently inhibits the synthesis of the C20 and C22 ω6 long-chain polyunsaturated fatty acids (ω6 LC-PUFA) metabolized from 18:3ω6.
3. However, increasing the dietary levels of 18:3ω3 from 1·6 to 4·0% has no further influence.
4. The results suggest that dietary 18:3ω6 is an efficient precursor for the ω6 LC-PUFA synthesis even in the presence of 18:3ω3.

Cell structural lipids of vertebrates contain the longer chain polyunsaturated fatty acids (LC-PUFA) of 20 and 22 carbon chain lengths with 4, 5 and 6 double bonds. Of these arachidonic acid (20:4ω6) and docosahexaenoic acid (22:6ω3) are the major LC-PUFA found in most tissue lipids, especially in the brain (Crawford, Casperd & Sinclair, 1976). They are derived from linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3) respectively, which vertebrates are unable to synthesize de novo, and must therefore be provided in the diet. The LC-PUFA are synthesized from these dietary 18 C essential fatty acids (EFA) by alternate desaturation and chain-elongation. The enzymes responsible for these metabolic processes are found mainly in the liver.

It has been concluded that the same enzymes are responsible for the synthesis of the LC-PUFA from both 18:2ω6 and 18:3ω3, but 18:3ω3 apparently inhibits the synthesis of the LC-PUFA derived from 18:2ω6 (Mohrhauer & Holman, 1963). When both 18:2ω6 and 18:3ω3 are present at the same concentration in the diets of laboratory rats there is a 40% reduction in the incorporation into liver phosphoglycerides of the LC-PUFA derived from 18:2ω6, and increasing the dietary levels of 18:3ω3 depresses it even further (Mohrhauer & Holman, 1963).

Our earlier dietary and radioisotope experiments have shown that γ-linolenic acid (18:3ω6), the desaturation product of 18:2ω6, is an efficient precursor for the synthesis of the LC-PUFA (Hassam, Sinclair & Crawford, 1975; Hassam & Crawford, 1976) and that it has a higher potency than 18:2ω6 in curing EFA deficiency (Hassam, Rivers & Crawford, 1977). In this paper a study is made of the influence of dietary 18:3ω3 on the efficiency of the metabolism of dietary 18:3ω6 to its longer chain derivatives (ω6 LC-PUFA).

## MATERIALS AND METHODS

Female albino rats (Wistar strain, Tuck & Sons, UK) were weaned on to a fat-free diet (Hassam, 1976) and after 160 d the animals showed the characteristic biochemical signs of EFA deficiency.

Table 1. *Liver and liver lipid weights of rats given γ-linolenic acid* (18:3ω6) *and varying amounts of α-linolenic acid* (18:3ω3)

(Values are the mean ± 1 SEM of four rats)

| Dietary energy (%) | | Liver wt. (g) | Liver lipids (mg/g liver) | Total phosphoglyceride fatty acids (mg) |
|---|---|---|---|---|
| 18:3ω6 | 18:3ω3 | | | |
| 2·0 | 0 | 8·6 ± 0·9 | 46 ± 2 | 124 ± 7 |
| 2·0 | 1·6 | 7·4 ± 0·9 | 46 ± 2 | 140 ± 19 |
| 2·0 | 2·8 | 7·1 ± 0·3 | 48 ± 6 | 116 ± 8 |
| 2·0 | 4·0 | 7·3 ± 0·9 | 49 ± 2 | 119 ± 13 |

Table 2. *The long-chain polyunsaturated fatty acid* (*LC-PUFA*) *contents of liver phosphoglyceride fraction of rats given γ-linolenic acid* (18:3ω6) *and varying amounts of α-linolenic acid* (18:3ω3)

(Values are mean ± 1 SEM of 4 rats)

| Dietary energy (%) | | Total liver phosphoglyceride fatty acids (mg/g) | |
|---|---|---|---|
| | | ω6 LC-PUFA | ω3 LC-PUFA |
| 18:3ω6 | 18:3ω3 | (20:3+20:4+22:4+22:5) | (20:5+22:5+22:6) |
| 2·0 | 0 | 0·406 ± 0·010 | 0·024 ± 0·002 |
| 2·0 | 1·6 | 0·331 ± 0·007 | 0·102 ± 0·003 |
| 2·0 | 2·8 | 0·324 ± 0·004 | 0·106 ± 0·003 |
| 2·0 | 4·0 | 0·314 ± 0·005 | 0·116 ± 0·005 |

They were then divided into groups of four animals and housed in groups. The fat-free diet was supplemented with 18:3ω6 (as *cis*-methyl-γ-linolenate, purity > 99 %, Bio-Oils Research Ltd, Nantwich, Cheshire) at 2 % of the dietary energy and varying amounts of 18:3ω3 (as *cis*-methyl-α-linolenate, purity > 99 %, Sigma Chemical Co, London) at 0, 1·6, 2·8 and 4·0 % of the dietary energy. Stearic acid (purity > 99 %, B.D.H. Ltd.) was included to give a total fat content of 6 % of the metabolizable energy total value of the diets.

The supplemented diets were given for a period of 7 d; the animals were killed, livers removed, washed in ice-cold saline (9 g NaCl/l) and lipids extracted. The methods for lipid extraction, isolation of the lipid fractions and the quantitation of the fatty acid methyl esters was carried out by the procedures described previously (Sinclair & Crawford, 1973).

## RESULTS AND DISCUSSION

There were no significant differences either in the liver weights or in the amount of liver lipids with increasing levels of 18:3ω3 in the diet (Table 1).

Since up to 80 % of the total fatty acids in the liver triglycerides were as 16 C and 18 C saturates and monounsaturates, the influence of dietary 18:3ω6 and 18:3ω3 supplementation on the fatty acid composition was reflected in the phosphoglycerides. The results presented here are, therefore, of this fraction.

The presence of 18:3ω3 in the diet led to changes in the proportions of the LC-PUFA derived from both 18:3ω6 and 18:3ω3 (Table 2). With 18:3ω3 in the diet at 1·6 % of the dietary energy, there was a 20 % reduction in the ω6 LC-PUFA content of the liver phosphoglyceride fraction. Increasing the dietary levels of 18:3ω3 from 1·6 % to up to 4 % of the energy only led to a further 4 % reduction in the ω6 LC-PUFA contents.

Table 3. *Fatty acid composition (expressed as wt. %) of liver phosphoglyceride fraction of rats given γ-linolenic acid* (18:3ω6) *(at 2 % dietary energy) and varying amounts of α-linolenic acid* (18:3ω3)

(Values are mean ± 1 SEM of four rats)

| Fatty acid | % dietary energy as 18:3ω3 | | | |
|---|---|---|---|---|
| | 0 | 1·6 | 2·8 | 4·0 |
| 16:0 } 18:0 } | 45·1 ± 2·1 | 46·2 ± 1·1 | 45·2 ± 1·6 | 43·2 ± 1·3 |
| 16:1ω7 } 18:1ω9 } | 9·7 ± 0·72 | 7·9 ± 0·50 | 8·9 ± 0·78 | 9·1 ± 0·59 |
| 20:3ω9 | 0·5 ± 0·15 | 0·3 ± 0·10 | 0·3 ± 0·04 | 0·3 ± 0·07 |
| 18:3ω6 | 0·9 ± 0·17 | 1·0 ± 0·17 | 0·9 ± 0·21 | 1·2 ± 0·20 |
| 20:3ω6 | 3·4 ± 0·61 | 4·0 ± 0·58 | 4·6 ± 0·53 | 4·8 ± 0·41 |
| 20:4ω6 | 30·9 ± 0·86 | 27·4 ± 1·1 | 26·6 ± 0·90 | 25·7 ± 0·82 |
| 22:4ω6 | 1·3 ± 0·15 | 0·7 ± 0·03 | 0·6 ± 0·03 | 0·4 ± 0·06 |
| 22:5ω6 | 5·1 ± 1·8 | 1·0 ± 0·06 | 0·6 ± 0·09 | 0·5 ± 0·04 |
| 18:3ω3 | Tr* | 0·3 ± 0·09 | 0·7 ± 0·16 | 1·5 ± 0·27 |
| 20:5ω3 | 0·5 ± 0·15 | 0·8 ± 0·18 | 1·6 ± 0·26 | 3·0 ± 0·34 |
| 22:5ω3 | Tr | 2·0 ± 0·58 | 2·1 ± 0·19 | 2·4 ± 0·21 |
| 22:6ω3 | 1·9 ± 0·06 | 7·2 ± 0·32 | 7·0 ± 0·41 | 6·4 ± 0·70 |

* Tr denotes < 0·05.

When 18:3ω3 was included in the diet, there was a fourfold increase in the ω3 LC-PUFA content and with increasing dietary levels there was increase in the ω3 LC-PUFA contents, although this was not statistically significant.

The specific ω6 LC-PUFA that fell with increasing dietary 18:3ω3 were 20:4ω6, 22:4ω6 and 22:5ω6 (Table 3). However, there was an increase in the 20:3ω6 with increasing levels of dietary 18:3ω3 and this could be due to an inhibition in the Δ5 desaturation of 20:3ω6 to 20:4ω6 by the ω3 fatty acids. Although 22:6ω3 increased when 18:3ω3 was included in the diet, there was no further increase in its level with increasing dietary 18:3ω3, the remaining increase in the ω3 LC-PUFA being due to rises in 20:5ω3 and 22:5ω3.

The results show that dietary 18:3ω3 has an influence on the metabolism of 18:3ω6 to the ω6 LC-PUFA. When both 18:3ω6 and 18:3ω3 are present in the diet at approximately equal amounts, there is a 20–25 % reduction in the ω6 LC-PUFA content of the liver phosphoglycerides but no further significant reduction when 18:3ω3 is increased in the diet to twice the amount. On the other hand, it has been shown that when 18:3ω3 is present in the diet at the same amount as 18:2ω6, the extent of incorporation into liver phosphoglycerides of ω6 LC-PUFA from 18:2ω6 is reduced by 40 % and increasing the dietary levels to twice the amount of 18:2ω6 leads to a reduction of 60 % (Mohrhauer & Holman, 1963). It would thus appear that dietary 18:3ω6 and 18:3ω3 do not compete to the same extent for the metabolic processes in the liver that convert them to the longer-chain polyunsaturated derivatives.

Therefore, in diets which provide both the ω6 and ω3 18 C parent acids, as most diets do, dietary γ-linolenic acid (18:3ω6) would be a more efficient precursor than linoleic acid (18:2ω6) for the LC-PUFA that are essential for cell structures and functions.

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