THE INCORPORATION OF LINOLENIC ACID AND DOCOSAHEXAENOIC ACID INTO LIVER AND BRAIN LIPIDS OF DEVELOPING RATS

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1. Introduction

Linolenic acid (18:3ω3) is a fatty acid produced by green plants [1] however it cannot be synthesised by animals [2]. In animals dietary linolenic acid is converted to longer chain, more unsaturated fatty acids in the liver [2] (fig. 1). The linolenate metabolites are consistently found in the structural lipids (phospholipids) of animal cells [3, 4].

The diet of carnivores therefore contains both the parent and derived acids whereas herbivorous diets contain only the parent acid [3]. The results of analyses of tissue fatty acids from large herbivores and carnivores reveal a greater concentration of docosahexaenoate (22:6ω3) in the tissues of the latter, which we have suggested is a reflection of the presence in the carnivore's diet of long chain polyenoic fatty acids [3, 5].

It has been assumed that the parent 18 carbon fatty acids are the major source of longer chain fatty acids in tissue phospholipids [2, 6] and until recently little attention has been paid to the 20 and 22 carbon fatty acids in the diet [3, 5, 7]. This report compares the incorporation of 18:3–^{14}C with 22:6–^{14}C into liver and brain fatty acids of the developing rat.

Parent fatty acid	Fatty acids derived from linolenic acid by chain elongation and desaturation		
18:3ω3 ———	20:5ω3 ——→	22:5ω3 ——→	22:6ω3

Fig. 1. Metabolism of linolenic acid in animals. Linolenic acid is an 18 carbon fatty acid with 3 methylene interrupted double bonds. The double bond sequence starts at carbon 3 from the methyl end of the molecule. Elongation and further desaturation occurs at the carboxyl end leaving the positioning of the double bonds unchanged relative to the methyl end.

2. Methods and materials

Rats were reared from weaning on a semi-synthetic diet containing 6% and 25% protein (w/w). The fat was a mixture of soya bean oil and linseed oil (5:1, v/v); this ensured that both linoleic and linolenic acids were present. After 6 months on the diet, the rats were mated and the pups reared by the dams for the first 21 days.

[1-^{14}C]Linolenic acid (The Radiochemical Centre, Amersham) was converted to its methyl ester and purified by thin-layer chromatography. Uniformly labelled docosahexaenoic acid was prepared as the methyl ester from lipids of *Crypthecodinium cohnii* which had been cultures with 2 mCi of [1-^{14}C]acetic acid [8, 9]. The radio-chemical purity of the 22:6ω3 as determined by gas-liquid chromatography was 92% 22:6ω3 and 7% 22:5ω3. The specific activities of the linolenic and docosahexaenoic preparations were 5.9 and 3.75 μCi/mg, respectively.

Two groups of five 16 day old pups were used; one group received 6.5 μCi of linolenate in 0.1 ml of olive oil intraperitoneally and the other group 0.9 μCi of docosahexaenoate made up and injected similarly. These groups will be referred to as the 18:3 group and the 22:6 group. After injection the pups were left with their mothers for 22 hr and then killed by decapitation. The livers and brains were removed quickly, washed in ice-cold saline and homogenised in chloroform:methanol (2:1). The lipids were extracted

and purified as described previously [7]. Aliquots of the total lipid extracts were assayed for radioactivity (scintillator: 0.2 g 1,4-di(2-(5-phenyloxazolyl))-benzene and 4 g 2,5-diphenyloxazole/l toluene) using a Packard liquid scintillation counter. The counting efficiency was 80%. Triglycerides and phospholipids were separated from the liver lipids by thin-layer chromatography and eluted from the chromatograms. The fatty acid methyl esters of the liver triglycerides and phospholipids and brain total lipids were prepared. The methyl esters were separated by gas-liquid chromatography [7] and the individual fatty acids were collected and assayed for radioactivity as described above.

3. Results

Radioactive linolenic and docosahexaenoic acids were incorporated into liver and brain lipids of the rats; the results are shown in table 1. In the 22:6 group a significantly greater percentage of the administered radioactivity was incorporated into the liver lipids compared with the 18:3 group, whereas the incorporation of radioactivity into the brain lipids was not significantly different. In both groups the liver triglycerides and phospholipids contained more than 94% of the total liver lipid radioactivity, but the distribution of radioactivity between triglycerides and phospholipids was different. In the

Table 1
Percentage of administered radioactivity in tissue lipids.

Tissue fraction	Lipid (mg)	Isotope administered		P
		18:3–^{14}C group (%)	22:6–^{14}C group (%)	
Total liver lipids	84.4 ± 5.6	1.941 ± 0.093	6.461 ± 1.520	< 0.025
Liver triglycerides	27.5 ± 1.9	1.288 ± 0.031	3.398 ± 0.807	< 0.050
Liver phospholipids	35.7 ± 2.1	0.438 ± 0.031	2.422 ± 0.867	< 0.050
Total brain lipids	72.9 ± 1.3	0.106 ± 0.025	0.204 ± 0.059	> 0.050

6.5 μCi of 18:3–1–^{14}C (as methyl ester) or 0.9 μCi of 22:6–U–^{14}C (as methyl ester) were injected intraperitoneally into 16-day old rat pups and the animals were killed 22 hr after injection. Results from five experiments with each isotope are reported as the mean ±S.E.M.

Table 2
Percentage distribution of fatty acids by weight and radioactivity in liver and brain lipids.

Fatty acid	Liver triglycerides			Liver phospholipids			Brain lipids		
	Weight* (%)	Radioactivity**(%)		Weight (%)	Radioactivity (%)		Weight (%)	Radioactivity (%)	
		18:3–^{14}C	22:6–^{14}C		18:3–^{14}C	22:6–^{14}C		18:3–^{14}C	22:6–^{14}C
18:3ω3	0.9	78.6	2.0	0.2	21.9	1.4	0.2	51.4	3.4
20:5ω3	2.0	3.5	1.9	0.4	13.0	1.1	0.5	2.8	1.9
22:5ω3	5.3	4.0	8.5	4.8	20.0	2.9	0.3	3.1	6.6
22:6ω3	12.3	2.4	85.4	20.6	17.7	89.8	15.0	8.0	79.3

Five experiments were performed with each isotope and the results are presented as the mean.

* Weight %: the areas of the individual fatty acids (from 14:0 to 22:6ω3) were calculated and the results are expressed as the percentage of the total area.

** Radioactivity %: the radioactivity was collected from the individual fatty acids (14:0 to 22:6ω3) and the results are expressed as the percentage of the total radioactivity collected.

18:3 group the triglyceride to phospholipid ratio was 2.9 compared with a ratio of 1.4 for the 22:6 group ($P < 0.01$).

In the 22:6 group more than 79% of the ^{14}C in the fatty acids of the liver triglycerides, phospholipids and brain lipid was associated with the 22:6ω3 (table 2). A different distribution of radioactivity was observed for the 18:3 group. In the liver triglycerides 75% of the radioactivity in the fatty acids was associated with 18:3ω3 and less than 12% with the longer chain metabolites. On the other hand in the liver phospholipids there was significantly more radioactivity in the metabolites of linolenic acid than in linolenic acid itself. In the brain fatty acids of the 18:3 group more than half of the ^{14}C was found in 18:3ω3 and only a small amount in the longer chain metabolites of linolenic acid.

4. Discussion

In mammals so far studied (including the rat) it is the 20 and 22 carbon polyenoic fatty acid that accumulate in the brain structural lipids, rather than their parent 18 carbon fatty acids [3]. In the rat, most of the 22:6ω3 in the brain accumulates during the suckling period [7]; this 22:6ω3 could be derived by synthesis from 18:3ω3 in the brain itself or by the direct incorporation of 22:6ω3 into the brain from the blood.

In 17 day-old rats the former route seems unlikely, since it has been shown recently that in the developing rat brain, the activity of the enzyme responsible for the conversion of 18 carbon fatty acids to 20 and 22 carbon fatty acids falls rapidly after birth and by the 11th day the activity is almost zero [10]. Also the results of the present experiments show that a more efficient route of incorporation of 22:6ω3 into brain lipids was via 22:6ω3 itself, rather than by metabolism from 18:3ω3.

The liver is the major site for the chain elongation and desaturation of fatty acids in the rat [11] and is also known to be responsible for the synthesis of plasma lipoproteins. Hence it is possible that dietary 18:3ω3 is converted to 22:6ω3 in the liver and is then transported to different tissues via the plasma lipoproteins. Dietary 22:6ω3 could also be a source of 22:6ω3 in the blood plasma. In the suckling rat, the diet is known to contain both 18:3ω3 and 22:6ω3 [7].

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