Essential fatty acid deficiency and evidence for arachidonate synthesis in the cat

BY A. J. SINCLAIR AND W. SLATTERY

Department of Agriculture, Veterinary Research Institute, Parkville, Victoria 3052, Australia

AND J. G. MCLEAN AND E. A. MONGER

School of Veterinary Science, University of Melbourne, Parkville, Victoria 3052, Australia

(*Received 10 November 1980 – Accepted 26 January 1981*)

1. There is controversy regarding the capacity of the cat to convert 18:2ω6 to 20:4ω6 and the ability of the essential fatty acid (EFA)-deficient cat to produce 20:3ω9.

2. This paper reports the isolation and identification of 20:3ω9 from kidney phospholipids of EFA-deficient cats.

3. The results suggest that the cat is capable of limited synthesis of 20:4ω6 using a Δ5- and Δ8-desaturase.

It has been suggested that the cat is unable to convert dietary linoleic acid to arachidonic acid (Rivers *et al.* 1975; Hassam *et al.* 1977). Studies by Sinclair *et al.* (1979) demonstrated that the cat has a deficiency in the synthesis of 20:4ω6 at the Δ6-desaturation step, that is in the conversion of 18:2ω6 to 18:3ω6. In these experiments it was shown that when cats were maintained on diets in which 18:2ω6 was the only essential fatty acid, the concentration of 20:4ω6 in the tissues gradually decreased while the 18:2ω6 increased and two unusual fatty acids (20:2ω6 and 20:3Δ5-*cis*, 11-*cis*, 14-*cis*) appeared. Administration of isotopically labelled 18:2ω6 or 20:3ω6 to cats showed that while there was no significant conversion of 18:2ω6 to 20:4ω6 there was transformation of the 20:3ω6 to 20:4ω6. When cats were fed 18:3ω6 the levels of 20:3ω6 and 20:4ω6 in the erythrocytes increased substantially.

Kuchel (1978) showed that cats fed on 18:2ω6 as the only dietary EFA appeared normal after 36 months on this diet and their tissues contained a finite but low amount of 20:4ω6. Whilst it is possible that the 20:4ω6 present in these cats resulted from tenacious retention by the tissues, the most likely explanation is that the cat is capable of limited synthesis of 20:4ω6 because signs of EFA deficiency did not appear.

In order to determine whether the cat is capable of 20:4ω6 synthesis, cats were placed on an EFA-deficient diet and their tissues examined for the presence of 20:3ω9. When other animals are fed EFA-deficient diets they accumulate 20:3ω9 in their tissues, and the presence of this triene is the classic indicator of EFA-deficiency (Holman, 1970). It is produced from 18:1ω9 by the same enzymic pathway that normally converts 18:2ω6 to 20:4ω6, and if 20:3ω9 is present when the cat is fed an EFA-deficient diet this would indicate that the cat must be capable of synthesizing 20:4ω6. Previous studies have reported that 20:3ω9 is not present in tissues from EFA-deficient cats (Rivers *et al.* 1976).

MATERIALS AND METHODS

Cats were maintained on two different diets; either a control diet (meat- and-fish-based commercial cat food), or an experimental semi-synthetic diet in which the only source of fat was hydrogenated beef fat (SAT diet). The composition of these diets has been previously described (Sinclair *et al.* 1979).

Ten cats were weaned at 8 weeks of age and half were placed on the control and half on the SAT diet for 6 months. The cats were then killed and the lipids were extracted from 1 g samples of liver, kidney, spleen, heart, plasma and erythrocytes by standard techniques (Sinclair, 1975). Lipids were also extracted from approximately 15 g kidney tissue from two control cats and two SAT-fed cats. The fatty acid methyl esters were formed as described previously (Sinclair *et al.* 1979) and separated by gas–liquid chromatography (GLC) using 2 m × 4 mm internal diameter glass columns packed with 100 g EGSS-X/kg Gas-Chrom P (Applied Science Laboratories Inc., State College, Pennsylvania, USA); the carrier-gas flow-rate was 40 ml/min and the column oven temperature was 193°.

The methyl esters were also separated by GLC using either a 45 m support-coated open tubular (SCOT) column packed with OV-275 (Sinclair *et al.* 1979), or a 45 m (0·5 m internal diameter) SCOT column packed with SP-1000 (SGE, Melbourne, Australia). The latter was used at a column oven temperature of 195° and a helium carrier gas flow of 100 mm/s. The fatty acid compositions were calculated from peak areas provided by an integrator (Spectraphysics, California, USA). Commercial standards (Nu-chek-Prep, Minnesota, USA) and standards of 20:3 Δ5-*cis*, 11-*cis*, 14-*cis* and 20:3ω9 were isolated from cat liver (Sinclair *et al.* 1979) and EFA-deficient rat liver respectively. Kidney phospholipids and neutral lipids were separated from the total lipids (Galanos & Kapoulas, 1962) and fatty acid methyl esters of these fractions were examined by GLC and the phospholipid fatty acid methyl esters were also separated by silver nitrate thin layer chromatography (Bandyapathyay & Dutta, 1975). Fractions eluted from the $AgNO_3$ plates were examined by GLC and the three-double-bond fraction was subjected to ozonolysis and reduction with triphenyl phosphine (Beroza & Bierl, 1967). The aldehyde and aldehydo esters resulting from this treatment were separated by GLC (Sinclair *et al.* 1979) and were identified by comparison with chromatograms of the ozonolysis and reduction products of standard methyl esters. The three-double-bond $AgNO_3$ fraction was also subjected to hydrogenation followed by GLC (Christie, 1973).

RESULTS AND DISCUSSION

The cats on the EFA-deficient (SAT) diet all developed the classical clinical signs of EFA-deficiency including general lethargy, retarded growth, rough dry coats with dandruff and skin lesions.

The tissue fatty acid methyl esters were examined by GLC using the packed EGSS-X column. In the SAT-fed cats it was consistently observed that there was a marked elevation of 16:1 and 18:1 and a decrease in the percentage of 18:0, 18:2ω6, 20:4ω6, and the C_{20} and C_{22} metabolites of 18:3ω3, when compared with the fatty acid profile from the tissues of the control cats. In all tissues from SAT-fed cats, there was the consistent appearance of small quantities (1–2% of total fatty acids) of an unknown fatty acid which had the same retention time as 20:3ω9. The fatty acid profile of the kidney is typical of the tissue changes described, and is shown in Table 1.

Using the packed EGSS-X column it was clear that there were at least three fatty acid methyl esters which chromatographed in the region of the unknown fatty acid, with little separation between the three components. Analysis of the tissue samples from SAT-fed cats using the OV-275 SCOT column resolved this area of the trace into four distinct peaks, which corresponded to 20:2ω6, the unknown, 20:3 Δ5-*cis*, 11-*cis*, 14-*cis* and 20:3ω6. All subsequent analyses were performed using this SCOT column. The unknown was in highest concentration in the kidney of SAT-fed cats (2·3%), but was not detected in the kidney lipids of the control cats (Table 1) and so the kidneys of SAT-fed cats were used as a source of the unknown.

Separation of the kidney lipids of SAT-fed cats into neutral lipids and phospholipids

Table 1. *Fatty acid composition (% total fatty acids) of kidney total lipids from control and SAT-fed cats*

Fatty acid	Control diet	SAT diet
16:0	13·2	12·7
16:1	2·3	6·0
17:0	2·6	3·7
17:1	0·6	1·2
18:0	25·5	20·1
18:1	20·0	38·5
18:2ω6	12·0	4·5
18:3ω3	0·2	0·1
20:0	0·8	0·7
20:1ω9	0·2	0·3
20:1	—	0·2
20:2	—	0·3
20:2	—	0·4
20:2ω6	0·2	—
Unknown	—	2·3
20:3*	—	0·3
20:3ω6	0·4	0·6
20:4ω6	14·3	4·0
22:0	0·7	0·8
20:5ω3	1·6	0·6
22:4ω6	0·2	0·1
24:0	0·8	0·7
24:1	0·8	1·7
22:5ω3	0·7	0·2
22:6ω3	3·0	0·1
Total PUFA	29·4	12·3

SAT, semi-synthetic diet in which the only source of fat was hydrogenated beef fat; PUFA, polyunsaturated fatty acids.

* 20:3 Δ5-*cis*, 11-*cis*, 14-*cis*.

showed that the polyunsaturated fatty acids (PUFA) and the unknown fatty acid were concentrated in the phospholipid fraction. $AgNO_3$-TLC separation of the phospholipid fatty acid methyl esters from the SAT-fed cat, showed five major bands which corresponded to the methyl ester standards of 20:0, 20:1, 20:2, 20:3 and 20:4. GLC examination of the fractions from the $AgNO_3$-TLC plate showed that the only band to contain the unknown fatty acid was that which corresponded to the three-double-bond standard. The unknown fatty acid constituted 62% of the three-double-bond fraction; the other significant components of this fraction were 20:3 Δ5-*cis*, 11-*cis*, 14-*cis* (8%) and 20:3ω6 (18%). Hydrogenation of the three-double-bond fraction revealed that the only significant peak (>85% of the total) corresponded to 20:0.

Examination by GLC of the fragments resulting from ozonolysis and reduction of the three-double-bond fraction showed the presence of two major components which must have been derived largely from the unknown fatty acid, a C-5 aldehydo ester and a C-9 aldehyde. Smaller amounts of a C-8 aldehydo ester and a C-6 aldehyde were also present which came from the other 20:3 isomers found in this fraction.

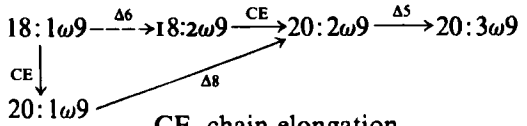
GLC examination of the phospholipid fatty acid methyl esters from the SAT-fed cats showed that on OV-275 the retention times of 20:3ω6, 20:3 Δ5-*cis*, 11-*cis*, 14-*cis* and the unknown relative to 20:4ω6 were 0·91, 0·86 and 0·83 respectively. The unknown had a relative retention time which was identical with that for the 20:3ω9 standard which had

been isolated from EFA-deficient rat liver lipids. On the SP-1000 SCOT column the relative retention times were 0·97, 0·95 and 0·93 respectively, and again the value for the unknown was identical with that of the 20:3ω9 standard.

These experiments showed that the unknown fatty acid was a C_{20} fatty acid with three double bonds. The retention time of this 20:3 fatty acid together with the ozonolysis studies support the identification of the unknown as 20:3 Δ5-*cis*, 8-*cis*, 11-*cis* (20:3ω9).

Rats fed on EFA-deficient diets convert 18:1ω9 to 20:3ω9 in the liver using Δ6- and Δ5-desaturases (Holman, 1970). This triene is the classic indicator of EFA deficiency and is produced by the same enzyme sequence that normally transforms 18:2ω6 to 20:4ω6.

In the cats fed on the EFA-deficient diet, the unknown fatty acid has been identified as 20:3ω9, implying that it has been synthesized using the Δ6- and Δ5-desaturases. However, since there appears to be little Δ6-desaturase activity in the cat (Hassam *et al.* 1977; Sinclair *et al.* 1979), the 20:3 may have been largely synthesized as shown, using a Δ8-desaturase together with a Δ5-desaturase which is known to be present in the cat (Sinclair *et al.* 1979).



CE, chain elongation.

Previous reports have indicated that cats are unable to convert linoleic acid to arachidonic acid (Rivers *et al.* 1975; Hassam *et al.* 1977), however, these results which identify 20:3ω9 in tissues from cats, show that cats do have some ability to synthesize long-chain PUFA from C_{18} dietary EFA. The synthesis of arachidonate from linoleate via the Δ8- and Δ5-desaturase pathways is not unique and for example accounts for significant synthesis of arachidonate in rat testicles (Albert & Coniglio, 1977) and human bladder and colon (Nakazawa *et al.* 1976).

The significance of these results is that they support the concept that the cat is capable of limited synthesis of arachidonate and other long-chain PUFA from dietary EFA. This means that the cat is not wholly dependent on dietary long-chain PUFA of animal origin to meet its requirements. This finding has important implications in the formulation of those cat diets which are extensively based on products of plant origin.

This work was supported by a grant from the Australian Research Grants Committee.

REFERENCES

Albert, D. H. & Coniglio, J. G. (1977). *Biochim. biophys. Acta* **489**, 390.
Bandyapathyay, G. K. & Dutta, J. (1975). *J. Chromatog.* **114**, 280.
Beroza, M. & Bierl, B. A. (1967). *Analyt. Chem.* **39**, 1131.
Christie, W. W. (1973). *Lipid Analysis.* New York: Pergamon Press.
Galanos, D. S. & Kapoulas, V. M. (1962). *J. Lipid Res.* **3**, 134.
Hassam, A. G., Rivers, J. P. W. & Crawford, M. A. (1977). *Nutr. Metab.* **21**, 321.
Holman, R. T. (1970). *Prog. Chem. Fats Lipids* **9**, 607.
Kuchel, T. R. (1978). Studies on the refeeding of essential fatty acid deficient cats with linoleic acid. MVS Thesis, University of Melbourne, Australia.
Nakazawa, I., Mead, J. F. & Yonemoto, R. H. (1976). *Lipids*, **11**, 79.
Rivers, J. P. W., Sinclair, A. J. & Crawford, M. A. (1975). *Nature, Lond.* **258**, 171.
Rivers, J. P. W., Sinclair, A. J., Moore, D. P. & Crawford, M. A. (1976). *Proc. Nutr. Soc.* **35**, 66A.
Sinclair, A. J. (1975). *Lipids* **10**, 175.
Sinclair, A. J., McLean, J. G. & Monger, E. A. (1979). *Lipids*, **14**, 932.

Printed in Great Britain