The nutritional and metabolic impact of γ-linolenic acid (18:3ω6) on cats deprived of animal lipid

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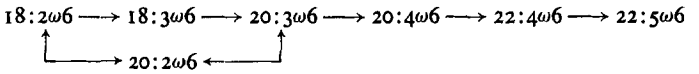
(*Received* 11 *August* 1977 – *Accepted* 23 *September* 1977)

1. The syndrome induced by depriving cats of animal lipid is partially cured by feeding 18:3ω6. This is associated with an increase in levels of 20:3ω6, but not 20:4ω6, in plasma phospholipids.

2. It is concluded that the cat lacks Δ5 desaturase activity and has a dietary requirement for 18:3ω6 and possibly 20:4ω6.

All vertebrate species so far studied have been shown to have a dietary requirement for polyunsaturated fatty acids. In most mammals this essential fatty acid (EFA) requirement is met by linoleic acid (18:2ω6) which is thought to be metabolized by alternate desaturation and chain elongation reactions according to the scheme below.



The fatty acids thus derived from 18:2ω6 form a homologous series in which increasing polyunsaturation parallels an increase in EFA potency. The production of these derived EFA from 18:2ω6 requires either the Δ6 desaturation of 18:2ω6 to γ-linolenic acid (18:3ω6) or the Δ8 desaturation of 20:2ω6 to dihomo-γ-linolenic acid (20:3ω6). Recent experiments suggest that the Δ8 desaturase may not be present in rats as had previously been assumed (Sprecher & Lee, 1975).

Biochemical and clinical lesions in cats given semi-purified diets in which 18:2ω6 was the source of EFA, and in which no derived EFA were present, have been described previously (Rivers, Sinclair & Crawford, 1975). Levels of 18:2ω6, and its chain elongation product 20:2ω6, rose in tissue phospholipids while levels of all other derived EFA fell. These observations were compatible with a lack of Δ6 and Δ8 desaturase activity in the cat, a hypothesis later confirmed by radio-isotope studies on non-deficient animals (Hassam, Rivers & Crawford, 1977*a*). Since the clinical changes observed were similar to those reported in EFA deficiency in other species and their remission paralleled normalization of tissue fatty acid patterns, it was therefore suggested that 18:2ω6 lacks EFA activity in the cat and that the syndrome encountered was a dietary deficiency in some or all of the derived EFA.

If the only defect in EFA metabolism in the cat were the lack of Δ6 and Δ8 desaturase activity, then dietary 18:3ω6 should have full EFA activity. The results presented in this paper clearly show that dietary 18:3ω6 possesses only partial EFA activity for the cat, and that although it is chain-elongated to 20:3ω6 the cat lacks activity of the Δ5 desaturase necessary to convert 20:3ω6 to 20:4ω6.

Table 1. *Composition of diets (g/kg) fed to cats*

Diet ...	CD5*	CD7†
Casein‡	400	400
Safflower Seed Oil§	250	125
Naudicelle‖	0	125
Glucose monohydrate¶	242	242
Vitamin mix**	64·5	64·5
Mineral mix††	43·2	43·2
Vitamin A‡‡	0·021	0·021
DL-α-tocopherol	0·25	0·25

* Fatty-acid composition of diet CD5 (as g/100 g total fatty acids): 16:0, 9·0; 18:0, 3·0; 18:1ω9, 17·9; 18:2ω6, 67·6; 18:3ω3, 2·5.

† Fatty-acid composition of diet CD7 (as g/100 g total fatty acids); 16:0, 8·8; 18:0, 3·1; 18:1ω9, 19·3; 18:2ω6, 64·6, 18:3ω6, 2·9; 18:3ω3, 1·3.

‡ Casein C, Glaxo Laboratories Ltd, Greenford, Middlesex, UK.

§ Healthilife Ltd, Bradford, UK.

‖ Bio-Oil Research Ltd, 30 Hornby Drive, Nantwich, Cheshire, UK.

¶ Frederick Allen and Sons Ltd, London E3, UK.

** Vitamin mix provides (mg/kg diet): thiamin HCl, 100; riboflavin, 100; nicotinamide, 500; pyridoxine HCl, 50; biotin, 25; pteroylglutamic acid, 1; *p*-aminobenzoic acid, 500; calcium pantothenate, 500; cyanocobalamin, 1; (g/kg diet) myoinositol, 1·0; choline chloride, 10·0; glucose monohydrate, 31·0; Solka Floc (Grade B.W. 40, Johnson, Jorgansen and Weltre Ltd, London EC4) 20·7.

†† Mineral mix provides (mg/kg diet): $AlK(SO_4)_2.12H_2O$, 6; KI, 7; $CuSO_4.5H_2O$, 10·5; $ZnCO_3$, 15; $MnSO_4.H_2O$, 22; NaF, 22; $CoCl_2.6H_2O$, 24; (as g/kg) $C_6H_5O_7Fe.5H_2O$, 1·5; NaCl, 1·0; $MgSO_4.7H_2O$, 3·4; Na_2HPO_4, 6·0; K_2HPO_4, 14·0; $CaCO_3$, 17·0.

‡‡ As retinyl palmitate, oily concentrate, approximate $1·7 \times 10^6$ i.u./g (0·5 g retinol equivalents/g) (manufacturer's stated analysis) from B.D.H. Ltd, Poole, Dorset, UK.

MATERIALS AND METHODS

Female cats (*Felis catus* L., domestic cross) bred in this laboratory were caged singly from weaning in an artificially lit room with constant 12 h day length; temperature and humidity were not controlled. Throughout the experiment cats were exercised as a group for 4 h/d and fed *ad lib.*

The diets, detailed composition of which is shown in Table 1, were modified from those used previously (Rivers *et al.* 1975). The two diets fed, CD5 and CD7, differed only in the type of oil they contained. Diet CD5 contained 25 % by weight safflower seed oil, while in diet CD7 half the safflower seed oil was replaced by an equal weight of evening primrose oil (Naudicelle, Bio-Oil Research Ltd). This modification did not significantly alter the linoleic acid content of the diets: 18:2ω6 provided 67·6 % of the fatty acids in diet CD5 and 64·6 % in diet CD7, but γ-linolenic acid, 18:3ω6, which was absent from diet CD5, provided 2·9 % of the fatty acids in diet CD7.

Six female kittens were fed for 18 months from weaning on diet CD5. They were then randomized into two equal groups, and one group (I) was given diet CD7, *ad lib.*, for 5 d, group II being kept on CD5 as controls. Seven weeks later group II was given a 5 d treatment with CD7 and group I kept on CD5 as controls. Blood samples were taken from the cephalic vein of cats in both groups in each experimental period on days 0 (before supplementation), 2, 5 and 8 (3 d after supplementation). Erythrocytes and plasma were separated and fatty acid patterns of phospholipids examined by techniques standard in this laboratory (Sinclair & Crawford, 1972).

RESULTS AND DISCUSSION

On day 0 of the first experimental period, that is, before diet CD7 had been given, all cats had dry staring coats, profuse dandruff, slow wound-healing with formation of skin ulcers and no behavioural evidence of oestrous cycles. In all these characteristics they contrasted with cats given our stock diet and closely resembled those described previously (Rivers *et al.* 1975). The consumption of CD7 for 5 d was associated with marked clinical improvement. In all animals coat and skin condition improved when diet CD7 was fed, slowly reverting when animals were returned to diet CD5. Three animals had superficial wounds which, despite treatment, had not healed; all had healed within 10 d of giving diet CD7. Animals had shown no previous signs of oestrus, but all came into unequivocal behavioural oestrus as they were given CD7 and some continued normal oestrous cycles after supplementation ended. The only animal to show behavioural oestrus during a control period was an animal from group I which had continued to cycle normally from the time it was first given CD7. Even so, the difference between the two groups was statistically significant ($P < 0{\cdot}01$ by the exact probability test).

There were no significant changes in body-weight or food intake with feeding on CD7. Before receiving CD7 cats had the same fatty acid pattern in blood phospholipids as previously described (Rivers *et al.* 1975), a high level of 18:2ω6 and 20:2ω6 and a reduction in all other derived EFA. Feeding on CD7 caused changes in the fatty acids of plasma choline phosphoglycerides (CPG). Since no effect of CD7 from period 1 was detectable at the start of period 2, results for the two periods are pooled in Table 2. As this shows, the level of 18:2ω6 and its chain elongation product 20:2ω6 did not vary significantly with feeding. On day 2, 18:3ω6 could be detected in plasma CPG, but by day 5 it was again below the threshold of detection. The level of 20:3ω6 rose steadily with feeding on CD7 and fell again when animals were returned to diet CD5, but no change whatsoever occurred in the level of arachidonic acid (20:4ω6) in plasma CPG during or after giving CD7. The unidentified fatty acid X, characteristic of cats given diets lacking derived EFA, was markedly reduced by giving CD7.

As the standard errors in Table 2 show, the level of 20:3ω6 in plasma CPG varied widely between animals. However, when results for individual animals were considered, a strong correlation was found between the amount of 18:3ω6 consumed and the level of 20:3ω6 in plasma CPG (on day 2, r 0·95, $P < 0{\cdot}02$; on day 5, r 0·87, $P < 0{\cdot}05$; results from both days pooled, r 0·93, $P < 0{\cdot}001$). The level of 20:3ω6 in plasma CPG had decreased by day 8, the decline being proportional to the level on day 5 (r 0·78, $P < 0{\cdot}05$).

Although fatty acid X has not been finally identified, it is apparently a straight-chain C_{20} trienoic acid chromatographically distinct from both 20:3ω6 and 20:3ω9. On a 10 % PEGA column fatty acid X had a retention time 86·96 % (SEM 0·15) of that of 20:4ω6, while 20:3ω6 had a retention time of 90·47 % (SEM 0·09) of that for 20:4ω6. In cats given CD7 there was a strong correlation between the increase in the level of 20:3ω6 in plasma CPG and the decrease in X (results of days 2, 5 and 8 pooled gave r 0·74, $P < 0{\cdot}001$).

Although the levels of 20:2ω6 and 18:2ω6 fluctuated they did not change systematically with giving CD7, and there were no significant correlations between incremental levels of 18:2ω6 or 20:2ω6 and 20:3ω6. There were no significant changes in the levels of fatty acids in erythrocyte phospholipids with feeding CD7 although wide day-to-day variations did occur.

The fatty acid changes observed in plasma CPG indicate that the 18:3ω6 of diet CD7 was metabolized by the cat. The relatively small change in levels of 18:3ω6 contrasts with the marked rise in 20:3ω6, indicating that the cat has an active chain elongase fully utilizing dietary 18:3ω6. However, the absence of any change in levels of 20:4ω6 in plasma

Table 2. *Fatty-acid composition of choline phosphoglycerides of plasma of cats fed diets CD5 and CD7**

(Results are means with their standard errors of groups I and II as g/100 g fatty-acid methyl esters eluting after 16:0; < 2% of total fatty acids eluted before 16:0)

	Day 0		Day 2		Day 5		Day 8	
No. of animals ...	CD5 (6)	CD7 (6)	CD5 (6)	CD7 (6)	CD5 (6)	CD7 (5)	CD5 (6)	CD7 (5)
18:2ω6	50·2 (±0·30)	51·0 (±0·40)	49·7 (±0·40)	49·8 (±0·55)	50·0 (±0·51)	50·2 (±0·47)	49·7 (±1·09)	51·1 (±0·57)
20:2ω6	3·6 (±0·15)	3·5 (±0·31)	3·3 (±0·28)	3·3 (±0·24)	2·8 (±0·24)	3·9 (±0·45)	3·5 (±0·47)	2·6 (±0·21)
18:3ω6	n.d.	n.d.	n.d.	0·13 (±0·06)	n.d.	n.d.	n.d.	n.d.
20:3ω6	n.d.	n.d.	n.d.	0·43 (±0·10)	n.d.	1·34 (±0·37)	n.d.	0·3 (±0·13)
20:4ω6	0·3 (±0·13)	0·4 (±0·16)	0·5 (±0·06)	0·4 (±0·05)	0·3 (±0·13)	0·4 (±0·10)	0·4 (±0·05)	0·4 ±0·04)
X	0·9 (±0·06)	0·8 (±0·32)	1·1 (±0·15)	0·2 (±0·12)	0·7 (±0·05)	0·4 (±0·23)	0·8 (±0·19)	0·8 (±0·12)

n.d., not detected (limit of detection 0·05 g/100 g).
* For details of composition see Table 1.

CPG is a marked contrast to the effects of feeding 18:3ω6 to rats, where there is a large rise in 20:4ω6 in plasma CPG (Larkin & Nye, 1975). In the present experiment levels of 22:4ω6 and 22:5ω6 were close to the limit of detection by gas–liquid chromatography (0·05%) and did not vary significantly during the experiment. Thus, the absence of any increase in levels of 20:4ω6 cannot be explained by its conversion to C22 polyenoics.

Hassam, Rivers & Crawford (1977*b*) have shown 18:3ω6 to be a very potent cure for EFA deficiency in the rat. The consumption of 18:3ω6 as diet CD7 was, in this experiment, associated with improvement in coat condition, wound healing, the onset of oestrus and, in plasma CPG, a rise in levels of 20:3ω6 and a decrease in the levels of X. All these changes occur when deficient cats are returned to the stock diet, rich in derived EFA (Rivers *et al.* 1975). But the absence of weight gain or improvement in erythrocyte membrane fatty-acid composition make it necessary to regard the remission in the present experiment as only partial. Subsequent long-term feeding experiments (Frankel & Rivers, unpublished results) confirm that 18:3ω6 cannot alone meet the EFA requirements of the cat.

That even partial remission occurs implies that dietary 18:3ω6 has some EFA activity for the cat. That remission is accompanied by only a small rise in levels of 18:3ω6 in blood phospholipids, and no change in levels of 20:4ω6, clearly shows that it is 20:3ω6 which is metabolically important.

It must be stressed that this does not imply that 20:4ω6 has no function in cats. It may be that 20:3ω6 and 20:4ω6 partially substitute for each other or that those characteristics such as body weight, which did not change, were specifically due to a deficiency in 20:4ω6 or one of its metabolites. On the basis of this experiment it must be concluded that the cat has a dietary requirement for 18:3ω6 and possibly also for 20:4ω6. Its requirement for other polyunsaturated fatty acids, including those of the ω3 series derived from 18:3ω3, remains totally unknown.

The multiple blocks on the metabolism of 18:2ω6 make the cat an attractive model for research on the physiological role of the EFA. The combined absence of the Δ8, Δ6 and Δ5 desaturases means that levels of dienoic, trienoic and tetraenoic fatty acids can, as in this experiment, be manipulated independently. This holds out the promise that, in con-

trast to other animal species, where only total EFA deficiency can be studied, in the cat the metabolic function of each of the EFA may be determined.

The specific absence of the Δ5 desaturase also suggests that the cat may be a useful model in prostaglandin research. Prostaglandins of the PGE_1 series are derived from 20:3ω6 while those of the PGE_2 type come from 20:4ω6. The prospect that tissue levels of 20:3ω6 and 20:4ω6 can be manipulated independently makes it possible that endogenous production of PGE_1 and PGE_2 can be altered by dietary means alone. Thus, the impact of endogenous production of prostaglandins, as opposed to their pharmacological effects, will be open to study.

We thank Dr M. A. Crawford for critical advice and Catherine Alderson for technical assistance. Bio-Oil Research Ltd, 30 Hornby Drive, Nantwich, Cheshire CW5 6JP provided generous supplies of Naudicelle. We gratefully acknowledge research grants from Pedigree Petfoods to T. F. and from Unilever, Vlaardingen to J.R.

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