ALTERATIONS IN BALANCE OF LONG CHAIN METABOLIC DERIVATIVES OF LINOLENIC ACID IN THE FAT DEFICIENT GUINEA PIG

M.A.CRAWFORD

Biochemistry Department, Nuffield Institute of Comparative Medicine, The Zoological Society of London, Regent's Park, London, N.W.1, England

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1. Introduction

The long chain polyenoics of 20 and 22 carbon chain lengths are incorporated into phospholipids and cell structures either from dietary sources or are produced by metabolism from the parent vegetable methylene interrupted polyenoics: linoleic with two double bonds commencing at *n*-6 and linolenic with three double bonds commencing at *n*-3. Elongation and further desaturation of these parent polyenoics occurs in animal tissues at the carboxyl end leaving the positioning of the double bonds unchanged relative to the methyl end. In this way two separate families (*n*-6 and *n*-3) of polyenoics are created for cell construction. Linoleic is mainly metabolized to arachidonic (C20:4, *n*-6) and linoleic to docosapentaenoate (C22:5 *n*-3) and docosahexaenoate (C22:6, *n*-3).

Comparative studies on primary protein structures, [1] and gross protein composition [2] demonstrated remarkable similarities from species to species regardless of dietary adaptation. However, our studies on muscle fats [3–7] indicate that although these were similar in related species, differences were demonstrable from one order to another [6].

Table 1
The balance of C22:6/C22:5 fatty acids in the liver lipids of guinea pig.

Age (months)	Diet	C22:5 and C22:6 (% of total ± S.E.)	Ratio $\frac{C22:6}{C22:5}$ ± S.E.	No. of animals
1	Stock	6.4 ± 0.3	3.2 ± 0.15	6
4	Stock	6.8 ± 0.4	3.5 ± 0.18	6
36	Stock	5.0 ± 0.6	2.0 ± 0.35	4
4	Fat free diet plus oil for last 3 months	7.6 ± 0.2	4.1 ± 0.2	6
4	Fat free diet for last 3 months	3.8 ± 0.4	0.37 ± 0.04	6

The one month old guinea pigs placed on a fat free diet for three months produced an inversion of the C22:6 to C22:5 ratio in the liver fatty acids as well as a reduction in the total amount of C22:5 + C22:6 combined. The difference is statistically significant ($p < 0.001$). The total liver lipid averaged 5.6 ± 0.7 in the experimental group with oil and 5.7 ± 0.9 in the fat free group.

In laboratory rats, mice and in humans, it is known that the docosahexaenoate is present in higher proportions but we found in all the large adult herbivores there appeared to be more of the docosapentaenoate than the docosahexaenoate (table 1). Several factors could affect the extent of unsaturation of the C22 acids in tissues and of these diet, rate of growth and ageing are of particular interest. As the richest natural source of parent vegetable acids in seed material, where they occur in association with tocopherol, we have examined the effect of removal of both parent polyunsaturated acids and tocopherols from the guinea pig diet and wish to report on the severe change which occurred in the balance of the C22:5. *n*-3 and C22:6, *n*-3 as a consequence.

2. Material and methods

Protein, (casein and meat meal 1:1 ether extracted) was provided at 20% of the fat free diet; water soluble vitamins and minerals were added in accordance with known requirements as described elsewhere [8], [8–10] vitamins A and D were supplied as oil free preparations. Potato starch, as a carbohydrate source, was used to complete the purified diets on an isocaloric basis. The source of polyunsaturates used to supplement the fat free diet was a mixture of sunflower and linseed oils (4:1) to ensure both linoleic and linolenic acids were present; 0.05 α tocopherol acetate was added and the mixture was supplied at 10% of the diet. Cellophane was included at 10% of the diet in both groups to provide bulk. Guinea pigs reared on the stock diet (Dixon's SG1) were used for comparison [10].

Random groups of animals were selected at one month of age from the stock colony; fed the fat free and the oil supplemented diets and then killed at 2½–3½ months when gross signs of deficiency became manifest in the fat free group. The external symptoms were loss of hair, inactivity and weakness. At this time continuation of the fat free diet resulted in death. Guinea pigs fed stock diet were also killed at the same age for comparison. Liver was taken for analysis after flushing with ice cold saline. The tissue was weighed and immediately homogenised in chloroform-methanol (2:1) as described previously [5, 7].

The metabolic long chain derivatives of linolenic acid were studied in the liver tissues of these animals by use of gas-liquid chromatography of their methyl esters on polyethylene glycol adipate, (PEGA) or on co-polymers of ethylene glycol and succinic acid with methyl silicone (EGSS-X) and apiezon. The carbon chain length was determined by logarithmic plots and confirmed by hydrogenation and mass spectrometry. The end carbon chain was determined by use of separation factors on PEGA [11, 12, 13].

3. Results

Liver lipids from the guinea pigs on fat free diets for three months showed alterations in fatty acids which might be expected from a fat deficient diet [14, 15] particularly with a loss of linoleic and linolenic acids and an increase in oleic. However, this report concerns the new finding of an alteration balance of the C22 polyenoics which changed radically in the terminal phase of the fat deficiency experiment. In small mammals and man there is usually more of the acid with six double bonds than the acid with five; in the terminal phase of the fat free experiment the ratio of the six to five double bonds in the C22 acids altered in favour of the acid with five double bonds (table 2) more typical large ruminant herbivores, collected data from which are summarised in table 2.

From table 1 it can be seen that at one month of age the liver lipids of normal guinea pigs contained a higher proportion of the acid containing six double bonds. This was unchanged in 3 month old guinea pigs fed oil or the standard laboratory diet for the next three months but after three months on a fat free diet there was more C22:5, *n*-3 than C22:6, *n*-3. This change in ratio was also associated with a diminuation of the total C22:5, *n*-3 and C22:6, *n*-3 acids present in the liver although the total liver lipid remained unchanged.

In the three year old normal guinea pigs the proportion of the docosahexaenoate fell although the balance was still in favour of the higher degree of unsaturation.

4. Discussion

Our data indicate that a loss of unsaturation occurs

Table 2
The average balance of C22:5/C22:6 in groups of some large and small animals.

Age group (years)	Range of body weight (kg)	Species	Tissue	22:5 + 22:6 % of total fatty acid	$\frac{22:6}{22:5}$
6–10	400–550	Syncerus caffer	Liver	8.0 ± 0.3	0.34 ± 0.08
4–10	50–550	Syncerus caffer Taurotragus oryx Bos indicus Kobus defassa Acephalus buselaphus Adenta kob Phacochoerus aethiopicus Rangifer L Cervus elaphus Odocoileus sp. Sus scrofa	Muscle	3.8 ± 0.04	0.17 ± 0.04
2–6	0.25–7.5	Procavia habessinica Cebidae saimiri sciureus Muridae cricetomys gambianus Leporidae oryctolagus cuniculus Cavea porcellus	Muscle	6.3 ± 0.4	4.0 ± 0.2

The balance of C22:5 and C22:6 acids is given for adult mammals ± S.E.

in the C22 acids with growth and ageing, and the experiment demonstrates that this change can be accelerated by dietary means. Although large herbivorous mammals have access to oil rich vegetation it is possible that time factors in metabolic elongation and desaturation combined with their rapid growth limits the accumulation of long chain metabolic derivatives.

It is known that the human red cell membrane contains docosahexaenoate as opposed to the pentaenoate [16]. The docosahexaenoate is an important constituent of phospholipid in human grey matter [18, 19]. As these long chain polyenic acids can be derived from dietary sources or in biochemical conversion from parent vegetable polyenoics it would seem important to degine the interaction of growth, dietary availability and biochemical conversion.

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