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# Prostaglandins, Leukotrienes and Essential Fatty Acids

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## Docosahexaenoic acid synthesis from alpha-linolenic acid is inhibited by diets high in polyunsaturated fatty acids

R.A. Gibson<sup>a,\*</sup>, M.A. Neumann<sup>b</sup>, E.L. Lien<sup>c</sup>, K.A. Boyd<sup>b</sup>, W.C. Tu<sup>a</sup><sup>a</sup> FOODplus Research Centre, School of Agriculture, Food and Wine, The University of Adelaide, South Australia, Australia<sup>b</sup> Child Nutrition Research Centre, Women's and Children's Health Research Institute, Flinders Medical Centre, Bedford Park, South Australia, Australia<sup>c</sup> Department of Food Science and Human Nutrition, University of Illinois, Urbana, Illinois, USA

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### ABSTRACT

The conversion of the plant-derived omega-3 (n-3)  $\alpha$ -linolenic acid (ALA, 18:3n-3) to the long-chain eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) can be increased by ALA sufficient diets compared to ALA deficient diets. Diets containing ALA above an optimal level result in no further increase in DHA levels in animals and humans. The present study evaluates means of maximizing plasma DHA accumulation by systematically varying both linoleic acid (LA, 18:2n-6) and ALA dietary level. Weanling rats were fed one of 54 diets for three weeks. The diets varied in the percentage of energy (en%) of LA (0.07–17.1 en%) and ALA (0.02–12.1 en%) by manipulating both the fat content and the balance of vegetable oils. The peak of plasma phospholipid DHA (> 8% total fatty acids) was attained as a result of feeding a narrow dietary range of 1–3 en% ALA and 1–2 en% LA but was suppressed to basal levels (~2% total fatty acids) at dietary intakes of total polyunsaturated fatty acids (PUFA) above 3 en%. We conclude it is possible to enhance the DHA status of rats fed diets containing ALA as the only source of n-3 fatty acids but only when the level of dietary PUFA is low (< 3 en%).

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### 1. Introduction

Docosahexaenoic acid (DHA, 22:6n-3) has a wide range of putative roles in both infants and adults. DHA is a major constituent of retinal and neural cells so that rat, primate and human infants fed diets deficient in n-3 fatty acids have low levels of tissue DHA. These low DHA levels result in reduced visual performance and impaired learning compared with those fed n-3 fatty acid sufficient diets [1–5]. Relative to diets low in n-3 fats, diets high in DHA are effective in enhancing visual and cognitive function in human infants [6–9] as well as preventing cardiac arrhythmia [10,11], reducing blood pressure [12] and modulating immune function [13] in adults. This has prompted regulatory authorities to recommend increased intakes of fish and oils rich in DHA for the general population [14]. If implemented on a global basis this may challenge fish stocks worldwide [15]. Thus, there is continued interest in the role of alpha-linolenic acid (ALA, 18:3n-3) in the diet since ALA is known to be the precursor of DHA and ALA is found in a range of vegetable oils.

The available evidence points to the fact that linoleic acid (LA, 18:2n-6) and ALA compete for a single set of desaturating and elongating enzymes (Fig. 1) and several *in vivo* studies have highlighted that the relative levels of n-6 and n-3 long chain

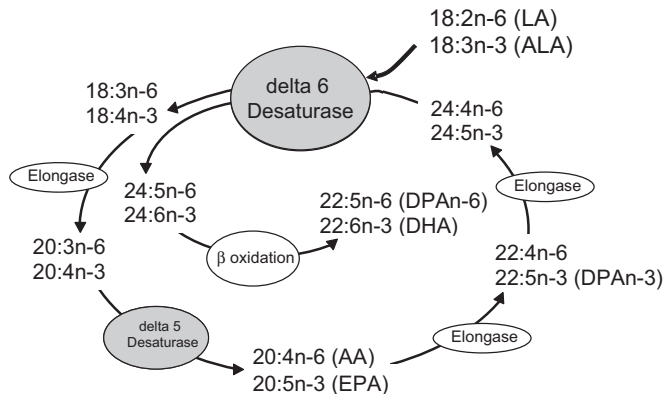
polyunsaturated fatty acid (LCPUFA) in animal tissues can be regulated by simply altering the balance of LA and ALA in the diet [16,17]. We have made several attempts to increase the tissue level of DHA in humans by increasing the level of ALA in the diet but have had limited success [18–21]. In an attempt to explain this, we fed a range of dietary ALA levels to piglets and rats and measured the response in tissues. While the levels of eicosapentaenoic acid (EPA, 20:5n-3) and docosapentaenoic acid (DPAn-3, 22:5n-3) increased dose-dependently with increasing dietary ALA, the DHA levels showed a curvilinear response to dietary ALA [16,17]. Because dietary LA is known to suppress DHA synthesis and accumulation [22,23], we hypothesized that DHA accumulation might be enhanced if the total levels of dietary LA were decreased. Data that support the role of LA in n-3 LCPUFA accumulation has recently been published [24]. The purpose of the present study was to test this hypothesis in rats fed diets that had a wide range of levels of both ALA and LA, using blends of natural oils.

### 2. Experimental procedures

#### 2.1. Animals

Three week old male weanling Hooded Wistar rats whose dams had been fed standard laboratory chow were assigned to one of the experimental diets *ad libitum* for 21 day. Groups of

\* Corresponding author. Tel.: +618 8303 4333; fax: +618 8303 7135.  
 E-mail address: [robert.gibson@adelaide.edu.au](mailto:robert.gibson@adelaide.edu.au) (R.A. Gibson).



**Fig. 1.** Schematic pathway of n-6 and n-3 fatty acid chain elongation/desaturation representing the contemporary view of the second utilization of the delta 6 desaturase for the synthesis of DHA (22:6n-3) and DPA n-6 (22:5n-6).

animals ( $n=5$ ) were started on dietary regimes with no more than two experimental groups started at any one time. The rats were maintained at room temperature of approximately 22 °C with a 12 h light: dark cycle. All animals were weighed at the beginning and at weekly intervals during the study. The experiments were approved by the Flinders University of South Australia animal ethics committee and were performed in accordance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

## 2.2. Diets

The dietary fat blends were designed to contain a range of LA:ALA ratios and total polyunsaturated fatty acid (PUFA) contents to result in a wide range of LA and ALA levels when expressed as energy percent (en%). Three major diet groups were prepared containing 5 wt% (wt%) fat (11.8 en%) 10 wt% fat (22.2 en%) and 20 wt% fat (39.4 en%). Subgroups within these major groups had different ratios of LA to ALA and different levels of total PUFA as outlined in Table 1, resulting in 54 distinct diets.

Fat blends were prepared in our laboratory by mixing fully hydrogenated coconut oil, (96.6% saturates, 1.3% trans fatty acids, 1.8% monounsaturates and 0.2% LA) (White Cloud, Meadow Lea Foods, North Ryde, NSW, Australia), Sunola (high oleic sunflower oil, 9.6% saturates, 83.1% monounsaturates, 6.9% LA and 0.4% ALA) (Meadow Lea Foods, North Ryde, NSW, Australia), safflower oil (11.1% saturates, 15% monounsaturates, 73.4% LA and 0.5% ALA) (Soyatech International, Mt Ommaney, Queensland, Australia) and flaxseed oil (10.8% saturates, 17.0% monounsaturates, 17.5% LA and 54.5% ALA) (Melrose Health Supplies, Mitcham, Victoria, Australia). The resulting fat blends were sent to Glen Forrest Stockfeeders (Glen Forrest, Western Australia, Australia) for incorporation into diets based on AIN93G rodent diet. Fat blends were designed to provide incremental levels of total PUFA (LA+ALA) ranging from 2%–45% of dietary fat with LA:ALA ratios of 10:1, 5:1, 2:1, 1:1, and 0.5:1. When included into the rodent chow the resulting LA:ALA ratios varied slightly from the intended levels (Table 1) and consequently data has been grouped as LA:ALA ratio of 7.4:1–11.3:1 (high), 5.3:1–6.6:1 (medium), 1.9:1–2.6:1 (moderate), 1.1:1–1.6:1 (moderately low) and 0.5:1–0.8:1 (low). Data from the moderately low and medium groups are not discussed in this paper but are included in Fig. 4. Some of the fat blends contained high levels of saturates, which were needed to achieve the very low levels of 18-carbon PUFA and low LA:ALA ratios. The nutrient composition of the experimental diets is found in Table 2.

## 2.3. Plasma collection and preparation

At the end of the feeding period, each rat was placed in a chamber containing Halothane. When the breathing rate had slowed, it was removed and toe pinch was used to determine if fully anaesthetized. At least 1 mL of blood was removed via cardiac puncture prior to euthanasia by injection of Lethobarb. The collected blood was immediately transferred to 4 mL Lithium Heparin tubes (Greiner Bio-one GmbH, A-4550 Kremsmunster, Austria). The plasma was collected after centrifugation of whole blood.

## 2.4. Lipid extraction

Total lipids were extracted from plasma samples with chloroform:methanol [25]. Phospholipids were separated from lipid extracts by thin layer chromatography (TLC) on silica gel plates (Silica gel 60H, Merck, Darmstadt, Germany). The solvent system for all TLC was petroleum spirit/diethyl ether/glacial acetic acid (180:30:2, by vol). Lipid classes were visualized with Fluorescein 5-Isothiocyanate against TLC standard 18-5 (Nuchek Prep Inc, Elysian, MN). The plasma phospholipids were methylated in 1% H<sub>2</sub>SO<sub>4</sub> in methanol at 70 °C for three hours. When cooled, the resulting methyl esters were extracted into n-heptane and transferred to vials containing anhydrous Na<sub>2</sub>SO<sub>4</sub> as the dehydrating agent.

## 2.5. Gas chromatography

Fatty acid methyl esters were separated and quantified using a Hewlett-Packard 6890 gas chromatography (Hewlett Packard, Palo Alto, CA) equipped with a 50-m capillary column (0.32 mm ID.) coated with BPX-70 (0.25 μm film thickness, SGE Pty Ltd, Ringwood, Victoria, Australia). The injector temperature was set at 250 °C and the detector (flame ionization) temperature at 300 °C. The initial oven temperature was 140 °C and was programmed to rise to 220 °C at 5 °C per minute. Helium was used as the carrier gas at a velocity of 35 cm per second. Fatty acid methyl esters were identified based on the retention time to authentic lipid standards obtained from Nuchek Prep Inc. (Elysian, MN).

### 2.5.1. Statistical analysis

All data are expressed as group mean ± SD. All analyses were performed using SPSS for Windows 11.0 (SPSS Inc, Chicago, IL). Data for key plasma fatty acids were plotted using SigmaPlot 2001 (v.7.101) (SPSS Inc, Chicago, IL) curve-fitting program. Curve selection for each plasma fatty acid was based on highest regression coefficient and lowest P value of all curves available.

## 3. Results

### 3.1. Growth

The average weight of all rat pups at weaning (week 3) was 43 ± 10 g. After three weeks of dietary treatment (week 6), the average final weight for all pups regardless of diet was 141 ± 18 g with an average weight gain of 98 ± 13 g. There were some differences between the groups in starting and final weight and total weight gain. To determine whether any of these differences were related to any dietary fat component, linear regression modeling was used with final weight and total weight gain as the dependent variables and starting weight, % fat, en% saturates, en% oleic acid (18:1n-9), en% LA, en% ALA, LA:ALA ratio and en% LA+ALA as variables. The most significant predictor of the final weight was the starting weight of the pup, which explained 55% of the variance while dietary fat components had little effect (< 8% of the variance).

**Table 1**  
Fatty acid composition of diets<sup>a</sup>.

Diet #	Total saturates	Total monounsaturates	LA	ALA	Total PUFA	LA:ALA ratio <sup>b</sup>
5% Fat						
1	71.9 (8.5)	23.8 (2.8)	2.84 (0.34)	0.30 (0.03)	3.1 (0.4)	10:1
2	75.3 (8.9)	20.1 (2.4)	3.02 (0.36)	0.46 (0.05)	3.5 (0.4)	7:1
3	80.9 (9.5)	14.4 (1.7)	2.64 (0.31)	0.76 (0.09)	3.4 (0.4)	3:1
4	86.0 (10.2)	9.2 (1.1)	2.30 (0.27)	1.12 (0.13)	3.4 (0.4)	2:1
5	91.4 (10.8)	3.8 (0.5)	1.81 (0.21)	1.45 (0.17)	3.3 (0.4)	1.2:1
6	43.8 (5.2)	49.5 (5.8)	5.38 (0.64)	0.66 (0.08)	6.0 (0.7)	8:1
7	42.1 (5.0)	51.6 (6.1)	4.75 (0.56)	0.88 (0.10)	5.6 (0.7)	5:1
8	55.4 (6.5)	38.0 (4.5)	4.41 (0.52)	1.77 (0.21)	6.2 (0.7)	2:1
9	67.6 (8.0)	24.4 (2.9)	4.27 (0.50)	2.63 (0.31)	6.9 (0.8)	2:1
10	82.5 (9.7)	9.7 (1.1)	2.81 (0.33)	3.56 (0.42)	6.4 (0.8)	0.8:1
11	43.7 (5.2)	45.7 (5.4)	8.70 (1.03)	1.32 (0.16)	10.0 (1.2)	7:1
12	42.4 (5.0)	45.2 (5.3)	10.03 (1.18)	1.79 (0.21)	11.8 (1.4)	6:1
13	42.3 (5.0)	46.2 (5.5)	7.61 (0.90)	3.36 (0.40)	11.0 (1.3)	2:1
14	41.4 (4.9)	47.4 (5.6)	5.93 (0.70)	4.67 (0.55)	10.6 (1.3)	1:1
15	67.9 (8.0)	19.9 (2.4)	4.59 (0.54)	6.89 (0.81)	11.5 (1.4)	0.7:1
16	41.1 (4.9)	42.4 (5.0)	14.43 (1.70)	1.38 (0.16)	15.8 (1.9)	10:1
17	39.8 (4.7)	43.5 (5.1)	13.60 (1.60)	2.55 (0.30)	16.1 (1.9)	5:1
18	40.3 (4.8)	43.1 (5.1)	11.48 (1.36)	4.47 (0.53)	16.0 (1.9)	3:1
19	40.8 (4.8)	42.7 (5.0)	8.36 (0.99)	7.60 (0.90)	16.0 (1.9)	1:1
20	55.0 (6.5)	27.7 (3.3)	6.44 (0.76)	10.04 (1.18)	16.5 (1.9)	0.6:1
21	33.1 (3.9)	36.4 (4.3)	27.32 (3.22)	2.69 (0.32)	30.0 (3.5)	10:1
22	33.2 (3.9)	36.3 (4.3)	25.33 (2.99)	4.65 (0.55)	30.0 (3.5)	5:1
23	32.7 (3.9)	36.7 (4.3)	20.43 (2.41)	9.67 (1.14)	30.1 (3.6)	2:1
24	33.0 (3.9)	36.2 (4.3)	15.68 (1.85)	14.61 (1.72)	30.3 (3.6)	1:1
25	32.6 (3.8)	35.2 (4.2)	11.04 (1.30)	20.72 (2.45)	31.8 (3.7)	0.5:1
26	26.5 (3.1)	29.3 (3.5)	39.17 (4.62)	4.62 (0.55)	43.8 (5.2)	8:1
27	25.8 (3.0)	29.1 (3.4)	37.57 (4.43)	7.05 (0.83)	44.6 (5.3)	5:1
28	25.2 (3.0)	29.4 (3.5)	31.02 (3.66)	14.05 (1.66)	45.1 (5.3)	2:1
29	25.4 (3.0)	29.1 (3.4)	23.96 (2.83)	21.11 (2.49)	45.1 (5.3)	1:1
30	25.1 (3.0)	29.0 (3.4)	16.60 (1.96)	28.95 (3.42)	45.6 (5.4)	0.6:1
10% Fat						
31	57.7 (12.8)	35.0 (7.8)	6.77 (1.50)	0.60 (0.13)	7.4 (1.6)	11:1
32	72.2 (16.0)	22.4 (5.0)	3.82 (0.85)	1.59 (0.35)	5.4 (1.2)	2:1
33	86.2 (19.1)	8.4 (1.9)	2.10 (0.47)	3.27 (0.73)	5.4 (1.2)	0.6:1
34	49.4 (11.0)	40.7 (9.0)	8.71 (1.93)	1.18 (0.26)	9.9 (2.2)	7:1
35	46.6 (10.3)	43.3 (9.6)	6.88 (1.53)	3.23 (0.72)	10.1 (2.2)	2:1
36	75.7 (16.8)	14.3 (3.2)	3.76 (0.83)	6.30 (1.40)	10.1 (2.2)	0.6:1
37	41.0 (9.1)	37.8 (8.4)	18.71 (4.15)	2.49 (0.55)	21.2 (4.7)	8:1
38	42.3 (9.4)	35.5 (7.9)	15.22 (3.38)	6.94 (1.54)	22.2 (4.9)	2:1
39	56.4 (12.5)	21.8 (4.8)	7.48 (1.66)	14.32 (3.18)	21.8 (4.8)	0.5:1
40	27.7 (6.2)	25.4 (5.6)	41.79 (9.28)	5.06 (1.12)	46.9 (10.4)	8:1
41	27.3 (6.1)	26.7 (5.9)	31.71 (7.04)	14.30 (3.17)	46.0 (10.2)	2:1
42	25.9 (5.8)	27.9 (6.2)	16.77 (3.72)	29.36 (6.52)	46.1 (10.2)	0.6:1
20% Fat						
43	61.1 (24.1)	33.6 (13.3)	4.80 (1.89)	0.50 (0.20)	5.3 (2.1)	10:1
44	71.8 (28.3)	22.8 (9.0)	3.63 (1.43)	1.75 (0.69)	5.4 (2.1)	2:1
45	88.0 (34.7)	6.6 (2.6)	1.86 (0.73)	3.51 (1.38)	5.4 (2.1)	0.5:1
46	43.5 (17.2)	45.8 (18.0)	9.69 (3.82)	1.04 (0.41)	10.7 (4.2)	9:1
47	44.7 (17.6)	44.9 (17.7)	6.89 (2.71)	3.42 (1.35)	10.3 (4.1)	2:1
48	73.0 (28.8)	16.8 (6.6)	4.20 (1.65)	6.00 (2.36)	10.2 (4.0)	0.7:1
49	40.9 (16.1)	37.3 (14.7)	19.42 (7.65)	2.39 (0.94)	21.8 (8.6)	8:1
50	41.4 (16.3)	36.3 (14.3)	14.69 (5.79)	7.65 (3.01)	22.3 (8.8)	2:1
51	58.4 (23.0)	20.6 (8.1)	6.84 (2.69)	14.18 (5.59)	21.0 (8.3)	0.5:1
52	27.1 (10.7)	24.2 (9.6)	43.98 (17.33)	4.66 (1.84)	48.6 (19.2)	9:1
53	27.0 (10.6)	27.2 (10.7)	31.00 (12.21)	14.82 (5.84)	45.8 (18.1)	2:1
54	26.2 (10.3)	28.1 (11.1)	14.92 (5.88)	30.78 (12.13)	45.7 (18.0)	0.5:1

<sup>a</sup> Results are expressed as % of total fatty acids with the energy % in parentheses.<sup>b</sup> LA:ALA ratio rounded to the nearest whole number (with the exception of 0.5:1 diets).

The only factor positively influencing total weight gain was en% ALA, but the effect was small explaining only 6% of the variance. This is important as change from a 5% fat diet to a 20% fat diet resulted in a 16% increase in the energy density of the diet.

### 3.2. Plasma phospholipid fatty acids

#### 3.2.1. Diet-induced changes in n-3 fatty acids

The level of ALA in the plasma phospholipid fraction was directly related to dietary ALA but the maximum level of ALA

attained was small, never exceeding 1.8% of total fatty acids (Fig. 2A). ALA incorporation into plasma phospholipids was also inversely related to the LA:ALA ratio with high ratios (7.4:1–11.3:1) resulting in almost complete inhibition of ALA incorporation.

Plasma phospholipid EPA and DPAn-3 had similar patterns of response to both increasing dietary ALA and the LA:ALA ratio (Fig. 2, B and C). When the LA:ALA ratios were low (0.5:1–0.8:1) and dietary ALA was less than 3 en%, the EPA and DPAn-3 increase was directly proportional to dietary ALA and rose to about 5% and

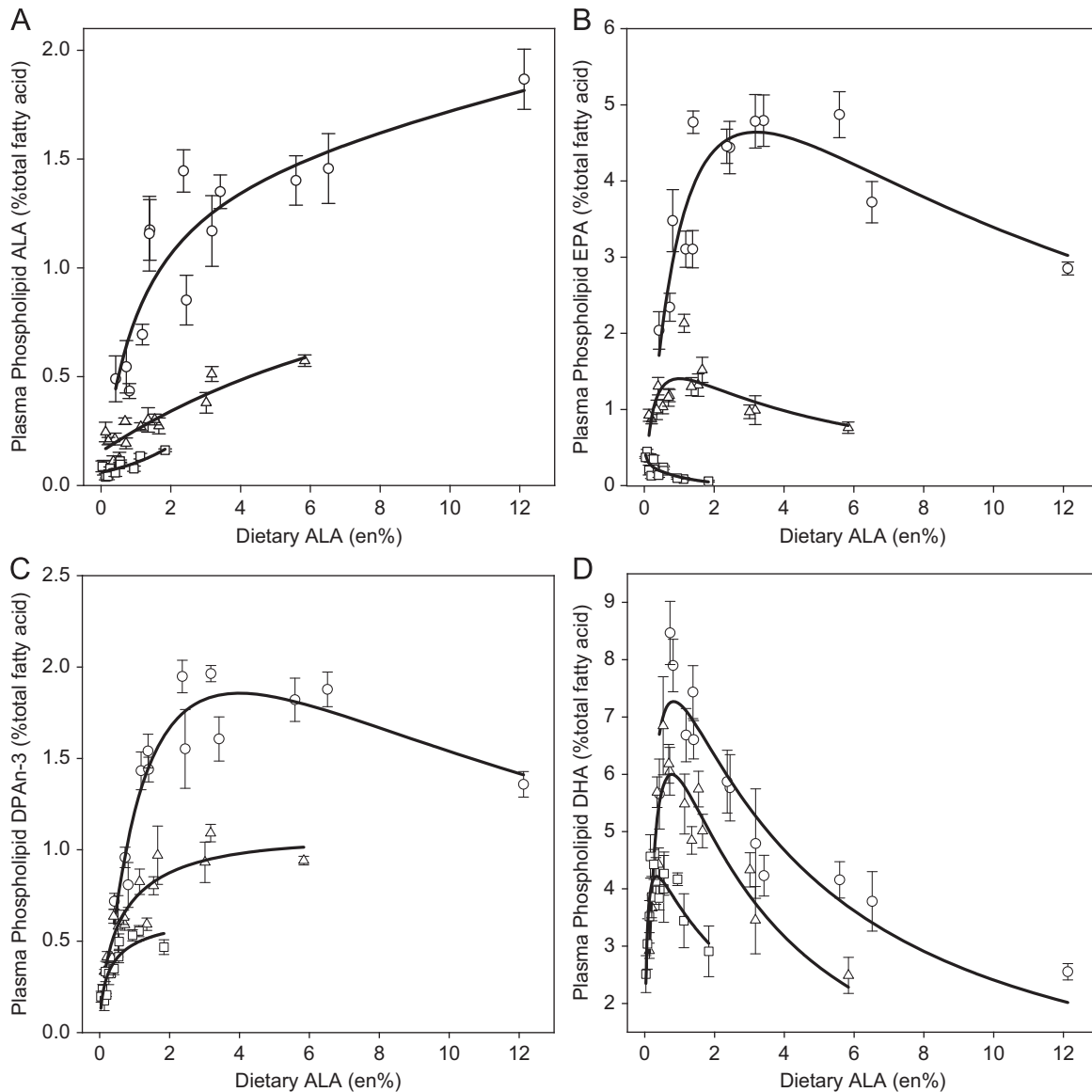
**Table 2**  
Ingredient profile of the experimental diets (g per 100 g).

	5 wt% fat diet	10 wt% fat diet	20 wt% fat diet
Fat blend (see Table 1)	5	10	20
Sucrose	10	10	10
Casein	20	20	20
Cellulose	5	5	5
Starch	41.75	36.75	26.75
Dextrinised starch	13.2	13.2	13.2
dl Methionine	0.3	0.3	0.3
AIN 93 minerals	3.5	3.5	3.5
AIN 93 vitamins	1	1	1
Choline chloride	0.25	0.25	0.25
Total E (KJ/100 g) <sup>a</sup> without cellulose	1569	1674	1884

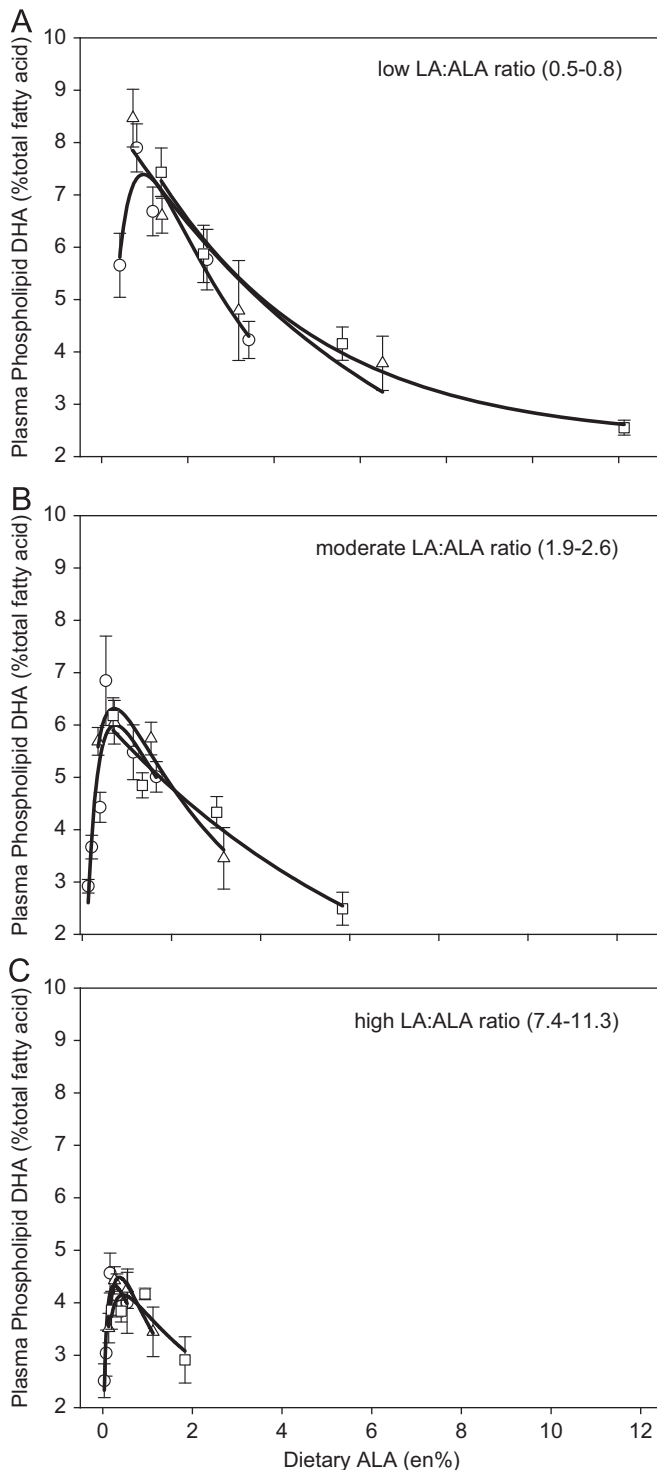
<sup>a</sup> Sucrose, Starch, Dextrinised Starch were calculated as CHO; Casein, dl Methionine were calculated as Protein; AIN 93 Minerals, AIN 93 Vitamins, Choline Chloride were ignored.

2% of total phospholipid fatty acids respectively (Fig. 2, B and C). Beyond dietary ALA intakes of 3 en% there was a modest decrease in the level of both EPA and DPAn-3. The proportion of these long chain PUFA in plasma phospholipids was also highly sensitive to LA in the diet. For example, at equivalent levels of dietary ALA, increasing the LA:ALA ratios from low (0.5:1–0.8:1) to moderate (1.9:1–2.6:1) resulted in a 50%–60% reduction in both metabolites. EPA incorporation into plasma phospholipids was almost completely suppressed at high LA:ALA ratios (7.4:1–11.3:1).

The accumulation of DHA into plasma phospholipids was sensitive to the level of both ALA and LA in the diet (Fig. 2D). At low ALA intakes there appeared to be a direct linear relationship between ALA and DHA. However, the peak of DHA accumulation was dependent on the LA:ALA ratio. The maximal DHA accumulation was observed at ~1.0 en% ALA when LA:ALA ratios were low, ~0.75 en% ALA when LA:ALA ratios were modest and ~0.3 en% when LA:ALA ratios were high. Above these levels of dietary ALA, tissue DHA levels were inversely related to dietary ALA regardless



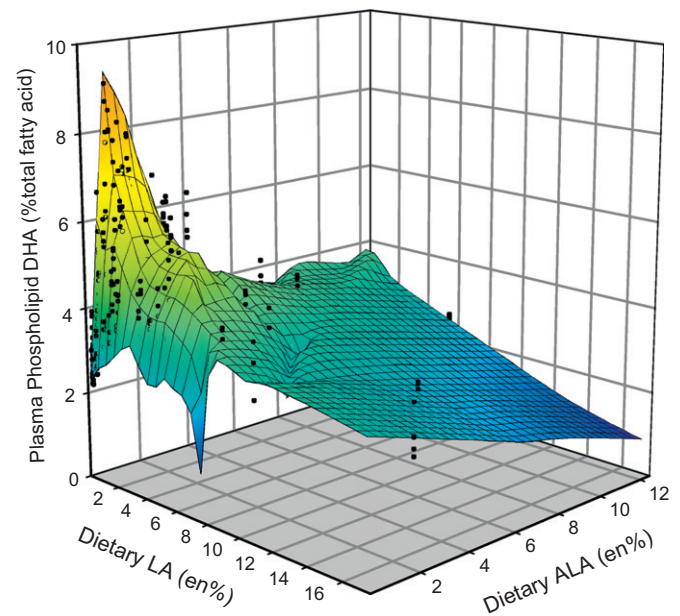
**Fig. 2.** Effect of dietary ALA level and LA:ALA ratio on plasma phospholipid fatty acids. LA:ALA ratios include low 0.5:1–0.8:1 ( $\Delta$ ), moderate 1.9:1–2.6:1 ( $\square$ ) and high 7.4:1–11.3:1 ( $\circ$ ). Fatty acids depicted are ALA (A), EPA (B), DPAn-3 (C) and DHA (D) compared to dietary ALA (as a percent of total dietary energy). Data are mean  $\pm$  SD ( $n=5$  rats per group).



**Fig. 3.** Effect of diets containing low (11.8 en%,  $\Delta$ ), medium (22.2 en%,  $\Delta$ ) and high (39.4 en%,  $\Delta$ ) levels of dietary fat on the distribution of plasma phospholipid DHA. The data are shown for three different LA:ALA ratios, low 0.5:1–0.8:1 (A), moderate 1.9:1–2.6:1 (B) and high 7.4:1–11.3:1 (C). Data are mean  $\pm$  SD ( $n=5$  rats per group). Dietary ALA is presented as a percentage of total dietary energy.

of the LA:ALA ratio. These data are interpreted to indicate that both dietary ALA and LA inhibit DHA accumulation as dietary levels of these 18-carbon PUFA are progressively increased.

Because the diets varied markedly in fat content, the effect of total dietary fat on DHA accumulation in plasma phospholipids was evaluated. Plots of the effect of dietary ALA on plasma DHA levels for low, moderate and high LA:ALA were consistent for the 3 levels of dietary fat, namely 11.8%, 22.2% and 39.9% energy, and provided



**Fig. 4.** Three dimensional (3D) plot of plasma phospholipid DHA relative to dietary LA and ALA (as percent dietary energy). Individual raw data values are represented by ( $\bullet$ ). The 3D surface was fitted to the raw data.

evidence that the effects of dietary ALA were independent of total fat in the diet (Fig. 3). In addition, regression analysis failed to detect an effect of dietary fat content on DHA accumulation.

The overall relationship between both dietary LA and ALA and plasma phospholipid DHA is illustrated in Fig. 4. A three-dimensional surface was fitted to 263 data points for individual animals (black dots) to better visualize the complex relationship between dietary LA, dietary ALA and plasma phospholipid DHA. The figure highlights the fact that high plasma phospholipid DHA levels are limited to a small area corresponding to low levels of both dietary ALA ( $\sim 1$ – $3$  en%) and LA ( $\sim 1$ – $2$  en%). Beyond 3 en% of either ALA or LA the rate of change in plasma DHA levels is reduced but DHA still decreases in response to dietary PUFA.

### 3.2.2. Diet-induced changes in n-6 fatty acids

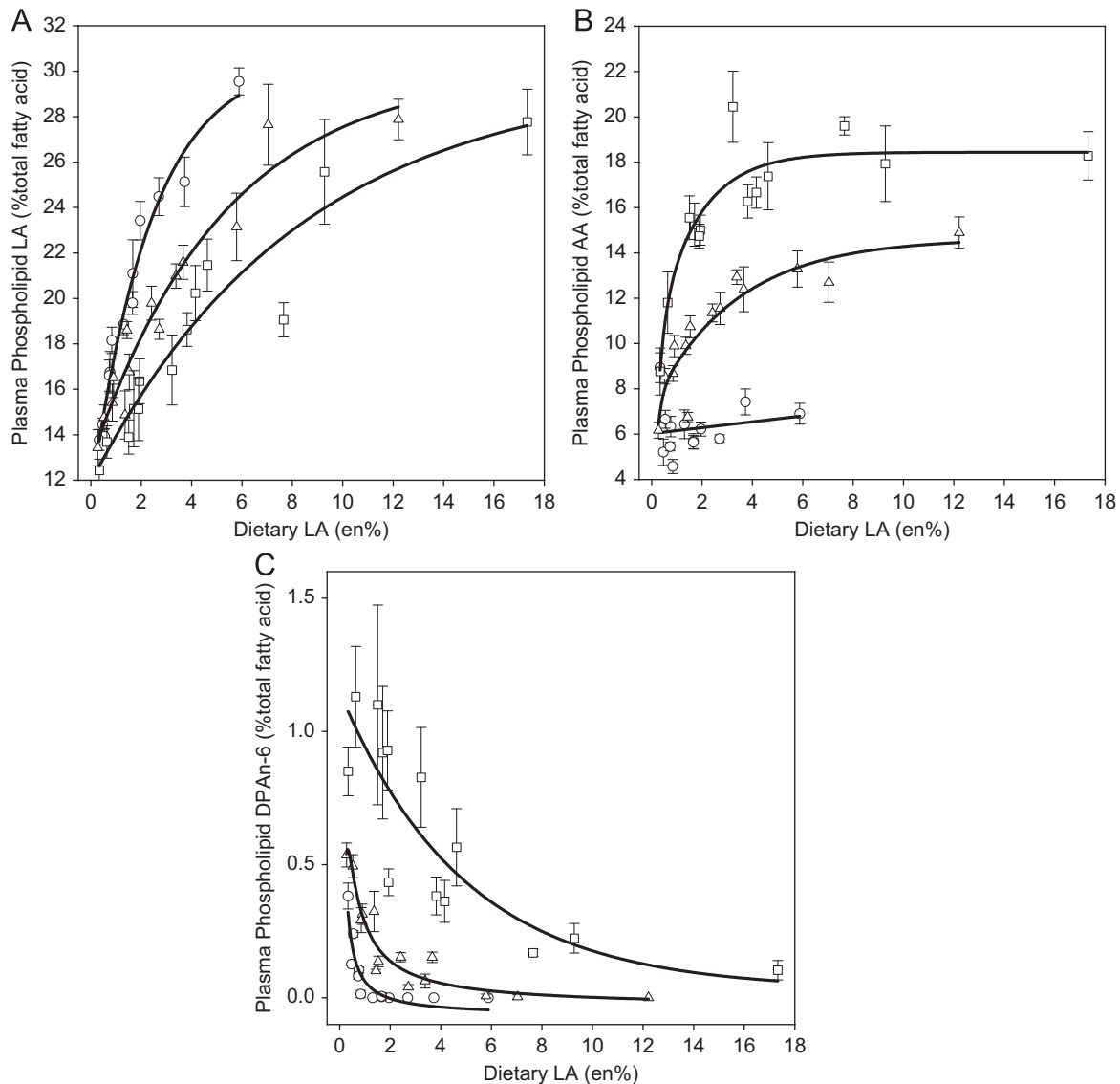
Plasma phospholipid LA levels increased in a curvilinear manner to between 28% and 30% of total fatty acids at all dietary LA:ALA ratios (Fig. 5A). Unexpectedly, ALA rich diets (low LA:ALA, 0.5:1–0.8:1) were the most efficient at increasing plasma phospholipid LA levels.

In contrast, the curves of plasma phospholipid AA appeared fully saturable reaching a maximum at relatively low levels of dietary LA of around 4 en% over the range of substrate tested (Fig. 5B). However, the absolute level of AA in the phospholipid fraction was directly related to the LA:ALA ratio, with AA curves increasing to a maximum of about 14% and 18% at moderate and high LA:ALA ratios respectively. Plasma phospholipid AA was unrelated to dietary LA when diets with a low LA:ALA ratio were fed.

Like its homolog DHA, plasma phospholipid DPAn-6 level was sensitive to the level of dietary PUFA with maximal levels occurring below  $\sim 2$  en% LA (Fig. 5C). Although the absolute maximum level of plasma phospholipid DPAn-6 was lower than DHA, it was dependent on the dietary LA:ALA ratio with a high ratio resulting in the highest levels.

## 4. Discussion

Our study has established that diets based on vegetable oils that contain the 18-carbon precursor fatty acid, ALA, as the only



**Fig. 5.** Effect of dietary LA level and LA:ALA ratio on plasma phospholipid n-6 fatty acids. LA:ALA ratios low 0.5:1–0.8:1 ( $\Delta$ ), moderate 1.9:1–2.6:1 ( $\square$ ) and high 7.4:1–11.3:1 ( $\circ$ ). Fatty acids depicted are LA (A), AA (B) and DPAn-6 (C) compared to dietary LA (as a percent of total dietary energy). Data are mean  $\pm$  SD ( $n=5$  rats per group).

dietary source of n-3 fats can result in a substantial accumulation of DHA into plasma phospholipids, but only when the level of dietary PUFA is low. The concentrations of DHA attained can be as high as those seen in rats fed 10% fish oil, containing preformed DHA [26]. A feature of the accumulation of DHA in the present study, which is presumed to be due to endogenous conversion from ALA, is that it is highly sensitive to substrate concentration. Below approximately 1 en% ALA (and 2 en% total PUFA) the relationship between dietary ALA and plasma phospholipid DHA was positive and linear. These results are consistent with those reported by Bourre et al. [27]. Our study further demonstrated that above 2 en% dietary PUFA, accumulation of plasma DHA was negatively related to dietary ALA. This may partially explain the inability of diets high in ALA to increase plasma and tissue DHA levels [16,18,19,21].

A possible explanation for these results was provided in 1991 by Sprecher et al. [28] who published the first of a series of papers that clearly demonstrated that the conversion of ALA to DHA involved two uses of the delta 6 desaturase (D6D), the first involving the conversion of ALA to stearidonic acids (SDA, 18:4n-3) and the second involving the conversion of 24:5n-3 to 24:6n-3 (Fig. 1). We interpret this to mean that both 18-carbon

polyunsaturated substrates, LA and ALA, have the capacity to inhibit the conversion of ALA through to DHA at two points. Firstly LA could compete with ALA for the D6D in the conversion of ALA to SDA and secondly both LA and ALA could compete with 24:5n-3 to reduce production of 24:6n-3. Our interpretation is supported by the findings that 18-carbon PUFA have a higher substrate affinity for the D6D than 24-carbon substrates [29]. Recently, a ALA study in rats [17] conducted by our group suggests that when increasing dietary ALA levels from 0.2 to 2.9 en% against a constant LA level (1 en%), the endogenous synthesis of n-3 LCPUFA from the precursor ALA is regulated independently of changes in the expression and of the synthetic enzymes or regulatory transcription factor, and provides evidence that n-3 LCPUFA synthesis is regulated more by substrate competition for existing enzymes than by an increase in their mRNA expression. In addition, it has been reported that DHA synthesis can be limited by the level of elongases [30] and by the limited accumulation of ALA into the hepatic phospholipid pool [31].

As our results reflect the net effect of synthesis and incorporation we are also aware that part of the decrease in the proportion of plasma phospholipid DHA with high PUFA diets (> 2 en%) could be due to competition with other fatty acids for

incorporation into phospholipids which is known to occur [32,33]. Furthermore, redistribution of DHA from one tissue to another has been reported in very young rats and our data may also partially reflect this process [34]. Interestingly, Blasbalg et al. [24] have recently pointed out that while the level of LCPUFA in the diet of Americans has not fallen over the last 50 years, the LCPUFA status of the population has fallen and suggest that this is due to the higher intakes of LA in the diet. We have pointed out in a primate model that high LA diets inhibit the incorporation of n-3 LCPUFA [33]. Clearly our results can partially be explained by PUFA preventing the incorporation of synthesized LCPUFA.

Regardless of the mechanisms involved, our study highlights the fact that plasma DHA levels are tightly controlled by dietary PUFA and our results help to explain a range of seemingly contradictory evidence from both animal and human studies. Support for our finding that DHA accumulation is maximal only when low PUFA (< 2 en%) diets are consumed comes from several sources. In his seminal studies Holman [35] showed significant increases in DHA of total liver fatty acids in rats fed 1% calories as ALA but only with levels of dietary LA less than 3% of energy. We have previously shown that Hooded Wistar rats fed a low PUFA olive oil diet (*i.e.* 0.9 en% LA and 0.12 en% ALA) had a plasma phospholipid DHA level 2.6 times that of rats fed a high n-6 PUFA sunflower diet (6.8 en% LA and 0.19 en% ALA) and equal DHA levels to those fed a high PUFA linseed oil diet (2.0 en% LA and 5.5 en% ALA) [26]. Similar results have been reported by Lands et al. [36] and Naughton et al. [37] who demonstrated that rats fed low PUFA diets had higher levels of DHA in plasma phospholipids than rats fed a basal diet containing higher levels of PUFA. More recently, Guesnet et al. [38] have demonstrated a clear linear inverse relationship between dietary LA and n-3 LCPUFA levels in rats. Finally, there are human data that support our observations. Courage et al. [39] showed that term infants fed evaporated milk (< 1 en% LA plus ALA, LA:ALA ratio 2:1) had a 45% higher level of DHA in erythrocyte membranes at 6 months of age compared to infants fed a standard formula with high PUFA levels (7 en% PUFA: LA:ALA=10:1).

Our current data may also explain the many animal and human studies that have failed to achieve a notable increase in DHA levels despite large increases in the level of dietary ALA. As it had previously been assumed that increasing DHA was simply a matter of increasing the amount of ALA in the diet, there have been many attempts to increase the content of DHA in mammalian tissues by this means [16,18–20,40]. We have previously conducted four studies, two in infants [19,21] one in piglets [16] and one in rats [17], that resulted in only marginally increased DHA levels in plasma phospholipids in response to increasing dietary ALA levels. In a study with adults [20] we increased dietary ALA levels to 5.3 en% with a LA:ALA ratio of 0.6:1 but saw no increase in the proportion of plasma phospholipid DHA relative to those on a standard Australian diet (LA:ALA=10:1; 6 en% total PUFA). An examination of these and other studies revealed that without exception they were conducted using diets with a total dietary PUFA level in excess of 3 en% and/or a dietary ALA level of greater than 1 en%. The data from the present study clearly show that at such a level of dietary PUFA, increases in dietary ALA, expressed either as a total amount or a changed LA:ALA ratio, would be without significant effect on DHA accumulation in plasma.

Tracer studies consistently show that ALA can be converted to DHA but the consensus is that the rate of conversion is low (1–5%). Our results suggest that the rate of synthesis will vary according to the fatty acid composition and total PUFA content of the background diet. For example, a stable isotope study by Pawlosky et al. [41] in adults found that there was a very low conversion of ALA to both EPA (0.2%) and DHA (~0.1%) but the

study was conducted against a background diet with a LA:ALA ratio of 7.4:1 and a low en% value of dietary ALA (0.72 en%). Goyens et al. have provided evidence that as little as 1% of ALA incorporated into plasma phospholipids was converted to DHA in subjects consuming high PUFA (7.5 en%, LA:ALA ratio 17:1) diets [42]. Our data show that within these dietary parameters, there would be little EPA or DHA synthesized and incorporated into plasma phospholipids. Other tracer studies conducted in human infants suffer from similar limitations [43,44]. There is a need to repeat such studies in animals and humans with diets that we estimate can maximize DHA synthesis *i.e.*, when the LA:ALA ratio is low and the total dietary PUFA is less than 2 en%. Such diets could be essential fatty acid replete but allow full expression of fatty acid synthesis and may give us new data to assess whether humans actually do have lower rates of desaturation than rats.

Our study provides *in vivo* evidence consistent with the fatty acid pathway proposed by Sprecher et al. [28] in which conversion of DHA from ALA and DPAn-6 from LA involves the D6D being utilized twice. Our results also support the *in vitro* work of Geiger et al. who showed that both dietary LA and ALA are strong inhibitors of the conversion of the 24-carbon D6D precursor fatty acids to subsequent metabolites [29]. As our *in vivo* studies have been conducted using natural oils, the results can be applied to general fatty acid metabolism and nutrition of monogastric animals and humans. While tracer and *in vitro* studies can be extremely useful, it may be that only *in vivo* models give a true indication of how tissues respond overall. Despite the fact that our study has the limitation that it measured the net effect of dietary fats and thus cannot necessarily distinguish between effects of synthesis and incorporation, it has the advantage of general applicability to animal and human diets.

Overall, our study highlights the fact that it is possible to enhance the level of DHA in plasma of animals fed diets containing only ALA as a source of n-3 fatty acids. If these data can be extended to the human situation it could explain why preformed DHA needs to be added to diets for both infants and adults. Both dietary regulations for infant formulas and the current intake of 18-carbon PUFA in adults greatly exceed the optimal window for endogenous DHA accumulation [45]. The implications of our results suggest the need to lower the level of dietary PUFA which is counter-intuitive and may be difficult to translate in the short to medium term. Thus the current dietary intake of dietary PUFA would seem to create a conditional essentiality of DHA which has special relevance to the animal and human food industry and to human health.

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## References

- [1] E.E. Birch, D.G. Birch, D.R. Hoffman, R. Uauy, Dietary essential fatty acid supply and visual acuity development, *Invest. Ophthalmol. Vis. Sci.* 33 (1992) 3242–3253.
- [2] R.D. Uauy, D.G. Birch, E.E. Birch, J.E. Tyson, D.R. Hoffman, Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates, *Pediatr. Res.* 28 (1990) 485–492.
- [3] M.S. Lamptey, B.L. Walker, Learning behavior and brain lipid composition in rats subjected to essential fatty acid deficiency during gestation, lactation and growth, *J. Nutr.* 108 (1978) 358–367.

- [4] M. Neuringer, W.E. Connor, C. Van Petten, L. Barstad, Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys, *J. Clin. Invest.* 73 (1984) 272–276.
- [5] S. Reisbick, M. Neuringer, E. Gohl, R. Wald, G.J. Anderson, Visual attention in infant monkeys: effects of dietary fatty acids and age, *Dev. Psychol.* 33 (1997) 387–395.
- [6] M. Makrides, R.A. Gibson, A.J. McPhee, et al., Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial, *Jama* 301 (2009) 175–182.
- [7] S.E. Carlson, A.J. Ford, S.H. Werkman, J.M. Peebles, W.W. Koo, Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin, *Pediatr. Res.* 39 (1996) 882–888.
- [8] M. Makrides, M. Neumann, K. Simmer, R. Gibson, J. Pater, Are long-chain polyunsaturated fatty acids essential nutrients in infancy? *Lancet* 345 (1995) 1463–1468.
- [9] P. Willatts, J.S. Forsyth, M.K. DiModugno, S. Varma, M. Colvin, Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age, *Lancet* 352 (1998) 688–691.
- [10] G.E. Billman, J.X. Kang, A. Leaf, Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs, *Circulation* 99 (1999) 2452–2457.
- [11] P.L. McLennan, Myocardial membrane fatty acids and the antiarrhythmic actions of dietary fish oil in animal models, *Lipids (suppl.)* 36 (2001) S111–114.
- [12] P. Nestel, H. Shige, S. Pomeroy, M. Cehun, M. Abbey, D. Raederstorff, The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans, *Am. J. Clin. Nutr.* 76 (2002) 326–330.
- [13] D.S. Kelley, I.L. Rudolph, Effect of individual fatty acids of omega-6 and omega-3 type on human immune status and role of eicosanoids, *Nutrition* 16 (2000) 143–145.
- [14] B. Meyer, N. Mann, J. Lewis, G. Milligan, A. Sinclair, P. Howe, Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids, *Lipids* 38 (2003) 391–398.
- [15] F. Domergue, A. Abbadi, E. Heinz, Relief for fish stocks: oceanic fatty acids in transgenic oilseeds, *Trends Plant Sci.* 10 (2005) 112–116.
- [16] C. Blank, M.A. Neumann, M. Makrides, R.A. Gibson, Optimizing DHA levels in piglets by lowering the linoleic acid to alpha-linolenic acid ratio, *J. Lipid Res.* 43 (2002) 1537–1543.
- [17] W.C. Tu, R.J. Cook-Johnson, M.J. James, B.S. Mühlhäusler, R.A. Gibson, Omega-3 long chain fatty acid synthesis is regulated more by substrate levels than gene expression, *Prostaglandins Leukot. Essent. Fatty Acids* 83 (2010) 61–68.
- [18] E. Mantzioris, M.J. James, R.A. Gibson, L.G. Cleland, Dietary substitution with an alpha-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues, *Am. J. Clin. Nutr.* 59 (1994) 1304–1309.
- [19] M. Makrides, M.A. Neumann, B. Jeffrey, E.L. Lien, R.A. Gibson, A randomized trial of different ratios of linoleic to alpha-linolenic acid in the diet of term infants: effects on visual function and growth, *Am. J. Clin. Nutr.* 71 (2000) 120–129.
- [20] E. Mantzioris, M.J. James, R.A. Gibson, L.G. Cleland, Differences exist in the relationships between dietary linoleic and alpha-linolenic acids and their respective long-chain metabolites, *Am. J. Clin. Nutr.* 61 (1995) 320–324.
- [21] K.J. Clark, M. Makrides, M.A. Neumann, R.A. Gibson, Determination of the optimal ratio of linoleic acid to alpha-linolenic acid in infant formulas, *J. Pediatr.* 120 (1992) S151–158.
- [22] H. Sprecher, Q. Chen, F.Q. Yin, Regulation of the biosynthesis of 22:5n-6 and 22:6n-3: a complex intracellular process, *Lipids* 34 (1999) S153–156.
- [23] H. Sprecher, D. Luthria, B. Mohammed, S. Baykousheva, Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids, *J. Lipid Res.* 36 (1995) 2471–2477.
- [24] T.L. Blasbalg, J.R. Hibbeln, C.E. Ramsden, S.F. Majchrzak, R.R. Rawlings, Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century, *Am. J. Clin. Nutr.* 93 (2011) 950–962.
- [25] E.G. Blish, W.J. Dyer, A rapid method for total lipid extraction and purification, *Can. J. Biochem. Physiol.* 37 (1959) 911–917.
- [26] R.A. Gibson, M.J. James, M.A. Neumann, J.S. Hawkes, L.G. Cleland, Incorporation of dietary oleate, linoleate, alpha-linolenate and eicosapentaenoate into the plasma lipid fractions of four strains of rat, *Biochim. Biophys. Acta* 1126 (1992) 49–52.
- [27] J.M. Bourre, M. Francois, A. Youyou, et al., The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats, *J. Nutr.* 119 (1989) 1880–1892.
- [28] A. Voss, M. Reinhart, S. Sankarappa, H. Sprecher, The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase, *J. Biol. Chem.* 266 (1991) 19995–20000.
- [29] M. Geiger, B.S. Mohammed, S. Sankarappa, H. Sprecher, Studies to determine if rat liver contains chain-length-specific acyl-CoA 6-desaturases, *Biochim. Biophys. Acta* 1170 (1993) 137–142.
- [30] M. Igarashi, J.C. DeMar Jr., K. Ma, L. Chang, J.M. Bell, S.I. Rapoport, Upregulated liver conversion of alpha-linolenic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet, *J. Lipid Res.* 48 (2007) 152–164.
- [31] P.L. Goyens, M.E. Spilker, P.L. Zock, M.B. Katan, R.P. Mensink, Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio, *Am. J. Clin. Nutr.* 84 (2006) 44–53.
- [32] L.G. Cleland, M.J. James, M.A. Neumann, M. D'Angelo, R.A. Gibson, Linoleate inhibits EPA incorporation from dietary fish-oil supplements in human subjects, *Am. J. Clin. Nutr.* 55 (1992) 395–399.
- [33] E.J. McMurchie, J.A. Rinaldi, S.L. Burnard, et al., Incorporation and effects of dietary eicosapentaenoate (20:5(n-3)) on plasma and erythrocyte lipids of the marmoset following dietary supplementation with differing levels of linoleic acid, *Biochim. Biophys. Acta* 1045 (1990) 164–173.
- [34] W. Lefkowitz, S.Y. Lim, Y. Lin, N. Salem Jr., Where does the developing brain obtain its docosahexaenoic acid? Relative contributions of dietary alpha-linolenic acid, docosahexaenoic acid, and body stores in the developing rat, *Pediatr. Res.* 57 (2005) 157–165.
- [35] R.T. Holman, Nutritional and metabolic interrelationships between fatty acids, *Fed. Proc.* 23 (1964) 1062–1067.
- [36] W. Lands, A. Morris, B. Libelt, Quantitative effects of dietary polyunsaturated fats on the composition of fatty acids in rat tissues, *Lipids* 25 (1990) 505–516.
- [37] J.M. Naughton, A.J. Sinclair, K. O'Dea, M.S. Steel, Effects of dietary butter enrichment on the fatty acid distribution of phospholipid fractions isolated from rat platelets and aortae, *Biochim. Biophys. Acta* 962 (1988) 166–172.
- [38] P. Guesnet, S.M. Lallemand, J.M. Alessandri, M. Jouin, S.C. Cunnane,  $\alpha$ -Linolenate reduces the dietary requirement for linoleate in the growing rat, *Prostaglandins Leukot. Essent. Fatty Acids* 85 (2011) 353–360.
- [39] M.L. Courage, U.R. McCloy, G.R. Herzberg, et al., Visual acuity development and fatty acid composition of erythrocytes in full-term infants fed breast milk, commercial formula, or evaporated milk, *J. Dev. Behav. Pediatr.* 19 (1998) 9–17.
- [40] C.L. Jensen, T.C. Prager, J.K. Fraley, H. Chen, R.E. Anderson, W.C. Heird, Effect of dietary linoleic/alpha-linolenic acid ratio on growth and visual function of term infants, *J. Pediatr.* 131 (1997) 200–209.
- [41] R.J. Pawlosky, J.R. Hibbeln, J.A. Novotny, N. Salem Jr., Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans, *J. Lipid Res.* 42 (2001) 1257–1265.
- [42] P.L. Goyens, M.E. Spilker, P.L. Zock, M.B. Katan, R.P. Mensink, Compartmental modeling to quantify alpha-linolenic acid conversion after longer term intake of multiple tracer boluses, *J. Lipid Res.* 46 (2005) 1474–1483.
- [43] N. Salem Jr, B. Wegher, P. Mena, R. Uauy, Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants, *Proc. Natl. Acad. Sci.* 93 (1996) 49–54.
- [44] T.U. Sauerwald, D.L. Hachey, C.L. Jensen, H. Chen, R.E. Anderson, W.C. Heird, Effect of dietary alpha-linolenic acid intake on incorporation of docosahexaenoic and arachidonic acids into plasma phospholipids of term infants, *Lipids (Suppl.)* 31 (1996) S131–135.
- [45] R. Gibson, M. Makrides, alpha-Linolenate reduces the dietary requirement for linoleate in the growing rat, *Prostaglandins Leukot. Essent. Fatty Acids* 85 (2011) 403–404.