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

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\textbf{A R T I C L E   I N F O}

Keywords:
Alpha linolenic acid (ALA)
Docosahexaenoic acid (DHA)
Linoleic acid (LA)
LA:ALA ratio

\textbf{A B S T R A C T}

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. Weaning 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 (\(>85\) 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 \textit{ad libitum} for 21 day. Groups of
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, 2.2 en% 20:4n-6, 0.8:1 (low), 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.
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 DPA-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 DPA-3 increase was directly proportional to dietary ALA and rose to about 5% and

<table>
<thead>
<tr>
<th>Diet #</th>
<th>Total saturates</th>
<th>Total monounsaturates</th>
<th>LA (en%)</th>
<th>ALA (en%)</th>
<th>Total PUFA (en%)</th>
<th>LA:ALA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.0 (5.5)</td>
<td>17.0 (4.5)</td>
<td>1.97 (0.4)</td>
<td>2.87 (0.5)</td>
<td>2.97 (0.5)</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>19.2 (5.5)</td>
<td>17.2 (4.5)</td>
<td>1.97 (0.4)</td>
<td>2.87 (0.5)</td>
<td>2.97 (0.5)</td>
<td>1:1</td>
</tr>
<tr>
<td>3</td>
<td>19.4 (5.5)</td>
<td>17.4 (4.5)</td>
<td>1.97 (0.4)</td>
<td>2.87 (0.5)</td>
<td>2.97 (0.5)</td>
<td>1:1</td>
</tr>
<tr>
<td>4</td>
<td>19.6 (5.5)</td>
<td>17.6 (4.5)</td>
<td>1.97 (0.4)</td>
<td>2.87 (0.5)</td>
<td>2.97 (0.5)</td>
<td>1:1</td>
</tr>
<tr>
<td>5</td>
<td>19.8 (5.5)</td>
<td>17.8 (4.5)</td>
<td>1.97 (0.4)</td>
<td>2.87 (0.5)</td>
<td>2.97 (0.5)</td>
<td>1:1</td>
</tr>
<tr>
<td>6</td>
<td>20.0 (5.5)</td>
<td>18.0 (4.5)</td>
<td>1.97 (0.4)</td>
<td>2.87 (0.5)</td>
<td>2.97 (0.5)</td>
<td>1:1</td>
</tr>
</tbody>
</table>

# Table 1

Fatty acid composition of diets.

a LA:ALA ratio rounded to the nearest whole number (with the exception of 0.5:1 diets).

b Results are expressed as % of total fatty acids with the energy % in parentheses.
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 \( \frac{1}{7} \) C24 en% ALA when LA:ALA ratios were low, \( \frac{1}{7} \) C24 en% ALA when LA:ALA ratios were modest and \( \frac{1}{7} \) C24 en% when LA:ALA ratios were high. Above these levels of dietary ALA, tissue DHA levels were inversely related to dietary ALA regardless

### Table 2

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

<table>
<thead>
<tr>
<th></th>
<th>5 wt% fat diet</th>
<th>10 wt% fat diet</th>
<th>20 wt% fat diet</th>
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</thead>
<tbody>
<tr>
<td>Fat blend (see Table 1)</td>
<td>5</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Starch</td>
<td>41.75</td>
<td>36.75</td>
<td>26.75</td>
</tr>
<tr>
<td>Dextrinised starch</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
</tr>
<tr>
<td>dl Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>AIN 93 minerals</td>
<td>3.5</td>
<td>3.3</td>
<td>3.5</td>
</tr>
<tr>
<td>AIN 93 vitamins</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Total E (KJ/100 g)*</td>
<td>1569</td>
<td>1674</td>
<td>1884</td>
</tr>
</tbody>
</table>

* 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.

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**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 (A), moderate 1.9:1–2.6:1 (A) and high 7.4:1–11.3:1 (A). 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 ± SD (n=5 rats per group).
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 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 (1–3 en%) and LA (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 ~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
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 n-3 LCPUFA 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 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, Guenst 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. Coward 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 linear inverse relationship between dietary LA and n-3 LCPUFA levels (7 en% PUFA; LA:ALA ratio 2:1). 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 Pawlowski 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.

Acknowledgments

We thank Ela Zielinski, Dani-Louise Bryan, Stuart Finlay and Roxanne Portolesi for their technical expertise. This study was supported in part from grants from Wyeth Nutrition International, Channel 7 Children’s Research Foundation, the Child Health Research Institute and the National Health and Medical Research Council (NHMRC). Robert Gibson is a NHMRC Senior Research Fellow.

References


