Differential Tissue Dose Responses of (n-3) and (n-6) PUFA in Neonatal Piglets Fed Docosahexaenoate and Arachidonoate

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Abstract

Docosahexaenoic acid (DHA) and arachidonic acid (ARA) are commonly added to infant formula worldwide; however, dietary concentrations needed to obtain optimal tissue levels have not been established. Hence, we studied tissue responses in piglets fed various doses of DHA and ARA. Doses were 0, 1, 2, and 5 times those used in U.S. infant formulas and DHA/ARA in Diet 0, Diet 1, Diet 2, and Diet 5 were 0, 4.1/8.1, 8.1/16.2, and 20.3/40.6 mg/100 kJ formula, respectively. Supplementation of dietary DHA and ARA increased DHA in brain, retina, liver, adipose tissue, plasma, and erythrocyte by 1.1- to 25.8-fold of Diet 0 (P-trend < 0.01). Tissue ARA (1.1- to 6.0-fold of Diet 0) responded to dietary ARA in liver, adipose tissue, plasma, and erythrocytes (P-trend < 0.05); brain and retina ARA was, however, unresponsive to dietary DHA and ARA. Plasma and erythrocyte DHA were positively associated with DHA in neural (brain and retina) and visceral (liver and adipose) tissues (r2 = 0.11–0.56; P < 0.001–P = 0.042). Plasma and erythrocyte ARA did not correlate with neural ARA. Only plasma ARA was associated with liver ARA (r2 = 0.222; P = 0.02) and adipose ARA (r2 = 0.867; P < 0.001) and erythrocyte ARA correlated with adipose ARA (r2 = 0.470; P < 0.001). We conclude that dietary DHA supplementation affords an effective strategy for enhancing tissue DHA, ARA in visceral but not neural tissues is sensitive to dietary ARA, and erythrocyte and plasma DHA can be used as proxies for tissue DHA, although blood-borne ARA is not an indicator of neural ARA. J. Nutr. 137: 2049–2055, 2007.

Introduction

The 2 long chain PUFA (LCPUFA),10 docosahexaenoic acid [DHA, 22:6(n-3)] and arachidonic acid [ARA, 20:4(n-6)], are common ingredients of infant formulas worldwide and are present in >75% of infant formula consumed in the United States. DHA and ARA constitute >25% of total fatty acids in the central nervous system (1). DHA is essential for retinal development (2) and ARA is important for ensuring normal growth (3). The most rapid period of human brain growth occurs from the last trimester in utero and continues up to y 2 of postnatal life (4). During this period, developing human infants satisfy their needs for DHA and ARA by placental transfer, breast milk, or formula, from which they obtain DHA or its precursors for endogenous synthesis. Meta-analysis also supports the efficacy of (n-3) LCPUFA intake in early visual system development (5).

Studies employing stable isotope have demonstrated that human term and preterm infants (6,7) as well as nonhuman primates (8) are capable of converting the (n-3) and (n-6) precursors, α-linolenic acid [LNA, 18:3(n-3)] and linoleic acid [LA, 18:2(n-6)], to DHA and ARA in vivo. However, these studies do not establish whether conversion is sufficient to meet the needs of the developing central nervous system. Regardless, the conversion ratio of shorter chain precursors to DHA or ARA during the perinatal period is highly variable (6–9).

Increasing LNA has not proven effective in increasing DHA levels (10). Supplementing DHA and eicosapentaenoic acid in absence of ARA (11,12) increases DHA but reduces the tissue ARA in association with poorer growth. Thus, it is generally agreed that both LCPUFA should be provided simultaneously (13). One recent report demonstrated that the amount of DHA currently added in U.S. infant formula does not to normalize cerebral cortex DHA to that of breast-fed infant baboons (1).
However, very few studies document the responses of tissue composition to increased dietary DHA and ARA, although there are studies in blood compartments of human infants (14,15) and adults (16). The piglet is an animal model highly relevant to humans because of the similarity of timing of the brain growth spurts in the 2 species (17) and the similarity of PUFA metabolism in the 2 omnivorous species.

Herein, we report on the effectiveness of incorporating DHA and ARA into piglet blood, liver, adipose, and neural tissue by increasing dietary DHA and ARA at levels 1, 2, and 5 times that commonly found in commercially available infant formula. This study focused on compositional changes in overall tissue DHA and ARA in response to dietary LCPUFA doses; tissue fatty acid accumulation was therefore analyzed based on a total lipid basis. We also investigated whether blood-borne DHA and ARA can be proxies for respective LCPUFA in various neural and peripheral tissues.

Materials and Methods

Experimental details were described in detail in a previous report (18) and will be outlined here. The animal protocol was approved by the Cornell University Institutional Animal Care and Use Committee (IACUC).

Experimental design. A randomized block design was used in this experiment. Twenty-four male piglets of normal gestation from 6 sows bred at the Cornell University swine farm were randomly assigned to 4 replicates of a factorial experimental design using 4 dietary fat blends per dose and 3 dietary DHA and ARA values corresponding to 1, 2, and 5 times the amount of DHA and ARA most commonly used in human infant formulas (19). Details for preparing liquid formula have been reported (18).

Fatty acid analysis-GC. Tissue lipids were extracted by the method of Bligh and Dyer (20) and they underwent derivatization to methyl ester using boron trifluoride-methanol. The methylated fatty acids were quantified using a Hewlett-Packard 5890 gas chromatograph equipped with a BPX-70 (SGE) capillary column (60 m × 0.25 μm), as reported previously (21).

Data analysis. Values are means ± SD. Group differences were tested using 1-way ANOVA and post hoc analysis with the least significant differences test was used to identify differences among 4 dietary treatment groups. Linear regression analysis was used to determine dose response (P-trend) of each fatty acid to dietary LCPUFA supplementation, relationships between DHA and ARA in the blood and in various tissues, and relationships between tissue DHA response and dietary LCPUFA doses administered. P-values < 0.05 were considered significant. All analyses were performed using SPSS version 11.5.

Results

(n-3) Fatty acid accretion. Accretion of DHA in the tissues examined increased with increasing doses of dietary DHA (Tables 3–5). Inclusion of preformed DHA elevated brain (visual cortex) (P-trend < 0.001), retina (P-trend = 0.001), liver (P-trend < 0.001), plasma (P-trend < 0.001), and erythrocyte (P-trend = 0.002) DHA to 1.1- to 2.9-fold of the Diet 0 group. In adipose tissue of the Diet 0 group, we detected only 0.06% DHA, whereas in piglets receiving LCPUFA supplements, we found 4.8- to 24.8-fold more DHA (P-trend < 0.001). In brain and retina, 18:3(n-3) was found in trace amounts; its accumulation in liver and adipose tissue was similar in the 4 diet groups. On the other hand, increasing the levels of dietary LCPUFA reduced plasma and erythrocyte 18:3(n-3) (P-trend = 0.014 and < 0.001, respectively). Accumulation of 22:5(n-3) in brain (P-trend = 0.012) and erythrocyte (P-trend = 0.003) decreased as LCPUFA increased in the diet. However, 22:5(n-3) in retina (P-trend = 0.014) and adipose tissue (P-trend = 0.001) increased with increasing LCPUFA.

TABLE 1 Fatty acid composition of formulas

<table>
<thead>
<tr>
<th></th>
<th>Diet 0</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA+MUFA</td>
<td>79.58 ± 0.42</td>
<td>80.12 ± 0.32</td>
<td>76.54 ± 0.35</td>
<td>76.22 ± 0.51</td>
</tr>
<tr>
<td>n-6</td>
<td>17.48 ± 0.36</td>
<td>16.63 ± 0.27</td>
<td>17.79 ± 0.30</td>
<td>19.14 ± 0.38</td>
</tr>
<tr>
<td>n-3</td>
<td>2.12 ± 0.07</td>
<td>2.33 ± 0.04</td>
<td>2.73 ± 0.05</td>
<td>3.65 ± 0.11</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>17.04 ± 0.11</td>
<td>15.52 ± 0.16</td>
<td>15.95 ± 0.04</td>
<td>14.98 ± 0.07</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>1.46 ± 0.01</td>
<td>1.33 ± 0.01</td>
<td>1.32 ± 0.01</td>
<td>1.22 ± 0.00</td>
</tr>
<tr>
<td>20:4(n-6)/18:3(n-3)</td>
<td>11.67 ± 0.00</td>
<td>11.67 ± 0.07</td>
<td>12.08 ± 0.07</td>
<td>12.29 ± 0.09</td>
</tr>
<tr>
<td>ARA</td>
<td>0.01 ± 0.00</td>
<td>0.03 ± 0.02</td>
<td>0.19 ± 0.00</td>
<td>3.15 ± 0.00</td>
</tr>
<tr>
<td>DHA</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.00</td>
<td>0.70 ± 0.01</td>
<td>1.66 ± 0.02</td>
</tr>
<tr>
<td>ARA/ARA</td>
<td>1.85 ± 0.01</td>
<td>1.84 ± 0.01</td>
<td>1.90 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 2.
TABLE 3  Accretion of (n-6) and (n-3) fatty acid in brain and retina of piglets fed different levels of DHA and ARA

<table>
<thead>
<tr>
<th>Diet</th>
<th>SFA</th>
<th>MUFA</th>
<th>18:2 (n-6)</th>
<th>20:4 (n-6)</th>
<th>22:4 (n-6)</th>
<th>22:5 (n-6)</th>
<th>18:3 (n-3)</th>
<th>22:5 (n-3)</th>
<th>22:6 (n-3)</th>
<th>P-trend2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 0</td>
<td>41.56 ± 9.53</td>
<td>29.02 ± 9.58</td>
<td>1.41 ± 0.20a</td>
<td>9.45 ± 1.25</td>
<td>3.85 ± 0.56</td>
<td>4.41 ± 0.64a</td>
<td>0.01 ± 0.01</td>
<td>0.55 ± 0.07a</td>
<td>8.11 ± 1.46c</td>
<td></td>
</tr>
<tr>
<td>Diet 1</td>
<td>47.91 ± 0.84</td>
<td>22.60 ± 2.50</td>
<td>1.10 ± 0.05b</td>
<td>9.05 ± 0.38</td>
<td>3.42 ± 0.17</td>
<td>3.27 ± 0.25b</td>
<td>0.02 ± 0.02</td>
<td>0.38 ± 0.07b</td>
<td>10.87 ± 1.72b</td>
<td></td>
</tr>
<tr>
<td>Diet 2</td>
<td>42.63 ± 7.67</td>
<td>25.81 ± 5.69</td>
<td>1.00 ± 0.20bc</td>
<td>9.94 ± 1.50</td>
<td>3.78 ± 0.58</td>
<td>3.06 ± 0.76b</td>
<td>0.01 ± 0.01</td>
<td>0.33 ± 0.06b</td>
<td>12.07 ± 1.56b</td>
<td>14.67 ± 2.28b</td>
</tr>
<tr>
<td>Diet 5</td>
<td>40.51 ± 7.73</td>
<td>25.59 ± 2.75</td>
<td>0.84 ± 0.10c</td>
<td>10.74 ± 1.95</td>
<td>3.76 ± 1.10</td>
<td>2.27 ± 0.59b</td>
<td>0.01 ± 0.01</td>
<td>0.35 ± 0.12b</td>
<td>16.47 ± 2.28b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TABLE 4  Accretion of (n-6) and (n-3) fatty acid in liver and adipose tissue of piglets fed different levels of DHA and ARA

<table>
<thead>
<tr>
<th>Diet</th>
<th>SFA</th>
<th>MUFA</th>
<th>18:2 (n-6)</th>
<th>20:4 (n-6)</th>
<th>22:4 (n-6)</th>
<th>22:5 (n-6)</th>
<th>18:3 (n-3)</th>
<th>22:5 (n-3)</th>
<th>22:6 (n-3)</th>
<th>P-trend2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 0</td>
<td>40.98 ± 3.49</td>
<td>15.45 ± 1.04</td>
<td>1.98 ± 0.39a</td>
<td>7.83 ± 0.66</td>
<td>2.01 ± 0.19a</td>
<td>2.47 ± 0.46a</td>
<td>0.06 ± 0.14</td>
<td>0.56 ± 0.04b</td>
<td>15.05 ± 1.24c</td>
<td></td>
</tr>
<tr>
<td>Diet 1</td>
<td>41.98 ± 3.56</td>
<td>13.75 ± 0.90</td>
<td>1.92 ± 0.77a</td>
<td>8.24 ± 1.16</td>
<td>1.58 ± 0.19a</td>
<td>1.07 ± 0.21b</td>
<td>0.01 ± 0.01</td>
<td>0.50 ± 0.08b</td>
<td>18.90 ± 2.87b</td>
<td></td>
</tr>
<tr>
<td>Diet 2</td>
<td>43.43 ± 1.04</td>
<td>12.94 ± 0.74</td>
<td>1.76 ± 0.32b</td>
<td>7.62 ± 0.73</td>
<td>1.73 ± 0.17b</td>
<td>1.05 ± 0.29b</td>
<td>0.00 ± 0.00</td>
<td>0.66 ± 0.08b</td>
<td>23.68 ± 1.53b</td>
<td></td>
</tr>
<tr>
<td>Diet 5</td>
<td>42.39 ± 1.42</td>
<td>11.84 ± 0.84</td>
<td>1.22 ± 0.21b</td>
<td>7.89 ± 0.60</td>
<td>1.54 ± 0.21b</td>
<td>0.62 ± 0.15c</td>
<td>0.01 ± 0.02</td>
<td>0.67 ± 0.14b</td>
<td>23.53 ± 5.53b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 6 per treatment diet. Means in a row with superscripts without a common letter differ, P < 0.05.
2 Relation between tissue fatty acid and dietary DHA+ARA doses; liver DHA responded to doses of supplemental DHA, P < 0.05; neural ARA were, however, unresponsive to supplemental ARA.

(n-6) Fatty acid accretion. Accumulation of ARA in neural tissues (brain and retina) was similar in the 4 groups (Table 3). In contrast, ARA accretion with dietary ARA in liver (P-trend = 0.018), adipose tissue (P-trend < 0.001), plasma (P-trend < 0.001), and erythrocytes (P-trend < 0.001) (Tables 4, 5). ARA levels were similar in the Diet 0, Diet 1, and Diet 2 groups; the Diet 5 group showed a 1.1-fold increase over Diet 0 group and caused the overall trend to be significant (P-trend = 0.018). In adipose tissue, ARA levels increased from 1.8-fold of the Diet 0 group in Diet 1 group to 6-fold in the Diet 5 group (Table 4).
Increased LCPUFA in the diet significantly reduced 18:2(n-6) incorporation in brain (P-trend < 0.001), retina (P-trend = 0.004), liver (P-trend < 0.001), plasma (P-trend < 0.001), and erythrocytes (P-trend < 0.001); adipose 18:2(n-6), however, did not differ among the 4 groups. For 22:5(n-6), increased LCPUFA in the diet decreased its incorporation in brain (P-trend < 0.001), retina (P-trend < 0.001), liver, (P-trend < 0.001), and erythrocytes (P-trend = 0.004). In plasma and adipose tissue, 22:5(n-6) accretion was unaffected by LCPUFA in the diet (Tables 3–5).

**Correlation between blood and tissue DHA or ARA.**
Overall, both plasma and erythrocyte DHA correlated (P < 0.001–P = 0.042; r² = 0.11–0.56) with all tissues examined (brain, retina, liver, and adipose tissue), whereas plasma and erythrocyte ARA did not correlate with brain or retina ARA. Plasma ARA showed a correlation with liver ARA (r² = 0.222; P = 0.02) and adipose tissue ARA (P < 0.001; r² = 0.867); erythrocyte ARA correlated only with adipose ARA (P < 0.001; r² = 0.470) (Tables 6,7). Plasma and erythrocyte DHA (P = 0.002; r² = 0.374) were correlated as well as ARA (P < 0.001; r² = 0.488) (Tables 6,7).

**Predicted equation for brain DHA subject to dietary DHA and ARA supplementation.** Equations were obtained to predict brain and retina DHA levels in response to formula supplementation with DHA and ARA at doses up to 5 times that of infant formula using simple linear regression. Both brain (P < 0.001; r² = 0.622) and retina (P = 0.001; r² = 0.390) DHA was linearly related to dietary LCPUFA supplementation according to the following equations:

- Brain DHA (%): 0.298 Diet 1 DHA (mg/100 kJ) + 9.015 (P < 0.001; r² = 0.622).
- Retina DHA (%): 0.379 Diet 1 DHA (mg/100 kJ) + 17.208 (P = 0.001; r² = 0.390).

Neither regression model had a significant litter effect; thus, only dietary treatments were included in the regression analysis.

**Discussion**
This study established the differential responses of DHA and ARA accretion in blood, neural, and peripheral tissues of neonatal piglets fed with increasing doses of dietary DHA (0.15–0.76% of energy) and ARA (0.30–1.53% of energy), equivalent to 1–5 times that found in human infant formula. The highest levels correspond to ~16 times the daily consumption estimated for human infants (18). Overall, we found that DHA levels of total lipids increased 1.1- to 2.9-fold of the Diet 0 group in brain, retina, liver, adipose tissue, plasma, and erythrocyte in a

**TABLE 6** Relationship between plasma or erythrocyte DHA and tissue DHA in piglets fed different levels of DHA and ARA

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>p</th>
<th>SE</th>
<th>P</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain DHA</td>
<td>1.08</td>
<td>0.35</td>
<td>0.005</td>
<td>0.303</td>
</tr>
<tr>
<td>Retina DHA</td>
<td>1.07</td>
<td>0.63</td>
<td>0.042</td>
<td>0.114</td>
</tr>
<tr>
<td>Liver DHA</td>
<td>1.50</td>
<td>0.37</td>
<td>&lt;0.001</td>
<td>0.431</td>
</tr>
<tr>
<td>Adipose tissue DHA</td>
<td>0.30</td>
<td>0.055</td>
<td>&lt;0.001</td>
<td>0.584</td>
</tr>
<tr>
<td>Erythrocyte DHA</td>
<td>0.90</td>
<td>0.25</td>
<td>0.002</td>
<td>0.374</td>
</tr>
</tbody>
</table>

1 Simple linear regression is used to illustrate correlations between blood (plasma and erythrocyte) and tissue DHA. P < 0.05 indicates significant correlation.
dose-dependent manner to supplementary dietary DHA (P-trend all < 0.05). ARA levels in the brain and retina were unaffected by increased doses of dietary ARA, whereas ARA in peripheral tissues (liver, adipose tissues, and blood) increased 1.1- to 2.9-fold of the Diet 0 piglets in response to increased ARA in the diet.

To date, there are very few controlled DHA and ARA dose-response studies in humans or animals. Consistent with our findings, Abedin et al. (22) studied phospholipid DHA and ARA profile in guinea pigs fed single cell DHA and ARA at 2 levels, 0.6/0.9% and 1.8/2.7%, corresponding to the Diet 2 and Diet 5 groups in our experiment. They found that DHA in the brain phosphatidylethanolamine and retina phospholipids increased to 1.8- and 2.6-fold of unsupplemented group but observed no changes in brain and retina ARA. Two other studies of neonatal pigs also showed that retinal phosphatidylcholine DHA rose significantly (10% greater) and that ARA in retinal phosphatidylethanolamine and phosphatidylcholine were unchanged after DHA and ARA supplementation at concentrations of 0.4-0.8% (weight percent) and 0.5–1.0%, which corresponded to our Diet 1 and Diet 2 groups (23,24). A recent study in baboons showed dose-dependent increases in cerebral cortex DHA using total lipid basis (25). Our results using tissue total lipid, as well as previous animal studies using tissue phospholipid (22,23), indicate that there was only a moderate 1.1- to 1.5-fold increase of the Diet 0 group in brain or retina DHA with dietary supplementation of 0.3–0.8% DHA and 0.5–1.0% ARA, whereas increases in the liver, erythrocyte, and plasma were more pronounced (1.4- to 14.4-fold of the Diet 0).

Besides phospholipid and total lipid, incorporation of DHA or ARA into triglyceride or specific phospholipid fractions of peripheral tissue (liver, heart, and kidney) and brain have also been examined in piglets fed 0.3% dietary DHA or 0.8% dietary ARA (26). Peripheral triglyceride and phospholipid DHA and ARA both responded to dietary DHA or ARA supplementation. In contrast to our results, their brain DHA and ARA in all phospholipid classes examined were resistant to supplemental DHA and ARA. Incorporation rates and turnover of LCPUFA in specific lipid fractions differ and thus may contribute to differential responses of tissue to dietary LCPUFA among study designs. Studies are needed to examine the impact of compositional change on functional outcomes for establishing optimal tissue LCPUFA levels.

In human infants and adults, the augmentation of formulas (Table 6), the β (slope) values for erythrocyte DHA in predicting tissue DHA (brain, retina, liver, and adipose) were all greater than those for plasma DHA. For example, we found that a 1% increase in erythrocyte fatty acid predicted a 2.42% increase in retina, whereas a 1% increase in plasma DHA only predicted a 1.07% increase in retina DHA. Although retina DHA was approximately twice as sensitive as brain DHA to erythrocyte levels, it also appeared that brain DHA and retina DHA had the same sensitivity to changes of plasma DHA. Plasma lipids are composed of different lipid classes (triacylglycerol, phospholipids, and cholesterol ester). Short-term changes in plasma levels of a specific class of lipid, for instance changes in triacylglycerol due to postprandial chylomicronemia, will influence overall fatty acid composition independent of long-term fatty acid intake. On the other hand, erythrocyte lipids are predominantly phospholipids, being considered as valid biomarkers for LCPUFA in the diet (31) and as an index for average tissue exposure to ω-3 LCPUFA, because they are less susceptible to postprandial lipemia than plasma (32). In this study, both plasma and erythrocyte DHA appeared to be valid proxies for prediction of tissue DHA changes. When basing estimates on total lipids, erythrocyte DHA is a better index of tissue LCPUFA than plasma DHA, because erythrocytes are much less sensitive to postprandial blood composition.

On the other hand, plasma and erythrocyte ARA did not correlate with ARA in neural tissues, brain, and retina; they were only significantly correlated with peripheral tissues such as liver and adipose. Because ARA serves as an immediate substrate for bioactive eicosanoids, ARA levels may be highly regulated in neural-related tissues. Thus, elevation of blood ARA may be only transient due to the role of blood as a nutrient transport medium, whereas tissue ARA may not respond to dietary manipulation.

In contrast to the dose-dependent relationship between tissue and dietary DHA, incorporation of DHA related to dietary LNA is in a curvilinear manner, with maximum DHA incorporation at LNA/LNA ratios between 2:1 and 4:1 (33). However, formula with LNA/LNA ratios reduced to even 3:1 are unable to elevate DHA comparable to breast-fed infants (10,34,35). Isotope studies illustrate that dietary DHA is 7-fold more efficacious than LNA in supplying brain DHA (36) and only 1.4% of LNA is converted to LCPUFA (37). This is partially explained by the
fact that β-oxidation is the major route for LNA disposition rather than conversion to DHA (38).

In conclusion, increasing dietary levels of microbial-derived dietary DHA and ARA supplements ranging from 1 to 5 times those added to human infant formula were efficacious in elevating DHA levels in brain, retina, liver, adipose tissue, plasma, and erythrocyte in a dose response manner. ARA accumulation in brain and retina was unresponsive to dietary ARA and accumulation in liver, adipose tissue, plasma, and erythrocyte responded positively to dietary ARA concentration. Erythrocyte and plasma total DHA correlated with neural (brain and retina) and peripheral (liver and adipose tissue) tissue DHA, indicating that blood-borne DHA were proxies for tissue levels and the correlations were fully elucidated by dosage effect of both DHA and ARA. Plasma and erythrocyte total ARA correlated to some peripheral tissues but not brain and retina ARA; thus, they cannot be used as markers to predict neural ARA.

Previously, we reported no adverse effects on clinical chemistry or histopathologic indications of toxic effects in the piglets fed single cell DHA and ARA up to levels (0.34–3.15%) corresponding to a daily consumption 16 times that of human infants (18). Our findings of tissue efficacy in this report add to our previous report regarding safety at the high dietary DHA and ARA levels. Thus, whether DHA supplementation higher than 0.36%, corresponding to concentrations in term infant formula, may confer additional benefits should be investigated further and the optimal intake of DHA with or without ARA should be determined for neurodevelopment.

**Literature Cited**


