Retinal Functions of Young Dogs Are Improved and Maternal Plasma Phospholipids Are Altered with Diets Containing Long-Chain n-3 Polyunsaturated Fatty Acids during Gestation, Lactation, and after Weaning 1–3

John E. Bauer, Kimberly M. Heinemann, George E. Lees, and Mark K. Waldron

* Department of Small Animal Clinical Sciences, † Comparative Nutrition Laboratory, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843 and ** Nestle-Purina Pet Care, St. Louis, MO

EXPANDED ABSTRACT

In humans and animals, brain and retinal functions are dependent on the n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA 22:6 n-3) during in utero development and postnatal life. The period of maximum brain growth in humans begins in the third trimester of gestation, peaks at birth, and continues throughout the first 18 months of neonatal life (1–4). It is during this crucial period that accretion of arachidonic acid (AA) and DHA in brain and retinal phospholipids proceeds at a rate 10 times faster than incorporation via chain elongation and desaturation of their respective precursors, linoleic acid (LA) and α-linolenic acid (ALA) (5,6). Retinal DHA is highly conserved and has an important role in neurologic function (7). Canine retina is capable of synthesizing DHA from its 22-carbon precursor, docosapentaenoic acid (22:5 n-3, DPA) (8). Bauer et al. reported DPA accumulation, but not DHA, in canine plasma phospholipids when the precursor ALA is fed (9). It therefore is likely that plasma DPA provides substrate for DHA synthesis in canine retina and other nervous tissues. Thus, a dietary source of preformed DHA or one of its precursors may be necessary during gestation and suckling for normal neural development in dogs.

The electroretinogram (ERG) is a sensitive measure of retinal function in humans and animals (10,11). It quantifies photoreceptor responses and their subsequent postsynaptic signals to a series of varying-intensity flash stimuli and includes the responses of many retinal cell types. Major ERG components have been studied in dogs (12), which appear to be a suitable model for studies of human retinal physiology and pathology (13).

The present study investigated ERGs of puppies born to mothers fed diets varying in marine (n-3) long-chain polyunsaturated fatty acid (LCPUFA) content. Maternal plasma phospholipid (PL) fatty acids were also evaluated during gestation and lactation to evaluate diet effects on fatty acid metabolism during canine reproduction.

MATERIALS AND METHODS

An existing breeding colony of dogs provided bred dogs and their puppies for this study as previously described (14). Dogs were individually maintained in kennels according to the American Physiological Society Guidelines for Animal Research. Protocols were approved by the Texas A&M University Animal Care and Use Committee. Twelve clinically normal female colony dogs were randomly assigned to 1 of 4 diet groups (n = 3 per group). The 4 diets were prepared by Nestle-Purina PetCare, St. Louis, MO and have been described previously (15). All diets contained adequate LA with varying amounts of ALA and (n-3) LCPUFA [i.e., 20/22 carbon (n-3) fatty acids]. The diets were designated Lo/Lo (control diet), Lo/Mod, Lo/Hi, and Hi/Lo, which refer to their ALA/(n-3) LCPUFA concentrations. Diet fatty acid compositions have been previously reported (15) (Table 1). The diets were fed from the time of estrus and artificial insemination.
throughout gestation, parturition, and lactation. Sufficient quantities were available during pregnancy to maintain weight gain of the bitches in the latter stages of gestation by adjusting amounts fed as necessary. Puppies born in each litter were normal and allowed to suckle ad libitum, which supplied exclusive nutrition during this time. At 21 d of age, gruel consisting of the mothers’ respective diets and water was offered to the puppies 3 times a day in addition to suckling. Gradually, the time puppies spent suckling was decreased until they were completely weaned by d 42. On weaning, puppies were continued on the same diet as their mothers until 12 wk of age.

Blood samples were collected into EDTA containing tubes from the mothers via jugular vena puncture at d 3, 7, 14, 28, 42, and 56 during gestation and d 10 and 28 during lactation. Food had been withheld from each dog overnight prior to taking the blood samples. Plasma was harvested from each sample immediately after collection. Plasma total lipids were extracted using chloroform:methanol (2:1, vol:vol) and subsequently separated into subclasses via thin-layer chromatography (16). Fatty acid methyl esters were prepared, and fatty acid profiles were determined via gas chromatography (16). Statistical analyses were performed by repeated-measures ANOVA with Bonferroni comparisons performed at \( P < 0.05 \) for plasma PL fatty acids (Statistix 8.0, Analytical Software).

At 12 wk of age, ERG assessment of puppy retinal development was performed. Each eye was tested separately using a series of square-wave flash stimuli. Details of the procedure have been described previously (15). The parameters used to assess ERG characteristics were a- and b-wave amplitudes, a- and b-wave implicit times, and threshold intensities. Statistical analyses were performed on ERG parameters using data obtained from the intensity of flash 8 via 1-way ANOVA with Bonferroni comparisons performed at \( P < 0.05 \). Because each eye was tested separately, sample sizes were twice the number of puppies in each group.

\section*{RESULTS}

\subsection*{Maternal plasma phospholipid fatty acids during gestation and lactation}

All dogs had similar plasma phospholipid (PL) fatty acid profiles at the beginning of the study. No time effects or time \( \times \) diet interactions \( (P < 0.05) \) were found. Consequently, the data from all time points during gestation were analyzed for main diet effects.

During gestation, LA was lower in dogs fed the Lo/Mod and Lo/Hi \( (P < 0.05) \) diets, and ALA was markedly higher in the Hi/Lo group compared with the other groups \( (\text{Table 1}) \). Plasma PL EPA, DPA, and DHA were higher with the marine oil PUFA diets than with controls \( (P < 0.05) \). Plasma EPA and DPA were also higher compared with controls when the Hi/Lo diet was fed \( (P < 0.05) \). However, it should be noted that no elevation of DHA was observed when dogs were fed this ALA-rich diet.

No time or time \( \times \) diet effects were observed in the fatty acid profiles between lactation d 10 and 28, and the data were again tested for main diet effects. During this period, plasma PL LA was higher in the Hi/Lo group than in the Lo/Hi group, and ALA was higher in the Hi/Lo group compared with all others \( (P < 0.05) \) \( (\text{Table 1}) \). Plasma PL EPA and DPA were higher in all \( (n-3) \)-enriched diet groups \( (P < 0.05) \) compared with the Lo/Lo diet. A further increase in EPA was also seen in dogs fed the Lo/Hi diet \( (P < 0.05) \) compared with the other \( (n-3) \)-enriched diet groups \( (\text{Table 1}) \). Plasma DHA was higher in dogs fed the Lo/Hi diet \( (P < 0.05) \), whereas elevations of DHA in the Lo/Mod and Hi/Lo groups failed to reach significance \( (P < 0.06 \text{ and } P < 0.07, \text{ respectively}) \).

Although plasma PL fatty acids were similar during both gestation and lactation, one most notable difference was that plasma PL AA was markedly lower during lactation \( (P < 0.05) \). This effect appeared to be independent of diet fed.

\section*{Electroretinography}

The ERGs of puppies revealed higher a-wave amplitudes in the Lo/Hi and Hi/Lo groups compared with the other groups \( (P < 0.05, \text{ Table 2}) \).

The a-wave implicit time \( (a_0) \) of the Lo/Hi group was shorter than that of the Lo/Lo group \( (P < 0.05) \), indicating a quicker response to light in the former group. The b-wave response \( (b\text{-amp}) \) in the Lo/Hi group was higher than that in the Lo/Mod group \( (P < 0.05) \). Puppies in the Hi/Lo group elicited a quicker

\begin{table}[h]
\centering
\caption{Effects of varying the \( (n-3) \) fatty acid content of diets on major plasma phospholipids polyunsaturated fatty acids during gestation in dams\textsuperscript{f}}
\begin{tabular}{lcccc}
\hline
\multicolumn{5}{c}{Diet} \\
\hline
\multicolumn{1}{c|}{Fatty acid} & \multicolumn{1}{c}{Lo/Lo} & \multicolumn{1}{c}{Lo/Mod} & \multicolumn{1}{c}{Lo/Hi} & \multicolumn{1}{c}{Hi/Lo} \\
\hline
\multicolumn{5}{c}{\textit{Gestation}} \\
18:2(n-6) & 12.03 ± 1.96\textsuperscript{ab} & 11.71 ± 1.59\textsuperscript{b} & 8.11 ± 1.21\textsuperscript{b} & 14.97 ± 1.96\textsuperscript{a} \\
20:4(n-6) & 16.80 ± 4.04 & 16.20 ± 3.52 & 12.60 ± 3.2 & 13.80 ± 2.8 \\
18:3(n-3) & 0.20 ± 0.20\textsuperscript{a} & 0.12 ± 0.07\textsuperscript{a} & 0.12 ± 0.08\textsuperscript{a} & 1.89 ± 0.72\textsuperscript{b} \\
20:5(n-3) & 0.52 ± 0.29\textsuperscript{b} & 2.64 ± 1.00\textsuperscript{b} & 4.23 ± 1.07\textsuperscript{b} & 2.91 ± 1.30\textsuperscript{b} \\
22:6(n-3) & 1.52 ± 0.35\textsuperscript{b} & 2.57 ± 0.40\textsuperscript{b} & 2.07 ± 0.35\textsuperscript{ab} & 3.77 ± 0.94\textsuperscript{c} \\
22:6(n-3) & 0.46 ± 0.19\textsuperscript{b} & 3.87 ± 0.98\textsuperscript{b} & 5.31 ± 0.95\textsuperscript{b} & 0.67 ± 0.31\textsuperscript{b} \\
\multicolumn{5}{c}{\textit{Lactation}} \\
18:2(n-6) & 15.50 ± 1.98\textsuperscript{ab} & 15.80 ± 1.11\textsuperscript{ab} & 9.52 ± 1.69\textsuperscript{b} & 18.84 ± 3.01\textsuperscript{b} \\
20:4(n-6) & 8.05 ± 1.36 & 5.47 ± 0.73 & 6.72 ± 0.97 & 5.80 ± 0.51 \\
18:3(n-3) & 0.16 ± 0.06\textsuperscript{a} & 0.28 ± 0.11\textsuperscript{a} & 0.09 ± 0.09\textsuperscript{a} & 2.97 ± 0.94\textsuperscript{b} \\
20:5(n-3) & 0.59 ± 0.13\textsuperscript{a} & 4.52 ± 0.80\textsuperscript{b} & 9.05 ± 1.15\textsuperscript{c} & 3.85 ± 0.88\textsuperscript{c} \\
22:6(n-3) & 0.90 ± 0.28\textsuperscript{a} & 2.40 ± 0.65\textsuperscript{b} & 3.40 ± 0.76\textsuperscript{b} & 2.51 ± 0.49\textsuperscript{b} \\
22:6(n-3) & 0.49 ± 0.17\textsuperscript{a} & 2.70 ± 0.59\textsuperscript{ab} & 7.50 ± 2.10\textsuperscript{b} & 1.85 ± 0.50\textsuperscript{c} \\
\hline
\textsuperscript{f} Values are means ± SD, \( n = 3 \) dogs/group, at 6 gestation time periods and 2 lactation time periods. Means in a row with superscripts without a common letter differ, \( P < 0.05 \).
\end{tabular}
\end{table}
TABLE 2
ERG parameters of puppies fed diets varying in (n-3) fatty acid types and amounts1,2

<table>
<thead>
<tr>
<th>Diet</th>
<th>Lo/Lo, 36 eyes</th>
<th>Lo/Mod, 26 eyes</th>
<th>Lo/Hi, 20 eyes</th>
<th>Hi/Lo, 30 eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-amp, μV</td>
<td>31.6 ± 3.3b</td>
<td>24.6 ± 1.7a</td>
<td>49.5 ± 3.6b</td>
<td>43.5 ± 3.4b</td>
</tr>
<tr>
<td>a, ms</td>
<td>6.1 ± 0.4b</td>
<td>5.6 ± 0.3ab</td>
<td>4.4 ± 0.3ab</td>
<td>5.0 ± 0.3ab</td>
</tr>
<tr>
<td>b-amp, μV</td>
<td>172.3 ± 10.0ab</td>
<td>153.2 ± 9.7a</td>
<td>197.8 ± 10.6b</td>
<td>169.7 ± 7.8ab</td>
</tr>
<tr>
<td>b, ms</td>
<td>35.5 ± 0.7b</td>
<td>34.9 ± 0.6b</td>
<td>33.0 ± 1.0ab</td>
<td>32.1 ± 0.6b</td>
</tr>
<tr>
<td>t, intensity</td>
<td>6.2 ± 0.2b</td>
<td>5.8 ± 0.2ab</td>
<td>5.3 ± 0.1a</td>
<td>5.9 ± 0.1b</td>
</tr>
<tr>
<td>unit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, number of eyes per puppy group are indicated. Means in a row with superscripts without a common letter differ, P < 0.05. Both eyes of each animal were tested and analyzed statistically, as were mean values for each puppy; the statistical results were the same.
2 Amplitudes and implicit times were determined at flash 8 (third greatest flash intensity). Data reproduced from Heinemann et al. (15) with permission.

The ERGs of puppies revealed significantly improved visual performance in the Lo/Hi group with superior rod response as indicated by improved a-wave amplitudes and implicit times. Puppies in the lowest dietary (n-3) LCPUFA group exhibited the poorest ERG response. Initial intensity at which the a-wave was detectable (threshold intensity) demonstrated that retinal response of the (n-3) LCPUFA puppies occurred at lower light intensity, exhibiting greater rod sensitivity, than that in other groups. Preformed dietary (n-3) LCPUFA versus ALA is a more effective means of enriching maternal plasma DHA and results in improved visual performance in their puppies.

DISCUSSION

Plasma PL fatty acid modifications caused by diet were similar during both gestation and lactation in this study with a few exceptions. Dietary LA varied 2-fold among the experimental diets and resulted in a dose response of LA in the plasma PL during both gestation and lactation. However, plasma PL AA amounts were not significantly different among the groups during both periods, and only a modest decrease in AA in the high-(n-3) PUFA groups was seen. It has been previously shown that tissue AA becomes saturated at low concentrations of dietary LA (17). Thus, dietary LA in all groups in this study was adequate to induce tissue saturation. Moreover, both dietary LA and ALA in the Hi/Lo group were notably high, yet AA content was somewhat blunted compared with the other groups. Therefore, it is also likely that high dietary LA limited elongation of LA via competition for Δ⁶ desaturation to some extent.

One notable difference between gestation and lactation in this study was a marked decrease in the amount of plasma PL AA in lactating dogs compared with the gestation period. Although plasma AA among groups remained unchanged during lactation per se, a marked decrease in plasma PL AA occurred in lactation compared with gestation. Reasons for this finding are unclear, but a decrease in plasma PL AA has been previously reported in both lactating women and rats (18,19). One explanation may relate to metabolic demand for the provision of AA in milk fat during suckling, causing tissue depletion during this time. Similar results among the (n-3) fatty acids were not apparent in this regard, presumably because of the greater amounts of (n-3) fatty acids concomitantly supplied in the diets.

Nonetheless, this finding indicates the need to remain aware of maternal (n-6) fatty acid status in addition to the current interest in providing (n-3) fatty acids during reproduction.

The increases in amounts of plasma PL EPA and DPA during both gestation and lactation in the Hi/Lo group indicate active conversion of ALA to its longer-chain derivatives in these dogs. Notable, however, is that no accumulation of DHA was found. This finding has been previously reported by Bauer et al. (9) in normal adult dogs fed high-ALA (10% total fatty acids) diets and thus appears to occur during reproduction as well. Thus, as far as DHA synthesis is concerned in adult animals, its final conversion from ALA may be limited at some regulatory point within either neuronal tissues (20) or those with active peroxisomes (21). The data presented here emphasize the importance of a dietary supply of (n-3) LCPUFA, rather than ALA, during gestation.

When the marine oil-containing diets were fed, dose effects were observed for EPA and DHA during both gestation and lactation. However, differences in plasma PL DPA values were not seen. Because DPA is an intermediate between EPA and DHA, it may be metabolically shunted between EPA retro-conversion and DHA synthesis, depending on tissue needs, which may subsequently modify its tissue accumulation at a given time.

ACKNOWLEDGMENT

The technical assistance of Karen Bigley and Mary Sanders is gratefully acknowledged.

LITERATURE CITED


