Maternal Dietary Fatty Acids Modify Canine Puppy Plasma Lipoprotein Distributions during the Suckling Period¹,²

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EXPANDED ABSTRACT

KEY WORDS: • canine • puppy • fatty acids • suckling • lipoprotein • plasma • cholesterol

Polysaturated fatty acids (PUFAs)⁴ are important for neonatal growth and development in all mammalian species studied to date. Normal fetal development requires the 18-carbon essential fatty acids as well as long-chain polysaturated fatty acids (LCPUFAs) with 20- and 22-carbon–chain lengths (1). Provision of both dietary (n-3) and (n-6) PUFAs in appropriate amounts are needed to optimize development of nervous system tissue and to promote kidney, liver, and skin functions. Two LCPUFAs, arachidonic acid of the (n-6) series and docosahexaenoic acid of the (n-3) series, are synthesized by chain elongation and desaturation of essential fatty acids, have received increasing interest recently because they are found in high concentrations in the phospholipids of normal cell membranes of the central nervous system (2). It was shown (3) that human infants who consume low LCPUFA levels also have lower LCPUFA levels in the phospholipids of the cerebral cortex. The clinical relevance of this finding is the subject of various growth and neurodevelopment studies.

Because the PUFAs are transported via plasma lipoproteins (LPs), the effects of dietary fatty acids on LP metabolism may play an important role in development. In dogs, high-density lipoproteins (HDLs) are the predominant plasma LP fraction (75–85%) (4). This differs from humans, where low-density lipoproteins (LDLs) predominate. For this reason, dogs are considered to be “HDL mammals” (5,6) and also exhibit atherosclerosis-resistance properties (4).

However, as in humans, diet can alter canine LP cholesterol distributions. For example, a study by Bauer (7) demonstrated that dogs fed a diet that is high in saturated fat (primarily from beef tallow) show an increase in both LDL and HDL fractions, and all LP fractions tend to shift toward a lower density. Dreon et al. (8) also reported that feeding saturated fat increases both LDL and HDL concentrations in humans. Replacing saturated fat with unsaturated fat in the diet decreases LDL more than HDL in humans. Polysaturated fatty acids are the most potent fatty acids for reducing LDL cholesterol (9).

An early study of canine LP metabolism showed that immature dogs had greater hepatic LDL receptor activity than mature dogs. This activity was undetectable by 24 mo of age. Canine liver contains two distinct LP receptors. There is an apo-protein-B,E (apo-B,E) receptor, which binds both LDL and HDL cholesterol, and an apo-E receptor, which binds only HDL cholesterol. The apo-B,E receptor is seen in immature, growing dogs, whereas only the apo-E receptor is found in adult dogs (10). This same study also revealed that feeding cholesterol can suppress apo-B,E receptors in immature dogs, whereas in mature dogs, prolonged fasting can induce expression of apo-B,E receptors. The apo-E hepatic membrane receptors remain constant in both immature and adult dogs or decrease only slightly with age (10). Thus, the possibility exists that immature dogs have greater concentrations of LDL fractions during early life, and dietary fatty acids may further modify plasma LP fractions overall.

The present study was conducted to investigate plasma LP distribution changes during suckling and early adolescence of puppies born to bitches that were fed diets that varied only in fatty acid composition and were otherwise identical.

1 Presented as part of the WALTHAM International Science Symposium: Nature, Nurture, and the Case for Nutrition held in Bangkok, Thailand, October 28–31, 2003. This symposium and the publication of the symposium proceedings were sponsored by the WALTHAM Centre for Pet Nutrition, a division of Mars, Inc. Symposium proceedings were published as a supplement to The Journal of Nutrition. Guest editors for this supplement were D’Ann Finley, James G. Morris, and Quinton R. Rogers, University of California, Davis.

2 This work was supported in part by Nestle-Purina PetCare and the Mark L. Morris Professorship in Clinical Nutrition at Texas A&M University.

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4 Abbreviations used: ALA, α-linolenic acid; HDL, high-density lipoprotein; LA, linoleic acid; LCPUFA, long-chain polysaturated fatty acids; LDL, low-density lipoprotein; LP, lipoprotein; PUFAs, polysaturated fatty acids.


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MATERIALS AND METHODS

Twelve clinically normal, sexually intact female dogs were randomly assigned to one of four diet groups. The diets were formulated to contain adequate amounts of linoleic acid (LA) that ranged from 1.2 to 3.5% dry matter, based on recommendations of the Association of American Feed Control Officials. The diets also contained varying amounts of α-linolenic acid (ALA) using linseed oil and (n-3) LCPUFAs (i.e., 20/22-carbon (n-3) fatty acids) from menhaden fish oil. The groups were designated Lo/Lo, Hi/Lo, Lo/Mod, and Lo/Hi, according to their ALA/(n-3) LCPUFA concentrations (Table 1). All other diet ingredients including protein, nitrogen-free extract, and vitamin and mineral sources were identical, which resulted in diets with similar nutrient concentrations except for fatty acid types. The diet compositions are described in a companion article (11). Each bitch was fed her randomly assigned diet from the time of insemination throughout gestation, parturition, and lactation according to her body condition. Sufficient quantities of the diets were fed to maintain a 25% weight gain of each bitch's nonpregnant body weight during the latter stages of gestation by adjusting the amounts fed as necessary. At 21 d of age, gruel consisting of the mothers' respective diets and water were offered to the puppies three times a day in addition to suckling. Gradually, the time the puppies spent suckling was decreased until they were completely weaned by d 42. Upon weaning, puppies were continued on the same diet as their mothers until 10 wk of age.

Blood samples were collected into EDTA-containing tubes from puppies at ages 4, 10, 28, and 70 d. Puppies were fasted for 2 h on d 4, 10, and 28 and were fasted overnight on d 70 before blood was taken. Plasma was harvested from the blood samples by slow-speed centrifugation. Samples were either analyzed fresh on the day of collection (via electrophoresis) or were frozen at −45°C for subsequent analysis.

LP distribution was determined by electrophoresis on 1% agarose gel each blood collection day and was quantified by scanning densitometry (12). Total cholesterol values were determined enzymatically using a microplate reader (12). Results were compared using Statistix software by two-factor ANOVA for diet, time, and diet × time interaction effects and subsequently with Tukey's comparison of means where necessary. A P-value was set at P < 0.05 for all data to determine statistical significance.

RESULTS

Both time and diet main effects were found, but no statistically significant diet × time interactions were observed. The concentrations of cholesterol in chylomicra and pre-β-LP in plasma were highest at d 4 and decreased progressively with time to d 28, which was not significantly different from d 70 (Fig. 1). Elevations in total and β-LP cholesterol levels were also observed on d 4 and 10 compared with the later times (Figs. 1 and 2). The α-LP cholesterol levels remained unchanged during the suckling period but decreased after weaning (Fig. 3). The only statistically significant diet effect observed was decreased total plasma cholesterol (Fig. 2) and all LP cholesterol fractions in puppies whose mothers were fed either of the menhaden fish–oil diets (Lo/Mod and Lo/Hi groups) compared with the diets of the Lo/Lo and Hi/Lo groups (Figs. 1 and 2).

<table>
<thead>
<tr>
<th>Fatty acid concentrations of diets</th>
<th>Concentration</th>
<th>Lo/Lo</th>
<th>Hi/Lo</th>
<th>Lo/Mod</th>
<th>Lo/Hi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated relative %</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Saturated</td>
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<td>23.28</td>
<td>37.31</td>
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<tr>
<td>Monounsaturated relative %</td>
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<td>37.16</td>
<td>38.44</td>
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<td>0.04</td>
<td>0.29</td>
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</tr>
<tr>
<td>22:5Δ6(n-3)</td>
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<td>0.02</td>
<td>1.46</td>
<td>2.78</td>
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<tr>
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<td>0.09</td>
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<td>0.37</td>
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<tr>
<td>18:3Δ9(n-3)</td>
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<td>19.20</td>
<td>1.80</td>
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<tr>
<td>18:2Δ6(n-6)</td>
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<td>17.30</td>
<td>14.12</td>
<td>6.58</td>
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</tr>
</tbody>
</table>

Figure 1: Time and diet main effects on β-LP cholesterol in puppy plasma. Values are means ± SD. Sample sizes within study groups were as follows. For d 4, 10, and 28: Lo/Lo, 26; Hi/Lo, 24; Lo/Mod, 22; and Lo/Hi, 18. For d 70: Lo/Lo, 18; Hi/Lo, 16; Lo/Mod, 12; and Lo/Hi, 10. Letters not in common are significantly different; P < 0.05.
DISCUSSION

This work is the first report of higher LDL fractions in canine puppies during the suckling period than in mature canines. However, the young of other species have demonstrated high LP cholesterol concentrations as well. A study performed by Mott et al. (13) showed that breastfed baboon infants had higher serum cholesterol due to increased HDL1 and HDL2 cholesterol concentrations. In suckling kittens, Butterwick et al. (14) observed significantly higher plasma cholesterol concentrations compared with the same kittens after weaning and compared with cats in older age groups. As these animals mature, their cholesterol concentrations begin to decline, and there are no differences in lipid and LP cholesterol concentrations among adolescents, adults, and seniors. It was also shown in Sprague-Dawley rats (15) that total plasma cholesterol concentrations were increased during the suckling period but decreased after weaning. The findings of the present study also reflect a similar decrease in total plasma cholesterol with maturation. This decrease in cholesterol concentration is probably due to high tissue demands for growth and development (14). Also, it is important to note that from d 21 to 42, the puppies were suckling and were also fed gruel made of their respective diets. This could also account for the differences seen between early (d 4 and 10) and later (d 28 and 70) times of the perinatal period.

In addition to age-related effects, the results of the present study also show alterations in LP metabolism due to differences in maternal dietary fatty acids. These findings are consistent with the cholesterol-lowering effects and reduction of post-prandial LP fractions of dietary marine oils that are reported for adult humans and other species (16–18).

In human neonates (which differ from canine neonates), only a minor part of total cholesterol is transported via the β-LP fraction (19). Immature canines have a high β-LP concentration during early life (d 4 and 10). As a dog matures, the β-LP fraction decreases. However, in humans, the β-LP fraction increases with maturity; adult humans carry ~75% of their cholesterol in the LDL fraction (4). Mao and Hamosh (15) concluded that the enzymes associated with the metabolism of cholesterol are well developed in Sprague-Dawley rats shortly after birth. Based on the findings of our study, it is reasonable to also suggest that cholesterol metabolic enzymes are also well developed in puppies shortly after birth. The reduction in total plasma cholesterol concentration as the puppies mature indicates that the receptors and enzymes necessary for lipid metabolism are active in young puppies.

Studies designed to investigate LP metabolism and diet modifications in dogs during the early neonatal period can provide useful new information for the growth and development of dogs used in service activities as well as provide a model for later gestational development of humans. Several studies suggest that in different species, dietary intake during early life may have long-term effects on metabolic systems such as cholesterol and LP metabolism and offer evidence that breastfed neonates are provided with a better defense against atherosclerosis than those fed formula (20–22). This is interesting, because breast milk contains higher cholesterol and saturated fat compared with formula, but that is apparently more beneficial in the long term (23,24).
LITERATURE CITED