Feeding Infant Piglets Formula with Long-Chain Polyunsaturated Fatty Acids as Triacylglycerols or Phospholipids Influences the Distribution of These Fatty Acids in Plasma Lipoprotein Fractions

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ABSTRACT Several sources of long-chain polyunsaturated fatty acids (LCP) are currently available for infant formula supplementation. These oils differ in their fatty acid composition, the chemical form of the fatty acid esters [triacylglycerols (TG) or phospholipids (PL)] and presence of other lipid components. These differences may affect LCP absorption, distribution and metabolic fate after ingestion. The purpose of the present study was to evaluate the influence of different chemical forms of dietary LCP on the composition of plasma, plasma lipoproteins, liver and jejunal mucosa in infant pigs. Thirty pigs (5 d old) were bottle-fed different diets for 4 wk: a control diet (C), a diet containing LCP as TG from tuna and fungal oils (TF-TG) or a diet containing LCP as PL from egg yolk (E-PL). We measured lipid and fatty acid composition of plasma and lipoproteins, as well as lipid composition of liver and intestinal mucosa. The arachidonic and docosahexaenoic acids in HDL-PL were significantly higher in piglets fed the E-PL diet than in those fed the TF-TG diet. Opposite results were found in the LDL-PL diet. No significant differences were found between groups in TG or cholesteryl concentrations of plasma or lipoproteins. Arachidonic acid in plasma PL and cholesteryl esters was significantly higher in the E-PL group than in the TF-TG group. The chemical form in which LCP esters are present in different dietary sources influences their distribution in plasma lipoproteins. This may be important for infant nutrition and suggests that not all LCP sources may be biologically equivalent.


KEY WORDS: • lipoproteins • long-chain polyunsaturated fatty acids • phospholipids • piglets • triacylglycerols

Human milk contains high levels of long-chain polyunsaturated fatty acids (LCP)1 (Jensen et al. 1995). LCP with 20 and 22 carbon atoms, mainly arachidonic acid (AA) and docosahexaenoic acid (DHA), are critical components of cellular membranes and are especially enriched in the developing retina and gray matter of the brain (Bazan et al. 1986, Clindinin et al. 1980). Some reports indicate that LCP content is improved in infants fed LCP-containing formulas (Clandinin et al. 1992, Koletzko et al. 1989). In addition, other studies show that such improvement could be correlated with improved visual acuity and cognitive development (Birch et al. 1992a, 1992b and 1998, Carlson et al. 1993 and 1996, Makrides et al. 1995, Uauy et al. 1990). Based on these observations, international committees have recommended that infant formula, especially for preterm infants, be supplemented with AA and DHA at levels normally found in human milk (British Foundation 1992, ESPGAN 1991, FAO/WHO 1994, ISSFAL 1994). On the other hand, the need to supplement formulas for term infants with preformed LCP continues to be a matter of debate (Auestad et al. 1997, Heird et al. 1997).

Lipid components of infant formulas are obtained from vegetable oils, which contain fatty acids of up to 18 carbon atoms in length. For this reason, LCP supplementation to the diet requires other lipid sources, generally of animal origin. There are several LCP-enriched sources available, such as fish oils, oils from unicellular organisms and egg oil fractions. Fish and unicellular oils are mainly composed of triacylglycerols (TG), and commercial egg oil fractions are rich in phospholipids (PL). Furthermore, these lipid sources also differ in fatty acid composition, presence of other lipid components and the molecular structure of their TG and PL. Because lipid digestion is a complex process involving enzymes, it is possible that these differences between lipid sources affect LCP absorption, distribution and metabolic fate.

Triacylglycerols provide >90% of the total energy of dietary fat; therefore, TG digestion and absorption have been extensively studied. On the other hand, little information is available about dietary PL. Intestinal hydrolysis of TG and PL yields different products: 2-monocacylglycerols and free fatty acids in the case of TG and 1-lyso phospholipids and free fatty acids in the case of PL (Pufal et al. 1995, Thomson et al. 1988). Those products are taken up by enterocytes, reesterified and secreted into lymph chylomicrons.

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3 Abbreviations used: AA, arachidonic acid; DHA, docosahexaenoic acid; LCP, long-chain polyunsaturated fatty acids; PL, phospholipids; TG, triacylglycerols.
A number of studies have focused on TG absorption and metabolism, mainly on the importance of the sn-2 position (Christensen et al. 1995, Jensen et al. 1994, Pufal et al. 1985). Less attention has been paid to dietary PL, but there is evidence of impaired TG absorption when the supply of exogenous PL is insufficient for micelle formation during fat digestion and absorption (Levy and Roy 1989). Moreover, clinical trials with premature infants found that DHA from egg PL was better absorbed than DHA from breast milk and DHA TG from single-cell oils (Carnielli et al. 1995). Other studies have also shown a positive effect of PL-LCP supplementation on fat absorption (Morgan et al. 1998). PL are also essential for intestinal lipoprotein formation and for fat distribution outside the enterocytes. Data from animal studies suggest that the intraduodenal infusion of triolein results in the formation of chylomicrons, whereas the infusion of egg phosphatidylcholine favors the formation of intestinal VLDL-size particles (Tso et al. 1984).

Based on previous work, we hypothesized that the chemical structure and composition of LCP sources used to supplement infant formula may influence the distribution of these important fatty acids and that therefore LCP sources may not be biologically equivalent. For this purpose, we evaluated the effects of dietary LCP as TG or PL on plasma and plasma lipoproteins in infant piglets. Because small intestine and liver are key organs in the metabolism of lipoproteins, we also determined the lipid composition of jejenum and liver.

**MATERIALS AND METHODS**

**Animals and diets.** Thirty 5-d-old Yorkshire piglets at term gestation (obtained from Ntra. Sra. de las Mercedes Farm, La Guardia, Jaen, Spain) were randomly assigned to each of three dietary groups. Each group of piglets was housed together and freely fed by bottle three times a day for 4 wk. Heating was provided with spot heat lamps attached above each cage. The study was approved by the Animal Care Committee at the University of Granada and conforms to the European Union Regulation of Animal Care for the care and use of animals for research.

Three powdered formulas, identical in all ingredients except for fat composition, were designed to meet the nutrient requirement of growing piglets (Miller and Ullrey 1987). The formulas were dissolved in warm water at a concentration of 188 g/L. The general composition of the control formula has been previously reported (Lopez-Pedrosa et al. 1998). Control fat was composed of a blend of olive, soy and coconut oils and milk fat. LCP were added by supplementation with either tuna and fungal oil (TF-TG diet) or egg yolk PL (E-PL diet). The final fatty acid composition of the diets is given in Table 1. Tuna oil with a low 20:5(n-3)/22:6(n-3) ratio was supplied by Mochida (Tokyo, Japan), and fungal oil was supplied by SunTory (Tokyo, Japan). Part of the vegetable fat blend in the control formula (2.7 g/100 g) was replaced by tuna and fungal oils to reach 0.6 and 0.3 g of AA and DHA/100 g, respectively. E-PL (Ovothin 160) was supplied by Lucas Meyer (Hamburg, Germany). For this diet, 13.2 g/100 g of liver and intestinal mucosa was homogenized in distilled water and extracted with hexane/isopropanol (3:2). Lipid extracts were dissolved in chloroform, aliquots were taken in duplicate for each measurement and the solvent removed under nitrogen. Isopropanol (100 µL) was added to facilitate mixing with enzymatic reagents, and total and free cholesterol and triacylglycerol concentrations were determined through spectrophotometry with commercial kits (Roche Diagnostic GmbH, Mannheim, Germany). PL concentrations were determined as inorganic phosphorous after sample mineralization (Zivesmit et al. 1950).

Cholesterol and triglycerides in plasma were measured directly with the commercial kits mentioned and according to the supplier’s instructions. In lipoproteins, those lipids were measured by the same method but adapted for a microplate assay. PL in plasma and lipoproteins were also measured in lipid extracts (Zivesmit et al. 1950).

Lipid fractions from plasma and lipoprotein extracts were separated by thin-layer chromatography on Silica Gel 60 plates (0.5 mm; Merck, Darmstadt, Germany) using hexane/isopropanol ether/acetic acid (75:25:1,5) according to the method previously described by Skipaki and Barclay (1969).

To analyze the lipid composition, 0.2 g of liver and intestinal mucosa was homogenized in distilled water and extracted with hexane/isopropanol (3:2). Lipid extracts were dissolved in chloroform, aliquots were taken in duplicate for each measurement and the solvent removed under nitrogen. Isopropanol (100 µL) was added to facilitate mixing with enzymatic reagents, and total and free cholesterol and triacylglycerol concentrations were determined through spectrophotometry with commercial kits (Roche Diagnostic GmbH, Mannheim, Germany). PL concentrations were determined as inorganic phosphorous after sample mineralization (Zivesmit et al. 1950).

**Fatty acid composition of adapted milk formula (control) and the same diet supplemented with (n-6) and (n-3) long-chain polyunsaturated fatty acids from fungal and tuna oils (TF-TG) or from egg yolk phospholipids (E-PL)**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>TF-TG</th>
<th>E-PL</th>
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<tbody>
<tr>
<td>8:0</td>
<td>2.3</td>
<td>1.9</td>
<td>1.7</td>
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<tr>
<td>10:0</td>
<td>2.8</td>
<td>2.5</td>
<td>2.2</td>
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<tr>
<td>12:0</td>
<td>1.6</td>
<td>1.7</td>
<td>1.8</td>
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<tr>
<td>14:0</td>
<td>5.6</td>
<td>6.2</td>
<td>6.2</td>
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<tr>
<td>16:0</td>
<td>22.3</td>
<td>23.4</td>
<td>24.9</td>
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<tr>
<td>18:0</td>
<td>8.6</td>
<td>8.4</td>
<td>9.9</td>
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<tr>
<td>16:1(n-7)</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
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<tr>
<td>18:1(n-9)</td>
<td>36.7</td>
<td>35.8</td>
<td>35.2</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>13.6</td>
<td>12.2</td>
<td>11.5</td>
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<td>18:3(n-3)</td>
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<td>1.1</td>
<td>1.2</td>
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<tr>
<td>20:4(n-6)</td>
<td>0.1</td>
<td>0.6</td>
<td>0.6</td>
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<tr>
<td>22:6(n-3)</td>
<td>—</td>
<td>0.3</td>
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homogeneity of variances was analyzed by Levene’s test. If variances were not homogeneous, Welch’s test was used to study group differences. When a significance difference was found (P < 0.05), Bonferroni’s test was used to examine individual comparisons. Values in the text are means ± SEM.

RESULTS

Growth and formula intake. The overall mean body weight at the beginning of the study was 2321 ± 46.9 g. Body weight increased exponentially over time. No significant differences were found between groups, with the overall body weight at the end of the study 5848 ± 195.4 g. The daily energy intake was 1239 ± 23 kJ/kg body.

Fatty acid composition of plasma and plasma fractions. Proportions of AA and DHA in plasma total lipids and its fractions generally were higher in both LCP-supplemented groups than in the control group (Table 2). Oleic acid in plasma and plasma PL and CE were higher in the control group than in both LCP-supplemented groups, except in PL of piglets fed TF-TG. Proportions of 18:2(n-6) in plasma PL of control piglets were also higher than those of the experimental groups.

The proportion of AA in PL and CE was significantly higher in the E-PL group than in the TF-TG group. No significant differences were found in the fatty acid composition of plasma TG among the three groups.

Fatty acid composition of lipoproteins. As in plasma lipids, proportions of DHA were significantly greater in both LCP-supplemented groups compared with the control group in all lipoprotein fractions (Table 3). AA was also greater in HDL lipids of LCP-supplemented groups and in VLDL and LDL lipids of the E-PL group. On the other hand, the VLDL and HDL 18:1(n-9) level was lower in the E-PL and TF-TG groups than in the control group. A lower proportion of 18:2(n-6) was present in HDL from both LCP-supplemented groups compared with the control group. Proportions of AA and DHA in HDL-PL were significantly higher in piglets fed the E-PL diet than in the control and TF-TG piglets (Fig. 1). In LDL-PL, the TF-TG group had higher proportions of both AA and DHA than the E-PL and control groups.

Lipid composition of plasma, lipoproteins, liver and jejunum. No significant differences were found between the control and LCP-supplemented groups or between the LCP-supplemented groups in the concentrations of TG, PL and total, free and esterified cholesterol of plasma and lipoproteins (data not shown). Total cholesterol concentrations of liver was significantly lower in both LCP-supplemented groups than in the control group (data not shown). Concentrations of cholesterol, TG, and PL in liver did not differ between the E-PL and TF-TG groups. Lipid composition of jejunal mucosa did not differ between the study groups (data not shown).

DISCUSSION

There have been many studies dealing with the influence of LCP-supplemented diets during the postnatal period in experimental animals (Arburckle et al. 1991, Foote et al. 1990) and in infants (Birch et al. 1992a, 1992b and 1998, Carlson et al. 1993 and 1996, Chandrin et al. 1992, Koletzko et al. 1989, Makrides et al. 1995, Uauy et al. 1990). However, to our knowledge, only two of these studies compared diets containing LCP from different sources. In one study, a comparison of LCP absorption in preterm infants fed either breast milk, infant formula (without LCP), formula with LCP derived from TG or formula with LCP from PL was reported (Canini et al. 1995); the other study reported the fatty acid composition of brain cortical areas and non-neural tissues in piglets fed either sow’s milk, a control formula, formulas enriched with (n-3) fatty acids or formulas enriched with (n-3) and (n-6) fatty acids from either egg yolk or pig brain PL (Guastard-Langelier et al. 1999). None of them studied lipoprotein composition.

In the present study, we report the influence of dietary

| TABLE 2 |

Selected fatty acid composition of plasma lipids and its fractions in infant piglets fed the control diet or diets supplemented with long-chain polyunsaturated fatty acids (LCP) as triacylglycerols (TG) or phospholipids (PL).

<table>
<thead>
<tr>
<th></th>
<th>16:0</th>
<th>18:0</th>
<th>16:1(n-7)</th>
<th>18:1(n-9)</th>
<th>18:2(n-6)</th>
<th>AA</th>
<th>20:4(n-6)</th>
<th>22:5(n-6)</th>
<th>18:3(n-3)</th>
<th>20:5(n-3)</th>
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<td>g/100 g total fatty acids</td>
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<td><strong>Total lipids</strong></td>
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<tr>
<td>Control</td>
<td>25.9 ± 0.5</td>
<td>27.2 ± 0.8</td>
<td>23.8 ± 0.3</td>
<td>24.4 ± 0.3</td>
<td>71.3 ± 0.3</td>
<td>0.9 ± 0.0</td>
<td>0.26 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.19 ± 0.0</td>
<td>13 ± 0.1</td>
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<tr>
<td>TF-TG</td>
<td>24.8 ± 0.7</td>
<td>21.7 ± 0.6</td>
<td>24.8 ± 0.7</td>
<td>8.1 ± 0.4</td>
<td>0.46 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.17 ± 0.0</td>
<td>2.6 ± 0.2</td>
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<td>E-PL</td>
<td>24.9 ± 0.7</td>
<td>29.3 ± 1.0</td>
<td>14.8 ± 1.1</td>
<td>12.8 ± 0.6</td>
<td>3.9 ± 0.4</td>
<td>0.4 ± 0.0</td>
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<td>0.9 ± 0.1</td>
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<td><strong>Phospholipids</strong></td>
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<tr>
<td>Control</td>
<td>26.9 ± 0.9</td>
<td>29.7 ± 0.8</td>
<td>13.5 ± 1.4</td>
<td>10.5 ± 0.7</td>
<td>4.5 ± 0.5</td>
<td>0.5 ± 0.2</td>
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<td>1.4 ± 0.3</td>
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<tr>
<td>TF-TG</td>
<td>26.5 ± 1.3</td>
<td>32.3 ± 1.0</td>
<td>9.9 ± 0.8</td>
<td>9.9 ± 0.8</td>
<td>6.4 ± 0.8</td>
<td>0.5 ± 0.1</td>
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<td>2.1 ± 0.4</td>
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<tr>
<td>E-PL</td>
<td>27.3 ± 0.5</td>
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<td>38.7 ± 0.7</td>
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<td><strong>Triacylglycerols</strong></td>
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<tr>
<td>Control</td>
<td>29.2 ± 0.3</td>
<td>9.6 ± 0.5</td>
<td>37.9 ± 0.6</td>
<td>8.8 ± 0.8</td>
<td>0.8 ± 0.2</td>
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<td>0.2 ± 0.1</td>
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<tr>
<td>TF-TG</td>
<td>29.5 ± 1.0</td>
<td>10.2 ± 0.6</td>
<td>33.9 ± 0.8</td>
<td>10.7 ± 1.3</td>
<td>1.6 ± 0.4</td>
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<td>0.5 ± 0.1</td>
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<tr>
<td>E-PL</td>
<td>16.2 ± 0.9</td>
<td>4.2 ± 0.4</td>
<td>1.2 ± 0.1</td>
<td>41.9 ± 1.2</td>
<td>25.7 ± 1.5</td>
<td>1.0 ± 0.2</td>
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<td>0.2 ± 0.0</td>
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<tr>
<td><strong>Cholesterol ester</strong></td>
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<tr>
<td>Control</td>
<td>19.9 ± 0.6</td>
<td>3.6 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>36.9 ± 1.0</td>
<td>25.6 ± 1.3</td>
<td>1.5 ± 0.3</td>
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<td>0.5 ± 0.1</td>
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<td>TF-TG</td>
<td>17.9 ± 0.9</td>
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<td>1.0 ± 0.1</td>
<td>33.0 ± 2.5</td>
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<td>0.7 ± 0.1</td>
<td>0.3 ± 0.0</td>
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1 Values are means ± SEM, n = 10. Means with a different superscript letter differ, P < 0.05.
2 AA, arachidonic acid; DHA, docosahexaenoic acid; Control, group fed adapted milk formula for piglets; TF-TG, group fed adapted milk formula for piglets supplemented with LCP from fungal and tuna oils; E-PL, group fed adapted milk formula for piglets supplemented with LCP from egg yolk phospholipids; —, not detected.
forms of LCP (either PL or TG) on the lipid and fatty acid composition of plasma and plasma lipoproteins and on the lipid composition of liver and jejunal mucosa in infant piglets.

The direct effect of fatty acids ingested with the diet on plasma and tissue fatty acid composition has been largely proved (Dougherty et al. 1987, Hrboticky et al. 1990). LCP-supplemented groups had lower proportions of 18:1(n-9) or 18:2(n-6) as free fatty acids in case of the E-PL diet, and DHA mainly as free fatty acid in the case of the TF-TG diet. 2-Monoacylglycerols are reestified to the NE, and AA mainly as free fatty acid in the newl formed TG, which would support in part our hypothesis that dietary LCP-TG may contribute to chylomicron TG. However, little information is available concerning lipids released as free fatty acids in the intestinal lumen (Pufal et al. 1985, Thomson et al. 1988). The metabolic fate of LCP from our experimental diets can be determined taking into account, on one hand, the digestion and absorption process, and on the other hand, the positional distribution of fatty acids in the lipid sources used to supplement the experimental diets. We previously reported that AA and DHA were mainly esterified to the sn-2 position of E-PL. For example, 50% of DHA acid was present in the sn-2 position of tuna oil, whereas nearly 80% of AA was esterified to the outer positions of the TG molecule in fungal oil (Amateis et al. 1999). Therefore, after digestion, LCP would be released as free fatty acids in case of the E-PL diet, and DHA mainly as 2-monoacylglycerols and AA mainly as free fatty acid in the case of the TF-TG diet. 2-Monoacylglycerols are restituted to newly form TG, which would support in part our hypothesis that dietary LCP-TG may contribute to chylomicron TG.

This different distribution of LCP in lipoprotein PL may be explained if after digestion and absorption, LCP are reesterified to the same chemical structure in which they were added to the diet (as PL or TG), being assembled mainly as PL on the chylomicron surface in the case of the E-PL group and mainly as TG in chylomicron core in the case of the TF-TG group. Plasma chylomicrons exchange some of their components with HDL during intravascular catabolism (Posner 1986); therefore, HDL from piglets fed an E-PL diet would contain a higher proportion of AA and DHA in HDL-PL, which is consistent with our observation.

The metabolic fate of LCP from our experimental diets can be determined taking into account, on one hand, the digestion and absorption process, and on the other hand, the positional distribution of fatty acids in the lipid sources used to supplement the experimental diets. We previously reported that AA and DHA were mainly esterified to the sn-2 position of E-PL. For example, 50% of DHA acid was present in the sn-2 position of tuna oil, whereas nearly 80% of AA was esterified to the outer positions of the TG molecule in fungal oil (Amateis et al. 1999). Therefore, after digestion, LCP would be released as free fatty acids in case of the E-PL diet, and DHA mainly as 2-monoacylglycerols and AA mainly as free fatty acid in the case of the TF-TG diet. 2-Monoacylglycerols are restituted to newly form TG, which would support in part our hypothesis that dietary LCP-TG may contribute to chylomicron TG. However, little information is available concerning lipids released as free fatty acids in the intestinal lumen (Pufal et al. 1985, Thomson et al. 1988). The metabolic fate of LCP from different dietary sources in lymph chylomicrons deserves further research.

LCP supplementation to the diet as both PL or TG lowered the hepatic cholesterol concentration. This result was in agreement with Ikeda et al. (1994), who also found less cholesterol in the livers of rats fed a DHA-containing diet. This effect may be due to a reduction in hepatic cholesterol synthesis, because (n-3) LCP inhibits HMG-CoA reductase activity (Choi et al. 1989). On the other hand, (n-3) LCP have been shown to lower plasma and liver TG (Harris 1996, Rusan et al. 1988). We did not find a lowering effect of (n-3) LCP on liver TG. However, it should be taken into account that the TG-lowering effect of (n-3) LCP has been shown in humans and experimental animals in studies of the prevention or treatment of cardiovascular disease using high doses of (n-3) LCP, mainly 20:5(n-3) (Rusan et al. 1988). Both the E-PL and TF-TG diets contained 0.3 g/100 g total fatty acids as DHA with no 20:5(n-3) (E-PL) or <0.05 g/100 g 20:5(n-3) (TF-TG). It is not clear whether DHA has the same effects as 20:5(n-3) on plasma and tissue lipids (Berge et al. 1999). Moreover, the presence of AA in our experimental diets may modulate the TG-lowering effects of (n-3) LCP; in fact, it has been reported that AA increases circulating levels of TG (Whelan et al. 1995).
Regarding comparisons between the group fed LCP as PL and the group fed LCP as TG, there have been two previous studies that showed beneficial effects of LCP-PL: one by Carlson et al. (1995) and one by our research group (Lopez-Pedrosa et al., 1995 and 1999). Carlson et al. (1995) reported a lower incidence of necrotizing enterocolitis in preterm infants fed a formula with E-PL. The authors justified their finding with one of several possible explanations: increased fatty acid oxidation of dietary n-3 fatty acids, increased bile acid synthesis, and decreased cholesterol absorption. However, the results of our study did not show an effect of LCP-PL on the incidence of necrotizing enterocolitis.

In summary, this study showed that the chemical form in which LCP are present in different dietary sources influences their distribution in plasma lipoproteins. This fact should be taken into consideration when LCP sources are selected for infant formula supplementation.

**ACKNOWLEDGMENTS**

The authors thank Maria Luisa Jimenez for her help in the care of animals and for her technical assistance. We also thank the Animal Nutrition Department of CSIC, especially D. José Aguilera, for providing animal facilities and good advice regarding piglet rearing.

**LITERATURE CITED**


acidity development in healthy preterm infants: effect of marine oil supple-
Carrielli, V. P., Luijendijk, I.H.T., Van Goudoever, J. B., Sulkers, E. J., Boerlage,
infants palmitic acid in amounts and stereoisomeric position similar to that of
1042.
Clandinin, M. T., Chappell, J. E., Leong, S., Heim, T., Swyer, P. R. & Chance,
Feeding preterm infants a formula containing C20 and C22 fatty acids simul-
lated plasma phospholipid fatty acid composition of infants fed human milk.
polyunsaturated fatty acids on cholesterol synthesis and degradation in rats
of different ages. Lipids 24: 45–50.
Christensen, M. S., Hoy, C. E., Becker, C. C. & Redgrave, T. G. (1995) Intes-
tinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosa-
hexaenoic (DHA), and docosahexaenoic acids: dependence on intramolecular triacyl-
quantitative isolation of plasma lipoproteins: rapid, single, discontinuous den-
phospholipids fatty acid composition of plasma, red blood cells, and platelets
and how they are affected by dietary lipids: a study of normal subjects from
ESPGHAN Committee on Nutrition, Aggett, P. J., Haschke, F., Heine, W., Hennel,
O., Koletzko, B., Launiala, K., Rey, J., Rubino, A., Schöch, G., Senterre, J. &
Tormo, R. (1991) Comment on the content and composition of lipids in infant
and Nutrition Paper No. 57: FAO, Rome, Italy.
synaptosomal, liver, plasma, and red blood cell lipids in piglets fed exclusively
on a vegetable-oil-containing formula with and without fish-oil supplements.
Goussard-Langelier, B., Guerret, P., Durand, G., Antoine, J. M. & Alessandri,
J. M. (1999) n-3 and n-6 fatty acid enrichment by dietary fish oil and
phospholipid sources in brain cortical areas and nonneural tissues of formula-
fed piglets. Lipids 34: 5–16.
Harris, W. S. (1996) n-3 fatty acids and lipoproteins: comparison of results
and positional selectivities of gastric lipase from premature infants. In:
structure on lipoprotein metabolism: a comparison of the effects of dioleoyl-
palmitoylgluceryl in which palmitate is esterified to the sn-2 or (3)-position
Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglyc-
erol by decreasing the activity of acyl-CoA:acyl carrier protein acyl-
191.
separate pathways of chylomicron and very low-density lipoprotein assembly
Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-
dietary arachidonic acid increases circulating triglycerides. Lipids 30: 425–
429.