The effect of 5 intravenous lipid emulsions on plasma phytosterols in preterm infants receiving parenteral nutrition: a randomized clinical trial

Sara Savini, Rita D’Ascenzo, Chiara Biagetti, Giulia Serpentini, Adriana Pompilio, Alice Bartoli, Paola E Cogo, and Virgilio P Carnielli

ABSTRACT

Background: Elevated plasma phytosterol concentrations are an untoward effect of parenteral nutrition (PN) with vegetable oil–based lipid emulsions (LEs). Phytosterols are elevated in neonatal cholestasis, but the relation remains controversial.

Objective: The objective was to study the effect of 5 LEs on plasma phytosterols in preterm infants.

Design: One hundred forty-four consecutive admitted preterm infants (birth weight: 500–1249 g) were studied. Patients were randomly assigned to receive 1 of 5 different LEs: S [100% soybean oil (SO)], MS [50% medium-chain triglycerides (MCTs) and 50% SO], MSF [50% MCTs, 40% SO, and 10% fish oil (FO)], OS [80% olive oil and 20% SO], or MOSF [30% MCTs, 25% olive oil, 30% SO, and 15% FO]. Phytosterols in the LEs and in plasma (on postnatal day 7 and day 14) were measured by gas chromatography–mass spectrometry.

Results: Patients in the S group had significantly higher total phytosterol intakes than did the other study groups. On PN days 7 and 14, plasma phytosterol concentrations were highest in the S group and lowest in the MOSF group. Despite similar β-sitosterol intakes between the MS and MSF groups, plasma concentrations were significantly lower in the MSF than in the MS group. Only 3 patients (2.1%) developed cholestasis: 1 in the MS, 1 in the MSF, and 1 in the MOSF group. No cases of cholestasis were observed in the S and OS groups.

Conclusions: In uncomplicated preterm infants receiving routine PN, we found a correlation between phytosterol intake and plasma phytosterol concentrations; however, cholestasis was rare and no difference in liver function at 6 wk was observed.

INTRODUCTION

Phytosterols are components of plant cell membranes similar in structure to cholesterol (1). The relative amount of cholesterol and phytosterols in a child’s diet depends on the relative amount of animal fats and vegetable oils. The intestinal absorption of phytosterols is much lower than that of cholesterol (2, 3).

In infants and children requiring parenteral nutrition (PN), the energy source is ordinarily provided by glucose and lipids. Lipids are administered intravenously as lipid emulsions (LEs) (4, 5). The most popular LEs contain soybean oil (SO) or olive oil and carry variable amounts of phytosterols (6). The association between plasma phytosterol concentrations and the severity of PN-associated cholestasis (PNAC) has been reported in several studies (7–11). Phytosterols are thought to be harmful, both because they are slowly metabolized in the liver and because they have been shown to inhibit cholesterol 7α-hydroxylase, which is the rate-limiting step in the conversion of cholesterol into bile acids (12–14).

In 1993 the onset of PNAC was reported after the increase in serum phytosterols, and in 1998 a correlation between serum phytosterols and PNAC was also reported (3, 14). To the best of our knowledge, no studies have compared the effect of different LEs with different phytosterol contents on plasma phytosterol concentrations and the possible association with PNAC. In the current study we randomly assigned preterm infants on routine PN to receive 1 of the 5 most commonly used LEs. We studied the effect of phytosterol intakes on plasma phytosterol concentrations and on liver function.

SUBJECTS AND METHODS

Trial design

In this single-center 5-arm, parallel-group, randomized clinical trial, preterm infants were recruited from the neonatal intensive care unit (NICU) of “G. Salesi” Children’s Hospital, Ancona, Italy, between January 2007 and October 2011. The

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4 Abbreviations used: FO, fish oil; GC-MS, gas chromatography–mass spectrometry; LE, lipid emulsion; MCT, medium-chain triglyceride; MOSF (SMOF; Fresenius Kabi), 30% MCTs, 25% olive oil, 30% SO, and 15% FO; MS (Lipofundin; B Braun), 50% MCTs and 50% SO; MSF (Lipidem; B Braun), 50% MCTs, 40% SO, and 10% FO; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; OS (ClinOleic; Baxter spa), 80% olive oil and 20% SO; PN, parenteral nutrition; PNAC, parenteral nutrition–associated cholestasis; S (Intralipid; Fresenius Kabi), 100% SO; SO, soybean oil.

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study was conducted in accordance with the principles of the Helsinki Declaration as revised in Edinburgh 2000 and was reviewed and approved by the local ethics committee. Written informed consent was obtained from both parents.

Participants

Neonates with a birth weight of 500 to 1249 g, who routinely received PN from the first hour of life, were consecutively enrolled. Preterm infants were randomly assigned in a 1:1:1:1:1 ratio to 1 of the 5 LEs studied following a simple randomization procedure with a sealed envelope system. The pharmacy received the enveloped randomization list with the patient codes and provided the allocated interventions. Exclusion criteria were severe malformations, inborn errors of metabolism, and severe congenital sepsis.

Interventions

Infants were randomly assigned to receive one of the following LEs: 1) S (Intralipid; Fresenius Kabi) containing 100% SO; 2) MS (Lipofundin; B. Braun) containing 50% medium-chain triglycerol (MCT) and 50% SO; 3) MSF (Lipidem; B Braun) containing 50% MCTs, 40% SO, and 10% fish oil (FO); 4) OS (ClinOleic; Baxter spa) containing 80% olive oil and 20% SO; and 5) MOSF (SMOF; Fresenius Kabi) containing 30% MCT, 30% SO, 25% olive oil, and 15% FO. The composition of the 5 LEs and their phytosterol contents are shown in Table 1. Phytosterols in the LEs were measured in our laboratory by gas chromatography–mass spectrometry (GC-MS).

The 5 different LEs prepared in the hospital pharmacy were of the same size and identical appearance. They were identified only by the patient number according to the randomization schedule. The clinician, the patient’s parents, and the individuals who assessed the study endpoints were blinded to the LEs.

The infants were started on PN with glucose, amino acids, and lipids at ~1 h after birth, according to the NICU protocol. The LEs were infused at a dose of 1, 1.5, 2, 2.5, and 3 g kg$^{-1}$ d$^{-1}$ from postnatal days 0 to 5, respectively, and were then kept constant from days 5 to 7, when PN tapering was begun, until day 21, when it was stopped. Minimal enteral feeding with human milk was provided from days 0 to 7; the maximum amount supplied was 8 mL kg$^{-1}$ d$^{-1}$ from days 1 to 4, and 16 mL kg$^{-1}$ d$^{-1}$ from days 5 to 8. Enteral feeding was gradually increased from day 9 to reach full oral feeding by day 18.

Primary outcomes

Plasma phytosterol concentrations were measured from 0.5 mL EDTA-treated blood collected on day 0 (cord blood), on day 7 (full PN), and on day 14 (when infants were receiving ~50% of energy intake from PN and 50% from enteral feeding).

Analytic methods

All the reagents were obtained from Sigma-Aldrich. Standard sterols were also obtained from Sigma and were prepared as a 1-mg/mL stock solution. The internal standard was 3β-hydroxy-24-ethyl-5,22-cholestadiene (Stigmasterol purity 96%), and it was prepared as a 2.40-mg/mL stock solution.

Lipid extraction was performed by using a modified version of the method of Folch et al (15) after the addition of the internal standard. Alkaline hydrolysis with potassium hydroxide/methanol (5 mol/L) was performed with 50 µL plasma, and sterols were extracted from the hydroalcoholic phase by liquid-liquid extraction with an equal volume of hexane for 15 min under shaking and after centrifugation. This operation was repeated a second time by adding diethyl ether instead of hexane. Hexane and diethyl ether were removed under a gentle stream of nitrogen.

Finally, sterols were derivatized by bis(trimethylsilyl)trifluoroacetamide in pyridine, and 1 µL was analyzed by GC-MS (16, 17). GC-MS analysis was performed with an Agilent Technologies apparatus (model GC7890A/MD5975) controlled by a work station with the use of Agilent ChemStation as software. Chromatographic separation was performed on a capillary column (SPB-5 30 m × 0.25 mm × 0.25 µm film thickness).

To obtain spectra of the sterol peaks, the mass spectrometer was operated in SIM mode. Quantitative analysis was performed monitoring the single ion: m/z 472, 484, and 486 were used to detect campesterol, internal standard, and β-sitosterol, respectively. The response ratio of each compound was calculated, and the concentrations of phytosterols were measured in relation to the amount of the internal standard. Phytosterol

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td>Composition of the 5 intravenous lipid emulsions used in the study$^1$</td>
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<tr>
<td></td>
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<tr>
<td>Medium-chain triglycerides (g/L)</td>
</tr>
<tr>
<td>Soybean oil (g/L)</td>
</tr>
<tr>
<td>Olive oil (g/L)</td>
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<tr>
<td>Fractionated fish oil (g/L)</td>
</tr>
<tr>
<td>Egg yolk phospholipids (g/L)</td>
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<tr>
<td>Glycerol (g/L)</td>
</tr>
<tr>
<td>Campesterol (mg/L)$^2$</td>
</tr>
</tbody>
</table>

$^1$S (Intralipid; Fresenius Kabi): 100% SO; MS (Lipofundin; B Braun): 50% MCTs and 50% SO; MSF (Lipidem; B Braun): 50% MCTs, 40% SO, and 10% FO; OS (ClinOleic; Baxter spa): 80% olive oil and 20% SO; MOSF (SMOF; Fresenius Kabi): 30% MCTs, 25% olive oil, 30% SO, and 15% FO. FO, fish oil; MCT, medium-chain triglyceride; SO, soybean oil.

$^2$Analyzed by gas chromatography–mass spectrometry in our laboratory.

$^3$Mean ± SD (all such values).
concentrations were obtained by using calibration curves as previously described by Corso et al (18). Total phytosterols were computed by adding the concentrations of campesterol and \( \beta \)-sitosterol.

**Secondary outcomes: clinical data and growth**

Body weight was measured daily according to a standard procedure of the NICU by using the same scale (precision: 5 g). Head circumference and length (crown-heel) were measured at birth and weekly thereafter with the use of a nonstretchable tape and a length board. SD scores were computed by using Italian reference data. Weight gain (\( g \cdot kg^{-1} \cdot d^{-1} \)) during PN was calculated weekly.

Liver-function tests were conducted as part of routine care at 6 wk of age, according to NICU policy. They were analyzed by using a Spectrophotometric Chemistry Analyzer (ADVIA 1200; Siemens) (19).

Cholestasis was defined as a conjugated bilirubin concentration >2.0 mg/dL, and it was assessed at 6 wk postnatal age according to the NICU protocol. All subjects were followed up until discharge. Incidence of the principal complications of prematurity occurred during the admission was recorded. Bronchopulmonary dysplasia is defined by the physiologic criteria of Walsh et al (20). Neonatal sepsis was defined as a positive blood culture result or as a clinical syndrome with systemic signs and symptoms of infection and abnormalities of laboratory findings (21). Necrotizing enterocolitis (NEC) was defined as Bell stage 2 or 3 (22). Other complications of prematurity were classified according to international and/or national definitions (23).

**Statistical analysis**

Plasma phytosterol concentrations were the primary endpoint. Power calculations were based on plasma phytosterol concentrations of preterm infants receiving LEs on S as previously studied by our group (SD: 19 \( \mu \)mol/L; data not published). A sample size of 28 in each arm, compared with the soybean-based emulsion as control arm, is capable of detecting a standardized difference of 1 with an \( \alpha \) of 0.01 and a power of 90%. Data were expressed as group median (IQR) for plasma sterols and were analyzed by using a Spectrophotometric Chemistry Analyzer (ADVIA 1200; Siemens) (19).

Liver function at 6 wk, growth data, and the incidence of major clinical complication of prematurity are reported in **Table 3**. Of the 144 study infants, only 3 patients had cholestasis (2.1%): 1 in the MS group, 1 in the MSF group, and 1 in the MOSF group; no cases of cholestasis occurred in the S and OS groups.

We found no differences in plasma conjugated bilirubin concentrations between the study groups at 6 wk of age. Moreover, we found no differences in bronchopulmonary dysplasia, patent ductus arteriosus, NEC, and sepsis between the study groups and no differences in growth. The cumulative phytosterol intakes (mg/kg) from birth to day 7 and from birth to day 14 are shown in **Table 4**.

Campesterol and \( \beta \)-sitosterol were consistently detected in the plasma of all patients. Plasma campesterol, \( \beta \)-sitosterol, and total phytosterols (sum of campesterol and \( \beta \)-sitosterol) at the start of the study and on PN days 7 and 14 are reported in **Table 5**. Individual plasma sterol concentrations were significantly greater on postnatal days 7 and 14 in the S group than in the other study groups.

The plasma campesterol concentration on day 7 was significantly lower in the MOSF and OS groups than in the S, MS, and MSF groups. On day 14, campesterol was significantly lower in the MOSF group than in the other study groups.

The plasma \( \beta \)-sitosterol concentration on day 7 was significantly lower in the MOSF and MSF groups than in the S, MS, and OS groups. \( \beta \)-Sitosterol intakes were similar in the MS and MSF groups; however, its plasma concentration was significantly lower in the MSF than in the MS group. On day 14, plasma \( \beta \)-sitosterol was similar between the MS and MSF groups; however, plasma concentrations in these 2 groups were significantly greater than in the MOSF group.

The relation between cumulative phytosterol intake (days 1–7) and plasma phytosterols on day 7 in all the study patients is depicted in **Figure 2**. A significant positive correlation was observed between plasma phytosterol concentrations and cumulative phytosterol intakes (\( r = 0.43, P = 0.01 \)). We found no significant correlations between phytosterol intakes, conjugated bilirubin, and liver-function test results at 6 wk (data not shown).

**DISCUSSION**

We measured the phytosterol content of the 5 most popular LEs on the market in Europe and reported plasma phytosterol concentrations in 144 preterm infants receiving routine PN. The phytosterol content of the LEs was measured by state-of-the-art GC-MS (24). Of interest, we found phytosterol concentrations similar to those published by Forchielli et al (24) for S, MS, and OS LEs. Furthermore, we measured the phytosterol content of MSF and MOSF, which were introduced in the market in more recent years. We did not study Omegaven (Fresenius Kabi) because, although devoid of phytosterols, it is a pure FO LE and
thus has a fatty acid profile markedly different from that of human milk lipids.

We calculated total phytosterol intakes and measured plasma phytosterol concentrations, which differed between the study groups. The highest phytosterol intakes (on days 7 and 14) were found in the infants receiving the S LE, and plasma phytosterols were significantly greater in this group than in the other study groups. Plasma phytosterol concentrations in the S group were rather similar to those published by Pianese et al (5) and by Iyer et al (9) in preterm infants without liver dysfunction. Notably, in our study, despite the highest phytosterol intake in the S group, none of the infants in this group developed cholestasis as defined by a conjugated bilirubin concentration \(>2\) mg/dL at 6 wk of age.

Total phytosterol intakes in the study groups other than the S group were not statistically different from each other (Table 4). Campesterol intakes were lower in the OS and MOSF (olive oil containing LE) groups than in the MS, MSF, and S

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>S (n = 30)</th>
<th>MS (n = 30)</th>
<th>MSF (n = 30)</th>
<th>OS (n = 29)</th>
<th>MOSF (n = 28)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>13/17</td>
<td>14/16</td>
<td>14/13</td>
<td>13/16</td>
<td>12/16</td>
<td>0.44</td>
</tr>
<tr>
<td>Gestational age (d)</td>
<td>198 ± 15</td>
<td>194 ± 13</td>
<td>198 ± 16</td>
<td>194 ± 17</td>
<td>193 ± 14</td>
<td>0.71</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>955 ± 202</td>
<td>937 ± 222</td>
<td>935 ± 202</td>
<td>905 ± 160</td>
<td>898 ± 199</td>
<td>0.73</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>35 ± 2</td>
<td>35 ± 3</td>
<td>35 ± 3</td>
<td>34 ± 3</td>
<td>34 ± 3</td>
<td>0.51</td>
</tr>
<tr>
<td>Birth head circumference (cm)</td>
<td>25 ± 1</td>
<td>25 ± 2</td>
<td>24 ± 1</td>
<td>24 ± 1</td>
<td>24 ± 2</td>
<td>0.35</td>
</tr>
<tr>
<td>Duration of parenteral nutrition (d)</td>
<td>21.6 ± 5.6</td>
<td>21.7 ± 6.9</td>
<td>20.9 ± 5.5</td>
<td>21.6 ± 6.5</td>
<td>19.0 ± 4.3</td>
<td>0.57</td>
</tr>
</tbody>
</table>

1 FO, fish oil; MCT, medium-chain triglyceride; MOSF (SMOF; Fresenius Kabi), 30% MCTs, 25% olive oil, 30% SO, and 15% FO; MS (Lipofundin; B Braun), 50% MCTs and 50% SO; MSF (Lipidem; B Braun), 50% MCTs, 40% SO, and 10% FO; OS (ClinOleic; Baxter spa), 80% olive oil and 20% SO; S (Intralipid; Fresenius Kabi), 100% SO; SO, soybean oil.

2 Significance \((P < 0.05)\) was determined by ANOVA; there were no significant differences.

3 Mean ± SD (all such values).
and campesterol also tended to be lower (P = 0.06) in the MSF group (the 2 LEs differ only because of 10% FO in MS), we observed that plasma β-sitosterol on day 7 was significantly lower (P < 0.02) and campesterol also tended to be lower (P = 0.06) in the MSF than in the MS group despite rather similar intakes. The reason why the MSF group had a significantly lower plasma phytosterol content of soy and olive oil (1).

Of interest when comparing the MS and MSF groups (the 2 LEs) is that the use of an LE with predominantly SO-based LE groups. The β-sitosterol intake was higher in the S and OS groups than in the MS, MSF, and MOSF groups. This findings can be explained by the phytosterol content of soy and olive oil (1).

Whether FO enhances the clearance of phytosterols is currently being studied.

We found no association between phytosterol intakes and abnormal liver function at 6 wk. We measured conjugated bilirubin at 6 wk because cholestasis, unless linked to a prenatal cause, normally develops sometime after birth and most often in association with sepsis or NEC (R D’Ascenzo, unpublished data, 2013). Of note all our infants were receiving minimal enteral feeding, which is known to reduce cholestasis (26).

We had only 3 cases of conjugated bilirubin >2 mg/dL in a total of 144 preterms (2.1%). PNAC has been reported in the literature with variable frequencies in preterm infants with a very-low body weight; it has been associated with PN duration, sepsis, NEC, bowel surgery, and lack of enteral feeding.

### TABLE 3
Laboratory and clinical outcome data for the 144 preterm infants

<table>
<thead>
<tr>
<th>Diagnosis (n [%])</th>
<th>S (n = 30)</th>
<th>MS (n = 30)</th>
<th>MSF (n = 27)</th>
<th>OS (n = 29)</th>
<th>MOSF (n = 28)</th>
<th>P^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.50 ± 1.3</td>
<td>1.53 ± 1.5</td>
<td>1.14 ± 0.9</td>
<td>1.54 ± 1.5</td>
<td>2.32 ± 2.5</td>
<td>0.21</td>
</tr>
<tr>
<td>Conjugated bilirubin (mg/dL)</td>
<td>0.49 ± 0.4</td>
<td>0.43 ± 0.4</td>
<td>0.53 ± 0.6</td>
<td>0.60 ± 0.5</td>
<td>0.45 ± 0.5</td>
<td>0.79</td>
</tr>
<tr>
<td>Weight loss [% of BW]</td>
<td>11 ± 6</td>
<td>12 ± 6</td>
<td>11 ± 5</td>
<td>14 ± 5</td>
<td>15 ± 6</td>
<td>0.23</td>
</tr>
<tr>
<td>Time to regain BW [d]</td>
<td>11 ± 5</td>
<td>12 ± 5</td>
<td>10 ± 5</td>
<td>14 ± 9</td>
<td>12 ± 5</td>
<td>0.20</td>
</tr>
<tr>
<td>Weight gain week 1 [g · kg^-1 · d^-1]</td>
<td>−17 ± 13</td>
<td>−18 ± 13</td>
<td>−16 ± 13</td>
<td>−23 ± 15</td>
<td>−24 ± 15</td>
<td>0.14</td>
</tr>
<tr>
<td>Weight gain week 2 [g · kg^-1 · d^-1]</td>
<td>18 ± 8</td>
<td>17 ± 13</td>
<td>16 ± 8</td>
<td>22 ± 7</td>
<td>16 ± 12</td>
<td>0.11</td>
</tr>
<tr>
<td>Weight gain week 3 [g · kg^-1 · d^-1]</td>
<td>16 ± 8</td>
<td>10 ± 9</td>
<td>12 ± 10</td>
<td>14 ± 12</td>
<td>16 ± 9</td>
<td>0.10</td>
</tr>
</tbody>
</table>

1 ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BCH, bronchopulmonary dysplasia; BW, birth weight; FO, fish oil; GGT, γ-glutamyl transpeptidase; MCT, medium-chain triglyceride; MOSF (SMOF; Fresenius Kabi), 30% MCTs, 25% olive oil, 30% SO, and 15% FO; MS (Lipofundin; B Braun), 50% MCTs and 50% SO; MSF (Lipidem; B Braun), 50% MCTs, 40% SO, and 10% FO; NEC, necrotizing enterocolitis; OS (ClinOleic; Baxter spa), 80% olive oil and 20% SO; PDA, patent ductus arteriosus; S (Intralipid; Fresenius Kabi), 100% SO; SO, soybean oil.

1 Significance (P < 0.05) was determined by ANOVA and chi-square tests; there were no significant differences.

1 Mean ± SD (all such values).

### TABLE 4
Cumulative phytosterol intakes of the 144 preterm infants receiving parenteral nutrition

<table>
<thead>
<tr>
<th>S (n = 30)</th>
<th>MS (n = 30)</th>
<th>MSF (n = 27)</th>
<th>OS (n = 29)</th>
<th>MOSF (n = 28)</th>
<th>P^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campesterol</td>
<td>4.4 ± 0.6\textsuperscript{a}</td>
<td>3.7 ± 0.5\textsuperscript{b}</td>
<td>3.8 ± 0.4\textsuperscript{a}</td>
<td>1.5 ± 0.2\textsuperscript{a}</td>
<td>2.4 ± 0.4\textsuperscript{a}</td>
</tr>
<tr>
<td>β-Sitosterol</td>
<td>10.2 ± 1.3\textsuperscript{a}</td>
<td>8.2 ± 1.1\textsuperscript{b}</td>
<td>7.2 ± 0.8\textsuperscript{a}</td>
<td>11.2 ± 1.4\textsuperscript{a}</td>
<td>7.6 ± 1.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Total phytosterol</td>
<td>14.6 ± 1.9\textsuperscript{b}</td>
<td>12.1 ± 1.6\textsuperscript{b}</td>
<td>11.4 ± 1.2\textsuperscript{b}</td>
<td>12.7 ± 1.6\textsuperscript{b}</td>
<td>10.0 ± 1.6\textsuperscript{b}</td>
</tr>
<tr>
<td>Campesterol</td>
<td>7.6 ± 1.4\textsuperscript{a}</td>
<td>6.9 ± 2.5\textsuperscript{a}</td>
<td>7.3 ± 2.1\textsuperscript{a}</td>
<td>3.4 ± 0.6\textsuperscript{a}</td>
<td>3.6 ± 0.6\textsuperscript{a}</td>
</tr>
<tr>
<td>β-Sitosterol</td>
<td>17.6 ± 3.2\textsuperscript{a}</td>
<td>14.4 ± 5.3\textsuperscript{a}</td>
<td>13.5 ± 3.5\textsuperscript{a}</td>
<td>18.8 ± 4.4\textsuperscript{a}</td>
<td>12.8 ± 1.9\textsuperscript{a}</td>
</tr>
<tr>
<td>Total phytosterol</td>
<td>25.2 ± 4.5\textsuperscript{b}</td>
<td>22.4 ± 7.8\textsuperscript{b}</td>
<td>20.8 ± 5.4\textsuperscript{b}</td>
<td>22.2 ± 4.9\textsuperscript{b}</td>
<td>16.4 ± 2.5\textsuperscript{b}</td>
</tr>
</tbody>
</table>

1 Values with different superscript letters are significantly different, P < 0.05 (Bonferroni). FO, fish oil; MCT, medium-chain triglyceride; MOSF (SMOF; Fresenius Kabi), 30% MCTs, 25% olive oil, 30% SO, and 15% FO; MS (Lipofundin; B Braun), 50% MCTs and 50% SO; MSF (Lipidem; B Braun), 50% MCTs, 40% SO, and 10% FO; OS (ClinOleic; Baxter spa), 80% olive oil and 20% SO; S (Intralipid; Fresenius Kabi), 100% SO; SO, soybean oil.

1 Significance (P < 0.05) was determined by ANOVA.

1 Mean ± SD (all such values).
(17, 26–28). The prevalence of cholestasis in our series appears to be lower than in other reports (29). Nearly identical mean conjugated bilirubin and transaminase values at 6 wk in all the study groups strongly suggest no effect of the LEs and of phytosterol intakes on liver function. Unfortunately, in the current study we did not measure serum bile acids. Studies using highly sensitive methods for the detection of subclinical cholestasis with repeated blood sampling and starting sooner after birth are perhaps worth performing (30). Because cholestasis is a rather rare event in our Unit, it would take an extraordinary number of patients to show a statistically significant difference. We cannot exclude a negative effect of phytosterols in cases of severe cholestasis.

In conclusion, this study provides comparative data on the phytosterol content of the 5 most popular LEs marketed in Europe. Neither intakes nor plasma phytosterol concentrations were associated with abnormal liver function at 6 wk of age.

We are grateful to the infants’ parents and to the NICU staff. The authors’ responsibilities were as follows—VPC: was responsible for the design of the study; SS, GS, and AP: contributed to the data collection and analysis; RD, CB, and AB: contributed to subject recruitment and to the data collection; and PEC: was in charge of the statistical analysis. All authors read and approved the final manuscript. No conflicts of interest were declared.

REFERENCES


