Applied nutritional investigation

Phytosterolemia in parenteral nutrition patients: Implications for liver disease development

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Abstract

Objective: Phytosterols present in parenteral nutrition (PN) lipid emulsions have been linked to phytosterolemia and cholestatic liver disease, although no direct relation has been established. We investigated the relation among plasma phytosterol (PY) infused, total plasma PY levels, and possible links to PN-associated liver disease.

Methods: Twenty-seven adult patients on home PN were enrolled in the study. PYs were measured in plasma and lipid emulsions by gas chromatography. Liver function tests and blood counts were assessed to identify hepatic impairment, and biopsies were performed in eight patients.

Results: Mean total plasma PY level was higher in patients than in controls (55.4 ± 6.2 versus 14.8 ± 2.3 µg/mL). Simple linear regression models showed a correlation among total plasma PY, liver function tests, and platelet counts, which was stronger for total bilirubin ($r^2 = 0.53, P = 0.0001$) and weaker for platelet counts ($r^2 = 0.158, P = 0.04$); between infused lipid and liver function tests, the correlation was significant for total bilirubin ($r^2 = 0.19, P = 0.038$) and aspartate aminotransferase ($r^2 = 0.164, P = 0.049$). In multiple linear regression analysis, a decreased oral diet ($b = -52.3, P = 0.001$) and infused PY ($b = 2.54, P = 0.093$) were risk factors for high plasma PY levels ($r^2 = 0.54$). Biopsies showed moderate to severe liver impairment in five patients.

Conclusion: Liver damage may be linked to high plasma PY levels and strengthened by lack of an oral diet in patients on home PN. © 2008 Elsevier Inc. All rights reserved.

Keywords: Parenteral nutrition; Phytosterols; Parenteral nutrition–associated liver disease; Lipid emulsions

Introduction

Long-term parenteral nutrition (PN) is a life-saving therapy for patients with permanent intestinal failure. PN, which was first used in the late 1960s, was initially administered in hospitals and later at home. Soon, several types of PN-related complications appeared, some of them life-threatening. Among these, the most severe and poorly understood is PN-associated liver disease (PNALD). The development of PNALD has been documented as progressive and irreversible in most cases [1,2].

Multiple factors have been invoked as responsible for PNALD [2]. Clinical data and research have shown that phytosterols (PYs), the naturally occurring equivalents of cholesterol (the mammalian sterol) in plants, may have an important role in the cause of PNALD [3,4]. The main sources of PYs are vegetable oils, nuts, seeds, and legumes [5,6]. Some of these fat sources (e.g., soybean, olive, and coconut oils) constitute the main component of the standard commercial lipid emulsions used in PN.
Unlike cholesterol, PYS are not synthesized in the body; the only sources are external. Whereas in normal Western diets PY ingestion is rather low, ranging from 150 to 350 mg/d, in vegetarian diets PY may reach up to 500 mg/d [5,7]. Regardless of the dietary intake, intestinal absorption of PYS is small, at <5%, compared with that of cholesterol, which is around 55% of ingestion [8]. PYS are metabolized in the liver, but apparently are not converted to C-24 bile acids; thus, they are excreted much faster from the liver into the bile [9]. Among the PY fractions, sitosterol, campes- terol, and stigmasterol account for up to 95% of all dietary PYS [5]. However, because the various fractions are metabo- lized at different rates, their proportions in serum differ. In healthy individuals, total PY (TPY) concentrations in serum tend to remain low and rather stable [5].

Although the intestinal barrier is effective to regulate PY intake, by delivering PYS directly into the bloodstream PN implies the need to metabolize a higher PY load. It is likely that increased PY first triggers an adaptive response to the higher intake. However, medium- to long-term high sus- tained intake leads to PY accumulation [10,11].

Clayton et al. [3] first reported a link between the PY in commercial lipid emulsions used in standard PN and PNALD (cholestasis) in children. Further research based on experimental and human models suggested that PN-administered PYS may indeed trigger PNALD by affect- ing bile acid synthesis and flow [12–14]. Yet, an accurate model of the actual mechanism is still lacking.

This study explored the association between infused PY and PNALD and possible links between liver disease and plasma TPY in a population of adult patients on medium- to long-term PN. The relation between liver function tests (LFTs) and plasma PY levels was investigated, and risk factors associated with PNALD were determined.

Materials and methods

Subjects

Twenty-seven patients with intestinal failure under treatment with home PN were recruited from five Spanish hospitals over a 2-y period and enrolled in the study. Their clinical characteristics are listed in Table 1. Nine patients were men and 18 were women, with a mean age of 48.4 ± 16.8 y (range 20–79). Mean duration of PN was 38.6 ± 41.1 mo (range 2–147), and mean body mass index was 20.8 ± 4.8 kg/m².

The underlying diagnoses were short bowel syndrome (n = 18), intestinal pseudo-obstruction (n = 5), and (non- resected) malabsorption syndrome (n = 4). The following eligibility criteria were applied: stable condition without

Table 1

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age (y)/sex</th>
<th>BMI (kg/m²)</th>
<th>Duration of PN (mo)</th>
<th>Diagnosis</th>
<th>Remnant bowel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50/M</td>
<td>17</td>
<td>30</td>
<td>SBS, intestinal ischemia</td>
<td>J: 20/C: left</td>
</tr>
<tr>
<td>2</td>
<td>37/F</td>
<td>21</td>
<td>147</td>
<td>SBS, iatrogenesis*</td>
<td>J: 20/C: all</td>
</tr>
<tr>
<td>3</td>
<td>57/F</td>
<td>19</td>
<td>132</td>
<td>SBS, mesenteric volvulus</td>
<td>J: 15/I: 10/C: all (no cecum)</td>
</tr>
<tr>
<td>4</td>
<td>20/M</td>
<td>16</td>
<td>6</td>
<td>SBS, mesenteric trauma</td>
<td>J: 15/C: left</td>
</tr>
<tr>
<td>5</td>
<td>52/M</td>
<td>20</td>
<td>26</td>
<td>SBS, mesenteric thrombosis</td>
<td>J: 10</td>
</tr>
<tr>
<td>6</td>
<td>39/M</td>
<td>24</td>
<td>52</td>
<td>SBS, mesenteric trauma</td>
<td>J: 40/C: left</td>
</tr>
<tr>
<td>7</td>
<td>62/M</td>
<td>24</td>
<td>9</td>
<td>SBS, colonic tumor</td>
<td>J: 100</td>
</tr>
<tr>
<td>8</td>
<td>52/M</td>
<td>14</td>
<td>67</td>
<td>Intestinal pseudo-obstruction</td>
<td>J: 100/C: all</td>
</tr>
<tr>
<td>9</td>
<td>25/F</td>
<td>12</td>
<td>36</td>
<td>Intestinal pseudo-obstruction</td>
<td>All</td>
</tr>
<tr>
<td>10</td>
<td>49/F</td>
<td>20</td>
<td>80</td>
<td>SBS, radiation enteritis</td>
<td>J: 120/C: left</td>
</tr>
<tr>
<td>11</td>
<td>68/F</td>
<td>23</td>
<td>4</td>
<td>SBS, intestinal lymphoma</td>
<td>J: 150/C: left</td>
</tr>
<tr>
<td>12</td>
<td>68/F</td>
<td>30</td>
<td>31</td>
<td>SBS, gynecologic tumor</td>
<td>J: 30</td>
</tr>
<tr>
<td>13</td>
<td>27/M</td>
<td>20</td>
<td>8</td>
<td>SBS, gun shot</td>
<td>J: 50/C: left</td>
</tr>
<tr>
<td>14</td>
<td>70/F</td>
<td>26</td>
<td>17</td>
<td>SBS, acute pancreatitis</td>
<td>J: 120/C: left 2/3</td>
</tr>
<tr>
<td>20</td>
<td>36/F</td>
<td>12</td>
<td>24</td>
<td>Malabsorption syndrome</td>
<td>All</td>
</tr>
<tr>
<td>16</td>
<td>33/F</td>
<td>17</td>
<td>18</td>
<td>SBS, adhesions</td>
<td>J: 50/C: left</td>
</tr>
<tr>
<td>17</td>
<td>29/F</td>
<td>17</td>
<td>51</td>
<td>Intestinal pseudo-obstruction</td>
<td>All</td>
</tr>
<tr>
<td>18</td>
<td>79/M</td>
<td>24</td>
<td>10</td>
<td>SBS, mesenteric thrombosis</td>
<td>J: 50/C: left</td>
</tr>
<tr>
<td>19</td>
<td>72/F</td>
<td>25</td>
<td>20</td>
<td>SBS, mesenteric ischemia</td>
<td>J: 25/C: left</td>
</tr>
<tr>
<td>20</td>
<td>53/M</td>
<td>26</td>
<td>2</td>
<td>SBS, mesenteric embolism</td>
<td>J: 50/C: left</td>
</tr>
<tr>
<td>21</td>
<td>20/F</td>
<td>12</td>
<td>8</td>
<td>Intestinal pseudo-obstruction</td>
<td>All</td>
</tr>
<tr>
<td>22</td>
<td>50/F</td>
<td>26</td>
<td>34</td>
<td>Malabsorption syndrome</td>
<td>J: all/I: 100/C: all</td>
</tr>
<tr>
<td>23</td>
<td>57/F</td>
<td>22</td>
<td>42</td>
<td>SBS, radiation enteritis</td>
<td>J: 150/C: left</td>
</tr>
<tr>
<td>24</td>
<td>63/F</td>
<td>21</td>
<td>7</td>
<td>Celiac disease</td>
<td>J: 150/I: all/ C: all</td>
</tr>
<tr>
<td>25</td>
<td>40/F</td>
<td>14</td>
<td>39</td>
<td>Malabsorption syndrome</td>
<td>All</td>
</tr>
<tr>
<td>26</td>
<td>37/F</td>
<td>20</td>
<td>7</td>
<td>Intestinal pseudo-obstruction</td>
<td>All</td>
</tr>
<tr>
<td>27</td>
<td>64/F</td>
<td>22</td>
<td>137</td>
<td>SBS, mesenteric thrombosis</td>
<td>J: 23/C: left</td>
</tr>
</tbody>
</table>

BMI, body mass index; C, colon; F, female; I, ileum; J, jejunum; M, male; PN, parenteral nutrition; SBS, short bowel syndrome

* Abortion maneuvers with complications.
active cancer and/or infection, no medical history suggesting liver disease and normal liver parameters at the start of PN; no alcohol abuse or administration of hepatotoxic drugs before or during PN; absence of hepatitis B surface antigen and hepatitis C antibodies for patients with abnormal liver parameters during PN; and the last surgical procedure at least 2 mo before sample collection. Only one patient (case no. 19) had severe renal insufficiency; none of the remaining patients had serum creatinine values $>135 \mu$mol/L in a stable condition.

Nutritional support

The ethics committee of each hospital approved the study and informed written consent for participation was obtained from all patients. Most patients were treated with mixed PN and oral/enteral nutrition; a few patients received only PN. PN was administered in all-in-one bags by cyclic infusion (12–18 h/d), except in two patients who received the infusion 24 h/d.

Patients received the following mean amounts of macronutrients per week: nitrogen $1.22 \pm 0.67$ g/kg (range 0.33–3.10) as amino acid solutions without glutamine or taurine, glucose $24.58 \pm 13.84$ g/kg (range 5–53), and lipids $5.33 \pm 3.38$ g/kg (range 1.14–12.96).

Vitamins and trace elements were given in the recommended doses and were included in the all-in-one bags. Electrolytes were also administered, but the requirements were tailored on an individual basis. No extra choline was given, and only one patient received carnitine.

The following lipid emulsions were administered: Lipofundina MCT + LCT 20% (Braun, Rubí, Spain) to 14 patients, Lipövenos 10% (Fresenius, Barcelona, Spain) to 6 patients, Intralipid 20% (Pharmacia, Barcelona, Spain) to 5 patients, and Ivelip 20% (Baxter, Valencia, Spain) to 2 patients. For the purposes of this study, we calculated the exact dose of lipids, dextrose, and amino acids (N2) infused during the 6-mo period before the blood sampling date.

Phytosterol intake was calculated by measuring PY concentrations in the lipid emulsions administered. Up to two PY measurements were performed on each brand and batch (Table 2). The measurement performed on the batch administered to the patient closest to sample collection was used to calculate TPY intake. Thus, mean PY intake per patient per week was $8.66 \pm 5.63$ mg/kg (range 1.70–20.4).

Parenteral nutrition was infused over a period of $\geq 12$ mo in 18 patients and over a shorter period in 9 patients. Oral feeding was always encouraged. Patients were classified into three categories depending on the presence and amount of oral/enteral feeding, without considering the absorption capability. Category 1 ($n = 3$) represents a lack or near absence of oral/enteral intake, category 2 ($n = 3$) represents a smaller amount of an oral diet and/or supplements, and category 3 ($n = 21$) represents a daily intake of an “ad libitum” diet adapted to the disease with nutritional supplements. In addition, extra doses of some minerals and vitamins were administered by mouth to patients included in categories 2 and 3.

Control group

Seven healthy subjects, five women and two men with a mean age of $39.5 \pm 13.2$ y (range 24–58 y), a body mass index of $22.4 \pm 1.5$ kg/m², and no history of hyperlipidemia served as controls for plasma PY assessment. Blood was drawn after a 12-h fast.

Analyses

Blood samples were drawn 4 h after cessation of PN infusion with lipids and after a 12-h oral fast. Samples were never drawn from the feeding line.

For sterol analysis, about 1 to 1.5 mL of blood was collected in a lithium heparin tube. Plasma samples were separated at $4^\circ$C by centrifugation at 2000 × g for 20 min and stored at $-80^\circ$C until analysis.

Sample preparation followed the method described by Clayton et al. [3], with some modifications to suit our laboratory equipment. The saponification solution was prepared with 12.5 mL of tetramethylammonium hydroxide plus 1 mL of a 5α-cholestanol standard solution (1.950 mg/L in diethyl ether), all filled up to 50 mL with isopropanol. Briefly, 200 μL of plasma was added to 1 mL of the saponification solution. The mixture was then heated to $80^\circ$C over 15 min. The saponified sterols and standard were then extracted into a hydrophobic lower phase by adding

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytosterol and cholesterol in parenteral lipid emulsions used in the study</td>
</tr>
<tr>
<td>Brand</td>
</tr>
<tr>
<td>Intralipid 20%</td>
</tr>
<tr>
<td>Intralipid 20%</td>
</tr>
<tr>
<td>Ivelip 20%</td>
</tr>
<tr>
<td>Ivelip 20%</td>
</tr>
<tr>
<td>Lipofundina MCT + LCT 20%</td>
</tr>
<tr>
<td>Lipofundina MCT + LCT 20%</td>
</tr>
<tr>
<td>Lipövenos 10%</td>
</tr>
<tr>
<td>Lipövenos 10%</td>
</tr>
</tbody>
</table>
500 μL of a mixture of tetrachloroethylene/methyl butyrate (1:3, vol/vol) and 2 mL of distilled deionized water. Finally, 200 μL of the lower phase was drawn and dried under vacuum at 60°C. The residue was further treated with 60 μL of a sialylating agent (Sylon HTP, Supelco/Sigma-Aldrich, Madrid, Spain) for 1 h at 60°C.

Five microliters of the derivatized solution was injected, by split-less mode, into a Hewlett-Packard 5890 gas chromatograph, equipped with an automatic sample injector, flame ionization detector, electronic integrator, and a data station with appropriate software (Hewlett-Packard, San Jose, CA, USA). A fused silica capillary column (30 m, 0.25-mm inner diameter, 0.25-μm SPB-5 stationary phase thickness; Supelco/Sigma-Aldrich) was used to separate sterols. Helium was used as a carrier gas at a column-head pressure of 145 kPa. The chromatographic conditions were an oven temperature of 265°C for 55 min, 280°C for injection, and a detector temperature of 300°C. All sterol peaks were identified by comparison of retention times with those of a reported standard (5α-cholestanate was the internal standard) and quantitation. Peak area integration was done by an electronic integrator.

The following sterols were determined: campesterol, stigmasterol, sitosterol, avenasterol, and brassicasterol. Other PYs, found in small amounts, were also determined and included the group of “other PYs.” TPY concentrations in plasma were calculated as the total sum of the determined sterols.

Phytosterol analysis in the lipid emulsions was carried out according to European Union Regulation (EEC) number 2568/91 for gas chromatography, with the instruments and conditions being the same as for plasma samples. Samples were analyzed twice. The coefficient of variation between replicate analyses was <7%.

The LFTs, which included γ-glutamyl-transferase, alkaline phosphatase, alanine transaminase, aspartate aminotransferase (AST), and total bilirubin, were determined in serum by molecular absorption spectrometry at each center under accredited conditions (ISO9001). Albumin, urea, creatinine, and electrolytes were analyzed in serum. Blood counts were also performed. These analyses were done on the same day as the sample collection.

Liver biopsies

Liver biopsy was not part of the protocol of this study because of the invasiveness of the procedure. Samples were obtained during abdominal surgical procedures, during diagnostic procedures in some cases of liver complications, from candidates for liver and small bowel transplantation, or postmortem. Specimens were stained with hematoxylin and eosin, Masson, periodic acid, and Perls stains.

Statistical analysis

In the statistical treatment, duration of PN and the three categories of oral nutrition described previously were used as patient variables; glucose intake (grams per kilogram per week), lipid intake, and PY intake (grams per kilogram per week) were used as the PN variables; and LFTs (γ-glutamyl-transferase, alkaline phosphatase, alanine transaminase, AST, and total bilirubin), platelet count, and plasma TPY were used as analytical variables.

To study the association between plasma PY levels and lipid intake with LFTs, we first built simple linear regression models. Correlation coefficient analysis was performed to test the linear relation between variables. Most variables did not show a normal distribution. Hence, their logarithmic transformation was used to build the models.

In the first model, we studied the association among LFTs, platelet count, and plasma TPY levels. Plasma TPY was taken as the independent variable, and LFT values and platelet count were taken as the dependent variables. In a second model, we investigated the association among LFTs, platelet count, and lipid intake. Lipid intake was taken as the independent variable, whereas LFT values and platelet count were taken as the dependent variables.

Student’s t test was performed to compare groups of patients as a function of TPY level in plasma and sitosterol percentages. To identify the risk factors for the presence of high TPY in plasma, stepwise multiple regression analysis was performed. In this model, plasma TPY was taken as the independent variable, whereas lipid intake and PY intake, presence or absence of oral feeding (qualitative), and duration of PN in months were taken as dependent variables. SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. All values are expressed as mean ± SD. In all tests, the significance level was set at P < 0.05, with the threshold for entry in multiple regression analysis set at P < 0.1.

Results

Plasma phytosterols

The mean ± SD value for plasma TPY was 55.4 ± 6.2 μg/mL in patients versus 14.8 ± 2.3 μg/mL in controls (P < 0.05). Twenty-one patients, the largest share of the group, showed intermediate TPY values (27.43 ± 11.18 μg/mL). Six patients showed high TPY plasma concentrations of 85–237 μg/mL (from 5- to 16-fold above normal), with a mean of 154.3 ± 51.9 μg/mL (Fig. 1).

Among the PY fractions, sitosterol showed the highest percentage and constituted the major contributor to plasma TPY, with a mean of 49.4% (range 29–59). This was followed by campesterol, with a mean of 21.9% (range 12–30). The remaining fractions included stigmasterol (mean 7.8%, range 6–10) and avenasterol (mean 4.6%, range 3–7), with minor amounts of brassicasterol and other PYs. Plasma TPY concentrations determined two groups of patients. Those with relatively low values (plasma TPY < 50 μg/mL, n = 21) constituted group A and those with
higher values (plasma TPY >85 µg/mL, n = 6) group B. None of the patients showed values from 50 to 85 µg/mL.

Sitosterol percentages were lower in group A (making up 42.6% of the TPY content) and higher values were found only in group B (making up 55.3% of the TPY content). This result was statistically significant by the t test (P = 0.039). In contrast, brassicasterol percentages decreased as plasma TPY increased.

Group B, with high PY levels, received the following lipid emulsions: case nos. 4, 5, and 9 received Lipofundina MCT+LCT 20%; case no. 11 received Lipövenos 10%; case no. 16 received Intralipid 20%; and case no. 19 received Ivelip 20%.

Liver function results

Fifteen patients, accounting for 55% of the total group, were found to have two or more elevated LFT findings with values two-fold above normal. Severe LFT alteration, defined as an increase over two-fold above normal in more than three parameters and/or total bilirubin levels >60 µmol/L, was found in eight of these patients. Moreover, within the study group, five patients (case nos. 8, 11, 16, 19, and 21) displayed a pattern of cholestasis, with two or three of the representative parameters elevated. In addition, four patients presented features linked to mixed liver injury (cholestasis and cytolysis). LFT results and TPY values for the six patients with phytosterolemia ≥85 µg/mL are listed in Table 3.

Liver biopsies

Liver biopsy was performed in a subset of eight patients. Time from blood sample collection to biopsy was 36 mo in case no. 8; <2 mo in case nos. 4, 9 and 16; and 14 mo in case no. 21. Among them, five showed histologic features of moderate to severe disease. Lesions suggestive of cholestasis (cholangiolar proliferation and/or canalicular cholestasis) were present in four patients, at varying degrees of severity. Features of severe liver impairment, with extensive fibrosis (marked bridging) and macrovesicular steatosis, were present in three patients (case nos. 8, 9, and 21). Nodulation was present in case no. 9. Moreover, the presence of portal inflammation in all cases, although of varying intensity, confirmed the severity of the lesions. All patients showed high plasma TPY levels. However, although case nos. 8 and 21 showed plasma TPY values up to two-fold above the control levels (36 and 33 µg/mL, respectively), case nos. 4, 9, and 16 showed values 10-fold above control levels (237, 158, and 162 µg/mL, respectively).

Simple linear regression analysis

Two simple linear regression models were developed. In the first (Table 4), the correlation between plasma TPY, LFTs (γ-glutamyl-transferase, alkaline phosphatase, alanine transaminase, AST, total bilirubin), and platelet counts were tested. The second model (Table 5) investigated the correlation between the same parameters and the amount of lipid infused. In the first model, all LFT variables and platelet counts showed a statistically significant association with plasma TPY. The strongest r² values were for total bilirubin (r² = 0.53, P = 0.0001) and AST (r² = 0.50, P = 0.0001).

Table 3
Liver function tests in patients with high TPY levels (≥85 µg/mL)

<table>
<thead>
<tr>
<th>Case nos.</th>
<th>TPY (µg/mL)</th>
<th>TB (µmol/L)</th>
<th>AP (µkat/L)</th>
<th>γ-GT (µkat/L)</th>
<th>ALT (µkat/L)</th>
<th>AST (µkat/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n ≤ 18)</td>
<td>(n ≤ 1.5)</td>
<td>(n ≤ 1)</td>
<td>(n ≤ 0.63)</td>
<td>(n ≤ 0.50)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>237</td>
<td>213</td>
<td>4.4</td>
<td>5.5</td>
<td>4.1</td>
<td>2.3</td>
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<tr>
<td>5</td>
<td>169</td>
<td>43</td>
<td>8.0</td>
<td>9.7</td>
<td>2.9</td>
<td>1.2</td>
</tr>
<tr>
<td>9</td>
<td>158</td>
<td>269</td>
<td>5.5</td>
<td>4.9</td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>11</td>
<td>115</td>
<td>54</td>
<td>6.5</td>
<td>4.0</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>16</td>
<td>162</td>
<td>42</td>
<td>8.3</td>
<td>2.6</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>19</td>
<td>85</td>
<td>17</td>
<td>12.2</td>
<td>4.8</td>
<td>0.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

ALT, alanine transaminase; AP, alkaline phosphatase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl-transferase; TB, total bilirubin; TPY, total phytosterol

* Conversion factor to milligrams per deciliter: divide by 17.1.
† Conversion factor to units per liter: divide by 0.01667.
In contrast, platelet counts showed a far weaker $r^2$ value ($r^2 = 0.15$, $P = 0.04$). The second model showed a statistically significant correlation between lipid infused and total bilirubin ($P = 0.038$) and AST ($P = 0.049$). However, $r^2$ values were weaker than in the first model, 0.19 for bilirubin and 0.16 for AST. Platelet counts were not significant in this model (Tables 5 and 6). Plasma TPY correlated with infused PY ($r = 0.54$, $P = 0.0038$).

**Multiple linear regression analysis**

Stepwise multiple regression models evaluated interactions among plasma TPY and the following variables: weekly dose of PY infused, duration of PN, and presence of oral nutrition (none, scarce, or regular; Table 6). The risk factors for high plasma TPY were the presence of an oral diet with a negative correlation ($b = -52.3$, $P = 0.001$) and PY infused ($b = 2.54$, $P = 0.093$).

**Discussion**

Total PY concentrations in plasma tend to remain stable in healthy individuals eating Western diets, and typically range from 3 to 17 μg/mL [15]. In contrast, individuals eating vegetarian diets and patients with hypercholesterolemia treated with extra PY are likely to have relatively high values, from 1.5 to 3 times above those of Western diets [5]. Thus, despite a high intake of PYs through the diet, plasma TPY values remain relatively low [16].

Phytosterolemia is a rare congenital disease involving intestinal hyperabsorption of sterols with an impaired ability to secrete biliary sterols [2,17]. Patients have high levels of TPY in plasma, up to 40 times above baseline, and sitosterol is the dominant PY fraction. Although some degree of liver damage has been described, most patients die prematurely from coronary disease.

In our study, average plasma TPY concentrations measured in patients with intestinal failure treated with home PN were significantly higher than those in healthy individuals who had low values, in agreement with published studies. The six patients with highest plasma TPY levels also presented severe liver dysfunction.

In a study of 29 children, Clayton et al. [3] found that patients receiving PN with lipids, some of them long term, had high plasma TPY concentrations and cholestatic liver disease. Patients who received higher lipid doses had higher plasma TPY levels and developed severe liver dysfunction. Conversely, patients who received low doses had variable plasma TPY concentrations and mild or undetectable liver dysfunction. Ellegard et al. [12], in their study of eight patients with short bowel syndrome receiving PN, found higher serum TPY levels than in 16 patients with short bowel syndrome without PN and controls. Although the number of patients studied was rather small to establish the significance of their results, they found that higher TPY levels in serum correlated with higher alkaline phosphatase levels. According to these findings, short bowel syndrome per se does not cause high serum TPY levels.

Excess of macronutrients [2,18] has been classically associated with PNALD. Two recent retrospective studies including a large number of adults receiving long-term PN have investigated the association between analytic and clinical variables and PNALD by multivariate analysis. In a study by Cavicchi et al. [19], patients received 88% of their basal metabolic rate requirements by PN. Regarding these results, a PN intake $>1$ g · kg$^{-1}$ · d$^{-1}$ of lipids is a significant risk factor for developing PNALD.

In a study by Luman and Shaffer [20], lower doses of lipids and a higher dose of glucose were administered, making up 62.7% of the patients’ calculated basal metabolic rate. Based on multivariate analysis, the investigators concluded that the only significant variable for PNALD is a small bowel syndrome shorter than 100 cm, which determines PN dependence.

Thus, in terms of energy intake, our patients fell between those of Luman and Shaffer [20] and Cavicchi et al. [19]. Although the importance of higher PN lipid doses has been stressed, the quality of the lipid emulsions is also relevant. Gerard-Boncompain et al. [21] and Sluiter et al. [22] described liver complications related to changing the brand of PN used for feeding. In both studies, patients improved and returned to their prior condition shortly after switching back to the former brand. They hypothesized a link between the composition of the emulsions and the clinical pictures, but no qualitative analyses were per-
The role of PY intake therefore remained to be elucidated.

A relation between perfused lipids and serum TPY levels has been demonstrated in healthy individuals [23,24]. The investigators showed that intravenous infusion of Intralipid 20% leads to a progressive increase of serum TPY, with slow decreases thereafter. Nevertheless, a literature review has suggested that such a relation may not always be apparent in patients. Because plasma TPY reflects the balance between input and biliary excretion, PYs are expected to start increasing significantly after liver damage [25,26].

In our study, lack of oral feeding and infused PY appeared as risk factors for high TPY in plasma. The benefits of oral feeding have long been recognized from the results of research in animal and human models. Among others, increased bile acid secretion, gallbladder contractility, bile salt pool, and bile salt formation may contribute to increasing PY excretion, thus lowering the risk of liver damage [2,26].

Phytosterol content in lipid emulsions has been shown to change according not only to the brand administered but also to the specific batch of the product. Moreover, PY load in lipid emulsions is mainly dependent on the source of the oil used in the emulsion [27], which makes the comparison of findings among studies even more complex.

The effects of a high PY load by vein on hepatic metabolism have been investigated in animal models. In a piglet model, Iyer et al. [11] found a progressive accumulation of PY in serum, liver, and bile, together with raised serum bile acid levels and decreased maximum bile acid excretion. They accordingly concluded that PYs can be a cause of cholestasis. In mice given a standard total PN lipid emulsion for 7 d, Tazuke et al. [14] observed alterations in mdr gene expression that may have contributed to the development of PNALD.

An important question is whether high plasma TPY may be a cause or a consequence of liver impairment in humans. In a recent study in 67 patients with end-stage primary biliary cirrhosis (PBC), Nikkila et al. [28] documented high serum levels of campesterol, sitosterol, and cholestanol. Interestingly, the levels dropped back to normal in all patients after liver transplantation. This suggests that, even when the intestinal barrier remains intact, a small amount of PYs arriving to the liver cannot be efficiently metabolized in the presence of liver damage. Although campesterol and sitosterol fractions are notably increased in patients with end-stage PBC, patients with PN-induced phytosterolemia tend to present much higher concentrations [3]. We found that the six patients with the highest plasma TPY levels also had the highest percentage of sitosterol and campesterol values. The higher sitosterol levels found in patients with PNALD may therefore suggest more comprehensive liver damage than that occurring in end-stage PBC. In part, this may reflect the different origin of the cholestatic liver disease condition. At this point it is clear that sitosterol and high serum or plasma TPY levels indicate some degree of non-specific liver damage. However, although there is evidence of direct liver toxicity by PY, further studies are needed to assess the mechanisms involved in the liver damage related to PNALD and PBC.

Recent evidence has been suggested that the administration of PY-free fish oil–based lipid emulsions, containing ω-3 fatty acids, may be useful to reverse PNALD. Research on animal models has shown that fish oil–based emulsions do not impair bile flow and may even prevent hepatic lipogenesis [29]. Moreover, Guru et al. [30,31] showed that their exclusive administration in pediatric patients may reverse the condition of PNALD, implying a significant shorter time to normalization of direct bilirubin and an improvement in survival rate. Therefore, fish oil–based emulsions may offer a good alternative to the use of standard soy-based emulsions in patients using home PN who are at risk of PNALD.

In conclusion, our findings are consistent with the results reported by Clayton et al. [3] in children and Ellegard et al. [12] in adults, thereby strengthening the link between high plasma PY levels and intravenous lipid emulsions administered to patients on long-term PN.

Moreover, the statistical correlation observed between all the LFTs and plasma TPY suggests that there is an associ-
ation between TPY and PNALD. Thus, in line with previous findings, liver damage may be linked to the presence of high PY levels in plasma.

A lack of oral feeding in patients on PN and the amount of intravenous PY intake appear to be risk factors of high plasma TPY. Therefore, oral and/or enteral feeding during PN treatment may be a key factor for preventing PNALD.

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References