Hepatic transport of bile salt and bile composition following total parenteral nutrition with and without lipid emulsion in the rat\textsuperscript{1-3}

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ABSTRACT The effect of total parenteral nutrition (TPN) on bile flow and composition and on hepatic bile acid transport maximum (T\textsubscript{m}) and bile salt-independent bile flow (BSIF) was studied in the rat following seven days TPN containing 33% calories from Intralipid (IL) or Liposyn (LP) or 0% calories from lipid. All TPN regimes markedly reduced bile flow. In no case did TPN cause an increase in bile cholesterol concentration or saturation relative to bile acid and phospholipid. Bile acid T\textsubscript{m} was reduced in rats receiving either 0% lipid or 33% IL; BSIF was reduced only in the 0% lipid group. Rats receiving 33% LP had a higher bile flow than the other TPN regimes while bile acid T\textsubscript{m} and BSIF were similar to controls. It is proposed that in established TPN, bile flow is reduced largely as a result of decreased hepatic bile acid excretion. In the rat, TPN has no deleterious effect on the molar concentration of cholesterol, phospholipid or bile acid in bile secreted by the hepatocyte. The significant differences between the effect of the two lipid emulsions on hepatobiliary function require further study. Am J Clin Nutr 1985;41:1283-1288.

KEY WORDS TPN, bile flow, bile composition, bile acid, cholestasis, parenteral lipid

Introduction

The development of hepatocellular dysfunction marked by abnormal plasma liver function tests (eg elevation of alkaline phosphatase, 5' nucleotidase or transaminases), cholestasis and gallbladder disease are the most common metabolic complications of total parenteral nutrition (TPN)\textsuperscript{(1-4)}. While no specific causal agent or formulation has been agreed on, it is clear that these problems are exacerbated by such factors as essential fatty acid deficiency\textsuperscript{(5)}, excessive dextrose and/or amino acid infusion\textsuperscript{(6)}, accumulation of hepatotoxic bile acid\textsuperscript{(7)} or amino acid degradation products\textsuperscript{(8)}, accompanying septis\textsuperscript{(9)} or ileal disease\textsuperscript{(3, 4)}. Most recent studies suggest that the inclusion of lipid in TPN does not increase the risk of gallbladder diseases\textsuperscript{(4, 10)}.

The most common explanation for TPN-induced choleystatic changes is failure of gallbladder emptying because of reduction or absence of the intestinal hormones which are normally released on oral food intake and serve to stimulate hepatobiliary secretion\textsuperscript{(1, 2)}. Reduced bile flow has been reported for humans receiving TPN\textsuperscript{(11)}. In the rat, a species lacking a gallbladder, a similar effect has been found\textsuperscript{(12)}, although, recently Heyman et al\textsuperscript{(13)} could find no effect of TPN on hepatic bile flow. Recent reports of studies in the prairie dog by Doty et al\textsuperscript{(14)}, used a comparison of the ratio of \textsuperscript{3}H cholic acid between gallbladder and hepatic bile to show that TPN had induced gallbladder stasis. In addition to bile flow per se, bile composition may also be important since the development of gallstones is well-known to be related to alteration in the molar content of the major organic compounds in bile; especially the

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degree of cholesterol supersaturation (15). Data on bile composition during TPN, however, is incomplete and contradictory with reports showing either increased cholesterol content (2, 12, 16) or bile of normal composition (14, 17, 18).

This report describes studies to define the effect of TPN on bile flow and composition in the rat, together with measurement of maximum hepatic capacity to excrete bile salt (Tm) and the fraction of bile flow generated by bile salt dependent and by bile salt-independent (BSIF) excretory pathways (15). Because administration of parenteral lipid emulsion causes elevation of plasma free cholesterol (19, 20) and altered hepatic cholesterol and bile acid metabolism (20), these studies used both lipid-free and lipid-containing TPN regimes. The rat was specifically chosen for study because it lacks a gallbladder. Thus, cannulation and collection of bile from the common bile duct allows the direct determination of quantity and composition of bile secreted by the hepatocyte.

Methods

Eight groups of male Wistar rats (175–225 g) were prepared for TPN, housed and maintained as described previously (20). A basal TPN solution containing dextrose, amino acids, vitamins and minerals was infused into six groups of rats at a rate of 48.2 ± 0.72 ml/day (± SD) and supplied approximately 2/3 total calories received as TPN. The composition of this solution was similar to that reported earlier (20) except that amino acids were supplied by 10% Travasol (Baxter Labs, Malton, Ontario) and vitamins as MVI-12 (USV Canada Inc., Mississauga, Ontario). Remaining TPN calories (approximately 33% total) were given by infusion of the following, one each to two groups of TPN rats; 25% dextrose (0% lipid), 10% Intralipid (33% IL, Pharmacia, Dorval, Quebec) or 10% Liposyn (33% LP, Abbott Labs, Montreal). Vitamin K was given midway through the course of TPN as vitamin K (Abbott Labs, Montreal), 0.02 mg in saline. Two groups of rats (control) were sham-operated, attached to the infusion apparatus and allowed free access to laboratory rat Purina Chow. Control rats were not fasted prior to study. The care and use of rats in these experiments followed the guidelines of the National Research Council and all procedures were approved by the University animal care committee.

After seven days one group from each treatment was anesthetized (sodium pentathol, 0.4 mg/kg ip), removed from the infusion apparatus and the common bile duct cannulated distal to the bifurcation as described earlier (21). Bile was quantitatively collected in 30 min aliquots for 2 h. As in previous studies (20), experiments were always commenced to coincide with the peak in hepatic diurnal cycle of cholesterol and bile acid synthesis. Bile samples were extracted (12), assayed for cholesterol (22), phospholipid (23) and bile acid (24) content. Data were calculated as mol excreted in bile/30 min/100 g body wt. The relative cholesterol saturation was calculated as the molar ratio of bile acid plus phospholipid to cholesterol, as used earlier by Gimmon et al (12). This calculation has been used in preference to lithogenic index because of the low concentrations of cholesterol normally present in rat bile.

Tm and BSIF were determined in the remaining four groups of rats, under sodium pentothal anesthesia, as described by Gonzalez et al (25). After cannulation of the common bile duct, one 30-min bile sample was collected to establish basal flow and composition. Rats were then stabilized to infusion via the jugular vein catheter by a 15-min infusion of 0.9% saline containing 4% BSA, during which bile was collected. The infusion was then changed to taurocholic acid (45 mM) in 0.9% saline plus 4% BSA at a rate of 1.2 μmol/min/100 g body wt as described in detail by Simon et al (25). Infusion rate was controlled by a Harvard infusion pump (Harvard Apparatus Co, S Natick, MA). Bile was collected in four 15-min aliquots. As reported by Simon et al (26), preliminary studies in this laboratory showed maximal Tm and bile flow was attained at 1.2 μmol taurocholic acid/min 100 g body wt, with significant decreases occurring at higher infusion concentration. All bile samples were extracted and analyzed as described above. Tm was calculated as the mean of the two highest consecutive hepatic bile acid outputs (26). BSIF was calculated according to the two component theory of bile flow (15) using the extrapolation of the linear regression of bile flow vs bile salt excretion to give bile flow at zero bile salt excretion. The slope, intercept (BSIF) and correlation coefficient was calculated on data for each rat, and additionally using pooled data from six rats in each group. Significant differences between treatments were determined using analysis of variance and Duncan’s Multiple Range Test.

Results

Body weight change during the seven-day experimental period was similar for all three TPN treatments; 3.3 ± 3.7 g, 5.7 ± 6.3 g, 6.8 ± 4.7 g (± SD) for 0% lipid, 33% IL and 33% LP, respectively. Weight gain of control rats was 20.2 ± 4.2 g. No significant difference was found among the groups in routine tests of liver function for alkaline phosphatase, SGOT or bilirubin. Data from studies on the effect of TPN on bile flow and composition showed no significant change among the four 30-min samples obtained over the 2 h collection period within a treatment group. Data for bile flow and molar content of cholesterol, phospholipid and bile acid is, therefore, given in Table 1 as the mean ± SE for each group for entire 2 h bile collection. All TPN treatments significantly reduced bile flow. This reduction was greater in rats which had received 0% lipid or 33%...
TABLE I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bile flow (mL/hr/100 g wt)</th>
<th>Cholesterol (μg/mL/100 g wt)</th>
<th>Phospholipid (μg/mL/100 g wt)</th>
<th>Bile acid (μg/mL/100 g wt)</th>
<th>Relative cholesterol concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.63 ± 0.02</td>
<td>1.6 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>30.0 ± 2.2</td>
<td>19.3 ± 0.9</td>
</tr>
<tr>
<td>0% Lipid</td>
<td>0.33 ± 0.02</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>46.1 ± 6.0</td>
<td>36.4 ± 6.4</td>
</tr>
<tr>
<td>33% IL</td>
<td>0.32 ± 0.03</td>
<td>1.2 ± 0.2</td>
<td>2.1 ± 0.3</td>
<td>24.1 ± 1.8</td>
<td>22.6 ± 4.4</td>
</tr>
<tr>
<td>33% LP</td>
<td>0.46 ± 0.04</td>
<td>1.2 ± 0.1</td>
<td>2.1 ± 0.4</td>
<td>28.7 ± 3.7</td>
<td>25.8 ± 3.9</td>
</tr>
</tbody>
</table>

*†‡ data within a column with a different superscript, are significantly different, p < 0.05, from one another, n = 6 for all groups.

Data given are ± SE for control rats and rats receiving TPN as described in Methods. † Relative cholesterol concentration of bile as the molar ratio bile acid + phospholipid/cholesterol.

IL than in rats which had received 33% LP. Lipid-free TPN for seven days had no effect on cholesterol or bile acid concentration but significantly decreased the phospholipid content of bile. The molar concentration of bile from rats receiving 33% IL was similar to controls while infusion of the other lipid emulsion, 33% LP, resulted in less cholesterol per mL bile than in the control rats. The composition of bile from rats receiving the two lipid preparations was similar (Table 1). When the data were expressed as μmol cholesterol or bile acid excreted/hr/100 g body wt, however, it was found that rats receiving 33% IL secreted approximately 50% less bile acid (data not shown). All rats receiving TPN secreted less cholesterol in bile per h from the body than controls. Calculation of the relative cholesterol saturation showed that in no case did TPN result in an increased content of cholesterol in bile relative to the content of bile acid plus phospholipid (Table 1).

The stimulation of bile flow caused by an intravenous infusion of taurocholic acid is shown in Figure 1. A 30-min bile sample collected prior to bile acid infusion confirmed data in Table 1 which demonstrated marked reduction of bile flow present in all TPN regimes, irrespective of the use of lipid. In control rats the infusion of taurocholic acid resulted in a prompt increase in bile flow which peaked between 15 and 30 min and remained constant thereafter. Rats receiving 0% lipid showed a significantly lower response in bile flow at all time points than controls. Bile flow generated in response to taurocholic acid infusion was, similarly, reduced by TPN with 33% IL, with the exception of the initial 15-min bile collection period (Fig 1). In marked contrast to 0% lipid and 33% IL, 33% LP rats produced a bile flow equivalent to that achieved in the sham operated controls.

Data for bile salt T_m, BSIF and the correlation coefficient of the linear regression plots of bile flow vs bile acid excretion used to calculate BSIF are given in Table 2. The T_m for bile salt was significantly reduced in rats receiving TPN with 0% lipid, 33% IL but not in rats receiving 33% LP. BSIF was reduced only in rats which had been given the lipid-free 0% lipid TPN formulation.

Discussion

The etiology of hepatocellular dysfunction and gallbladder disease which frequently accompany TPN is unknown. Conceivably, prolonged absence of oral food intake in the patient nutritionally supported by TPN results in alterations in cycling frequency and composition of the enterohepatic circulation. Reduced hepatic bile secretion may be expected to lead to accumulation of potentially hepatotoxic levels of metabolites normally excreted in bile. Gallbladder disease may, similarly, be caused by bile stagnation or, on the other hand, it may be related to an alteration in the molar composition of the major organic compounds cholesterol, phospholipid and bile acid present in the bile itself. Information on the effects of TPN on bile flow and composition in man or animals is incomplete and conflicting. The present studies have attempted to supply this information by determination of hepatic bile flow and composition, together with maximum hepatic bile acid secretory capacity in the rat following seven days TPN.

In these experiments TPN, irrespective of the use of lipid emulsion uniformly decreased
bile flow (Table 1). A similar 50% reduction in bile flow has been reported for the rat following 12 days TPN (12). Recently, Heyman et al (13) reported that eight days lipid-free TPN in the rat had no effect on bile flow. Bile flow in the control group in these studies, however, was 0.30 ± 0.04 ml/hr/100 g body wt (13). Bile flow of control rats in the present study (0.63 ± 0.02 ml/hr/100 g body wt, ± SE) are much higher and similar to those reported by others for rats of the same weight range (26). In other species, TPN was found to reduce bile flow by approximately 25% in the adult rabbit (27) but to have no effect on hepatic bile flow in the prairie dog (18). Possibly these differences relate to diurnal cycle or time elapsed in the control group from last food intake when the bile duct was cannulated.

The TPN formulations used in the present studies resulted in neither increased cholesterol molar concentration or relative concentration in hepatic bile nor in a reduced bile acid concentration. Data have been reported for the human indicating no effect of TPN on bile acid concentration in bile samples

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bile salt Tm (μmol/hr/100 g wt)</th>
<th>BSIF (ml/hr/100 g wt)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74.9 ± 5.8*</td>
<td>0.38 ± 0.04*</td>
<td>0.93</td>
</tr>
<tr>
<td>0% Lipid</td>
<td>39.6 ± 2.9†</td>
<td>0.24 ± 0.03†</td>
<td>0.88</td>
</tr>
<tr>
<td>33% IL</td>
<td>48.7 ± 4.0†</td>
<td>0.33 ± 0.03†</td>
<td>0.90</td>
</tr>
<tr>
<td>33% LP</td>
<td>65.5 ± 6.4*</td>
<td>0.41 ± 0.04*</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*† Data within a column with a different superscript are significantly different, p < 0.05, n = 6 for all groups.

Data given are x ± SE for bile salt Tm (transport maximum) and BSIF (bile salt-independent bile flow) calculated using all data for each group. Data calculation and rat treatment is described in Methods. § correlation coefficient for linear regression analysis.
obtained by duodenal intubation (7, 16). Previous studies in animals maintained by TPN have reported increased bile lithogenicity due to increased concentration of cholesterol and reduced concentration of bile acid (12), normal biliary bile acid concentration for the rat (13), and decreased molar concentration of cholesterol and lithogenicity of bile (17) or unaltered bile lithogenic index in the prairie dog (18). The reason for the discrepancy in these findings is unclear.

Data from the present studies (Table 1) are interpreted to suggest that the most significant effect of TPN is on bile flow rather than on bile composition. These studies, although they were performed in the rat which lacks a gallbladder and normally has a much lower cholesterol content in bile than man, support the hypothesis (1, 2, 4) that gallbladder disease develops initially through failure to maintain bile flow in the absence of oral food intake. In this regard Messing et al (2) used ultrasonography to demonstrate that gallbladder sludge was a prerequisite to gallstone formation in adults receiving TPN. Similar findings of biliary sludge and gallbladder stasis have been observed in the prairie dog after TPN irrespective of the use of parenteral lipid emulsion (14). Conceivably, prolonged storage of bile in the gallbladder results in progressive concentration (18) with the generation of a thick bile, stone growth and cholelithiasis. The present studies can be extrapolated only to TPN with an intact enterohepatic circulation. The greater risk of gallbladder disease with parenteral nutritional support of adults or children with ileal disease than in patients receiving TPN for other reasons (4, 10) clearly suggests that the return of bile acids from the ileum via the portal vein is important; possibly for generation of hepatic bile flow (15) or for maintenance of normal bile acid concentration.

The process of bile acid excretion, although not fully understood, is known to involve uptake of bile acids across the sinusoidal membrane by a specific carrier-mediated process requiring maintenance of a sodium gradient by Na*, K*-ATPase, their subsequent transport across the hepatocyte and finally excretion across the canalicular membrane by an energy-independent carrier (15). The rate limiting step is believed to be the carrier-mediated transport across the canalicular membrane (28). Bile flow is generated as water flows down the osmotic gradient arising from the excretion of bile acid into the bile canaliculus (15). It is recognized that bile flow does occur in the absence of any bile acid excretion, this fraction of the bile flow is termed BSIF (15). The present studies demonstrated that following 7 days TPN with either 0% lipid or 33% IL, the rat has a reduced ability to excrete an intravenous load of bile acid into bile and consequently generates a lower bile flow (Table 1, Fig 1). The data suggest the reduced bile flow in these two groups of rats is, therefore, at this time in TPN, primarily the result of decreased bile acid excretion. Theoretically, the reduced bile acid transport maximum could be due to reduced uptake by the sinusoidal membrane, trans-hepatocyte movement or excretion across the canalicular membrane. In this regard Back et al (29) proposed, on the basis of serum tests of liver function representing either sinusoidal membrane function or canalicular function, that after 1 week of TPN in infants the effect was on the canalicular membrane with no change occurring in sinusoidal membrane function or integrity. No difference was present between infants who received Intralipid and those who did not (29). Similarly, the present studies found no difference in total bile flow, F, or BSIF (Tables 1 and 2) between rats receiving TPN with 0% lipid and 33% IL. Further studies are required before the mechanism and site of decreased bile acid excretion can be clearly defined.

Rats receiving 33% LP maintained a higher bile flow (Table 1) and bile acid Tm (Table 2) after 7 days TPN than rats receiving either 0% lipid or 33% IL. The response of the 33% LP group to taurocholic acid infusion was, furthermore, similar to sham-operated controls suggesting that, over a relatively short-term infusion period, bile acid excretory function is better maintained with Liposyn than with either Intralipid or a lipid-free TPN regime. The reason for this is not known. The two lipid emulsions, Liposyn and NutralseaLipid, differ from one another primarily in the vegetable oil on which they are based and in their emulsion particle size. It is not unreasonable to suggest that the different fatty acid composition, particularly the
level of, and ratio between, the two essential fatty acids, linoleate and linolenate, may affect their metabolism and subsequent function in lipoprotein metabolism, structural phospholipids or prosta-glandin synthesis. Recently, the infusion of Liposyn into infants has been shown to result in enhanced hyperlipidaemia of longer duration than found with Nutralipid infusion (30); thus further implying that important differences may be present in the metabolic handling of these two parenteral lipid products. Clarification of the apparent difference between the emulsions on both plasma triglyceride metabolism (30) and bile flow and bile acid excretion (Tables 1 and 2) is obviously a subject requiring further study.

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References

27. Zahavi I, Shafer EA, Gall DG. Total parenteral nutrition (TPN) associated cholestasis in infant and adult rabbits. Gastroenterology 1982;82:1217A.