Liver Membrane Composition after Short-Term Parenteral Nutrition With and Without Taurine in Guinea Pigs: The Effect to Taurine (43617)

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Abstract. **Having recently demonstrated that taurine supplementation prevents total parenteral nutrition (TPN)-induced cholestasis, we chose to use this model to examine plasma membrane composition in relation to bile formation. Male guinea pigs received daily a mixture of glucose and of the amino acid solution Travasol with or without added taurine (1.2 m*M*). After 3 days, bile was collected and liver plasma membrane fractions enriched in sinusoidal lateral membrane and bile canalicular membrane domains were isolated. In animals receiving TPN alone, bile flow and biliary secretory rate of bile acid and bicarbonate decreased significantly compared with controls. Although membrane ATPases (Na^+K^+ and Mg^+) were unchanged, TPN induced an increase in the lipid to protein ratio and a decrease of polyunsaturated fatty acids, in conjunction with a higher content of diene conjugates in sinusoidal lateral membrane fractions. Taurine corrected these changes and, in addition, reduced significantly the cholesterol to phospholipid ratio in both membrane fractions. The data show that changes in liver cell membranes occur in TPN-induced cholestasis and suggest that free radical injury may play a role. As taurine prevented cholestasis as well as membrane changes, it is suggested that taurine should be added to amino acid solutions used for parenteral nutrition.**

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Total parenteral nutrition (TPN) is the most important development in clinical nutrition of the past two decades. Although the incidence of technical and catheter-related complications has decreased significantly, hepatic complications remain a constant threat (1).

TPN-induced intrahepatic cholestasis has been attributed to the various components of TPN regimens, i.e., an excess or an imbalance of amino acids (2, 3), deficiency of carnitine (4), and deficiency of cystine or taurine (5). Abnormalities in bile salt composition that result in the formation of hepatotoxic bile salts, namely, lithocholic acid, have also attracted attention in both infants and adults on TPN (6–8). Lithocholate-induced cholestasis is known to be associated with hepatic plasma membrane changes (9). However, no studies have yet examined the effect of TPN on liver cell membrane composition.

Taurine has been shown to increase bile formation and secretion through an increase in bile acid synthesis (10–12) in guinea pigs as well as to protect against the cholestatic effect of sulfated lithocholate both in its free and conjugated form (13). The aim of this study was to evaluate the effect of TPN and taurine supplementation on plasma membrane composition in a guinea pig model of TPN-induced cholestasis.

Materials and Methods

Male Dunkin-Hartley guinea pigs (400–450 g) were obtained from Charles River (La Prairie, Quebec) and allowed 3 to 4 days of acclimatization in a room maintained at 22°C with a 12:12-hr light:dark cycle. Purina guinea pig chow and water were offered *ad libitum.*

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Table I. Nutrients Provided Daily by the TPN Solution

Amino acids (mmol/kg/day)	
Essential	
L-Leucine	4.19
L-Phenylalanine	3.33
L-Methionine	3.46
L-Lysine	3.56
L-Isoleucine	3.26
L-Valine	3.50
L-Histidine	2.52
L-Threonine	3.14
L-Tryptophane	0.79
Nonessential	
L-Alanine	20.78
Glycine	24.66
L-Arginine	5.31
L-Proline	3.25
L-Tyrosine	1.96
Carbohydrates (mmol/kg/day)	
Dextrose	144.28
Electrolytes (mmol/liter)[a]	
Sodium	70
Chloride	70
Potassium	60
Phosphate	30
Calcium	23
Magnesium	5
Vitamins (ml/day)[b]	
Multivitamins	0.08

[a] Electrolytes were administered at 2 ml/hr.

[b] Multivitamins (U.S. vitamins) supply: vitamin A, 53.0 UI; vitamin D, 3.0 UI; vitamin E, 0.2 UI; ascorbic acid, 0.33 mg; niacinamide, 0.64 mg; pantothenic acid, 0.24 mg; pyridoxine, 0.06 mg; riboflavin, 0.06 mg; thiamine, 0.05 mg.

After a 2-hr fast, the animals were anesthesized and silastic tubing (PE-50; Dow Corning Corp., Mississauga, Canada) was positioned in the distal part of the superior vena cava, tunneled subcutaneously, and exteriorized in the scapular region, where it was threaded through a metal swivel to permit free movement of the animals. Some of the animals equipped with a central catheter were given saline 0.45% at 2 ml/hr but continued to have free access to food and water. The two experimental groups received a mixture of glucose and of the amino acid mixture Travasol (Canada, Inc., Mississauga, Ontario) with and without added taurine (1.2 m*M*) for a period of 3 days at a perfusion rate of 2 ml/hr. The nutrients provided daily are shown in Table I.

After 3 days, the animals were anesthesized for placement of a common bile duct cannula, and bile collections were initiated within 1 hr of interrupting the TPN feeding. After 30 min of bile drainage, two periods of 15-min collections were obtained. Some aliquots were collected under mineral oil for immediate measurement of bicarbonate (Microgazometer Corning 965). Other specimens were frozen at −60°C until further analysis. Bile flow was determined gravimetrically assuming 1 ml of bile to have a density of 1 g. Cholesterol, phospholipid, and bile acids were determined enzymatically (14–16). Sodium, potassium, chloride, and calcium were measured in our clinical biochemistry laboratory using the parallel analyzer from Rupp and Bowman Co. (Farmington Hills, MI).

After completion of bile collections and before removing the liver, a small liver sample was taken for light microscopy and electron microscopic (EM) examination (17). The liver was then flushed with cold (4°C) saline (0.9%) through a needle in the portal vein after interruption of the arterial circulation. Rapid removal and homogenization of the liver was then carried out in 2 v/w of 1 m*M* bicarbonate buffer. The homogenate was centrifuged at 50*g* for 5 min. Supernates were then centrifuged at 500*g* for 10 min. The resuspended pellet was centrifuged at 1,700*g* for 10 min. Sinusoidal membranes (SM) and bile canalicular membranes (BCM) were isolated by a method in use in our laboratory and described previously (18). Briefly, membranes were gently mixed with a sucrose solution (d = 1.26 g/ml) in order to reach a final density of 1.22 g/ml. A second sucrose solution (d = 1.18 g/ml) was added and followed by a 1 m*M* $NaHCO_3$ buffer. Ultracentrifugation at 60,000*g* was carried out for 1 hr. The SM fraction was identified at the interface between the two sucrose solutions while the BCM fraction was identified at the interface between the lower density sucrose solution and the $NaHCO_3$ buffer. Each membrane fraction was then diluted with a $NaHCO_3$ (1 m*M*)-$CaCl_2$ (0.5 m*M*) buffer followed by centrifugation at 10,000*g* for 20 min. The same procedure was repeated twice. Aliquots of membranes were kept for EM

Table II. Bile Flow, Bile Acid Secretory Rate, and Bile Composition[a]

	Bile flow (μl/min/g liver)	BASR (nmol/min/g liver)	Biliary lipid constituents (m*M*)			Biliary electrolytes (m*M*)			
			Ch	PL	BA	Na^+	K^+	Cl^-	HCO_{3-}
Controls (*n* = 6)	6.0 ± 0.4	14.23 ± 2.4	0.005 ± 0.001	0.04 ± 0.004	2.7 ± 0.3	147 ± 2	3.9 ± 0.2	59 ± 2	78.5 ± 2.3
TPN (*n* = 6)	2.8 ± 0.2[b]	6.90 ± 1.0[b]	0.005 ± 0.001	0.05 ± 0.007	3.2 ± 0.5	142 ± 4	3.8 ± 0.1	57 ± 4	69.9 ± 2.2[b]
TPN-taurine (*n* = 6)	3.9 ± 0.3[b,c]	12.6 ± 1.9[c]	0.016 ± 0.003[b,c]	0.04 ± 0.007	3.5 ± 0.7	141 ± 2	3.5 ± 0.1	58 ± 5	73.7 ± 3.4

[a] Values represent the mean ± SE. Abbreviations used in table: BASR, bile acid secretory rate; Ch, cholesterol; PL, phospholipids; BA, bile acids.

[b] $P < 0.05$ vs controls.

[c] $P < 0.05$ vs TPN.

Table III. Activities of NA^+K^+-ATPase and Mg^{2+}-ATPase in Sinusoidal and Bile Canalicular Domains[a]

	Homogenate	SM	BCM
Na^+K^+-ATPase			
Controls	0.36 ± 0.10	1.45 ± 0.29	1.41 ± 0.72
TPN	0.53 ± 0.06	1.47 ± 0.30	1.54 ± 0.26
TPN-taurine	0.53 ± 0.09	1.30 ± 0.17	1.80
Mg^{2+}-ATPase			
Controls	2.23 ± 0.27	5.62 ± 1.41	5.96 ± 0.93
TPN	2.23 ± 0.27	4.59 ± 0.80	7.60 ± 1.84
TPN-taurine	1.87 ± 0.28	3.29 ± 0.86	5.94 ± 0.14

[a] Values (μmol phosphate/mg protein/hr) represent the mean ± SE from five to six individual animals, except for the BCM of the TPN-taurine group, for which enough membrane material was available only from two guinea pigs.

studies and the rest was frozen at −70°C until analysis. Membranes were prepared for EM by overnight immersion in the universal fixative (glutaraldehyde 1%, formaldehyde 4%, and Na^+ phosphate buffer [pH 7.2]) followed by 2% osmium tetroxide in a 0.1 *M* cacodylate buffer. After dehydration with alcohol, embedding was done in Araldite resin (17).

Lipids were extracted from membranes using the Folch procedure (19). Phospholipids were determined (20) and their fatty acid pattern was obtained by capillary gas chromatography (21). The cis, trans form of linoleic acid, 18:2 (9, 11), a marker of lipid peroxidation, was quantitated and expressed as a ratio of the cis, cis form, 18:2 (9, 12) (22). Cholesterol was measured with an enzymatic kit (14). The activity of the BCM enzyme marker, leucine amino peptidase, was measured according to Goldbarg and Rutenberg (23). Glucose-6-phosphatase was assayed by the method of Kakis and Yousef (24) published from our laboratory. The specific activities of Na^+K^+-ATPase as well as of Mg^{2+}-ATPase were obtained in each membrane fraction by the technique of Boyer and Reno (25).

All values were expressed as the mean ± SE. Statistical analysis was carried out by one-way analysis of variance and Scheffe's multiple comparison procedure for groups of unequal size (26). The accepted level of significance was $P < 0.05$.

Results

In agreement with a previous study from this laboratory using the same experimental model (27), there was no significant difference in body or liver weight among the three groups (control, TPN, TPN-taurine). No difference was observed in liver weight. There was also no difference in the water, protein, and lipid content of the livers among the three groups. No light microscopy nor EM changes were observed.

Bile Flow and Bile Composition. As shown in Table II, in both TPN groups, bile flow was lower than in controls. However, animals on TPN-taurine had a larger bile flow than those in the group on TPN alone. The bile acid secretory rate, which decreased by 50% in the TPN group compared with controls, was restored in the group on the TPN solution with added taurine. Furthermore, taurine brought about a 3-fold increase in the concentration of cholesterol recovered from hepatic bile without changing other lipid constituents. The only modification observed in the biliary electrolyte composition involved HCO_3^-. The net decrease noted in the TPN group was not seen in the group on TPN-taurine.

Plasma Membrane Composition. In the membrane fractions, there was little glucose-6-phosphatase activity (<0.04 μmol phosphate/mg protein/hr) compared with the homogenate (2.31 ± 0.27 μmol phosphate/mg protein/hr), indicating that membrane preparations were only slightly contaminated by microsomes. Electron microscopy corroborated this finding. The activity of leucine amino peptidase, a marker enzyme of BCM fraction, was enriched 10- to 12-fold when compared with the activity recovered in the homogenate. There was no change in the specific activities of Na^+K^+-ATPase or of Mg^{2+}-ATPase in the homogenates and in the two membrane fractions obtained from the three groups (Table III). On the other hand, changes in lipid composition were observed but they were limited to the sinusoidal membranes (Table IV). TPN led to an increase of the ratio of total lipids over total proteins ($P < 0.05$) in the SM fraction. On the other hand, addition of taurine to the TPN solution resulted

Table IV. Lipid Composition of Liver Membranes[a]

	Controls		TPN		TPN-taurine	
	SM	BCM	SM	BCM	SM	BCM
Cholesterol (nmol/mg protein)	94.2 ± 10.2	194.0 ± 23.7	107.2 ± 5.5	191.8 ± 19.9	59.1 ± 4.1[b]	160.2 ± 19.3
Phospholipids (nmol/mg protein)	255.3 ± 18.7	287.2 ± 25.1	318.5 ± 17.1	283.5 ± 24.2	261.0 ± 22.3	321.7 ± 9.7
Cholesterol/phospholipids	0.39 ± 0.06	0.70 ± 0.09	0.35 ± 0.02	0.65 ± 0.10	0.24 ± 0.03[b,c]	0.45 ± 0.06[c]
Lipid/protein (μmol total lipid/mg total membrane protein)	0.35 ± 0.03	0.50 ± 0.05	0.43 ± 0.02[c]	0.46 ± 0.03	0.31 ± 0.02[b]	0.53 ± 0.06

[a] Values represent the mean ± SE from four to six individual samples of sinusoidal membrane and of bile canalicular membrane domains.
[b] $P < 0.05$ vs TPN.
[c] $P < 0.05$ vs controls.

Table V. Fatty Acid Composition of Liver Membranes[a]

	Controls		TPN		TPN-taurine	
	SM	BCM	SM	BCM	SM	BCM
$\frac{\text{SAT + MONOUNSAT}}{\text{PUFA}}$	1.2 ± 0.2	2.2 ± 0.5	1.5 ± 0.1	2.1 ± 0.4	1.1 ± 0.1[b]	2.0 ± 0.3
n6 Family (% of total)	39.0 ± 3.3	21.5 ± 4.2	30.6 ± 1.7[c]	22.8 ± 1.3	38.5 ± 1.9[b]	23.0 ± 4.8
20:4 n6 (% of total)	7.8 ± 1.0	3.9 ± 1.4	5.7 ± 0.6	3.8 ± 0.8	7.7 ± 0.7[b]	3.9 ± 1.6
$\frac{18{:}2\ (9{,}11)\text{LA}}{18{:}2\ (9{,}12)\text{LA}} \times 100$	0.4 ± 0.1	2.3 ± 0.5	1.2 ± 0.06[c]	2.7 ± 0.5	0.3 ± 0.1[b]	1.6 ± 0.3

[a] Values represent the mean ± SE from four individual samples of sinusoidal membrane and of bile canalicular membrane domains. Abbreviations used in table: SAT, saturated; MONOUNSAT, monounsaturated; PUFA, polyunsaturated fatty acids; LA, linoleic acid.
[b] $P < 0.05$ vs TPN group.
[c] $P < 0.05$ vs controls.

in a 45% reduction in the cholesterol content of the SM as well as to a decrease in the ratios of cholesterol to phospholipid and lipid to protein when compared with values in the TPN group. The fatty acid composition of SM membrane phospholipids was also altered by TPN (Table V). The ratio of saturates and monounsaturates over polyunsaturates was increased while the percentage of fatty acids accounted by the n-6 family and arachidonic acid was decreased. Furthermore, TPN led to a 3-fold increase in the ratio of 18:2 (9 cis, 11 trans):18:2 (9 cis, 12 trans) when compared with controls. In contrast, the animals infused with the TPN-taurine solution did not present these changes.

Discussion

Having shown previously that TPN-treated guinea pigs had a decreased bile flow and bile secretion rate corrected by the addition of taurine to the amino acid solution (27), the aim of the present study was to explore the effect of TPN on liver cell membrane composition and Na^+K^+-ATPase activity using the same experimental model.

Results confirm our initial findings with regard to bile flow as well as bile acid secretory rate and show that TPN increased the lipid to protein ratio and reduced the arachidonic acid content as well as the entire n-6 fatty acid family of SM. These SM fatty acid changes were associated with an increase in the ratio of 18:2 (9 cis, 11 trans):18:2 (9 cis, 12 cis). Taurine prevented these functional changes in bile secretion as well as the alterations in membrane composition and decreased significantly the cholesterol to phospholipid ratio when compared with both the TPN without taurine group and controls. It is interesting to note that this lower cholesterol to phospholipid ratio in the SM membranes of the TPN-taurine group was associated with an increase of cholesterol in bile, suggesting that taurine may induce movement of cholesterol from the membrane to bile. Despite these modifications of the membrane lipid constituents, Na^+K^+-ATPase activity was not affected by TPN and was unchanged by taurine.

Changes in liver cell membrane composition and in enzymatic activities, especially of Na^+K^+-ATPase, have been suggested as a mechanism of intrahepatic cholestasis (28, 29). The activity of Na^+K^+-ATPase is implicated in the bile acid uptake via a cotransport mechanism with Na^+ (30). However, in the guinea pig, the activities of both Na^+K^+-ATPase and Mg^+-ATPase in liver cell membranes are very low compared with rats.[2] Thus, in the guinea pig, this enzyme may not play a major role in bile formation nor in this model of TPN-induced cholestasis.

The only report of membrane changes associated with TPN before the present one has shown that TPN altered rat hepatic microsomal membrane composition and fluidity (32). There was a reduced percentage of phospholipid fatty acids as linoleic acid, as well as a reduced total percentage of polyunsaturated fatty acids with a decrease in membrane fluidity. Although no measurement of fluidity was obtained in the present study, it can be surmised that the protective effect of taurine on SM membranes are consistent with reports showing that taurine stabilizes membranes (33) and thereby preserves membrane fluidity and function (34).

The decrease in membrane arachidonic acid and other members of the n-6 family in conjunction with the increase in diene conjugates in the SM is consistent with the hypothesis that lipid peroxidation could be responsible for the changes in fatty acid composition. Lipid peroxidation has been described in very low birth weight infants with respiratory distress syndrome (35). These observations have been assigned to peroxidation and generation of free radicals in lipid emulsions used in parenteral nutrition (36). The present study suggests that, in response to the amino acid and glucose TPN solution, there is free radical injury characterized by peroxidation of sinusoidal membranes, impairing their ability to transport water, ions, and bile acids. The data presented imply that the protection offered by taurine against TPN-induced changes in membrane composi-

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[2] Guertin F, Loranger A, Roy CC, Yousef I, Tuchweber B. Bile formation and liver membrane composition in guinea pig and rat. Submitted for publication.

tion and bile secretion may be mediated by its role as an antioxidant (37-39) and as a free intracellular amino acid that regulates calcium homeostasis by increasing the binding affinity of Ca^{2+} to biologic membranes (40).

These data show that changes in liver cell membranes occur in TPN-induced cholestasis and that lipid peroxidation may play a role. The fact that taurine prevented changes in bile secretion as well as the membrane alterations suggests that taurine should be added to amino acid solutions used for parenteral nutrition.

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