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Effect of lipid infusion on bile composition and lithogenicity in patients without cholesterol gall stones

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Abstract

A prospective study was performed to investigate the effect of short term lipid infusion on bile composition and its lithogenicity in humans. Thirty five patients shown to be free of cholesterol gall stones participated in the study. Starting 48 hours before surgery they were infused randomly with a lipid emulsion of either long chain triglycerides (LCT) or a mixture of medium and long chain triglycerides (MCT/LCT) (50%/50%) for six hours each 24 hours. A group of patients infused with a solution of 5% glucose in NaCl 0·9% served as a control. Bile samples were obtained by puncture of the gall bladder during operation. Both lipids caused an increase in biliary cholesterol and phospholipids but this effect was more pronounced and significant ($p<0·001$) only with the MCT/LCT emulsion. The fatty acid composition of biliary phospholipids was not affected by either lipid infusion. The cholesterol saturation index increased significantly ($p<0·005$) with the MCT/LCT emulsion and there was shortening in the nucleation time but this was not significant. There was no effect on the distribution of cholesterol between micelles and vesicles. This study shows that infusion of MCT/LCT lipid emulsion can cause lithogenic changes in bile composition in humans and may thus contribute to sludge formation and cholelithiasis during long term parenteral nutrition.

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Several previous reports have shown an increased incidence of gall bladder disease in patients on long term total parenteral nutrition.[1 2] In addition to biliary sludge, they develop gall stones and symptomatic biliary disease. The prevalence of biliary sludge in patients on long term total parenteral nutrition is as high as 100%,[1] and is a predisposing factor to cholelithiasis. The most common explanation for these effects is bile stasis caused by failure of gall bladder emptying as a result of reduced secretion of intestinal hormones. These hormones are normally released after oral food intake and serve to stimulate hepatobiliary secretion.[3 4] Bile composition during total parenteral nutrition has not been studied systematically. A few reports dealing with this subject in animal models show contradictory results.[5 6]

In view of the major role of bile composition in gall stone formation,[7 8] we found it of interest to study the effect of intravenous lipid infusions on the composition and lithogenicity of human bile. In addition to the conventional 100% long chain triglyceride (LCT) lipid emulsion we studied the effects of the newly developed mixture of 50%:50% (w:w) medium and long chain triglyceride (MCT/LCT), in comparison with a control group who received infusions of 5% glucose and normal saline. Our results indicate that lipid emulsions containing MCT/LCT induce changes in the composition of human bile.

Methods

SUBJECTS

The 35 patients without cholesterol gall stones included in this study were part of a larger group (n=58) requiring elective laparotomy for gall stones and other diseases who had consented to participate in the study. They were randomised to receive the saline and lipid infusions. Those found at laparotomy to have cholesterol gall stones (n=23) were not included in this study. The clinical characteristics of the 35 patients are presented in Table I. All the patients were well nourished and had normal hepatic, renal, and thyroid function, and none were receiving medications (including oral contraceptives) that could affect the parameters studied.

Ultrasound examination showed no evidence of gall stones or sludge in any of the patients, with the exception of the 11 with documented pigment stones. In these 11 patients, the gross appearance and chemical analysis of the stone, as well as the absence of cholesterol monohydrate crystal in the sediment,[9] were used to classify stone composition. All the results remain significant when the statistical tests were performed without the pigment stone patients. Criteria for a priori exclusion from the study were obesity (>125% ideal body weight), recent weight loss, hyperlipidaemia (plasma cholesterol >250 mg/dl, plasma triglycerides (>200 mg/dl), or diabetes mellitus.

The patients were randomly divided into three

TABLE I *Patient characteristics*

	Group		
	Control (A)	*LCT (B)*	*MCT/LCT (C)*
Sex (M/F)	5/11	2/8	5/4
Age (yr)	64·2 (14·7)	56·3 (15·7)	57·4 (13·1)
Weight (kg)	64·3 (14·1)	63·1 (8·2)	67·2 (7·1)
Height (cm)	165·4 (8·2)	160·8 (10·1)	163·6 (7·2)
Fasting plasma lipids (mg/dl):			
Triglyceride	102·2 (34·2)	96·7 (31·3)	101·4 (34·2)
Cholesterol	173·8 (33·9)	165·9 (41·4)	156·1 (28·6)
Esterified	114·6 (25·3)	113·2 (35·6)	115·9 (22·4)
Free	59·2 (8·6)	52·7 (14·7)	60·2 (7·8)
Phospholipid	169·1 (23·4)	165·2 (34·5)	173·9 (39·1)
Diagnosis			
Pigment stones	5	5	1
Gastrointestinal cancer	8	3	4
Others	3	2	4

LCT=long chain triglyceride; MCT=medium chain triglyceride.

groups in accordance with their infusion regimen (Table I). Patients of group A (n=16) were infused with glucose/saline and served as a control group; patients of group B (n=10) received LCT lipid infusions, while those of group C (n=9) received MCT/LCT lipid infusions. Although the groups were randomly divided in accordance with their infusion regimen, group B incidentally proved to contain a larger number of younger women. However, there was no significant difference in bile composition between the men and women in the control group, or between the men and women in the other two groups. It seems therefore that the fact that group B has a greater number of women is not important with regard to the effect of lipid infusion on bile composition. No statistically significant differences were found between the three groups of patients except for the male/female ratio (Table I). One patient with a non-functioning gall bladder (total lipid concentration <4 g/dl)[10] from group C was withdrawn from the study as were five each from groups B and C for various technical reasons.

LIPID EMULSIONS

Endolipid 20% (LCT) (B Braun Melsungen), contains 20% w/v of soybean oil (C14–C24), egg yolk lecithin (1·2%), and glycerol in sterile water. Lipofundin 20%, (MCT/LCT) (B Braun Melsungen), is a 20% w/v fat emulsion containing soybean oil LCT (10% w/v) (C14-C24), MCT (10% w/v) (C6-C12), egg yolk lecithin (1·2%), and glycerol in sterile water.

STUDY PROTOCOL

The subjects were admitted to hospital after an overnight fast 48 hours before elective laparotomy, and oral intake was restricted to water. All the patients were infused with 2500 ml of solutions per 24 hours at a constant rate for two days. The solution used for patients of group A was glucose 5% in NaCl 0·9%. Patients of groups B and C were infused with fat emulsions LCT 50%:50% w:w MCT/LCT, respectively. Lipids were infused at the rate of 0·2 g triglyceride/kg per hour over a period of six hours followed by 18 hours of glucose 5% NaCl 0·9% infusion. The infusion regimens for groups B and C were identical in all respects, except for the type of triglycerides.

Blood samples were obtained before and at the end of the six hours lipid infusion on the second day for determination of plasma lipids. Bile samples were obtained 18 hours after the last lipid infusion by puncture of the gall bladder[11] at the beginning of the operation before manipulation of the gall bladder. After aspiration of all the bile, the gall bladder was palpated for stones, to confirm the preoperative ultrasound examination findings.

BILE ANALYSIS

The aspirated bile was shaken and samples were immediately frozen at −70°C for subsequent lipid analysis. The remaining bile was ultracentrifuged at 192 000 *g* for 40 minutes at 25°C to remove cell debris. Samples of the supernatant bile were separated into the vesicular and micellar phases, as described previously.[12] The distribution of biliary cholesterol between the vesicular and micellar carriers was determined as described previously.[8 12] Nucleation time of bile samples at 37°C was determined as previously described.[13] The chemical composition of whole bile frozen at −70°C was determined within four weeks. The bile acid concentration was determined enzymatically,[14] cholesterol was determined by the method of Abbell *et al*[15] and phospholipids as described by Bartlett.[16] The chloesterol saturation index (CSI) was calculated using the critical tables of Carey.[17]

ISOLATION AND IDENIFICATION OF FATTY ACID COMPOSITION

Frozen bile samples were thawed and extracted with methanol-chloroform according to Bligh and Dryer.[18] To purify the necessary lipids for further analysis, the chloroform extracts were passed through a silicic acid column. Neutral lipids were eluted with chloroform; this was followed by acetone, which removed most of the bile pigments. Phospholipids were isolated from the chloroform eluate by thin layer chromatography on silica gel G plates employing hexane-diethylether-acetic acid-methanol (90:20:2:3 by volume) as solvent. Phosphatidylcholine was isolated from the phospholipid eluate by thin layer chromatography using chloroform-methanol-acetic acid-water (100:20:12:5, by volume) as solvent. Lipids were eluted from the plates with a mixture of chloroform-methanol-water (1:1:0·1 by volume). Phosphatidylcholine was saponified with 0·5 M methanolic-KOH at 50°C, and the fatty acids recovered after acidification by hexane extraction. The fatty acids were methylated with diazomethane[19] and separated by gas liquid chromatography on a 25 M fused silica column crossed linked with methylsilicone (0·52 m film thickness) at a temperature range of 185°C–230°C employing a Hewlett Packard 5990A gas chromatograph equipped with a flame ionization detector. The relative composition of a fatty acid mixture was calculated from the areas under the peaks employing a Hewlett-Packard 3390A integrator. For quantitative analysis, heptadecanoic acid was added as internal standard, assuming that the response of the detector for all methyl-fatty acids was identical.

PLASMA LIPID ANALYSIS

Ten ml of blood drawn into glass tubes containing the anti-coagulant ethylene diamine tetra-acetic acid (1 mg/ml of blood) were transferred to the laboratory in iced water for immediate separation by low speed centrifugation (1000 *g*) for 10 minutes at 4°C using a refrigerated centrifuge. Sodium azide (1 mg/ml plasma) was added to the plasma, and the plasma was then kept at 4°C for up to five days before determination of total plasma lipids. Triglyceride concentrations were determined enzymatically by Test-Combination: triglycerides without free glycerol (Boehringer Mannheim). Total and free cholesterol were determined by an enzymatic colorimetric test

TABLE II *Plasma lipid composition after lipid infusions*

	Group	
Plasma lipids (mg/dl)	*LCT (B)*	*MCT/LCT (C)*
Triglyceride	688·3 (45·4)	418·3 (20·2)*
Cholesterol ester	120·1 (22·4)	114·1 (26·6)
Free Cholesterol	57·2 (5·8)	66·6 (10·8)
Phospholipid	285·1 (15·9)	260·9 (14·3)

*p<0·05; LCT = long chain triglyceride; MCT = medium chain triglyceride.

using the kits Cholesterol Monotest CHOP-PAP high performance and Test-Combination (Boehringer Mannheim) respectively. Phospholipids were analysed enzymatically by Test Combination: colorimetric test (Boehringer Mannheim).

STATISTICAL ANALYSIS

Data are presented as means (SD). The significance of differences between various groups was analyzed using the Student's unpaired *t* tests and Fisher's exact test.

The protocol of this study was approved by the hospital ethical committee. All participants gave their written informed consent before the study.

Results

CHANGES IN PLASMA LIPIDS

The plasma lipid concentrations at the end of the second infusion period are shown in Table II for the two patient groups. The baseline values are given in Table I. Plasma lipids were not affected by infusing saline and dextrose in the control group. Infusion of both types of lipid emulsion, whether LCT or MCT/LCT, resulted in a noticeable increase in the plasma triglyceride and phospholipid values (p<0·001). LCT induced a much higher level of triglyceride than MCT/LCT (p<0·05). No changes were observed in total, free, or esterified plasma cholesterol concentrations with either lipid emulsion.

CHANGES IN BILE LIPIDS

Infusion of the LCT fat emulsion did not affect bile salt concentrations but caused a 25% increase in phospholipid and a 33% increase in cholesterol concentrations (Table III). However, these effects were not significant (0·10>p>p0·50).

The effects of the MCT/LCT infusion were in the same direction but more pronounced. This infusion resulted in a 50% increase in phospholipids and a twofold increase in cholesterol concentrations (p<0·001). The 25% increase in bile salts was not significant (Table III).

TABLE III *Effect of infusions on bile composition*

	Group			p*	
	Control (A) (n=16)	*LCT (B) (n×10)*	*MCT/LCT (C) (n=9)*	*C-A*	*C-B*
Cholesterol (mM)	14·9 (7·7)	19·9 (6·4)	31·4 (13·2)	0·001	0·05
Phospholipids (mM)	34·4 (11·1)	42·6 (10·9)	53·8 (15·4)	0·005	NS
Bile salts (mM)	117·1 (40·7)	113·5 (28·4)	141·1 (33·9)	NS	NS
Total lipid (g/dl)	8·9 (3·0)	9·6 (0·3)	12·3 (3·2)	0·025	0·05
CSI	1·3 (0·3)	1·5 (0·1)	1·7 (0·3)	0·005	NS
NT (days)	22·5 (9·7)	26·6 (6·8)	17·0 (11·7)	NS	0·05
Cholesterol in vesicles (%)	8·4 (10·3)	6·9 (4·5)	7·2 (6·2)	NS	NS

*None of the measured parameters showed significant difference between long chain triglyceride (LCT) treated patients and the control group. Significance of differences between patients infused with medium chain/long chain triglyceride MCT/LCT and those in the control group are given in the C-A column; significance of the differences between patients treated by the two lipid infusions is given in the C-B column. NT = nucleation time; CSI = cholesterol saturation index.

The CSI increased after infusion of both lipid emulsions but this was significant only with the MCT/LCT emulsion. The increase in the CSI in patients infused with MCT/LCT was greater than that observed for patients infused with LCT, despite a rise in bile salt concentration in the former group. The increased CSI in the MCT/LCT group was accompanied by an apparent shortening of the nucleation time, but this change was not statistically significant.

There were no significant differences between the three groups in the fraction of cholesterol contained in the vesicles. A mean of 7·5 (8·0) of the cholesterol was found in vesicles in the bile of all the patients.

The fatty acids profile of biliary phospholipids was not altered after the infusion of the different lipid emulsions (Table IV).

In analysing the relationships between infusion induced changes in bile and blood, the only correlation found was that between the rise in plasma triglycerides and biliary cholesterol for both emulsions (T=0·535; p<0·050).

Discussion

Long term total parenteral nutrition has been reported to induce biliary sludge and cholelithiasis.[1,2] This is commonly attributed to bile stasis in the absence of oral feeding.[3] This study examines the possibility that lipid induced changes in bile composition and lithogenicity also contribute to this complication of total parental nutrition. The data presented here indicate that short term infusion of MCT/LCT lipid emulsion caused significant changes in bile composition and lithogenicity. The lithogenic effect of long term infusion of this emulsion, coupled with gall bladder stasis, might lead to a greater risk of gall stone formation. Nonetheless, this speculation will have to be tested epidemiologically.

Infusion of LCT, the commonly used fat emulsion, caused only a moderate and insignificant increase in biliary cholesterol and phospholipids as well as in the CSI, without affecting the nucleation time. This does not rule out the possibility that a long term infusion of LCT emulsions could have a more pronounced effect on the lithogenicity of bile and eventual stone formation. Bile stasis may still be considered as a major factor, but supersaturation due to an increased level of biliary cholesterol may also contribute to stone formation.

The mechanism by which fat emulsions increase the biliary cholesterol concentration is not clear. The possibility that lipid infusion somehow results in increased water absorption from the gall bladder, hence increasing the concentrations of all the bile components, seems unlikely in view of the finding that the various bile components were affected differently by the lipid infusion (Table III). Two possible alternative mechanisms were considered: (I) enhanced mobilisation of cholesterol from peripheral tissues to the liver; and (II) enhanced cholesterol synthesis in the liver.

TABLE IV *Fatty acids composition of biliary lecithin with medium chain/long chain triglyceride (MCT/LCT) and LCT**

	Group		
Fatty acid	*Control (A)*	*MCT/LCT (C)*	*LCT (C)*
C16:1	2·36 (0·76)	1·16 (0·32)	1·31 (0·23)
C16:0	35·39 (7·47)	35·15 (0·07)	36·20 (1·22)
C18:2	28·52 (8·37)	34·24 (2·31)	36·31 (2·40)
C18:1	5·17 (3·41)	4·75 (1·29)	4·31 (0·57)
C18:0	4·85 (2·70)	6·24 (2·37)	3·67 (1·92)
C20:4	9·13 (1·70)	6·90 (4·30)	8·86 (1·95)
C20:5	2·29 (1·24)	1·40 (0·75)	1·21 (2·07)
C22:6	2·88 (1·45)	3·22 (1·41)	2·57 (0·00)

*Fatty acids comprising less than 1% of total acyl fatty acids in any of the three groups were not included in this table.

The LCT emulsion particles have been reported to acquire cholesterol ester and free cholesterol by exchange with endogenous lipoprotein[20] and cell membranes[21 22] and to deliver the excess cholesterol to the liver in the form of cholesterol enriched emulsion remnants. This process may involve a mechanism similar to that by which endogenous chylomicrons are cleared by the liver.[23 24] Theoretically, a part of this excess may be excreted into the bile, thus increasing biliary cholesterol and consequently its lithogenicity. This mechanism is consistent with the high frequency of gall stones in patients suffering from type IV and type IIB hyperlipoproteinaemia,[25] which are both characterised by an increased concentration of triglyceride rich very low density lipoprotein. This mechanism, however, seems an unlikely explanation for the changes observed in bile composition and lithogenicity with MCT/LCT infusion. Since MCT is characterised by a lesser ability to acquire cholesterol ester,[26] the infusion of MCT/LCT would have been expected to have a less pronounced effect on bile composition via cholesterol mobilisation. However, our data indicate than MCT/LCT infusion has a more pronounced effect on bile composition than LCT, suggesting that this is due to the alternative mechanism. Specifically, the increase in the plasma triglycerides concentration caused by infusion of MCT/LCT emulsion was smaller than that caused by LCT, in agreement with a previous report,[27] which attributed this difference to the rapid hydrolysis of the MCT. The medium chain fatty acids resulting from this hydrolysis are rapidly oxidised in the mitochondrial matrix, causing an increased production of acetyl CoA.[28] Acetyl CoA follows various metabolic pathways, which include de novo synthesis of fatty acids, cholesterol and phospholipids.[29 30] This may explain, at least partially, the more pronounced effect of MCT/LCT on bile phospholipids and cholesterol observed in our study. This hypothesis of enhanced de-novo synthesis is also supported by the finding that the fatty acid profile of bile phospholipids was not significantly altered by the lipid infusions, in spite of the very large difference between the fatty acid profile of the emulsions infused and the bile.

In conclusion, these results show that acute lipid infusions affect biliary lipid composition within a short period of two days. This effect is more marked with MCT/LCT infusions than LCT alone and results in an increase in bile lithogenicity. These data may contribute to our understanding of the development of sludge and gall stones during total parenteral nutrition containing lipid emulsion.

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