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Effects of Bile Acids, Lecithin, and Monoolein on Amino Acid Absorption from the Human Duodenum[1](39664)

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Bile acids inhibit water absorption and electrolyte transport from the colon of humans (1) and canines (2) and from the jejunum of hamsters (3); and they inhibit glucose absorption from the human jejunum (4, 5). Although taurochenodeoxycholic acid (2.5 m*M*) has been said to inhibit absorption of L-leucine and L-lysine from the human jejunum (6), and the unconjugated bile acid deoxycholate inhibits absorption of lysine and leucine from the rat intestine (7, 8), the effects of other bile acids on absorption of essential amino acids (EAAs) have not been studied. The addition of lecithin abolishes the effects of bile acids upon water movement and electrolyte transport in the human jejunum, but the addition of oleate does not restore normal glucose absorption. The effect of lecithin and monoolein on EAA absorption has not been reported.

Since intraduodenal concentrations of EAAs are not equal, we elected to study the absorption of unequal molar mixtures of EAAs, similar to the composition of casein or egg (9) protein hydrolysate. The absorption rate of such EAA mixtures from the human and transplanted human duodenum (10) and from the human jejunum (11) has been reported previously. The jejunal absorption rates of individual amino acids in these mixtures are very similar to the rates for equimolar EAA mixtures (12). Again, the effects of bile acids, lecithin, or monoolein on absorption of individual amino acids in these mixtures of EAAs have not been reported.

The aims of our study were: (1) to establish the duodenal absorption kinetics of EAAs; (2) to determine the effects of taurine conjugated bile acids (cholic, chenodeoxycholic, and deoxycholic) on duodenal EAA absorption; (3) to determine whether monoolein alters duodenal EAA absorption; and (4) to determine whether the addition of lecithin or monoolein to the perfusion mixture of bile acid and EAAs may affect duodenal EAA absorption.

Materials and methods. Fifty healthy male volunteers participated in these studies. After an overnight fast, a double-lumen polyethylene tube was placed intraduodenally under fluoroscopic observation. Isotonic saline or test solutions containing a non-absorbable marker – polyethylene glycol (PEG), 5 g/liter – were perfused into the second part of the duodenum at a constant rate (10 ml/min). During steady-state conditions, specimens of duodenal contents were siphoned from near the ligament of Treitz, 20 cm distal to the infusion site. Perfusates were collected over ice and pooled at 20-min intervals. In each study, isotonic saline was perfused 60 min initially for cleansing; and then either a control (isotonic saline) or test solution was perfused for 100 min. The test perfusates contained EAAs alone; EAAs in combination with sodium taurocholate (TC), sodium chenodeoxycholate (TCDC), or sodium taurodeoxycholate (TDC); and EAAs in combination with TC + monoolein (MO) and with TC + lecithin. The 78 m*M* EAA perfusate contained (in m*M*) 10.65 threonine (Thr), 12.95 valine (Val), 5.15 methionine (Met), 11.25 isoleucine (Ileu), 16.8 leucine (Leu), 6.7 phenylalanine (Phe), 13.45 lysine, and 1.05 tryptophan. Tryptophan was not measured by our techniques and not included in our study. In all studies, gastric juice was aspirated continuously from the antrum by an additional tube.

The volumes of gastric and duodenal aspirates and their pH and PEG concentrations (13) were measured. Duodenal EAA concentrations were determined by methods previously described (10) and EAA absorptions were calculated from their concentra-

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tions relative to PEG in duodenal samples. Only aspirates collected after the first 40 min of test perfusion were utilized for these determinations.

Since the rate of perfusion and the composition of the perfusate in each trial were known constants, the rates of administration of EAAs could be expressed as μM/min; and the calculated amounts recovered during collection intervals of known length could be expressed in the same terms. The difference between the rates of perfusion and recovery represented the rate of absorption by the duodenum. In addition, we have expressed our results of percent absorption of individual amino acid and the total EAA by the formula:

$$\text{Percent absorption} = \frac{\text{Rate of EAA absorbed in } \mu M/\text{min}}{\text{Rate of EAA perfused in } \mu M/\text{min}} \times 100.$$

For determining intrastudy variability in the absorption of each EAA, the last three 20-min collections from each of five subjects were compared; and the average coefficient of variance was between 7 and 15%.

EAAs were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.; sodium TC, sodium TCDC, and sodium TDC were synthesized from taurine (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N.Y.) and cholic acid, chenodeoxycholic acid, and deoxycholic acid, respectively (Matheson Coleman & Bell, East Rutherford, N.J.) by methods of Hofmann (14). The products migrated as single spots on thin-layer chromatography. Emulsified monoolein consisted of 10 mM of 1-MO (Eastman Organic Chemicals Div.) in dilute 1 mM sodium TC. This solution was sonicated for 60 min with a Biosonik (Will Scientific, Inc., Rochester, N.Y.) to emulsify the MO into solution. Thin-layer chromatography showed that the preparation was more than 95% monoglyceride, and recoveries from the perfusates showed that more than 90% of the monoglyceride was hydrolyzed to fatty acid during the study. Lecithin (chromatographically pure, Schwartz/Mann Division, Becton, Dickinson and Co., Orangeburg, N.Y.) was used as purchased. When EAA, MO, lecithin, or bile acids were combined in test solutions, the original molar concentrations were preserved. All perfusates were prepared immediately before use, made isotonic with sodium chloride, adjusted to pH 6.3, and warmed to 37°C.

The validity and reproducibility of the perfusion technique and coefficients of variations of PEG determinations have been detailed elsewhere (13, 15).

Experimental design. 1. To determine EAA duodenal absorption, five subjects each were given four 100-min perfusions which (in random order) contained 13, 26, 52, and 78 mM EAA mixture. 2. To determine intrastudy variation, three collections made during the 78 mM EAA perfusion of each of the five subjects were analyzed. 3. To test the effect of bile acid upon absorption of 78 mM EAA from the duodenum, 10 mM TC, TCDC, and TDC were perfused with 78 mM EAA in 7, 3, and 3 subjects, respectively; and the collections were compared with those from 11 subjects who had received 78 mM EAA alone. In addition, the effect of 5 mM TC was tested in five studies. 4. To determine the effect of fat upon duodenal absorption of 78 mM EAA, 10 mM monoolein emulsified with 1 mM TC was perfused with 78 mM EAA in six subjects. 5. To test whether the effect of 10 mM TC upon EAA absorption could be abolished, 10 mM MO was added to the mixture of 10 mM TC and 78 mM EAA in five cases and 2.5 mM lecithin was added to the same mixture in five other cases. 6. Lastly, to determine how influx of EAA into the duodenum is influenced by pancreozymatic and cholecystokinetic effects of intraluminal fat and EAA (13, 16), EAA output into the duodenum was measured during normal isotonic saline perfusion and during perfusion with normal isotonic saline and cholecystokinin-pancreozymin (CCK) intravenous stimulation in three subjects.

Results. Absorption of EAA mixture from duodenum. Absorption of the total EAA mixture and of individual amino acids in the mixture is given as percentages in Table I. The duodenum absorbed 50% of the total mixture. Individually, the absorption of valine, isoleucine, leucine, and phenylalanine approximated this rate; that of methionine was slightly more; and that of lysine and

TABLE I. ABSORPTION RATES OF EACH PERFUSED EAA AND TOTAL MIXTURE: IN μM/MIN (AND PERCENTAGE), MEANS ± SE.

Perfusate (all units mM)	N	Thr	Val	Met	Ileu	Leu	Phe	Lys	Total
					Non-paired studies				
Endogenous EAA,[a] with									
Isotonic saline	3	−1.5 ± 0.3[b]	−2.1 ± 0.6[b]	−0.7 ± 0.2[b]	−1.7 ± 0.5[b]	−2.6 ± 0.7[b]	−1.3 ± 0.3[b]	−1.9 ± 0.5[b]	−11.7 ± 3.2[b]
		(−4 ± 1)	(−3 ± 1)	(−2 ± 1)	(−3 ± 1)	(−3 ± 1)	(−4 ± 1)	(−3 ± 1)	(−3 ± 1)
Isotonic saline + iv CCK	3	−8 ± 3[b]	−11 ± 3[b]	−7 ± 4[b]	−7 ± 2[b]	−10 ± 5[b]	−7 ± 2[b]	−14 ± 4[b]	−70 ± 15[b]
		(−21 ± 8)	(−16 ± 5)	(−7 ± 2)	(−11 ± 3)	(−11 ± 5)	(−24 ± 7)	(−23 ± 6)	(−18 ± 4)
Control:	11	38 ± 4	67 ± 5	31 ± 2	62 ± 4	93 ± 7	31 ± 2	58 ± 4	381 ± 27
78 EAA		(36 ± 4)	(53 ± 4)	(60 ± 4)	(56 ± 4)	(55 ± 4)	(46 ± 4)	(44 ± 3)	(50 ± 4)
Tests:									
78 EAA, with									
+ 1 TC	6	33 ± 9	66 ± 13	30 ± 5	65 ± 8	87 ± 18	28 ± 6	41 ± 16	351 ± 67
10 MO		(31 ± 8)	(51 ± 10)	(57 ± 10)	(57 ± 7)	(52 ± 11)	(43 ± 9)	(41 ± 12)	(46 ± 9)
+ 10 TC	5	36 ± 11	59 ± 14	26 ± 7	51 ± 14	76 ± 21	30 ± 5	50 ± 15	345 ± 89
10 MO		(34 ± 10)	(46 ± 11)	(50 ± 13)	(45 ± 13)	(45 ± 13)	(45 ± 8)	(51 ± 12)	(48 ± 12)
+ 10 TC	5	32 ± 6	52 ± 9	26 ± 3	50 ± 8	79 ± 10	25 ± 4	61 ± 12	325 ± 44
2.5 lecithin		(30 ± 5)	(40 ± 7)	(51 ± 5)	(45 ± 7)	(47 ± 6)	(37 ± 6)	(46 ± 9)	(42 ± 6)
+ 10 TCDC	3	31 ± 9	46 ± 13	24 ± 5	53 ± 17	67 ± 16	20 ± 6	41 ± 13	282 ± 79
		(29 ± 8)	(36 ± 10)	(47 ± 10)	(47 ± 15)	(40 ± 10)	(30 ± 10)	(31 ± 10)	(37 ± 10)
+ 10 TC	7	13 ± 3[b]	36 ± 5[b]	16 ± 2[b]	34 ± 4[b]	48 ± 6[b]	15 ± 3[b]	33 ± 6[b]	193 ± 27[b]
		(12 ± 3)	(28 ± 4)	(31 ± 4)	(30 ± 4)	(28 ± 4)	(21 ± 5)	(24 ± 4)	(25 ± 4)
+ 5 TC	5	16 ± 5[b]	23 ± 7[b]	12 ± 6[b]	27 ± 6[b]	42 ± 10[b]	13 ± 4[b]	26 ± 9[b]	161 ± 9[b]
		(15 ± 5)	(29 ± 6)	(23 ± 12)	(24 ± 5)	(25 ± 6)	(19 ± 5)	(19 ± 6)	(21 ± 5)
+ 10 TCDC	3	13 ± 5[b]	20 ± 7[b]	14 ± 3[b]	20 ± 6[b]	51 ± 15[b]	9 ± 2[b]	15 ± 8[b]	141 ± 31[b]
		(12 ± 4)	(15 ± 5)	(27 ± 6)	(18 ± 6)	(30 ± 9)	(13 ± 3)	(11 ± 7)	(18 ± 4)
					Paired studies				
EAA: 13	5	5 ± 1[b]	8 ± 2[b]	4 ± 1[b]	7 ± 2[b]	11 ± 3[b]	3 ± 1[b]	6 ± 2[b]	46 ± 12[b]
26	5	10 ± 2[b]	18 ± 4[b]	8 ± 2[b]	16 ± 4[b]	24 ± 6[b]	8 ± 2[b]	15 ± 4[b]	100 ± 22[b]
52	5	22 ± 2[b]	42 ± 3[b]	19 ± 1[b]	39 ± 2[b]	57 ± 3[b]	18 ± 1[b]	30 ± 3[b]	229 ± 14[b]
78	5	34 ± 4	61 ± 4	30 ± 3	58 ± 4	83 ± 7	28 ± 2	45 ± 9	346 ± 25

[a] Minus sign indicates endogenous EAA secreted under saline perfusion, without and with iv CCK stimulation.
[b] Significantly different ($P < 0.01$) from control (78 mM EAA), by rank sum methods.

threonine was somewhat less.

In kinetic studies (Table I) of the five subjects who received mixtures totaling 13, 26, 52, and 78 m*M* EAA, a direct linear relationship was found between the total perfused and the total absorbed. Of individual EAAs (Fig. 1), it was observed that methionine was absorbed at the most rapid rate, while the branch-chained amino acids —isoleucine, leucine, and valine—were absorbed slightly less rapidly, and lysine and threonine at the slowest rates.

Effect of taurine-conjugated bile acids on EAA (Table I). TC and TDC significantly depressed EAA absorption ($P < 0.01$). Both 5 and 10 m*M* concentrations of TC depressed total EAA absorption by 50%, while TDC decreased it 65%. TCDC depressed EAA absorption by only 26%, which was not statistically significant. The inhibition of EAA absorption by TC and TDC affected all individual amino acids similarly.

Effect of MO and lecithin on EAA absorption (Table I). Emulsified MO alone had no significant effect on EAA absorption. The addition of MO to the perfusion mixture of EAA and 10 m*M* TC restored EAA absorption to normal. Similarly, the addition of 2.5 m*M* lecithin to the mixture of 78 m*M* EAA and 10 m*M* TC restored EAA absorption to normal.

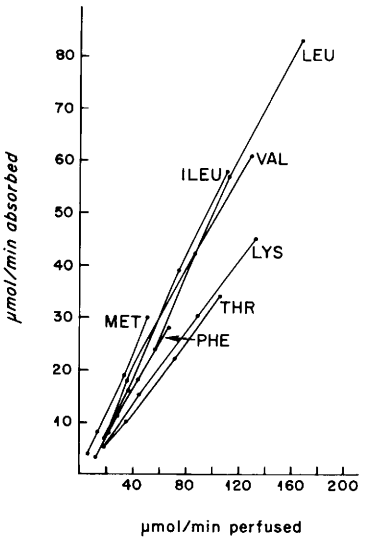

FIG. 1. Duodenal absorption of component EAAs from mixture perfused (see Table I for composition): Means from five subjects.

Secretion of endogenous EAA into duodenum (Table I). The total endogenous secretion of EAA into the duodenum of three subjects under basal conditions (saline perfusion alone) was minimal (12 μM/min), equaling only 3% of the total amount of EAA absorbed and <2% of EAA perfused. In an additional two subjects, EAA influx into the duodenum during 10-m*M* TC perfusion was measured and found to be as negligible as that observed with perfusion of saline alone.

When the pancreas was stimulated with exogenous CCK (intravenously) the total endogenous EAA secretion was 70 μM/min, equaling 9% of the EAA perfused and 18% of that absorbed.

Discussion. Our results differ from all other reported data on amino acid absorption in the human, since we have investigated EAA absorption from the duodenum rather than jejunum. As in the studies of jejunal absorption (9, 11, 12), the rate of duodenal absorption of individual EAAs in a mixture varies greatly. Generally our results agree with others (11, 12) in that methionine was absorbed most rapidly, while the branch-chained neutral amino acids (isoleucine, leucine, and valine) were absorbed slightly less rapidly, phenylalanine and lysine less rapidly, and threonine least rapidly. However, in the jejunal studies lysine had the slowest absorption. This minor difference may have resulted because, whereas we perfused only EAAs, the other investigators included nonessential amino acids with them. Their nonessential amino acid mixture contained arginine, which has a greater affinity for the basic amino acid transport site than lysine and competitively inhibits lysine absorption. Adibi and associates (9) found that absorption of perfused lysine was significantly greater when arginine was not perfused with it.

The proportion of the EAA mixture absorbed by the duodenum in our studies (50%) was slightly less than that absorbed from the jejunum (53–72%) (11, 12). These data appear to support findings that indicate absorption of methionine (17) and glycine (18) from the human duodenum is less than from the jejunum. However, our studies most likely underestimate EAA absorption rates when EAA was perfused

without BA. It is known that EAA perfusion of the duodenum stimulates biliary and pancreatic secretion. When we stimulated the pancreas with exogenous CCK, EAAs were secreted endogenously. Therefore, it is likely that our percent absorption of EAA is 5–10% lower than the true value and that the percent absorptions in the human duodenum and jejunum are very similar for this particular EAA mixture.

In contrast, taurine-conjugated bile acids perfused with the EAAs inhibit pancreatic and biliary secretion (16); and the reported absorption rates of the bile acid–EAA combinations are believed to be accurate and relatively unaffected by endogenous EAA.

In our studies conjugated trihydroxy (TC) and dihydroxy (TDC) bile acids inhibited EAA absorption. This observation with TDC is in agreement with results of *in vivo* (8, 19) and *in vitro* (7) animal experiments. However, inhibition of amino acid absorption has not been observed with TC bile acids (7, 19). Although contamination of bile salt preparations with deoxycholate has been incriminated in the past as a cause for inhibition of water-soluble nutrients (7), our preparations of TC were at least 99% pure by thin-layer chromatography. Furthermore, identical preparations of TC in other studies from this laboratory (20, 21) failed to exhibit any effect on net water movement. Therefore, it is extremely unlikely that TDC contamination of TC caused the diminution of EAA absorption when we perfused EAA with TC.

How bile acids inhibit EAA absorption and how lecithin and MO abolish this inhibition are not known and have not been elucidated by our studies. Bulk water flow, effects on adenosine triphosphatase (ATPase), and disruption of cell membranes may be involved.

Convection (solvent drag, bulk water flow) is probably important in EAA absorption. It has been demonstrated that convection in the direction of absorption is important in carbohydrate absorption; but the convection component of carbohydrate absorption during osmotic secretion is very small (22). Glucose absorption is depressed by TDC, which induces net water movement (5); but it is not depressed significantly when water movement is enhanced with mannitol (5). Furthermore, TC—which inhibits EAA absorption—does not have any effect on net water movement (20). Therefore it is unlikely that water movement induced by bile acids is the only or major factor that inhibits EAA absorption.

Bile acids may influence chemical mechanisms proposed for active transport of electrolytes (4). Recently it has been shown that glycine- and taurine-conjugated bile salts inhibit Na^+,K^+-stimulated rat intestinal mucosal ATPase (23) and stimulate adenylate cyclase activity of human colonic mucosa *in vivo* (24). Whether these properties of bile acids cause inhibition of EAA absorption is unknown. However, the effects of individual bile acids upon ATPase activity do not correlate well with their effects upon water and electrolyte absorption (4, 25). Therefore, the assumption that inhibition of active transport by bile acids is related to their effects on ATPase is not well substantiated.

It is well known that bile acids interrupt cell membranes (25). Altered surface morphology of intestinal mucosa exposed to deoxycholate has been demonstrated (2, 3); and these changes may be related to reduction of EAA absorption observed with the EAA + TDC perfusion. Lecithin or MO may abolish the bile salt inhibition of EAA absorption by causing less bile salt to be available at the surface of the mucosal cells. Recently, it has been demonstrated by an equilibrium-dialysis method that intermicellar bile salt concentration decreases as phospholipid content of mixed micelles increases (26). Therefore, it is postulated that when either MO or phospholipid (lecithin) is in the perfusing solutions, micelles are enlarged—and consequently incorporate more bile acids within them and allow less bile salt to act at the surface of mucosal cells. Disruption of cell membranes is thereby prevented, and EAA absorption proceeds normally.

Summary. The effects of bile acid, lecithin, and monoolein (MO) on essential amino acid (EAA) absorption from the human duodenum were determined. It was found that: (1) methionine was absorbed at the most rapid rate while isoleucine, leucine, and valine were absorbed slightly less rapidly and lysine and threonine at the slowest rates; (2) duodenal EAA absorption

rates are very similar to previously reported rates for jejunal absorption of EAA; (3) taurocholate (TC) and taurodeoxycholate significantly depressed EAA absorption; and (4) although emulsified MO had no significant effect on EAA absorption, the addition of MO or lecithin to the EAA + 10 m*M* TC perfusion mixture restored EAA absorption to normal. How bile acids inhibit EAA absorption and how lecithin and MO abolish this inhibition are not known and have not been elucidated by our studies. However, it is postulated that when either lecithin or MO is present, less bile salt is available to act at the surface membranes of intestinal cells, disruption of cell membranes is prevented, and EAA absorption proceeds normally.

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