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Effects of Dietary Nutrients on Intestinal Taurocholic Acid Absorption[1] (41272)

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Abstract. In order to assess the intraluminal event that may be responsible for bile acid malabsorption in cystic fibrosis, taurocholic acid absorption was determined in the presence and absence of representative unhydrolyzed dietary nutrients in animal models. Triglyceride (corn oil) significantly reduced taurocholic acid uptake by villi isolated from hamster ileum. Likewise, when combinations of nutrients were studied, only those combinations of nutrients containing triglyceride inhibited taurocholic acid absorption. Neither starch nor albumin or a combination of these two substrates altered this process. Triglyceride also produced significant reductions in taurocholic acid absorption in perfused segments of terminal ileum of rats as determined by reduced biliary recovery of absorbed bile acid. Again, starch and albumin had no effect *in vivo*. These findings support an "intraluminal theory" of bile acid malabsorption in cystic fibrosis limited to only the adverse influence of unhydrolyzed lipid on this normal physiological process.

The enterohepatic circulation of bile acids allows the efficient recycling of these sterols in animals and man and ensures an adequate bile acid pool necessary for the proper digestion and absorption of fats (1). A crucial step in the maintenance of this enterohepatic circulation is the absorption of bile acids by the small intestine. Bile acids are passively absorbed throughout the small and large intestine, while an active bile acid transport process is present in the terminal ileum (2–4). This active component is of prime importance since distal ileal dysfunction or resection causes severe bile acid malabsorption with a reduction in the total bile acid pool (1).

Bile acid malabsorption is also known to occur in cystic fibrosis in cases where pancreatic insufficiency is a consistent finding (5). The presence of undigested dietary nutrients throughout the lumen of the small intestine is believed to inhibit bile acid absorption. It is thought that these large macromolecules in some way bind or sequester bile acids, thereby preventing their absorption (5–7). This "intraluminal theory," however, has not been studied extensively. Recently we reported that phospholipid inhibits bile acid absorption *in vitro* when bile acid levels are above the critical micellar concentration (4). Also, others have demonstrated *in vivo* that monoglyceride and fatty acid inhibit taurocholic acid absorption (8).

Utilizing both *in vivo* and *in vitro* techniques in animal models, the present study was undertaken to determine the effects of representative undigested dietary nutrients on intestinal taurocholic acid absorption. In this way, an assessment can be made of the possible intraluminal events responsible for bile acid malabsorption in cystic fibrosis as related to pancreatic insufficiency.

Material and Methods. *Chemicals and solutions.* Taurocholic acid (TC), starch, albumin, and triolein were purchased from Sigma Chemical Company, St. Louis, Missouri. TC was shown to be greater than 98% pure by thin-layer chromatography. Tritium-labeled TC ([^{3}H]TC) was purchased from New England Nuclear, Boston, Massachusetts. The triglyceride source was Mazola Corn Oil (Best Foods Company). This product is 98.8% triglyceride and contains less than 0.0001% phospholipid and less than 0.01% monoglyceride as determined by the manufacturer.

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Krebs–Ringer bicarbonate (KRB) was the physiologic solution used throughout this study (9). TC absorption was determined in all studies from a physiologic solution containing an initial TC concentration of 10.0 m*M*. When nutrients were included, the total amount of a specific nutrient in the incubation solution was equal to the amount (in mg) of TC present at 10.0 m*M*. When in combination, the nutrients were in equal proportion so that the total amount of combined nutrients equaled the amount (in mg) of TC at 10.0 m*M*. Prior to each experiment, appropriate amounts of starch or albumin, as obtained from the supplier, were dissolved along with TC and [^{3}H]TC in KRB and these served as stock solutions. When corn oil or triglyceride was included, the mixture was sonicated for 15 min before being used as incubation medium. All solutions were maintained at 37°C.

Animals. Male golden hamsters, 140–150 g (Engel, Farmersburg, Ind.), were used in the *in vitro* studies while male Sprague–Dawley rats, 250–300 g (Charles River, Wilmington, Mass.), were utilized in the *in vivo* experiments. All animals were housed in the institutional animal facility and provided with standard laboratory chow and tap water. Prior to each experiment animals were fasted for 24 h with water allowed *ad libitum*.

In vitro experiments. Intestinal TC absorption and the influence of dietary nutrients were examined *in vitro* using the villus technique (10). Tissue samples taken from anesthetized hamsters consisted of full-thickness antimesenteric disks of ileum about 5 mm in diameter. No more than five tissue samples were taken from one animal. Tissue was kept in an oxygenated holding bath of KRB until all five samples were taken. After the transfer of one piece of tissue to each incubation vessel (containing 4.9 ml of KRB, pH 7.4) and an equilibration period of 30 sec at 37°C, 100 μl of a concentrated stock solution of TC, with [^{3}H]TC, was pipetted into the vessel and time 0 recorded. Nutrients were included where appropriate. Tissue was incubated for 1 min at 37°C with constant shaking in an atmosphere of 5% CO_2 in O_2. Tissue was then frozen in KRB and lyophilized overnight. Following removal of villi, subvillus tissue as well as villi were dissolved separately and radioactivity in all tissues and incubation media was determined using a Beckman Model LS-333 liquid scintillation counting system. TC uptake *in vitro* is expressed as micromoles per gram (dry wt) per minute. Using [^{3}H]inulin we have determined the distribution of extracellular space in this preparation to be less than 0.1%.

In vivo experiments. Absorption of TC and the influence of dietary nutrients on this process *in vivo* were determined using a slight modification of the ileal perfusion/biliary recovery technique of Heaton and Lack (11). Rats were anesthetized with sodium pentobarbital (50 mg/kg ip) and the small intestine was carefully exposed through an abdominal incision. Inflow and outflow cannulas were placed in the proximal and distal ends of a 10-cm segment of distal ileum for perfusion of the segment with appropriate substrates. Following a 15-min KRB perfusion, segments were perfused with either (a) 10 m*M* TC and [^{3}H]TC or (b) 10 m*M* TC with [^{3}H]TC plus nutrient for 1 hr. This was followed by a 1-hr KRB perfusion before beginning perfusion of the second solution. This order was reversed in one-half the studies. In this way, each animal served as its own control. Bile was continuously collected in 10-min intervals via a bile duct cannula (PE 10) and radioactivity in aliquots of each sample was determined as above. In these studies, a triglyceride, triolein, was substituted for corn oil. Following each experiment the perfused segment of ileum was removed and lyophilyzed, and the dry weight was determined. TC absorption is expressed as nanomoles of TC recovered in bile per gram (dry wt) of ileal segment perfused.

The significance of alterations in TC absorption was determined using the pooled *t* test in the *in vitro* studies and the paired *t* test in the *in vivo* experiments. Significant differences were accepted at a *P* value of less than 0.05 in all studies.

Results. *In vitro experiments.* The control value for TC uptake by villi isolated from hamster ileum was 28.6 ± 2.3 (mean ±

SEM) μmol/g (dry wt) · min^{-1} in 16 determinations in which the initial concentration of TC in the incubation medium was 10.0 m*M*. In 8 experiments each, the addition of starch or albumin to the incubation did not significantly alter TC absorption (Fig. 1). TC uptake in the presence of starch was 25.9 ± 1.2 μmol/g (dry wt) · min^{-1} while with albumin it was 25.2 ± 1.3 μmol/g (dry wt) · min^{-1}. By contrast, when corn oil was included in the incubation medium TC absorption was significantly reduced to 14.3 ± 0.7 μmol/g (dry wt)·min^{-1} in 8 determinations (Fig. 1). This represents approximately a 50% inhibition.

TC uptake by hamster villi was also measured in the presence of various combinations of these nutrients. When starch and albumin were included in the incubation medium in 8 studies, TC absorption was 27.2 ± 1.0 μmol/g (dry wt) · min^{-1}. This value was not different from the control value for TC alone of 28.6 ± 2.3 (Fig. 2). However, when corn oil was present in any combination of nutrients used, TC uptake was significantly reduced. These values were 19.8 ± 0.6, 19.2 ± 0.5, and 19.2 ± 0.9 μmol/g (dry wt) · min^{-1} for the combinations of starch + albumin + corn oil, albumin + corn oil, and starch + corn oil, respectively, in 8 determinations each. The average percentage inhibition in these studies was 32%.

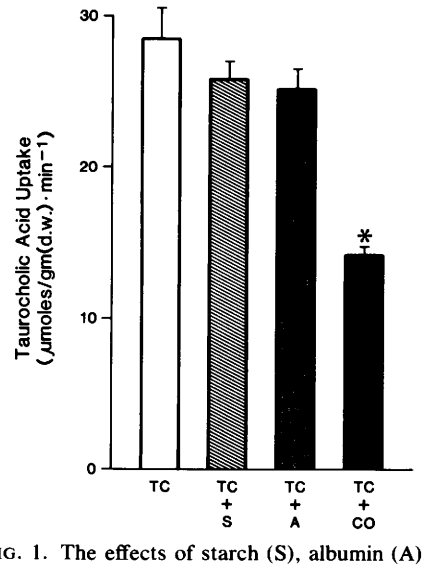

FIG. 1. The effects of starch (S), albumin (A), and corn oil (CO) on taurocholic acid (TC) uptake by villi isolated from hamster ileum. $n = 8$ in all groups except TC alone, where $n = 16$. *Significantly less than TC alone.

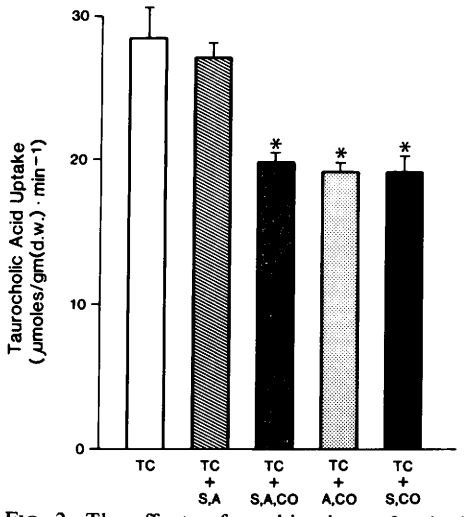

FIG. 2. The effects of combinations of unhydrolyzed nutrients on TC uptake by villi isolated from hamster ileum. Abbreviations are the same as in Fig. 1. $n = 8$ in all groups except TC alone, where $n = 16$. *Significantly less than TC alone.

In vivo experiments. To substantiate these findings, whole animal experiments were conducted. In rats, perfusion of the distal ileum with TC plus starch did not significantly alter the recovery of [^{3}H]TC in bile during a 1-hr period in six separate experiments (Fig. 3). Likewise when albumin

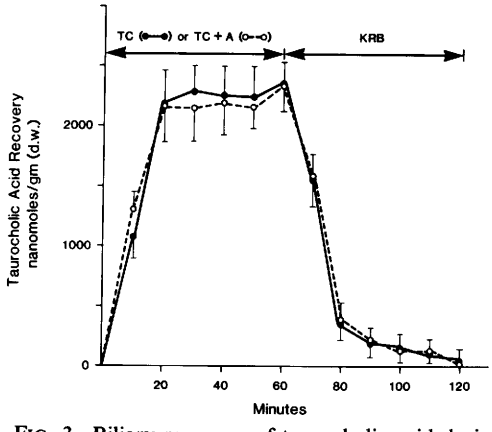

FIG. 3. Biliary recovery of taurocholic acid during perfusion of the distal ileum of rats with solutions of TC alone or TC plus S. $n = 6$ paired observations. Abbreviations as in Fig. 1 or text.

was present in the TC perfusion medium, [^{3}H]TC recovery in bile in six experiments was not different from control perfusion of TC alone (Fig. 4). However, when a TC solution containing triglyceride (triolein) was perfused through the ileal segment, significant reduction in [^{3}H]TC recovery was observed in all six studies at all but the 10-min time point of the perfusion period. The average percentage inhibition of TC recovery produced by addition of triglyceride was 21%.

Discussion. The present study was undertaken to determine the influence of a representative group of unhydrolyzed dietary components on bile acid absorption by the ileum. Experiments were carried out in both *in vitro* and *in vivo* systems to fully assess the affects of these macromolecules on this process and to perhaps more precisely define the intraluminal theory of bile acid malabsorption in cystic fibrosis.

The results of the present study indicate that neither starch nor albumin alters the uptake of TC by villi from hamster ileal mucosa *in vitro* at an initial bile acid concentration of 10.0 m*M* (Fig. 1). Likewise neither of these compounds adversely affected the ileal absorption of this bile acid in the *in vivo* studies as indicated by unaltered biliary recovery curves (Figs. 3 and 4). These results are similar to those of Harries *et al.* (12), who demonstrated in the rat a similar lack of effect of albumin on TC absorption using a different *in vivo* technique.

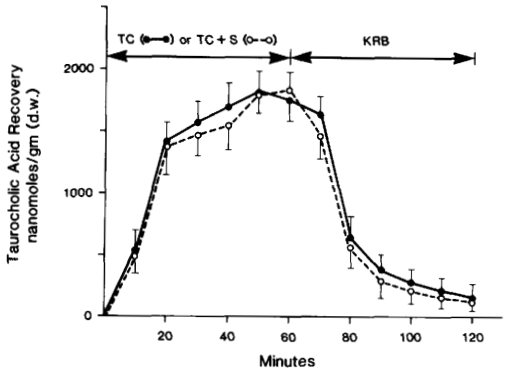

FIG. 4. Biliary recovery of taurocholic acid during perfusion of the distal ileum of rats with solutions of TC alone or TC plus A. n = 6 paired observations. Abbreviations as in Fig. 1 or text.

Our laboratory earlier reported similar findings with cholic acid absorption (13). Sklan and his co-workers (14) showed that casein inhibited the uptake of taurocholic acid in isolated duodenal loops of chick intestine. The discrepancy between our results and those of Sklan *et al.* (14) may be explained by the fact that their incubation medium also contained oleic acid, which Roy *et al.* (8) reported to inhibit bile acid uptake. Furthermore, duodenal absorption rates of bile acid are slow compared to similar rates reported in the ileum. This difference may account for the conflicting results. Despite the fact that others have reported inhibition of TC absorption by albumin and casein (14), our findings and those of others (12) suggest that carbohydrate and protein do not interact with trihydroxy bile acids in the incubation medium or intestinal lumen nor do they interfere in any way with the bile acid absorption site on the luminal membrane of the intestine.

In contrast, however, corn oil significantly decreased the uptake of TC by ileal villi (Fig. 1). Not only was this inhibition of uptake seen when corn oil alone was used but also in those combinations of nutrients where corn oil was present (Fig. 2). Since this brand of corn oil is 98.8% triglyceride, it is most likely that this moiety is adversely

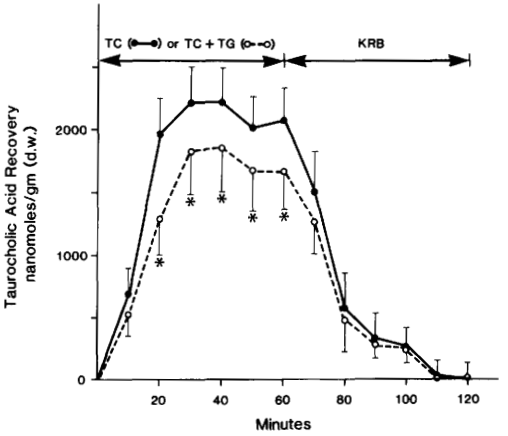

FIG. 5. Biliary recovery of taurocholic acid during perfusion of the distal ileum of rats with solutions of TC alone or TC plus triglyceride (TG). n = 6 paired observations. *Significantly less than TC alone. Abbreviations as in Fig. 1 or text.

influencing TC absorption. In the *in vivo* studies, triglyceride (triolein) was substituted for corn oil and it was found that TC absorption was inhibited by about 21% as determined by the biliary recovery technique (Fig. 5). We have previously shown that phospholipid inhibits TC uptake by villi from hamster ileum when the TC concentration is above 2.0 m*M* (4). However, Roy *et al.* (8) using an *in vivo* model were unable to demonstrate significant inhibition of bile acid absorption by unhydrolyzed triglyceride. This discrepancy is difficult to explain other than by the fact that different experimental preparations and/or bile acid concentrations were used. Certainly the data presented here and those from our previous work indicate that unhydrolyzed triglyceride and phospholipid inhibit intestinal bile acid absorption.

The studies reported here in which combinations of nutrients were tested are of some interest. Since in the postprandial state many types of nutrients are simultaneously present in the small intestine, the effects of various combinations of undigested nutrients on TC absorption were examined. Our results indicate TC uptake was reduced only in experiments in which corn oil was present. The combination of starch and albumin had no effect on TC absorption. These findings are consistent with studies involving individual nutrients, that is, corn oil was the only substrate tested which significantly reduced TC absorption.

From the results of these studies, it is possible to develop two hypotheses for the mechanism of inhibition of TC absorption by triglyceride. Since bile acids are amphipathic molecules, it may be that triglyceride binds at the lipid-soluble site of these sterols. This would in effect decrease the intraluminal concentration of monomeric bile acid, thus reducing the driving force for passive diffusion of these molecules across mucosal cell membranes. This would also prevent the binding of the bile acid molecule to the carrier system of the active transport component of ileal bile acid absorption. This theory, however, is unlikely in view of earlier results from our laboratory which indicated that at low concentrations of taurocholic and cholic acids, presumably when only monomeric bile acid is present, equimolar concentrations of phospholipid did not inhibit uptake of these bile acids by hamster villi *in vitro* (4). A more likely explanation for what is observed here may involve bile acid micelle formation. At the taurocholic acid concentration used in this study (10.0 m*M*), bile acid micelles were undoubtedly present. With triglyceride in the incubation medium or perfusion solution, micelles would be expected to become more bulky and complex as has been reported for mixed micelles containing lecithin (15). This increase in the physical size of micelles would retard their movement toward the mucosal cells of the intestine, thus reducing the concentration of bile acid at the absorptive surface. Also, more bile acid molecules would be expected to be incorporated into these expanded micelles, thereby reducing the concentration of monomeric bile acid in the lumen. This effect would reduce the driving force for passive diffusion and decrease substrate availability for active bile acid transport.

Bile acid malabsorption in cystic fibrosis is a potential clinical problem in this disease and may compound the already serious nutritional compromises in these patients. The pathophysiology of this malabsorption is unknown but is believed to be caused by some intraluminal event, namely, the binding or sequestering of bile acids by undigested dietary nutrients present due to pancreatic insufficiency. Our studies indicate that unhydrolyzed lipids may be the only nutrients to produce this effect since nonlipid nutrients did not alter TC absorption *in vivo* or *in vitro*.

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