

Hydrolysis and Absorption of Medium-Chain Triglycerides and Soy Oil (34033)

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Medium-chain triglyceride oil (MCT) contains octanoic and decanoic acid and is absorbed at least four times as rapidly as olive oil in normal, unanesthetized rats (1). The medium-chain fatty acids (MCFA) are transported from the intestine via the portal circulation as free fatty acids with only small amounts in the thoracic duct lymph as free fatty acids or triglycerides (2). MCT is well absorbed in a number of malabsorption syndromes (3-6) in which conventional dietary fats are poorly absorbed.

Greenberger *et al.* (7) demonstrated that small amounts of labeled trioctanoin could be absorbed in isolated intestinal loops of the rat in the absence of pancreatic lipase. They suggested that intact MCT may enter the mucosal cell without intraluminal hydrolysis and that this triglyceride could be hydrolyzed within the cell by a mucosal lipase which is active for MCT, but has little activity for tripalmitin. Valdivieso and Schwabe (8, 9) found that deficiency of pancreatic enzyme or diversion of bile acids in rats markedly reduced the amount of $^{14}\text{CO}_2$ in expired air of animals given trioctanoin labeled with ^{14}C ; they concluded that luminal hydrolysis of MCT was essential for its absorption. However, the use of olive oil as a vehicle for administering the trioctanoin in these studies may have interfered with its utilization. Clark and Holt (10) have shown that diversion of pancreatic juice decreases absorption of trioctanoin to one-third of the normal rate in the rat, but that bile diversion has no effect on MCT absorption.

The present study was designed to further examine the role of pancreatic lipase and bile salts in the hydrolysis and absorption of MCT as compared with digestion and absorption of long chain triglycerides. Soy oil was used as a natural mixture of long chain triglycerides.

Materials and Methods. In vitro. MCT

and soy oil emulsions were prepared in a hand homogenizer (C. W. Logeman Co.) using 15 g of oil plus 85 ml of a 2% solution of gum arabic (Fisher Scientific Co.) or Tween 80 (Sigma Chemical Co.). For the enzymic studies, 2 ml of emulsion, 8 ml of 0.005 *M* Tris-hydroxymethyl aminomethane (THAM) (Fisher Scientific Co.) were used. This was diluted to a total volume of 30 ml, the pH adjusted to 8.2 and the solution brought to 37° prior to addition of 1 ml of pancreatic lipase solution prepared from Steapsin (Sigma Chemical Co.), according to the method of Desnuelle (11). The fatty acids liberated during a 10-min incubation period at 37° and pH 8.2 were automatically neutralized with 0.1 *N* NaOH and the volume recorded using a recording pH Stat (E. H. Sargent and Co.). When the effects of bile salt were studied, 1 ml of an aqueous solution containing 100 mg sodium taurocholate (Sigma Chemical Co.) and 5 mg of sodium linoleate (Nutritional Biochemical Corp.) was added to the buffered fat emulsion before dilution and pH adjustment. Preliminary studies showed that linoleate enhanced the effect of bile salts in reversing inhibition of lipolysis of MCT by Tween 80. The small amount of free fatty acid may create physical conditions which assist in initiating lipolysis.

In vivo. Studies were carried out in fasted, ether-anesthetized adult female rats. Three rats were used to determine each value. In some of the studies the small intestine was first flushed with 50 ml of physiological saline in order to reduce the intraluminal concentration of lipase and bile salts.

In the normal rats the small intestine was isolated by ligatures just distal to the pyloric valve and proximal to the ileocecal junction. In the rats with rinsed intestine, the proximal ligature was placed below the entrance of the bile duct into the small intestine.

One milliliter of a 30% fat emulsion in 2%

TABLE I. *In Vitro* Hydrolysis of MCT and Soy Oil Emulsion by Pancreatic Lipase.^a

Emulsifier	Fat	Other additions	Hydrolysis (μ moles of fatty acid liberated/10 min)
Gum arabic	MCT	None	560
	Soy oil		42
	MCT	100 mg taurocholate	558
	Soy oil	and 5 mg linoleate	140
Tween 80	MCT	None	0
	Soy oil		0
	MCT	100 mg taurocholate	512
	Soy oil	and 5 mg linoleate	62

^a Hydrolysis conditions: All incubations at 37°; 2 ml 15% o/w emulsions, 8 ml 0.005 M THAM, pH 8.2, diluted to 30 ml plus 1 ml enzyme solution.

gum arabic solution or in 2 or 4% Tween 80 solution was instilled into the duodenum of these rats through the pyloric valve before this ligature was secured. After 0, 2, 4, or 6 hr, animals were sacrificed by cervical dislocation and the isolated section of small intestine removed. Twenty milliliters of saline were instilled to wash the luminal contents into a flask containing 2.2 ml of 25% HCl. Fluid remaining in the intestine was carefully expressed with the aid of a soft rubber roller and added to the intestinal contents. Esterified and free fatty acids were determined by a modification of the method of Van de Kamer *et al.* (12). Recovery of MCFA was facilitated by reducing the concentration of ethanol in the aqueous phase from 60% to 15%. This modification increased partitioning of MCFA in the petroleum ether phase from approximately 40% to over 90%.

Results. *In vitro*, MCT was hydrolyzed more rapidly than soy oil when emulsified in gum arabic alone. In the 10-min period studied, 560 μ moles of fatty acids were liberated from MCT compared to 42 μ moles from soy oil (Table I). The addition of taurocholate and linoleate increased the hydrolysis of soy oil to produce 140 μ moles fatty acids but had no effect on the splitting of MCT.

However, when MCT and soy oil were emulsified in 2% Tween 80 solution, hydrolysis by pancreatic lipase was completely inhibited. Minard (13) has reported that Tween 80 inhibits hydrolysis of corn oil by pan-

creatic lipase, and that this can be reversed by the addition of appropriate concentrations of bile salts. Addition of taurocholate partially reversed inhibition of lipolysis of MCT; linoleate plus taurocholate almost completely restored pancreatic hydrolysis of MCT to normal (Table I). In the presence of taurocholate plus linoleate, soy oil emulsions in Tween 80 were hydrolyzed more rapidly than soy oil in gum arabic alone but not as effectively as found with gum arabic plus bile salts.

In vivo, MCT was rapidly absorbed from the gum arabic emulsion in the isolated intestinal segments (Table II). After 2 hr, 50% of the administered oil was absorbed and after 4 hr, about 95%. At 2 hr, almost half of the remaining fat in the intestine was present as free fatty acids and at 4 hr, the little remaining fat was all free fatty acids. Saline flushing prior to administration of the emulsion to reduce the intraluminal concentration of lipase and bile salts reduced MCT absorption. In 2 hr after instillation of the gum arabic emulsion, 39% of the fat was absorbed and after 4 hr, 53%. The proportions as free fatty acids in the fat remaining in the intestine were also much less than in the normal intestine at 2 hr.

Absorption of soy oil from the normal gut was less rapid than that of MCT; 19 and 23% of the administered oil was absorbed during the 4- and 6-hr test periods. At both time periods, approximately 2/5 of the fat re-

TABLE II (continued)

Fat emulsion admin.	Hr after admin.	4% Tween 80 vehicle					
		Normal intestine		Rinsed intestine		Total fat absorbed (%)	Total fat absorbed (%)
		Fat recovered (%)	Total fat absorbed (%)	Fat recovered (%)	Fat recovered (%)		
Soy oil	0	8.1	0	4.3	0	0	
		94.1		96.6			
	4	38.6	21.1	4.2	4.7		
		40.3		91.2			
	6	28.9	31.8	6.0	3.9		
		39.3		90.2			

maining was present as free fatty acids. Washing the intestine decreased lipolysis and impaired absorption of soy oil; 8 and 14% of the administered fat was absorbed 4 and 6 hr after instillation; only about 1/3 of the fat recovered from the lumen was present as free fatty acids.

In the normal animal, absorption of MCT from 2% Tween 80 was quite similar to that from the gum arabic emulsion. The 4% Tween 80 emulsion decreased MCT absorption at 4 hr (Table II). After 2 and 4 hr respectively, 58 and 92% of MCT were absorbed from the 2% Tween 80 emulsion; 52 and 72% of the MCT were absorbed from the 4% Tween 80.

Hydrolysis and absorption of soy oil in the normal intestine was apparently unaffected by Tween 80. Absorption was similar to findings with gum arabic emulsions. After 4 and 6 hr, respectively, 23 and 26% of soy oil were absorbed from 2% Tween 80 and 21 and 32% from 4% Tween 80 emulsion, compared to 19 and 23% from gum arabic emulsions (Table II).

After the intestine was washed to reduce concentrations of lipase and bile salts, Tween 80 markedly inhibited absorption of both MCT and soy oil. With both fats lipolytic activity was decreased, as judged by the low levels of free fatty acids found in the luminal contents.

Discussion. *In vitro* studies with gum arabic emulsions clearly demonstrate that MCT is much more rapidly hydrolyzed by pancreatic lipase than the long-chain triglycerides of soy oil. This difference may be related to the finding that long-chain fatty acids, liberated from long-chain triglycerides, inhibit further pancreatic lipase activity, whereas medium-chain fatty acids do not inhibit this enzyme (14). The long-chain fatty acids have a marked affinity for the oil phase of triglyceride emulsions, whereas the relatively water-soluble medium-chain fatty acids do not attach themselves to the triglyceride emulsions (15). The ability of a variety of agents (bile salts, ionized calcium, and albumin) to increase the rate of hydrolysis of triglycerides by pancreatic lipase may be

related to their ability to combine, either chemically or physically, with fatty acids in the aqueous phase. Removal from the oil-water interface of the long-chain fatty acids facilitates triglyceride hydrolysis.

With the concentration of Tween 80 used in the present *in vitro* studies, hydrolysis of both MCT and soy oil by pancreatic lipase were completely inhibited. With lower concentrations of Tween 80, in studies not reported here, it was found that less Tween 80 was required to inhibit hydrolysis of MCT than that of soy oil; with soy oil, Tween 80 often caused a lag in start of hydrolysis rather than inhibition related to level of Tween 80, as found with MCT. Tween 80, which inhibits lipoprotein lipase (16, 17), as well as pancreatic lipase (13), apparently surrounds the oil droplets and alters the availability of substrate to lipase.

In vivo, MCT was absorbed much more rapidly than was soy oil from gum arabic emulsions in both normal and washed intestine. Washing the intestine moderately reduced absorption of both oils. When the intraluminal bile salt and lipolytic enzyme concentrations were markedly reduced by washing the intestine, over half of the administered MCT was absorbed in 4 hr, but only 1/10 of the soy oil was absorbed in this period.

In the rinsed intestine, only a small portion of the MCT was recovered as free fatty acids, suggesting that absorption of MCT was limited mainly by the ability to hydrolyze this triglyceride. This is in agreement with the finding that MCT is well absorbed in rats and man fed high levels of cholestyramine which sequester intestinal bile acids (18, 19). This is also in accord with the findings of Clark and Holt (10) who reported that absorption of MCT in bile-diverted rats was essentially normal, but absorption of MCT was reduced to 1/3 when both enzymes and bile were diverted. The rapid absorption of MCFA results, in part, from their greater water solubility. This favors partitioning of MCFA away from the oil (emulsion) phase into the aqueous phase of digestive chyme from which they apparently diffuse into the mucosa.

It is very difficult to completely assure the absence of luminal lipolytic enzymes in the intestine in order to study their role in triglyceride hydrolysis and absorption (20). Washing a segment of intestine does not necessarily remove all enzyme activity; with a pancreatic fistula as used by Clark and Holt (10) some lipolytic activity may remain in the small intestine (20). In addition, a gastric lipase which hydrolyzes MCT (21) may introduce luminal lipolytic activity in the intestine.

In the present experiments Tween 80, which inhibits lipase activity in the absence of bile salts, was used to emulsify preparations of MCT and soy oil introduced into normal and washed segments of rat intestine. In the normal intestine, absorption of MCT and soy oil was not significantly affected by the presence of Tween 80. In the washed intestine, 19% of the MCT administered in 2% Tween 80 was absorbed in 4 hr as compared with 53% of the MCT given in gum arabic emulsions in similar animals. Higher levels of Tween 80 virtually prevented absorption of any MCT. Soy oil hydrolysis and absorption was also blocked by Tween 80. The data suggest that absorption of MCT is inhibited concomitant with the block in hydrolysis of MCT. However, it is possible that Tween 80 inhibits mucosal lipase or otherwise alters membrane permeability, in addition to its effect on pancreatic lipase. It must be emphasized that the use of Tween 80 as a tool which completely blocks lipolytic activity may not provide a model analogous to pancreatic deficiency in man, since in the absence of pancreatic lipase, lipolytic activity may arise from gastric lipolytic enzymes or intestinal lipase.

Summary. Studies were conducted on *in vitro* hydrolysis of MCT and soy oil emulsified in gum arabic and Tween 80 solutions by pancreatic lipase and *in vivo* hydrolysis and absorption of these emulsions in normal and washed isolated sections of the small intestine of rats.

In gum arabic emulsions, MCT was hydrolyzed ten times more rapidly than soy oil *in vitro*. Addition of taurocholate and linoleate had no effect on MCT hydrolysis but in-

creased the rate of hydrolysis of soy oil three-fold. Tween 80 inhibited action of lipase on both MCT and soy oil. Taurocholate and linoleate completely reversed the inhibition of MCT hydrolysis and partially overcame the inhibition of soy oil hydrolysis.

When instilled into the normal intestine, emulsions of MCT in gum arabic were more rapidly hydrolyzed and more completely absorbed than those of soy oil; Tween 80 had little effect on absorption of both oils in the normal intestine. Absorption of both oils from gum arabic emulsions was somewhat less in the washed intestine than in the normal intestine. However, Tween 80 almost completely inhibited hydrolysis and absorption of both oils from the washed intestine, suggesting that intraluminal hydrolysis of MCT is necessary for rapid absorption of this fat.

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