THE UNIVERSITY OF CALGARY

THE DIFFERENTIAL ABSORPTION OF TRIGLYCERIDES IN THE PANCREATIC LIPASE-FREE STATE.

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "The differential absorption of triglycerides in the pancreatic lipase-free state" submitted by Bill P. C. Chow in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Medium chain triglycerides (MCT) are considered to be readily absorbed intact in the absence of lipase, unlike long chain triglycerides. Commercial MCT oils may be comprised of various medium chain fatty acids from 6 to 12 carbons (C) resulting in differences in size and molecular weights (MW). The effect of molecular weight and fatty acid (FA) chain length on the efficiency of intact MCT absorption is unknown. Intestinal absorption was therefore measured by single pass, marker perfusion in anesthetized Sprague-Dawley rats. A 10 cm segment of jejunum was isolated below the ligament of Trietz and perfused with 154 mM NaCl at 0.075 ml/min/100g body weight for 75 min to remove any residual pancreatic lipase. Then a 7.7 mM emulsion containing 5 different MCT and ^{3}H-PEG 4000 marker or a 2.0 mM emulsion of LCT and ^{3}H-PEG 4000 marker was perfused at 0.13 ml/min for 80 min following a 40 min equilibration period. MCT were analyzed by capillary gas liquid chromatography and confirmed by GC-MS. LCT were analyzed by radioisotope techniques.

Results: $\bar{X}\pm SE$. In the MCT perfused group, 10 of the 13 rats showed no lipase activity in the effluent after saline washout, while the other 3 had 3.8±1.2 U/L. Lipase was reduced to 0 U/L in the 5 rats of the LCT perfused group. The absence of lipase activity and the presence of MCT rather than MCFA in the effluent suggest intact MCT absorption.

Table 1. The types of triglycerides used for perfusion.

EMULSION	TRIGLYCERIDE	No. FA CARBONS	FA COMPOSITION (C)			MW.	% in PERFUSATE*
	TRIOCTANOIN	24	8:0	8:0	8:0	470.7	25.5 ± 0.4
	DECADIOCTANOIN	26	8:0	8:0	10:0	498.8	29.0 ± 0.3
MCT	OCTADIDECANOIN	28	8:0	10:0	10:0	526.8	11.0 ± 0.1
	TRIDECANOIN	30	10:0	10:0	10:0	554.9	18.7 ± 0.4
	TRIDODECANOIN	36	12:0	12:0	12:0	639.0	15.8 ± 0.4
LCT	TRIOLEIN	54	18:1	18:1	18:1	885.4	100

* Values are $\bar{X}$±SE.

All 5 MCT were well absorbed. There was no difference in % absorption between the 5 MCT. LCT was not absorbed.

Conclusion: In the perfused rat jejunum, 1) LCT is not absorbed in the absence of pancreatic lipase. 2) The MCT molecule is absorbed intact. 3) Absorption of MCT with MW from 470.7 to 639.0 and FA carbon chain length of 24 to 36 was excellent in the absence of lipase. 4) The molecular weight of the MCT does not affect its intact absorption by the small intestine.

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To my parents

Lie King and Siu Lun

One's kite will rise on the wind as far as ever one has string to let it go.

D.H. Lawrence ("The Rainbow")

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

REVIEW OF LIPID ABSORPTION

1.1 WHAT ARE MEDIUM AND LONG CHAIN TRIGLYCERIDES?

Lipids are complex water insoluble organic substances found in cells of plants and animals. One special class of lipid is the neutral fat triacylglycerol or simply, triglyceride. Triglycerides consist of three fatty acid molecules esterified to the hydroxyl groups of a glycerol molecule. Long chain triglycerides (LCT) are composed of long chain fatty acids. These are fatty acids with chain lengths of 14 carbon atoms or greater and can be either saturated or unsaturated. Medium chain triglycerides (MCT) are composed of medium chain fatty acids (MCFA). These have chain lengths varying between 6 and 12 carbon atoms and are exclusively saturated (Figure 1). Triglycerides are polar, insoluble, nonswelling amphiphiles,[1] forming a monolayer film across the surface of aqueous solutions. Triglycerides in solid crystalline state, are in a tuning-fork configuration with the sn-1 and sn-3 fatty acids pointing in one direction and the sn-2 in the opposite direction. In the liquid state, the fatty acids are thought to be less rigid as atoms rotate around all single-bonded aliphatic chain carbons. At the oil-water interface, the hydrophilic glycerol faces the water phase and all three hydrophobic fatty acid moieties extend back into the oil phase.[2] In general the triglycerides with a smaller molecular weight and shorter fatty acid chain lengths are more water soluble.[6] Also,

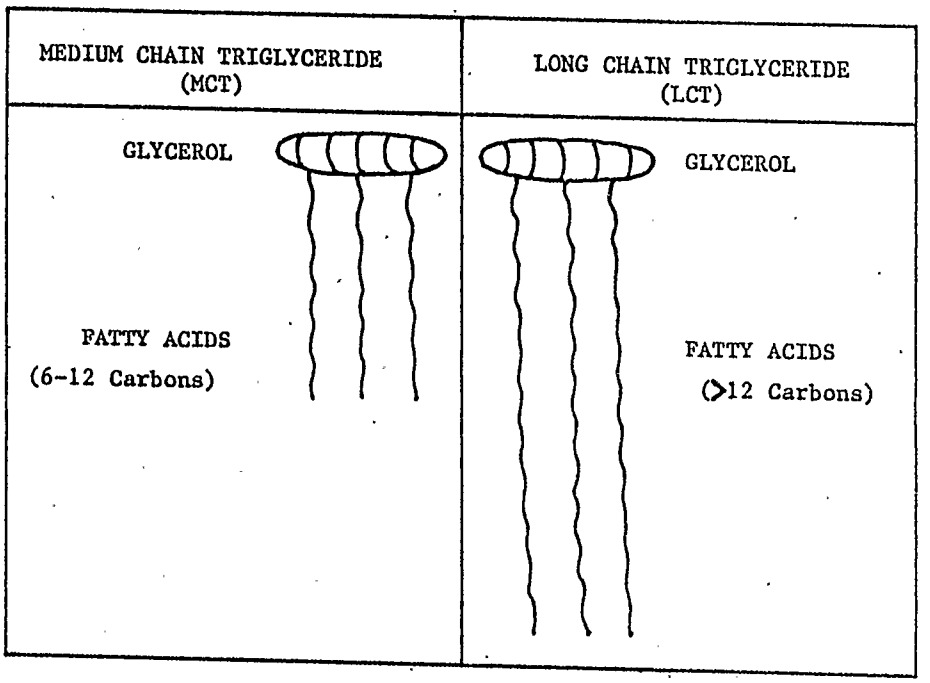

Figure 1. A comparison of medium chain triglyceride (MCT) and long chain triglyceride (LCT).

melting point increases with the number and length of saturated fatty acid components.[3] Some physiochemical properties of a MCT, trioctanoin, and a LCT, triolein, are listed in Table 2.

Naturally occurring dietary fats are composed of 95% triglycerides.[4,5] In the average diet, oleic acid (C18:1) comprises half the fatty acids ingested. The remaining fatty acid consists predominantly of stearic (C18:0), linoleic (C18:2), palmitic (C16:0), palmitoleic (C16:2), and myristic (C14:0) acids.[6] Very little ingested triglyceride is composed of MCFA.[4] This reflects upon the relative scarcity of MCT and MCFA in foods. Coconut oil however is a rich source of MCT. It contains approximately 65% MCT.[6] Ingested medium chain fatty acids include caproic (hexanoic, C6:0), caprylic (octanoic, C8:0), capric (decanoic, C10:0), and lauric (dodecanoic, C12:0) acids. Vigen Babayan of the E. F. Drew Chemical Corporation was primarily responsible for the manufacture of MCT. MCT were synthesized by re-esterifying glycerol and free fatty acids of mainly 8 and 10 carbons in length having been liberated from coconut oil. These MCFA were released from the oil by steam hydrolysis and fractionation.[7,8] Most naturally occurring triglycerides are mixed in that they may be composed of more than one fatty acid type.[6] Long chain triglycerides as long chain fatty acids (LCFA) are important sources of energy, storage, and hepatic lipid synthesis in the body.[8] LCT as an energy source provides 9 calories/g.[6] Medium chain triglycerides are not normally stored

Table 2. Physiochemical properties of triolein and trioctanoin.*

PROPERTIES	TRIOLEIN	TRIOCTANOIN
M.W.	885.4	470.7
M.P. (^{o}C)	- 5.5	- 22
B.P. (^{o}C)	235-240	233.1
DENSITY	0.8988	0.9540
n_D	1.4621	1.4481
SOLUBILITY	eth, chl, peth	al, eth, bz, chl, lig
Water	insoluble	insoluble
Alcohol	slightly	slightly
Ether	very	very
Acetone	--	--
Benzene	--	very

* Data from references 64-66.

A comparison of the properties of a LCT (triolein) and a MCT (trioctanoin).

Abreviations:
al - alcohol (ethanol)
B.P. - boiling point
bz - benzene
chl - chloroform
eth - ether (diethyl ether)
lig - ligroin
M.P. - melting point
M.W. - molecular weight
n_D - refractive index
peth - petroleum ether

in adipose tissue in humans and storage has only been shown in rats with portocaval shunts [9] and in pigs fed a 99% MCT diet.[10] However, Sarda et al[11] have recently shown that in the human infant, MCT when given orally was deposited into subcutaneous adipose tissue, storing up to 12% of MCFA. Also MCT are rarely used in hepatic liver synthesis.[8,12] The majority of MCT therefore is oxidized in the liver for energy, providing 8.3 calories/g.[6]

1.2 HISTORICAL BACKGROUND

The history of the research into the lipid absorption was extensively covered by Senior[13] and again by Johnson.[14] The search for the mechanism of triglyceride absorption, although not as colourful as the story of the discovery of insulin,[15] was from its infancy embroiled in controversy. Fat absorption generally means LCT absorption. Scientific literature and discussions often degenerated to personal attacks on integrity and character. The subject was first opened by Olof Rudbeck in 1653 with his observation that the thoracic duct took on a milky appearance after a fatty meal.[13] In 1843, Gruby and Delafond observed the presence of fine fat droplets in the intestinal epithelial cells.[13] Then in 1856, two hundred years after Rudbeck's initial findings, Claude Bernard, considered by many to be the father of physiology, again documented the phenomenon of fat absorption in the rabbit.[16] He observed how following the ingestion of a fat

meal that the mesenteric lymphatics located distally to the pancreatic duct became cloudy.[14] He also observed this in dogs given a fat meal.[13] From this he postulated the lymphatic system to be the route of transport of absorbed fat from the intestine.[14] He also suggested the involvement of pancreatic secretions in digesting neutral lipids.[13,14]

Two physiologists, Munk and Pfluger, in the late nineteenth and early twentieth century championed conflicting theories of fat absorption. These began heated exchanges for the next 60 years. Munk presented his findings beginning in 1880 that demonstrated lymphatic triglyceride levels in the rat increased following ingestion of a fatty meal of either free fatty acids, amyl cetyl esters, or triglycerides. He concluded that the majority of the ingested triglyceride was taken up by the intestinal mucosa intact in the fine emulsion droplets and transported in the lymph. This hypothesis was coined the "particulate theory." Pfluger later published a conflicting model in 1900 based on Munk's observations which he termed the "lipolytic theory". He suggested that ingested triglycerides were first hydrolyzed to glycerol and fatty acids and that these lipolytic end products were subsequently absorbed by the intestine as soaps or dissolved in bile. Pfluger was sucessful in swaying the opinion of the scientific community and eventually the lipolytic theory gained acceptance and remained in favour for the next thirty years.[14]

The resurgence of the particulate theory was led by Frazer in

1938. He put forth a modified model termed the "partition theory" which encompassed all the evidence at that time. That some triglyceride hydrolysis occurred in the intestine was acknowledged, but was thought to be minimal, forming only small amounts of LCFA, monoglycerides and diglycerides. These products then combined with endogenous bile acids to better emulsify remaining ingested triglycerides into fine emulsion droplets. Consistent with the old particulate theory, these droplets then passed into the epithelium. Frazer's theory was that fatty acids entered the hepatic portal system for transport, whereas mono-, di-, and tri-glycerides entered the hepatic lymphatic system. The particulate theory was once again put into disfavour by experiments published by Bloom et al in 1950-1951. They cannulated the thoracic duct in rats and recovered 80-90% of ingested LCFA in triglyceride form in the lymph. They also recovered a large amount of MCFA and SCFA (chain lengths less than 6 carbon atoms) in the portal venous blood. These results contradicted the partition theory. It was concluded that LCFA must be re-esterified after absorption and before delivery into the lymph.[14]

1.3 LONG CHAIN TRIGLYCERIDE DIGESTION AND ABSORPTION

Triglyceride absorption can be divided into a number of steps (a) intralumenal emulsification and digestion, (b) micellar solubilization, (c) penetration into the mucosal cell, (d) intracellular triglyceride re-esterification, (e) chylomicron

formation, and (f) chylomicron transport to the lymphatic circulation.[1] This review will cover steps (a) to (c).

The average western food supply in 1980 had a fat contentof 169 g per capita per day.[17] Animal fat constituted 58% of the fat in the diet while vegetable fat amounted to 42%.[17] Although the fat content of the diet was 160 g in the late 1970's, the average consumption was 82.8 g/day.[17] Dietary fat supplies 40% of the caloric intake.[17] About 95% of ingested fat is in the form of triglycerides which consist mainly of long chain saturated and unsaturated fatty acids.[4,5] So what is normally refered to as fat is LCT. However, about 5-10% of the ingested triglycerides are composed of MCFA.[4,5] Other important ingested lipids are phospholipids and the sterols which include cholesterol and plant sterols.[1] The process of fat absorption is very efficient with less than 5% of ingested triglycerides being excreted.[4] Fat digestion and absorption occurs mainly in the upper gastrointestinal tract and in the human is almost complete in the proximal 100 cm of the jejunum. In the hamster, fat absorption is mainly a jejunal function.[16]

1.3.1 INTRALUMINAL EMULSIFICATION AND DIGESTION

The primary purpose of lipid emulsification and digestion is to convert an insoluble lipid like triglyceride into a more soluble form that will form a finer emulsion increasing the available surface area for the hydrolytic action of pancreatic

lipase producing fatty acids. Lipid digestion and absorption is a complex process that starts in the mouth, where large pieces of fat are cleaved to smaller pieces forming a crude emulsion. It is then swallowed into the stomach along with lingual lipase secreted from lingual serous glands called von Ebner glands[2,18,19] in response to oral triglycerides and mastication.[4] It has recently been determined that a gastric lipase is secreted into the stomach from the gastric glands of the gastric mucosa.[18,20] Lingual and gastric lipase partially hydrolyze triglycerides into limited amounts of fatty acids and diglycerides. The fatty acids liberated supply polar groups that aid in emulsification of fat droplets. Once incorporated into the emulsion droplet, the fatty acids rise to the oil-water interface. At the surface, fatty acids reduce the interfacial tension thus further stabilizing the emulsion and producing finer (smaller) droplets.[2] The churning grinding action of the stomach and the shearing forces of gastric emptying also facilitate emulsification into fine droplets of less than 0.5 μm in diameter when the lipids enter the duodenum.[2,21]

Lingual and gastric lipases are very similar in their respective pH optima, substrate specificity and interaction with bile salts. Both enzymes are acid lipases. Their maximal lipolytic activty occurs in an acidic medium, such as in the stomach. The pH optimum of lingual lipase is 3.0-5.5 in humans[22] and 2.0-6.0 in rats.[18] Gastric lipase is similar.[18] These lipses hydrolyze MCT more rapidly than LCT.[2,18] There is preferential cleavage of the sn-3

position fatty acid rather than the sn-1 ester bond.[2,18] There is also a preference to release polyunsaturated LCFA rather than saturated fatty acids.[18] Both lipases do not require bile acids for lipolysis,[18] and the reaction is inhibited by bile salt micelles.[1] It is still not clear whether there are two distinct lipases with similar characteristics involved in intragastric lipolysis or whether the same enzyme is produced in two separate sites.[1,18]

Fatty acids, amino acids and hydrochloric acid entering the duodenum stimulate the release of the gut hormone cholecystokinin (CCK), which in turn stimulates pancreatic enzyme secretion, and gallbladder contraction delivering bile to the small intestine.[4,5] Secretin, released gastric acid within the first few centimetres of the duodenum stimulates a bicarbonate-rich secretion from the pancreatic ducts and in some species, the bile ducts.[4,5] Pancreatic secretions are high in bicarbonate and contain pancreatic lipase, procolipase, prophospholipase A_2 and cholesterol esterase (nonspecific lipase). Biliary secretions contain bile salts, cholesterol and phospholipids.[4] The physiological concentration of bile salts in the duodenum is 8-10mM.[23] The lipid droplets are further emulsified in the duodenum by intestinal contractions[2] and endogenously secreted biliary phospholipids (as phosphatidyl choline or its partially hydrolytic produced, lyzolecithin) and bile salts. The decrease in droplet size and increase surface area facilitate efficient

lipolysis.[4] Pancreatic lipase and colipase are the major enzymes involved in triglyceride digestion.

Pancreatic lipase is secreted from pancreatic acinar cells in the active form.[1,2] It has a pH optimum of 8 in the absence of bile salts but this value drops to 6 when bile salts are present.[1] The molecular weight of human pancreatic lipase is 67,000, as determined by SDS (sodium dodecyl sulphate)-polyacrylamide gel electrophoresis.[1] It functions to hydrolyze triglycerides in the intestine. The enzyme is specific for the primary ester bonds, at the sn-1 and sn-3 positions, resulting in two free fatty acids and a 2-monoglyceride.[1,2,5,21,23] Since pancreatic lipase is a water soluble entity and its substrate is water insoluble, its site of action is at the oil-water interface.[4] Emulsification of fat is extremely important for rapid digestion as smaller emulsion particles present larger surfaces for lipase to interact with. Although pancreatic lipase must act at the oil-water interface, its enzymatic activity can be irreversibly inhibited by coming too close to the oil surface resulting in hydrophobic binding between lipase and triglyceride emulsion.[1] Lypolysis can also be inhibited by bile salts at concentrations above the critical micellar concentration.[1,21] It is thought that bile salts, act as detergents displacing pancreatic lipase from the interface.[24]

The possible existence of pancreatic colipase was first discovered in 1910 by Rosenheim who found a factor in pancreatic

juice that facilitated lipolysis. It was again observed by Basky[1,23] and confirmed by Morgan.[23] It was found to have a molecular weight of 10,000.[1,23] Its primary function is the anchoring of pancreatic lipase to the emulsion particle.[4,5,24] Colipase is secreted from the pancreas as a procoenzyme normally in a 1:1 molar ratio with lipase. This procolipase is activated by tryptic hydrolysis of the arginine-glycine bond in the N-terminal chain[2] of the initial 102 to 107 amino acid molecule producing an activated 96 amino acid molecule.[1,23] Colipase has a binding site for pancreatic lipase and forms a 1:1 complex with it.[1,24] It also has a binding site for bile salt micelles and forms 1:1 complexes.[1,24]

The model of intraluminal triglyceride digestion most widely accepted is the "sequence theory."[23] In the duodenum, the pancreatic procolipase activation is initiated by enterokinase secretion from the brush border of intestinal epithelial cells stimulated by bile salts. The enterokinase releases trypsin from trypsinogen which activates the pancreatic colipase.[6,16] Pancreatic lipase functions to breakdown triglycerides into two moles of fatty acids and one mole of monoglyceride. Lipase only function at oil-water interfaces between oil emulsion droplets and the bulk water phase.[4] Endogenous bile salts, being detergents, aid fat digestion by clearing any hindering proteins enveloping the emulsion particle.[4,23] This frees the emulsion surface for lipase to have greater access to substrate. However, these same bile

salts while freeing the surface also could displace lipase from the interface.[24] This inhibitory effect is overcome by the action of colipase. Colipase alone first binds the diglyceride (or monoglyceride) subtrate possibly by hydrophobic interaction despite the presence of bile salts.[1] It then forms a 1:1 complex with pancreatic lipase, facilitating the anchoring of lipase to the oil-water interface.[4,5,24] This interaction also prevents lipase from being inactivated by the emulsion. Colipase also forms a 1:1 complex with bile salt micelles.[1,24] The products of lipolysis migrate from the oil phase to the water phase aided by Ca^{2+} [5,21] and form mixed micelles[4,24] with bile salts by combining with the bile salt micelle complexed to colipase. This proccess prevents end-product inhibition of lipolysis.

1.3.2 MICELLAR SOLUBILIZATION

Micellar solublilization is an important step in fat absorption. The products of lipase activity are mainly LCFA since LCT constitute the majority of the fats ingested. LCFA have a low solubility in water,[1] so in order to pass through the aqueous medium, that is the bulk water phase of the intestinal lumen, they must be incorporated into micelles. At low concentrations, bile salts exist as monomers.[1] But at levels above the critical micellar concentration in solution, the bile salts tend to aggregate together into structures called micelles.[1,25] A micelle composed of only bile salts is called a "simple

micelle."[26] A simple micelle that have incorporated lipids into its structureis is called a "mixed micelle."[25]

Micelles are spherical in shape.[26] Bile salts form the outer shell surrounding a hydrophobic core. The bile salt molecule is oriented with the hydrophillic hydroxyl and amino groups facing outward to the water and the hydrophobic steroid hydrocarbon moiety directed inward into the core.[25,26] Phospholipids mainly of endogenous origin and of exogenous origin insert into the core between the bile salt molecules with the polar head pointing outward and the nonpolar hydrocarbon chains pointing inward.[25] This enhances micelle stability. Other lipids such as cholesterol, fat soluble vitamins and perhaps fatty acids and monoglycerides are solubilized into the hydrophobic core.[1,4] Fatty acids and monoglycerides probably remain at the surface to stabilize the particle. When phospholipids combine with bile salts in a mixed micelle, the structure swells with water spreading the polar head groups of the phospholipid, lecithin, enabling solubilization of more lipid.[25] Such mixed micelles are considered to be the major route of delivery for fat digestion products to the absorptive cell.[4]

1.3.3 ENTEROHEPATIC CIRCULATION OF BILE SALTS

The physiological concentration of bile salts in the intestine is 8-10 mM.[23] It was observed that in the rat, greater than 90% of the bile salt pool was present in the intestinal lumen

at any given time.[27] In humans and other animals with gallbladders, most of the bile salt pool is located in the gallbladder during the interdigestive period.[28] However, the amount of bile salts required for daily fat absorption greatly exceeds the total endogenous bile salt pool. The capacity for **de novo** bile salt synthesis from cholesterol by the liver (maximum being a 5-10 fold increase) cannot meet daily requirements if all bile salts were lost following biliary output into the upper small intestine. The body must therefore recover intestinal bile salts and minimize loss by excretion. Bile salts are recycled in the "enterohepatic circulation of bile salts." Recycling is extremely efficient with only a 3-5% loss from the bile salt pool each cycle.[25] The rate of bile salt synthesis is dependent on and accurately replaces the amount loss in the feces each day, maintaining a constant pool size of about 50 mg/kg body weight.[25] The liver regulates synthesis by the amount of bile salt return in a negative feedback fashion.[25] Low bile salt return stimulates synthesis and a high rate inhibits it. The entire bile salt pool is recirculated between 5 to 10 times a day.[25]

The enterohepatic ciculation of bile salts starts in the liver. Bile salts are secreted from hepatocytes into bile canaliculi and hence into the biliary tree and the gallbladderin those animals possessing one. There bile is concentrated and stored.[28] When stimulated by CCK[4,5] in response to chyme entering the duodenum, the gallbladder contracts and the sphincter of Oddi relaxes to

allow bile to enter the intestinal lumen. There is even intermittent delivery of gallbladder bile during fasting. Bile salts are then reabsorbed in the terminal ileum by active transport and delivered into the portal venous system. Some passive absorption occurs in the small bowel and colon. In the liver, the bile salts are efficiently reclaimed for the next cycle.

1.3.4 PENETRATION INTO MUCOSAL CELL

The major barriers to absorption are the unstirred water layer and the cell membrane.[29] The unstirred water layer is believed to be an unequilibrated area that covers the surface of the intestinal mucosa with an estimated thickness of 100-500 μm ***in vitro***,[24] and perhaps greater ***in vivo***.[26] Structurally, the unstirred water layer is composed of a series of unequilibrated lamellae extending from the cell membrane outward each becoming progressively more stirred until blending with the stirred bulk water phase.[26] The membrane of the intestinal epithelial cell is a lipid bilayer embedded with proteins. The degree of resistance posed by each barrier on the passive diffusion of a solute from the bulk water phase into the cell is dependent on the water solubility properties of the molecule, the thickness of the unstirred water layer, and the permeability characteristics of the cell membrane.

The rate of diffusion across the unstirred water layer varies with many factors: (i) the concentration gradient, (ii) the ability of the probe molecule to diffuse, ie. the diffusion coefficient

(D), (iii) the thickness of the unstirred water layer (d), and (iv) the effective surface area of the unstirred water layer (S_w). Assuming C_1 and C_2 represents the concentration of the molecule in the bulk water phase and in the area immediately adjacent to the surface of the cell membrane respectively, then the flux of a probe across the unstirred water layer will be given by the formula:

$$J = (C_1 - C_2)\ (D/d)\ (S_w)$$

The rate of diffusion across the cell membrane is dependent on many factors: (i) the concentration gradient, and (ii) the ability of the probe molecule to penetrate the membrane ie. the permeability coefficient (P). Assuming C_2 and C_3 represents the concentration of the molecule in the area immediately adjacent to the surface of the cell membrane and inside the cell respectively, then the flux of a probe across the unstirred water layer will be given by the formula normalized for membrane surface area:

$$J = P\ (C_2 - C_3)$$

The concentration at C_3 closely approximates zero so $C_2 >> C_3$ because absorbed substances are quickly metabolized in the cell or rapidly transported from it.[30] Therefore the equation can be simplified to:

$$J = PC_2$$

Under steady state absorptive conditions, the flux across the

unstirred water layer is equal to the flux across the cell membrane.

$$J = (C_1 - C_2)\,(D/d)\,(S_w) = PC_2$$

The question of which barrier offers most resistance to a penetrating molecule primarily depends on the properties of the molecule. A small, polar molecule is membrane limited. It can easily diffuse through the unstirred water layer as it has a high diffusion coefficient. But, it is blocked at the cell membrane because of a low permeability coefficient. Conversely, a large nonpolar molecule is limited by the unstirred water layer because of its very low diffusion coefficient. However once across, the large hydrophobic molecule easily penetrates the membrane into the cell due to a high permeability coefficient. Essentially, substances with similar properties to the particular barrier can pass through that barrier.

The unstirred water layer is believed to be the major barrier to lipid absorption.[30] Micellar solubilization of lipolytic products allows them to traverse the unstirred water layer to be absorbed by intestinal epithelial cells. Lipolytic products are absorbed in the proximal small intestine, whereas conjugated bile salts are actively absorbed in the ileum as a part of the enterohepatic circulation. This suggests that micelles are not absorbed intact. In the absorptive process, fatty acids and monoglycerides are brought in close proximity to the absorptive cell by the mixed

micelles. They dissociate from the micelles and readily pass through the brushborder membrane.[24] Uptake of lipids into the cell is probably a passive process. Simmonds[30] and Westergaard and Dietschy[31] presented evidence supporting the monomeric absorption of lipids rather than the direct transfer of lipids from the micelle to the microvilli of mucosal cell.[32] They suggested that the importance of micellar solubilization of lipids was to increase the concentration gradient for diffusion[30] overcoming the resistance of the unstirred water layer.[31] In essence, the absorption of monomeric free fatty acid from the unstirred water layer adjacent to the mucosal cell promotes further release of fatty acid from the mixed micelle.[31]

Once inside the mucosal cell, fatty acids and monoglycerides are transported by a fatty acid binding protein (FABP) to the smooth endoplasmic reticulum where triglycerides are resynthsized.[5] Migration of the triglycerides to the endoplasmic reticulum results in the addition of apoproteins to the triglycerides to form lipoproteins.[4,24] Droplets of these lipoproteins are called chylomicrons. The chylomicrons are secreted into the intercellular space by reverse pinocytosis and pass to the mesenteric lymphatics where they ultimately reach the general circulation via the thoracic duct.[4,5,24]

1.4 ABSORPTION OF MEDIUM CHIAN TRIGLYCERIDES

The above mechanism is the major route of absorption for

long chain triglycerides, and operates only in the presence of pancreatic lipase, colipase and bile salts. Medium chain triglycerides, however, have two mechanisms of absorption.

The first mechanism is through hydrolysis by pancreatic lipase as decribed for long chain triglycerides. But MCT, unlike LCT, behave somewhat differently. In man, ingested MCT do not elicit any change in pancreatic secretion while LCT produced a greatly increased output.[8] Intraluminal enzymatic hydrolysis of MCT is more rapid and more complete than LCT.[6,8] Pancreatic lipase cleaves all three ester bonds efficiently resulting in glycerol and free MCFA. MCT are mainly absorbed as MCFA.[8] In mixed triglycerides, pancreatic lipase preferentially hydrolyzes glycerides with MCFA over LCFA.[8] Medium chain fatty acids being relatively water soluble[1] are more easily absorbed by the mucosal cell,[5] and the lipolytic products do not require incorporation into micelles so bile salts are not required.[6] MCT do not undergo re-esterification or chylomicron formation for release from the intestinal mucosal cell.[4] Instead the MCFA pass directly into the portal venous circulation[5] transported as soluble fatty acids bound to serum albumin to the liver.[8] This stems from differences in metabolism in the intestinal mucosa. The primary step in triglyceride re-esterification is activation to acyl-CoA by acyl-CoA synthetase. This enzyme is specific for fatty acids with chain lengths of greater than 12 carbon atoms.[1,8] MCFA consequently are not re-esterified to MCT.[7,8] Since re-

esterification is completely random producing mixed triglycerides, MCFA are also not incorporated into mixed triglycerides. Resynthesized triglycerides are almost exclusively long chained. The intracellular fatty acid binding protein has a low affinity for MCFA, compounding the difficulty in MCFA esterification.[8] Therefore, ingested MCT reach the liver more rapidly than LCT as they are digested faster, absorbed more quickly, leave the mucosal cell unchanged and transported directly to the liver via the portal system.

The second mechanism of MCT absorption is the ability of MCT to be absorbed into intestinal cells intact as whole triglycerides.[33,34,35] This process is thought to be independent of pancreatic lipase and bile. There may even be a mucosal lipase in the epithelial cell that hydrolyzes absorbed intracellular MCT to free fatty acids[36] before delivery to the portal vein for transport to the liver.[5]

CHAPTER II

ABSORPTION OF TRIGLYCERIDES IN THE ABSENCE OF LIPASE

2.1 INTRODUCTION

Fat absorption is a complex process which involves coordinated gastric, intestinal, biliary and pancreatic function. Dietary fat is primarily triglycerides containing long chain fatty acids. Emulsification of dietary triglyceride begins in the stomach and continues in the upper small intestine aided by bile and fatty acids. Triglyceride is hydrolyzed into free fatty acids and monoglyceride by the activity of lipase and colipase from the pancreas. These products combine with bile acids and phospholipids from the liver to form mixed micelles for transport to the mucosal cells for absorption.

Medium chain triglycerides (MCT) are neutral lipids consisting of fatty acid (FA) molecules with chain lengths from 6 to 12 carbons. It has been shown that MCT is successful in the clinical treatment of lipid digestion disorders resulting from biliary atresia, obstructive jaundice, primary biliary cirrhosis,[37] blind-loop syndrome, pancreatitis,[38] cystic fibrosis[39,40,41] and pancreatectomy.

Medium chain triglycerides can be absorbed by the small intestine either as fatty acids or intact, as a complete triglyceride molecule. In the presence of pancreatic enzymes, MCT are hydrolyzed into medium chain fatty acids (MCFA) before

absorption.[4,5,6] However in their absence, MCT are thought to be absorbed as intact unhydrolyzed triglycerides[33,34,35], unlike long chain triglycerides (LCT) which require hydrolysis and micellar solubilization.[4,5,24] However, when these experiments were performed, the elimination of pancreatic lipase from the small intestinewas not varified by analysis for any residual lipase. MCT absorption is unaffected by bile[5] and occurs faster than LCT,[4] suggesting shorter chain triglycerides may be absorbed more rapidly than longer chain triglycerides. The original work indicating MCT might be absorbed intact and not require pancreatic lipase lead to its clinical use as an energy source in patients with pancreatic insufficiency. Further, commercial MCT oils, used in nutritional therapy,[33,34,35,36,37] may be comprised of various MCFA resulting in differences in size and molecular weights (MW). The effect of molecular weight and fatty acid chain length on the efficiency of intact MCT absorption is unknown and untested. Recently, several studies have attempted to determine the range in molecular weights that are absorbed by the intestine.[42,43,44,45,] These studies used different sizes of polyethylene glycol (PEG) molecules. Tagesson and Bengtsson[42] used PEGs with molecular weights 326-634, 1000 and 3000. Good absorption was observed over PEGs 326-634 with values decreasing with size from 25% to 12% over this range. PEG 1000 showed a poorer absorption at 5% and PEG 3000 showed no absorption. Therefore, our hypothesis is smaller molecular weight triglycerides can be absorbed more rapidly than larger ones.

Moreover, in the pancreatic difficient state, MCT with smaller molecular weight may be absorbed more efficiently than larger MCT or LCT molecules.

The purpose of this study was to:

1) develop an animal model that simulated pancreatic insufficiency that closely approximated the physiologic state.

2) demonstrate absorption of whole unhydrolyzed MCT under these conditions.

3) determine the relative rates of absorption of the various MCTs present in MCT oil and LCT in the absence of pancreatic lipase to test the hypothesis.

Overall, this study was an attempt to investigate the most readily absorbed size of MCT in an animal model that mimics pancreatic insufficiency and to assess the efficacy of administration of a oil rich in smaller MCT.

2.2 METHODS AND MATERIALS

2.2.1 MATERIALS

The MCT oil was purchased from Mead-Johnson of Bristol Myers, Inc., Belleville, Ont. Trinanaoin, tridecanoin and tridodecanoin were purchased from Nu Chek Prep, Inc., Elysian, MN. Triolein , sodium glycocholate, sodium taurocholate and urethane were purchased from Sigma Chemical Co., St. Louis, MO. Sodium chloride, sodium bicarbonate, potassium chloride, calcium choride, sodium pyrophosphate, semicarbazide hydrochloride, sodium fluoride,

chloroform, methanol and pesticide grade n-hexane were purchased from Fisher Scientific Co., Fair Lawn, NJ. Polyethylene glycol (PEG) 4000 was purchased from J.T. Baker Chemical Co., Phillipsburg, NJ. B-nicotinamide-adenine dinucleotide (NAD) was purchased from Boehringer Mannheim, Inc., Dorval, Que. and 3-alpha-hydroxysteroid dehydrogenase from Cooper Biomedical, Malvern, PA. ^{3}H-PEG 4000 and [carboxyl-^{14}C-] triolein were purchased from New England Nuclear Corp., Boston, MA. The kit for lipase determination of lipase was purchased from Sigma Diagnostics, St. Louis,MO, and the autotitrator consisted ofthe Radiometer Copenhagen ABU80 autoburette, PHM82 standard pH meter and TTT80 titrator (Radiometer Copenhagen, Copenhagen, Denmark). The SE54 capillary column and thermogreen LB-2 septa were purchase from Supelco, Ltd., Bellefonte, PA. The gas liquid chromaograph (GLC) was a Varian Vista 6000 gas chromatograph with an 8000 series autosampler mated to a Vista 402 chromatography data system (Varian, Inc., Walnut Creek, CA). The gas chromatograph-mass spectrometer (GC-MS) was the Hewlett-Packard model 5985B automated gas chromatograph / quadrupole mass spectrometer system (Hewlett-Packard Co., Avondale, PA). The infusion/withdraw pump was the Harvard model 944 (Harvard Apparatus Co. Inc., South Natick, MA). Glass(50 ml) and plastic syringes,and needles (18 and 23gauge) were purchased from Becton Dickinson and Co., Rutherford, NJ. The polyethylene tubing, PE280 (I.D. 2.15 mm, O.D. 3.25 mm), PE240 (I.D. 1.67 mm, O.D. 2.42 mm) and PE190 (I.D. 1.19 mm, O.D. 1.70 mm)

were obtained from Clay Adams Division of Becton Dickinson and Co., Parsippany, NJ. The Vis/UV spectrophotometer was a Beckman model DU-50 and the liquid scintillation counter was a Beckman model LS9800 (Beckman Instruments, Inc., Fullerton, CA). Liquid scintillation fluid was Ready-Solv HP/b purchased from Beckman Instruments, Inc. The sonicator was the Fisher Scientific Co. model 300 sonic dimembrator (Artek Systems Corp., Farmingdale, NY). The small animal body temperature regulator was the YSI model 73ATA (Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

All glassware were acid-washed and rinsed in chloroform to remove any residual lipids and soaps.

2.2.2 METHODS

2.2.2.1 INFUSION MIXTURE

The 7.7 mM MCT mixture consisted of 5 mM MCT oil (approximate MW 510) 1.4 mM tridecanoin, 1.3 mM tridodecanoin, 10 mM sodium taurocholate and 1.0 μCi ^{3}H-labelled polyethylene glycol (PEG) 4000 dissolved in buffered electrolyte solution sonicated to an uniform emulsion. The 2 mM LCT mixture was of 2 mM triolein, 10 mM sodium taurocholate, 1.0 μCi ^{3}H-PEG 4000 and 1.5 μCi [carboxyl-^{14}C-] triolein dissolved in buffered electrolyte solution sonicated to an uniform emulsion. This buffered electrolyte solution was a modified Krebs-Henseleit solution of 110 mM NaCl, 45 mM $NaHCO_3$, 6 mM KCl, 0.2 mM $CaCl_2$ and 6 mM PEG 4000. ^{3}H-PEG 4000 was used as a

nonabsorbable marker to monitor fluid movement across the intestinal mucosa.[46,47,48,49,50,51,52]

2.2.2.2 IN VIVO PERFUSION TECHNIQUE

The intestinal perfusion technique with preliminary clearing of intralumenal debris was based on the method of Sols and Ponz,[53,54] as described by Parsons [55] and modified from Perdue, Chung and Gall.[56]

The experimental model is illustrated in Figure 2. Male Sprague-Dawley rats weighing 250-350 g were fasted overnight (allowed only water) and anesthetised with urethane at 1.5 g/kg body weight i.m. Body temperature was maintained at 37°C with a heating pad controlled by a temperature regulator with rectal probe. Tracheotomy, employing a 3 cm length of PE240 tubing, was performed through a sagittal incision in the neck at a level caudal to the thyroid gland. A ventral midline incision was made in the abdomen and a 10cm length of jejunum was isolated with polyethylene catheters 5 cm caudad to the ligament of Trietz. The proximal infusion line consisted of a 2 cm legnth of PE280 joined to 60 cm of PE190 by a PE240 connector. The distal outflow line consisted of 20 cm PE280. The abdominal cavity was then closed. The upper half of the incision was closed with surgical silk while the lower half was clamped closed with a hemostat to allow post-surgical access. Perfusate was administered at constant rate by 50 ml glass syringe on a Harvard Apparatus infusion/withdraw pump.

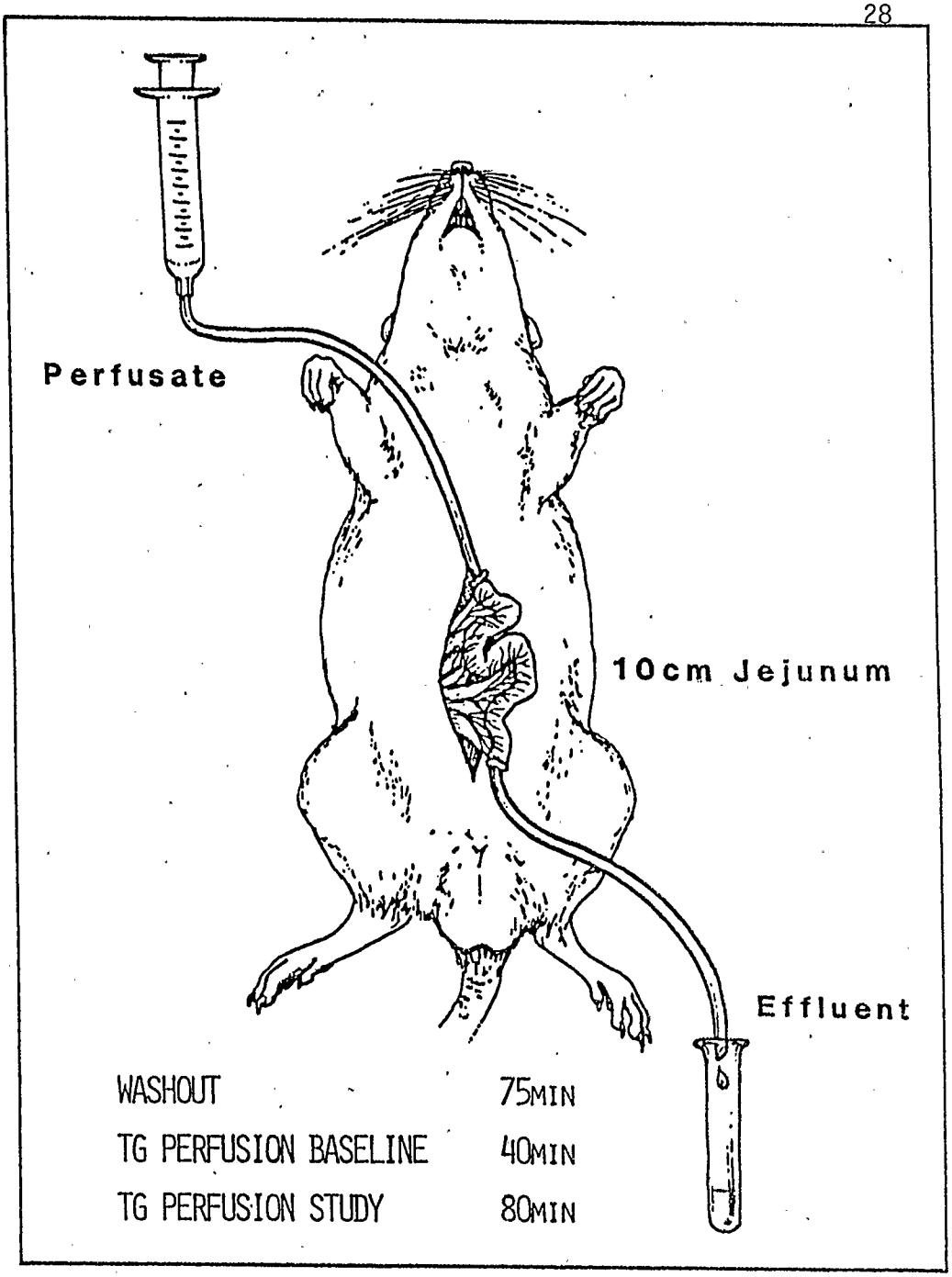

Figure 2. An illustration of the experimental model. Male Sprague-Dawley rats with 10cm isolated jejunum.

The segment was first perfused with 154 mM NaCl at 75 µl/min/100 g body weight for 75 min to remove any residual pancreatic lipase and bile salts. Ten minute samples were collected initially and at the conclusion of washout to confirm clearance. The lipid emulsion was then perfused at 0.13 ml/min for 120 min: 40 min for baseline equilibration, then an 80 min experimental period. Twenty minute samples of effluent were collected for later analysis of lipid composition. At the termination of the perfusion, the intestinal segment was emptied and flushed with cold isotonic NaF to remove any lipids adhering to the intestinal surface.(34) The effluent line was flushed with water,cleared,then flushed again with 6 ml chloroform to remove lipids adhering to its lumenal surfaces. The chloroform was evaporated by ultrapure nitrogen and the sample assayed for lipid content. Another sample of the lipid perfusate was collected at the conclusion of the experiment using identical technique as previously noted. This allowed for any change in the stable lipid emulsion during perfusion.

Two lipid emulsions were employed in separate perfusion studies, a 7.7 mM MCT emulsion on 13 rats and a 2 mM LCT (triolein) emulsion on 5 rats.

2.2.2.3 ANALYSIS

1) Bile acids were quantified by method of Talalay(57) as modified by Admirand and Small.(58) This is a colorimetric assay employing a spectrophotometer to monitor the conversion of NADH to

NAD, which absorbs (optical density) at 340 nm wavelength visible light versus reference sample without the correcting enzyme. NADH provides the reducing power for 3-alpha-hydroxysteroid dehydrogenase enzymatic breakdown of bile acid. Concentrations were derived from the standard curve for glycocholic acid.

2) Pancreatic lipase were determined by Sigma Lipase Kit 800-B modified by the use of an autotitrator (Radiometer Copenhagen) with endpoint at pH 9.9 (for thymothalein). This assay is based on the ability of lipase to hydrolyze triglcerides present in the lipid substrate made from olive oil into free fatty acids. The titration of these fatty acids with dilute NaOH (0.05 M) results in a measure of lipase activity.

3) MCT were extracted by method of Folch et al[59] and stored in hexane. MCT was analyzed by capillary gas liquid chromatography (GLC) with flame ionization detector (FID)[60] (Varian Vista 6000) and confirmed by gas liquid chromatography-mass spectrometry (GC-MS). Trinonanoin (27 carbon, 3 of C:9, MW 512.8) was used as internal standard. The GLC conditions were 1 µl sample, start temperature 150°C, hold 2min at 150°C, rate of temperature increase 5°C/min, end temperature 300°C, SE54 fused silica capillary column (12.5 m length, I.D. 0.25 mm, film thickness 0.25 µm), carrier gas N_2 at 1-2 ml/min flow rate, gas pressure head 10 psi, FID detector gasses medical air and H_2, FID range 10^{-11} and attenuation 4. The confirmation of MCT was by an automated gas gromatograph/quadrupole mass spectrometer system (Hewlett-Packard

5985B) using a 12 m SE54 fused silica capillary column.

4) ^{3}H-PEG 4000 and [carboxyl-^{14}C-] triolein was determined by liquid scintillation, corrected for quench. A 0.1 ml aliquot of sample was used for counting disintegration of beta particles per minute (DPM).

5) Paired t-tests for testing for difference between two means were performed on the data concerning pancreatic lipase and bile salt washout. One-way analysis of variance with multiple comparisons by the Bonferroni method[61,62] was performed on the lipid absorption data. A two-tailed t-test was uased to compare the LCT (triolein) data.

2.2.2.4 CALCULATIONS

1) Bile Acid Concentration

Variables:
- a) Enzyme methanol blank absorbance (O.D.)
- b) Non-enzyme methanol blank O.D.
- c) Absorbances of 12 glycocholic acid standards (STD) with enzyme (test)
- d) Absorbances of 12 glychocholic acid standards without enzyme (control)
- e) Concentrations (conc.) of each glycocholic acid standard (mg%, ie. mg/dl)
- f) Absorbances of samples with enzyme
- g) Absorbances of samples without enzyme
- h) Dilution factor (1)

i) Conversion factor (10 dl/L)

j) Molecular weight glycocholic acid standard (487.6)

(i) Correction for the background of pyrophosphate buffer.

Blank O.D. = Enz. Blank O.D. - Non. Blank O.D.

(ii) Standard (STD) O.D.

O.D.for each STD = Enz.STD O.D. - Non. STD O.D - Blank O.D.

(iii) Generation of standard curve.

Slope Point for each STD (mg%) = Conc. STD / O.D. STD

(iv) Slope of standard curve.

Standard Curve Slope = Mean of STD Slope Points

(v) Calculation of bile salt concentration from standard curve.

Sample O.D. = Enz. Sample O.D. - Non. Sample O.D.

Sample Bile Acid Conc. (mg%) = (Sample O.D.) (Slope) (Dilution Factor)

Sample Bile Acid Conc. (mM) = (Sample mg%) (10) / MW STD

2) Lipase Activity

Calculations as per the Sigma lipase kit modified by the variable (e). Titration of fatty acids allows quantifying of lipase activity.

Variables: a) Actual concentration of NaOH titrant (M)

b) Exact concentration of NaOH titrant required for assay (0.05 M)

c) Volume NaOH to titrate blank (control) (ml)

d) Volume NaOH to titrate test (ml)

e) Volume correction factor for 5 ml aliquot used for titration from 17.5 ml sample (3.5)

f) Conversion factor (280) for U/L

Lipase (U/L) = (3.5) (Vol. NaOH Test - Vol. NaOH Blank) (Conc. NaOH/0.05M) (280)

3) Corrected Volume Effluent

The corrected volume is the actual volume of effluent when the flux of water is taken into account.

Variables: a) DPM/0.1ml ^{3}H-PEG of Perfusate at beginning and conclusion of experiment

b) DPM/0.1ml ^{3}H-PEG of Effluent

c) Perfusion rate (0.13 ml/min)

d) Collection time (20 min)

(i) Average of perfusate counts at the beginning and end of experiment.

Perf. ^{3}H-PEG Counts (DPM/0.1ml) = Average Counts Perf. at Beginning and at End

(ii) Corrected volume of effluent sample.

Corrected Vol. Effl. (ml) = [(DPM/0.1ml Perf.) / (DPM/0.1ml Effl.] [Rate] [Time]

4) Absorption of MCT

The equations calculate the mass of MCT in the perfusate and in the effluent from GLC analysis. The uptake is the difference between these two values.

For each type of MCT:

Variables: a) GLC area counts of perfusate at beginning and end of experiment

b) GLC area counts of effluent samples (no MCT adhered to tubing or intestine)

c) Mass trinonanoin standard (100 μg)

d) Molecular weight of MCTs (Table 1)

e) Volume sample used in assay (0.25 ml)

f) Perfusion rate (0.13 ml/min)

g) Collection Time (20 min)

h) Corrected volume effluent (ml)

(i) Amount of MCT perfused into gut.

Conc. MCT (mM) = ([100µg(Area MCT / Area STD)] / MW STD) / 0.25ml

Conc. MCT Perfusate (mM) = Mean of Perfusate Conc. at Beginning and End

Mass MCT Perfused (µmol) = (Conc. MCT Perfusate) (Rate) (Time)

(ii) Amount of MCT coming out of gut recovered in effluent. No MCT were adhered to the tubing or wall of intestine.

Mass MCT Effl. Sample (µmol) = (Conc. MCT Effl.) (Corrected Vol. Effl.)

Total Mass MCT Recovered (µmol) = Sum of Amounts Effluent MCT

(iii) Percent MCT absorbed.

% MCT Absorbed = [100] [(MCT Perfused - Total MCT Recovered) /MCT Perfused]

5) LCT Absorption

The amount of LCT absorbed was calculated from radioactivity counts of the label in the perfusate, effluent, and adhered to the

intestinal wall and to the tubing.

Variables: a) DPM/0.1ml [carboxyl-^{14}C-] triolein in perfusate samples at beginning and conclusion of experiment

b) DPM/0.1ml [carboxyl-^{14}C-] triolein in 20min effluent samples

c) DPM/0.1ml [carboxyl-^{14}C-] triolein adhering to intestinal lumen in NaF flush

d) DPM [carboxyl-^{14}C-] triolein adhering to the lumen of tubing

e) Volume sample used for liquid scintillation (0.1 ml)

f) Perfusion rate (0.13 ml/min)

g) Total collection time (80 min)

h) Corrected volume effluent (ml)

i) Volume NaF (4 ml)

j) Volume correction factor (10)

(i) Amount LCT into gut.

Counts Perfusate (DPM/0.1ml) = Mean of Perf. Counts at Beginning and End

DPM Perfused = (10) (Perf. DPM/0.1ml) (Rate) (Total Time)

(ii) Amount LCT coming out recovered.

DPM in Effluent Sample = (10) (Effl. DPM/0.1ml) (Corr. Vol. Effl.)

Total DPM Effluent = Sum of DPM in Effluent Samples

DPM Intestine = (DPM/0.1ml) (Volume NaF)

Total DPM Recovered = Total DPM Effluent + DPM Intestine + DPM Tubing

(iii) Percent LCT absorbed.

% LCT Absorbed = [100] [(DPM Perf. - Total DPM Recovered) / DPM Perf.]

2.3 RESULTS

2.3.1 VALIDATION OF INTESTINAL WASHOUT

The initial perfusion of saline through the 10 cm segment of isolated jejunum significantly reduced lipase and bile acid content in the lumen. Pancreatic lipase activity in the 13 rats that underwent subsequent MCT perfusion was reduced from 72.3±17.6 U/L in the initial phase of intestinal washout to 0.9±0.5 U/L ($p<0.001$) by 75 min. 10 of the 13 rats exhibited no lipase activity in the effluent while the other 3 had 3.8±0.9 U/L. Bile

acid concentrations were decreased from 2.1±0.4 mM to 0.3±0.1 mM ($p<0.001$) (Figure 3). In the 5 animals that underwent subsequent triolein perfusion, pancreatic lipase was also eliminated by 75 min of washout from 31.5±8.4 U/L initially to 0 U/L ($p<0.01$). Bile acids were significantly reduced from 1.2±0.3 mM to 0.1±0.01 mM ($p<0.02$) (Figure 4).

2.3.2 MCT ABSORPTION

After 40 min of equilibration, jejunal absorption reached steady state as indicated by a constant rate of MCT absorption (Figure 5). MCT were analyzed by capillary GLC and confirmed by GC-MS. The MCT emulsion contained 5 different MCT (Table 1): (a) trioctanoin, (b) decadioctanoin, (c) octadidecanoin, (d) tridecanoin and (e) tridodecanoin. Figure 6 is a typical chromatogram of the intestinal outflow. The absence of lipase activity and the presence of MCT rather than MCFA and monoglyceride in the effluent suggest intact MCT absorption. Absorption was measured as the per cent disappearance of perfused lipid from the intestinal lumen for the 10 cm length of gut. All 5 MCT were well absorbed and there was no difference in the percent absorption between them (Figure 7) as indicated by analysis of variance. Figure 8 is a graph of the rate of MCT absoption versus the concentration of each MCT in the infusate. There was a proportionate relationship between absorption rate and its concentration in the perfusate. The loss of infused MCT by

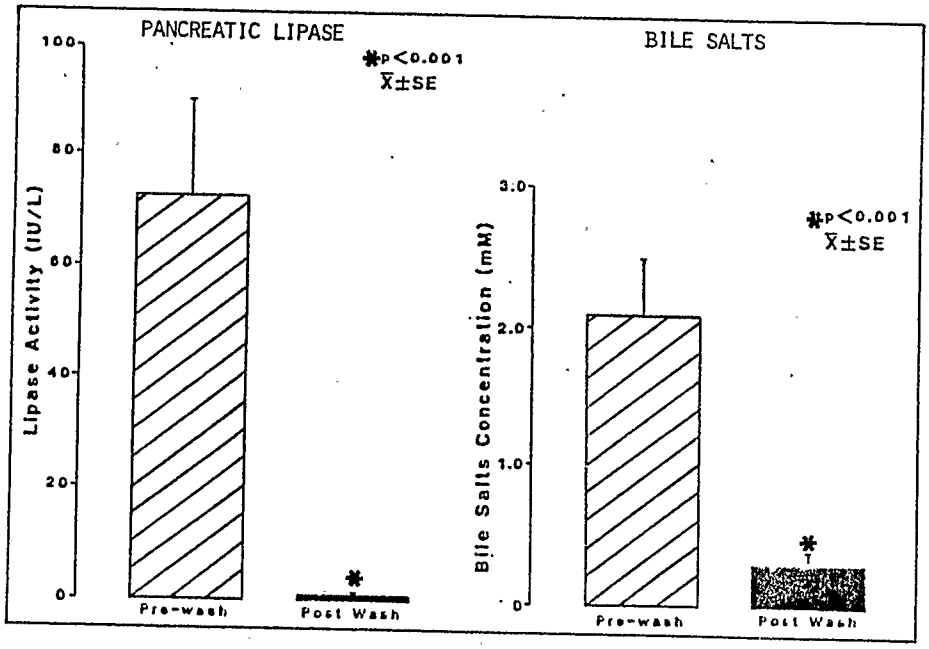

Figure 3. The efficiency of the washout period on the clearance of intraluminal pancreatic lipase and bile salts in the MCT perfused group.

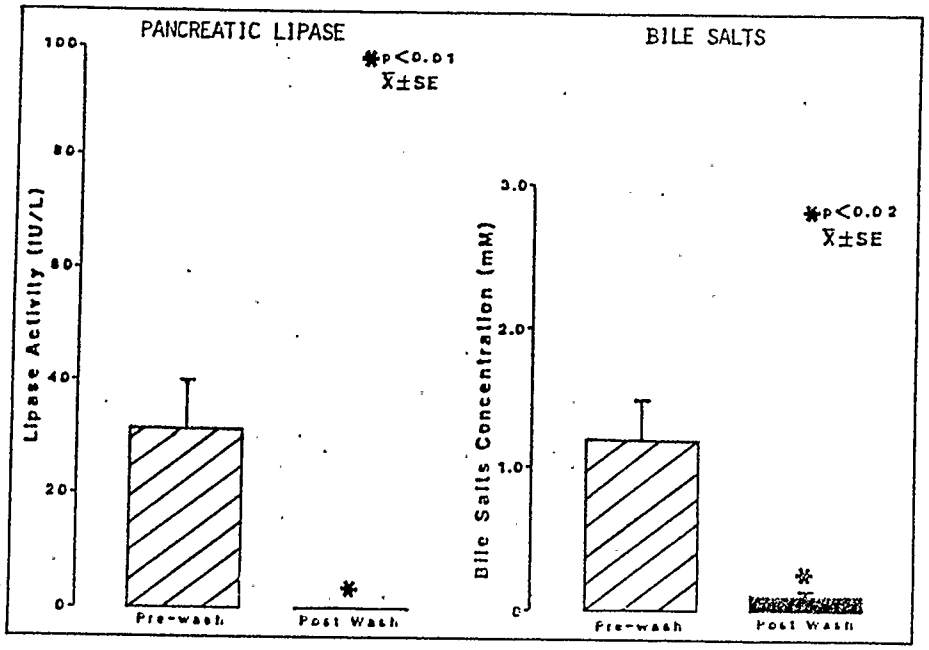

Figure 4. The efficiency of the washout period on the clearance of intraluminal pancreatic lipase and bile salts in the LCT perfused group.

Table 1. The types of triglycerides used for perfusion.

EMULSION	TRIGLYCERIDE	No. FA CARBONS	FA COMPOSITION (C)			MW	% in PERFUSATE*
	TRIOCTANOIN	24	8:0	8:0	8:0	470.7	25.5 ± 0.4
	DECADIOCTANOIN	26	8:0	8:0	10:0	498.8	29.0 ± 0.3
MCT	OCTADIDECANOIN	28	8:0	10:0	10:0	526.8	11.0 ± 0.1
	TRIDECANOIN	30	10:0	10:0	10:0	554.9	18.7 ± 0.4
	TRIDODECANOIN	36	12:0	12:0	12:0	639.0	15.8 ± 0.4
LCT	TRIOLEIN	54	18:1	18:1	18:1	885.4	100

* Values are $\overline{X} \pm SE$.

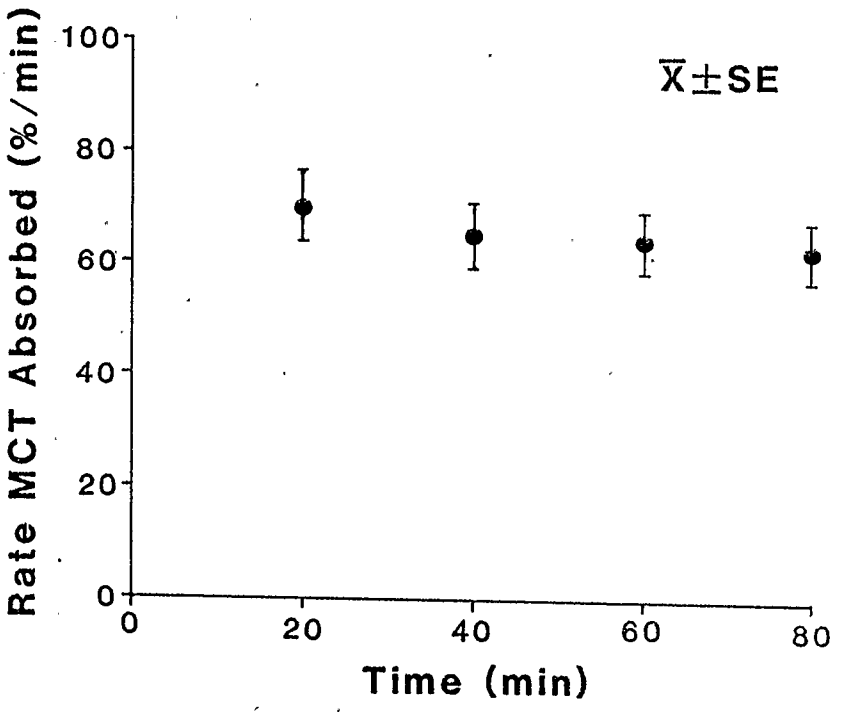

Figure 5. Steady-state absorption of MCT during 80min MCT emulsion perfusion after equilibration.

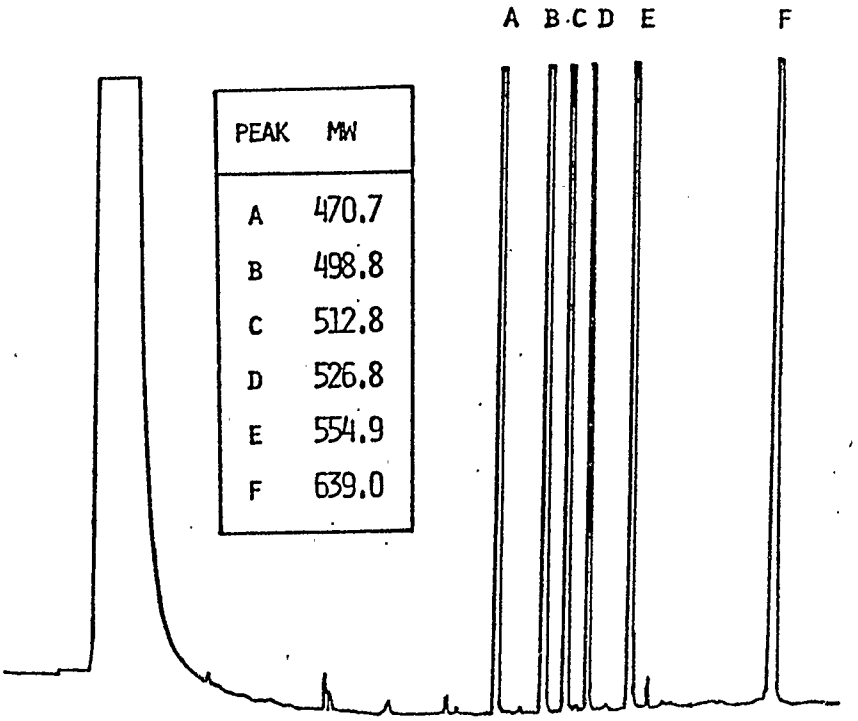

Figure 6. Capillary GLC chromatogram of MCT in the effluent. No MCFA or monoglycerides were detected. The six peaks are intact MCT: (A) trioctanoin, (B) decadioctanoin, (C) trinonanoin (internal standard), (D) octadidecanoin, (E) tridecanoin, (F) tridodecanoin.

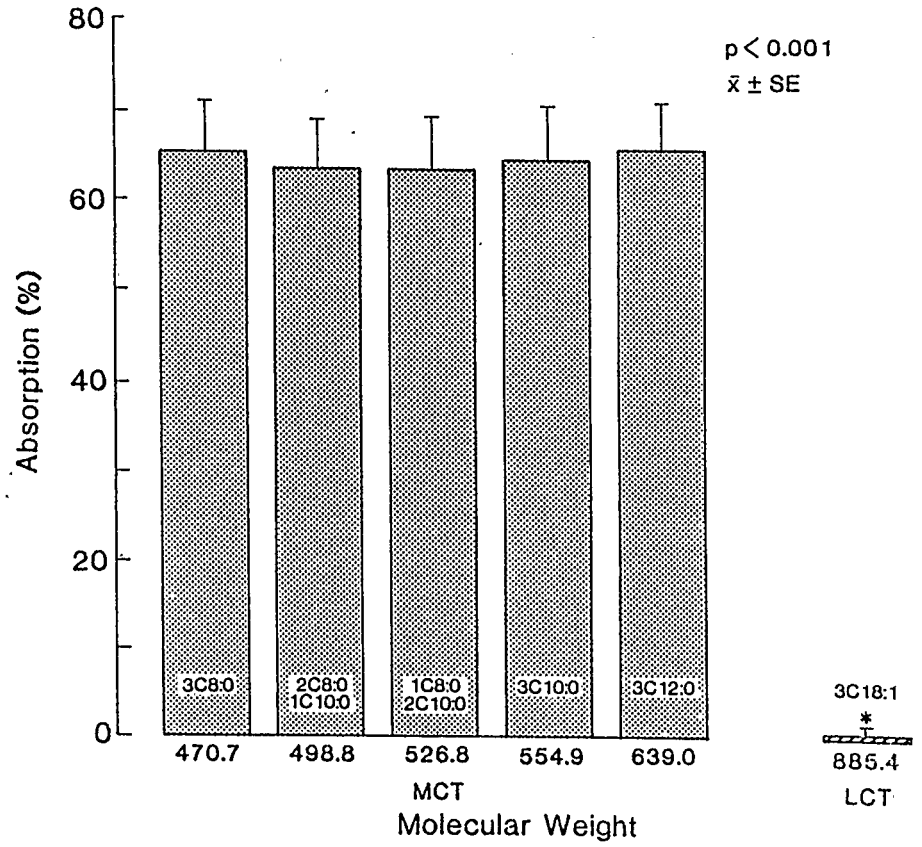

Figure 7. The percentage of infused triglyceride absorbed.

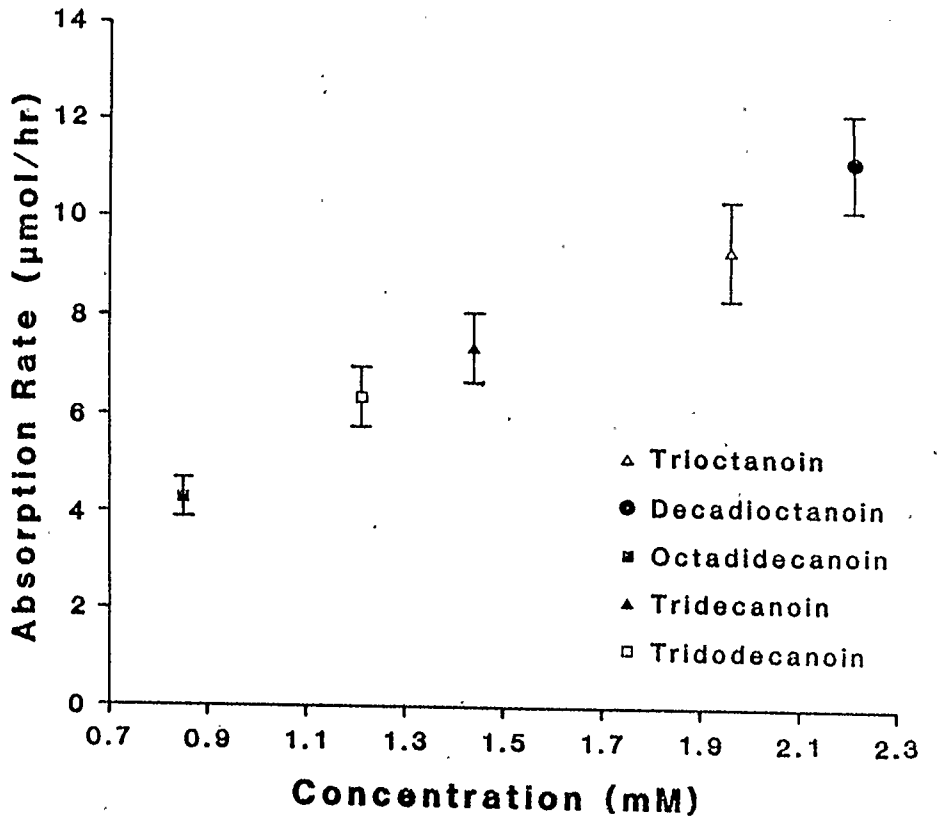

Figure 8. The dependence of the absorption rate of individual MCT on its absolute concentration in the perfusate. Ordinate: absorption rate in 10cm jejunum. Abscissa: absolute concentration of each of the 5 MCT administered.

adherence to the polyethelene tubing was insignificant: only 0.2±0.04% of total MCT infused recovered in the tubing at the end of the infusion period.

2.3.3 LCT ABSORPTION

The LCT perfusate was a preparation of a single triglyceride, triolein. The method of analyzing triolein concentrations was by radiolabelling techniques measuring disappearance of ^{14}C-triolein from the effluent. Triolein was not absorbed from the intestinal lumen as indicated by 0.2±1.6% absorption Figure 7. Statistical analysis by t-test indicated that there was no significant difference between the mean triolein absorbed and the null hypothesis that 0% absorption occurred. There was a significant difference in absorption between the 5 MCTs and the triolein ($p<0.001$) as determined by the one-way analysis of variance with multiple comparisons by the Bonferroni method.[61,62] The mass of MCT adhering to the polyethelene tubing was insignificant as only 0.7 ± 0.2% of total triolein infused waslost to tubing.

2.4 DISCUSSION

Triglycerides are neutral lipid molecules. They consist of three fatty acids esterified to a glycerol. Medium chain triglycerides (MCT) have fatty acid chain lengths between six and twelve carbon atoms. MCT are more water soluble and are smaller

than their long chain triglyceride (LCT) counterparts (Figure 1). Pancreatic lipase breaks down triglycerides by hydrolysis into one mole of glycerol and three moles of fatty acids. MCT have been used in a variety of nutitional regimens. Early studies on lipid absorption have demonstrated that MCT may be absorbed as whole unhydrolyzed triglycerides in the absence of pancreatic lipase[32,33,34] Although these studies did show intact MCT absorption, they did not demonstrate directly the elimination of intralumenal lipase. Modern MCT oils contain MCT of various sizes. Generally, a triglyceride with shorter fatty acid chains have lower molecular weights. This led to MCT being used as a supplement in the treatment of pancreatic insufficiency.

The intent of this study was to investigate lipid absorption in a pancreatic insufficient model. Then, to use it to confirm intact absorption of unhydrolyzed MCT and that LCT was not absorbed under these conditions. And finally to use it to test the hypothesis that smaller MCT may be absorbed faster in the absence of lipase by determining any differences the absorption of the various MCT in MCT oil.

Our study was performed with an **in vivo** single-pass PEG marker perfusion of an isolated 10 cm segment of jejunum previously excluded of intraluminal bile and pancreatic lipase. Analysis of pancreactic lipase and bile salt content was performed to ensure no hydrolysis of MCT occurred within the lumen. Lipid concentration was analyzed by capillary gas liquid chromatography (GLC) on MCT

and by radioisotope techniques on ^{14}C-labelled LCT. Preliminary enteroclysis of the jejunal segment was successful in removing intraluminal pancreatic lipase. The 75 min washout period used in our study reduced lipase activty to virtually zero (Figure 3 and 4). Combined gas chromatography-mass spectrometry of the medium chain triglceride (MCT) preparation revealed five types of MCT present in the MCT oil: 24, 26, 28, 30 and 36 fatty acid carbons (1). Steady-state conditions were achieved during perfusion of MCT and LCT emulsions (Figure 5). Jejunal lipid absorption showed no difference in the amount of MCT absorbed in the absence of pancreatic lipase regardless of molecular size (Figure 7). All were well absorbed. In contrast, LCT was not absorbed (Figure 7). The rate of individual MCT absorption was proportionate to its concentration in the initial emulsion (Figure 8).

The method of analyzing MCT by gas liquid chromatography (GLC)[60] is a simple and efficient technique of quantifying MCT. The merits of this approach include the ability to separate and identify many different triglycerides combined in a mixture without resorting to using radio-labelled MCT. With limited labels, a mixture of MCT could not be studied properly. Previous methods used to quantify triglycerides have employed radioisotope labelling techniques.[33,34,35] A known amount of radio-labelled triglyceride was added to cold triglyceride to form the test emulsion. After the absorption study, the lipids in the samples to be analyzed were extracted by the method of Folch, Lees and Sloane-

Stanley[59] then separated by thin-layer chromatography. The triglycerides were scraped and analyzed by liquid scintillation corrected for quench for to determine the amount of radioisotope present. From this, the concentration of the lipid was calculated. The most common radiolabels available are carbon 14 (^{14}C) and tritium (^{3}H). Thus the number of different triglycerides that can be used at one time is limited by the availability of radiolabels. Analysis of triglycerides by capillary GLC employed in this study appears to be a more versitile and efficient method than the radioisotpe technique. Several triglycerides can be used simultaneously in one mixture. This is only limited only by the boiling point of the substance.

Lipids are notorious for adhering to the walls of plastic tubing and containers. Therefore, the lipid content of infusion mixtures may be significantly altered by absorption into the plastic tubing. Large amounts of lipid (oleic acid and glyceryl-1-monooleylether) in a bile salt solution were found to be taken up by silicone rubber and polyvinyl chloride tubing. But very little lipid is lost to the walls of polyethylene (PE) tubing.[63] This study used only polyethylene tubing to minimize the amount of triglceride lost. Our results showed insignificant amounts of MCT and LCT adhering to the tubing at only 0.2±0.04% and 0.7±0.2% of total infused respectively.

Greenberger, Rodgers and Isselbacher[33] studied lipid absorption **in vivo** in isolated intestinal loops previously

irrigated to reduce pancreatic enzymes and bile salts. They neglected, however, to be secure that no residual pancreatic lipase remained. The loop was a segment of lower duodenum-jejunum bound at both ends by ligatures creating an intestinal sac into which lipid solutions were injected and subsequently withdrawn for analysis. They found that the absorption of the MCT trioctanoin, at 37%, was significantly less impaired than the absorption of the LCT tripalmitin, at 23%. Their method of thin-layer chromatography (TLC) and liquid scintillation was adequate for analyzing lipids since they used triglycerides labelled at the carboxyl end of the fatty acid. This would allow for the radiolabel to be detected in both the triglyceride, monoglyceride (LCT hydrolysis only) and fatty acid TLC bands. This method is not as good as the state-of-the-art gas liquid chromatography. They suggested that unhydrolyzed triglyceride was absorbed.

Clark and Holt[34] employed continuous intraduodenal infusion of trioctanoin in conscious unrestrained rats via indwelling duodenal cannulae with either pancreatic flow diverted from entering the intestinal lumen, bile flow diverted, or both. They observed that in the rats with only the bile flow diverted, lipolysis was slightly decreased but the overall fat uptake was unimpaired. However, in the pancreatic and bile diverted rats, absorption was one third that of control rats with pancreatic and bile flow intact. The lipid recovered in the intestinal mucosa was found to be mainly triglyceride. Lipid analysis was similar to that of

Greenberger et al.[33] It was adequate but not as precise as GLC. Restoring bile flow to the intestine in these rats had no effect on absorption. Thus it was concluded that pancreatic lipase is more important to trioctanoin absorption than bile salts and that trioctanoin was absorbed as unhydrolyzed triglyceride in the absence of pancreatic lipase, confirming Greenberger's[33] findings.

Valdevieso[35] first raised the possibility that pancreatic lipase may not have been completely removed in the prior studies performed by Greenberger et al[33] and Clark and Holt[34]. He used rats with induced pancreatic atrophy. Radio-labelled trioctanoin was injected into an isolated loop of jejunum similar to the method of Greenberger et al.[33] He concluded that the MCT was absorbed intact since the rats had pancreatic atrophy, and the loop contents contained predominately MCT.

The results of these early studies indicated absorption of unhydrolyzed MCT in the absence of pancreatic lipase. However, they did not directly assay intralumenal pancreatic lipase to demonstrate its elimination from the gut. If hydrolysis did occur, the observed absorption of MCT in the gut would be of both MCT and of the lypolytic products, glycerol and medium chain fatty acids, not solely of the MCT. That is, they would not have detected any hydrolyzed MCT as its lipolytic products would have disappeared ie. absorbed. It may indicate that whole triglycerides may not have been absorbed as effectively as reported.

This study attempted to develop an animal model that mimics pancreatic insufficiency. Initial washout with 154 mM NaCl was performed to remove intralumenal contents. The elimination of pancreatic lipase was to prevent lypolysis of triglyceride thus create the pancreatic insufficient state. This ensured that the triglycerides would remain intact and absorbed as such by the intestine. Bile was excluded so it would not affect MCT absorption. Although it was demonstrated that bile salts alone do not influence MCT absorption, other components of bile may.[6,34] This study showed the complete removal of pancreatic lipase and greatly reduced bile salt at the conclusion of washout (Figures 3 and 4). Therefore, the model similates pancreatic insufficiency in the 10 cm segment of isolated jejunum.

The results suggest that MCT was absorbed intact in the absence of pancreatic lipase. Evidence was from the determination that no lipase was present in the lumen during MCT perfusion (Figure 3). GLC analysis of the effluent from the perfused segment of jejunum revealed only the presence of the five MCT types administered that were not absorbed (Figure 6). No lipolytic products, ie. monoglycerides or free medium chain fatty acids, were found. This indicated that no intraluminal lipolysis had occurred and that the MCT absorbed was in the form of neutral lipids. Absorption was th erefore independent of pancreatic enzymes. Figure 7 shows the absorption of MCT with molecular weight from 470.7 to 639.0 and fatty acid carbons of 24 to 36 in the rat jejunum was excellent in

the absence of lipase. This confirmed the findings of earlier studies.[33,34,35] The LCT triolein was not absorbed by the intestine (Figure 7) confirming the theory that LCT are only absorbed after hydrolysis as long chain fatty acids and long chain monoglyceride. However, Greenberger et al[33] showed LCT absorption in the absence of lipase although at a much reduced level compared to MCT and to controls with lipase. This may have been due to residual lipase adhering to the epithelial brushborder that were not removed by prior irrigation.

Previous studies have shown MCT are absorbed more efficiently than LCT,[33,34] indicating that shorter chained triglycerides are absorbed more rapidly than longer chained triglycerides. However the relative rates of absorption among MCT are not known. The results from our MCT absorptive studies did not support the notion that the smaller molecular weight MCT are absorbed faster than longer chain MCT. The relative percentages of absorption for each chain length as seen in Table 1 show a fairly equal absorption for all lengths. Clinically, this study suggests no absorptive benefit from administrating a dietary supplement of MCT oil preferrentially high in shorter chain MCT than longer chain MCT.

The emulsions infused through the jejunum were triglyceride emulsions with taurocholic acid, a bile salt. Therefore, in our system triglycerides were present in three phases, in emulsion particles, in the monomeric phase, and incorporated in bile salt micelles. It was observed that MCT were absorbed while LCT was not

(Figure 7). The likely explanation is that absorption in this experimental design was limited by diffusion. The perfusion rate was slow, approximating physiologic conditions. The unstirred water layer would remain intact acting as the major barrier to absorption. Since lipid permeation through the cell membrane is a rapid process, uptake is dependent on simple diffusion across the unstirred water layer. Uptake of MCT in this study occurred under steady-state conditions (Figure 5). Thus during stedy-state absorption, the following equation applies:

$$J = (C_1 - C_2)\ (DS_w/d) = P\ C_2$$

Since the permeability coefficient for lipids is high, uptake is diffusion-limited. And since in a diffusion limited model, C_2 approaches zero. Therefore, the equation becomes:

$$J = C_1 DS_w/d$$

Thus, MCT were absorbed by two mechanisms. First, MCT being more water soluble, would more readily go from the emulsion phase into the monomeric phase in the bulk water phase of the lumen comprising the concentration at C_1. These monomers of MCT may then diffuse along the concentration gradient across the unstirred water layer, as C_2 would be low, then to pass through the epithelial cell membrane. There is the possibility of a putative brushborder lipase allowing local hydrolysis and absorption. But it would be expected that some products of this lipolysis would have been

detected in the effluent. The second mechanism of MCT could have been mediated by micellar solubilization. MCT incorporated into micelles diffuse across the unstirred water layer. Since the uptake of nutrients occur monomerically,[30,31] MCT then leave the micelle and enter the unstirred water layer as monomers , C_2, for uptake into the cell. It is difficult to speculate the degree to which each process operates. However, Clark and Holt[34] found bile did not effect trioctanoin absorption in the absence of lipase. Westergaard and Dietschy[26,31] suggested that there was no significant effect of bile salt micelles on the mucosal uptake of medium chain fatty acids.

The absorption of LCT can be analysized similarly. Since they are water insoluble molecules, very little or no LCT would be present as monomers in the bulk water phase of the intestinal lumen. Thus, there would be little or no diffusion of LCT monomers across the unstirred water layer. LCT incorporated into micelles may penetrate the unstirred water layer but may be limited here by its partition coeffient. The partition coefficient (K)[26] is the ratio of lipid in the micellar phase to that in the monomeric phase.

$$K = (M_{monomer})(V_{aqueous}) \; / \; (M_{micelle})(V_{micelle})$$

where, M = mass, V = volume

Perhaps the explanation for the lack of absorption of LCT observed through micellar solubilization is due to a very low partition

coefficient. So in the unstirred water layer, LCT may tend to remain solubilized in the micellar phase instead of entering the monomeric aqueous phase. Thus the concentration at C_2 would be very low and no absorption of LCT would be expected since the event requires a monomeric phase.

There was no significant difference in the percentage of each MCT absorbed observed (Figure 7). This may be because the five perfused MCT were quite close in molecular weight and size that they behaved similarly. Another factor variable concentrations of each MCT in the emulsion (Table 1) might have influenced the results. Figure 8 shows the dependence of the observed rate of absorption on concentration. The rate of absorption increased proportionately to the increase in concentration. This relationship indicates that although the absolute mass of each individual MCT absorbed was different, the rate of absorption corrected for concentration was equal. This is illustrated by Figure 7, as the amount of lipid absorbed during perfusion expressed as a percentage of that infused. So the standardized rate of uptake are equal among the five MCT infused simply because the flux increased proportionately with increasing concentration. This adhered to the principle that a larger diffusion gradient produces a greater movement of diffusing material.

Under normal conditions, biliary secretions and pancreatic enzymes have a major role in lipid digestion and absorption and the uptake of intact triglycerides is relatively insignificant.

However, it is reasonable to speculate in patients with impaired intraluminal digestion of fat (eg. pancreatic insufficiency) that absorption of intact triglyceride dominates. The present study indicates that significant amounts of MCT can be absorbed intact in the absence of pancreatic enzymes, but not LCT. There was no difference in the efficiency of absorption of MCT of varying sizes. The hypothesis of more efficient absorption of lower molecular weight and shorter chain MCT was disproved. Thus an MCT oil with a greater composition of smaller molecular weight MCT does not offer any absorptive advantage over conventional MCT oil.

2.5 CONCLUSION

This study of triglyceride absorption demonstrates that in the perfused rat jejunum, 1) Long chain triglyceride (LCT) is not absorbed in the absence of pancreatic lipase. 2) The medium chain triglyceride (MCT) molecule is absorbed intact as neutral lipid. 3) Absorption occurred in the absence of pancreatic enzymes. 4) Absorption of MCT with molecular weight from 470.7 to 639.0 and fatty acid carbon chain length of 24 to 36 was excellent in the absence of pancreatic lipase. 5) The molecular weight of MCT does not affect its intact absorption by the small intestine.

Although the transport of intact triglycerides under normal circumstances is of limited value as the presence of pancreatic lipase ensures complete lypolysis and lipid absorption, its significance increases in the abnormal condition of pancreatic

insufficiency. Therefore, the clinical significance of this study is, for pancreatic insufficient patients, the administration of medium chain triglyceride oil selectively high in shorter medium chain fatty acids, offers no absorptive advantage over the administration of conventional MCT oil preparations.

CHAPTER III

FURTHER QUESTIONS RAISED BY THIS STUDY

3.1 FURTHER INVESTIGATION INTO INTACT TRIGLYCERIDE ABSORPTION

The results of this study raises some questions of interest in future investigations. This study demonstrated that MCT can be absorbed in the absence of lipase in sufficiently large amounts and that this occurred as intact molecules. Therefore, at what point in terms of chain length anb aqueous solubility are triglycerides not absorbed intact? Would there be an abrupt demarcation or a gradation in this ability? These questions could be answered using a mixture of triglycerides of varying sizes ranging from medium to long chain triglycerides perfused in the same manner as this study. Analysis could be by capillary GLC using an on-column injection gas chromatograph. Thus separation of triglycerides would not be limited by the temperature of the injection port as the sample would be injected directly into the column. The only limit to the triglycerides that can be administered therefore are those whose boiling points exceed the maximum temperature of the column or gas chromatograph.

3.2 TRANSPORT EVENTS OF MCT

Transport of fatty acids have been extensively studied by many researchers. It may be interesting to determine the transport rates of MCT across the mucosal epithelial membranes and unstirred

water layer and the events that occur during transport. Also, to investigate the degree of resistance posed by each barrier to the passage of triglyceride from the lumen to the mucosa.

3.3 ABSORPTION OF A STRUCTURED TRIGLYCERIDE

A study of the absorption of a structured lipid should also prove interesting, similar to the structured lipids of the Captex 810 Series (Capital City Products Co., Columbus, OH). The structured lipid of interest would be a triglyceride with two medium chain and one long chain fatty acids. The objective would be to investigate whether this lipid would adopt MCT characteristic and be absorbed intact in the jejunum so that a preparation of this lipid may prove helpful to patients suffering from pancreatic insufficiency. However, the problem at this point is that no such pure mixed triglyceride exists. The lipids of the Captex Series contain a multitude of mixed triglycerides of long and medium chain fatty acids making analysis and assessment difficult. This study would have to wait until lipid chemistry progresses to the point where triglycerides with specific fatty acids at each of the three ester bonds can be feasibly synthesized.

3.4 MUCOSAL LIPASE AND TRANSPORT OF ABSORBED MCT IN THE PORTAL SYSTEM

Playoust and Isselbacher[36] demonstrated the existence of a mucosal lipase system that hydrolyzes MCT in the intestinal

mucosa. It was distinct from the intralumenal pancreatic lipase system. They found that MCT in the epithelial cell was hydrolyzed before entry into the portal blood. However, not all MCT was hyrolyzed as some appeared in the portal blood as unhydrolyzed MCT but mostly as free MCFA.[33,36] This was not due to triglyceride resynthesis as the enzyme responsible for the activation of fatty acids, acyl-CoA synthetase, required by the two resterification mechanisms (monoglyceride and phosphatidic acid pathways) are only specific for LCFA.[24] Valdevieso[35] confirmed this by his observation that MCT are hydrolyzed in the cell by mucosal lipase and were transferred to the portal blood partly as triglyceride and partly as free fatty acids. A study by Clark and Holt[34] reported results that dissagreed with Playoust and Isselbacher[33,36] and Valdevieso[35]. They found only free MCFA passed into the portal blood and concluded that intramucosal lypolysis was complete.

It would be of interest to investigate further the mucosal lipase system and to resolve whether absorbed MCT is transported in portal blood as MCT and MCFA, or exclusively as MCFA. If it were transported in both forms, then in what proportion if any and would this be a fixed ratio. The method to study this would be to employ the Ussing chamber. A section of intestine would be mounted in the usual manner with the mucosal side facing one chamber and the serosal side facing the other. A MCT mixture can be placed on the mucosal side and the appearance of lipids monitored on the serosal side.

3.5 MEMBRANE LIPASE

The question of membrane lipases is not resolved. Are there lipases on the mucosal side of intestinal epithelial cells that cause local lipolysis? This may be examined by making membrane vesicles from brushborder membranes and incubation of these vesicles in a triglyceride mixture. Then isolating the vesicles to evaluate their contents.

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