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*J Clin Invest.* 1976;**58**(2):493-499. <https://doi.org/10.1172/JCI108493>.

### Research Article

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# Regulation of Pancreatic and Gallbladder Functions by Intraluminal Fatty Acids and Bile Acids in Man

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**ABSTRACT** The effects of intraduodenal glycerol, fatty acid (FA) chain length and FA loads, and bile acid (BA) concentrations on pancreatic and gallbladder function were investigated in 31 healthy volunteers by a perfusion method. FA absorption rates in the duodenum and proximal jejunum were measured simultaneously.

Pancreatic and gallbladder responses were augmented by increasing FA chain length and FA loads until the "maximal" secretory capacity of the pancreas and gallbladder emptying was attained. Glycerol had no effect. Raising BA concentrations above the critical micellar concentration accelerated FA absorption rates but decreased the magnitude of pancreatic and gallbladder responses to FA. Higher BA concentrations exerted an opposite effect, slowing FA absorption and increasing pancreatic and gallbladder responses. Indeed, a significant, inverse correlation was found between FA absorption and pancreatic and gallbladder responses to FA, suggesting a relationship between the length of intestine exposed to FA and the amount of cholecystokinin (and/or other neurohormonal factors) released, which stimulates pancreatic secretion and gallbladder contraction.

## INTRODUCTION

Fatty acids (FA)<sup>1</sup> in the lumen of the proximal small bowel provide a critical stimulus for pancreatic enzyme secretion and gallbladder contraction, mediated by neurohormonal mechanisms (1), especially release of cho-

lecystokinin (CCK). In man, we earlier reported that high luminal concentrations of bile acids (BA) inhibit the stimulatory action of monoolein and certain other digestive derivatives on CCK release, pancreatic enzyme secretion, and gallbladder contraction (2).

We have now investigated additional factors which can affect intraluminal FA-BA interactions and thus, perhaps through hormone secretion, influence responses of the pancreas and gallbladder.

First, we examined the actions of different FA carbon chain lengths, since they affect micellar solubilization and absorption of FA (3, 4). Second, we studied varying duodenal loads of FA and luminal FA concentrations to establish dose-related responses of the pancreas and gallbladder to FA. Third, we compared the effects of intraduodenal infusions of different combinations of FA and BA, in concentrations similar to those found postprandially in the duodenum, to further characterize intraluminal FA-BA interactions which might influence pancreatic and gallbladder function. Finally, we measured rates of jejunal absorption of FA and correlated these with secretory responses of the pancreas and gallbladder. These studies further elucidate the regulatory actions of small bowel contents on functions of the pancreas and gallbladder.

## METHODS

31 healthy male volunteers (aged  $31.3 \pm 2.4$  yr, mean  $\pm$  SE) participated in these studies after giving informed consent. Small bowel perfusion, as earlier described (2), quantified total pancreatic enzyme and biliary outputs in response to intraluminal stimuli and absorption rates of perfused FA and water movement were simultaneously measured. After an overnight fast, individuals were intubated (under fluoroscopic control) with a duodeno-jejunal tube consisting of three small polyethylene tubes (inside diameter, 2 mm) cemented together. The infusion site, used for either normal saline (control) or test solutions containing polyethylene glycol (PEG 5 g/liter) as a nonabsorbable marker perfused at a constant rate of 10 ml/min, was located at the second portion of the duodenum, just proximal to the papilla of Vater; aspiration sites were located 20 and 50

Presented in part at the Annual Meeting of the American Gastroenterological Association, New York, 1973.

Received for publication 1 August 1975 and in revised form 20 April 1976.

<sup>1</sup>Abbreviations used in this paper: BA, bile acid; C8, octanoic acid; C12, lauric acid; C18, oleic acid; CCK, cholecystokinin; FA, fatty acid; PEG, polyethylene glycol 4000; TC, sodium taurocholate.

TABLE I  
PEG Recovered in Gastric Aspirates during Intestinal  
Perfusion of Control and FA Solutions  
Containing PEG

Perfusate			% of PEG perfused into the intestine recovered in gastric aspirates		
FA	mM	TC	Mean	SE	Range
Control (normal saline)	—	—	3.7	1.0	0-23.5
C8	10	1	3.4	3.0	0-15.5
C8	10	10	1.8	0.2	0-2.7
C8	20	10	3.8	1.9	0-8.5
C12	20	10	2.3	0.9	0-4.7
C18	10	1	4.0	3.0	0-15.5
C18	10	10	2.8	1.5	0.5-8.9
C18	10	20	1.5	1.1	0-6.9
C18	20	10	1.7	0.5	0-3.4
C18	30	10	1.6	1.2	0-7.4

cm distally (approximately at the angle of Treitz and the proximal jejunum).

Effluents were collected by siphonage from both aspiration sites, 15 min apart to compensate for transit time (5), and the samples were pooled over ice at 20-min intervals. Collections from the proximal aspiration site were never more than 25% of the volume perfused in the period. Aliquots were analyzed immediately for trypsin and lipase concentrations and, after heating to 70°C for 20 min, for FA concentration. The remaining portions were analyzed for PEG and BA concentrations.

Stomach contents were continuously aspirated by a second tube placed in the gastric antrum. Samples were analyzed for PEG concentration. Percent duodenal PEG refluxed into the stomach (Table I) was small and similar for the different test solutions employed.

**Perfusates.** Perfusates (Table II) included normal saline, as a control solution to determine basal pancreatic and biliary outputs, and three test solutions. These contained sodium taurocholate and FA: oleic (C18), lauric (C12), or octanoic (C8), all selected because of their different carbon chain lengths. Both medium chain FA (C12 and C8) were saturated, but an unsaturated long chain FA (C18) was used to achieve optimal solubilization at a physiological intraluminal pH. Glycerol was also perfused, to determine the effect of this common hydrolytic product of fat digestion.

All perfusates were prepared immediately before use, made isotonic with sodium chloride, adjusted to pH 6.3, and warmed to 37°C. Glycerol was obtained from Fisher Scientific Co., Pittsburgh, Pa. Oleic, lauric, and octanoic acids were purchased from Nu Chek Prep, Elysian, Minn. and found to be  $\geq 99\%$  when tested by gas-liquid chromatography. They were labeled with [ $^{14}\text{C}$ ]oleic, [ $^{14}\text{C}$ ]lauric, and [ $^{14}\text{C}$ ]octanoic acids, respectively (New England Nuclear, Boston, Mass.), all yielding a final specific activity of 1  $\mu\text{Ci/mol}$ . Sodium taurocholate was synthesized from taurine (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.) and cholic acid (Matheson, Coleman & Bell, East Rutherford, N. J.) as described by Hofmann (6). The synthetic product migrated as a single spot on thin-layer chromatography.

**Experimental design.** All individuals received two different test solutions during separate 100-min perfusion periods. Test perfusions were preceded by a 100-min per-

TABLE II  
Composition of the Perfusates, Outputs of Pancreatic Enzymes and Bilirubin, and Duodenal BA Concentrations

Objective	Perfusate			Per- fusions n	Output			Duodenal bile acids
	FA	mM	TC mM		Trypsin	Lipase	Bilirubin	
					$U \times 10^3/h$	$U \times 10^3/h$	$mg/h$	$mM$
Control values	NS	0	0	61	$8.1 \pm 1.2$	$34.1 \pm 4.2$	$5.2 \pm 0.7$	$1.4 \pm 0.4$
Effect of glycerol	Glycerol	10	1	4	$6.9 \pm 2.9$	$29.5 \pm 8.9$	$3.9 \pm 0.7$	$1.3 \pm 0.3$
	Glycerol	10	10	4	$8.7 \pm 2.6$	$38.1 \pm 6.8$	$2.0 \pm 0.7$	$10.0 \pm 0.6$
Effect of FA chain length	C18	20	10	6	$51.6 \pm 6.9$	$268.3 \pm 54.7$	$31.8 \pm 3.1$	$10.3 \pm 0.6$
	C12	20	10	6	$36.4 \pm 6.2$	$181.4 \pm 36.5$	$22.6 \pm 4.2$	$9.3 \pm 0.6$
	C8	20	10	6	$14.1 \pm 2.7$	$69.6 \pm 18.3$	$3.3 \pm 1.8$	$9.5 \pm 0.6$
Effect of luminal FA concentration	C18	10	10	6	$27.7 \pm 4.2$	$136.3 \pm 24.8$	$7.9 \pm 2.1$	$9.9 \pm 0.5$
	C18	20	10	6	$51.6 \pm 6.9$	$268.3 \pm 54.7$	$31.8 \pm 3.1$	$10.3 \pm 0.6$
	C18	30	10	6	$52.5 \pm 11.4$	$263.9 \pm 57.5$	$38.2 \pm 5.3$	$9.5 \pm 0.7$
	C8	10	10	6	$13.5 \pm 2.2$	$78.9 \pm 14.6$	$5.3 \pm 1.8$	$9.9 \pm 0.4$
	C8	20	10	6	$14.1 \pm 2.7$	$69.6 \pm 18.3$	$3.3 \pm 1.8$	$9.5 \pm 0.6$
Effect of luminal BA concentration	C18	10	1	6	$42.7 \pm 4.7$	$236.5 \pm 28.1$	$46.6 \pm 4.0$	$4.2 \pm 0.3$
	C18	10	10	6	$27.7 \pm 4.2$	$136.3 \pm 24.8$	$7.9 \pm 2.1$	$9.9 \pm 0.5$
	C18	10	20	5	$47.7 \pm 3.9$	$228.9 \pm 35.1$	$26.3 \pm 6.3$	$17.8 \pm 1.4$
	C8	10	1	6	$23.5 \pm 4.6$	$111.2 \pm 27.4$	$18.7 \pm 4.6$	$2.2 \pm 0.3$
	C8	10	10	6	$13.5 \pm 2.2$	$78.9 \pm 14.6$	$5.3 \pm 1.8$	$9.9 \pm 0.4$

TABLE III  
Composition of FA Perfusates, Luminal FA Loads, Concentrations and Absorption Rates,  
and Duodenal BA Concentrations

Perfusate			FA Load			FA concentration		FA absorption rates		FA unabsorbed	
FA	TC		Duod.	Jejun.	Distal Jejun.	Duod.	Jejun.	Duod.	Jejun.	Duod.	Jejun.
	mM	mM		$\mu\text{mol/min}$		mM		$\mu\text{mol/cm/min}$		% of infused	
C18	20	10	200	121.8 $\pm$ 27.3	87.6 $\pm$ 20.9	7.7 $\pm$ 1.0	4.4 $\pm$ 0.6	3.78 $\pm$ 1.30	1.14 $\pm$ 0.30	61.2 $\pm$ 12.3	43.6 $\pm$ 9.3
C12	20	10	200	89.6 $\pm$ 1.8	37.1 $\pm$ 3.8	10.3 $\pm$ 0.2	4.9 $\pm$ 0.4	5.46 $\pm$ 0.07	1.74 $\pm$ 0.17	43.4 $\pm$ 2.9	23.5 $\pm$ 2.0
C8	20	10	200	44.1 $\pm$ 9.4	15.2 $\pm$ 5.2	4.5 $\pm$ 0.7	0.9 $\pm$ 0.4	7.73 $\pm$ 0.56	0.96 $\pm$ 0.16	22.3 $\pm$ 4.9	7.8 $\pm$ 2.7
C18	10	10	100	22.3 $\pm$ 6.1	12.0 $\pm$ 4.4	1.9 $\pm$ 0.5	1.0 $\pm$ 0.3	3.90 $\pm$ 0.31	0.34 $\pm$ 0.07	22.3 $\pm$ 6.0	12.1 $\pm$ 4.4
C18	20	10	200	121.8 $\pm$ 27.3	87.6 $\pm$ 20.9	7.7 $\pm$ 1.0	4.4 $\pm$ 0.6	3.78 $\pm$ 1.30	1.14 $\pm$ 0.30	61.2 $\pm$ 12.3	43.6 $\pm$ 9.3
C18	30	10	300	267.2 $\pm$ 24.6	210.4 $\pm$ 34.9	14.4 $\pm$ 1.1	8.3 $\pm$ 1.5	3.70 $\pm$ 1.10	1.53 $\pm$ 0.73	78.6 $\pm$ 56.4	65.1 $\pm$ 9.5
C8	10	10	100	11.1 $\pm$ 2.6	4.1 $\pm$ 1.2	0.8 $\pm$ 0.2	0.3 $\pm$ 0.2	4.43 $\pm$ 0.12	0.23 $\pm$ 0.05	11.1 $\pm$ 2.6	4.1 $\pm$ 1.2
C8	20	10	200	44.1 $\pm$ 9.4	15.2 $\pm$ 5.2	4.5 $\pm$ 0.7	0.9 $\pm$ 0.4	7.73 $\pm$ 0.56	0.96 $\pm$ 0.16	22.3 $\pm$ 4.9	7.8 $\pm$ 2.7
C18	10	1	100	57.9 $\pm$ 4.1	27.3 $\pm$ 3.4	4.6 $\pm$ 0.6	2.5 $\pm$ 0.3	2.08 $\pm$ 0.21	1.02 $\pm$ 0.13	57.9 $\pm$ 4.0	27.3 $\pm$ 3.4
C18	10	10	100	22.3 $\pm$ 6.1	12.0 $\pm$ 4.4	1.9 $\pm$ 0.5	1.0 $\pm$ 0.3	3.90 $\pm$ 0.31	0.34 $\pm$ 0.07	22.3 $\pm$ 6.0	12.1 $\pm$ 4.4
C18	10	20	100	57.5 $\pm$ 8.1	37.7 $\pm$ 6.2	5.0 $\pm$ 0.4	3.2 $\pm$ 0.7	2.60 $\pm$ 0.34	0.66 $\pm$ 0.17	47.9 $\pm$ 6.6	31.3 $\pm$ 4.8
C8	10	1	100	25.0 $\pm$ 5.5	9.7 $\pm$ 1.1	2.5 $\pm$ 0.3	0.8 $\pm$ 0.2	3.76 $\pm$ 0.27	0.51 $\pm$ 0.15	25.4 $\pm$ 5.5	9.7 $\pm$ 1.8
C8	10	10	100	11.1 $\pm$ 2.6	4.1 $\pm$ 1.2	0.8 $\pm$ 0.2	0.3 $\pm$ 0.2	4.43 $\pm$ 0.12	0.23 $\pm$ 0.05	11.1 $\pm$ 2.6	4.1 $\pm$ 1.2

Loads are expressed as amounts of FA entering the duodenal perfusion segment (Duod.), the jejunal perfusion segment (Jejun.), and the jejunum just beyond the distal aspiration port (Distal Jejun.).

fusion with normal saline and were randomized using an incomplete block design (7).

During all perfusions (test or control), only those aspirates recovered during the last 60 min were used for determination of total luminal BA and FA concentrations and for calculations of pancreatic enzyme output and FA absorption. This avoided both the initial nonspecific "wash-out" effect on pancreatic enzyme secretion (8) and allowed FA absorption rates to be measured under steady-state conditions. Since gallbladder contraction started during the first few minutes of perfusion with some FA, this was determined from "peak" bilirubin output per hour as described by us (2).

**Analysis of samples.** PEG, trypsin, lipase, bilirubin, and total bile acid concentrations were measured by methods previously described (8-10) and their outputs were calculated from concentrations in duodenal samples relative to PEG recovery (11). FA were extracted from duodenal and jejunal aspirates and titrated with tetrabutylammonium hydroxide (12).  $^{14}\text{C}$  radioactivity was measured in a liquid scintillation counter (model LA-250, Beckman Instruments, Inc., Fullerton, Calif.) with Ready-Solv solution. Control experiments showed less than 5% variation in FA radioactivity measured in extracted, as compared to nonextracted, samples.

**Calculation of FA absorption rate.** FA absorption rates during the last hour of perfusion of FA solutions were calculated by reference to PEG concentrations (13). Since [ $^{14}\text{C}$ ]FA specific activity in duodenal and jejunal effluents during test periods was only  $9.2\pm 2.8$  (mean $\pm$ SE) lower than the specific activity of the [ $^{14}\text{C}$ ]FA solution perfused into the intestine, we considered endogenous FA contamination of perfusates to be small and therefore estimated FA absorption rates from disappearance of labeled FA from each test segment. These comprised a 20-cm duodenal (perfusion site to first aspiration site at 20 cm) and a 30-cm jejunal (from first to second aspiration site) segments. Results were expressed (Table III) as: (a) FA load, representing the amount of FA perfused or FA leaving one test segment and entering the adjacent segment per unit

time (micromoles per minute); (b) FA absorption rate, representing the rate of disappearance of FA from the gut lumen (micromoles per centimeter per minute); and (c) % FA unabsorbed, representing the fraction of FA load remaining unabsorbed in the lumen of the intestine at each aspiration site.

**Calculation of jejunal water movement.** Volume changes in the jejunal segment representing net water movements were estimated during steady-state perfusion (last hour) of control and FA solutions by the formula: PEG concentration entering jejunal perfusion segment/PEG concentration in jejunal aspirates  $\times 100$ . Using this formula, 100% represents no net water movement. Deviations above or below this value reflect net secretion and absorption of water, respectively.

## RESULTS

**Effect of glycerol or FA of varying chain length on pancreatic enzyme secretion and gallbladder contraction (Table II).** Glycerol (10 mM) perfused with either 1 or 10 mM sodium taurocholate (TC) did not increase pancreatic enzyme or bilirubin outputs above control (normal saline).

The effect of three different fatty acids (C18, C12, and C8) was tested at the same concentration (20 mM) and at constant TC concentration (10 mM). Pancreatic enzyme outputs evoked by each FA were above basal values ( $P < 0.05$ ). C18 was more potent than C12 and both were more potent than C8 ( $P < 0.01$ ). Bilirubin outputs were greater than control values with C18 and C12 ( $P < 0.01$ ) but not with C8. Thus, increases in pancreatic and gallbladder responses were observed with increasing FA chain length.

**Effect of perfused FA concentration on pancreatic enzyme secretion and gallbladder contraction (Table II).** The influence of luminal FA concentration on

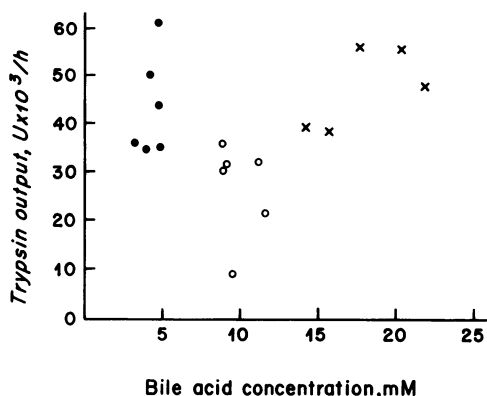


FIGURE 1 Influence of luminal bile acid concentration on individual pancreatic enzyme response to intraduodenal oleic acid (10 mM). All perfusates contained TC either 1 mM (●), 10 mM (○), or 20 mM (×).

pancreatic and gallbladder responses was investigated with two different FA: C18 (perfused at 10, 20, or 30 mM concentration) and C8 (at 10 or 20) mM. All perfusates contained TC 10 mM. C18 (10 mM) produced a significant increase in pancreatic enzyme output above basal levels, as previously. When the concentration was increased to 20 mM, enhancement in enzyme output was observed ( $P < 0.05$ ), but no further increase occurred after raising the concentration to 30 mM.

C8 (10 mM) elicited a smaller but significant pancreatic enzyme response ( $P < 0.05$ ), but no enhancement in enzyme output was observed when concentrations were raised from 10 to 20 mM.

Bilirubin outputs during perfusion of C18 (10 mM) were not significantly above control values, but higher concentrations of C18 (20 or 30 mM) caused a striking elevation in bilirubin output ( $P < 0.01$ ). During perfusion of C8 (at 10 or 20 mM) bilirubin outputs did not differ significantly from control values.

**Effect of intraluminal BA concentration on pancreatic and gallbladder responses to FA.** The influence of varying TC concentrations on FA actions was investigated by perfusing 10 mM of C18 (with either 1, 10, or 20 mM TC) or 10 mM of C8 (with either 1 or 10 mM TC) (Table II).

Increasing TC concentration in the perfusate from 1 to 10 mM decreased the pancreatic enzyme outputs otherwise stimulated by C18 ( $P < 0.02$ ). When TC was increased to 20 mM, pancreatic enzyme outputs in response to C18 increased ( $P < 0.01$ ), being similar to those obtained at 1 mM TC concentration. Responses to different BA concentrations during perfusion of standard concentrations of C18 (10 mM) are depicted (Fig. 1). As with C18, increasing TC from 1 to 10 mM (Table II) decreased enzyme response to C8 ( $P < 0.05$ ). The effect of 20 mM TC on C8 was not tested.

Gallbladder contraction occurred under circumstances similar to those stimulating pancreatic enzyme secretion (Table II). High bilirubin outputs (gallbladder contraction) were obtained with C18 perfused with 1 mM TC ( $P < 0.01$ ) compared with control, but outputs declined to basal levels when concentrations of TC were increased to 10 mM and increased again when TC concentration was augmented to 20 mM ( $P < 0.05$ ). Bilirubin outputs during perfusion of C8 with 1 mM TC were higher than control ( $P < 0.05$ ) although smaller than with C18 with 1 mM TC ( $P < 0.01$ ). An increase in TC concentration from 1 to 10 mM abolished gallbladder response to C8 as it did with C18.

**Effect of luminal BA concentration on FA absorption.** Luminal BA concentrations influenced rates of absorption of long chain FA (C18) and, to a lesser extent, of medium chain FA (C8). At constant C18 concentration (10 mM), increasing TC concentration from 1 to 10 mM (Table III) enhanced the rate of absorption of C18 ( $P < 0.01$ ) in the duodenum. However, a further increase in TC concentration in the perfusate from 10 to 20 mM decreased the rate of absorption of C18 ( $P < 0.02$ ). Comparison of duodenal C18 absorption rates and luminal BA concentrations suggests an optimal molar ratio between BA and C18 perfusate concentrations for maximal C18 absorption. Under our conditions, this ratio was about 1.0 (TC: 10 mM/C18: 10 mM). Either a BA excess or deficit from this ratio slowed absorption of C18 (Fig. 2). Further, luminal BA concentrations exerted opposite influences upon C18 absorption and pancreatic responses to this FA (Fig. 1 vs. 2). Thus, BA concentrations which enhanced C18 absorption resulted in smaller enzyme outputs and vice versa.

Absorption of C8 was increased by augmenting the concentration of TC perfused from 1 to 10 mM ( $P < 0.05$ ), although the increase was less than when C18

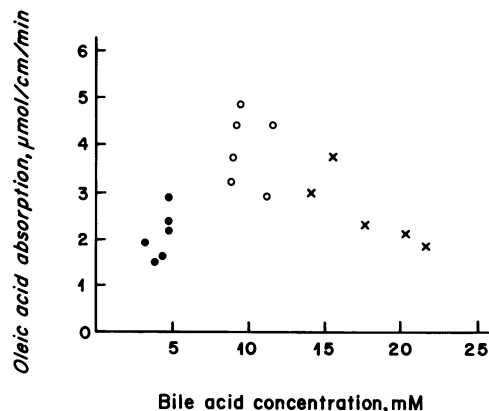


FIGURE 2 Influence of luminal bile acid concentration on duodenal oleic acid (10 mM) absorption. All perfusates contained TC either 1 mM (●), 10 mM (○), or 20 mM (×).

was tested. Enhancement of C8 absorption by TC was associated, as with C18, with a significant decrease in trypsin output ( $P < 0.05$ ). At similar TC concentration there was an inverse relationship between FA chain length and absorption rate (Table III).

**Relationships between FA absorption and pancreatic and gallbladder function.** Pancreatic enzyme outputs were compared with the fraction of unabsorbed FA remaining at the jejunal aspiration site. The mean amounts of unabsorbed C8, C12, and C18 in the jejunum correlated well with increasing pancreatic enzyme outputs ( $r = 0.96$ ), before "maximal" (14) pancreatic enzyme output was attained (Fig. 3). Since the amount of perfused FA dispersed through the small bowel decreases until absorption is completed, this correlation between enzyme output and the fraction of unabsorbed FA in the jejunum is consistent with a relationship between the length of proximal small bowel exposed to unabsorbed FA and the magnitude of pancreatic secretion.

Bilirubin outputs in response to perfused FA (Table II) roughly paralleled pancreatic enzyme responses. However, those FA solutions providing only a weak stimulus to pancreatic secretion (trypsin outputs less than  $15.0 \text{ U} \times 10^3/\text{h}$ ) did not increase bilirubin outputs, suggesting failure to trigger gallbladder contraction. Conversely, FA solutions producing maximal pancreatic enzyme outputs elicited comparable bilirubin outputs suggesting similar gallbladder contraction and emptying. Mean bilirubin responses to FA solutions

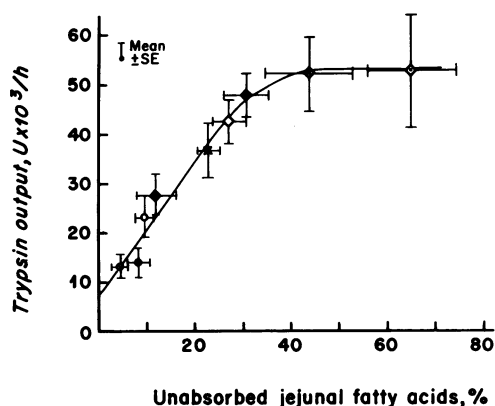


FIGURE 3 Relationship between pancreatic enzyme output and the fractional absorption of perfused fatty acids. All perfusates contained TC and either oleic acid (C18), lauric acid (C12), or octanoic acid (C8) at the following concentrations: ( $\diamond$ ) C18: 30 mM, TC: 10 mM; ( $\diamond$ ) C18: 20 mM, TC: 10 mM; ( $\blacklozenge$ ) C18: 10 mM, TC: 20 mM; ( $\diamond$ ) C18: 10 mM, TC: 1 mM; ( $\times$ ) C12: 20 mM, TC: 10 mM; ( $\blacklozenge$ ) C18: 10 mM, TC: 10 mM; ( $\circ$ ) C8: 10 mM, TC: 1 mM; ( $\bullet$ ) C8: 20 mM, TC: 10 mM; ( $\odot$ ) C8: 10 mM, TC: 10 mM.

TABLE IV  
Jejunal Water Movement during Control and FA Perfusion

Perfusate			% Water movement		Significance (vs. control)
FA	TC		Mean	SEM	
	<i>mM</i>	<i>mM</i>			
Control (normal saline)			85.0	2.2	—
C8	10	1	92.0	6.9	NS
C8	10	10	84.1	3.4	NS
C8	20	10	90.9	8.4	NS
C12	20	10	113.5	6.0	<i>P</i> < 0.01
C18	10	1	94.2	6.4	<i>P</i> < 0.05
C18	10	10	90.3	4.0	NS
C18	10	20	94.8	3.1	<i>P</i> < 0.01
C18	20	10	111.8	8.2	<i>P</i> < 0.01
C18	30	10	132.8	9.0	<i>P</i> < 0.01

% Water movement = (PEG concentration entering jejunal perfusion segment)/(PEG concentration in jejunal aspirates)  $\times 100$ .

stimulating submaximal outputs correlated well with the fraction of unabsorbed jejunal FA ( $r = 0.81$ ).

**Net water movement during perfusions (Table IV).** FA solutions which increased jejunal FA concentrations above 2 mM produced a linear increase in net water movement into the gut lumen ( $r = 0.98$ ).

## DISCUSSION

Our results show that three luminal factors can influence FA absorption and pancreatic enzyme and gallbladder responses to FA: first, FA chain length, next the amount of FA delivered into the gut, and last BA/FA concentration ratios. We suggest that all these factors may mainly operate through the common mechanisms of CCK release.

Longer FA chain lengths elicited greater pancreatic and gallbladder responses than shorter ones and were also absorbed at slower rates, this being attributable to differences in luminal solubilization, metabolism, and transport of the lipids (3, 4, 15, 16). Indeed, Meyer and Jones (17) found a direct relationship between the chain length of luminal FA (nine carbons and longer) and the amount of pancreatic secretion in the dog. However, since they did not measure FA absorption rates, no estimates of gut length exposed to FA can be made.

The amounts of FA delivered into the gut also affected both FA absorption rates and pancreatic and gallbladder responses. In addition, intraluminal FA induced net water movement towards the gut lumen, confirming previous studies in our laboratory (18). Greater FA loads resulted in higher luminal FA concentrations and increased FA absorption rates. However, when the "maximal" capacity of the intestinal mucosa (4) was exceeded, absolute FA absorption rates

did not rise proportionally and FA were presumably dispersed over longer segments of gut.

FA absorption rates and pancreatic and gallbladder responses were also influenced by luminal BA concentration, but not in a linear fashion. Moderate increases in BA concentration enhanced FA absorption, whereas this was inhibited by higher BA concentrations. The opposite effect was observed on pancreatic and gallbladder responses. Thus, pancreatic and gallbladder functions were ultimately inversely related to luminal BA concentration. If micellar solubilization of FA accelerates transport of FA across the intestinal unstirred water layer, this may explain the increase in FA absorption at luminal BA values above the critical micellar concentration (13). Further, a large BA excess beyond that necessary to form micelles may, by analogy with the model of Withington and Collett (19), "retain" FA in micelles and thereby retard transfer of FA across the intestinal cell membranes. Our findings, like those of Linscheer (20) in the rat, suggest the existence of optimal ratios between luminal FA and BA for FA absorption. Such a ratio may produce a level of saturation of BA micelles with FA which facilitates both transport of FA across the unstirred layer and rapid transfer across the epithelial membrane.

Inhibition of FA absorption by a relative excess of luminal BA may play a physiologic role. If high duodenal FA concentrations with fully saturated BA micelles ordinarily accelerate absorption of FA, FA/BA concentration ratios must be reduced more distally in the gut by preferential absorption of FA (21). Consequently, the excess of intraluminal BA would retard FA absorption and disperse FA over a larger segment of intestine.

We found that the factors which tend to increase the length of small intestine required to absorb perfused FA comprised: higher luminal BA concentrations, longer FA chain length (both retarding FA absorption), and FA loads exceeding the absorptive capacity of the gut. All of these also resulted in greater stimulation of pancreatic and gallbladder secretion. In fact, there was a significant relationship between pancreatic and gallbladder responses and the fraction of FA unabsorbed which indirectly represents the length of intestine presumably exposed to FA. Therefore, FA chain length, FA load, and BA concentration could all influence pancreatic and gallbladder functions by determining the surface area of proximal small bowel stimulated by FA and secreting CCK in response. CCK-releasing cells are probably uniformly distributed along the proximal 100 cm of small bowel (22). The greater the surface area of gut exposed to the stimulus of FA, the larger the amounts of CCK released and the greater the responses of the target organs (pancreas and gall-

bladder). This interpretation of our data accords with the work of Meyer and Grossman (1) who showed that in dogs CCK release by L-phenylalanine is mainly a function of "load" (amount introduced into duodenum per unit time) rather than luminal concentration, suggesting that the capacity of each cell releasing CCK is limited and that the total number of cells stimulated determine the amount of CCK released.

Our data, however, cannot completely exclude the possibility that FA chain length may influence pancreatic and gallbladder functions not only through differences in FA absorption rates but also through specific effects on intestinal regulatory mechanisms. Indeed, in the dog, Meyer and Jones (17) found that the composition of pancreatic secretion (bicarbonate to protein ratios) stimulated by luminal FA varied, depending upon FA chain length.

In previous studies (2) we observed that BA inhibited pancreatic and gallbladder responses to intraduodenal 1-monoolein and essential amino acids. Our present observations may explain this inhibitory effect on 1-monoolein (rapidly hydrolyzed to C18) on the basis of faster C18 absorption. However, it seems unlikely that BA modified essential amino acid absorption to the degree needed to almost suppress essential amino acid stimulation of pancreas and gallbladder as found (2). Thus, BA may inhibit essential amino acid action through an, as yet, unrecognized mechanism whereas they may affect FA action mainly by determining the amount of CCK released through changes in FA absorption rate.

Assuming constant CCK release, the response of target organs must also be taken into consideration. We have earlier found that the stimulatory threshold for gallbladder contraction in response to exogenous (i.v.) CCK is higher than that of the pancreas (14). Since we have now shown that weak FA solutions stimulate pancreatic enzyme secretion without causing gallbladder contraction, it appears that pancreatic and gallbladder responses to endogenous CCK can be similarly dissociated. Thus, it is theorized that after eating, intraluminal factors, including BA concentrations and total amounts or chain length of FA liberated by fat hydrolysis, regulate CCK release by determining the surface area of proximal small bowel exposed to FA. This hormone, in turn, stimulates pancreatic secretion and gallbladder emptying in relation to the sensitivity of each target organ. These interrelated events presumably participate in adjusting pancreatic and gallbladder responses to intraluminal requirements for digestion.

#### ACKNOWLEDGMENTS

The authors are indebted to Mrs. Judy Duenes, Miss Bonita Barnes and Mr. Richard Tucker for their technical

assistance; to Miss Gaurdis Grube for secretarial help; and to Dr. Neville Hoffman for his valuable advice.

This work was supported in part by National Institutes of Health grants AM-6908 and AM-5259.

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