# Gallbladder and Small Intestinal Regulation of Biliary Lipid Secretion during Intraduodenal Infusion of Standard Stimuli

GREGORY T. EVERSON, MICHAEL J. LAWSON, CAROL MCKINLEY, RADENE SHOWALTER, and FRED KERN, JR., *Division of Gastroenterology, University of Colorado School of Medicine, Denver, Colorado 80262*

ABSTRACT The gallbladder and small intestine are reservoirs for the bile acid pool during its enterohepatic circulation and, as such, may regulate biliary secretion of bile acid. During studies of biliary bile acid secretion, a stimulus to gallbladder contraction is continuously infused into the duodenum. Under these conditions, it is assumed that the gallbladder is tonically contracted and that the rate of bile acid secretion into the duodenum equals the hepatic bile acid secretion rate. However, secretion rates vary by as much as 100%, depending upon which of two standard stimuli is used. Therefore, we studied the role of gallbladder emptying and small intestinal transit in determining biliary lipid secretion rate and composition during infusion of these stimuli in five healthy subjects. Each subject was studied with a liquid formula containing 40% of calories as fat, and with an amino acid solution for 10 h. Bile acid, phospholipid, cholesterol, and markers were measured in duodenal bile and hourly secretion rates were calculated by marker dilution technique. Real-time gallbladder sonographs and serum pancreatic polypeptide levels were obtained every 30 min. Small bowel transit time was estimated by the breath hydrogen response after giving lactulose intraduodenally.

During liquid formula infusion, gallbladder emptying was more complete, small intestinal transit was faster, and pancreatic polypeptide levels were higher. Secretion rates of all lipids were greater and molar percent cholesterol was lower. For the combined data from both infusions, the secretory relationships of cholesterol to bile acid, cholesterol to phospholipid, and phospholipid to bile acid were curvilinear.

We conclude that more complete gallbladder emptying and faster intestinal transit increase the enterohepatic cycling of bile acids and lower the molar percent cholesterol of bile. Some of the fluctuation observed in biliary lipid secretion rates, especially during amino acid infusion, is due to gallbladder refilling and emptying.

Dr. Everson was supported by a National Institutes of Health research training grant (gastrointestinal diseases), AM 07038. Address reprint requests to Dr. Everson. Dr. Lawson is a recipient of a Fullbright Postdoctoral Fellowship and a Royal Australasian College of Physicians travelling grant. His present address is Gastrointestinal Division, Queen Elizabeth Hospital, Woodville, South Australia.

*Received for publication 24 June 1982 and in revised form 22 November 1982.*

## INTRODUCTION

In 1972, Grundy and Metzger (1) introduced the study of biliary lipid secretion in intact human subjects using a continuous intraduodenal infusion of a complex liquid formula, containing 40% of calories as fat. Others have used a mixed amino acid solution (2–4) or intermittent liquid meals (5–9) to stimulate biliary lipid secretion. It has been assumed that these stimuli tonically and completely contract the gallbladder, removing it from the enterohepatic circulation, and stimulate small intestinal motility, so that the rate of biliary lipid output into the duodenum would equal the rate of hepatic secretion. Reported biliary lipid secretion rates in similar population groups, however, vary by as much as 100%.

In healthy individuals, ileal bile acid absorption from the intestinal lumen (10) and hepatic bile acid extraction from portal blood are highly efficient (11). Because bile acid pool size is constant in the steady state and no bile acid is stored in the liver, the rate of hepatic bile acid secretion is directly proportional to the frequency of enterohepatic cycling, which is largely determined by the rate of delivery of bile acid from the extrahepatic biliary tree to the terminal

ileum. The delivery of bile acid to the terminal ileum, in turn, is determined by two mechanical pumps, the gallbladder and small intestine. Increased storage of bile acid in the gallbladder or slowed small intestinal transit would slow the delivery of bile acid to the terminal ileum and lower the bile acid secretion rate. Because bile contains relatively more cholesterol when bile acid secretion rate is low (6, 12–15), the lower rates of bile acid secretion during gallbladder storage and slowed intestinal transit would be expected to increase the cholesterol saturation of bile.

This study was designed to investigate the role of the gallbladder and small intestine in regulating the rate of biliary lipid secretion and biliary lipid composition.

TABLE I
*Characteristics of Infusates*

| | Liquid formula | Amino acid solution |
|---|---|---|
| Composition, *g/dl* | Fat, 6.4<br>CHO, 14.1<br>Protein, 4.7 | Fat, 0<br>Glucose, 5.0<br>Amino acid, 4.3 |
| Calories, *cal/ml* | 1.3 | 0.4 |
| Osmolality, *mosmol/liter* | 543 | 420 |
| Marker | β-Sitosterol | BSP |
| Infusion rate, *ml/min* | 3.2±0.2 | 3.7±0.9 |

*CHO*, carbohydrate. Osmolality was measured by freezing point lowering.

## METHODS

This study was approved by the Human Subjects Committee of the University of Colorado School of Medicine. All subjects gave written informed consent, were paid volunteers, and were hospitalized in the Clinical Research Center for study.

Five healthy, nonobese women, ages 21 to 28, were each studied twice. After they fasted overnight, a triple lumen polyvinyl tube was passed through the nose and positioned in the duodenum by fluoroscopy with genital shielding, so that the proximal infusion orifice was adjacent to the ampulla of Vater, 12 cm from the distal collecting orifice. The third lumen was filled with mercury from proximal to distal orifice and sealed at both ends to aid in fluoroscopic guidance.

After the tube was in position, either a mixed amino acid solution with 5 mg BSP/100 ml as marker or liquid formula with β-sitosterol as marker was continuously infused through the proximal lumen. The amino acid solution contained 5% (wt/vol) glucose and 4.3% (wt/vol) of mixed amino acids (2). Each liter of liquid formula contained 132 g of powdered skimmed milk, 810 ml of distilled water, 140 ml of polycose (Ross Laboratories, Columbus, OH), and 50 ml of corn oil. The mixture was sonicated with a sonifier cell disrupter (Branson Sonic Power, Plainview, NY) for 30 min just before infusion. The resultant uniform emulsion was agitated every 30 min throughout the study. The concentration of β-sitosterol in the infusate was measured in aliquots taken every hour during the period of infusion and was constant. Liquid formula contains 40% of calories as fat. It has three times more carbohydrate, three times more calories, and is slightly more hypertonic than the amino acid solution (Table I).

In earlier studies, 10–20% of subjects became nauseated and vomited during infusion of the amino acid solution (3). To ensure paired data, each subject was first studied with amino acid so that only those completing the amino acid infusion without incident were studied with liquid formula. Two of seven subjects did not complete the amino acid study because of nausea and vomiting. The interval between the two infusions was 5–10 d. Duodenal bile was continuously aspirated from the distal orifice at a rate of 0.5 ml/min and each 30-min sample was treated separately. At the bedside, duplicate aliquots of each sample were extracted with 2:1 chloroform/methanol for phospholipid measurement, while other aliquots were refrigerated at 4°C for analysis of cholesterol, bile acid, and markers. Real-time sonographs of the gallbladder were obtained every 30 min by using an ADR model 2131 real-time scanner with a 3.5-mHz multiplexed linear array transducer, 13.5-cm length (Advanced Diagnostic Research Corp., Tempe, AZ). Blood samples for measurement of human pancreatic polypeptide were drawn when sonographs were obtained.

Small intestinal transit was measured by the lactulose breath test (16). At the 5th h of study 10 g of lactulose in 100 ml of water was given as a bolus through the distal orifice. Breath samples for measurement of hydrogen were collected before and every 15 min for 4 h after the lactulose was given.

*Analytical techniques.* Infusate and each 30-min sample were analyzed for bile acid, phospholipid, cholesterol, and marker. Bile acid concentration was measured spectrofluorometrically by the enzymatic method of Talalay (17), phospholipid, colorimetrically by the method of Bartlett (18), and BSP, colorimetrically (19). Cholesterol and β-sitosterol were measured by gas-liquid chromatography by using coprostanol (5-β-cholesten-3-ol) as internal standard (20). The amino acid solution contained no cholesterol or phospholipid. Liquid formula contained cholesterol (~0.1 μmol/ml) and phospholipid (~0.3 μmol/ml). The cholesterol and phospholipid concentration of each duodenal sample was corrected for infusate cholesterol and phospholipid using the calculation: $[CH]_{net} = [CH]_D - ([CH]_I\ [\beta S]_D/[\beta S]_I)$, where $[CH]_{net}$ = concentration of cholesterol in duodenal bile sample due to biliary secretion (micromoles per milliliter), $[CH]_D$ = measured cholesterol concentration in duodenal samples (micromoles per milliliter), $[CH]_I$ = infusate cholesterol concentration (micromoles per milliliter), $[\beta S]_I$ = infusate β-sitosterol concentration (micromoles per milliliter), and $[\beta S]_D$ = duodenal sample β-sitosterol concentration (micromoles per milliliter). $[CH]_{Dnet}$ (or $[PL]_{Dnet}$) is used in subsequent calculations of secretion rates (as $[X]_D$, see below) by the standard marker dilution equation, $X_{sec} = ([X]_D/[M]_D) \cdot [M]_I \cdot R \cdot 60$ min, where $X_{sec}$ = secretion rate of a particular biliary lipid, X, (micromoles per hour). $[X]_D$ = duodenal concentration of $X$ (micromoles per milliliter), $[M]_D$ = duodenal concentration of marker (micromoles per milliliter), $[M]_I$ = infusate marker concentration (micromoles per milliliter), and $R$ = rate of infusion (milliliters per minute). Based on previous studies (1, 21), it was assumed that negligible cholesterol and β-sitosterol absorption occurred over the 12-cm perfused segment of bowel.

Gallbladder volume was measured from each gallbladder sonograph by a previously validated technique (22).

Human pancreatic polypeptide was measured by radioimmunoassay by Dr. Ian Taylor, Sepulveda Veterans Administration hospital, Sepulveda, CA.

Breath hydrogen concentration was measured with a Quintron model S gas chromatograph (Quintron Instrument Company, Inc., Milwaukee, WI), equipped with a molecular sieve column and thermal conductivity detector. The column was maintained at room temperature, and argon (18 ml/min) was used as the carrier gas. Small bowel transit time was defined as the time of rise in breath hydrogen concentration from base line. In each experiment, this was subjectively evaluated by eight observers blinded to the results of gallbladder emptying and biliary lipid secretion. Transit time was determined from the appearance of the plot of hydrogen concentration (parts per million) vs. time, and reported as the mean (±SD) of these eight estimations.

*Analysis of data.* Differences in gallbladder emptying, small bowel transit, serum levels of human pancreatic polypeptide, mean biliary lipid secretion rates, and mean molar percent cholesterol during the two infusions were analyzed by paired $t$ tests. Data from all five subjects were included in all statistical analyses. Biliary lipid secretory relationships during each infusion were evaluated by linear and nonlinear regression analysis; linear: $y = ax + b$; nonlinear: $y = x/(b + ax)$, where $y$ and $x$ are hourly lipid secretory rates and $a$ and $b$ are constants.

## RESULTS

Investigators have usually ignored biliary lipid output during the first 4 h of study, because of its fluctuation, and have calculated secretion rates only after this period. Accordingly, we divided our study results into the first 4 and last 6 h.

*Biliary lipid secretion.* With both infusions, secretion rates were lower in the last 6 h than in the first 4 h due to the high secretion rates in the first 2 h after initiating gallbladder contraction. During both the first 4 and last 6 h of study, the mean hourly secretion rates of bile acid, phospholipid, and cholesterol showed considerable variability but were greater during the liquid formula than during the amino acid infusion (Table II).

The rates of bile acid and phospholipid secretion were increased more than that of cholesterol during liquid formula infusion, resulting in a lower molar percent cholesterol than during the amino acid infusion in four of five subjects during the first 4 h ($P < 0.15$) and in all subjects during the last 6 h ($P < 0.05$) (Fig. 1). The increments in cholesterol secretion coincident with increments in either bile acid or phospholipid secretion were far smaller during liquid formula infusion (Fig. 2A–C). For these reasons, biliary lipid secretory relationships were quite different with the two infusions. More cholesterol was secreted per micromole bile acid or phospholipid during the amino acid infusion. The best fit of the combined data

TABLE II
*Biliary Lipid Secretion Rates*

| | BA | | PL | | Ch | |
|---|---|---|---|---|---|---|
| Subject | AA | LF | AA | LF | AA | LF |
| | μmol/h | | μmol/h | | μmol/h | |
| First 4 h* | | | | | | |
| 1 | 1,815±363 | 2,761±2720 | 491±95 | 771±917 | 107±23 | 157±217 |
| 2 | 1,895±849 | 4,868±1979 | 349±218 | 1,003±356 | 152±78 | 282±131 |
| 3 | 2,353±821 | 2,951±790 | 546±245 | 1,023±390 | 191±77 | 303±222 |
| 4 | 1,479±470 | 3,160±1391 | 246±119 | 663±357 | 115±55 | 122±93 |
| 5 | 902±343 | 3,908±1035 | 148±105 | 697±171 | 55±21 | 198±59 |
| $P$ | <0.025 | | <0.005 | | <0.05 | |
| Last 6 h‡ | | | | | | |
| 1 | 1,258±382 | 2,084±951 | 238±93 | 516±231 | 57±23 | 64±21 |
| 2 | 1,258±508 | 3,205±1,151 | 193±92 | 613±144 | 79±29 | 136±38 |
| 3 | 1,265±399 | 2,690±740 | 208±105 | 801±182 | 97±34 | 205±41 |
| 4 | 1,382±861 | 3,888±1,042 | 207±189 | 661±176 | 95±72 | 128±31 |
| 5 | 986±1,020 | 4,527±1,745 | 157±154 | 654±206 | 45±17 | 182±68 |
| $P$ | <0.02 | | <0.001 | | <0.05 | |

BA, total bile acid; PL, phospholipid; CH, cholesterol; AA, amino acid infusion; LF, liquid formula infusion.
* Secretion rates measured during the first 4 h of infusion of stimulus.
‡ Secretion rates measured during the last 6 h of the infusion of stimulus. The higher secretion rates during the first 4 h of stimulus infusion are due to the high secretion rates that occur in the first 2 h after initiation of gallbladder contraction.

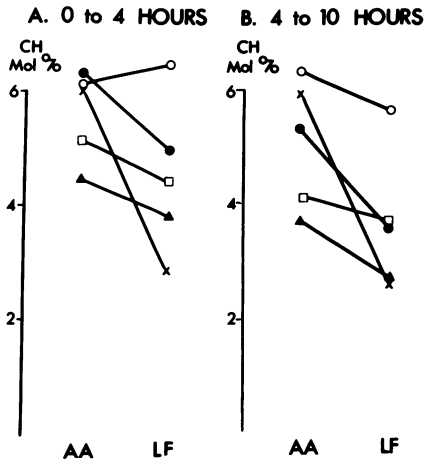
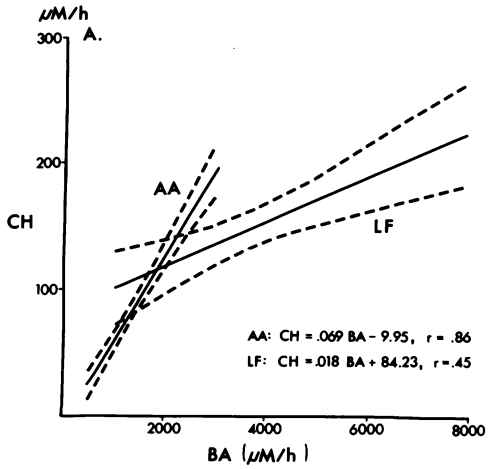
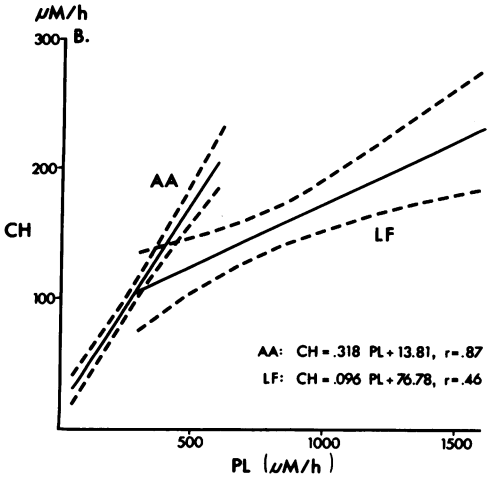



FIGURE 1 The mean molar percent cholesterol (CH mol %) was lower with liquid formula during the first 4 (panel A) and last 6 h (panel B) of infusion. AA, amino acid infusion; LF, liquid formula infusion.

(both amino acid and liquid formula) for cholesterol vs. bile acid, cholesterol vs. phospholipid, or phospholipid vs. bile acid was the equation for a rectangular hyperbola, $y = x/(b + ax)$ (Table III). However, as bile acid secretion rate increases there is considerable divergence of cholesterol and phospholipid secretion, $CH\ Sec_{max} = 298$ and $PL\ Sec_{max} = 5555$ (Table III). As bile acid secretion increased there was little change in the ratio of phospholipid to bile acid but the ratio of cholesterol to bile acid decreased considerably.

*Gallbladder emptying.* In each subject gallbladder emptying was more complete with liquid formula infusion during both the first 4 ($P < 0.05$) and last 6 h ($P < 0.02$) (Fig. 3). Gallbladder volumes during amino acid infusion fluctuated considerably, and there were periods of apparent refilling. On the other hand, during liquid formula infusion gallbladder emptying was prompt and more complete, and showed little fluctuation (Fig. 4).



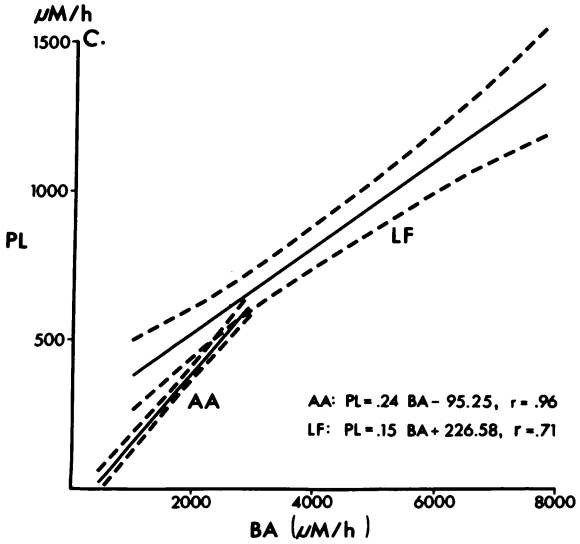




FIGURE 2 Hourly secretion rates of cholesterol (CH), bile acid (BA), and phospholipid (PL) are plotted against each other. The solid regression lines represent the fit of the data to the equation $y = ax + b$, where $y$ and $x$ are secretion rates and $a$ and $b$ are constants. The 95% confidence interval for the slope of each regression is given by the dotted lines. A. Increments in BA secretion were associated with greater increments of CH secretion during AA infusion ($n = 52$) than during LF infusion ($n = 57$). B. Increments in PL secretion were associated with greater increments of CH secretion during AA infusion ($n = 47$) than during LF infusion ($n = 58$). C. Approximately the same relationship was observed between PL and BA secretion during infusion of either AA ($n = 47$) or LF ($n = 58$).

TABLE III

*Regression Analysis of Secretory Relationships of Combined Data from Amino Acid and Liquid Formula Infusions*

| | | Equations* | |
|---|---|---|---|
| | | $y = x/(b + ax)$ | $y = ax + b$ |
| CH-BA‡ | RSS§ | 2.19 | 2.55 |
| | Ch $Sec_{max}$‖ | 298 | — |
| CH-PL | RSS | 2.10 | 2.38 |
| | CH $Sec_{max}$ | 269 | — |
| PL-BA | RSS | 4.11 | 4.26 |
| | PL $Sec_{max}$ | 5,555 | — |

CH, cholesterol; BA, bile acid; PL, phospholipid.

* $y = ax + b$ describes a linear relationship between $x$ and $y$. $y = \frac{x}{b + ax}$ describes a hyperbolic relationship between $x$ and $y$.

‡ The secretion rate (micromolars/hour) of the lipid to the left of the hyphen is always plotted on the y-axis while that of the lipid to the right of the hyphen is plotted on the $x$-axis.

§ RSS, residual sum of squares $\times 10^{-5}$. The equation that better fits the data is the one with the lower RSS.

‖ $Sec_{max}$ is the maximum secretion rate (micromolars/hour) of $y$-axis lipid as the secretion rate of the $x$-axis lipid approaches infinity. $Sec_{max}$ equals $1 \div a$ in the equation, $y = x/(b + ax)$.

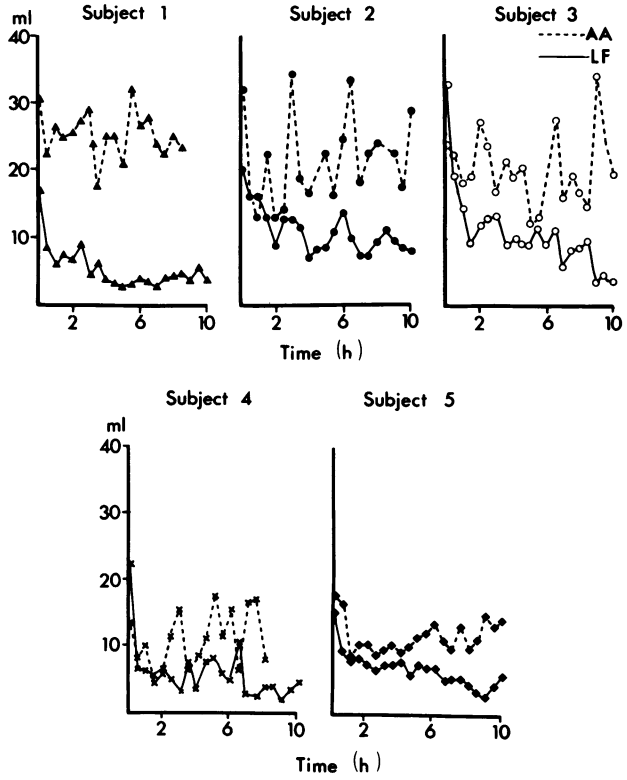



FIGURE 4 Gallbladder volume is plotted against time. Volumes during both amino acid (dotted line) and liquid formula (solid line) infusion are shown for each subject.

In all studies, including both amino acid and liquid formula infusions, there were 23 periods, excluding initial gallbladder emptying, in which gallbladder emptying was >5 ml. During 22 of these periods there was a concomitant increase in bile acid output.

*Small bowel transit time.* In four of five subjects, small bowel transit was slower during amino acid infusion (Table IV). Since subject 4 produced hydrogen in response to lactulose during liquid formula infusion, the lack of increase in breath hydrogen during the amino acid infusion is most consistent with a markedly prolonged transit time. However, there may be other explanations for the lack of hydrogen response. The nasoduodenal tube may have slipped back into the

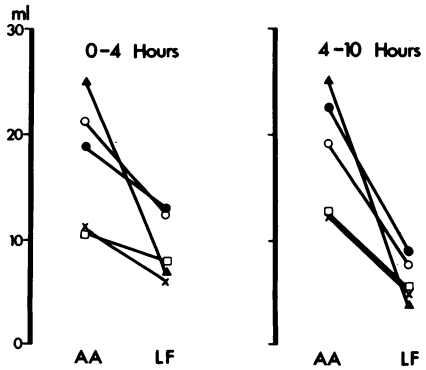



FIGURE 3 Gallbladder volume (milliliters) during both the first 4 (panel A) and last 6 h (panel B) was always less during infusion of liquid formula (LF).

TABLE IV

*Small Intestinal Transit Times during Infusion of Stimuli*

| Subjects | Transit time, *min* | |
|---|---|---|
| | AA | LF |
| | *Mean±SD* | |
| 1 | 39±14 | 47±15 |
| 2 | 90±0 | 45±0 |
| 3 | 99±8 | 21±8 |
| 4* | 240±0 | 68±8 |
| 5 | 45±0 | 30±0 |
| *P* | <0.15 | |

Mean±standard deviation of the transit time as determined by eight observers.

* No hydrogen rise from base line was detected after 240 min. Since this subject was capable of producing hydrogen, as observed during the liquid formula infusion, the lack of hydrogen rise during amino acid infusion indicates that her transit time was >240 min.

stomach, the subject may have been colonized with colonic bacteria incapable of making hydrogen, or this may represent day to day variation. The first seems unlikely as bile was continuously aspirated during the study and there was no drop in secretion rate in the 4 h after lactulose was given. The second is also unlikely as a dramatic change in colonic flora would have had to occur within 1 wk without the administration of drugs or antibiotics. The third is extremely unlikely as the day-to-day variation in transit time measured by this technique has been defined and is minimal, especially with intraduodenal infusion of lactulose (23). Subject 1, who had the biggest difference in gallbladder emptying with the two infusions, had no change in transit time. Interestingly, she had a smaller increase in bile acid secretion compared with the remaining subjects.

*Human serum pancreatic polypeptide.* In four of five subjects, levels of pancreatic polypeptide were two- to threefold higher during both the first 4 and last 6 h with liquid formula infusion (Table V). Subject 5 had no change in pancreatic polypeptide levels from base line with either infusion. Nonetheless, this subject had findings which were similar to the rest of the group: increased gallbladder emptying, faster intestinal transit, and increased bile acid secretion in response to liquid formula. In the other subjects, the pattern of the pancreatic polypeptide response was that of a sustained elevation from base line with minimal fluctuation.

## DISCUSSION

When bile acid pool size is constant, the rate of biliary bile acid secretion is dependent on the enterohepatic cycling frequency of bile acid. Cycling frequency, in turn, is dependent on two mechanical pumps, the gallbladder and small intestine. Thus, differences in gallbladder storage and small intestinal transit of bile acid would be expected to alter biliary bile acid secretion rate. Biliary bile acid secretion rate is lower when stimulated by intermittent feeding (5–9) than by continuous intraduodenal infusion (1, 5, 23–30) of a standard liquid formula (Table VI). In addition, bile acid secretion rate during intermittent liquid formula infusion is similar to that of postcholecystectomy subjects (2, 31–33). Bile acid secretion rate during continuous intraduodenal amino acid infusion (2, 3) is less than that measured during continuous intraduodenal liquid formula infusion (1, 5, 23–30) and similar to that measured during intermittent liquid formula feedings (5–9) and in postcholecystectomy subjects (2, 31) (Table VI). When similar groups of subject are compared (references 24 and 25 compared with reference 3), bile acid secretion rates during liquid formula infusion are more than twice those during amino acid infusion. In the present study, during liquid formula infusion the rate of biliary lipid secretion was higher, gallbladder emptying was prompt and more complete, and small intestinal transit was faster. We conclude that the higher rate of biliary lipid secretion achieved during liquid formula infusion in this and other studies is due to increased enterohepatic cycling of bile acid caused by more complete gallbladder emptying and faster small intestinal transit. Biliary lipid secretion rate during amino acid infusion is submaximal, due to incom-

TABLE V

*Human Pancreatic Polypeptide Response to Infusion of Stimuli*

| | HPP, *pg/ml* | | | |
|---|---|---|---|---|
| | First 4 h | | Last 6 h | |
| Subject | AA | LF | AA | LF |
| | *Mean±SD* | | | |
| 1 | 91±21 | 319±161 | 93±16 | 424±94 |
| 2 | 150±60 | 264±104 | 108±22 | 198±59 |
| 3 | 91±36 | 221±262 | 107±38 | 213±86 |
| 4 | 43±16 | 187±80 | 56±21 | 263±97 |
| 5 | 28±7 | 26±3 | 36±13 | 38±11 |
| *P* | <0.025 | | <0.005 | |

HPP, human pancreatic polypeptide; AA, amino acid infusion; LF, liquid formula infusion.

TABLE VI

*Reported Studies of Biliary Bile Acid Secretion in Subjects with Intact Gallbladders and Subjects Status Postcholecystectomy*

| | Bile acid secretion, *μmol/h* | |
|---|---|---|
| | Mean | Range |
| Subjects with gallbladders | | |
| Liquid formula, continuous (1, 5, 23–30) | 2,200 | 1,550–2,920 |
| Liquid formula, intermittent (5–9) | 1,650 | 1,470–2,015 |
| Amino acid, continuous (2, 3) | 1,150 | 1,073–1,236 |
| Subjects postcholecystectomy‡ (2, 31) | 1,390 | 1,149–1,588 |

All data were converted to μmol/h by using the published weights of subjects and, where applicable, by assuming an average molecular weight of conjugated bile acids of 500. Mean is the average of reported control means; numbers in parentheses are references.

* The only criterion for inclusion in this heading was that the group served as a control population, i.e., they had no liver or gallbladder disease. Most "control" populations were quite heterogeneous: age ranges 18–87, sex ratios (male:female) from 0:1 to 1:0, percent of ideal body weight from 86 to 281%.

‡ Studies were done by duodenal perfusion technique after patients had completely recovered from surgery.

plete gallbladder emptying and slower intestinal transit.

Biliary lipid composition is dependent on the rate of biliary bile acid secretion. In patients with bile fistulae the molar percent cholesterol of bile increases as the bile acid secretion rate drops (12–14). In subjects with gallbladders, fasting sequesters part of the bile acid pool in the gallbladder, induces a lower bile acid secretion rate (6, 8, 15) and increases cholesterol saturation of bile (6, 15).

When similar groups of subjects from different studies are compared, the molar percent cholesterol during continuous liquid formula infusion (2.9±0.9% [24], 3.4±0.4% [25]) is less than that during continuous amino acid infusion (4.3±0.5% [3]). In the present study we have shown a similar difference between the two infusates in the same individual. We conclude that the decreased molar percent cholesterol during liquid formula infusion is the result of a higher rate of bile acid secretion due to more complete gallbladder emptying and more rapid small intestinal transit.

Since the molar percent cholesterol of bile varies with bile acid secretion rate, the relationship of cholesterol secretion to bile acid secretion must necessarily be curvilinear over the full range of bile acid secretion rates. However, if only a portion of the range of bile acid secretion rates is examined, the relationship of cholesterol to bile acid secretion may appear to be linear. In our study, when data from both the amino acid and liquid formula infusions were combined, the relationship of cholesterol secretion to either bile acid or phospholipid secretion was curvilinear (Table II). On the other hand, when data from each infusion are analyzed separately (Fig. 3), these relationships are linear, as only a fraction of the range of bile acid (or phospholipid) secretion rates is evaluated. This may explain why some authors have found the secretory relationships of cholesterol to phospholipid or bile acid to be linear (3, 7, 9, 23, 28, 34, 35), whereas others found them to be curvilinear (12, 13).

Gallbladder volume fluctuated during most studies and the gallbladder never emptied completely during any infusion, but large (10–20 ml) fluctuations were common only during amino acid infusion. Pulses of gallbladder bile periodically join the stream of hepatic bile entering the duodenum and are responsible for some of the apparent fluctuation in the secretion of biliary lipids in secretion studies. When this occurs the measured secretion rate is not the actual hepatic secretion rate.

Small intestinal transit is also an important determinant of bile acid cycling frequency (35), and thus the rate of biliary lipid secretion (29, 36). Faster small intestinal transit increases bile acid cycling frequency and the rate of biliary lipid secretion. In the present study faster intestinal transit occurred during liquid formula infusion and was associated with higher rates of biliary lipid secretion.

The more complete gallbladder emptying and more rapid intestinal transit during liquid formula infusion are probably related to greater stimulation and release of humoral mediators of gallbladder contraction and intestinal peristalsis. Pancreatic polypeptide release from the pancreas parallels that of cholecystokinin and motilin (37, 38), two hormones thought to be responsible for gallbladder emptying and small intestinal peristalsis. Reliable assays for the latter hormones were not available to us, but the two- to threefold greater rise in serum pancreatic polypeptide in four of five subjects during liquid formula infusion indicates greater humoral stimulation.

In summary, we have shown that biliary lipid secretion rates are dependent on stimulation of gallbladder emptying and small intestinal transit. Reported differences in mean secretion rates between groups of subjects from different studies or between different groups of subjects within the same study may be due to differences in stimulation of gallbladder emptying and intestinal transit. Monitoring gallbladder emptying and intestinal transit during biliary lipid secretion studies is important for the interpretation of results. In addition, in healthy individuals gallbladder emptying and small intestinal transit affect biliary lipid composition.

## ACKNOWLEDGMENTS

The authors are grateful to Dr. Richard Jones and Dr. Philip Archer, Department of Biometrics, University of Colorado School of Medicine, Denver, CO, for their help with the statistical analysis of data, and thank the Advanced Diagnostic Research (ADR) Corp., Tempe, AZ, for providing the real-time scanner (model 2131) used in these studies. The authors also wish to thank Dr. Ian Taylor, Sepulveda Veterans Administration, Sepulveda, CA, for performing the pancreatic polypeptide radioimmunoassay.

This work was supported by grant AM 19605 from the National Institutes of Arthritis, Metabolism, and Digestive and Kidney Disease, National Institutes of Health; and by the Clinical Research Center, University of Colorado School of Medicine, grant RR-0051 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.

## REFERENCES

1. Grundy, S. M., and A. L. Metzger. 1972. A physiological method for estimation of hepatic secretion of biliary lipids in man. *Gastroenterology.* **62:** 1200–1216.
2. Shaffer, E. A., and D. M. Small. 1977. Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. *J. Clin. Invest.* **59:** 828–840.
3. Kern, F., Jr., G. T. Everson, B. DeMark, C. McKinley, R. Showalter, W. Erfling, D. Z. Braverman, P. Szczepanik-vanLeeuwen, and P. D. Klein. 1981. Biliary lipids, bile acids, and gallbladder function in the human female. *J. Clin. Invest.* **68:** 1229–1242.

4. Go, V. L. W., A. F. Hofmann, and W. H. J. Summerskill. 1970. Pancreozymin bioassay in man based on pancreatic enzyme secretion: potency of specific amino acids and other digestive products. *J. Clin. Invest.* **49:** 1558–1564.
5. Mok, H. Y. I., K. vonBergmann, and S. M. Grundy. 1979. Effects of continuous and intermittent feeding on biliary lipid outputs in man: application for measurements of intestinal absorption of cholesterol and bile acids. *J. Lipid Res.* **20:** 389–398.
6. Northfield, T. C., and A. F. Hofmann. 1975. Biliary lipid output during three meals and an overnight fast. I. Relationship to bile acid pool size and cholesterol saturation of bile in gallstone and control subjects. *Gut.* **16:** 1–17.
7. LaRusso, N. F., P. A. Szczepanik, and A. F. Hofmann. 1977. Effect of deoxycholic acid ingestion on bile acid metabolism and biliary lipid secretion in normal subjects. *Gastroenterology.* **72:** 132–140.
8. Mok, H. Y. I., K. von Bergmann, and S. M. Grundy. 1980. Kinetics of the enterohepatic circulation during fasting: biliary lipid secretion and gallbladder storage. *Gastroenterology.* **78:** 1023–1033.
9. LaRusso, N. F., N. E. Hoffman, A. F. Hofmann, T. C. Northfield, and J. L. Thistle. 1975. Effect of primary bile acid ingestion on bile acid metabolism and biliary lipid secretion in gallstone patients. *Gastroenterology.* **69:** 1301–1314.
10. Krag, E., and S. F. Phillips. 1974. Active and passive bile acid absorption in man: perfusion studies of the ileum and jejunum. *J. Clin. Invest.* **53:** 1686–1694.
11. Reichen, J., and G. Paumgartner. 1980. Excretory function of the liver. *In* Liver and Biliary Tract Physiology. I. International Review of Physiology, Chapter 3. W. B. Javitt, editor. University Park Press, Baltimore, MD. **21:** 103–150.
12. Lindblad, L., K. Lundholm, and T. Schersten. 1977. Influence of cholic and chenodeoxycholic acid on biliary cholesterol secretion in man. *Eur. J. Clin. Invest.* **7:** 383–388.
13. Wagner, C. I., B. W. Trotman, and R. D. Soloway. 1976. Kinetic analysis of biliary lipid excretion in man and dog. *J. Clin. Invest.* **57:** 473–477.
14. Nilsson, S., and T. Schersten. 1969. Importance of bile acids for phospholipid secretion into human hepatic bile. *Gastroenterology.* **57:** 525–532.
15. Metzger, A. L., R. Adler, S. Heymsfield, and S. M. Grundy. 1973. Diurnal variation in biliary lipid composition. Possible role in cholesterol gallstone formation. *N. Engl. J. Med.* **288:** 333–336.
16. Bond, J. H., Jr., and M. D. Levitt. 1975. Investigation of small bowel transit time in man utilizing pulmonary hydrogen ($H_2$) measurements. *J. Lab. Clin. Med.* **85:** 546–555.
17. Hurlock, G., and P. Talalay. 1957. Principles of the enzymatic measurement of steroids. *J. Biol. Chem.* **227:** 37–52.
18. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
19. Seligson, D., J. Marino, and E. Dodson. 1957. Determination of sulfobromophthalein in serum. *Clin. Chem.* **3:** 638–645.
20. Kern, F., Jr., H. Eriksson, T. Curstedt, and J. Sjovall. 1977. Effect of ethinyl estradiol on biliary excretion of bile acids, phosphatidyl cholines, and cholesterol in the bile fistula rat. *J. Lipid Res.* **18:** 623–634.
21. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1968. Dietary $\beta$-sitosterol as an internal standard to correct for cholesterol losses in sterol balance studies. *J. Lipid Res.* **9:** 274–387.
22. Everson, G. T., D. Z. Braverman, M. L. Johnson, and F. Kern, Jr. 1980. A critical evaluation of real-time ultrasonography for the study of gallbladder volume and contraction. *Gastroenterology.* **79:** 40–46.
23. Bond, J. H., and J. D. Levitt. 1977. Use of breath hydrogen ($H_2$) to quantitate small bowel transit time following partial gastrectomy. *J. Lab. Clin. Med.* **90:** 30–36.
24. Schwartz, C. C., H. R. Almond, Z. R. Vlahcevic, and L. Swell. 1979. Bile acid metabolism in cirrhosis. V. Determination of biliary lipid secretion rates in patients with advanced cirrhosis. *Gastroenterology.* **77:** 1177–1182.
25. Bennion, L. J., D. M. Mott, and B. V. Howard. 1980. Oral contraceptives raise the cholesterol saturation of bile by increasing biliary cholesterol secretion. *Metab. Clin. Exp.* **29:** 18–22.
26. Grundy, S. M., W. C. Duane, R. D. Adler, J. M. Aron, and A. L. Metzger. 1974. Biliary lipid outputs in young women with cholesterol gallstones. *Metab. Clin. Exp.* **23:** 67–73.
27. Bennion, L. J., and S. M. Grundy. 1975. Effects of obesity and caloric intake on biliary lipid metabolism in man. *J. Clin. Invest.* **56:** 996–1011.
28. Mabee, T. M., P. Meyer, L. DenBesten, and E. E. Mason. 1976. The mechanism of increased gallstone formation in obese human subjects. *Surgery (St. Louis).* **79:** 460–468.
29. Valdivieso, V., R. Palma, F. Nervi, C. Covarrubias, C. Severin, and C. Antezana. 1979. Secretion of biliary lipids in young Chilean women with cholesterol gallstones. *Gut.* **20:** 997–1000.
30. Mok, H. Y. I., K. vonBergmann, and S. M. Grundy. 1978. Effects of interruption of enterohepatic circulation on biliary lipid secretion in man. *Dig. Dis.* **23:** 1067–1075.
31. Key, P. H., G. G. Bonorris, J. W. Marks, A. Chung, and L. J. Schoenfield. 1980. Biliary lipid synthesis and secretion in gallstone patients before and during treatment with chenodeoxycholic acid. *J. Lab. Clin. Med.* **95:** 816–826.
32. Adler, R. D., A. L. Metzger, and S. M. Grundy. 1974. Biliary lipid secretion before and after cholecystectomy in American Indians with cholesterol gallstones. *Gastroenterology.* **66:** 1212–1217.
33. Almond, H. R., R. Vlahcevic, C. C. Bell, D. H. Gregory, and L. Swell. 1973. Bile acid pools, kinetics and biliary lipid composition before and after cholecystectomy. *N. Engl. J. Med.* **289:** 1213–1216.
34. Einarsson, K., and S. M. Grundy. 1980. Effects of feeding cholic acid and chenodeoxycholic acid on cholesterol absorption and hepatic secretion of biliary lipids in man. *J. Lipid Res.* **21:** 23–34.
35. Einarsson, K. A., S. M. Grundy, and W. G. M. Hardison. 1979. Enterohepatic circulation rates of cholic acid and chenodeoxycholic acid in man. *Gut.* **20:** 1078–1082.
36. Mok, H. Y. I., K. vonBergmann, and S. M. Grundy. 1977. Regulation of pool size of bile acids in man. *Gastroenterology.* **73:** 684–690.
37. Floyd, J. C. 1980. Pancreatic polypeptide. *Clin. Gastroenterology.* **9:** 657–678.
38. Keane, F. B., E. P. Di Magno, R. R. Dozius, V. L. W. Go. 1980. Relationships among canine interdigestive exocrine pancreatic and biliary flow, duodenal motor activity, plasma pancreatic polypeptide and motilin. *Gastroenterology.* **78:** 310–316.