

# Effects of fasting on bile acid metabolism and biliary lipid composition in man

William C. Duane<sup>1</sup>, Ronald L. Ginsberg, and Lynn J. Bennion

Phoenix Clinical Research Section, National Institute of Arthritis, Metabolism and Digestive Diseases, Phoenix Indian Medical Center, Phoenix, Arizona 85016

**Abstract** The effects of a four to six day fast on gallbladder bile lipid composition, bile acid pool size, bile acid composition, and cholic acid metabolism have been determined in normal human subjects. Total bile acid pool size and cholic acid pool size were measured before and after fasting by a one-sample technique previously validated in our laboratory. The rate of synthesis of cholic acid and its fractional turnover rate before fasting were measured using standard techniques. Estimates of fasting cholic acid synthesis rate and fractional turnover rate were calculated as daily averages from the change in cholic acid pool size, in combination with the change in cholic acid specific activity, during the fasting period. Since these estimates are approximate, a maximum value for cholic acid synthesis rate during fasting was also determined by assuming that the entire change in cholic acid specific activity during the fasting period occurred instantaneously.

The molar percent of cholesterol in gallbladder bile was reduced in eight of nine subjects after a four to six day fast ( $P < .01$ ; mean reduction 30.5%). The molar percents of bile acid and phospholipid were not significantly altered by fasting. The cholesterol saturation index, calculated on the basis of these data, was reduced by an average of 31.0% after a four to six day fast ( $P < .02$ ). The average daily cholic acid synthesis rate and the fractional turnover rate were reduced in all six subjects on whom isotope kinetic studies were carried out. The mean decrease in synthesis rate was 68.5% ( $P < .05$ ; range 55.2–79.8%) while the mean decrease in fractional turnover rate was 64.4% ( $P < .05$ ; range 30.2–100%). Reduction in synthesis rate was confirmed by the determination of maximum fasting synthesis of cholic acid, which averaged 61.1% lower than synthesis in the fed period. Fasting had no consistent effect on total bile acid pool size, cholic acid pool size, or bile acid species composition.

**Supplementary key words** cholesterol · fasting

Studies in animal models during the fasting state have provided information regarding the regulation of bile acid metabolism (1–3). In the rat, hepatic cholesterol synthesis falls ten-fold within 24–48 hr of complete caloric restriction (3, 4). Similarly, bile acid synthesis and secretion rates in the Rhesus monkey are reduced by a 1–5 day fast (1). Even in

the relatively short fasts of a regular feeding cycle in the rat, both 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and cholesterol 7 $\alpha$ -hydroxylase, the rate limiting enzymes in cholesterol and bile acid synthesis, respectively, show rises in activity with feeding and reductions in activity to a plateau during fasting (2, 5, 6).

Few studies have been reported regarding bile acid metabolism in humans during fasting. Stanley (7) demonstrated a reduced fecal excretion of isotopic cholic acid in fasting versus fed human subjects, providing evidence for a decreased fractional turnover rate of bile acids during fasting. Several studies have demonstrated that the molar percent of cholesterol (relative to bile acids and phospholipid) in human hepatic and gallbladder bile is significantly higher after a 6–12 hr fast than in the fed state (8–10). This has led to speculation that more prolonged fasting may promote cholesterol gallstone formation; however, the effect of several days of fasting on the lipid composition of gallbladder bile has not been examined.

In the present investigation, isotope dilution techniques have been employed in human subjects to estimate cholic acid synthesis rate, fractional turnover rate, pool size, and total bile acid pool size before and after a 4–6 day fast. In addition, the effect of fasting on bile acid composition and gallbladder bile lipid composition has been examined.

## METHODS

### Subjects

Nine healthy volunteers ranging in age from 18 to 35 years were studied on the metabolic ward of the

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; SA<sub>c</sub>, cholic acid specific activity.

<sup>1</sup> Present address: Department of Internal Medicine, Veterans Administration Hospital, 54th St. and 48th Ave. South, Minneapolis, Minnesota 55417.

Phoenix Clinical Research Section. Three were male, six were female. Three were American Indian (LH, FBa, JB) and the remainder were Caucasian. None had evidence of hepatobiliary or other abnormalities as judged by medical history, physical examination, and screening laboratory procedures including chest X-ray, complete blood count, urinalysis, serum glutamic-oxaloacetic transaminase, alkaline phosphatase, bilirubin, electrolytes, thyroxine, albumin, plasma cholesterol, and triglycerides. Informed consent was given by all volunteers prior to study.

### Study design

Initially each subject lived on the metabolic ward eating a repetitive, standardized diet for two to three weeks. After this stabilization period, the study was begun as follows. In the evening, 6–8 hr after a light meal, each subject swallowed a polyvinyl tube weighted with metal aspiration tip and mercury bag. About 20  $\mu\text{Ci}$  of [2,4- $^3\text{H}$ ]cholic acid (New England Nuclear, Boston, Mass.) was administered via the tube which was then flushed with 50 ml of sodium bicarbonate solution. The following morning, the position of the tube was checked by X-ray to insure that the aspiration tip was adjacent to the ampulla of Vater. Gallbladder contraction was then induced by intraduodenal infusion of an amino acid solution (5% protein hydrolyzate, Cutter Laboratories, Berkeley, Calif.) and bile collected on ice for 45–60 min by siphonage from the duodenum. A 1–3 ml aliquot of this bile was retained for analysis; the remainder was diluted 3–5 fold and returned to the subject by slow drip through the duodenal tube. In three of the nine subjects the fasting period was begun immediately after collection of this bile sample and removal of the tube.

The remaining six subjects were designated to have bile acid kinetic studies. Therefore bile samples were collected from these six subjects on the three subsequent days as well. Between these multiple control samplings, the tube was removed and the six subjects ate normally throughout the day. After the collection on the fourth control day the tube was removed, and each of the six subjects began the fasting period. Although the bile collection of the fourth day marks the beginning of the period during which there is no artificial induction of gallbladder contraction, it is important to note that at this point the six subjects had actually been fasting for about 15 hr. During the fasting period all subjects were restricted to the ward and were allowed access to only water, coffee, tea, and 2–3 cans per day of diet soda containing less than 3 calories per can. Urinary ketones were measured daily and serum electrolytes, blood

urea nitrogen, uric acid, and blood sugar were measured every other day. All subjects had strongly positive urinary ketones after the second day of the fast. Side effects during the fast were hunger, occasional postural hypotension, and mild weakness. None of these were severe enough to warrant discontinuation of the study.

On the fourth evening of the fast each of the nine subjects except LH again swallowed the polyvinyl tube. About 2  $\mu\text{Ci}$  [carboxyl- $^{14}\text{C}$ ]cholic acid (New England Nuclear) were administered via the tube and bile samples were collected the following two mornings as described above. LH consented to a longer fast and received [ $^{14}\text{C}$ ]cholate on the sixth evening of the fast instead of the fourth evening. After the second collection the fast was ended.

Each of the six bile samples was analyzed in duplicate for total bile acids by an automated procedure based on Talalay's enzymic assay (11), and for phospholipid and cholesterol as previously described (12). In addition duplicate aliquots of each sample were taken for determination of acid composition and cholic acid specific activity as follows. Bile acids were deconjugated by a 3 hr hydrolysis in 1.25 N NaOH at 125°C and 15 psi. The hydrolysate was acidified and extracted with 2.5 volumes of  $\text{CHCl}_3$ -MeOH 8:3. An aliquot of this chloroform extract was used to determine bile acid composition by gas-liquid chromatography of the acetate derivatives of the bile acid methyl esters on 1.5% QF-1. The remaining extract was applied to thin-layer chromatography plates which were subsequently developed in isooctane-ethyl acetate-acetic acid 5:5:1. The cholic acid band was identified with  $\text{I}_2$  vapor and eluted with  $\text{CHCl}_3$ -MeOH 8:3. One aliquot of the eluate was assayed for radioactivity. Another aliquot was evaporated and redissolved in a known volume of 0.01 N NaOH for enzymatic determination of cholic acid mass. These eluates were periodically analyzed by gas-liquid chromatography to insure that they contained only cholic acid. This method is accurate to  $\pm 3.8\%$  as determined on standard mixtures of unconjugated bile acids of known cholic acid specific activity ( $\text{SA}_c$ ).

### Calculations

Statistical comparison of data from control and testing periods was done by non-paired Student's *t* test.

Lipid composition of gallbladder bile is expressed as molar percent bile acids, phospholipid, and cholesterol obtained by summing the total moles of these three lipids and dividing that number into the moles of each individual lipid. Lipid composition of gallbladder bile for the control period was obtained by

averaging the lipid compositions of the first four bile specimens. Lipid composition of gallbladder bile for the fasting period was obtained by averaging the data for the last two bile specimens. The mean precision of the molar percent bile acid, phospholipid, and cholesterol measurements were 1.80, 1.39, and 0.75 mole percent, respectively, in subjects during the control period.

For a set of  $n$  replicate measurements of a variable,  $x$ , precision is defined as:

$$\frac{\bar{x} - x_1 + \bar{x} - x_2 + \bar{x} - x_3 + \dots + \bar{x} - x_n}{n}$$

Where  $\bar{x}$  is the mean of the  $n$  replicate measurements and  $x_1, x_2, x_3, \dots, x_n$  are the individual measurements. In each subject precision for the measurement of each biliary lipid was calculated. For the control period  $n = 4$  and for the fasting period  $n = 2$ . Mean precision for each biliary lipid was calculated by averaging the precision of all subjects.

During the fasting period these mean precision figures were 1.90, 1.94, and 0.29 molar percent for bile acids, phospholipid, and cholesterol, respectively. The saturation index for cholesterol solubility was obtained by the method of Thomas and Hofmann (13) using the line of maximum cholesterol solubility determined by Holzbach (14) and Hegardt and Dam (15).

Individual bile acid composition is expressed as weight percent, obtained by summing the total mass (mg) of all species of bile acid and dividing that number into the mg of each individual bile acid. Again the bile acid composition for the control period was obtained by averaging the data on the first four bile specimens, while bile acid composition for the fasting period was obtained by averaging the data on the last two bile specimens.

For the control period, precision of cholic, chenodeoxycholic, deoxycholic, and lithocholic acids, respectively, were 2.48, 1.81, 1.28 and 0.28. In the fasting period, these figures were 0.95, 1.06, 1.33, and 0.64, respectively. These values were derived only from data on subjects who had bile acid kinetic studies.

Total bile acid pool size before the fast was calculated on the basis of the administered [ $^3\text{H}$ ]-cholic acid according to the equation:

$$P = \text{dpm}_a(B/\text{dpm})$$

where

- $P$  = the total bile acid pool size.
- $B$  = the concentration of total bile acids in the first sample of gallbladder bile.

dpm = the concentration of radioactivity in the same bile sample.

$\text{dpm}_a$  = the dose of radioactivity administered the evening prior to sampling.

Total bile acid pool after the fast was calculated in similar fashion on the basis of [ $^{14}\text{C}$ ]cholic acid in the first fasting sample of gallbladder bile.

Cholic acid pool was calculated from the [ $^3\text{H}$ ]-cholic acid specific activity in the first control bile sample and from the [ $^{14}\text{C}$ ]cholic acid specific activity in the first fasting bile sample according to the equation:

$$C = \text{dpm}_a/\text{SA}_c$$

where  $\text{dpm}_a$  has the same meaning as above,  $C$  is the cholic acid pool, and  $\text{SA}_c$  is the cholic acid specific activity.

This one-sample measurement of bile acid pool size is precise to  $\pm 2.6\%$  as determined in our laboratory by repeat measurements on fifteen normally fed subjects (16). A discussion of assumptions involved with this method in comparison to the Lindstedt method has been published previously (16).

Cholic acid synthetic rate ( $s_1$ ) and fractional turnover rate ( $k_1$ ) in the prefasting control period were obtained by calculating a regression line of  $\ln(\text{SA}_c)$  versus time for the four control period samples. The fractional catabolic rate,  $k_1$ , is the slope of this line, having the units of inverse time. Cholic acid synthetic rate is calculated by multiplying the fractional catabolic rate by the cholic acid pool size or  $s_1 = k_1 C$ .

Synthetic rate and fractional catabolic rate during the fasting period were calculated as average synthesis per day ( $\bar{s}_2$ ) and average fractional catabolic rate per day ( $\bar{k}_2$ ). It should be emphasized that these values are approximate daily averages and do not necessarily represent actual synthesis or removal rate for any given hour or day of the fasting period. For purposes of calculation, these daily averages are treated as constants. Then for a single pool model of cholic acid and [ $^3\text{H}$ ]cholic acid pools the following differential equations apply:

$$dC/dt = \bar{s}_2 - \bar{k}_2 C \quad \text{Eq. 1}$$

$$dC^*/dt = -k_2 C^* \quad \text{Eq. 2}$$

where  $C$  is the cholic acid pool and  $C^*$  is the [ $^3\text{H}$ ]cholic acid pool. Solving these two equations:

$$C(t) = (C_0 - \bar{s}_2/\bar{k}_2)e^{-k_2 t} + \bar{s}_2/\bar{k}_2 \quad \text{Eq. 3}$$

$$C^*(t) = C^*_0 e^{-k_2 t} \quad \text{Eq. 4}$$

TABLE 1. Gallbladder bile lipid composition during control and fasting periods<sup>a</sup>

Subject	Bile Acid			Phospholipid			Cholesterol			Saturation Index		
	Control Period	Fasting Period	% Change	Control Period	Fasting Period	% Change	Control Period	Fasting Period	% Change	Control Period	Fasting Period	% Change
MM	79.6	77.8	- 2.26	13.8	14.6	+ 5.80	6.70	7.66	+14.3	1.38	1.51	+ 9.42
ST	80.0	72.7	-10.0	12.4	22.8	+83.9	6.85	4.52	-34.0	1.50	.667	-55.5
TF	74.6	76.0	+ 1.88	17.4	19.5	+12.1	7.96	4.57	-42.6	1.39	.755	-45.7
MD	75.4	78.5	+ 4.11	19.1	18.2	- 4.71	5.45	3.32	-39.1	.907	.581	-35.9
LH	64.7	70.8	+ 9.43	26.8	24.0	-10.4	8.49	5.14	-39.5	1.10	.729	-33.7
FBr	73.9	65.5	-11.4	20.9	31.1	+48.8	5.22	3.35	-35.8	.816	.413	-61.6
FBa	74.7	77.9	+ 4.28	18.2	15.1	-17.0	7.10	7.00	- 1.41	1.21	1.36	+12.4
JB	63.3	72.8	+15.0	29.2	23.4	-19.9	7.50	3.80	-49.3	.932	.554	-40.6
BH	77.5	87.4	+12.8	16.2	9.30	-42.6	6.28	3.30	-47.5	1.17	.845	-27.8
Mean	73.8	75.5	+ 2.65	19.3	19.8	+ 6.22	6.84	4.74	-30.5	1.16	.824	-31.0
SEM	2.02	2.04	3.09	1.86	2.14	12.84	0.36	0.54	7.30	0.080	0.123	8.66
	NS			NS			<i>P</i> < 0.01			<i>P</i> < 0.02		

<sup>a</sup> Values given as molar percent.

where

$t$  = the time of fasting, usually 4 days.

$C(t)$  = the cholic acid pool at time  $t$  calculated as above on the basis of [<sup>14</sup>C]cholic specific activity of the first fasting bile sample.

$C^*(t)$  = the [<sup>3</sup>H]cholic acid pool at time  $t$  calculated by multiplying  $C(t)$  by the [<sup>3</sup>H]cholic acid specific activity in the first fasting bile sample.

$C_0$  = the cholic acid pool at the beginning of the fast, i.e., the control period cholic acid pool.

$C^*_0$  = the [<sup>3</sup>H]cholic acid pool at the beginning of the fast calculated by multiplying  $C_0$  by the [<sup>3</sup>H]cholic acid specific activity in the bile sample taken just before the fasting period began.

Then:

$$\bar{k}_2 = \frac{\ln(C^*(t)/C^*_0)}{t} \quad \text{Eq. 5}$$

$$\bar{s}_2 = \frac{\bar{k}_2 [C(t) - C_0 e^{-\bar{k}_2 t}]}{1 - e^{-\bar{k}_2 t}} \quad \text{Eq. 6}$$

As stated above, these values are daily averages and are to some extent approximations. Therefore, to confirm a reduction in synthesis during the fasting period, a value for maximum cholic acid synthesis during the fasting period ( $s_{\max}$ ) was derived. The following reasoning was applied to this calculation. During the fasting period there is a decline in specific activity of [<sup>3</sup>H]cholic acid from  $SA_c(0)$  to  $SA_c(t)$  as shown in Table 2. The fall in  $SA_c$  occurs because of isotope dilution by newly synthesized cholic acid. The absolute amount of [<sup>3</sup>H]cholic acid available for dilution is maximal at time zero. Thus total cholic acid synthesized during the fast would be maximal if all

synthesis occurred instantaneously at time zero. If all cholic acid synthesis occurs instantly at time zero, there would be a change in cholic acid pool size from  $C_0$  (Table 2) to a hypothetical value,  $C'$ , and a fall in [<sup>3</sup>H]cholic acid specific activity from  $SA_c(0)$  to  $SA_c(t)$ . Since the quantity of isotope remains unchanged:

$$[SA_c(0)][C_0] = [SA_c(t)][C'] \quad \text{Eq. 7}$$

Rearranging this equation:

$$C' = [SA_c(0)/SA_c(t)][C_0] \quad \text{Eq. 8}$$

The total amount of synthesized cholic acid is then:

$$s_{\max} = C' - C_0 = [C_0][SA_c(0)/SA_c(t) - 1] \quad \text{Eq. 9}$$

In order to compare maximum cholic acid synthesis during the fast to cholic acid synthesis in the control period,  $s_{\max}$  was normalized to average maximum daily synthesis ( $\bar{s}_{\max}$ ). Thus  $\bar{s}_{\max} = s_{\max}/t$

It should be emphasized that the calculation of  $s_{\max}$ ,  $s_2$ , and  $k_2$  was performed using a single piece of data from the control period and a single piece of data from the fasting period. Therefore these values may be subject to more error than if calculation had been performed using a larger data set.

## RESULTS

The effect of a 4–6 day fast on the lipid composition of gallbladder bile is summarized in Table 1. The molar percent of cholesterol decreased in all but one of the nine subjects during fasting, the average decrease being 30.5% of control ( $P < .01$ ). No significant change in the molar percent of either bile acid or phospholipid occurred during fasting. Cholesterol saturation index, calculated on the basis of these data, was reduced in seven of the nine subjects ( $P < .02$ ) with a mean reduction of 31%.

TABLE 2. Cholic acid pool size, tritiated cholic specific activities and tritiated cholic acid pool sizes before and after fasting

Subject	C <sub>0</sub> <sup>b</sup>	SA <sub>c</sub> (0)	C* <sub>0</sub>	t <sup>a</sup>	C(t) <sup>c</sup>	SA <sub>c</sub> (t)	C*(t)
	mg	dpm/mg	dpm × 10 <sup>6</sup>	days	mg	dpm/mg	dpm × 10 <sup>6</sup>
MD	1286	17,260	22.2	4.0	1376	11,890	16.4
LH	804	11,520	9.26	6.0	645	5648	3.64
FBr	1541	10,840	16.7	4.0	1029	8253	8.49
FBa	513	38,980	20.0	4.0	476	31,840	15.2
JB	454	2332	1.06	4.0	900	1194	1.07
BH	1385	15,000	20.8	4.0	1786	11,450	20.4
Mean	997	-----	NS	-----	1035	-----	-----
SEM	191	-----	-----	-----	197	-----	-----

<sup>a</sup> Time between the last control sample and the first fasting sample is denoted by t.

<sup>b</sup> Values at the beginning of the fast are denoted by 0.

<sup>c</sup> C is the cholic acid pool, C\* is the [<sup>3</sup>H]cholic acid pool, SA<sub>c</sub> is cholic acid specific activity.

Both average daily cholic acid synthesis rate and fractional turnover rate were decreased during fasting in all six of the subjects who had isotope kinetic studies (Tables 2 and 3). The decrease in synthesis and fractional turnover were both significant at the  $P < .05$  level. The magnitude of reduction in fractional turnover rate was variable, ranging from 30.2% to 100% with a mean reduction of 69.2%. The magnitude of reduction in cholic acid synthesis rate was more constant, ranging from 51.8% to 79.8%, with a mean reduction of 68.5%.

Reduction of synthesis rate in response to fasting was confirmed by calculation of the maximum cholic acid synthesis compatible with the observed change in [<sup>3</sup>H]cholic acid specific activity. Each individual subject had a maximum fasting synthesis rate at least 47% lower than his synthesis rate in the control period (Table 3). For the entire group, maximum cholic acid synthesis during fasting was 61% lower than during the control period. Although these values for maximum synthesis rates during the fast probably overestimate true synthesis, they are close to our values for average daily synthesis rates (mean reduction 68.5%). This similarity occurred because so little labeled cholic acid was lost during the fast. Thus, the assumption of zero loss of labeled cholic acid used in the calculation of maximum synthesis rates was nearly met by actual fasting conditions.

Mean cholic acid pool size of our six subjects prior to fasting was not significantly different from mean pool size after a 4–6 day fast (Table 2). Some individual subjects however, showed substantial changes in cholic acid pool size during fasting. For example, in one subject (JB) the cholic acid pool increased during fasting by 98% while in another (FBr) the pool size decreased by 33%. Thus fasting does not appear to have any regular or consistent effect on the cholic acid pool.

Average total bile acid pool size of our nine sub-

jects was not significantly changed by fasting (Table 4). For individual subjects, the magnitude of change in total bile acid pool size was less variable than the magnitude of change in cholic acid pool size; however, five subjects increased their total bile acid pool and four subjects decreased their pool during fasting. Thus, as was the case with the cholic acid pool, fasting did not appear to have any consistent effect on total bile acid pool size.

Mean bile acid composition prior to fasting was nearly identical to mean bile acid composition after a 4–6 day fast (Table 4). Since total bile acid pool size also remained relatively constant, fasting did not appear to have any overall effect on the pools of chenodeoxycholic or deoxycholic acid.

## DISCUSSION

Perhaps the most surprising finding in the present investigation is that a 4–6 day fast reduced the molar percent of cholesterol, relative to bile acids and lecithin, in human gallbladder bile. Short fasts (3–12 hr) have been consistently associated with the opposite effect, namely an increase in molar percent cholesterol of both hepatic and gallbladder bile (8–10). Eight of our nine fasted subjects, however, showed a reduction in the molar percent cholesterol of gallbladder bile by the fourth day of fasting, and this reduction was greater than 30% in seven of these eight subjects. Thus, contrary to what might be expected, a 4 day fast appears to reduce the relative cholesterol content of gallbladder bile.

The mechanism of this reduction in molar percent cholesterol of gallbladder bile is unknown. It seems reasonable, however, to assume that the reduction of hepatic cholesterol synthesis known to occur in fasting animals (3, 4, 17) also occurs in fasting man. This reduced cholesterol production, in combination with

TABLE 3. Cholic acid synthesis and fractional turnover rates in control and fasting periods

Subject	Synthesis					Fractional Turnover			$r^a$
	Control Period ( $s_1$ )	Fasting Period ( $\bar{s}_2$ )	Change	Fasting Period Maximum ( $\bar{s}_{max}$ )	Change <sup>b</sup>	Control Period ( $k_1$ )	Fasting Period ( $\bar{k}_2$ )	Change	
	mg/day		%	mg/day	%	day <sup>-1</sup>		%	
MD	274	123	-55.2	145	-47.1	0.213	0.076	- 64.3	0.997
LH	414	83.7	-79.8	139	-58.1	0.514	0.156	- 69.6	0.997
FBr	373	84.5	-77.4	121	-67.6	0.242	0.169	- 30.2	0.998
FBa	75.8	25.0	-67.0	28.0	-62.0	0.148	0.069	- 53.4	0.990
JB	546	110	-79.8	108	-80.2	1.200	0.000	-100.0	0.997
BH	222	107	-51.8	107	-51.8	0.161	0.004	- 97.5	0.998
Mean	317	88.9	-68.5	108	-61.1	0.413	0.079	- 69.2	
SEM	66.8	14.2	5.1	17.1	4.8	0.167	0.029	10.9	

$\left[ \begin{array}{c} \text{Control Period} \\ \text{Fasting Period} \end{array} \right] P < 0.05$

$\left[ \begin{array}{c} \text{Control Period} \\ \text{Fasting Period} \end{array} \right] P < 0.05$

<sup>a</sup> Correlation coefficient of  $\ln(SA_c)$  vs time for the control period.

<sup>b</sup> Change in comparison of control synthesis rate.

complete absence of dietary cholesterol, would substantially reduce the amount of cholesterol available for secretion into bile. It is certainly conceivable that, under these circumstances, hepatic cholesterol secretion would be reduced relative to bile acid and/or lecithin secretion. Thus the effect of a 4 day fast, unlike that of a short overnight fast, may be to reduce the molar percent cholesterol of hepatic bile. Final conclusions on these possibilities, however, will require further investigation.

Several previously published studies appear to be at variance with our finding of a reduced molar percent cholesterol in gallbladder bile during fasting. Redinger, Hermann, and Small (1) have reported

that a 1-7 day fast increased the relative cholesterol content of bile in two Rhesus monkeys. They noted, however, that bile acid secretion reached a plateau within 24 hr of starting the fast while cholesterol secretion continued to fall throughout the entire fasting period. Thus, an eventual reduction in the molar percent cholesterol in bile by more prolonged fasting could not be excluded. It is possible that the effect of fasting in man is similar to that in the Rhesus monkey except that man is able to decrease the relative cholesterol level of bile more rapidly in response to fasting. Alternatively there may be a qualitative difference in the effect of fasting on bile lipid composition in these two different species.

TABLE 4. Total bile acid pool size and bile acid composition in control and fasting periods

Subject	Total Bile Acid Pool			Control Period				Fasting Period			
	Control Period	Fasting Period	Change	Cholic	Cheno	Deoxy	Litho	Cholic	Cheno	Deoxy	Litho
	mg	mg	%	mg%				mg%			
MM	2732	2670	- 2.3	53.0	39.4	7.60	0.02	46.6	38.0	15.4	0.75
ST	2587	2827	+ 9.3	43.6	33.9	22.0	0.54	46.4	33.5	19.2	0.58
TF	2360	2497	+ 5.8	51.5	37.9	10.4	0.16	48.8	40.4	10.6	0.07
MD	2810	3178	+13.1	42.7	45.5	10.9	0.86	46.3	45.6	8.01	0.14
LH	2742	2489	- 9.2	22.2	39.6	31.0	7.19	19.4	41.3	30.3	8.82
FBr	3613	3170	-12.3	48.4	41.7	9.38	0.50	41.3	42.7	15.7	0.35
FBa	2170	2143	- 1.2	24.9	62.6	11.9	0.71	30.4	60.9	8.45	0.23
JB <sup>a</sup>	2115	2344	+10.8	29.7	60.5	0.00	0.00	42.7	49.9	4.67	0.03
BH	3384	4364	+29.0	47.6	45.1	6.81	0.50	45.9	47.6	6.45	0.05
Mean	2724	2854	+ 4.78	40.4	45.1	12.2	1.16	40.9	44.4	13.2	1.22
SEM	169	222	4.22	3.91	3.32	3.03	0.76	3.24	2.64	2.67	0.95

NS

<sup>a</sup> Subject JB had ursodeoxycholic acid present in his bile. This bile acid accounted for 9.8% and 2.7% of bile acids in the control and fasting periods respectively.

Cheno, chenodeoxycholic acid; deoxy, deoxycholic acid; litho, lithocholic acid.

Finally, Schreiber et al. (18) have shown that prolonged reduction of caloric intake is associated with an increase in gallbladder bile molar percent cholesterol. Although their subjects were in a catabolic state, all subjects consumed some food everyday. Their diet provided dietary cholesterol and, in addition, probably stimulated hepatic cholesterol synthesis and gallbladder contraction. Thus the effects of this reduced, but positive, caloric intake on lipid composition of gallbladder bile would not be expected to simulate the effects of complete caloric restriction.

Use of the conventional Lindstedt method (19) to estimate bile acid synthesis and fractional removal rate during fasting might introduce a significant artifact into these measurements. Artificial stimulation of gallbladder contraction, which is necessary for sampling from humans in the Lindstedt method, would represent an important disruption of the fasting state. This potential source of artifact was avoided in the present study by collection of gallbladder bile only before and after the fasting period.

A possible source of error in this approach is that only two measurements of cholic acid specific activity together with two measurements of one-sample pool size are used in the estimation of synthesis and fractional removal rates during the fast. However, our measurements of one-sample pool size and cholic acid specific activity are both reproducible to within 5% (16). This error is small relative to the observed changes in synthesis and fractional turnover rates (about 70%, Table 3). It is unlikely, therefore, that measurement error could account for the observed reduction in synthesis and fractional turnover even in a single experimental subject. To further minimize the possibility of significant measurement error these measurements were performed on six individual subjects.

It should be noted that the reproducibility of data for our one-sample method of pool size determination was obtained on subjects who were eating normally. It is conceivable, therefore, that this method may not be as highly reliable in subjects who have fasted 4 days. On the other hand, if fasting impaired the reliability of this method, it is surprising that our values for maximum fasting cholic acid synthesis (calculation of which is independent of fasting pool size) are so similar to the values for average daily cholic acid synthesis during fasting (calculation of which depends on fasting pool size). Moreover, the near identity of total bile acid pool size in fed and fasting states (Table 4) would be an unexpected finding if fasting significantly impaired the reliability of this technique.

A disadvantage of the methods used in the present

investigation is that cholic acid synthesis and fractional turnover rates during the fast could be only approximated as daily averages. Since both of these variables may change progressively during the fasting period, our data do not permit a rigorous statement of synthesis or fractional turnover for any given hour or day of the fast. In the strictest sense we can say only that overall synthesis and fractional removal rates were reduced during a 4–6 day fast.

On the other hand, if cholic acid synthesis and fractional turnover rates were constant during the fasting period, our calculated daily averages would rigorously reflect synthesis and fractional turnover for any given day of the fast. It is possible that such constancy is the case since the prefasting bile sample was obtained in the morning after each subject had undergone his usual overnight fast. This 15 hr overnight fast may have been sufficient to allow fractional turnover and synthesis to level off at constant values. Fractional turnover rate is largely dependent on the loss of bile acid during enterohepatic circulation. It seems reasonable to suppose that this rate of loss would reach a plateau after all food had been absorbed from the gut. Moreover, Stanley's (7) study of fecal output of isotopic bile acid in response to fasting provides experimental justification for this assumption since the rate of excretion of isotope became constant within 1 day of fasting. The possibility that bile acid synthesis rate reaches a plateau by the 15th hour of fasting is supported by *in vitro* measurements of  $7\alpha$ -hydroxylase and HMG-CoA reductase. Activity of both of these enzymes fell rapidly within 6–8 hr of cessation of caloric intake, and reached a minimum by about 14 hr after the last meal (2, 5, 6, 20, 21). If fasting is continued for longer periods of time, the activity of both enzymes may vary, but to a much lesser extent than during a normal feeding cycle (20–23). Thus, changes in bile acid synthesis rate induced by fasting may occur almost entirely in the first 15 hr of the fast.

Although the time course of decline in bile acid synthesis has not been ascertained, the present study demonstrates that overall bile acid synthesis does decline during a 4 day fast in normal man. Fasting resulted in a mean reduction of 68.5% in average daily cholic acid synthesis. Even when synthesis during fasting was calculated as a maximum value, cholic acid synthesis during the fast averaged 61% lower than synthesis in the control period (Table 3). The magnitude of this reduction in synthesis is almost identical to the 68% reduction in bile acid synthesis found by Redinger et al. (1) in Rhesus monkeys fasted 3–5 days. Similarly, in the rat, cholesterol  $7\alpha$ -hydroxylase activity is known to decline in response

to fasting (2, 5, 23). In view of these animal data, it is not surprising that a 4 day fast reduces bile acid synthesis in humans as well.

The reduction in fractional turnover rate of cholic acid observed in our fasting subjects confirms a previous report by Stanley (7) who demonstrated a reduced fecal output of radioactive bile acid in fasting subjects given [<sup>14</sup>C]cholate intravenously. He reported values for cholate fractional turnover during fasting that ranged from 0.005 to 0.05 day<sup>-1</sup> while the range for our subjects was from 0.000 to 0.169 day<sup>-1</sup>. Both sets of data, therefore, suggest considerable biological variability in the response of cholate fractional turnover rate to fasting; however, a decrease of fractional turnover rate in fasting man would appear to be a uniform finding.

Neither the cholic acid pool nor the total bile acid pool showed any overall change during fasting in our subjects. Several individual subjects, however, had substantial changes in cholic acid pool during fasting. Total bile acid pool, while less variable than cholic acid pool, increased slightly in five subjects and decreased slightly in four subjects. Thus bile acid pool size does not appear to change in any consistent way during fasting, but rather may increase or decrease according to the relative magnitudes of reduction in synthesis rate and fractional turnover rate.

Numerous studies in animal models and in man indicate that bile acid synthesis is, at least in part, under negative feedback control by bile acids returning to the liver (24–27). Fasting, which is known to be accompanied by a reduction in hepatic bile secretion (1, 9), as well as a reduction in serum bile acid levels (28), is presumably associated with a decreased flux of bile acid through the liver. If bile acid synthesis in man were controlled only by negative feedback inhibition by bile acids returning to the liver, then fasting should increase, not decrease, bile acid synthesis rate. The demonstrated reduction in bile acid synthesis during fasting, therefore, strongly suggests that some additional regulatory mechanism of bile acid synthesis rate exists in man.

Further investigation will be required to elucidate this additional regulatory mechanism. In some animal species increased cholesterol input, in the form of added dietary cholesterol, enhances bile acid synthesis (29, 30). This effect has been difficult to demonstrate in man, however, because of limited absorption of dietary cholesterol (31–33). In the present investigation decreased cholesterol input probably occurred during the fasting period, both by deprivation of exogenous cholesterol and perhaps by diminished hepatic cholesterol synthesis. This decreased cholesterol input may have been responsible

for the observed reduction in bile acid synthesis during the fasting period. Alternatively, one of the many hormonal and metabolic adjustments which occur in response to fasting could play a regulatory role in bile acid synthesis. ■

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