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Caloric Restriction and Bile Salt Dynamics in Intact and Cholecystectomized Syrian Hamsters†

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In vivo studies of bile salt absorption and distribution using ^{14}C-taurocholate were made in fed and fasted, intact and cholecystectomized Syrian hamsters. There was no significant difference in the rate of ileal ^{14}C-bile salt absorption or the distribution of ^{14}C-bile salts among the compartments of the enterohepatic circulatory system in any of the animals. The results showed that the sphincter of Oddi is patent in fasting, intact and cholecystectomized hamsters. They also suggested that the diminished bile salt pool seen in fasted, cholecystectomized but not in intact hamsters arises from a) a primary, fasting-induced decrease in liver enzyme activity involved in bile acid synthesis; b) the lack of a gallbladder to store a portion of the bile acids in the enterohepatic circulatory pool; and c) losses of bile acids into the large gut during each cycle of the enterohepatic circulation.

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Several observations on the effects of fasting on bile physiology and bile acid and cholesterol metabolism in humans and lower animals have been reported. In Rhesus monkeys fasting results in depressed bile acid synthesis and a tendency toward lithogenic bile.[1] In rats[2] caloric restriction results in a tenfold decrease in the rate of hepatic cholesterol synthesis. Caloric restriction in intact humans[3,4] causes decreases in bile acid synthesis and excretion which persist during at least 16 days of fasting.

Data concerning the effects of fasting on bile acid metabolism in cholecystectomized animals have until recently been confined to observations during overnight fasts in humans. The results of several of these studies are conflicting. There is disagreement as to whether the circulation of the bile salt pool is continuous or interrupted.[4-7] The distribution of bile salts among the pool compartments during fasting and feeding is also largely speculative.[5-7] To explore these problems we studied bile acid metabolism in fed and fasted Syrian hamsters,[8] a species whose bile lipid composition and bile physiology are similar to those of humans. These studies showed that there were no significant differences in composition, size, or fractional turnover time of the bile salt pool in intact and cholecystectomized hamsters. In intact hamsters, fasting caused a shift in bile salts from the small intestine, cecum, and liver to the gallbladder, while in cholecystectomized animals there was a moderate shift in the percentage of bile salts from the liver and small intestine to the cecum. Interestingly, intact hamsters were able to maintain the size of their total bile salt pool during a fast, while the pools of cholecystectomized hamsters decreased.[9] Two hypotheses can be proposed to explain the mechanism responsible for this important difference.

The first hypothesis involves the opening and closing of the sphincter of Oddi, which presumably controls bile flow. If the patency of this sphincter is under hormonal control,[10] it is reasonable to expect that it would be closed during a fast and bile flow would cease most of the time since little or no chyme enters the duodenum to stimulate hormone production and sphincter opening. Under these conditions, in

intact animals fasting would result in cessation of enterohepatic circulation, rapid accumulation of bile salts in the gallbladder, and an increase in hepatic bile salt concentration which would cut off or diminish bile acid synthesis through negative feedback. Because of the capacity of the gallbladder, the size of the bile salt pool would be maintained despite the drop in the rate of hepatic bile acid synthesis. In cholecystectomized, fasted hamsters, closure of the sphincter of Oddi would result in a rapid increase in the concentration of hepatic bile salts due to new synthesis and return of the salts from the small intestine and cecum to the liver. When the concentration rose sufficiently, bile acid biosynthesis would again stop or diminish markedly due to negative feedback, but without a gallbladder cholecystectomized hamsters would accumulate only small amounts of bile salts in the liver-bile duct reservoir. If this condition were maintained for some time, it is conceivable that the bile salt pool would be diminished due to losses from the small intestine.

In the second hypothesis, the sphincter of Oddi is always patent and the effects seen in fasting are due primarily to a decrease in enzyme activity within the liver itself. Short fasts have been known to reduce hepatic enzyme activity and alter the diurnal pattern of rate-limiting enzymes of cholesterol and bile acid biosynthesis.[11] Diminished liver production of bile salts would result in an inability to restore normal losses that occur in the intestine as the pool circulates due to the patency of the sphincter of Oddi. While intact, fasted hamsters would maintain their bile salt pool size via storage of bile salts in the gallbladder, cholecystectomized hamsters would be unable to store sufficient bile salts to overcome the direct, fasting-induced effect of lower liver bile acid production and would thus exhibit a loss of pool size.

To examine these hypotheses we investigated the absorption and distribution of taurocholate-24-^{14}C introduced into the enterohepatic circulation of intact and cholecystectomized, fed and fasted Syrian hamsters.

Materials

Animals

The Syrian hamsters were three-month-old females weighing about 100 g at the start of the experiment (Charles River Breeding Laboratories, Inc, Wilmington, Mass).

The 48 animals used (intact and cholecystectomized) were maintained on Purina lab chow *ad libitum* for at least one month before the experiments. Their weights and food intake were recorded and their general health monitored.

Reagents and solvents

All chemicals used were reagent grade and all solvents were distilled before use. Sodium taurocholate was purchased from Calbiochem, San Diego, Calif, and sodium taurocholate-24-^{14}C from New England Nuclear, Boston, Mass. Thin-layer chromatography and scintillation counting showed that the radiochemical purity of this substance was 99+%, and its specific activity was 44.5 mCi/mmol.

The buffer solution used for injection into the ileal sac was prepared by adding 50 μCi of taurocholate-24-^{14}C and 8 x 10^4 mμ moles of sodium taurocholate to 100 ml of a solution that contained 10 mMoles $NaHCO_3$, 6 mMoles KCl, and 131 mMoles NaCl per liter. The pH was then adjusted to 7.1-7.2. A mixture of 5% CO_2 in O_2 was bubbled through the solution for 10 minutes before injection.[12]

The mixture used for scintillation counting was prepared by dissolving 10 g of 2,5-diphenyloxazole and 0.6 g of 1,4-bis-2-(methyl-5-phenyl-oxazole)-benzene in two liters of toluene and adding 266.6 ml of absolute ethanol.

Preparation of cholecystectomized hamsters

After the hamsters had been anesthetized with a mixture of oxygen and methoxyflurane, a midline incision was made above the liver. The gallbladder was carefully separated from the liver and the cystic duct ligated with 5-0 suture. The bladder was excised, and the incision closed using suture and wound clips. A few hamsters were sham operated to check for the effects, if any, of surgical shock. All animals were ready for use in two to three weeks.

Method

Forty-eight hamsters were divided equally into the following groups: a) fed intact; b) 48-hr fasted intact; c) fed cholecystectomized; and d) 48-hr fasted cholecystectomized. They were anesthetized with a mixture of oxygen and methoxyflurane. A 5 cm ventral midline incision was made from the distal thoracic cavity to an area immediately proximal to the external urethral orifice. During the entire protocol the animal's internal organs were kept moist by periodic wetting with isotonic saline solution. The small intestine was ligated and then transected immediately proximal to the point of ligation at the ileocecal junction. Another ligation of the small intestine was made 15 cm above the ileocecal junction with a measured marker. A large bore needle connected to a hose that joins a bulb suspended 60 cm in air was inserted into the small intestine distal to the point of this second ligation. Intestinal content was washed out with 30-40 ml of isotonic saline solution (maintained at

37°C in a water bath) added to the bulb-hose-needle apparatus. When washing was complete, a 10 cm segment of ileum measured back from the cecum was ligated at both ends. One ml of the prepared solution containing taurocholate-24-^{14}C and sodium taurocholate in buffer was introduced into the sac and absorption allowed to occur for 30 minutes, a period determined from a plot of ^{14}C-taurocholate activity at a number of time intervals in the *in vivo* preparation. During this time urine was collected via an indwelling catheter.

After the 30-minute period the animals were killed by cutting the heart muscle. The sac, remainder of the small intestine and stomach, liver and bile ducts, kidney, gallbladder (when present), and urinary bladder were removed and quick frozen. ^{14}C-activity in the sac, small intestine-stomach, kidney and liver was determined by lyophilization followed by combustion in oxygen, absorption of the resulting $^{14}CO_2$ in 40% ethanolic ethanolamine, and scintillation counting.[8] The ^{14}C activity in the urine, urinary bladder and gallbladder (plus content) was determined by ethanolic extraction followed by scintillation counting. ^{14}C activity is a valid indicator of bile acid mass, since bile acids are metabolic end products and maintain their basic structure until excreted.[13]

Statistics

Standard deviations were calculated for all grouped data. Student's *t* test was used to determine the significance of differences between different groups of data. The limit of significance was set at $p = 0.01$.

Results

The accompanying table shows the percentage distribution of ^{14}C-bile acids in the various compartments of the enterohepatic circulation and the kidney and urinary bladder plus content 30 minutes after ^{14}C-taurocholate was introduced into the ileal sac.

There was no significant difference in the percentage of ^{14}C-bile acid activity remaining in the sac in any of the groups (A-B, $p = 0.05$; A-C, $p = 0.50$; A-D, $p = 0.10$; B-C, $p = 0.50$; B-D, $p = 0.80$; C-D, $p = 0.40$). Thus, equal amounts of ^{14}C-taurocholate passed across the ileal wall in 30 minutes in each group. Likewise, there was no significant difference in the percentage of ^{14}C-bile acid activity in the stomach and intestine up to the point of ligation in any of the animals (A-B, $p = 0.90$; A-C, $p = 0.70$; A-D, $p = 0.40$; B-C, $p = 0.70$; B-D, $p = 0.40$; C-D, $p = 0.60$). These results would be possible only if the sphincter of Oddi were patent in all cases. The percentage of ^{14}C-bile acid activity found in the livers at the end of 30 minutes of absorption was the same in all cases (A-B, $p = 0.03$; A-C, $p = 0.90$; A-D, $p = 0.10$; B-C, $p = 0.10$; B-D, $p = 0.50$; C-D, $p = 0.30$). Interestingly, the gallbladder plus content showed very little ^{14}C activity in any group. The percentage of ^{14}C-bile acid found in kidneys and urinary bladders plus content was very small in each group. This finding lends support to the hypothesis that the sphincter of Oddi was patent in all cases, because if it had been closed and bile acids had been absorbed from the ileal sac, bile acid concentrations would have increased in the systemic circulation and ^{14}C activity would appear in the urine. Tests showed that there was a negligible amount of ^{14}C-activity in blood, skin, skeletal muscles, lungs, diaphragm, and large intestine plus content in each group.

Distribution of ^{14}C-Bile Acid Activity 30 Minutes After Introduction of ^{14}C-Taurocholate into the Ileal Sac

Type of hamster	Tissue	% ^{14}C-Bile Acid Activity	
		(A) — fed	(B) — fasted
Intact	Ileal sac	72.6 ± 18.8*	80.3 ± 21.2
	Intestine + stomach to ligation	18.8 ± 6.3	14.8 ± 3.4
	Liver	8.5 ± 2.0	4.9 ± 1.8
	Gallbladder	0.1	0.1
	Kidney	0.2	0.3
	Urinary bladder	0.1	0.1
		(C) — fed	(D) — fasted
Cholecystectomized	Ileal sac	72.6 ± 9.5	80.2 ± 7.8
	Intestine + stomach to ligation	15.7 ± 3.0	13.8 ± 5.9
	Liver	11.7 ± 5.2	6.0 ± 3.0
	Kidney	0.1	0.1
	Urinary bladder	0.2	0.1

*Standard deviation

Discussion

There are several important points among the results shown in the table. One is that the recorded ^{14}C activity in the stomach and intestine up to the point of ligation is the same in fed or fasted, intact, or cholecystectomized hamsters. These results run counter to the common notion[14] that the sphincter of Oddi closes during a fast in intact animals, since such action would interrupt the enterohepatic circulation and prevent passage of bile salts in the intestinal lumen, producing a significant decrease in recorded ^{14}C activity. It follows that the circulation of the bile salt pool continues during fasting in both intact and cholecystectomized hamsters. This observation supports data in humans,[15] hamsters,[9] and baboons[16] which suggest that at least some enterohepatic recycling of bile salts occurs without dietary stimuli.

Having determined that the sphincter of Oddi is patent during fasting, it is possible to choose between the hypoth-

eses proposed earlier to explain why intact hamsters are able to maintain the size of their bile salt pool during a fast while cholecystectomized hamsters cannot. During fasting, bile acid synthesis in both intact and cholecystectomized animals decreases because of primary decreases in liver enzyme activity. This concept is supported by studies[17-20] showing that fasting reduces the activities of both 3-hydroxy-3-methylglutaryl-CoA reductase and the 7α-hydroxylase of cholesterol, the rate-limiting enzymes of cholesterol and bile acid biosynthesis, respectively, in the liver. As the fast progresses, the enterohepatic circulation of bile salts continues, bceause of the patency of the sphincter of Oddi. In both types of animals, 5 to 10% of the pool is lost during each cycle.[13] However, there is an important difference. Intact hamsters can maintain the size of their pool by storing a fraction in the gallbladder during each cycle, but because cholecystectomized hamsters have no storage reservoir, the size of their bile salt pool decreases. It is interesting to speculate that the rat, which lacks a gallbladder normally yet maintains its bile salt pool during a fast,[8] may have evolved enzyme systems that are less sensitive to the direct, diminishing effects of food deprivation.

There was no significant difference in the percentage of ^{14}C-taurocholate activity remaining in the ileal sac after 30 minutes in fed or fasted, intact or cholecystectomized hamsters. *In vitro* studies of intestinal bile salt absorption rates[21] have established that the net flux of bile acids and salts across the ileal wall depends on luminal bile salt concentration and pH. Since both these factors were constant in our experiments, the results show that fed and fasted hamsters, whether intact or cholecystectomized, absorb taurocholate from the ileal lumen at about the same rate. If there were significant differences in the rate of transport of bile acids through the liver and/or sphincter of Oddi, they did not limit the rate of ileal bile salt absorption.

Although no gallbladder-filling kinetic studies were done, observation during our experiments indicates that the gallbladder fills rapidly in the absence of food. Since our animals were fasted for 48 hrs before injection, it was no surprise that we found virtually no ^{14}C activity in the gallbladder of intact animals. The gallbladder had no doubt filled and concentrated bile salts to its maximum capacity and therefore had no further volume available to accept ^{14}C-taurocholate originating in our injected solution.

In conclusion, our data tend to support the hypothesis that the sphincter of Oddi is always patent. It does not, however, necessarily follow that the sphincter is simply a passive opening, since the degree of patency may in fact change under various physiological conditions. One study[22] suggests that the sphincter may open and close continuously in a rhythmic manner during fasting in patients who have undergone cholecystectomy. In the intact subject, this rhythmic opening and closing may help an apparently patent sphincter establish back pressure that aids in filling the gallbladder.

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