Cholesterol catabolism in the rabbit in fasted and fed states

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Abstract Urinary and fecal endogenous steroid excretion of fed or fasted New Zealand white rabbits was determined by the isotopic steady state method after subcutaneous implantation of radioactive cholesterol. While plasma cholesterol was increasing during a 9-day fast, fecal steroid excretion decreased to 10% of the excretion rates in the fed state. Refeeding the fasted rabbits led to a decrease in plasma cholesterol and an increase in fecal endogenous steroid excretion. Urinary steroid excretion, which represented 18% of total endogenous steroid excretion for fed animals, decreased during fasting and increased during refeeding, but these changes were relatively small. The small intestine, cecum, and colon of fed or fasted rabbits had similar endogenous steroid contents. The predominant form of fecal endogenous steroid was acidic steroid. During attempts to alter the circulating bile acid concentration by supplying deoxycholate (200 mg/day) to fed rabbits or cholestyramine (2 g/day) to fasted rabbits, plasma cholesterol concentration did not change to the same extent as during fasting or refeeding, respectively. The decreased cholesterol catabolism and the hypercholesterolemia that are seen in the fasting rabbit may result from decreased clearance of plasma cholesterol.

Supplementary key words fecal acidic steroids · fecal neutral steroids · urinary steroids · β-sitosterol · cholestyramine · hypercholesterolemia · plasma bile acids

Plasma cholesterol levels of rabbits increase during a prolonged fast (1–3). This increase in plasma cholesterol is associated primarily with an elevation of low density lipoprotein (3, 4). Although force-feeding glucose or calcium salts to fasted rabbits has been shown to prevent the increase in plasma cholesterol (2), the mechanism involved in fasting hypercholesterolemia has not been determined. In our previous report (3) we could not find an increased influx of cholesterol into plasma and suggested that fasting hypercholesterolemia resulted from a decreased removal of plasma cholesterol. In order to support this suggestion we have used the excretion of cholesterol and its metabolites from the body as a gauge for the removal of plasma cholesterol in fed and fasted rabbits.

MATERIALS AND METHODS

Adult New Zealand white rabbits were fed 100 g of Purina laboratory rabbit chow daily except when fasted. In one experiment, the chow was coated with 0.2% sodium deoxycholate (Mann Research Laboratories, Orangeburg, N.Y.) by spraying the chow with an aqueous solution of the bile salt. In another study, cholestyramine (Questran, Mead Johnson Laboratories, Evansville, Ind.) was administered to fasted rabbits by intragastric intubation.

In order to study steroid excretion by the isotopic steady state method, gelatin capsules containing 50 mg of cholesterol labeled with 50 μCi of either [1,2-^{3}H]cholesterol or [4-^{14}C]cholesterol (Amersham/Searle Corp., Arlington Heights, Ill.) were implanted subcutaneously into rabbits weighing 3.6–5.0 kg. After implantation, the rabbits were injected with a priming dose of labeled cholesterol[2] that was dissolved in 25 μl of ethanol and dispersed in 1 ml of 0.9% saline. In one study, 1 μCi of [22,23-^{3}H]β-sitosterol (New England Nuclear, Boston, Mass.) dissolved in 3 ml of corn oil (Mazola) was given by stomach intubation. All isotopes were purified to at least 98% purity by thin-layer chromatography (silica gel H; hexane–ether 1:1, v/v). In all steroid excretion studies, the rabbits were collared with polyethylene discs to minimize coprophagy, and they were housed in metabolic cages that allowed separate collection of urine and feces. Urine and feces were always collected in 3-day pools. Feces were air dried for 3 days; urine and dried feces were frozen until analyzed. Blood was taken from the marginal ear vein with 0.4 M EDTA (10 μl/ml of blood) as an anticoagulant. The plasma was analyzed immediately or frozen for later analysis. Intestinal contents were washed into jars by saline perfusion of the removed segments. Bile was aspirated from the gall bladder with a syringe.

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[2] 3 μCi of either [1,2-^{3}H]cholesterol (100 mCi/mg) or [4-^{14}C]cholesterol (143 μCi/mg) was injected.

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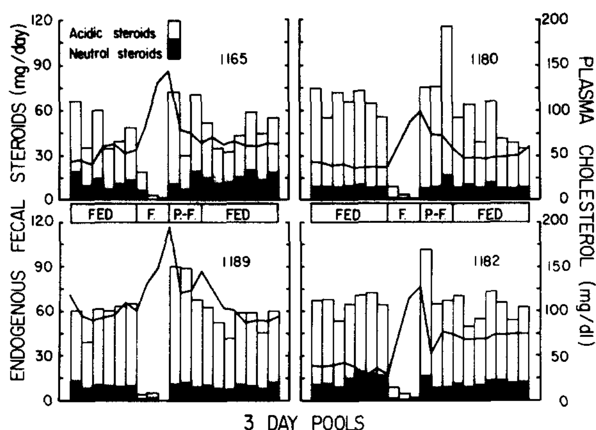

Fig. 1. Changes in plasma cholesterol concentration and endogenous fecal steroid excretion of rabbits during fasting and feeding. The four rabbits, identified by number in the upper right-hand corners, were fed their usual chow diet during the fed and postfasted (P.-F.) periods. Only water was supplied during the fasted (F.) period. Plasma cholesterol is represented by line graphs and endogenous fecal steroids by bar graphs.

Total radioactivity of ground feces, homogenized intestinal contents, and bile was determined by combustion of duplicate aliquots in a Harvey biological material oxidizer. Tritiated water was counted in Bray's solution (5), and [^{14}C]carbon dioxide was trapped and counted in a solution containing 250 ml of phenethylamine, 350 ml of ethylene glycol monomethyl ether, 400 ml of toluene, 6.0 g of 2,5-diphenyloxazole (PPO), and 0.2 g of *p*-bis-[2-(5-phenyloxazolyl]benzene (POPOP). Small corrections were made for combustion efficiency, which was determined with similar samples of known radioactivity. If replicates differed by more than 6%, combustions were repeated. Neutral steroids were obtained by hexane extraction after mild saponification of the sample according to Miettinen, Ahrens, and Grundy (6). The neutral steroid extract was combusted as described above or a chloroform solution was bleached under an ultraviolet lamp, the chloroform was evaporated, and the residue was counted directly in toluene scintillator (0.4% PPO and 0.01% POPOP). In order to determine plasma bile acid radioactivity, plasma was diluted 1:9 with 0.1 M sodium hydroxide in saline and was passed through a column of Amberlite XAD-2 (Rohm and Haas Co., Philadelphia, Pa.). The column was then washed with water until neutral, and the bile acids were eluted from the column with ethanol (7). Using this procedure, 100% recovery of radioactivity was obtained from plasma that was labeled in vitro with [^{3}H]glycochclate. After saponification of a bile acid fraction obtained from plasma labeled in vivo with [4-^{14}C]cholesterol, less than 1% of the bile acid radioactivity was extracted with hexane; therefore, neutral steroid radioactivity did not contaminate this fraction. Urine radioactivity was determined by counting aliquots directly in a dioxane-based solution containing Cab-O-sil (8). Steroid mass was calculated by dividing the total radioactivity by the average of the plasma cholesterol specific activities at the beginning and end of each sample collection period. This calculation depends on the assumption that excreted steroids are derived from a cholesterol pool in rapid equilibrium with plasma cholesterol. If the validity of this assumption is approximated to different extents in the fasting and fed states, the comparison of excreted steroid mass by this method may be subject to error. Acidic steroids were determined as the difference between total endogenous and neutral endogenous steroids; all steroid values were expressed as cholesterol equivalents.

Plasma cholesterol concentration was determined after saponification and hexane extraction (9). Aliquots of the hexane extract were taken for radioactivity and colorimetric (10) determinations. All radioactivity was measured with a Packard Tri-Carb model 3375 liquid scintillation spectrometer, and quenching was corrected by internal or external standards.

RESULTS

1 to 2 months after subcutaneous implantation of radioactive cholesterol, plasma cholesterol specific activity remained constant. Fecal excretion of endogenous cholesterol and its metabolites was then determined from plasma cholesterol specific activity and fecal radioactivity throughout periods of fasting and feeding (for representative examples see **Fig. 1**). Plasma cholesterol concentration increased during fasting without a significant change in specific activity while fecal endogenous steroid excretion decreased. As plasma cholesterol decreased after refeeding, the excretion of fecal steroids increased, usually to levels above that for the normal fed state. Because three distinct fecal excretion patterns appeared to exist, excretion rates were grouped into the fasted, postfasted, and fed states, which included, respectively, the samples collected throughout the 9-day fast, the 9-day period immediately after the fast, and the fed period, which consisted of 18–21 days before the fast plus 21 days after the postfasted period (**Table 1**).

Although total fecal endogenous steroid excretion was similar for fed female and male rabbits, female rabbits excreted significantly more neutral steroids than male rabbits. Both sexes excreted more acidic steroids than neutral steroids in feces. Fasting decreased total fecal steroid excretion to about 10% of the excretion rates of fed animals even though changes in plasma cholesterol concentration were variable. Acidic steroids decreased to a greater extent than neutral steroids, as shown by the decrease in the acidic steroid:neutral steroid ratio from 10.0 during the fed state to 6.5 during fasting for male rabbits and from 4.0 to 2.6 for female rabbits. When the plasma cholesterol concentrations decreased during the postfasted period, fecal steroid excre-

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TABLE 1. Effects of a 9-day fast and refeeding on plasma cholesterol and on fecal excretion of endogenous steroids

Sex	Dietary State	No. of Animals	Endogenous Fecal Steroids[a] Total	NS	AS	Plasma Cholesterol[b]
			mg/day	*mg/day*	*mg/day*	*mg/dl*
M	Fed	3	64.2 ± 4.9	6.1 ± 0.7	58.1 ± 5.5	40.7 ± 2.6
	Fasted	3	4.5 ± 0.7	0.6 ± 0.1	3.9 ± 0.6	+63.4 ± 29.4
	Postfasted	3	92.1 ± 12.3	8.1 ± 0.9	84.0 ± 11.4	−39.7 ± 30.2
F	Fed	6	62.0 ± 7.4	12.6 ± 1.3	49.3 ± 6.7	68.1 ± 8.3
	Fasted	6	6.7 ± 1.2	1.9 ± 0.3	4.8 ± 0.9	+90.1 ± 6.2
	Postfasted	5	73.2 ± 6.2	11.3 ± 0.3	61.9 ± 6.4	−60.3 ± 8.0

[a]Averages ± SE for total steroids, neutral steroids (NS), and acidic steroids (AS) are all expressed as cholesterol equivalents.

[b]Fed: the average value during feeding. Fasted: the increment above the fed value after 9 days of fasting. Postfasted: the decrease from the 9-day fasted value until day 9 of refeeding.

tion rates exceeded the excretion rates of fed animals. This excess of fecal steroid excretion during the postfasted period was usually less than the decline in fecal steroid excretion during fasting.

In some animals, urinary excretion of cholesterol and its metabolites was also measured (**Table 2**). Urinary steroid excretion rates of male and female rabbits were similar and represented 18% of the combined endogenous fecal and urinary steroid excretion of fed animals. Significant changes in urinary excretion during fasted and postfasted periods occurred in the same directions as fecal excretion but were relatively small. The concentration of plasma bile acids also followed similar variations in response to different dietary states (Table 2).

Because fecal mass excreted during the 9-day fast was only 8% of the fecal mass during 9 days of feeding, the possibility existed that fecal mass and steroids were held up in the intestine; therefore, the intestinal steroid contents of fed and 9-day fasted rabbits were determined (**Table 3**). Although fecal steroid excretion of fasted rabbits was about 20% of that excreted by fed rabbits, the endogenous steroid contents of the small intestine, cecum, and colon were similar for fed and fasted rabbits. The gallbladder appeared to have a higher content of steroids during fasting. More than 99% of the biliary steroids were acidic steroids for fed or fasted rabbits. When contents from more distal parts of the digestive tract were examined, the ratio of acidic steroids to neutral steroids decreased, which demonstrates both the reabsorption of the bile acids along the digestive tract and the contribution of cholesterol by the sloughing off of intestinal epithelium.

Because bacterial degradation of neutral steroids may have occurred in fasting or fed rabbits, trace amounts of β-[^{3}H]sitosterol were dissolved in corn oil that inherently contained about 18 mg of β-sitosterol (11), and the oil solution was intubated into the stomachs of fed rabbits (**Table 4**). In fed rabbits, recovery of the label in the feces was obtained within 6 days, with 80% recovered in the first 3 days. After 21 days had passed and any remaining label was cleared, the same animals were fasted for 3 days, intubated with a similar dose of β-[^{3}H]sitosterol, and fasted for 6 additional days. Almost no radioactivity or feces were excreted during the 6 fasting days after intubation. Collection

TABLE 2. Effect of a 9-day fast and refeeding on urinary excretion of steroids and on plasma bile acid concentration

Dietary State	No. of Animals	Urinary Steroids[a]	Plasma Bile Acids[a]
		mg/day	*mg/dl*
Fed	5	14.4 ± 0.8	1.72 ± 0.15
Fasted	5	11.4 ± 0.7	1.07 ± 0.12
Postfasted	4	18.3 ± 1.2	2.33 ± 0.30

[a] Averages ± SE are expressed as cholesterol equivalents. Data from three males and two females are presented for fed and fasted states. One of the females was not studied in the postfasted condition.

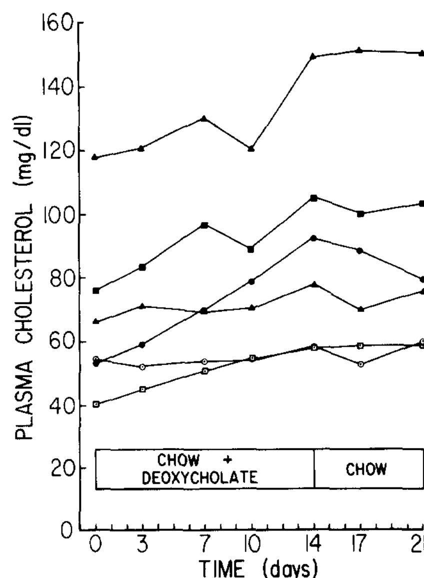

Fig. 2. Effect of feeding sodium deoxycholate (200 mg/day) on the plasma cholesterol concentration of chow-fed rabbits.

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TABLE 3. Distribution of cholesterol and its metabolites in the rabbit digestive system during 9 days of fasting or feeding

Sex	Dietary State	Steroid Type	Endogenous Steroid Content[a]				Endogenous Fecal Steroids[a,b]
			Gallbladder	Small Intestine	Cecum	Colon	
			mg				*mg*
F	Fasted	Total	357 ± 44	176 ± 24	177 ± 49	29.7 ± 9.6	108 ± 14
		NS	2.4 ± 0.3	19.5 ± 5.8	37.6 ± 8.6	8.3 ± 3.0	41.4 ± 10.2
		AS	355 ± 44	156 ± 19	139 ± 42	21.4 ± 7.0	66.4 ± 6.4
		AS/NS	158 ± 57	12.3 ± 5.6	3.7 ± 0.6	3.1 ± 0.6	1.9 ± 0.3
M and F	Fed	Total	148 ± 96	194 ± 18	290 ± 29	47.3 ± 11.9	495 ± 32
		NS	0.85 ± 0.25	22.5 ± 3.5	47.8 ± 20.5	8.6 ± 2.2	95.4 ± 29.6
		AS	147 ± 68	171 ± 22	242 ± 50	38.6 ± 14.2	400 ± 62
		AS/NS	165 ± 35	7.8 ± 2.2	6.9 ± 4.1	5.2 ± 3.0	4.8 ± 2.2

[a]Averages ± SE for total steroids, neutral steroids (NS), and acidic steroids (AS) are all expressed as cholesterol equivalents. Fasted: four females. Fed: one male and one female.
[b]Total excretion for 9 days.

TABLE 4. Fecal recovery of radioactivity (%) after intubation of β-[^{3}H]sitosterol into the stomachs of fed and fasted rabbits

Rabbit	Days Fed							Cumulative
	1–3	4–6	7–9	10–12	13–15	16–18	19–21	
1254	78.0	14.9	2.4	0.7	0.3	0.2	0.2	96.5
1256	78.0	23.2	2.3	0.6	0.1	0.3	0.1	104.6
1258	83.9	19.1	1.8	0.7	0.3	0.2	0.1	106.1
Avg	80.0	19.1	2.2	0.7	0.2	0.2	0.1	102.4

Rabbit	Days Fasted[a]	Days Fed						Cumulative
	1–6	7–9	10–12	13–15	16–18	19–21	22–24	
1254	0.5	1.4	17.2	15.6	14.1	10.6	6.8	66.1
1256	2.2	33.7	26.2	10.7	6.3	3.9	2.9	85.9
1258	0.0	16.0	49.9	15.2	5.8	3.5	1.0	91.5
Avg	0.9	17.0	31.1	13.8	8.7	6.0	3.6	81.2

[a]Animals were intubated after 3 days of fasting and were fasted for an additional 6 days.

of feces for 18 additional days resulted in recovery of about 90% of the label for two rabbits but only 66% for rabbit 1254. This rabbit had a more delayed excretion of label than the other rabbits and excreted 6.8% of the dose during the last collection period (22–24 days). Only 73% of the fecal label of rabbit 1254 could be recovered as neutral steroid while other rabbits had greater than 90% of the fecal label in neutral steroids. Rabbits 1256 and 1258 also demonstrated a delayed excretion of label, but 77% of the dose was excreted in the first 9 days of refeeding.

Because our steroid excretion studies showed that plasma cholesterol concentration and bile acid excretion rates changed in opposite directions, we tried to determine the effect of bile acid pool size on plasma cholesterol in the rabbit. In order to increase the bile acid pool size of fed rabbits, 200 mg of deoxycholate was fed daily for 2 wk (**Fig. 2**). Only small increases in plasma cholesterol were seen during this bile acid feeding period (**Table 5**). In another experiment, water or 2 g of cholestyramine, a bile acid sequestrant, which was suspended in water, was intubated daily into rabbits that had been fasted for 5 days previously (**Fig. 3**). Although intubation of cholestyramine halted or reversed the increment in plasma cholesterol in response to

TABLE 5. Changes in plasma cholesterol with alteration of circulating bile acid mass

Expt. No.	No. of Animals	Treatment[a]	Days	Plasma Cholesterol after Treatment[b] (% of day 0)
1	6	Deoxycholate	0–14	134 ± 10
		Chow	15–21	130 ± 7
2	7	Fasting	0–5	217 ± 28
		Cholestyramine	6–8	188 ± 37
3	7	Fasting	0–5	180 ± 14
		Water	6–8	200 ± 19

[a]In expt. 1, rabbits were fed chow coated with 0.2% sodium deoxycholate for 14 days and then returned to their normal chow diet for 7 days. In expts. 2 and 3, rabbits were fasted for 5 days and then either water or an aqueous suspension of 2 g of cholestyramine was intubated daily for 3 days while the animals were maintained in the fasting state.
[b]Values are means ± SE.

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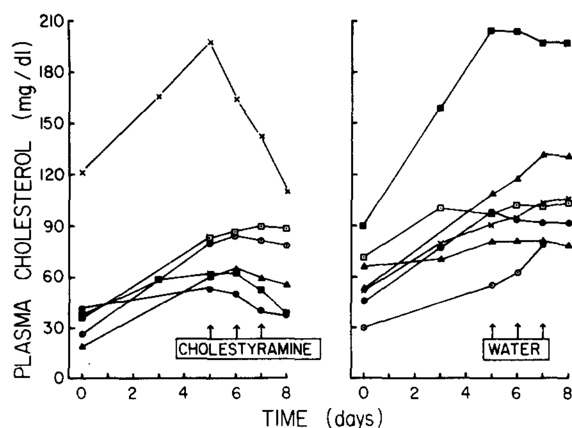

Fig. 3. Effect of intragastric intubation of cholestyramine on the rise in plasma cholesterol concentration of fasting rabbits. The rabbits were fasted on day 0 and either water or an aqueous suspension of 2 g of cholestyramine was intubated at times indicated by the arrows.

fasting, our control animals that received water without cholestyramine did not consistently increase their plasma cholesterol concentrations throughout the experiment (Table 5). The plasma cholesterol levels decreased dramatically in only one rabbit after cholestyramine treatment.

DISCUSSION

The rabbit can dispose of cholesterol by excreting cholesterol and its metabolites not only in feces but also in urine. Although most of the endogenous steroids were excreted in feces, about 20% of the total excretion was in urine. This relatively high urinary steroid excretion was probably due to the high concentration of bile acids in rabbit plasma compared with other species (12–14). Assuming that urinary steroid excretion occurred only as acidic steroids, the combined fecal and urinary acidic steroid excretion was 72.7 mg/day[3] for 3–4 kg male rabbits. In 2–3 kg male rabbits fed a commercial diet, Hellström and Sjövall (12) obtained a 69.1 mg/day[3] production rate for deoxycholate, the main bile acid of rabbits, by following the decay of biliary deoxycholate specific activity. The rates of endogenous fecal neutral steroid excretion, 6.1 mg/day for male rabbits weighing 3–4 kg and 12.6 mg/day for female rabbits weighing 4–5 kg, were lower than the 21 mg/day total neutral sterol excretion reported by Hellström (15). The latter values were determined in feces of 2–3 kg rabbits by gas–liquid chromatography and possibly included intestinal sterols that were not in isotopic equilibrium with plasma cholesterol. Endogenous fecal neutral steroid excretion rates of 11.1 mg/day for female rabbits have been obtained in this laboratory with a single intravenous injection of radioactive cholesterol.[4] This low fecal neutral steroid excretion was not the result of losses due to degradation of neutral steroids by intestinal bacteria, for only during fasting was recovery of intubated β-[^{3}H]sitosterol not quantitative. The poorer recoveries realized with fasted rabbits may be due to the delayed excretion of the β-[^{3}H]sitosterol, because the degree of neutral steroid degradation in man has been shown to increase with time spent in the colon (16).

The rate of excretion of endogenous steroids or the whole body cholesterol synthesis rate of the fed animals in Tables 1 and 2 was about 80 mg/day. This rate was considerably less than the synthesis of plasma cholesteryl ester, which was found to be almost 200 mg/day in previous studies with labeled mevalonate (3). Similar experiments in men with normal plasma cholesterol levels have shown that the synthesis rate of plasma esterified cholesterol is about 4 g/day (17), whereas cholesterol production rate, which includes dietary cholesterol intake, is about 1 g/day (18). This greater synthesis of plasma esterified cholesterol indicates that plasma cholesteryl ester is recirculated between tissues and plasma several times before it is irreversibly excreted.

Although an 80–90% decrease in total steroid excretion was observed during the 9-day period of fasting, this decrease is not a good measure of the decrease in the irreversible disposal of cholesterol from the body in the fasting state because it does not take into account the retention of acid and neutral steroids in the gastrointestinal tract and the enterohepatic circulation of the fasting animal (Table 3). In the rabbit, 97–98% of the bile acid pool is known to be present in the gallbladder, liver, and gastrointestinal tract, with the liver representing only a very small portion of the total (12). Although gallbladder steroids continue to recirculate before final removal from the enterohepatic circuit, the bile acids, which constitute 99% of the gallbladder steroids, are products of cholesterol degradation and have therefore already been removed from the body's cholesterol pool.

According to the data in Table 3, the total amount of endogenous steroid contained in the gallbladder and the entire length of intestine is 740 mg for the fasting animals and 679 mg for the fed animals, a difference of 61 mg. In 9 days, the fed animals excreted 625 mg of endogenous steroids in feces and urine (Tables 2 and 3), whereas the fasting animals excreted 211 mg and retained an extra 61 mg. Thus, the difference in cholesterol disposal rate during a 9-day period is 625 − (211 + 61) mg, i.e., 353 mg, or 39 mg/day. This shows that in the fasting animals about a 50% reduction in cholesterol disposal (39 out of 80 mg/day) has occurred. Since these fasting animals accumulated 20 mg of plasma cholesterol per day, it would appear that a reduction of cholesterol biosynthesis of 39 − 20, or 19, mg/day might take place in the fasting rabbits. This would represent a reduction in cholesterol biosynthesis of 19 out of 80 mg/day, or 24%.

[3] Values are expressed as cholesterol equivalents.
[4] Ross, A. C., and D. B. Zilversmit. Submitted for publication.

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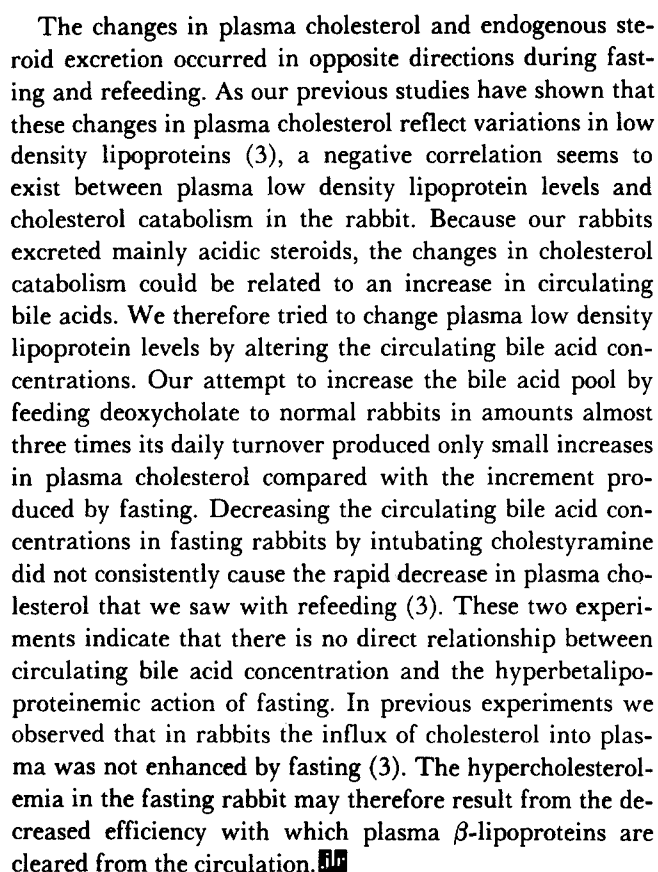

The changes in plasma cholesterol and endogenous steroid excretion occurred in opposite directions during fasting and refeeding. As our previous studies have shown that these changes in plasma cholesterol reflect variations in low density lipoproteins (3), a negative correlation seems to exist between plasma low density lipoprotein levels and cholesterol catabolism in the rabbit. Because our rabbits excreted mainly acidic steroids, the changes in cholesterol catabolism could be related to an increase in circulating bile acids. We therefore tried to change plasma low density lipoprotein levels by altering the circulating bile acid concentrations. Our attempt to increase the bile acid pool by feeding deoxycholate to normal rabbits in amounts almost three times its daily turnover produced only small increases in plasma cholesterol compared with the increment produced by fasting. Decreasing the circulating bile acid concentrations in fasting rabbits by intubating cholestyramine did not consistently cause the rapid decrease in plasma cholesterol that we saw with refeeding (3). These two experiments indicate that there is no direct relationship between circulating bile acid concentration and the hyperbetalipoproteinemic action of fasting. In previous experiments we observed that in rabbits the influx of cholesterol into plasma was not enhanced by fasting (3). The hypercholesterolemia in the fasting rabbit may therefore result from the decreased efficiency with which plasma β-lipoproteins are cleared from the circulation.

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