EFFECT OF FAT INTAKE ON CHOLESTEROL TURNOVER AND BILE ACID FORMATION

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Summary Three groups of male rats were maintained on diets containing different amounts of fat. After one week on such regimens, they were injected intraperitoneally with cholesterol-3H. During the following 28 days, the radioactivity and quantity of fecal cholesterol and its metabolites were determined. The coprostanol excretion was about the same in all groups and the bile acids excretion increased with increasing fat intake; however, compared to the fat-free group, the excretion of the injected cholesterol-3H was greater in the 3% fat group and less in the 10% fat group. Consequently, the specific radioactivity of bile acids was lower in the 10% group than in the others. The half-life of labelled cholesterol was 12.6, 16.0 and 22.2 days for the 3%, 10% fat and fat-free groups, respectively. Rather than a fat-free diet, a low-fat diet of 3% or so, would be of more advantage in eliminating cholesterol by increasing the formation of bile acids to emulsify the fat. In the 10% fat group, however, the enlarged pool size of bile acid probably delayed cholesterol metabolism to bile acids.

The influence of fat feeding on cholesterol metabolism has been reported by many investigators. The absorption of cholesterol increases with the amount of fat intake with an upper limit (1), although the fatty acid composition of the carrier fat affects the extent of sterol absorption (2). Several workers have reported that fat influenced the biosynthesis and excretion of cholesterol, but with conflicting results. In the experiments of Sabine et al. (3) and Jansen et al. (4) with mice, the high-fat diet suppressed cholesterol synthesis; whereas with rats, Hill et al. (5, 6), using similar diets, found an increase in hepatic synthesis of cholesterol from acetate after feeding fat. Bortz (7) also observed that fat feeding to rats maintained on a fat-free diet brought about a decrease in fatty acid synthesis and an increase in cholesterol synthesis. Further, the composition of fatty acids such as linoleate, may influence cholesterol metabolism. However, previous investigations have not always reproducibly demonstrated the increased
excretion of cholesterol and the decreased synthesis of cholesterol due to linoleate (8–19). We found that linoleate had no effect on the half-life of cholesterol, though it lowered plasma cholesterol (20).

On the other hand, the relation between the amount of fat intake and cholesterol metabolism is not so clear. Bile acids, the metabolites of cholesterol, are necessary for the digestion and absorption of fat, so fat intake may accelerate the metabolism of cholesterol. The present experiments were therefore designed to examine the effects of varied amounts of dietary fat on overall cholesterol metabolism, using fat with a balanced composition of fatty acids.

MATERIALS AND METHODS

Male Wistar rats weighing about 100 g were fed three kinds of sucrose diets: one fat-free, one containing 3% fat and one containing 10% fat. The fat consisted of a composition of olive oil, corn oil and hydrogenated coconut oil (1:1:1). The fatty acid compositions of the fat were caproic acid, 0.11%; caprylic acid, 0.22%; capric acid, 2.52%; lauric acid, 15.5%; myristic acid, 3.92%; palmitic acid, 8.94%; stearic acid, 3.11%; oleic acid, 47.1%; linoleic acid, 17.0%; and others. The fat-free diet consisted of 68.4% sucrose, 18% casein, 9.5% cellulose, 4% salt mixture (21), 0.1% choline chloride and vitamin mixture.1 As the vitamin mixture contained linoleic acid, the fat-free diet actually contained 0.5% linoleate, which would be enough to rats as vitamin (22). In the 3% fat diet, 3 g fat replaced 1.5 g cellulose and 1.5 g sucrose, and in the 10% fat diet, 10 g fat replaced 5.5 g cellulose and 4.5 g sucrose. The three diets gave growth comparable to that with a complete stock diet. Rats were injected intraperitoneally with 5 µCi cholesterol-3H(G)2 dissolved in 1 ml of 0.3% Tween 80 per 100 g body weight one week after being separated into three dietary groups.

Feces collected for 1- to 7-day periods (collected pooled feces at day 2, 4, 7, 10, 14, 18, 22 and 28) after injection were dried in an oven at 105°C to 110°C for 3 hr and ground into powder. The fecal excretion of neutral sterols and bile acids, and their radioactivity were determined. On day 28 the rats were killed. The remaining radioactivity and the cholesterol content in plasma, liver and carcass, excluding blood and liver, were measured. The carcasses were autoclaved with 2 N NaOH at 120°C for 3 hr before extraction of total lipids. Radioactivity measurements of fecal cholesterol and its metabolites were measured as described in a previous paper (23). Sterols and bile acids were analyzed by gas chromatography. The half-life of cholesterol was determined according to the

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1 Vitamins furnished per 100 g diet of ration: retinyl palmitate, 2,000 IU; calciferol, 500 IU; (mg) methyl linoleate, 500; α-tocopheryl palmitate, 10.0; thiamine-HCl, 1.0; riboflavin, 1.0, pyridoxine, 1.0; nicotinic acid, 8.0; folic acid, 0.25; calcium pantothenate, 2; biotine, 0.5; inositol, 4.0; menadione, 0.25.

2 Cholesterol-3H(G) obtained from The Radiochemical Centre, Amersham, England, had a specific activity of 8.93 Ci/mmole and a radiochemical purity of over 99%.
method of LINDSTEDT (24). Plasma and liver lipids were extracted according to FOLCH et al. (25) and their cholesterol content was determined using the color reagent of ZAK et al. (26). Other details were the same as those described previously (21, 23, 27).

Animals, about 250 g, fed diets similar to those above for 2 hr after one-day starvation were used for bile cannulation. Bile ducts were cannulated under anesthesia. Bile was collected for the following 2 hr. Bile acids were extracted from bile and analyzed by gas chromatography according to UCHIDA et al. (28). Cholesterol was analyzed by gas chromatography as previously (23).

RESULTS

Turnover of injected radioactive cholesterol

The time course of the specific activity of cholesterol-3H in feces was plotted on semilogarithmic graph paper as shown in Fig. 1. The half-life of cholesterol in the second phase, calculated by the least square method, was 22.2, 12.6 and 16.0 days, respectively, for rats fed the fat-free, the 3% fat and the 10% fat diets. The half-life of cholesterol was shortest in the 3% fat diet group and was somewhat longer in the fat-free group than in the fat fed group.

Excretion of cholesterol

The cumulative amount of injected cholesterol-3H, excreted as fecal cholesterol and its metabolites during the 0–28 day period are shown in Table 1. The
Table 1. Sterols excretion in rats after injection of cholesterol-3H. After the rats were fed three kinds of diets for one week, they were injected intraperitoneally with 5 μCi cholesterol-3H and killed 28 days after the injection. Meantime the feces of one group were pooled on a tray and collected for a 1-7 day period. So, variabilities and statistically significant differences of excretion data could not be calculated. However, standard deviations in such excretion data usually were in the range of about 7-15% of means.

| Dietary fat (%) | 0          | 3         | 10        |
|----------------|------------|-----------|-----------|
|                | mg/28 days | cpm x 10^3/28 days |
| Cholesterol    | 33.9       | 35.6      | 27.4      |
| Coprostanol    | 50.6       | 54.6      | 54.0      |
| Bile acids     | 27.7       | 33.6      | 42.1      |
| Total          | 112.2      | 123.8     | 123.5     |
| Sterols        | 1,091.2    | 1,051.9   | 942.8     |
| Bile acids     | 327.4      | 375.9     | 260.5     |
| Total          | 1,418.6    | 1,427.8   | 1,203.3   |

Mean (5 rats).

excretion of sterols and bile acids by the 3% fat group was roughly similar to that of the fat-free group, but that of the 10% fat group was less than the others. However, the amount of fecal excretion of bile acids increased with increasing fat content in the diet, though the amount of fecal sterols was fairly constant. The average specific radioactivities of total bile acids were 11.8, 11.2 and 6.2 (cpm x 10^3/mg), respectively, for the fat-free, the 3% and the 10% fat groups. Clearly, the average specific radioactivity of the bile acid excreted by the 10% fat group was much lower than that of the others.

An example of a gas chromatogram of fecal bile acids is shown in Fig. 2.

![Gas chromatogram of fecal bile acids](Fig. 2. Gas chromatogram of fecal bile acids. The retention times of Peak 1, 2, 3, 4 and 5 corresponded to lithocholic acid, deoxycholic acid, chenodeoxycholic acid, hyodeoxycholic acid and cholic acid, respectively.)
The major component was hyodeoxycholic acid, which was 30–35% of total bile acids in all groups. The amount of bile acids was calculated on the basis of all peaks. The relative composition of fecal bile acids did not change much on increase of fat intake, except for the excretion of cholic acid, which was 0.94, 1.90 and 12.2% of total bile acids, respectively, for rats fed fat-free, 3% fat and 10% fat diets.

**Retained cholesterol**

The total amount and radioactivity of cholesterol in plasma and liver of rats in the fat-free, 3% and 10% fat groups 28 days after the injection of cholesterol-\(^3\)H are shown in Table 2. No statistically significant differences in liver or plasma cholesterol levels or the amount of radioactivity present in liver or plasma were observed. However, the total radioactivity of the carcasses, excluding blood and liver, of the 3% fat-fed group were significantly lower than those of the other two groups.

**Excretion of bile acids into the bile**

The results are shown in Table 3. Bile acid excretion into the bile increased somewhat with increases in fat content of the diet. Biliary bile acids were cholic acid, deoxycholic acid, hyodeoxycholic acid, chenodeoxycholic acid, lithocholic acid and others, which decreased in that order. For the first 30 min after cannulation, cholic acid excreted 30.2, 29.6 and 44.2% (mean of 5–6 rats) of total bile acids, respectively, for fat-free, 3% and 10% fat groups. Other bile acids did not experience such change due to the diets. Cholesterol excretion was similar in the three groups for 2 hr after the cannulation.
DISCUSSION

The results of the present study demonstrated that the fecal excretion of bile acids increased with increased fat intake. In the acute feeding experiment, it was shown that a 10% fat diet increased the excretion of bile acids into bile (Table 3). This could be due to the mobilization of a bile acid pool on fat feeding. Thus, in the chronic feeding experiments, the enterohepatic circulation of bile acids was probably accelerated. However, the fecal excretion of radioactive bile acids was higher in the 3% fat group, but lower in the 10% group, than in the fat-free group. The average specific radioactivity of fecal bile acids (total radioactivity of bile acids fraction/total amount of bile acids) was lower in the 10% fat group than in either the 3% fat group or the fat-free group. In the group on a 10% fat diet, the slow rate of conversion of exogenously injected radioactive cholesterol could be explained as the result of feedback inhibition of 7α-hydroxylase of cholesterol caused by the accelerated enterohepatic circulation of bile acids.

With the 3% fat group, the fecal excretion of radioactive bile acids increased as shown in Table 1 and the cholesterol activity remaining in the carcass 28 days after the injection of cholesterol-3H was lower than in the fat-free group. The half-life of the second phase of the cholesterol decay curve, representing the overall turnover of cholesterol in the whole body, was shorter than the value of the fat-free group. These results suggest that the 3% fat diet can cause the induction of 7α-hydroxylase in the liver.

From the results of the present experiments, it could be concluded that fat feeding in rats caused an increase in fecal excretion of bile acids likely due to the accelerated enterohepatic circulation of bile acids. On the other hand, the excessive fat decreased the catabolism of cholesterol. The influence of a high fat diet on cholesterol metabolism should be further studied by more direct methods, before a final conclusion is arrived at.

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