Stimulation of Cholesterol Metabolism in Pyridoxine-Deficient Rats

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Summary  The effect of pyridoxine deficiency on cholesterol catalolism was studied in rats. The concentrations of bile lipid components were higher in pyridoxine-deficient rats than in controls. A decreased ratio of taurine to glycine conjugates was observed in the deficient rats. No change in the neutral sterol content, but an increase in the bile acid content of the feces was observed in the deficient rats. Increased cholesterol catabolism in pyridoxine-deficient rats was also shown by the shorter half-life of the $[^{14}C]$cholesterol injected into these animals.

Key Words  cholesterol metabolism, bile acids, bile components, fecal sterol, taurine, glycine, pyridoxine deficiency

Since Rinehart and Greenberg first reported atherosclerosis in Rhesus monkeys fed on a pyridoxine-deficient diet (1), discrepant observations on cholesterol metabolism in pyridoxine deficiency have been made. There are reports of hypercholesterolemia in pyridoxine-deficient rats (2), chicks (3) and rabbits (4) as well as no change (5) or even a decrease (6) in the plasma sterol level of rats (6). Lupien et al. (7-9) and Shah et al. (10) reported no change in the cholesterol content but an increase of cholesterogenesis in pyridoxine-deficient rat liver. Recently we found that rats fed on a high(70%)-protein and pyridoxine-deficient diet accumulated triglyceride and cholesterol ester in the liver (11,12). We also obtained evidence in vivo (13) and in vitro (14) of increased hepatic cholesterogenesis from acetate in rats fed on pyridoxine-deficient diet. This increased cholesterogenesis was found to be correlated with an increase in hepatic 3-hydroxymethyl glutaryl coA reductase activity (15).

In the present work we studied the catabolism of cholesterol in pyridoxine-deficient rats.
MATERIALS AND METHODS

Animals. Male Wistar strain rats weighing 50 to 60 g were fed on 20% casein diet for 7 weeks or 70% casein diet for 5 weeks and then used for study. The rats were divided into two groups: the pyridoxine-deficient group received diet ad libitum while control animals were pair-fed with the deficient animals on pyridoxine-supplemented diets. The diet used contained 20% or 70% vitamin-free casein (NBC), 55% or 5% corn starch, 10% sucrose, 8% oil mixture (cod liver oil-soybean oil, 1:4), 4% mineral mixture (Oriental 2), 1% vitamin mixture (Oriental 2), 2% cellulose powder and 0.2% choline chloride. Animals were starved for about 17 hr before the experiments. For collection of bile, rats were anesthetized with ketamine hydrochloride and a catheter of PE-50 polyethylene tubing was placed in the common bile duct. Bile was collected for 2 hr from anesthetized rats and used for chemical analyses. The turnover rate of cholesterol was determined by i.v. injection into rats of 1 μCi of [4-14C]cholesterol per 100 g body weight.

Chemicals. [4-14C]Cholesterol was purchased from Daiichi Chemicals Co. Scintillator and Carbo-sorb were obtained from Packard Co. Ketamine hydrochloride (Ketalar 50) was obtained from Sankyo Co. All other chemicals were secured from Nakarai Co. or Wako Pure Chemicals Co.

Analysis of bile. Bile lipids were extracted and fractionated by the method of Shioda et al. (16). Cholesterol was determined by the method of Zak (17). Bile acids were fractionated by thin layer chromatography (butanol:acetic acid:water, 10:1:1), hydrolyzed, and then analyzed by measuring the color developed with sulfuric acid (18). Phospholipid was measured as inorganic phosphate after oxidation with 60% HClO₄ by a modification of the method of Fiske-Subbarrow (19).

Analysis of serum. Serum bile acids were extracted by the method of Eastwood et al. (20), hydrolyzed (21, 22) and determined fluorometrically (23).

Analysis of feces. Fecal sterol was extracted by the method of Miettinen et al. (24), fractionated by thin layer chromatography and determined by the method of Zak (17). Cholesterol and coprosterol in feces were also analyzed by gas liquid chromatography in a Hitachi 073 instrument as trimethylsilyl ethers after separation by thin layer chromatography (25, 26). Fecal bile acids were extracted by the method of Grundy et al. (27) and determined fluorometrically as described above.

Analysis of radioactivity. Radioactivities of [14C]cholesterol and its metabolites in various tissues and feces were determined in a scintillator (Permafluor V, Packard) with an Aloka liquid scintillation counter after oxidation of samples with a sample oxidizer (Packard Model 306 Tri-Carb).

RESULTS

Secretion and composition of bile

The flow rate and lipid composition of bile were determined in rats on 20% or 70% casein diet with or without pyridoxine. As shown in Table 1 the rate of bile secre-
Table 1. Secretion and composition of bile of rats on 20 or 70% casein diet with or without pyridoxine.

| Diet Casein | PIN | Bile flow (ml/100 g/hr) | Bile acid (μmol/ml (%)| Free cholesterol (μmol/ml (%)) | Phospholipids (μmol/ml (%)) |
|-------------|-----|-------------------------|------------------------|---------------------------------|----------------------------|
| 70% (7)*    | +   | 0.62 ± 0.10             | 20.60 ± 3.72 (90.0)    | 0.34 ± 0.07 (1.5)              | 1.94 ± 0.85 (8.5)          |
| 70% (7)     | −   | 0.78 ± 0.18             | 27.55 ± 8.79 (85.8)    | 0.51 ± 0.04* (1.6)             | 4.06 ± 0.93* (12.6)        |
| 20% (8)     | +   | 0.42 ± 0.09             | 14.47 ± 3.35 (82.0)    | 0.24 ± 0.04 (1.3)              | 2.94 ± 0.93 (16.7)         |
| 20% (8)     | −   | 0.41 ± 0.14             | 21.97 ± 11.38 (80.8)   | 0.37 ± 0.08* (1.4)             | 4.84 ± 0.93* (17.8)        |

*Number of rats. Values are means ± SD. Other conditions are described in the text.

* p<0.05, significantly different from the control.

Table 2. Composition of bile acids of rats on 20 or 70% casein diet with or without pyridoxine.

| Diet Casein | PIN | Taurine conjugates (%) | Glycine conjugates (%) | Triol/Diol ratio |
|-------------|-----|------------------------|------------------------|------------------|
|             |     | Triol | Diol | Total | Triol | Diol | Total | Triol/Diol |
| 70% (7)     | +   | 66.8  | 26.7 | 93.5  | 5.1  | 1.4  | 6.5   | 2.56   |
| 70% (7)     | −   | 21.4  | 11.2 | 32.6* | 49.7 | 17.7 | 67.4* | 2.46   |
| 20% (8)     | +   | 58.9  | 29.3 | 88.2  | 9.4  | 2.4  | 11.8  | 2.15   |
| 20% (8)     | −   | 35.3  | 17.5 | 52.8* | 35.4 | 11.8 | 47.2* | 2.41   |

Figures in parentheses are numbers of rats used. * p<0.05, significantly different from the control.

Cholesterol metabolism in B6 deficiency.

Cholesterol metabolism in B6 deficiency. The secretion and composition of bile of rats on 20 or 70% casein diet with or without pyridoxine was higher in rats on 70% casein diet than in the rats on 20% casein diet regardless of whether the diet contained pyridoxine, and there was not much difference between values for the pyridoxine-deficient and control groups. The content of bile acid was higher in the deficient groups on either 20 or 70% casein diet. The deficient rats excreted more bile acid than the controls. The levels of cholesterol and phospholipids which are known to be necessary for mixed micelle formation in the bile, were proportional to that of bile acids in all groups. In all cases examined, analysis showed that the bile acids consisted of 30% diol and 70% triol (Table 2). This ratio has been reported to influence micelle formation and, subsequently, cholesterol absorption. Pyridoxine deficiency influenced the proportions of taurine and glycine conjugates: bile acids were mainly excreted as taurine conjugates in control rats, whereas only one-third and one-half thereof were excreted as taurine conjugates in rats on 70% and 20% casein pyridoxine-deficient diet respectively, the remainder being excreted as glycine conjugates. These changes in the proportions of conjugates reflect the hepatic contents of glycine and taurine: the glycine and taurine contents of the liver in rats on 70% casein pyridoxine-deficient diet were 10.23 μmol/g and 1.25 μmol/g, respectively, whereas those of control rats were 3.24
Table 3. Lipid composition of serum of portal blood from rats on 20 or 70% casein diet with or without pyridoxine.

| Casein   | PIN | Bile acids (µmol/ml) | Cholesterol (µmol/ml) | Phospholipids (µmol/ml) |
|----------|-----|----------------------|-----------------------|-------------------------|
| 70% (7)  | +   | 0.52 ± 0.11          | 2.45 ± 0.23           | 1.72 ± 0.23             |
| 70% (7)  | -   | 1.02 ± 0.03*         | 1.89 ± 0.24*          | 1.11 ± 0.07*            |
| 20% (8)  | +   | 0.42 ± 0.08          | 2.04 ± 0.34           | 1.87 ± 0.28             |
| 20% (8)  | -   | 0.58 ± 0.09*         | 2.12 ± 0.49           | 1.75 ± 0.28             |

Figures in parentheses are numbers of rats. *p<0.05, significantly different from the control.

Table 4. Neutral and acidic sterol excretion in feces of rats on 20 or 70% casein diet with or without pyridoxine.

| Casein   | PIN | Feces (mg/100 g/day) | Cholesterol (mg/100 g/day) | Coprosterol (mg/100 g/day) | Bile acids (mg/100 g/day) |
|----------|-----|----------------------|-----------------------------|-----------------------------|-----------------------------|
| 70% (8)  | +   | 223 ± 36             | 1.25 ± 0.47                 | 1.14 ± 0.33                 | 3.62 ± 0.38                 |
| 70% (8)  | -   | 268 ± 31             | 1.36 ± 0.42                 | 1.18 ± 0.47                 | 5.50 ± 0.69*                |
| 20% (10) | +   | 231 ± 23             | 0.66 ± 0.19                 | 1.69 ± 0.25                 | 2.56 ± 0.27                 |
| 20% (10) | -   | 242 ± 39             | 0.81 ± 0.13                 | 2.08 ± 0.51                 | 3.58 ± 0.85*                |

Figures in parentheses are numbers of rats. *p<0.05, significantly different from the control.

Since this difference in the proportions of conjugates of bile acid induced by pyridoxine deficiency might result in a poor absorption of bile acid in the deficient animals, we analyzed the lipid compositions of the serum of portal blood. Unexpectedly, the bile acid contents of the portal serum were higher in pyridoxine-deficient rats on either 20 or 70% casein diet than in controls. However, the levels of cholesterol and phospholipids were low in the serum of rats on 70% casein pyridoxine-deficient diet (Table 3).

**Analysis of fecal sterols**

The excretion of cholesterol and its metabolites was examined by analyzing fecal sterols and bile acids in rats fed on the various diets (Table 4). Differences were found in the cholesterol levels of the feces of rats fed on diets containing different levels of casein, but no difference was found in fecal sterols in pyridoxine-deficient and control rats. On the other hand, the bile acid levels differed in the feces of pyridoxine-deficient and control rats: pyridoxine-deficient rats excreted more bile acids in the feces than did the controls.
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Table 5. Radioisotopic distribution in rats after [4-¹⁴C]cholesterol injection.

| Diet  | PIN | Plasma (%) | Liver (%) | S. Intestine (%) | L. Intestine (%) | Fecal excretion (%) |
|-------|-----|------------|-----------|------------------|------------------|---------------------|
| 70% (6) | +  | 2.2 ± 0.5  | 9.7 ± 2.4 | 23.0 ± 2.1       | 9.9 ± 1.2        | 55.2 ± 4.5          |
| 70% (6) | -  | 1.2 ± 0.6* | 11.5 ± 3.0| 15.8 ± 3.8*      | 6.9 ± 2.5*       | 64.6 ± 7.4*         |
| 20% (6) | +  | 2.8 ± 0.9  | 8.8 ± 1.5 | 11.9 ± 2.6       | 8.9 ± 1.9        | 67.5 ± 5.0          |
| 20% (6) | -  | 1.0 ± 0.5* | 4.0 ± 1.9*| 6.5 ± 2.8*       | 7.3 ± 2.7        | 81.2 ± 7.3*         |

Figures in parentheses are numbers of rats. Fecal excretion is shown as the amount excreted in 10 days and 14 days by rats on 70% and 20% casein diet, respectively. *% of dose injected. *p<0.05, significantly different from the control.

Table 6. Turnover rate of [4-¹⁴C]cholesterol injected into rats on pyridoxine-deficient or control diet.

| Diet  | Half-life (days) |
|-------|------------------|
|       | Control | Deficient |
| 70% Casein | 8.3 ± 3.3 (6) | 5.8 ± 1.7 (6) |
| 20% Casein  | 13.3 ± 1.5 (6) | 7.9 ± 3.2* (6) |

Figures in parentheses are numbers of rats. *p<0.05, significantly different from the control.

Metabolism of cholesterol

[4-¹⁴C]Cholesterol was injected intravenously into rats in the different groups; 10 or 14 days later the rats were killed and the distribution of radioactivity in their tissues was examined (Table 5). In all tissues tested, especially the plasma radioactivity was lower in pyridoxine-deficient rats than in controls. Pyridoxine-deficient rats excreted more radioactivity in their feces than controls. Determination of the rate of excretion of [4-¹⁴C]cholesterol showed that its half-life was shorter in pyridoxine-deficient rats (Table 6).

DISCUSSION

Previously, we reported that hepatic cholesterogenesis is increased in pyridoxine-deficient rats on 20 or 70% casein diet, although fatty liver due to accumulation of triglyceride and cholesterol ester is induced only in rats fed on 70% casein diet. The rate of cholesterogenesis is regulated by nutritional and hormonal factors (28). Cholesterol 7α-hydroxylase, which is considered to be a rate-limiting enzyme in bile acid synthesis, is also known to be influenced by the rate of cholesterol synthesis (29). We found that in pyridoxine-deficient rats the rate of bile
secretion was normal, but that the levels of bile acids, cholesterol and phospholipids in the bile were increased (Table 1). Increased cholesterogenesis in pyridoxine deficiency might result in increased secretion of bile acids. The data obtained here are not consistent with the observations of others (30, 31) who reported no difference between the bile acid contents of bile from pyridoxine-deficient and control rats. The reason for this discrepancy is unknown. Bile acids are conjugated with either glycine or taurine in the liver and then secreted into the bile. Normally, rat bile contains mainly taurine conjugates of bile acids (32), but the proportions of glycine and taurine conjugates vary under certain conditions. Pyridoxine deficiency disturbs amino acid metabolism, increases the glycine content of the liver and decreases the excretion of taurine in the urine (33–35). In pyridoxine-deficient rats, the proportions of the two conjugates of bile acids also change (Table 2): in the normal condition about 90% of the bile acids were conjugated with taurine whereas only one-half and one-third were conjugated with taurine in pyridoxine-deficient rats on 20% and 70% casein diet, respectively. These changes in the proportions of the two conjugates may reflect the relative concentrations of glycine and taurine in the liver, because the formations of these conjugates are catalyzed by a common enzyme (36, 37). It has been reported that hepatic cholesterogenesis is regulated by the level of bile acid in the portal blood (38) or by the level of absorption of cholesterol from the small intestine (39). We measured the lipid components in serum of portal blood and found that the level of bile acids was higher in pyridoxine-deficient rats than in controls (Table 3).

Fecal sterols were analyzed in rats on 20 or 70% casein diet with or without pyridoxine to determine whether pyridoxine deficiency affected the excretion of bile acids. An increase in bile acids in the feces was observed in pyridoxine-deficient rats (Table 4). The increased cholesterol catabolism in pyridoxine deficiency was confirmed by determination of the half-life of [4-14C]cholesterol injected into rats (Table 6).

Further studies are required to determine whether increased cholesterogenesis or increased cholesterol catabolism is the primary cause of the disturbance of cholesterol metabolism in pyridoxine deficiency. Studies are also necessary to discover how this increased cholesterol metabolism is related to fatty liver in pyridoxine-deficient rats.

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