We have reported that supplemental dietary vitamin B6 markedly suppresses colon tumorigenesis in azoxymethane-treated mice (1, 2). It has been suggested that the anti-tumor effect of dietary vitamin B6 is mediated by lowering cell proliferation, inflammation, angiogenesis, oxidative stress, and DNA damage (3–7). Recently, we observed that dietary vitamin B6 suppresses colonic cell damage as well as expression of heat shock protein 70 and heme oxygenase-1 (targets of anti-tumor agents) in rats exposed to 1,2-dimethylhydrazine (8).

In agreement with these findings, accumulating epidemiological evidence has strongly suggested an inverse association between vitamin B6 status and colon cancer (9–11), although the data on associations between ovary, bladder, and mammary cancers, and vitamin B6 status are controversial. Our animal studies have indicated a reduction in mammary tumorigenesis in rats receiving high amounts of vitamin B6, but the effect appears modest rather than remarkable (1, 2). Furthermore, our animal study has suggested that the level of pyridoxal 5′-phosphate (PLP) in the colon is relatively susceptible to dietary supplementation of vitamin B6 as compared with that in other organs (Kabo et al., unpublished data). Thus, we considered that the anti-tumor effect of dietary vitamin B6 may be specific for the colon.

Secondary bile acids, which are the intestinal microbial metabolites of primary bile acids, have been suggested to be highly cytotoxic and to induce DNA damage and thus, to promote the development of colon cancer (12, 13). The high production of intestinal organic acids, the fermentation products by microflora, has been associated with a lower risk of colon cancer (14). A high-fat (HF) diet increases the production of fecal secondary bile acids, decreases the production of cecal organic acid (13, 15), and is considered to increase the risk of colon cancer (16, 17). The high production of intestinal immunoglobulin A (IgA) and mucins, both of which play a role in the maintenance of gut barrier function, have been considered to be associated with a lower risk of colon cancer (18, 19). Thus, it was of interest to examine whether dietary vitamin B6 modulates these colonic luminal variables under the HF diet condition. This study examines the effect of dietary vitamin B6 on colonic luminal variables, including secondary bile acids, IgA, and mucins, in rats fed a HF diet.

**Materials and Methods**

Male Sprague Dawley rats (4 wk of age) were purchased from Hiroshima Laboratory Animal Center (Hiroshima, Japan) and maintained according to the

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**Note**

**Consumption of Vitamin B6 Reduces Fecal Ratio of Lithocholic Acid to Deoxycholic Acid, a Risk Factor for Colon Cancer, in Rats Fed a High-Fat Diet**

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**Summary**

To examine the effect of supplemental dietary vitamin B6 on the colonic luminal environment, growing male rats were fed a high-fat diet containing 1, 7, or 35 mg pyridoxine HCl/kg diet for 6 wk. Food intake and growth were unaffected by the dietary treatment. Supplemental dietary vitamin B6 significantly reduced the production of a fecal secondary bile acid, lithocholic acid (the most toxic secondary bile acid and a risk factor for colon cancer), and markedly reduced the ratio of lithocholic acid to deoxycholic acid (a less toxic secondary bile acid) in feces (p<0.05). Increasing dietary vitamin B6 increased fecal mucin levels (a marker of intestinal barrier function) in a dose-dependent manner (p<0.05) but did not affect fecal immunoglobulin A levels (an index of intestinal immune function). Cecal levels of organic acids were not significantly affected by supplemental dietary vitamin B6. These results suggest the possibility that dietary vitamin B6 affects the colonic luminal environment by altering the production of secondary bile acids and mucins.

**Key Words**

vitamin B6, colonic luminal environment, high-fat diet, rats

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were extracted by the method of Bovee-Oudenhoven et al. (25) and quantitated using a fluorometric assay (26). Cecal organic acids were measured by the internal standard method using an HPLC equipped with an Aminex HPX-87H ion exclusion column (7.8 mm i.d. x 30 cm, Bio-Rad, Richmond, CA) (27, 28). Activities of harmful fecal enzymes, including β-glucosidase, β-glucuronidase, tryptophanase, and urease, were determined according to the methods described elsewhere (29). Data were expressed as the means±SE. Statistical analysis was conducted by one-way analysis of variation (ANOVA) and Scheffe’s multiple-range test (Excel Statistics 2006 for Windows, Social Survey Research Information Co., Ltd., Tokyo, Japan). Statistical significance of the difference among means was estimated at p<0.05.

**Results and Discussion**

Growth and food intake did not differ among the groups (Table 1). Fecal dry weight was also unaffected. The serum level of PLP was significantly increased by supplemental dietary vitamin B6 in a dose-dependent manner. The concentration of PLP in the colon was significantly higher in the 7- and 35-mg PN HCl/kg diet groups than in the 1-mg PN HCl/kg diet group. These results suggest that supplementation of the diet with vitamin B6 modulates serum and colonic vitamin B6 status.

The production of a fecal secondary bile acid, lithocholic acid, was significantly lower in the 35-mg PN HCl/kg diet group than in the 1-mg PN HCl/kg diet group (Table 1, p<0.05), whereas that of fecal deoxycholic acid was significantly higher in the 35-mg PN HCl/kg diet group than in the 1-mg PN HCl/kg diet group (p<0.05). The concentration of fecal hyodeoxycholic acid (a metabolite of lithocholic acid) and the primary bile acid, cholic acid, were unaffected by dietary vitamin B6. A HF diet is associated with increased excretion of fecal enzymes, including β-glucosidase, β-glucuronidase, tryptophanase, and urease, were determined according to the methods described elsewhere (29). Data were expressed as the means±SE. Statistical analysis was conducted by one-way analysis of variation (ANOVA) and Scheffe’s multiple-range test (Excel Statistics 2006 for Windows, Social Survey Research Information Co., Ltd., Tokyo, Japan). Statistical significance of the difference among means was estimated at p<0.05.
acid and cholic acid, respectively. The mechanisms by which vitamin B₆ affects bile acid metabolism under HF diet conditions are currently unknown. Lithocholic acid strongly activates pregnane X receptor (PXR) and farnesoid X receptor (FXR), but deoxycholic acid has weak effects (37, 38). Therefore, a question was raised if the altered ratio of lithocholic acid to deoxycholic acid leads to modulation of the signaling of these receptors in the intestine.

Mucins can provide the first defense barrier between the luminal contents and the intestinal mucosal cells, and protect the underlying epithelium from potential pathogens and antigens (39). Compared with the 1-mg PN HCl/kg diet group, the 35-mg PN HCl/kg diet group had significantly increased fecal mucin levels (Table 1), implying a novel effect of dietary vitamin B₆ on intestinal barrier function. The increased production of intestinal mucins has been suggested to be associated with a lower risk of colon carcinogenesis (19). Thus, it was of interest to test the possibility that the anti-colon tumor effect of dietary vitamin B₆ is mediated by increased mucin production. Intestinal mucins provide binding sites for secretory IgA (40), and it has been suggested that high production of IgA is associated with a lowered risk of colon cancer (18). Thus, we examined the possibility that dietary supplemental vitamin B₆ may increase fecal IgA as well as mucin levels. However, our result indicates no influence on fecal IgA. It is necessary to examine the underlying mechanisms of increased production of mucin by supplemental vitamin B₆.

The weights of cecal digesta and cecal organic acids did not differ among the groups (Table 2). Thus, the elevation of the colonic B₆ level by dietary vitamin B₆ apparently does not affect intestinal fermentation. Tryptophanase degrades tryptophan to indole, ammonia, and pyruvate, and its increased activity is related to the incidence of colon cancer (41). In this study, fecal tryptophanase activity tended to be lower in the 7- and 35-mg PN HCl/kg diet groups (0.36 ± 0.04 and 0.34 ± 0.04 units per g dry weight, respectively) than in the 1-mg PN HCl/kg diet group (0.49 ± 0.08 units per g dry weight) (p = 0.092 and p = 0.057, respectively). Other harmful fecal enzymes, such as β-glucosidase,

| Pyridoxine HCl (mg/kg diet) | 1    | 7    | 35   |
|----------------------------|------|------|------|
| Weight of cecal contents (g) | 2.20 ± 0.19 | 2.10 ± 0.18 | 2.29 ± 0.24 |
| Cecal organic acid (μmol/g cecal digesta) | | | |
| Succinic acid | 4.2 ± 1.2 | 7.5 ± 1.3 | 6.7 ± 2.4 |
| Formic acid | 2.7 ± 0.8 | 1.7 ± 0.4 | 1.3 ± 0.3 |
| Acetic acid | 19.5 ± 1.3 | 23.2 ± 1.4 | 20.4 ± 2.1 |
| Propionic acid | 5.1 ± 0.4 | 5.9 ± 0.6 | 5.2 ± 0.4 |
| Isobutyric acid | 1.4 ± 0.1 | 1.8 ± 0.1 | 1.3 ± 0.2 |
| Butyric acid | 5.7 ± 0.7 | 6.1 ± 0.3 | 5.0 ± 0.5 |
| Isovaleric acid | 1.9 ± 0.2 | 2.5 ± 0.1 | 2.3 ± 0.2 |
| Valeric acid | 1.6 ± 0.1 | 1.8 ± 0.1 | 1.8 ± 0.3 |
| Total organic acids | 41.8 ± 3.0 | 50.4 ± 2.2 | 41.1 ± 5.0 |

1 Values are presented as means ± SE (n=7–8).
β-glucuronidase, and urease, were unaffected by the dietary level of vitamin B₆ (data not shown).

In conclusion, the present study provides evidence that dietary vitamin B₆ decreases the ratio of fecal lithocholic acid to deoxycholic acid and increases the production of fecal mucins in rats fed a HF diet. These findings imply a novel effect of dietary vitamin B₆ that may be favorable for colon health under HF diet conditions. Deoxycholic acid has been reported to be an inducer of gene expression of MUC2, a major intestinal mucin, but the role of lithocholic acid on the gene expression is unknown (42). Thus, the relation between the decreased ratio of the lithocholic acid to deoxycholic acid and increased mucins remains to be examined.

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