Effect of Dietary Fat and Fiber on Fecal Flora, Bacterial Metabolites, and Fecal Properties in Japanese Volunteers

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Summary The effects of dietary fat and dietary fiber (DF) levels in diet on fecal flora, activities of three fecal enzymes, putrefactive metabolites, fecal mutagenicity and fecal properties were studied in eight healthy volunteers. They were given low fat and low DF diet (LF: fat energy ratio was 13.9%, and DF intake was 9.0 g/day) for 10 days, high fat and low DF diet (HF: fat energy ratio was 52.7%, and DF intake was 7.1 g/day) for 10 days, and high fat and high DF diet (HFF: fat energy ratio was 52.0%, and DF intake was 24.8 g/day) for 10 days. No change of fecal flora at the bacterial group level was observed throughout the experimental period, except that the population of lactobacilli showed a tendency to increase in HF period. Fecal activities of ẞ-glucuronidase, ẞ-glucosidase and nitroreductase and some putrefactive products were unchanged between LF and HF, while these values decreased in HFF period. No significant change of fecal properties was observed between LF and HF, while by HFF supplementation fecal weight increased and fecal pH value was lower than that in LF and HF. Excretions of iron, zinc and calcium in feces did not increase by high DF supplementation.

Key Words fecal flora, putrefactive metabolites, corn dietary fiber, fat, mineral excretion, fecal mutagenicity, fecal enzymes

Many epidemiological studies have reported regional differences of the frequency of adult chronic diseases (1, 2). In European and American people, diseases...
such as obesity, diabetes, coronary heart disease and some cancers occurred frequently, while there was a small occurrence of these diseases in Africa and Asia (3). The European and American diets have high fat and low dietary fiber (DF) content, while the Afro-Asian diets have low fat and high DF content. Fat and DF contents in the daily diet have been considered important factors in such chronic diseases according to many of the epidemiological or experimental studies (1, 2).

Several studies (4–9) have been carried out on the effect of DF and fat intake on fecal flora and on fecal metabolites, but the results are not always consistent with each other. Such variance of these results might be attributed to differences of the experimental conditions such as source of DF and fat supplementation and volunteers.

Diet composition of Japanese people has been westernized in recent years, and geriatric diseases have increased. Benno and Mitsuoka reported the effect of an experimental western diet on fecal flora in Japanese volunteers (10). This experimental diet was a high meat diet; however, we considered that the western diet is not so much a high meat diet as a high fat diet.

In this paper, we described an experimental Japanese diet, a western diet, and a DF-supplemented western diet given to Japanese healthy volunteers, and described the influences of fat and DF contents in the diet on fecal flora, fecal properties, fecal enzyme activities and fecal putrefactive products.

EXPERIMENTAL

Experimental design. The volunteers were given an experimental Japanese diet that was a low fat and low DF diet (LF), an experimental western diet that was a high fat and low DF diet (HF) and an experimental DF-supplemented western diet that was a high fat and high DF diet (HFF), in that order, for 10 days each. The effect of dietary fat level was determined in the two periods of LF and HF, and the effect of DF level was determined in the two periods of HF and HFF. In each dietary period, all fecal samples were collected from subjects for 5 days (from Day 6 to Day 10), and these samples were used for measurement of fecal weight, pH and mutagenicity. Fecal flora, moisture, enzyme activities (β-glucosidase, β-glucuronidase and nitroreductase), putrefactive products and mineral excretions in feces were determined twice (Days 8 and 10). This study was performed in accordance with the Helsinki Declaration as updated in Tokyo, Japan (1975).

Subjects. Eight healthy adults (26–46 years old, 6 males and 2 females) were used in this study. No subject had taken antibiotics or other therapy for at least 4 weeks prior to the experiment.

Experimental diet. Daily menus of each subject were recorded, and nutritional compositions of experimental diet were calculated. The diet compositions are shown in Table 1. In HF and HFF diets, the supplementation of fat was animal fat such as butter, lard and cream. HFF diet was the HF diet supplemented with 18 g/day of corn fiber (Cellfer #200, Nihon Shokuhin Kako Co., Ltd., Tokyo),

Table 1. The composition of test diets.

<table>
<thead>
<tr>
<th>Experimental period</th>
<th>LF</th>
<th>HF</th>
<th>HFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal/day)</td>
<td>1823±278</td>
<td>2697±465</td>
<td>2574±301</td>
</tr>
<tr>
<td>Fat energy (%)</td>
<td>13.9±2.7</td>
<td>52.7±6.8</td>
<td>52.0±5.5</td>
</tr>
<tr>
<td>Protein energy (%)</td>
<td>16.0±2.0</td>
<td>16.4±2.1</td>
<td>15.0±2.1</td>
</tr>
<tr>
<td>Carbohydrate energy (%)</td>
<td>70.0±4.3</td>
<td>30.9±7.2</td>
<td>33.0±6.3</td>
</tr>
<tr>
<td>Animal fat total fat (%)</td>
<td>53.5±9.5</td>
<td>72.3±5.6</td>
<td>73.5±6.7</td>
</tr>
<tr>
<td>Dietary fiber intake (g/day)</td>
<td>9.0±3.2</td>
<td>7.1±3.2</td>
<td>24.8±7.3</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>320±120</td>
<td>887±260</td>
<td>802±202</td>
</tr>
<tr>
<td>Iron intake (mg/day)</td>
<td>12.2±6.2</td>
<td>14.5±8.6</td>
<td>14.2±7.6</td>
</tr>
</tbody>
</table>

Values are mean±SD. LF, low fat and low dietary fiber diet; HF, high fat and low dietary fiber diet; HFF, high fat and high dietary fiber diet.

contained in a cracker. Corn fiber, which is high in DF content and low in phytin content, is a commercial DF source prepared mechanically from a corn hull which is a by-product in the corn wet-milling starch production process. Corn fiber contained about 90% of the total DF analyzed by the method of Prosky et al. (11).

Analysis of fecal flora. Bacterial analysis of the fecal flora was carried out using the method of Mitsuoka et al. (12, 13). The culture media used in this study were four non-selective agar plates (M10, EG, BL and TS) and ten selective agar plates (BS, ES, NBGT, NN, LBS, DHL, TATAC, PEES, PD and NAC). The bacterial identification was performed with colonial and cellular morphologies, Gram-reaction, spore-formation and aerobic growth. The bacterial counts per gram of wet feces were calculated and converted to a common logarithmic equivalents.

Measurement of fecal properties. The fecal weight was determined by a balance and pH was determined directly by inserting a glass-electrode into feces. The fecal moisture content was determined by the measurement of fecal weight decrease after drying by a vacuum drying oven (70°C, 75 cmHg, for 2 days); Ca, Fe and Zn contents in the feces were measured by using a plasma emission spectrometer.

Measurement of fecal enzyme activities. Fecal activities of β-glucosidase, β-glucuronidase and nitroreductase were measured by the method of Rowland et al. (14). Fresh fecal samples were suspended in anaerobic phosphate buffer (pH 7.0). The suspensions were incubated with each substrate, i.e., p-nitrophenyl β-D-glucoside, p-nitrophenyl β-D-glucuronide and p-nitrobenzoic acid in the test tubes replaced with oxygen-free nitrogen gas. Enzyme activities were measured as time-related appearance of p-nitrophenol or p-aminobenzoic acid over 60 min. Each enzyme activity concentration was expressed in mol of products per hour per gram of wet feces, and daily activity output was expressed in mol of products per hour per day.

Measurement of fecal putrefactive products. Putrefactive products such as
indole, p-cresol, phenol and skatole were analyzed by a gas chromatography (15), and polyamines such as putrescine, cadaverine, spermidine and spermine were analyzed by a high-pressure liquid chromatography (16). Each fecal putrefactive product is also shown as concentration in feces and as daily output.

**Mutagenicity assay.** The assay of mutagenicity was carried out by the in vivo method of Heddle et al. (17), and Suzuki and Bruce (18), and by the in vitro method of Ames et al. (19). For in vivo method, fecal material extracted by dichloromethane was injected in the rectum of male mice (C57BL/6J, Charles River), aged 7–8 weeks. After 24 h, mice were killed, and the appearances of nuclear aberration in rectum crypt epithelial cell were counted (17). For in vitro method, fecal material extracted by dichloromethane containing 20% of acetone was used for Ames test (19).

**Statistical analysis.** Number of fecal flora was analyzed statistically by the Wilcoxon’s rank-sum test, and frequency of occurrence of fecal flora was analyzed statistically by the Fisher’s exact test. Other data such as fecal properties, enzyme activities and putrefactive products were analyzed statistically by the paired Student’s t-test.

**RESULTS**

**Fecal flora**

Table 2 shows the fecal flora. No significant change in fecal flora at the bacterial group level was observed throughout each experimental period, although the number of lactobacilli in HF and HFF tended to be higher than that in LF.

**Fecal properties**

Fecal pH and fecal moisture are shown in Fig. 1. No remarkable change of fecal moisture was observed. Fecal pH in HF showed a tendency to be higher than that in LF, while that in HFF was significantly lower than that in HF (p < 0.05). Fecal weight is shown in Fig. 2. Fecal weight tended to increase with the three consecutive periods. In this study, daily energy intake in LF was different from that in HF and HFF. Fecal weight per 1,000 kcal of energy intake in HF was lower than that in LF, and that value in HFF increased to the level in LF. Daily mineral excretion in feces is shown in Fig. 3. The excretion (both the concentration in feces and the amount of daily output) of fecal Ca and Zn in HF was more than those in LF significantly (p < 0.05), while those values in HFF decreased to the levels in LF. Fecal excretion of Fe decreased slightly with the three consecutive periods.

**Fecal enzyme activities**

Figure 4 shows the changes of fecal enzyme activities. Concentration of β-glucuronidase and β-glucosidase activities tended to decrease with consecutive periods. Daily output of β-glucuronidase and β-glucosidase activities in HF showed a tendency to be higher than those in LF, while those values in HFF decreased to
Table 2. Effect of dietary fat and fiber levels on the human fecal flora.

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>LF period</th>
<th></th>
<th>HF period</th>
<th></th>
<th>HFF period</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 8</td>
<td>Day 10</td>
<td>Day 8</td>
<td>Day 10</td>
<td>Day 8</td>
<td>Day 10</td>
</tr>
<tr>
<td>Bacteroidaceae</td>
<td>10.6±0.3 (8/8)*</td>
<td>10.7±0.2 (8/8)</td>
<td>10.5±0.3 (8/8)</td>
<td>10.5±0.1 (8/8)</td>
<td>10.6±0.2 (8/8)</td>
<td>10.4±0.2 (8/8)</td>
</tr>
<tr>
<td>Eubacteria</td>
<td>10.3±0.2 (8/8)</td>
<td>10.2±0.3 (8/8)</td>
<td>10.2±0.3 (8/8)</td>
<td>10.4±0.3 (8/8)</td>
<td>10.4±0.2 (8/8)</td>
<td>10.1±0.3 (8/8)</td>
</tr>
<tr>
<td>Peptococcaceae</td>
<td>9.8±0.3 (8/8)</td>
<td>9.8±0.5 (8/8)</td>
<td>9.9±0.4 (8/8)</td>
<td>9.8±0.4 (8/8)</td>
<td>9.5±0.5 (8/8)</td>
<td>9.8±0.3 (7/8)</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>10.0±0.3 (8/8)</td>
<td>10.1±0.4 (8/8)</td>
<td>10.3±0.3 (8/8)</td>
<td>10.3±0.3 (8/8)</td>
<td>10.2±0.3 (8/8)</td>
<td>10.0±0.2 (8/8)</td>
</tr>
<tr>
<td>Veillonellae</td>
<td>6.1±1.3 (8/8)</td>
<td>6.1±0.6 (8/8)</td>
<td>6.6±0.8 (8/8)</td>
<td>6.2±1.3 (8/8)</td>
<td>6.3±0.6 (8/8)</td>
<td>6.0±1.7 (7/8)</td>
</tr>
<tr>
<td>Megasphaerae</td>
<td>8.3 (1/8)</td>
<td>7.0±1.5 (4/8)</td>
<td>6.9±0.6 (2/8)</td>
<td>7.9±1.4 (4/8)</td>
<td>5.4 (2/8)</td>
<td>5.3 (2/8)</td>
</tr>
<tr>
<td>*Clostridium perfringens</td>
<td>4.0 (2/8)</td>
<td>4.2 (2/8)</td>
<td>&lt;2.0 (0/8)</td>
<td>&lt;2.0 (0/8)</td>
<td>6.5 (2/8)</td>
<td>5.3 (2/8)</td>
</tr>
<tr>
<td>*Clostridium-others</td>
<td>9.4±0.5 (8/8)</td>
<td>9.5±0.5 (8/8)</td>
<td>9.3±0.6 (8/8)</td>
<td>9.5±0.3 (8/8)</td>
<td>9.7±0.3 (8/8)</td>
<td>9.5±0.2 (8/8)</td>
</tr>
<tr>
<td>*Lactobacilli</td>
<td>5.7±1.4 (7/8)</td>
<td>5.6±1.1 (7/8)</td>
<td>6.6±1.9 (8/8)</td>
<td>6.5±1.7 (8/8)</td>
<td>6.7±1.5 (8/8)</td>
<td>6.6±1.4 (8/8)</td>
</tr>
<tr>
<td>*Enterobacteriaceae</td>
<td>7.8±1.3 (8/8)</td>
<td>7.5±1.3 (8/8)</td>
<td>6.9±0.9 (8/8)</td>
<td>7.3±0.9 (8/8)</td>
<td>6.8±1.2 (8/8)</td>
<td>6.6±0.8 (8/8)</td>
</tr>
<tr>
<td>*Enterococci</td>
<td>7.0±0.9 (8/8)</td>
<td>6.6±0.9 (8/8)</td>
<td>6.5±1.3 (8/8)</td>
<td>6.6±1.6 (8/8)</td>
<td>6.9±1.4 (8/8)</td>
<td>7.0±1.0 (8/8)</td>
</tr>
<tr>
<td>*Staphylococci</td>
<td>&lt;2.0 (0/8)</td>
<td>&lt;2.0 (0/8)</td>
<td>&lt;2.0 (0/8)</td>
<td>2.6 (2/8)</td>
<td>2.3 (2/8)</td>
<td>2.8 (2/8)</td>
</tr>
<tr>
<td>*Bacilli</td>
<td>4.2 (1/8)</td>
<td>&lt;2.0 (0/8)</td>
<td>&lt;2.0 (0/8)</td>
<td>&lt;2.0 (0/8)</td>
<td>&lt;2.0 (0/8)</td>
<td>&lt;2.0 (0/8)</td>
</tr>
<tr>
<td>Yeasts</td>
<td>4.0±0.4 (6/8)</td>
<td>3.3±0.8 (7/8)</td>
<td>3.3±0.8 (7/8)</td>
<td>2.5±0.2 (3/8)</td>
<td>3.4±0.8 (3/8)</td>
<td>2.6±0.3 (4/8)</td>
</tr>
<tr>
<td>Total counts</td>
<td>11.0±0.1</td>
<td>11.0±0.2</td>
<td>10.9±0.1</td>
<td>11.0±0.2</td>
<td>11.0±0.1</td>
<td>10.8±0.2</td>
</tr>
</tbody>
</table>

Values are means±SD of log bacterial counts per gram of wet feces (n=8). *Frequency of occurrence.
Fig. 1. Fecal moisture content and fecal pH during the period of LF (low fat and low dietary fiber diet), HF (high fat and low dietary fiber diet) and HFF (high fat and high dietary fiber diet). Each plot represents mean±SD. *Significantly different from HF period (p<0.05).

Fig. 2. Fecal weight during the period of LF, HF and HFF. Each column represents 8 subjects' data as mean±SD.

Fig. 3. Excretions of Fe, Ca and Zn in feces during the period of LF, HF and HFF. Each column represents 8 subjects' data as mean±SD. *Significantly different from LF period (p<0.05).

Fig. 4. Fecal β-glucosidase, β-glucuronidase and nitroreductase activities during the period of LF, HF and HFF. Each column represents 8 subjects’ data as mean±SD. *Significantly different from HF period (p<0.05).

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Fig. 5. Fecal phenol, p-cresol, indole and skatole excretion in feces during the period of LF, HF and HFF. Each column represents 8 subjects’ data as mean±SD. *Significantly different from HF period.

the levels in LF. Both concentration and daily output of nitroreductase activities in HF showed a tendency to be higher than those in LF, while those values in HFF were smaller than those in LF significantly (p<0.05).
Fig. 6. Polyamines excretion in feces during the period of LF, HF and HFF. Each column represents 8 subjects' data as mean±SD.

Fig. 7. Fecal mutagenicity changes during the period of LF, HF and HFF. Mutagenicity assays were carried out by the method of Heddle et al. (17), and Suzuki and Bruce (18) using the rectum of male mice (C57BL/6J, Charles River). ○, average.

**Fecal putrefactive products**

Putrefactive products in feces are shown in Figs. 5 and 6. No remarkable effects by HF supplementation were observed, while fecal contents (both concentration in feces and amount of daily output) of p-cresol, indole and total putrefactive products without polyamines in HFF were significantly smaller than those in HF.
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(p < 0.05). The fecal polyamine contents (both concentration in feces and amount of daily output) in HF did not change remarkably from those in LF, and polyamines without putrescine in HFF showed a tendency to be smaller than those in HF. On the other hand, fecal putrescine in HFF did not change from that in HF.

Fecal mutagenicity

*In vitro* tests by the Ames method were all negative. The change of fecal mutagenicity *in vivo* is shown in Fig. 7. All *in vivo* cytogenetic data were also normal, while fecal mutagenicity in HF tended to be higher than that in LF, and that in HFF was recovered to the levels in LF.

DISCUSSION

The relationship between the intestinal flora and dietary factors have been studied by some workers, but their results do not always agree with each other (20–22). Benno and Mitsuoka reported that the numbers of bifidobacteria, eubacteria and lactobacilli in high meat diet intake were decreased (10). Reddy *et al.* reported that the numbers of bacteroidaceae, bifidobacteria, peptococcaceae and lactobacilli in the high-risk diet of which nutritional composition was high protein and high fat were higher than those in the low-risk diet that was a no meat diet (9), and these results agreed with Aries *et al.* (20) or Hill *et al.* (23). Our result on high fat supplementation in this study is that the number of *Lactobacillus* tended to increase, and this result is in accord slightly with that obtained by Reddy *et al.* Drasar *et al.* found no changes of fecal flora by wheat bran intake (8), while Fuchs *et al.* found an increase in the number of total anaerobes, *Clostridium* and *Streptococcus*, and a decrease in the number of *Lactobacillus* and *Eubacterium* (22). Baird *et al.* reported that there was no significant change of fecal flora by intake of a bagasse which was the dregs of a sugar cane (4). Wheat bran, corn fiber and bagasse are composed from a plant cell wall tissue, and they contain large percentage of hemicelluloses that are arabinoxylan. Our result on high DF supplementation is that there were no remarkable changes; this is in accord with that obtained by Baird *et al.*

It is widely known that fecal enzymes, such as β-glucuronidase, β-glucosidase and nitroreductase catalyze the reactions of carcinogen production and activation in the gut (24). In particular, β-glucuronidase catalyzes the deconjugation of glucuronic acid conjugates which are made from harmful compounds by detoxication in the liver, and then harmful compounds are retained for a longer time in the body as a result of the enterohepatic circulation.

Some of the putrefactive products produced in the amino acid metabolism of intestinal bacteria are absorbed and play a role in mutagenesis and carcinogenesis (25).

Reddy *et al.* reported significant increase of fecal β-glucuronidase activity by high meat diet (9). Cummings *et al.* reported no change in β-glucuronidase activity...
when male volunteers consumed either 62 or 152 g/day of dairy fat (26). By corn fiber supplementation, we reported the decrease of β-glucuronidase activity in Japanese volunteers in our previous paper (27). A similar observation was made by London et al., who found glucuronide hydrolysis decreased by 80 to 90% in mice fed a purified diet containing 20% of soya bean bran or corn bran (28).

In this study, the inhibition of harmful compound productions, that is, fecal enzyme activities, putrefactive products and mutagenicity by high DF diet supplementation would be important to prevent carcinogenesis of the host. Since the fecal flora at the bacterial group level did not change in HFF, the decrease of harmful metabolites might have resulted from either the changes of fecal flora in the genus level, or the metabolic activity changes of microbes. Additionally, harmful enzymes and products are diluted by increased feces in the gut, and harmful influences to host might decrease.

The detrimental influence of high DF supplementation is considered to be inhibition of mineral absorption in the gut (5). The increased Ca excretion on HF supplementation is considered to result from increased dietary Ca input that originates from milk products used for the fat supplementation. Ca intake in HFF was about equal to that in HF, but Ca excretion in HFF decreased. In this study, mineral excretion did no increase by high DF supplementation; this might have resulted from the low phytin content of corn fiber.

To summarize our interpretation of the results, there were no significant effects by high fat supplementation. High DF supplementation did not influence fecal flora at the bacterial group level but any bacterial harmful metabolites were decreased. These changes of bacterial metabolites suggested that high DF supplementation resulted in improvement of intestinal environment.

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