Hypocholesterolemic Effect of Dietary Fiber: Relation to Intestinal Fermentation and Bile Acid Excretion

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Summary Rat cecal contents were static-cultured with three kinds of dietary fibers and examined for the production of short-chain fatty acids after 24 h of cultivation. The total amount and molar ratio of acetate, propionate and butyrate (n=8) were 7.20±0.62 mM and 38:19:43 with indigestible dextrin (ID), a low-viscosity, water-soluble dietary fiber, 10.88±0.46 mM and 49:5:46 with pectin (PE), a high-viscosity, water-soluble dietary fiber, and 1.83±0.19 mM and 64:11:25 with corn fiber (CF), a water-insoluble dietary fiber, respectively (the corresponding values obtained with glucose were 10.59±0.37 mM and 15:27:58). Next, rats were kept on a cholesterol- and bile acid-free high-sucrose diet. At the completion of the 8-week feeding period, the serum total-cholesterol levels were significantly lower, at 57.6±3.8 (n=8), 63.2±4.67 (n=7), and 77.8±3.7 mg/dl (n=9), in the ID-, PE-, and CF-supplemented diet groups, respectively, than in the control group given no dietary fiber (92.7±3.8 mg/dl, n=7). The cecal propionate production was significantly increased in both the ID and PE groups, while the fecal excretion of bile acids was increased in all three fiber groups compared to the control group. In addition, there was a significantly negative correlation between the serum total-cholesterol level and cecal propionate production in the ID group, between the serum cholesterol level and bile acid excretion in the CF group, and between the serum cholesterol level and cecal propionate production or bile acid excretion in the PE group. These results suggest that the degree of intestinal fermentation and bile acid excretion, which are considered to be associated with the hypocholesterolemic action of dietary fiber, varies with each kind of dietary fiber.

Key Words dietary fiber, hypocholesterolemic effect, fermentation, bile acid, short-chain fatty acid, propionate

Dietary fibers are believed to lower the serum cholesterol level by inhibiting the absorption of neutral and acidic steroids from the small intestine (I). However,
this hypothesis can not be generalized for all kinds of dietary fiber because the effect of different types of fiber is altered to varying degrees by ion-exchange capability or viscosity (1). On the other hand, Wright et al. (2) and Illman et al. (3) have recently reported that propionate inhibits the synthesis of cholesterol in the liver. Considering that the production of short-chain fatty acids by microflora varies with the type of ingested dietary fiber, which serve as main materials for fermentation, it is feasible that propionate is intimately involved in the hypocholesterolemic effect of dietary fiber (4–8). Furthermore, it has been suggested that dietary fibers may affect the lipid metabolism in the liver and peripheral tissues by modifying the secretion of gastrointestinal hormones, insulin, and glucagon (1). Taking into account the hypocholesterolemic effect of dietary fiber, these individual factors seems to act alone or together in varying degrees to lower the serum cholesterol level.

The present investigation was undertaken to elucidate the mechanism by which dietary fiber lowers the serum cholesterol level. First, three kinds of dietary fibers which have more or less hypocholesterolemic effects and differ in physicochemical properties (e.g., water solubility and viscosity) (9–11) were examined for fermentation under static culture conditions with cecal leavings from rats. Second, the serum cholesterol levels were plotted against the propionate-producing capacities of cecal leavings or the amounts of bile acid excretion into the feces in rats given a cholesterol- and bile acid-free diet supplemented with these dietary fibers for 8 weeks.

MATERIALS AND METHODS

1. Dietary fibers. Water-soluble indigestible dextrin (hereinafter called ID) is a product “Fibersol®-2” of Matsutani Chem. Ind. Ltd., Itami, which is a corn starch-derived fiber with a mean molecular weight of 1,600. Its viscosity was 15 cps, as measured in 30% aqueous solution at 30°C with a B type viscometer. Citrus pectin (hereinafter called PE) was obtained from Wako Pure Chem. Ind. Ltd., Osaka, which had the viscosity of 200 cps in 1% aqueous solution, and corn fiber (hereinafter called CF) being Cellfer® from Nihon Shokuhin Kako Co., Ltd., Tokyo, was used as a water-insoluble fiber.

2. Fermentation experiments with various dietary fibers by static culture. Male Sprague-Dawley rats 6 weeks old were purchased from Japan CLEA Co., Ltd., Osaka, and were fed on a commercial stock diet (CE-2, Japan CLEA Co., Ltd.). Two weeks later, the rats were anesthetized by an injection of sodium pentobarbital (25 mg/kg of body weight) to excise the cecum. A part of the cecal content was suspended with 4 volumes of phosphate-buffered saline (pH 7.4, PBS) which had been autoclaved at 120°C for 20 min, and filtered through 4 layers of gauze in an anaerobic box (Galaxy, Iuchiseieido Co., Ltd., Tokyo). The filtrate was used as a culture medium. On the other hand, glucose (control), ID, PE, and CF were individually dissolved in PBS to obtain their substrate solutions (500
mg/dl), of which a 1-ml portion was infused together with each milliliter of the culture medium into a screw-vial (Product No. 224802, Wheaton Co., Ltd., New Jersey, U.S.A.). Immediately, nitrogen gas was blown into the screw-vial, which was incubated at 37°C for 30 h under an airtight condition. The culture medium alone, without adding the substrate solution, was used as a blank. The culture was stopped by freezing it in dry ice acetone, and liquid paraffin was layered over the frozen sample to avoid any loss of short-chain fatty acids by volatilization. All samples were kept at −80°C until analysis. Prior to analysis, each sample was thawed and centrifuged at 1,000 × g for 10 min. The supernatant was filtered through a membrane filter (pore size: 0.22 μm, Millipore Japan Co., Ltd., Tokyo). Crotonic acid (Wako Pure Chem. Ind. Ltd.) was added to the filtrate as an internal standard. The mixture was ultracentrifuged (Ultrafree-C3 with the filtration limit of 5,000 molecular weight, Millipore Japan Co., Ltd.) and short-chain fatty acids were determined using a Shimadzu GC-14A gas chromatograph equipped with a hydrogen flame ionization detector; column, fused silica WCOT (0.58 mm × 30 m); carrier gas, helium at a flow rate of 10 ml/min; programmed temperature, 95 to 140°C at a rate of 2.5°C/min.

Acetate, propionate, and butyrate (GL Science Co., Ltd., Tokyo) were used as reference standards. The coefficient of variation (CV) of this analysis system was below 4% about these three kinds of fatty acids and their recovery was almost quantitative (97.4, 105.4, and 105.0% with acetate, propionate, and butyrate, respectively). The production amount of short-chain fatty acids from the respective substrates was corrected by subtracting the blank production with the substrate-free culture medium.

Experiment 1 (Time-dependent changes in the propionate production): One milliliter of the culture medium obtained by mixing 45 g of cecal contents with 180 ml PBS and incubating with 1 ml of substrate solution (i.e. 500 mg/dl glucose or different kinds of dietary fibers) under static culture conditions for 30 h to assess the time course of propionate production.

Experiment 2 (Production of short-chain fatty acids and lactate from dietary fibers): One milliliter of the culture medium obtained by mixing 50 g of cecal contents with 200 ml PBS and incubating with 1 ml substrate solution (i.e. 500 mg/dl glucose or different kinds of dietary fibers) under static-culture conditions. Six or 24 h later, the samples were analyzed for acetate, propionate and butyrate (gas chromatographically), and lactate (enzymatically using lactate dehydrogenase).

3. Feeding experiment. Male Sprague-Dawley rats 3 weeks old were fed on a "high-sucrose" diet containing sucrose, casein, corn oil, mineral mix (MM-2), vitamin mix (Harper), choline chloride, and vitamin E in the weight % ratio of 64.75:25:5:4:1:0.2:0.05% (11, 12). Two weeks later, the rats were divided into 4 groups: group I was fed the high-sucrose diet, group II to IV were fed 95% high-sucrose diets supplemented with either 5% ID (group II), PE (group III), or CF (group IV). They were given free access to the respective diet, which were
replaced with fresh ones every 2 days (the amount of food intake was measured each time). The animals were housed in wire-bottomed cages in an air-conditioned (23±1°C) room with controlled lighting (20:00–8:00 h). At the end of the 8th week, the animal was placed in a stereotaxic device (Model MAC-1S, CFK Research Institute) and blood was collected via the right jugular vein under consciousness (13) to measure the serum levels of total-cholesterol, HDL-cholesterol, and triacylglycerol using commercial assay kits (Wako Pure Chem. Ind. Ltd.). The cecal contents were weighed and the amounts of short-chain fatty acids produced were determined in the feces for 3 days before sacrifice, along with determination of bile acids in the manner previously described (14).

4. Statistical analysis. Data are expressed as the mean±SEM. Differences between groups were assessed by Student’s t-test or the Duncan’s multiple range test after preliminary analysis of variance (ANOVA) and were considered statistically significant at p<0.05.

RESULTS

1. Fermentation with various types of fiber

Experiment 1. Figure 1 shows the time courses of propionate production from glucose and different kinds of dietary fiber. When glucose was used as the substrate, the propionate production first increased steeply reaching a plateau (3.02 mM) in 15 h and showing 2.91 mM even after 30 h. The propionate production in the ID group remained at a low level relative to the control value obtained with glucose, but increased gradually with time, reaching 65% of the control value at 30 h. That in the PE group reached a maximum at 6 h, 14% of the control value. That in the CF group appeared after 9 h of cultivation, reaching no more than 5% of the control value at 30 h.

Fig. 1. Time-dependent production of propionate from glucose (●), indigestible dextrin (ID: ○), pectin (PE: △), and corn fiber (CF: □).
Fig. 2. Short-chain fatty acid (SCFA) production from glucose (G), indigestible dextrin (ID), pectin (PE), and corn fiber (CF) at 6 (□) and 24h (□). Total SCFA was the sum of acetate, propionate, and butyrate.

Experiment 2. The amounts of short-chain fatty acid production from glucose or different kinds of dietary fiber at 6 and 24h are shown in Fig. 2. The production amount of total short-chain fatty acids in 24h, which was the sum of acetate, propionate, and butyrate, was comparable between both groups of glucose (10.59 ± 0.37 mM) and PE (10.88 ± 0.46 mM), the value being significantly higher in them than in the ID group (7.20 ± 0.62 mM). In the CF group, the production of total short-chain fatty acids in 24h (1.83 ± 0.19 mM) was significantly lower than in the ID group. The production of individual short-chain fatty acids and their molar ratio varied in different substrates. The molar ratio of acetate, propionate, and butyrate was 15:27:58 for the glucose group, 38:19:43 for the ID group, 49:5:56 for the PE group, and 64:11:25 for the CF group. The lactate production by anaerobic fermentation from glucose and from different kinds of dietary fiber is shown in Table 1. The amount of lactate production from glucose increased about threefold at 6h compared with the pre-culture, but was reduced by half of value at 6h at 24h. With respect to dietary fiber, lactate production was significantly lower at 6 or 24h than the pre-culture despite the pH change below 7.0 to 5.6, 5.4, and 6.2.

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Table 1. Lactate production (mM) from glucose and different kinds of dietary fiber and changes in pH.

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>ID</th>
<th>PE</th>
<th>CF</th>
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<tbody>
<tr>
<td>0 h</td>
<td></td>
<td></td>
<td></td>
<td>0.37±0.03&lt;nsup&gt;1&lt;/nsup&gt;</td>
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<tr>
<td>(pH) 5.0</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>6 h</td>
<td>1.25±0.04&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.19±0.01&lt;sup&gt;Bbc&lt;/sup&gt;</td>
<td>0.12±0.01&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>0.26±0.02&lt;sup&gt;Bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>(pH) 5.4</td>
<td>(5.4)</td>
<td>(5.8)</td>
<td>(5.5)</td>
<td>(6.2)</td>
</tr>
<tr>
<td>24 h</td>
<td>0.56±0.01&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>0.13±0.01&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>0.13±0.01&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>0.23±0.03&lt;sup&gt;Bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>(pH) 5.3</td>
<td>(5.6)</td>
<td>(5.4)</td>
<td>(5.4)</td>
<td>(6.2)</td>
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</table>

Cecal contents were incubated with various substrates for 6 or 24 h. The details are described in the text. Values (mean±SE) not sharing a common superscript letter in the same column or line are significantly different from each other (p<0.05). ID, indigestible dextrin; PE, pectin; CF, corn fiber.

Table 2. Effect of dietary fiber on serum lipid levels in rats.

<table>
<thead>
<tr>
<th></th>
<th>Total-cholesterol (mg/dl)</th>
<th>HDL-cholesterol (mg/dl)</th>
<th>Atherogenic index</th>
<th>Triacylglycerol (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td>Control 7</td>
<td>92.7±3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.5±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57±0.06</td>
<td>218±30</td>
</tr>
<tr>
<td>ID 8</td>
<td>57.6±3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.2±4.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.68±0.11</td>
<td>134±28</td>
</tr>
<tr>
<td>PE 7</td>
<td>63.2±4.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.7±3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65±0.07</td>
<td>166±39</td>
</tr>
<tr>
<td>CF 9</td>
<td>77.8±3.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.3±5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54±0.12</td>
<td>147±33</td>
</tr>
</tbody>
</table>

Rats were kept for 8 weeks on a high-sucrose diet supplemented with or without dietary fiber at the 5% level. Mean±SE: values not sharing a common superscript letter in the same column are significantly different from each other (p<0.05). Control, fiber free; ID, indigestible dextrin; PE, pectin; CF, corn fiber.

2. Feeding experiment

The body weight gain throughout 8 weeks was significantly greater in the ID group (307.3±6.6 g) and the PE group (310.4±9.8 g) than in the control group (278.2±6.0 g) and CF group (282.2±5.6 g), but there were no significant differences in feed efficiency (0.24 to 0.26) among these groups.

The serum lipid levels in the rats of these groups are summarized in Table 2. The total-cholesterol level was significantly lower in the fiber groups than in the control group, with a significance difference between the ID or PE group and the CF group. Although there were no significant differences in the HDL-cholesterol level between the CF and the control groups, the ID and PE groups gave lower values than the control group. The atherogenic index in the ID and PE groups tended to be higher than the control and CF groups, although the difference was not significant. There were no significant differences in the triacylglycerol level among all the groups, although the ID and PE groups gave lower values (p<0.10) than the other groups.

Table 3 shows the results of measuring the cecal content (g), including the
Table 3. Production of short-chain fatty acids in cecal contents, weight of cecal contents, and bile acid excretion in feces.

<table>
<thead>
<tr>
<th></th>
<th>Short-chain fatty acid (µmol/cecum)</th>
<th>Cecum contents (g)</th>
<th>Bile acid excretions (µmol/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
<td>Propionate</td>
<td>n-Butyrate</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>48.8±7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.5±1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ID</td>
<td>8</td>
<td>93.4±7.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.5±2.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PE</td>
<td>7</td>
<td>96.8±13.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.2±8.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CF</td>
<td>9</td>
<td>64.1±7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.6±2.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Rats were kept for 8 weeks on a high-sucrose diet with or without dietary fiber at the 5% level. Values (mean±SE) not sharing a common superscript letter in the same column are significantly different from each other (p<0.05). Control, fiber free; ID, indigestible dextrin; PE, pectin; CF, corn fiber.

Fig. 3. Relationship between serum total-cholesterol and cecal propionate production in rats. Rats were fed for 8 weeks on a high-sucrose diet with or without dietary fiber at the 5% level. ●, control, fiber free, n=7; ○, ID, indigestible dextrin, n=8; △, PE, pectin, n=7; □, CF, corn fiber, n=9. Regression line and coefficient correlation for each fiber group+control: ID+control (n=15) \( Y = -1.442X + 117.5, r=0.870, p<0.001; \) PE+control (n=14) \( Y = -0.611X + 97.4, r=0.649, p<0.02; \) CF+control (n=16) \( Y = -0.109X + 86.6, r=0.057, \) not significant. \( Y \) is the serum total-cholesterol level and \( X \) is the cecal propionate production.

amount of short-chain fatty acids produced (µmol/cecum), and the fecal bile acid excretion (µmol/day). The amounts of short-chain fatty acids produced were much greater in the ID and PE groups than in the control group. On the other hand, the
Fig. 4. Relationship between serum total-cholesterol and bile acid excretion in feces. Rats were fed for 8 weeks on a high-sucrose diet with or without dietary fiber at the 5% level. ●, control, fiber free, n=7; ○, ID, indigestible dextrin, n=8; △, PE, pectin, n=7; □, CF, corn fiber, n=9. Regression line and coefficient correlation for each fiber group + control: ID + control (n=15) $Y = -0.790X + 88.6$, $r=0.314$, not significant; PE + control (n=14) $Y = -1.283X + 110.9$, $r=0.890$, $p<0.001$; CF + control (n=16) $Y = -0.742X - 101.7$, $r=0.649$, $p<0.01$. $Y$ is the serum total-cholesterol levels and $X$ is the bile acid excretion in feces.

CF group was not significantly different in the production of short-chain fatty acids from the control group. The fecal excretion of bile acids was significantly higher in all the dietary fiber groups, especially in the PE and CF groups.

Figure 3 shows the correlation between the serum total-cholesterol level and the cecal propionate production in the dietary fiber groups and in the control group. A regression line with a correlation coefficient of $r = -0.586$ was obtained ($n=31$), indicating a significant correlation between the serum total-cholesterol and the cecal propionate production ($p<0.05$). When the plots were restricted to those from each fiber group plus the control group, the correlation coefficient was obtained as $-0.870$ for ID + control ($n=15$), $-0.649$ for PE + control ($n=14$), or $-0.057$ for CF + control ($n=16$). This implies that the propionate production may be intimately involved in the regulation of serum cholesterol in the ID group ($p<0.001$) as well as in the PE group ($p<0.02$).

In Fig. 4, the serum total-cholesterol levels are plotted against the daily excretion of bile acids into the feces. A regression line with correlation coefficient of $r = -0.357$ ($n=31$) was obtained, where a significant correlation ($p<0.05$) was valid between the serum total-cholesterol and the bile acid excretion. With respect
to the individual dietary fiber groups, the correlation coefficient was $r = -0.314$ for ID + control ($n = 15$), $r = -0.890$ for PE + control ($n = 14$), and $r = -0.649$ for CF + control ($n = 16$). A significant correlation held for the PE + control group ($p < 0.001$) and CF + control group ($p < 0.01$).

**DISCUSSION**

In the present study, different types of dietary fiber with different physicochemical properties were incubated together with rat cecal contents under static culture conditions, and examined for fermentation changes into short-chain fatty acids (acetate, propionate, and butyrate) and lactate as their precursor (15). The lactate production did not differ so much among the types of fiber, but the total yield of short-chain fatty acids and their molar ratio varied with the type of fiber. Briefly, less short-chain fatty acids as a whole were produced from ID in 24 h than from PE, and the reverse was observed for the propionate production. Considering that fermentative products, including short-chain fatty acids, largely depend on species of microflora, the difference in fermentability between types of dietary fiber may have reflected different patterns of fiber-assimilating microflora. Although highly viscous PE was considered to be insensitive to the action of microflora, much more short-chain fatty acids were produced from PE than from ID. Further studies are needed to clarify the relation of physicochemical properties of dietary fibers (e.g., viscosity, water holding capacity, molecular weight, molecular structure, and constitutive sugar) to their assimilation by microflora. The production of short-chain fatty acids from an insoluble fiber “CF” was extremely low, as described elsewhere (16).

Next, the relationship between the serum lipid level and the cecal short-chain fatty acid production or fecal bile acid excretion was investigated with rats which had been fed the cholesterol- and bile acid-free diets containing 5% dietary fiber for 8 weeks. Rats absorb dietary cholesterol better than humans, so their serum cholesterol level is easily affected by dietary cholesterol (17). In this regard, it has recently been reported that a high sucrose diet is not only hypercholesterolemic per se (11,12) but also favorable for precluding the effect by dietary cholesterol (14). The intake of high-sucrose diets supplemented with dietary fibers led to a lowering of the serum cholesterol level, though the hypocholesterolemic effect varied with the type of dietary fiber. In addition, large amounts of short-chain fatty acids were produced in the ID and PE groups compared with the control group. Even so, it should be noted that, unlike the in vitro experiment, there was no significant difference in propionate production between the ID and PE groups. The molar ratio of acetate, propionate, and butyrate and their total amounts were not necessarily consistent with the other observation. This difference may be accounted for by a difference in microflora due to different diets (standard chow and high-sucrose diet) and a difference in digesta movement in the intestine (in vivo and in vitro). Moreover, it can be considered that the accumulation of short-chain fatty...
acids in the cecum is limited to some extent because of the tissue size, but that these short-chain fatty acids are readily absorbed from the intestine (18). A detailed study is further required in this respect. The fecal bile acid excretion was significantly increased in all three dietary fiber groups. To consider to what extent such an increase in propionate production or enterohepatic circulation contributes to the hypocholesterolemic action of dietary fibers, the serum total-cholesterol levels were plotted against the cecal propionate production or fecal bile acid excretion. As the result, a significant negative correlation was found between the serum total-cholesterol level and cecal propionate production in the ID group, between the serum total-cholesterol level and cecal propionate production or fecal bile acid excretion in the PE group, and between the serum total-cholesterol level and fecal bile acid excretion in the CF group. These findings indicate that factors responsible for the hypocholesterolemic action differ somewhat with the type of fiber. In any case, much more information is needed regarding the mechanism of hypocholesterolemic action of dietary fiber.

REFERENCES

HYPOCHOLESTEROLEMIC EFFECT OF DIETARY FIBER


