Effects of Different Types of Dietary Fiber Preparations Isolated from Bamboo Shoots, Edible Burdock, Apple and Corn on Fecal Steroid Profiles of Rats

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Summary This study was conducted to evaluate the effects of different types of dietary fibers (DF) under the conditions with or without cholesterol (Chol) loading on the amount and composition of steroids in rat feces. Rats were fed Chol-unsupplied diets containing 10% lard and 5% DF preparation isolated from four kinds of food, bamboo shoots, edible burdock, apple and corn, for three weeks. The respective diets were supplemented with 0.5% Chol and then given to the rats for a further two weeks. The excretion of total bile acid (BA) and several major BAs increased significantly in the apple group with or without Chol loading when compared with that in the cellulose (CP) or other DF groups. The tendency in the apple group was more noticeable when the diet was supplemented with Chol. This is presumably a major reason for the tendency of decrease in serum and liver Chol concentrations in the apple group. The ratio of secondary BAs to total BA in the feces was significantly low in the apple group. Although the lithocholic acid (LCA)/deoxycholic acid (DCA) ratio, a risk index for colorectal cancer, was significantly lower in the bamboo, burdock and apple groups than in the CP or corn groups when given the diet without Chol, the differences disappeared with the addition of Chol. The proportion of coprostanol, a secondary metabolite of Chol, was smaller in the former three groups than in the CP or corn groups. These results suggest that the intake of some DF by host animals works beneficially for the microbial conversion of BA and Chol in the large intestine but that the addition of Chol acts to cancel such beneficial effects.

Key Words dietary fibers, fecal steroid profiles, secondary bile acids, neutral sterols, lithocholic acid/deoxycholic acid ratio

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It is assumed, from various experiments using animal models (1,2) or in vitro studies (3–5), that secondary metabolites produced from bile acids (BA) in the large intestine act as promoters of colorectal carcinogenesis. It is also held that deoxycholic acid (DCA) and lithocholic acid (LCA) are particularly potent promoters (3–5), and that LCA is more promotive than DCA (5,6). The LCA/DCA ratio is therefore regarded as important. A rise in this ratio (5,7,8) infers a higher cancer risk. On the other hand, fecal BA excretion is affected not only by the quality and quantity of dietary lipids (9,10) but also by the quantity and type of dietary fiber (DF) (11–13). It has been observed that the composition of BA excreted is also dependent on the presence or absence of cholesterol (Chol) in the diet (13). Past studies suggest that the fecal BA composition is affected differently by different types of DF and experimental conditions, and that it does not necessarily show a similar spectrum. It is more likely that the differences are involved in the chemical structures as well as the physical properties of the DF, but the details are unclear as yet. We previously found that DF preparations isolated from several foods were effective in suppressing the chenodeoxycholic acid (CDCA) metabolic system and lowering the fecal LCA/DCA ratio irrespective of the dietary fat level as compared to cellulose (CP), but that Chol added to any diets containing various DF preparations promoted the CDCA metabolic system, thus elevating the fecal LCA/DCA ratio (14). This study was intended to further investigate whether such a difference in the response of fecal BA composition to the difference in DF types and the effect of Chol addition would be exhibited by other types of DF. In the experiment, we used two kinds of vegetable DF preparations which had not yet been thoroughly studied, as well as fruit and cereal preparations on which some information is available.

METHODS

Preparation and composition of DF. We used four kinds of DF preparations. Two of them were prepared from vegetables in our laboratory and the other two were commercially available DF products. Takenoko (bamboo shoots, Phyllostachys heterocycla (Carr.) Mitf., raw) and gobo (edible burdock, Arctium lappa L., raw) were purchased in a store. The fresh vegetable samples were cut into fine strips, freeze-dried in a vacuum environment, powdered with a coffee mill and passed through a 30 mesh sieve. The DF preparations were extracted from the powdered samples by the modified AOAC enzymatic gravimetric method (15), and the alcohol precipitates were dried after washing with alcohol and acetone. The resulting powder samples were used for the subsequent tests. As fruit and cereal DF preparations, commercially available “apple” pulp powder (Apple Fiber N from Del Monte Japan, Tokyo) and refined “corn” bran (Cellfer from Nippon Shokuhin Kako Co., Ltd., Tokyo) were used without further purification. Cellulose powder (CP) (Toyo Roshi Kaisha, Tokyo) was used as a control. The general components of the samples were routinely determined and the DF compositions...
were analyzed according to the Southgate method (16). As shown in Table 1, the apple DF preparation is low in fiber content and rich in carbohydrate (dextrin). Each sample has a characteristic DF composition.

**Animals and rearing method.** Male Sprague-Dawley rats aged four weeks (the initial body weight, 90–100 g; CLEA Japan, Inc., Tokyo) were used in the study, and six animals were allotted to each group. The rats were reared in the manner described in a previous report (14). Following 9-days' preliminary rearing, the rats were fed respective diets composed of (in %); casein (25), lard (10), sucrose (44.8), mineral mixture (AIN-76™) (3.5), vitamin mixture (AIN-76™) (1.0), choline bitartrate (0.2) and DF (5). The apparent amount of DF addition was as follows: CP, 5%; takenoko, 6.9%; gobo, 6.7%; apple, 15.2%; and corn, 6.1%. The difference was corrected by reducing the sucrose level. The corrections for protein content in the DF preparations were made by casein adjustment.

Three weeks later, the diets were exchanged for those containing 0.5% Chol, which were continued for two weeks. The feces were collected for the last five days in the third and fifth weeks, and weighed before and after freeze-drying to obtain the water content. The dried feces were pulverized and kept at −30°C until analysis.

**Analytical method.** Blood and liver lipids and fecal acidic and neutral steroids were determined in the manner described in a previous report (14).

**Statistical processing.** Individual comparisons among groups were made using Duncan's new multiple range test. Data were also analyzed by two-way ANOVA using the SAS computer package. A separate analysis was carried out to determine the significance of fiber or Chol, and fiber × Chol (during weeks 3 and 5).

**RESULTS**

**Body weight gain, food intake, liver weight, and serum and liver lipid concentrations**

The results obtained are summarized in Table 2. There were no substantial differences among groups in terms of body weight gain and food intake up to week 5. Serum total Chol (TC), triglyceride (TG) and phospholipid (PL) concentra-
Table 2. Effect of several dietary fiber preparations on body weight gain, food intake, liver weight and serum and liver lipid concentrations.\(^1\)

| Items                        | Cellulose (g/day) | Takenoko (g/day) | Gobo (g/day) | Apple (g/day) | Corn (g/day) |
|------------------------------|-------------------|-------------------|--------------|---------------|--------------|
| Body weight gain             | 6.94±0.54         | 7.57±0.31         | 7.86±0.34    | 7.20±0.26     | 7.63±0.20    |
| Food intake                  | 18.9±0.59\(^{ab}\) | 19.9±0.74\(^{ab}\) | 20.1±0.40\(^a\) | 19.8±0.34\(^{ab}\) | 18.4±0.24\(^b\) |
| Liver                        | 18.1±1.34\(^{ab}\) | 19.2±1.61\(^{ab}\) | 21.1±1.11\(^a\) | 16.7±0.16\(^b\) | 19.7±0.75\(^{ab}\) |
| Serum (mg/100 ml)            |                   |                   |              |               |              |
| Total cholesterol            | 140±8.5\(^{ab}\)  | 149±9.2\(^{ab}\)  | 154±9.5\(^a\) | 123±5.5\(^b\) | 164±13.5\(^a\) |
| Triacylglycerol              | 227±57.1\(^a\)   | 138±12.9\(^{ab}\) | 146±20.6\(^{ab}\) | 100±17.8\(^b\) | 144±18.3\(^{ab}\) |
| Phospholipids                | 182±13.0\(^a\)   | 174±5.7\(^{ab}\)  | 184±15.7\(^a\) | 145±7.4\(^b\) | 196±11.9\(^a\) |

\(^{1}\)M±SEM (n=6): values in the same line with different superscript letters are significantly different at \(p<0.05\).

Fecal weight and water content

The results are shown in Table 3. The fecal wet weight was significantly higher or tended to be higher in the takenoko group than in other groups in both weeks 3 and 5. The addition of Chol to the diets increased the fecal wet and dry weights in all the groups. The fecal water content, on the other hand, was significantly greater in the takenoko and apple groups than in the CP and other DF groups in both weeks 3 and 5. The fecal water content in all of the DF groups tended to increase when the Chol-supplemented diets were given.

Fecal steroid excretion

The results of fecal BA excretions are shown in Table 4. The supplemental effect of both fiber and Chol was conspicuous in the excretion of all BA except \(\omega\)-muricholic acid. The interaction between fiber and Chol was noted in \(\alpha\)-muricholic acid (\(\alpha\)-MCA), cholic acid (CA), \(\beta\)-muricholic acid and total BA. The excretion of DCA, \(\alpha\)-MCA and CA was significantly higher in the apple group than in the CP group, but it was significantly lower or tended to be lower in the corn group than in the other groups.

Table 5 shows the ratio of secondary BAs to total BA, the CDCA/CA ratio and the LCA/DCA ratio. The ratio of secondary BAs to total BA was significantly smaller in the apple group than in the CP and other DF groups whether Chol was added or not. The CDCA/CA ratio was significantly low in the apple and gobo.
Table 3. Effect of several dietary fiber preparations on fecal weight and moisture, and statistical analysis data.¹

| Items                  | Week | Cellulose | Takenoko | Gobo  | Apple | Corn  | Pooled SEM | Two-way ANOVA (p value)² |
|------------------------|------|-----------|----------|-------|-------|-------|------------|-------------------------|
| Fecal wet weight      | 3    | 1.63ᵇᵃ  | 2.04ᵃ    | 1.53ᵇ | 1.84ᵇ | 1.82ᵇ | 0.06       | Fiber 0.0009            |
| (g/day)                | 5    | 2.00ᵃ    | 3.02ᵇ    | 2.17ᵃ | 2.40ᵃ | 2.39ᵃ | 0.09       | Chol 0.0001             |
|                        |      |           |          |       |       |       |            | Fiber × Chol NS         |
| Fecal dry weight      | 3    | 1.39ᵇᵃ  | 1.36ᵇᵃ   | 1.23ᵃ | 1.40ᵇ | 1.55ᵇ | 0.03       | Fiber 0.0308            |
| (g/day)                | 5    | 1.67     | 1.85     | 1.68  | 1.76  | 1.97  | 0.05       | Chol 0.0001             |
|                        |      |           |          |       |       |       |            | Fiber × Chol NS         |
| Fecal moisture        | 3    | 14.2ᵃ    | 30.8ᵇ    | 19.2ᵃ | 23.0ᵇ | 14.7ᵃ | 1.32       | Fiber 0.0001            |
| (%)                   | 5    | 16.2ᵃ    | 37.4ᵇ    | 21.4ᶜ | 25.9ᶜ | 17.3ᵃ | 0.91       | Chol 0.0459             |
|                        |      |           |          |       |       |       |            | Fiber × Chol NS         |

¹ Means (n = 6) in the same line with different superscript letters are significantly different at p < 0.05.
² Overall p values from two-way ANOVA for differences by fiber, Chol or fiber × Chol (data analysis of weeks 3 and 5). NS, not significant; Chol, cholesterol.
Table 4. Effect of several dietary fiber preparations on fecal bile acid excretion, and statistical analysis data\(^1\) (mg/g dry feces).

| Bile acid       | Week | Cellulose | Takenoko | Gobo  | Apple | Corn  | Pooled SEM | Two-way ANOVA (p value)\(^2\) | Fiber | Chol | Fiber × Chol |
|-----------------|------|-----------|----------|-------|-------|-------|------------|--------------------------------|-------|------|-------------|
| Lithocholic acid| 3    | 0.06\(^{ab}\) | 0.02\(^{b}\) | 0.04\(^{ab}\) | 0.08\(^{c}\) | 0.05\(^{ab}\) | 0.00 | Fiber | 0.0083 | Chol | 0.0001 |
|                 | 5    | 0.27\(^{ab}\) | 0.12\(^{a}\) | 0.23\(^{ab}\) | 0.40\(^{b}\) | 0.30\(^{ab}\) | 0.03 | Fiber × Chol | NS |      |             |
| Deoxycholic acid| 3    | 0.15\(^{a}\) | 0.16\(^{a}\) | 0.20\(^{a}\) | 0.55\(^{b}\) | 0.12\(^{a}\) | 0.02 | Fiber | 0.0001 | Chol | 0.0001 |
|                 | 5    | 0.43\(^{a}\) | 0.30\(^{a}\) | 0.48\(^{a}\) | 0.72\(^{b}\) | 0.31\(^{a}\) | 0.03 | Fiber × Chol | NS |      |             |
| α-Muricholic acid| 3   | 0.04\(^{ac}\) | 0.06\(^{a}\) | 0.05\(^{ac}\) | 0.13\(^{b}\) | 0.02\(^{c}\) | 0.00 | Fiber | 0.0001 | Chol | 0.0001 |
|                 | 5    | 0.18\(^{a}\) | 0.16\(^{a}\) | 0.16\(^{a}\) | 0.60\(^{b}\) | 0.03\(^{c}\) | 0.02 | Fiber × Chol | NS |      |             |
| Hyodeoxycholic acid| 3    | 0.87\(^{a}\) | 0.70\(^{ab}\) | 0.53\(^{ab}\) | 0.42\(^{b}\) | 0.79\(^{ab}\) | 0.06 | Fiber | NS |      |             |
|                 | 5    | 0.98 | 1.62 | 1.60 | 0.83 | 2.16 | 0.19 | Fiber × Chol | NS |      |             |
| Cholic acid     | 3    | 0.03\(^{a}\) | 0.06\(^{a}\) | 0.03\(^{a}\) | 0.38\(^{b}\) | trace | 0.02 | Fiber | 0.0001 | Chol | 0.0008 |
|                 | 5    | trace | 0.20\(^{a}\) | 0.26\(^{a}\) | 1.11\(^{b}\) | trace | 0.06 | Fiber × Chol | 0.0015 |      |             |
| β-Muricholic acid| 3   | 0.11\(^{ab}\) | 0.06\(^{b}\) | 0.10\(^{ab}\) | 0.65\(^{a}\) | 0.01\(^{b}\) | 0.08 | Fiber | 0.0001 | Chol | 0.0016 |
|                 | 5    | 0.03\(^{a}\) | 0.67\(^{a}\) | 0.96\(^{a}\) | 4.83\(^{b}\) | 0.01\(^{a}\) | 0.32 | Fiber × Chol | 0.0009 |      |             |
| ω-Muricholic acid| 3   | 0.14 | 0.14 | 0.15 | 0.28 | 0.04 | 0.04 | Fiber | NS |      |             |
|                 | 5    | 0.94 | 0.13 | 0.28 | 0.83 | 0.31 | 0.16 | Fiber × Chol | NS |      |             |
| Total bile acid | 3    | 1.53\(^a\) | 1.39\(^a\) | 1.21\(^a\) | 2.92\(^b\) | 1.12\(^a\) | 0.14 | Fiber | 0.0001 | Chol | 0.0001 |
|                 | 5    | 3.18\(^a\) | 3.33\(^a\) | 4.13\(^a\) | 9.96\(^b\) | 3.29\(^a\) | 0.31 | Fiber × Chol | 0.0001 |      |             |

\(^1\) Means (n = 6) in the same line with different superscript letters are significantly different at p < 0.05.

\(^2\) See footnote to Table 3.
Table 5. Effect of several dietary fiber preparations on fecal bile acid parameters, and statistical analysis data.  

| Parameter                  | Week | Cellulose | Takenoko | Gobo  | Apple | Corn  | Pooled SEM | Two-way ANOVA (p value) |
|----------------------------|------|-----------|----------|-------|-------|-------|-------------|------------------------|
| Secondary/total bile acid  | 3    | 0.78a     | 0.70ab   | 0.72a | 0.51b | 0.90a | 0.03        | Fiber 0.0001            |
|                            | 5    | 0.81ac    | 0.65a    | 0.69ac | 0.31b | 0.94c | 0.04        | Chol NS                 |
|                            |      |           |          |       |       |       |             | Fiber x Chol NS         |
| CDCA/CA\(^1\)             | 3    | 6.91ad    | 4.95ab   | 3.81bc | 1.78c | 7.57d | 0.32        | Fiber 0.0001            |
|                            | 5    | 6.39ab    | 5.40a    | 5.02a  | 4.48a | 9.17b | 0.50        | Chol 0.0001             |
|                            |      |           |          |       |       |       |             | Fiber x Chol NS         |
| LCA/DCA\(^1\)             | 3    | 0.36a     | 0.12b    | 0.18b  | 0.14b | 0.45a | 0.02        | Fiber 0.0001            |
|                            | 5    | 0.64a     | 0.36a    | 0.48a  | 0.54a | 0.92b | 0.04        | Chol NS                 |
|                            |      |           |          |       |       |       |             | Fiber x Chol NS         |

\(^1\)Means (n = 6) in the same line with different superscript letters are significantly different at p < 0.05.

\(^2\)See footnote to Table 3.

\(^3\)CDCA, chenodeoxycholic acid and its metabolites; CA, cholic acid and its metabolites; LCA, lithocholic acid; DCA, deoxycholic acid.
Table 6. Effect of several dietary fiber preparations on fecal neutral sterol excretion, and statistical analysis data (mg/day).

| Neutral sterol | Week | Cellulose | Takenoko | Gobo | Apple | Corn | Pooled SEM | Two-way ANOVA (p value) |
|----------------|------|-----------|----------|------|-------|------|------------|------------------------|
| Coprostanol    | 3    | 3.37a     | 2.63ab   | 6.56a| 0.57c | 0.18 | 0.0003     | Fiber                  |
|                | 5    | 9.58a     | 8.47a    | 6.64a| 4.67a | 1.2  | 0.0001     | Fiber x Chol           |
| Cholesterol    | 3    | 6.45b     | 6.66a    | 6.13b| 3.23a | 0.57c | 0.0285     | Fiber                  |
|                | 5    | 46.0      | 65.8     | 65.6 | 10.4  | 27.8 | 0.27       | Fiber x Chol           |
| Cholestrol     | 3    | 1.00a     | 0.65a    | 0.96 | 0.83  | 0.06 | 0.0001     | Fiber                  |
|                | 5    | 5.12a     | 9.96     | 9.0  | 0.87  | 0.12 | 0.0001     | Fiber x Chol           |
| Total neutral  | 3    | 9.10a     | 12.9a    | 9.16b| 11.94a| 0.86b| 0.0005     | Fiber                  |
| sterol         | 5    | 59.1      | 77.6     | 74.9 | 80.4  | 52.3 | 0.49       | Fiber x Chol           |

1 Mean (n=6): values in the same line with different superscript letters are significantly different at p<0.05.

2 See footnote to Table 3.
groups as compared to the CP and corn groups. While no difference was noted between the CP and corn groups in the LCA/DCA ratio in week 3, the ratios in the takenoko, gobo and apple groups were significantly lower than those in the former two groups. After the addition of Chol, the ratio rose significantly only in the corn group when compared with the other DF groups. The addition of Chol elevated the ratio significantly in all the groups.

The amounts of various neutral sterols (NS) excreted into the feces per day are shown in Table 6. Coprostanol varied most markedly, indicating an interaction between fiber and Chol in addition to the effect of fiber or Chol. The excretion of coprostanol was significantly smaller in the takenoko and apple groups than in the CP and corn groups. Among the fecal sterols in week 5, Chol was the largest component. In this respect, there were no significant differences among the CP and DF groups. The proportion of coprostanol was significantly high in the corn group as compared to the other groups including the CP group. The total NS excretion was strongly affected by the addition of Chol. The interaction between fiber and Chol as well as the effects of fiber and Chol were seen in the coprostanol/Chol ratio. Before the addition of Chol, the total NS excretion was low in the takenoko and apple groups as compared to that in the CP group, while it was significantly high in the corn group. After the addition of Chol, the total NS excretion in the corn group was significantly high as compared to that in other groups.

DISCUSSION

The wet weight of feces excreted per day was higher in the takenoko group than in the CP or other DF groups both in weeks 3 and 5. This is presumably related to the fact that the fecal water content was highest in this group. Since the fecal water content in the group given corn, which contains an abundance of insoluble hemicellulose, was comparable to that in the CP group, the matrix structure of the fiber itself rather than the quantity of insoluble hemicellulose is probably responsible for the increased fecal bulk.

Serum and liver TC concentrations tended to be lower in the apple group than in the CP group. Sugano et al. (17) reported that apple fibers suppressed hypercholesterolemia. Egashira et al. (18, 19) obtained similar findings, and suggested that the effective component itself ought to be pectin because it exists in a soluble fraction of apple fibers and consists mainly of uronic acids. Pectin has long been known to be effective in lowering serum and liver Chol concentrations (19, 20). The amount of total BA excretion in the apple group was significantly larger than in the CP and other DF groups both before and after the addition of Chol. This is presumably related to the decrease of serum Chol in the apple group. The capacity of DF to adsorb BA (21–23) is known to vary with the types of DF. According to an in vitro study by Ebihara et al. (23), takenoko fiber has been shown to adsorb taurocholate 24 times as much as cellulose and twice that of gobo or corn fiber despite no difference in serum or liver Chol concentrations in hypercholesterole-
lemic rats. An analysis of fecal BAs revealed that most BAs except HDCA were significantly larger or tended to be larger in the apple group than in other DF groups before the addition of Chol. Conversely, the proportion of HDCA, which is a major metabolite of CDCA, was significantly small or tended to be small in the apple group as compared to the CP or other DF groups. This is also indicated by the fact that the ratios of CDCA/CA and LCA/DCA in the feces were significantly lower in the apple group than in the CP group. This may indicate that the CA pathway, rather than the CDCA pathway, in the lower intestinal tract was promoted in the apple group.

If this is true, it is possible that colon cancer risks can be lowered by the intake of apple fiber pulp preparation. Such a phenomenon (the reduction of secondary BAs) was not observed in case of a single administration of pectin (24,25). According to the Jacobs' review (26) of eight studies regarding the effect of pectin on inducing colon cancer, no effect was observed in three, carcinogenesis was more or less stimulated in four and the suppressive effect was apparent only in one study. Therefore, the effect observed in this study may be attributable to the insoluble hemicellulose fraction of the apple fiber preparation, the inclusion of dextrin or to their interaction. It is interesting that the LCA/DCA ratio was significantly low even in the groups given vegetable fibers such as takenoko and gobo without the addition of Chol as compared to the CP group. The corn group, however, showed an even higher LCA/DCA ratio than the CP group. No difference was observed between the CP and corn groups in the ratio of secondary BAs to total BA in either of weeks 3 and 5. Story (12) also observed that corn bran increased fecal excretion of the CDCA derivative. When Chol was added to the diets, LCA excretion increased in all the groups, as was reported in a previous study (14), and no significant difference in the LCA/DCA ratio was observed between the CP group or any other DF group. This indicates that the addition of Chol acts to reduce the effect of the DF preparations from vegetables or fruits on lowering the LCA/DCA ratio.

Beher et al. (27,28) reported that Chol-supplemented diets decreased the CA pool and increased the CDCA pool in rats. Uchida et al. (29) also observed a similar effect and reported that fecal CDCA metabolites increased. Although the level of Chol supplement in our study was only 0.5%, or one-fourth of the level used by Uchida et al. (29), we also noted stimulation of the CDCA metabolism. It was revealed that the Chol metabolism in the large intestine was not modified by the type of DF in rats given Chol-supplemented diets. Enteric bacteria and their metabolic abilities are influenced by the relative difficulty of fermentation based on the compositions and chemical structures of fibers, and this is presumably the reason for the difference in the response of the fecal BA indexes to fibers.

We studied the relation between hemicellulose and the BA composition or its ratio. Although the number of DFs analyzed was limited, a significant correlation was obtained between the water soluble hemicellulose and secondary BA/total BA ratio ($r = -0.449, p < 0.0128$) or the CDCA/CA ratio ($r = -0.689, p < 0.0001$) in the feces.
week 3. Further studies using a larger number of samples are warranted to clarify these relationships.

On the other hand, the fecal excretion of coprostanol, a major secondary metabolite of Chol, was larger in the CP and corn groups than in the other three DF groups, and this tendency was unaffected by the addition of Chol. A response contrary to the excretion of fecal coprostanol was seen in the fecal Chol excretion. This suggests that the conversion of Chol is promoted in the large intestine of rats given insoluble DF such as CP and corn bran. It is inappropriate to explain the BA metabolism in the large intestine by the ratio of soluble DF and insoluble DF alone. It is assumed, however, that a single administration of non-fermentative DF will act to increase the secondary metabolites of BA and Chol. Corn fiber, however, is characterized by its property to adsorb various environmental mutagens (30,31), and its ability to adsorb pyrolysate mutagens is known to be stronger than that of apple fiber (31). This may indicate that the value of DFs against colon cancer risk should be evaluated not only using BA metabolites but also from other physiological aspects.

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