Evaluation of the Relative Available Energy of Several Dietary Fiber Preparations Using Breath Hydrogen Evolution in Healthy Humans

Tsuneyuki OKU and Sadako NAKURA

Graduate School of Human Health Science, University of Nagasaki, Siebold, Nagasaki 851–2195, Japan

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Summary  A standardized simple, indirect method for assessing the relative energy of dietary fiber carbohydrates is not yet established. There is a need for a standardized in vivo assay. The objective of the present study was to evaluate the relative available energy (RAE) for 9 major dietary fiber materials (DFMs) based on fermentability from breath hydrogen excretion (BHE) in subjects. Fructooligosaccharide (FOS) was used as a reference. The study was conducted using a within-subject, repeated measures design and approved by the Ethical Committee of University of Nagasaki. After DFM ingestion, end-expiratory gas (750-mL) was collected at 1-h intervals for 8 h, as well as at 2-h intervals between 8 h and 14 h, and 30 min after waking up and 24 h after DFM ingestion. Breath hydrogen concentration was assessed with a gas chromatograph. The RAE of DFMs tested was evaluated based on the area under the curve (AUC) of BHE of FOS. Based on the ratio of AUC for 8 h, the RAE of polydextrose, partially hydrolysed guar gum, resistant maltodextrin and partially hydrolysed alginate was 1 kcal/g, and that of glucomannan, heat-moisture treatment and high-amylose cornstarch and cellulose was 0 kcal/g, while the RAE of all tested DEMs including cellulose and glucomannan was 1 kcal/g in the calculation based on AUCs for 14 h and 24 h in subjects. We suggest that a breath hydrogen collection period of 14 h or more could be used to measure RAE for a range of fiber preparations in vivo.

Key Words  relative available energy, dietary fiber, fermentability, breath hydrogen

Dietary fiber (i.e., largely indigestible carbohydrates) has beneficial effects for health and is actively used in health foods and functional foods (1, 2). Dietary fiber taken orally reaches the large intestine without being digested by α-amylase or disaccharidases present in the mucosa of the small intestine. In the large intestine, dietary fiber is fermented to varying degrees by intestinal microbes and metabolized into short chain fatty acids, carbon dioxide, hydrogen, methane, and bacterial cell components (3–6). Among them, short-chain fatty acids are absorbed from the large intestine and used solely as the energy source of the host. That is, even carbohydrates that are not digested and not absorbed provide available energy to a living body by being fermented and absorbed in the large intestine (5).

Energy values for fermentable fibers are an important matter for regulatory bodies as they are incorporated into on-pack labels, and for weight control products, as a help to direct consumer choice (2). The available energy of nondigestible carbohydrate in humans is evaluated using balance studies and other methods (7, 8).

However, this is not practical, because balance studies are time-consuming, expensive, and very stressful for subjects. Although an indirect and simple method to evaluate the available energy of nondigestible carbohydrates is desirable, it has not been established yet.

The amount of available energy of nondigestible and fermentable carbohydrates is dependent upon the amount of short-chain fatty acids produced in the fermentation by intestinal microbes. Thus, energy production is dependent on the fermentability of nondigestible carbohydrates which reach the colon. We estimated the relative available energy value (RAE) of dietary fiber materials (DFMs) based on the breath hydrogen excretion (BHE) and calculated the relative ratio versus the amount of hydrogen excretion from the ingestion of fructooligosaccharide (FOS), which is fermented completely by intestinal microbes. Therefore, the profile of BHE seems to be different from the fiber sources. But, FOS is a convenient reference because it is fermented completely by intestinal microbes. Besides, FOS has been classified as a nondigestible carbohydrate of which the energy value is 2 kcal/g by the Japanese Health Promotion Law (9). In this study we expressed the energy values derived from the indirect method as the relative ratio to FOS.

In the nutrition labelling for processed foods, the indication of energy is an essential item. The available energy derived from digestible carbohydrates is 4 kcal/g. However, that of nondigestible carbohydrates is not known because the available energy derived from non-di-
gestible and fermentable carbohydrates such as oligosaccharides cannot be evaluated for common foods listed in standard food tables. To estimate the available energy from nondigestible and/or nonabsorbable carbohydrates such as oligosaccharides and sugar alcohols, we proposed a unique and simple method which enables calculation from the fermentation equations with the ratio of short-chain fatty acids produced by intestinal microbes ($10^{-13}$), and demonstrated that a carbohydrate which is fermented completely has an energy available value of 2 kcal/g. This value is used for nutrition labelling in The Japanese Health Promotion Law ([9]). The estimated available energy based on BHE is classified into three energy categories, 0 kcal/g, 1 kcal/g, and 2 kcal/g, which are expressed as integers for the necessity of practical use in the calculation of nutritional intake.

We have proposed an indirect and simple method and tried to evaluate the energy available from some DFMs based on the fermentability from BHE for 8 h ([13]). The results obtained were different from the energy coefficients of DFMs based on the fermentability obtained from animal and human experiments in the Japanese Health Promotion Law. Furthermore, the values of available energy obtained were not correct because the period of breath-gas collection for 8 h to calculate the fermentability was too short. Therefore, in the present study, we collected breath gas for 24 h after the ingestion of DFMs, and tried to evaluate again the RAE of DFMs in healthy human subjects.

**MATERIALS AND METHODS**

All of the experiments complied with the code of ethics of the World Medical Association (Declaration of Helsinki, Oct. 2008). The study protocol was approved by the Ethical Committee of Siebold University of Nagasaki (received No. 41–1, approval No. 39, Nagasaki, Japan). All subjects provided written informed consent to participate in the study. All experiments were carried out in the Public Health Nutrition Laboratory of the Graduate School of Human Health Science, Siebold University of Nagasaki.

**Subjects.** Nine healthy females, aged $21.8 \pm 0.6$ y and with a BMI of $20.1 \pm 2.4$ kg/m$^2$, voluntary participated in this study. The exclusion criteria were a history of gastrointestinal diseases, carbohydrate malabsorption, obesity, or pulmonary disease. The average and standard deviation (SD) values of fasting blood glucose levels of the subjects was $82.7 \pm 4.1$ mg/100 mL. Prior to the experiments, we ensured that all of the subjects were hydrogen producers and not methane producers. The subjects had not taken antibiotics or laxatives for $\geq 2$ wk prior to the experiments.

**Materials.** To evaluate the available energy, DFMs which are frequently used in foods for specified health uses and functional foods were chosen. The water soluble dietary fiber materials were polydextrose (PD), resistant maltodextrin (RMD), partially hydrolysed guar gum (PHGG), partially hydrolysed sodium alginate (PHAA) and glucomannan (GM); water insoluble dietary fiber materials were heat-moisture treated high amylose cornstarch (HMT-HACS) and cellulose.

PD (Lytes, Danisco Japan Ltd., Tokyo, Japan) was a mixture of polymers in which glucose, sorbitol and citrate (90 : 9 : 1) were condensed under high pressure and high temperature, and had an average molecular weight of 1,500. It contained $\sim 25\%$ of monosaccharides and oligosaccharides. The available energy of PD has been estimated as 0.8–1.2 kcal/g by other methods, administered orally as $^{14}$C-polydextrose to rats and humans ([14, 15]). RMD (Fibersol-2, Matsutani Chemical Industry Co., Ltd., Osaka, Japan) is a mixture of monosaccharides, oligosaccharides and glucose polymers, and contains $93.73\%$ dietary fiber (by the Association of Official Analytical Chemists (AOAC) method). The aver-
Special meals for experiments. The period over which the subjects were restricted with regard to food intake was very long. Hence, special meals and snacks from which hydrogen is not produced when ingested were given to participants during the experiment. All food-stuffs used in the meals had been confirmed not to produce breath hydrogen. The combinations of a cookie (Butter Cookie Sable 60 g, University Coop., Tokyo), canned tuna fish (Sea-chicken Mild, 80 g, Hagoromo Food Co., Ltd., Shizuoka, Japan), boiled egg (50 g), and sport drinks (Pocari Sweat 250 mL) were used as food materials for special meals (breakfast, lunch, supper, snacks). The amount of energy and protein intake is shown in Table 2.

On the day of the experiment, all subjects took the special meals and snacks given at the indicated time in a day (Fig. 1). If needed, they could drink green tea or water. If the subject had an empty stomach, a sports drink containing sugars was given. In addition, each subject was given a multivitamin tablet (Nature Made, Otsuka Pharm. Co., Ltd.) to supply some vitamins every day.

Experimental protocol. The present study had a within-subject, repeated-measures design. All test substances were given in a random order with intervals of ≥1 wk. Experiments were carried out under the direction of a physician (Fig. 1). The experimental protocol was carried out in accordance with the methods employed in our previous study (13, 18).

Table 2. Energy and protein of experimental meals.

<table>
<thead>
<tr>
<th></th>
<th>Energy (kcal)</th>
<th>Protein (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal-1</td>
<td>437</td>
<td>9.7</td>
</tr>
<tr>
<td>Meal-2</td>
<td>587</td>
<td>22.2</td>
</tr>
<tr>
<td>Snack-1</td>
<td>366</td>
<td>3.5</td>
</tr>
<tr>
<td>Meal-3</td>
<td>412</td>
<td>9.4</td>
</tr>
<tr>
<td>Snack-2</td>
<td>336</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2,138</strong></td>
<td><strong>48.0</strong></td>
</tr>
</tbody>
</table>

Per person, per day.
Cookie 60 g, canned tuna fish 80 g, boiled egg 50 g, sport drink 250 mL were used as food materials for special meals. The energy value and protein content were used from the food labelling.

![Fig. 1. Experimental protocol of breath hydrogen gas test.](image-url)
After an overnight fast, the subject's health status was examined, the food intake for the previous week was reviewed, and blood pressure and pulse rate were measured. Then 750-ml samples of end-expiratory gas were collected. After ingestion of the test substances, the end-expiratory gas was collected at 1-h intervals for 8 h, and then at 2-h intervals between 8 h and 12 h after the ingestion, with a special collection bag after the removal of dead-space gas. The sleeping period was between 14 h and 20 h after ingestion of the test substance. Breath gas was collected 30 min after waking up and 24 h after ingestion.

Participants consumed their usual diet but were prohibited from ingesting foods containing nondigestible carbohydrates starting 3 d before each experimental day. We provided a supper, which consisted of cooked paddy white rice, fried chicken, a small amount of cabbage, and soup, on the day before each experiment. Previously, we evaluated that breath hydrogen was detected negligibly by the ingestion of these foodstuffs given for supper. During the experiment, they were also prohibited from ingesting foods or beverages except for water, as well as from sleeping or smoking. Subjects were required to sit on a chair and were prohibited from exercising with hyperventilation until the final collection of expiratory gas (13, 18). Participants ingested an experimental meal from which breath hydrogen was not produced, so that they would not feel hungry.

**Analyzes of breath hydrogen.** Five millilitres of end-expiratory gas was sucked into a plastic syringe and loaded into a simple gas chromatograph (Breath Gas Analyzer BGA1000D, Laboratory for Expiration Biochemistry Nourishment Metabolism Co., Ltd.) to measure the concentrations of hydrogen and methane gas.

**Calculations and statistical analyses.** BHE was calculated as mean values and SDs; normal distribution was tested. Evaluation of the available energy of DFMs was calculated based on a ratio. The ratio was calculated based on the areas under the curve of hydrogen excretion by the ingestion of test materials versus that of FOS. The result of the ratio was classified into three energy categories based on the Japanese Health Promotion Law. It was evaluated to the category of 2 kcal/g if the ratio of test material versus FOS was $\geq 0.75$; the category of 0 kcal/g if the ratio was $\leq 0.25$; the category of 1 kcal/g if the ratio was between 0.25 and 0.75. The AUC was considered to be significant if $p<0.05$ was obtained by two-sided analysis using ANOVA and Dunnett's post hoc test using SPSS for Windows (Japan version) 12.0 (SPSS Inc., Tokyo, Japan).

**RESULTS**

**Subject participation**

No subject dropped out of the study and none of the subjects experienced side effects. Their health status throughout the entire study period was good. The average intake of energy by a subject was 2,048±220 kcal/d and that of protein was 45.2±6.0 g/d.

**Breath hydrogen excretion (BHE) following consumption of dietary fiber test foods**

The profiles of BHE values with mean values and SDs of 9 subjects are shown in Figs. 2–4.

a) **FOS.** When each subject was given FOS (5 g), all subjects excreted breath hydrogen gas and did not produce methane gas. Breath hydrogen gas (which is produced through only the fermentation caused by intestinal microbes) started to be excreted $\sim 3$ h after the intake of FOS. The amount of hydrogen gas excreted reached the peak at 5–6 h and decreased gradually until 14 h after ingestion. BHE was maintained 24 h after ingestion and did not recover to basal levels. The concentration of hydrogen in the first collection of breath gas after waking up was slightly higher than that taken before sleeping, but that of hydrogen after 24 h was small (Fig. 2). The amount of breath hydrogen produced from DFMs was markedly lower than that from FOS.

b) **PD, RMD and PHGG.** When PD (5 g) was ingested by subjects, BHE started to increase 2–3 h after ingestion and reached a peak 5 h after ingestion (Fig. 2). Thereafter, BHE decreased gradually and was excreted little by

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Fig. 2. Profiles of breath hydrogen excretion by oral ingestion of polydextrose (PD), resistant maltodextrin (RMD), and partially hydrolyzed guar gum (PHGG) in healthy subjects. Data are expressed as the mean and SD ($n=9$). a–c: significantly different between the same letters at each time point, at $p<0.05$ by Dunnett’s post hoc test. FOS: fructooligosaccharide used as a reference.
little until the end of experiment. PD contains ~20% of low-molecular-weight saccharides which are fermentable. The total amount of breath hydrogen excreted was markedly lower than that for FOS.

When RMD (5 g) was digested by subjects, breath hydrogen started to be excreted 2–3 h after ingestion and reached a maximum 5–6 h after ingestion (Fig. 2). Thereafter, BHE decreased gradually until 14 h after ingestion. A part of RMD was readily fermented by intestinal microbes, but was less than that of FOS. The total amount of breath hydrogen excreted for 14 h was between that of PD and that of FOS. For the ingestion of PHGG (5 g), breath hydrogen started to be excreted 2–3 h after ingestion and reached a maximum 6–8 h after ingestion (Fig. 3). Thereafter, BHE decreased gradually until 14 h after ingestion as in the case of RMD. The fermentation of PHGG by intestinal microbes was poorer than that of RMD, but was much more than that of PD.

c) PHAA and GM. When PHAA (5 g) was ingested by subjects, breath hydrogen started to be excreted ~3 h after ingestion and reached a peak at 5 h (Fig. 3). PHAA was very resistant to fermentation by intestinal microbes even though it has a low molecular weight and is water-soluble. GM was very resistant to fermentation by intestinal microbes, and the start of BHE was very late (7–8 h after ingestion). However, the total amount of breath hydrogen excreted for 14 h was similar to that of PHAA (Fig. 3).

d) HMT-HACS and cellulose. When HMT-HACS (5 g), which is very poorly soluble in water, was ingested by subjects, the start of BHE was late and very low. BHE started to increase 8 h after ingestion and showed a peak at 10 h. The fermentability was lower than that of PHAA and GM (Fig. 4). When cellulose (5 g), which is water-insoluble, was ingested, the excretion profile of breath hydrogen was similar to that of HMT-HACS, and the total amount of breath hydrogen excreted for 14 h was similar to that of HMT-HACS (Fig. 4).

Area under the curve (AUC) of BHE 24 h after ingestion of DFMs

The rate of fermentation by intestinal microbes was
different according to the type of DFM, so the AUC of BHE 8 h, 14 h and 24 h after ingestion was calculated using the results in Fig. 2 to Fig. 4, and the results of AUC are shown in Table 3 and the relative ratios versus FOS are shown in Table 4.

The AUC of the reference of FOS (the available energy of which has been evaluated to be 2 kcal/g) was 6,000 ppm for 8 h, 12,000 ppm for 14 h, and 13,000 ppm for 24 h. The increment in the AUC between 14 h and 24 h after ingestion was slight. To calculate the relative ratio, these AUC values of FOS were set at 100% at 8 h, 14 h and 24 h, respectively.

The AUC of PD was 2,000 ppm for 8 h, 3,900 ppm for 14 h, and 5,000 ppm for 24 h (Table 3). The relative ratios versus FOS were 33.3 for 8 h, 32.8 for 14 h and 38.5% for 24 h (Table 4). These values were significantly smaller than those of FOS (p<0.05). Using the same method, the AUC and the relative ratios of each test material were calculated.

**Evaluation of relative available energy (RAE) of each dietary fiber material (DFM) based on the available energy of FOS**

When FOS (which is not digested in the small intestine) is ingested, it is completely fermented by intestinal microbes and not excreted to feces (16, 17). The available energy of FOS is 2 kcal/g. The RAE of DFMs based on the AUC of FOS is shown in Table 4. Thus, in the calculation based on AUC for 8 h, the RAE value for PD, PHGG, RMD, and PHAA was 1 kcal/g and that for GM, HMT-HACS and cellulose was 0 kcal/g. However, in the calculation based on the AUC for 14 h and 24 h, the RAE of all the DFMs tested became 1 kcal/g. Thus, the RAE of all DFMs tested was similar. However, all RAEs of the DFMs obtained from a short period after ingestion were lower than those of the long period after ingestion, and were different according to the type of dietary fiber.

In terms of appropriate experimental periods, these results demonstrate that even GM (which is a water-soluble dietary fiber with a high molecular weight) and cellulose (which is a water-insoluble dietary fiber with a high molecular weight) are slowly fermented by intestinal microbes and produce short chain fatty acids. Therefore, we must use the fermentability value obtained from a long period (>14 h) after the ingestion of test materials, when the available energy of the dietary fiber is

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**Table 3. Areas under the curve versus time of breath hydrogen gas excretion.**

<table>
<thead>
<tr>
<th>Fructooligosaccharide</th>
<th>AUC (ppm/8 h)</th>
<th>AUC (ppm/14 h)</th>
<th>AUC (ppm/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Glucomannan</td>
<td>1,300±1,100</td>
<td>3,100±2,500</td>
<td>4,100±3,000</td>
</tr>
<tr>
<td>Guar gum</td>
<td>2,700±1,900</td>
<td>5,500±3,700</td>
<td>6,600±4,300</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>1,600±1,000</td>
<td>3,600±3,100</td>
<td>4,100±3,300</td>
</tr>
<tr>
<td>Polydextrose</td>
<td>2,000±600</td>
<td>3,900±1,400</td>
<td>5,000±2,200</td>
</tr>
<tr>
<td>Resistant maltodextrin</td>
<td>3,500±1,600</td>
<td>7,100±3,900</td>
<td>8,300±4,300</td>
</tr>
<tr>
<td>Cellulose</td>
<td>1,400±800</td>
<td>4,000±2,100</td>
<td>4,900±2,700</td>
</tr>
<tr>
<td>HMT-HACS</td>
<td>1,000±600</td>
<td>4,400±2,400</td>
<td>4,900±2,700</td>
</tr>
</tbody>
</table>

Data are expressed mean and SD (n=8). AUC: areas under the curve versus time. HMT-HACS: heat-moisture treated, high amylose corn starch.

**Table 4. Energy estimation from ratio of AUC versus FOS.**

| FOS | 8 h-collection | | 14 h-collection | | 24 h-collection | | Estimation by Health Promotion Law (kcal/g) |
|-----|----------------|----------------|----------------|----------------|----------------|----------------|
|     | Ratio (%)      | Estimated energy (kcal) | Ratio (%)      | Estimated energy (kcal) | Ratio (%)      | Estimated energy (kcal) |     |
| FOS | 100.0          | 2               | 100.0          | 2               | 100.0          | 2               | 2   |
| Glucomannan | 21.7          | 0               | 26.1          | 1               | 31.5          | 1               | 2   |
| Guar gum    | 45.0          | 1               | 46.2          | 1               | 50.8          | 1               | 2   |
| Sodium alginate | 26.7          | 1               | 30.3          | 1               | 31.5          | 1               | 0   |
| Polydextrose          | 33.3          | 1               | 32.8          | 1               | 38.5          | 1               | 0   |
| Resistant maltodextrin | 58.3          | 1               | 59.7          | 1               | 63.8          | 1               | 1   |
| Cellulose             | 23.3          | 0               | 33.6          | 1               | 37.7          | 1               | 0   |
| HMT-HACS              | 16.7          | 0               | 37.0          | 1               | 37.7          | 1               | 2   |

Data are expressed mean and SD (n=8). AUC: areas under the curve versus time. HMT-HACS: heat-moisture treated, high amylose corn starch.
A DFM that is not fermented by intestinal microbes does not produce short-chain fatty acids, so the available energy is 0 kcal/g. Therefore, the available energy of DFMs which are fermentable becomes between 0 kcal/g and 2 kcal/g. However, in the present study, we used integers after rounding off because complicated numerical values are not practical in nutrition education or nutrition labelling. Nevertheless, the value of available energy for each DFM is calculated based on the fermentability from BHE and is not an integer. In addition, the category of 0 kcal/g, 1 kcal/g and 2 kcal/g was used in energy evaluation of the Japanese Health Promotion Law (9).

To calculate the RAE from the AUC for each DFM, we pre-supposed that the AUC of BHE for FOS was 100%, so the available energy was 2 kcal/g. The estimated RAES of PHGG, PHAA, PD, and RMD by 14-h collection were 1 kcal/g and they were in the same category after 8-h collection. However, although the RAES of GM, cellulose, and HMT-HACS by 8-h collection were 0 kcal/g, they increased to 1 kcal/g after 14-h collection. The value of RAE was different according to the collection period of breath gas, because the velocity of fermentation by intestinal microbes is different in the materials tested.

The available energy of RMD has been already discussed and evaluated as 1 kcal/g using the CO₂ and O₂ ratio in a balance study (7). In the present study using an indirect and simple method based on BHE, the RAE of RMD was estimated as 1 kcal/g, and the value conformed to that of the balance study (21). This supports the validity of the indirect method using BHE. Furthermore, Livesey et al. reported that the fermentation of PD was low and that there were interactions between in vitro and in vivo study (21). Moreover, Figdor and Rennhard and Anchour et al. evaluated the available energy of PD as 1 kcal/g in human and rat experiments using [14C]-PD (14, 15). These results are not contradictory to the value obtained from the present study.

Cellulose is not hydrolyzed by α-amylase or small intestinal enzymes. Although cellulose has very low fermentability and yields few short chain fatty acids in in vitro incubation using human feces (22, 23), cellulolytic bacterium has been isolated in the human gut microbial community (24–26) and it was different between presence and absence of methanogen (26). These reports support our result that BHE was detected in cellulose ingestion and the RAE was not 0 kcal/g in this study.

BHE by the ingestion of GM was clearly lower than that of FOS in this study, and the RAE of GM was evaluated as 1 kcal/g. Matsuzawa reported that glucosan was degraded almost 100% by soluble enzymes in human feces in an in vitro study (27). But, GM increases the fecal volume and accelerates the defecation (28). These results suggest that the fermentation or degradation by intestinal microbes is different between in vivo studies using humans and in vitro study.

HMT-HACS, which is similar to high amylose maize starch, showed incremental changes between 8 h and 14 h with regard to the AUC. It was reported that the excretion of breath hydrogen by high amylose maize starch in healthy humans increased markedly (29), and high amylose maize starch is partly digested in the human intestine by ileostomy (30) and in pigs (31). However, the fermentability is affected by source, and particle size (29, 32). HMT-HACS employed in this study comprised 64% resistant starch, and the remaining carbohydrates were difficult to be digested by intestinal enzymes. Thus, 1 kcal/g is valid for HMT-HACS.

The fermentability of nondigestible carbohydrate is dependent on the preparations, its molecular weight

DISCUSSION

Dietary fiber reaches the large intestine where it is fermented to varying degrees by the resident microbial population. Short-chain fatty acids produced by fermentation are utilized solely as the energy source of the host. However, an indirect and simple method used to evaluate the available energy of nondigestible and fermentable carbohydrates has not been established. In the present study, we tried to evaluate the available energy of several DFMs using a method that calculates from the fermentation equations which consist of the ratio of short-chain fatty acids produced by intestinal microbes (10–12). The fermentability of DFMs was estimated by measuring the excretion of breath hydrogen in healthy subjects. It was an indirect but very simple method and had potential to estimate the available energy of DFMs.

Fermentability reflects the degree of breakdown of fiber carbohydrates and the production of short chain fatty acids. It is affected by the quantity as well as the type of DFM consumed, because intestinal microbes have the fermentation capacity, and the amount of available energy is changed by fermentability. In the present study, 5 g of each test material was ingested by the subjects, because ≥10 g of tested materials could not be tolerated by the subjects. Furthermore, the amount of DFM which is added to processed foods with health benefits is <5 g. We consider 5 g of test material to be an appropriate dose to evaluate the available energy of DFMs.

It has been stated in the detailed enforcement regulations for the application of the Japanese Health Promotion Law (9) that carbohydrate that is not digested in the small intestine and which is completely fermented in the large intestine has 2 kcal/g of available energy. The value is derived from some proposed fermentation equations (11–13). FOS is a typical nondigestible oligosaccharide with such properties as well as lactulose (16–18). Hence, we used FOS (2 kcal/g) as a reference to calculate the RAE of several DFMs in the present study. Livesey reported that the available energy of digestive carbohydrate is 4 kcal/g and that of full-fermentable carbohydrate is a half of that of available carbohydrate, 2 kcal/g (19). In addition, the EU, Canada and Australia/New Zealand established 2 kcal/g of the energy value for fermentable fiber (20). Therefore, it is reasonable that the energy value of FOS as a reference is 2 kcal/g, as used in the present study.

A DFM that is not fermented by intestinal microbes does not produce short-chain fatty acids, so the available energy is 0 kcal/g. Therefore, the available energy of DFMs which are fermentable becomes between 0 kcal/g and 2 kcal/g. However, in the present study, we used integers after rounding off because complicated numerical values are not practical in nutrition education or nutrition labelling. Nevertheless, the value of available energy for each DFM is calculated based on the fermentability from BHE and is not an integer. In addition, the category of 0 kcal/g, 1 kcal/g and 2 kcal/g was evaluated based on BHE.
and the particle size of ingested test materials. Furthermore, using a within-subject, repeated-measures design is important, because the population of intestinal microbes is different according to individual and also ethnicity. Therefore, the present study in which the available energy of dietary fibers is expressed as RAE appears to be suitable.

Thus, the evaluated available energy of some DFMs was different according to the collection period of BHE. However, the available energy which was based on the 24-h AUC was identical to that of 14-h AUC. In terms of the detection of increasing hydrogen at 24 h after ingestion as shown in the figures, our examination was based on a 36 h continuous experiment, and we concluded that the detection of hydrogen in the morning was artifact. These results demonstrated that the collection period of breath hydrogen must be ≥14 h because the velocity of fermentation by intestinal microbes is different according to the type of DFM. Hence, we recommend a 15-h collection period to evaluate the available energy and to alleviate the stress of subjects. However, the available energy of GM, PHAA, PD and RMD was not different among values based on 8-h AUC, 14-h AUC and 24 AUC because these materials are readily fermented by intestinal microbes, as is FOS.

In the detailed enforcement regulations for the application of The Japanese Health Promotion Law (9), the values of available energy (energy coefficient) used are 2 kcal/g for GM, PHGG and HMT-HACS, 1 kcal/g for RMD, and 0 kcal/g for PHAA, PD and cellulose, respectively. Apart from RMD, the value for DFMs obtained from the present study were not in accordance with the values noted in the detailed enforcement regulations for the application of The Japanese Health Promotion Law. The reason is that fermentability values were obtained from animal experiments and in vitro experiments (not human experiments) and then used to evaluate the available energy. In the present study, the evaluation of available energy was carried out based on the results from human experiments alone. Therefore, the energy coefficients of DFMs in the present study are recommended for the nutrition labelling.

We tried to evaluate the RAE of nondigestible and fermentable carbohydrates using the fermentability based on BHE for ≥14 h, and found that the collection period 8 h after ingestion of the test material is too short to evaluate the available energy. The method which we propose in the present study is indirect but very simple and valid. The energy coefficient of several DFMs should be used for the nutrition labelling of processed foods.

Acknowledgments

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REFERENCES


