The Metabolizable Energy of Dietary Resistant Maltodextrin Is Variable and Alters Fecal Microbiota Composition in Adult Men

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Abstract

Resistant maltodextrin (RM) is a novel soluble, nonviscous dietary fiber. Its metabolizable energy (ME) and net energy (NE) values derived from nutrient balance studies are unknown, as is the effect of RM on fecal microbiota. A randomized, placebo-controlled, double-blind crossover study was conducted (n = 14 men) to determine the ME and NE of RM and its influence on fecal excretion of macronutrients and microbiota. Participants were assigned to a sequence consisting of 3 treatment periods (24 d each: 0 g/d RM + 50 g/d maltodextrin and 2 amounts of dietary RM (25 g/d RM + 25 g of maltodextrin/d and 50 g/d RM + 0 g/d maltodextrin)) and were provided all the foods they were to consume to maintain their body weight. After an adaptation period, excreta were collected during a 7-d period. After the collection period, 24-h energy expenditure was measured. Fluorescence in situ hybridization, quantitative polymerase chain reaction, and 454 titanium technology–based 16S rRNA sequencing were used to analyze fecal microbiota composition. Fecal amounts of energy (544, 662, 737 kJ/d), nitrogen (1.5, 1.8, 2.1 g/d), RM (0.3, 0.6, 1.2 g/d), and total carbohydrate (11.1, 14.2, 16.2 g/d) increased with increasing dose (0, 25, 50 g) of RM (P < 0.0001). Fat excretion did not differ among treatments. The ME value of RM was 8.2 and 10.4 kJ/g, and the NE value of RM was −8.2 and 2.0 kJ/g for the 25 and 50 g/d RM doses, respectively. Both doses of RM increased fecal wet weight (118, 148, 161 g/d; P < 0.0001) and fecal dry weight (26.5, 32.0, 35.8 g/d; P < 0.0001) compared with the maltodextrin placebo. Total counts of fecal bacteria increased by 12% for the 25 g/d RM dose (P = 0.17) and 18% for the 50 g/d RM dose (P = 0.019). RM intake was associated with statistically significant increases (P < 0.001) in various operational taxonomic units matching closest to ruminococcus, eubacterium, lachnospiraceae, bacteroides, holdemania, and faecalibacterium, implicating RM in their growth in the gut. Our findings provide empirical data important for food labeling regulations related to the energy value of RM and suggest that RM increases fecal bulk by enhancing the excretion of nitrogen and carbohydrate and the growth of specific microbial populations. J. Nutr. 144: 1023–1029, 2014.

Introduction

Around the turn of the 19th century, Rubner and Atwater proposed metabolizable energy (ME)2 values for macronutrients, and, for the most part, their work has stood the test of time. An exception is dietary fiber; research continues today to determine the ME value of dietary fiber and its interactions with other macronutrients (1–3). Although there are several studies in the literature describing the effects of fiber on energy value of the overall diet (4–6), there are few published reports on the energy value of fiber specifically (7,8). Research efforts to develop novel fibers (9–11) and their incorporation into functional foods present an opportunity to increase fiber intake, which typically falls below dietary recommendations, especially in Western populations (12). One such fiber is resistant maltodextrin (RM), which is produced by the heat and enzymatic treatment of cornstarch. It is a fine, water-soluble, nonviscous, and tasteless powdered mixture of...
oligosaccharides (3–10 degrees of polymerization) and polysaccharides (≥11 degrees of polymerization). RM is heat, freeze, and acid stable, making it suitable for a wide variety of food applications. In addition to improving dietary fiber intake, it is reported to improve glycemic response (13) and lower postprandial TG elevation (14) and may increase intestinal regularity (15).

Moreover, this novel type of dietary fiber may affect gut microbiota. RM is a substrate for fermentation by the commensal microbiota, primarily in the proximal large intestine (10). It may have a prebiotic effect by enhancing the growth of potentially beneficial microbes rich in glycohydrolases (16). Indeed, a bifidogenic effect was shown previously for resistant starch-type 3 polymorph B (17), and a bifidogenic trend associated with bulking was reported for RM (15,18). Another study showed that *Ruminococcus bromii* numbers increased during consumption of resistant starches (19). A recent double-blind feeding study of types 2 and 4 resistant starches had differential effects on microbiota composition, including increases in *Bifidobacterium adolescentis* and *R. bromii* (20). Release of energy from dietary fiber substrates may also be dependent on the kinds of microbiota present (21).

Energy values attributed to RM vary between 0.8 kJ/g (China) and 15.9 kJ/g (United States) because of differing country-specific nutrition labeling regulations for dietary fiber. Animal studies suggest that RM is not well absorbed and has an ME value of 2.2 kJ/g (22). One study conducted in healthy adults reported an energy value of 5.2 kJ/g, but these data were based only on 8-h indirect calorimetric measurements and breath hydrogen production (23). Furthermore, participants in this study were not adapted to the diet, and fecal collections to assess nutrient balance were not conducted. Hence, the primary objective of the present study was to determine the ME and net energy (NE) of RM from 24-h indirect calorimetry and urinary and fecal balance collections in participants well adapted to a controlled diet. Secondary objectives were to assess the impact of RM on the balance and digestibility of other macronutrients and its effect on gut microbiota.

**Participants and Methods**

**Participants.** Fifteen nonsmoking, weight-stable males aged 35–55 y were recruited from the Beltsville, Maryland area. Exclusion criteria included the following: 1) history or presence of kidney disease, liver disease, certain cancers, gastrointestinal, pancreatic, or other metabolic diseases, or malabsorption syndromes; 2) history of bariatric or other surgeries related to weight control; 3) antibiotic use during the intervention or for 3 wk before any intervention period; and 4) history of eating disorders or other dietary patterns that are not consistent with the dietary intervention (e.g., vegetarians, very low-fat diets, high-protein diets). Participants signed an informed consent that was reviewed and approved by the Medstar Research Institute (Washington, DC) institutional review board [institutional review board of record for the Beltsville Human Nutrition Research Center (BHNRC)]. Males were selected to eliminate variability in energy expenditure associated with the menstrual cycle (24). Participants were compensated for their participation in the study.

**Experimental design and treatments.** The study was designed as a randomized, double-blind, placebo-controlled, crossover study with 3 treatment periods of 24 d each separated with a minimum washout period of 2 wk. Each participant was assigned to a sequence that consisted of 3 treatment diets: a placebo control diet (0 g/d RM + 50 g/d maltodextrin) and 2 amounts of RM (25 g/d RM + 25 g/d maltodextrin and 50 g/d RM + 0 g/d maltodextrin). RM (Fibersol-2; Matsutani Chemical Industry) and maltodextrin (TK-16; Matsutani Chemical Industry) were mixed with water and consumed as a beverage. The amounts of RM were selected to provide 1 intake amount similar to the adequate intake amount of 25 g/d dietary fiber and a second amount of intake double the first. One-half of the daily treatment (RM and/or maltodextrin) was consumed at breakfast at the Human Studies Facility at the BHNRC, and the remaining half of the daily treatment was consumed at dinner at the same site under the observation of a dietitian or investigator to verify compliance. Participants were instructed to add the treatment to a drink (juice, milk, or water).

**Diets.** All foods and beverages consumed by the participants during the treatment periods were prepared, weighed, and provided to them at the BHNRC. Protocols for providing meals were used as described previously (25). All participants were instructed to avoid food and beverages containing caffeine, except those provided by the BHNRC. Alcohol consumption was not allowed during the intervention. Study participants were fed at weight maintenance (25). Initial energy intake was set to equal the energy expenditure determined during the preliminary calorimeter measurement (see below). No diet adjustments were made during the balance collection periods. The diets contained −14% of energy from protein, 22% of energy from fat, and 64% of energy from carbohydrate. This macronutrient composition was selected to provide a control diet low in fiber and high in carbohydrate while maintaining protein intake of the typical American diet. As a result, fat content was similar to a National Cholesterol Education Program Step 2 diet (26).

**Biologic sample collection and chemical analyses.** Fecal collections were initiated on day 14 using previously described methods (25). Participants recorded the date and time of each bowel movement and provided this information when the samples were delivered to the BHNRC and processed (25,27). The number of weekly bowel movements was calculated as the sum of the daily bowel movements during the 7-d collection period. Diets were collected and prepared for chemical analysis (25,27). For microbiota analysis, separate fecal samples were collected on days 1, 13, and 24 of each intervention, for a total of 9 fecal samples per participant. Participants obtained a cooler filled with ice for storage of the sample until delivery to the laboratory; all samples were delivered usually within 4 h of defecation. Samples were processed by kneading in a strong plastic bag immediately on arrival in the laboratory. A small portion of the sample was fixed for fluorescence in situ hybridization (FISH) analysis as described below, and the remainder was stored at −80°C. Urine was collected as described previously (25).

Diets, feces, and urine were analyzed for gross energy by adiabatic bomb calorimetry (Parr Instrument Company) and for nitrogen by combustion (Leco Corp.). Diets and feces also were analyzed for fat (methylene chloride extraction; CEM Corp.) and ash (muffle furnace). RM was analyzed in food and feces using an enzymatic–gravimetric method and liquid chromatography (28).

**Calorimetry.** On day 24 of feeding, 24-h energy expenditure was measured by respiratory exchange using a room-sized calorimeter. After a practice run in the calorimeter for adaptation, participants reported to the facility and entered the calorimeter at 0800. Measurements occurred over the following 23.5 h, and the participants were released from the calorimeter the next day at 0730. While in the calorimeter, the participants followed a fixed activity schedule, including an exercise period with a controlled and defined amount of work. Energy expenditure, oxygen consumption, and carbon dioxide production were continuously recorded, and all urine was collected during the calorimeter measurements. Gas consumption and production was measured by determining the mass and composition (by mass spectrometer) of air flow into and out of the calorimeter. Energy expenditure and substrate oxidation were calculated based on the equations of Livesey and Elia (29,30). Nitrogen content of the 24-h urine collection was determined as described above.

**Calculations.** Total carbohydrate (dry matter basis) was calculated as

\[
\text{Total carbohydrate (g)} = 100 - (\% \text{fat} + \% \text{protein} + \% \text{ash}).
\]

Non-RM carbohydrate in the diet and feces was calculated as

\[
\text{Non-RM carbohydrate (g)} = \text{Total carbohydrate (g)} - \text{RM (g)}.
\]
The ME value of RM was calculated (7) as

\[
ME_{RM} (kJ/g) = \frac{GE_{RM} \times \left( \frac{ME_{RM}}{I_{RM}} \right) - \left( GE_{RM \text{ diet}} - GE_{RM} \right) \times \left( \frac{ME_{RM \text{ diet}}}{I_{RM}} \right)}{4 \cdot RM \text{ Intake}},
\]

where \( ME_{RM} \) is the ME value of RM, \( ME_m \) is the ME requirement for maintenance [retained energy (RE) = 0], and I is the intake (grams per day) of RM. The ME requirement for maintenance is calculated for each individual with the assumption that the ME efficiency is 0.95 for negative energy balance and 0.90 for positive energy balance (7,31). The NE value was calculated between the 0 and 25 g/d RM treatments and between the 0 and 50 g/d RM treatments.

RE is calculated as (32)

\[
RE (kJ/d) = EE (kJ/d) - ME (kJ/d),
\]

where EE is energy expenditure (kilojoules per day).

**Microbiota analysis.** Fecal microbiota composition was analyzed using a 16S rRNA approach that included denaturing gradient gel electrophoresis (DGGE)–based profiling, qPCR, FISH analysis, and 454 titanium technology–based 16S rRNA sequencing as described previously (33).

**Statistical analyses.** To determine the effect of the treatment on wet weight, dry weight, the number of bowel movements, and chemical composition of the diet and excreta, a mixed-model ANOVA with repeated measures was performed using the participant as the random term (SAS version 9; SAS Institute). The statistical model included terms for age, BMI, treatment sequence, and period and their interactions. Results are reported as least-square means and SEMs. Differences among treatments were determined using Tukey-Kramer test. Differences between the ME and difference between the NE values of the 2 RM-containing diets were determined by paired \( t \) test. For the bacterial profiles generated by DGGE, we used imaging software (Quantity One) to scan the gel images. Background was subtracted from each lane, and the profiles were subjected to Gaussian modeling. We then calculated a similarity matrix based on Pearson’s correlation coefficients and generated phylogenetic trees based on various algorithms (Ward’s Universal Primer from Multiple Alignment). The amounts of bacteria as determined by FISH were analyzed as total counts per gram of feces and \( \log_{10} \) transformed counts per gram of feces. The effect of RM on bacterial numbers was defined as the mean during the RM intervention minus the mean during the placebo period subtracted by the mean when participants were consuming their usual diet (i.e., baseline and washout periods). Effect of treatment on amount of bacteria was determined by ANOVA. qPCR data were analyzed as genome equivalents per nanogram of DNA. Changes in amounts of bacterial groups of interest were determined by comparing the change of the genome equivalents per nanogram of DNA between day 1 and day 24 of the RM and placebo periods. A paired \( \chi^2 \) test followed by Fisher combining was used to test for a difference in the prevalence of operational taxonomic units (OTUs). We adjusted for an expected high false-discovery rate by increasing the requirement for statistical significance to \( P < 0.05 \) (Tukey-Kramer test). RM, resistant maltodextrin.

**Results**

Fourteen of the 15 participants who began the study completed the entire study protocol. One participant failed to complete the study because of scheduling conflicts. Only data from those 14 participants who completed the intervention were included in the data analysis. There were no self-reported deviations from the dietary protocol based on a review of the daily questionnaires. Physical characteristics of the participants are reported in Table 1. With the exception of self-reported increases in flatus, the treatments were well tolerated by the participants.

The dry weight and gross energy of food and treatment consumed did not differ among treatment periods (Table 2). Mean RM intake was 0.5, 22.6, and 47 g/d RM for the 3 treatments. These amounts were close to the planned amounts of 0, 25, and 50 g/d RM. Because RM was replaced with maltodextrin in the 0 and 25 g/d RM treatments, total carbohydrate intake did not differ among the 3 treatments. Nitrogen and fat intake were similar among all 3 treatments, as planned.

Total fecal wet and dry weight increased from the 0 to the 25 g/d RM treatments (0.05 but were not significantly different among the 0 and 25 g/d RM treatments (Table 3). There was no effect of treatment on urinary energy excretion; however, fecal energy excretion increased with the increasing consumption of RM. There was no effect of treatment on the number of bowel movements during the 7-d collection period.

**TABLE 1** Baseline physical characteristics of participants enrolled in a randomized crossover study of resistant maltodextrin

<table>
<thead>
<tr>
<th>Characteristic Value Range</th>
<th>n = 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>86.8 ± 2.5</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.8 ± 1.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.9 ± 0.8</td>
</tr>
</tbody>
</table>

1 Values are means ± SEs. Labeled means in a row not sharing a superscript letter differ, \( P < 0.05 \) (Tukey-Kramer test). RM, resistant maltodextrin.

**TABLE 2** Daily food, energy, and nutrient intake of participants consuming maltodextrin or resistant maltodextrin as part of controlled diets

<table>
<thead>
<tr>
<th>Amount of dietary RM² (g/d)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>SEM</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (dry matter), g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>567</td>
<td>566</td>
<td>566</td>
<td>19</td>
<td>0.74</td>
</tr>
<tr>
<td>Treatment⁴</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total intake</td>
<td>617</td>
<td>616</td>
<td>616</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake, kJ/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>11,560</td>
<td>11,560</td>
<td>11,560</td>
<td>390</td>
<td>0.74</td>
</tr>
<tr>
<td>Treatment</td>
<td>820</td>
<td>820</td>
<td>820</td>
<td>10</td>
<td>0.95</td>
</tr>
<tr>
<td>Total energy</td>
<td>12,380</td>
<td>12,380</td>
<td>12,380</td>
<td>390</td>
<td>0.93</td>
</tr>
<tr>
<td>Carbohydrates, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-RM³</td>
<td>443³</td>
<td>421³</td>
<td>397³</td>
<td>13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>13.7</td>
<td>13.5</td>
<td>13.6</td>
<td>0.4</td>
<td>0.60</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
<td>0.0</td>
<td>0.60</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>443</td>
<td>443</td>
<td>443</td>
<td>13</td>
<td>0.74</td>
</tr>
<tr>
<td>Fat, g/d</td>
<td>65</td>
<td>65</td>
<td>65</td>
<td>2</td>
<td>0.74</td>
</tr>
<tr>
<td>Nitrogen, g/d</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>1</td>
<td>0.74</td>
</tr>
</tbody>
</table>

1 Data are expressed as least-square means, \( n = 14 \). Labeled means in a row not sharing a superscript letter differ, \( P < 0.05 \) (Tukey-Kramer test). RM, resistant maltodextrin.

2 Listed is the nutrient content of the background diet, to which RM was added at the doses indicated.

3 Probability of treatment effect based on mixed-model, repeated-measures ANOVA.

4 Maltodextrin or RM.

5 Difference between total carbohydrates and RM. For additional details on methodology, see Participants and Methods.
TABLE 3  Daily fecal excretion frequency and composition of participants consuming maltodextrin or resistant maltodextrin as part of controlled diets

<table>
<thead>
<tr>
<th>Amount of dietary RM (g/d)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>SEM</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowel movements, times/wk</td>
<td>7.0</td>
<td>8.5</td>
<td>7.9</td>
<td>0.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Excreta weight, g/d</td>
<td>1810</td>
<td>1880</td>
<td>1910</td>
<td>170</td>
<td>0.58</td>
</tr>
<tr>
<td>Urine weight</td>
<td>118a</td>
<td>148b</td>
<td>161c</td>
<td>7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Feces wet weight</td>
<td>26.5a</td>
<td>32.0b</td>
<td>35.8b</td>
<td>1.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Excreta energy, kJ/d</td>
<td>448</td>
<td>480</td>
<td>462</td>
<td>29</td>
<td>0.07</td>
</tr>
<tr>
<td>Urine energy</td>
<td>544a</td>
<td>662b</td>
<td>733c</td>
<td>29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total energy</td>
<td>992a</td>
<td>1140b</td>
<td>1200b</td>
<td>50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Carbohydrate excretion, g/d</td>
<td>0.3a</td>
<td>0.6b</td>
<td>1.2c</td>
<td>0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RM</td>
<td>10.8a</td>
<td>13.6b</td>
<td>15.0b</td>
<td>0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-RM (^1)</td>
<td>11.1a</td>
<td>14.2b</td>
<td>16.2b</td>
<td>0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>11.2</td>
<td>13.3</td>
<td>13.4</td>
<td>0.6</td>
<td>0.18</td>
</tr>
<tr>
<td>Nitrogen excretion, g/d</td>
<td>1.5a</td>
<td>1.8b</td>
<td>2.1c</td>
<td>0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urine</td>
<td>12.8</td>
<td>13.3</td>
<td>13.4</td>
<td>0.6</td>
<td>0.18</td>
</tr>
<tr>
<td>Feces</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>0.1</td>
<td>0.94</td>
</tr>
</tbody>
</table>

\(^1\) Data are expressed as least-square means, \( n = 14 \). Labeled means in a row not sharing a superscript letter differ, \( P < 0.05 \) (Tukey-Kramer test). RM, resistant maltodextrin.

\(^2\) Probability of treatment effect based on mixed-model, repeated-measures ANOVA.

\(^3\) Difference between total carbohydrate and RM. For additional details on methodology, see Participants and Methods.

RM fecal excretion ranged from 0.3 to 1.2 g/d. Despite the low recovery of RM in the feces, the increase in RM fecal excretion from the 0 to 25 g/d RM treatments (\( P < 0.05 \)) and from the 25 to 50 g/d RM treatments (\( P < 0.05 \)) was significant. A similar pattern occurred for fecal total carbohydrate excretion. Fecal excretion of the non-RM carbohydrate fraction was significantly (\( P < 0.05 \)) higher after consumption of the 25 and 50 g/d RM treatments compared with the 0 g/d RM treatment. Fecal nitrogen excretion from the 0 to 25 g/d RM treatment (\( P < 0.05 \)) and from 25 to 50 g/d RM treatment (\( P < 0.05 \)) increased, whereas urinary nitrogen excretion and total nitrogen excretion did not differ among the treatments. Urinary nitrogen excretion represents >80% of the total nitrogen excretion. Given the large proportion of nitrogen excretion in urine, total nitrogen excretion did not differ among treatments despite the significant changes in fecal nitrogen excretion.

Energy expenditure (24-h) and respiratory quotient did not differ among treatments (Table 4). There was no detectable dose-dependent increase in total energy expenditure between the 25 and 50 g/d RM intake of RM (10.8 and 10.8 MJ/d, respectively). The ME value of RM calculated between the 0 and 25 g/d RM doses was 8.2 kJ/g, and the ME value of RM, between the 0 and 50 g/d RM doses, resulted in a value of 10.4 kJ/g. Among individuals, there was a wide range in the measured ME value of RM (Supplemental Fig. 1A, B). The NE calculated between the 0 and 25 g/d RM doses resulted in a value <1 (−8.2 kJ/g), and the calculated NE value of RM between the 0 and 50 g/d RM doses was 2.0 kJ/g. The ME and NE values of RM were not different at the 2 amounts of RM intake (Table 4).

DGGE, a simple but efficient method for initial profiling of fecal microbiota, suggested that each individual harbored a unique microbiota. A distinct band was detected that was consistently and dose dependently increased after 24 d of RM supplementation in 12 of 14 participants. DNA from 2 of these bands was isolated, cloned, and sequenced. Both resulting sequences matched closest to sequences in the family Lachnospiraceae in the phylum Firmicutes.

To perform a more targeted quantitative microbiota analysis, the microbiota was analyzed by FISH with probes and primers directed against major groups of bacteria. Total bacteria per gram of feces increased during RM supplementation (Fig. 1). This association was dose dependent and statistically significant on the linear scale (\( P = 0.02 \)) during consumption of the 50 g/d RM treatment. Using FISH, we did not detect a difference in total bifidobacteria counts per gram of feces, partially because of an apparent bifidogenic effect of the 0 g/d RM diet. Conversely, qPCR analysis indicated an 80% increase in bifidobacteria (\( P = 0.03 \)) when comparing days 1 and 24 during the 50 g/d RM treatment, whereas during the 0 g/d RM period, the bifidobacteria increase was not significant (\( P = 0.48 \); data not shown).

To expand our microbiota analysis from the limited number of targeted groups of gut bacteria, high-throughput microbial community 16S rRNA sequencing using 454 titanium technology was performed. After removal of low-quality and short readings, a total of 466,622 sequences were analyzed, with a mean of 5622 sequences per participant and a mean length of 296 nucleotides (range of 200–529). At the phylum level, Firmicutes represented 49%, bacteroidetes 28%, proteobacteria 0.6%, actinobacteria 0.5%, and others (unidentified and sequences with ambiguous matches), 20% of all sequences. Chao1-based rarefaction curves indicated that overall numbers of estimated bacterial OTUs did not differ among the treatments. UniFrac-based principal component

TABLE 4  Mean energy expenditure, respiratory quotient, metabolizable energy intake, retained energy, and calculated metabolizable and net energy of RM

<table>
<thead>
<tr>
<th>Amount of dietary RM (g/d)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>SEM</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy expenditure, kJ/d</td>
<td>10.50</td>
<td>10.80</td>
<td>10.80</td>
<td>200</td>
<td>0.26</td>
</tr>
<tr>
<td>Respiratory quotient</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.01</td>
</tr>
<tr>
<td>Total metabolizable energy intake, (^2) kJ/d</td>
<td>11,400a</td>
<td>11,200b</td>
<td>11,200b</td>
<td>370</td>
<td>&lt;0.001 (^2)</td>
</tr>
<tr>
<td>Retained energy, (^2) kJ/d</td>
<td>883</td>
<td>409</td>
<td>361</td>
<td>356</td>
<td>0.08</td>
</tr>
<tr>
<td>Metabolizable energy, kJ/g</td>
<td>Not applicable</td>
<td>8.3 ± 1.4</td>
<td>10.4 ± 0.8</td>
<td>0.06 (^3)</td>
<td></td>
</tr>
<tr>
<td>Net energy, (^2) kJ/g</td>
<td>Not applicable</td>
<td>−8.2 ± 10.5</td>
<td>2.0 ± 5.0</td>
<td>0.38 (^4)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Data are expressed as least-square means, \( n = 14 \). Labeled means in a row not sharing a superscript letter differ, \( P < 0.05 \) (Tukey-Kramer test). RM, resistant maltodextrin.

\(^2\) Probability of treatment effect based on mixed-model, repeated-measures ANOVA.

\(^3\) For details on the calculation of metabolizable, retained, and net energy, see Participants and Methods.

\(^4\) Probability of difference between consumption of 25 and 50 g/d RM based on paired \( t \) test.
analysis also did not detect differences in overall microbiota composition by treatment status. However, the distribution of OTUs across treatment suggested that many were increased or decreased by RM (Supplemental Fig. 2). The OTUs with the greatest magnitude of change during the consumption of RM matched closely (>95%) to lachnospiraceae, clostridia, bacteroides, parabacteroides, and coprococcus; other OTUs that increased had no close matches in the database.

Discussion

Defining the ME value of fiber is important for accurate food labeling. Nevertheless, a uniform approach for assessing this value is lacking and is dependent on country-specific regulatory requirements. Given the heterogeneous nature of fiber and the complexities of fermentation, empirical data become important. In this study, we used nutrient balance to determine the ME content of RM, a highly soluble, nonviscous fiber, and combined these data with indirect calorimetry to determine its NE value. Although energy expenditure did not differ between RM consumption of 25 or 50 g/d RM, the increase in energy expenditure when compared with 0 g/d RM suggests that the energetic cost of adding RM to the diet results in a NE value of <0 kJ/g.

The observed ME value of RM was similar to that reported for other fibers, including mixed fibers (1), sugar beet fiber (7), inulin (7,34), and low-digestible carbohydrates (8). The ME value of RM measured in the present study was higher than reported previously for animals (2.2 kJ/g) and humans (5.2 kJ/g) and may reflect differences in digestive physiology between humans and rats (22) and study design (23). Importantly, the significant variation in ME value of RM observed among participants suggests that participant characteristics, including differences in rate of digesta passage and resident gut microbiota can, at least after a short period of RM consumption, affect energy digestibility. This observation would suggest the possibility that ME value over time could vary in an individual based on adaptation of the gut microbiota. Interestingly, there was less variability in the ME value of RM at the higher amount compared with the lower amount of intake, and, for some individuals, the ME value was consistent between the 2 amounts of intake, whereas for others it was variable. Physiologic and microbial bases for this variability remain unclear.

Consuming RM had a significant effect on fecal weight, although there was no effect on the number of weekly bowel movements. The increase in fecal bulk is consistent with the findings of Fastinger et al. (15) that RM consumption significantly increased fecal organic matter and showed a trend toward increasing fecal dry matter. Our results are also similar to another low-digestible carbohydrate fed at 100 g/d in which the number of bowel movements did not change despite an increase in the amount of wet and dry fecal weight (8). The increase in fecal weight per unit of RM consumed was similar to that of pectin, a naturally occurring, soluble fiber that is a fermentable polymer of α-(1-4)-linked D-galacturonic acid (35,36). Doubling RM intake from 25 to 50 g/d RM had only a small impact on wet and dry fecal mass (P = 0.06). Despite the increase in fecal weight between the diet with 0 g/d RM and the diets containing RM, the amount of RM recovered in the feces was small, suggesting that this fiber was highly fermentable. The increase in fecal weight (wet and dry) was primarily associated with an increase in non-RM carbohydrate excretion. The chemical composition of this carbohydrate is not currently known, but 1 possibility is that this carbohydrate fraction is associated with microbes and could be bacterial extracellular polysaccharides. This observation is consistent with the increased mass of microbes excreted based on the increase in microbial concentration measured by FISH. The data suggest that, with the production of short-chain FAs from the fermentation of RM (15), these serve as an energy source for microbial growth.

Increased fecal nitrogen excretion also suggests an increased microbial mass. The effect of RM on nitrogen digestibility is consistent with that of other fiber sources. The decrease in nitrogen digestibility per unit of fiber (percentage per gram of fiber) ranges from 0.02% (4) to 0.7% (6). In this study, nitrogen digestibility decreased 0.1%/g RM, and the effect was due to changes in fecal and not urinary nitrogen excretion. Overall, there was no effect on total nitrogen excretion. Because the analytical methods used in this study cannot distinguish between microbial and nonmicrobial nitrogen, it is not clear whether the dietary nitrogen was converted to microbial protein and excreted as such in the feces.

Bacterial groups or sequences that were increased by RM included lachnospiraceae, bacteriodes, parabacteriodes, bifidobacteria, clostridia, and coprococcus. Several of these bacteria are known human commensals that possess extensive endoglycolytic and exoglycolytic enzymes (16,37) that can break down...
complex carbohydrates and that help them outcompete other microbes in this ecologic niche. Many of the other sequences associated with RM supplementation did not match closely to any cultivated gut bacteria or sequences deposited in the database. Bifidobacteria represented <2% of the total bacteria observed in our studies. This suggests that we are still only at the beginning of exploring the vast amount of microbial diversity associated with the human gut. Great attention has been focused on increasing amounts of bifidobacteria and lactic acid bacteria to improve or maintain health (38,39). The health contributions of individual bacterial species are far from completely understood. Individuals in our study showed functional gut microbiota differences in the ability to break down the complex carbohydrate found in RM. Additional research is needed to determine whether the specific strains of gut bacteria associated with RM intake in this study vary among other groups of individuals and whether ME values differ as a function of this. There are limitations of this study to consider. Fermentable carbohydrates result in the production of volatile FAs (e.g., acetate, propionate, and butyrate) that may be used by the body as a source of energy, as well as methane and hydrogen, which can be lost from the body. Potential loss of energy in the form of hydrogen and methane gas production was not measured in the study. Although fecal microbiota are not equivalent to that which is active in the proximal colon, it is closely related (40,41), and feces represent a convenient sample for repeated collections. In this study, the adaptation period was 14 d before collection of samples for the measurement of ME. Many previous studies suggest that a 2–3 wk run-in period is sufficient for host adaptation to new diets. However, the time needed to adapt or change the microbiota of the large intestine is unknown. This study provides empirical data on the ME and NE value of RM, a highly soluble, nonviscous fiber of 8.3 and −8.2 kJ/g, respectively, at a dietary intake consistent with current dietary recommendations. The ME and NE values were not significantly different for the 2 amounts of RM tested. These empirical data should become the basis for food labeling of the energy value of RM for the global marketplace. Consumption of this novel fiber increased fecal bulk, likely through the excretion of total carbohydrate and nitrogen and fecal microbiota. RM intake appeared to promote the growth of lachnospiraceae, bacteroides, parabacteroides, bifidobacteria, clostridia, and coprococcus. The strengths of this study are its double-blind, placebo-controlled, randomized crossover design with adaptation to a highly controlled diet and the ability to evaluate dose response.

Acknowledgments

The authors thank Dr. Steve Radecki for his help with experimental design and statistical analysis. D.J.B., V.M., W.V.R., K.S.S., T.H., D.R.P., K.O., H.T., S.K., D.T.G., M.U., and T.C. designed the research; D.J.B., W.V.R., K.S.S., D.R.P., and T.H. conducted the research; D.J.B., V.M., W.V.R., K.O., H.T., S.K., D.T.G., M.U., T.C., and X.W. analyzed the data; D.J.B. and V.M. wrote the paper; and D.J.B. and V.M. had primary responsibility for final content. All authors read and approved the final manuscript.

References


