Interplay between dietary fibers and gut microbiota for promoting metabolic health

Mistry, Rima

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 08-02-2021
Chapter 3

Dietary isomalto/malto-polysaccharides increase fecal bulk and microbial fermentation


*These authors contributed equally to this work

Submitted
Abstract

The prevalence of metabolic syndrome-related disease including obesity and type 2 diabetes mellitus has strongly increased. Nutritional intervention appears as an attractive strategy, and particularly novel prebiotics receive growing attention. Isomalto/malto-polysaccharides (IMMP) form one such class of promising novel prebiotics which promote proliferation of beneficial bacteria \textit{in vitro}. The present study investigated for the first time the \textit{in vivo} effects of IMMP (10%, w/w) on various metabolic parameters, microbial fermentation products and microbiota composition in C57BL/6 wildtype mice. At the end of a three-week dietary intervention mice receiving IMMP had significantly more fecal bulk (+26%, p<0.05), higher plasma non-esterified fatty acids (+10%, p<0.05) and lower fecal dihydrocholesterol excretion (-50%, p<0.05), compared to control mice. Plasma and hepatic lipid levels were not influenced by dietary IMMP, as were other parameters of sterol metabolism including bile acids. IMMP was mainly fermented in the cecum and large intestine, where it was associated with a higher relative abundance of \textit{Bacteroides} and butyrate producers (\textit{Lachnospiraceae}, \textit{Rosiburi}, \textit{Odoribacter}). Combined our results demonstrates that IMMP administration to mice increases fecal bulk and induces potentially beneficial changes in the intestinal microbiota.
Introduction

Increased consumption of “Western-style” diets and a sedentary lifestyle are considered major contributing factors to the increasing prevalence of the metabolic syndrome, comprising obesity, insulin resistance, type 2 diabetes, hypertension, non-alcoholic fatty liver disease and cardiovascular diseases.(1) Epidemiological studies have indicated a relationship between low dietary fiber intake and risk of developing metabolic syndrome.(2,3) Whether the epidemiologically beneficial effects of fiber intake are a result of lower glycemic index, reduced energy content or the fact that dietary fibers stimulate gastrointestinal (GI) microbiota fermentation is not currently clear. Nevertheless, the American Heart Association recommends a daily dietary fiber intake of 25-38 g/day (14g/1000kcal/day).(4,5) Accumulating evidence on the potential benefits of dietary fibers has generated an increasing interest in foods that are low in glycemic index, slowly degraded or completely escape digestion.

Isomalto/malto-polysaccharides (IMMP) are a novel class of dietary fibers with a prebiotic potential.(6,7) IMMP contain a high proportion of α-(1→6) glycosidic linkages. Earlier studies showed that IMMP can stimulate proliferation and activity of Bifidobacterium and Lactobacillus during in vitro fermentation using adult human fecal inoculum as a microbial source.(7,8) In vitro studies showed that this modulatory effect on microbial communities was accompanied by the accumulation of succinate and short-chain fatty acids (SCFA), in particular acetate and propionate in the media. (8) These studies, as well as research on similar substrates, such as dextran and isomalto-oligosaccharides (IMOs), suggest that IMMP may have beneficial effects on metabolism and health.(9) However, currently no in vivo data are available supporting this assumption. Therefore, we designed the first in vivo study to investigate the effects of a particular IMMP in a mouse model. We studied the utilization of the dietary IMMP substrate throughout different regions of the GI of mice as well as the metabolic impact of IMMP supplemented diet with a special focus on bile acid and cholesterol metabolism.

Materials and Methods

Animal experiments

C57BL/6 female mice were obtained from Harlan (Horst, The Netherlands). All mice were nine weeks old at the start of the dietary intervention. Animals were individually housed in a light- and temperature-controlled facility (12 h light-dark cycle, 21°C). All animal experiments were approved by the Committee of Animal Experimentation at
the University of Groningen and performed in accordance with the Dutch National Law on Animal Experimentation as well as international guidelines on animal experimentation. IMMP-94 contained 94% \( \alpha-(1 \rightarrow 6) \) linkages (Avebe, Veendam, The Netherlands) and was made by enzymatic conversion of potato starch using the GTFB 4,6-\( \alpha \)-glucanotransferase enzyme purified from \textit{E.coli} and pullulanase (Promozyme D2, Novozymes, Bagsvaerd, Denmark) kindly supplied by Dr. Hans Leemhus (Avebe) as described previously.(7) Animals were fed \textit{ad libitum} with either control (n=6) or IMMP (10% w/w, n=6) supplemented diet for 21 days. Control baseline diet (Safe Diets, Augy, France) contained 60.94% corn starch, 0.06% cholesterol, 20% caseinate, 0.3% L-cystine, 7% carbohydrate mix (sucrose:maltodextrin, 50:50), 7% soya bean oil, 0.2% choline bitartrate, 3.5% mineral mixture, 1% vitamin mixture (w/w). A modified diet containing 10% IMMP was obtained by replacing an equal amount of corn starch (50.94% corn starch, 0.06% cholesterol, 20% caseinate, 0.3% L-cystine, 7% carbohydrate mix, 7% soya bean oil, 0.2% choline bitartrate, 3.5% mineral mixture, 1% vitamin mixture).

Fecal samples were freshly collected from the animals at time points 0 h, 24 h, 48 h, 3 days, 7 days, 14 days and 21 days and stored at \(-80^\circ\text{C}\) until further analysis. Animals were weighed weekly. Food intake and fecal output were recorded on days 7, 14 and 21. At day 21 the gall bladder of mice from both groups was cannulated under anesthesia (hypnorm 1mg/kg body weight; diazepam 10 mg/kg body weight). Bile was collected continuously for 20 minutes and the rate of secretion was determined gravimetrically.(10) After termination, the GI was excised and the entire contents of stomach, small intestine, cecum and large intestine were individually collected and immediately stored at \(-80 ^\circ\text{C}\) for later analysis.

**Analysis of bile, plasma and liver**

At the time of termination, a large blood sample was collected by heart puncture. Plasma was isolated and aliquots were stored at \(-80 ^\circ\text{C}\) until further analysis. Liver was excised at termination and homogenized. Extraction of lipids was performed from the homogenates using the Bligh and Dyer procedure and redissolved in water containing 2% Triton X-100.(10) Liver as well as plasma total cholesterol and triglycerides were measured using commercially available reagents (Roche, Diagnostic, Basel, Switzerland). Biliary bile acid and cholesterol concentrations were analyzed as described earlier.(11)
Fecal sterol and bile acid measurements

Feces were collected over a period of 24 h. Fecal samples were dried, weighed and ground. 50 mg of feces were used to extract neutral sterols and bile acids which were measured using gas-liquid chromatography as published.(12) Plasma bile acids were methylated with a mixture of acetyl chloride and trimethylsilylated with pyridine, N, O-Bis (trimethylsilyl) trifluoroacetamide and trimethylchlorosilane. Plasma bile acids were then determined using liquid-chromatography mass spectrometry.

Fecal DNA extraction and microbiota analysis using next generation sequencing

Between one and three fecal pellets, or approximately 0.1g of intestinal content sample were used for DNA extraction. Total bacterial DNA was extracted according to a previously described protocol with minor modifications.(8) Fecal pellets were homogenized in 350 µL STAR buffer, with cooling at room temperature and the bead-beating step was repeated using 200 µL of fresh STAR buffer. The V4 region of 16S ribosomal RNA (rRNA) genes was amplified using uniquely barcoded primers 515F-n (5’-GTGCCAGCMGCCGCGGTAA-) and 806R-n (5’-GGACTACHVGGGTWTCTAAT) (200 nM each).(13) Purified PCR products were pooled into libraries and sent for adapter ligation and HiSeq sequencing (GATC-Biotech, Konstanz, Germany). Data processing and analysis was carried out using NG-Tax.(13) In brief, libraries were filtered to contain only read pairs with perfectly matching barcodes that were subsequently used to separate reads by sample. Operational taxonomic units (OTUs) were assigned using an open reference approach and the SILVA_111_SSU 16S rRNA gene reference database (https://www.arb-silva.de/).(14)

Analysis of fecal short-chain fatty acids

Feces were kept frozen before being processed for SCFA analysis. Approximately 50 mg of feces were mixed with 0.35 mL of 50 mmol/L sulfuric acid and 0.025 mL of 4 mg/mL 2-ethylbutyric acid. The mixture was homogeneously suspended by mixing by vortex in the presence of glass beads (rinsed with Millipore water beforehand) in an Eppendorf tube. Subsequently the samples were centrifuged for 20 min at 18,600 g and 4 °C, and the supernatant was analyzed by high performance liquid chromatography – refractive index (HPLC-RI) method published previously.(15) The dry matter content of mouse feces was estimated by comparing the weight differences before and after freeze-drying the feces.
Analysis of oligosaccharide profiles of murine digesta by HPAEC-PAD

The digesta samples from stomach, small intestine, cecum and large intestine of the mice were freeze-dried and then mixed in Millipore water at a concentration of 2.5 mg/mL. After mixing thoroughly by vortex, the tube containing the suspension was put in boiling water for 5 min and then centrifuged at 18,600 g for 20 min. The supernatant was taken and analyzed by high performance anion exchange chromatography – pulsed amperometric detection (HPAEC-PAD). 10 µL of sample was injected into a Dionex ICS 5000 system (Dionex) with a CarboPac PA-1 column (250 mm x 2 mm ID) and a CarboPac PA guard column (25 mm x 2 mm ID). The temperature of the column was set at 20°C. The flow rate of the two mobile phases (A) 0.1 M NaOH and (B) 1 M NaOAc in 0.1 M NaOH was set to 0.3 mL/min. The gradient elution was applied as follows: 0 – 40 min, 0 – 40% B; 40 – 40.1 min, 40 – 100% B; 40.1 – 45 min, 100% B; 45 – 45.1 min, 100 – 0% B; 45.1 – 60 min, 0% B. PAD (Dionex ISC-5000 ED) was used to monitor elution. HPAEC data was processed using ChromeleonTM 7.1 software (Dionex).

Statistics

Statistical analysis on metabolic parameters was performed using GraphPad Prism software (San Diego, CA). All data are presented as means ± SEM. Statistical differences between groups were assessed using the Mann-Whitney U-test. Statistical significance for all comparisons was assigned at P<0.05. Microbial composition data was expressed as a relative abundance of each genus level taxon obtained with NG-Tax. A 5000 reads per sample rarefraction cut-off was used in alpha diversity indices (Shannon, Chao1, and PD Whole Tree) calculations and group comparisons were done using nonparametric two-sample t-tests with Monte Carlo permutations in QIIME. (16) The association between microbiota composition and the dietary treatment group was investigated with RDA analysis in Canoco5, with significance assessed using a permutation test.(17) Beta diversity analysis, including weighted and unweighted unifrac distances estimates, and ANOSIM group comparisons were calculated in QIIME using rarefied data. Genus level taxa that differed significantly between different treatment groups were identified with Kruskal-Wallis analysis using QIIME. (16,18)
Results

IMMP utilization along the GI tract

The digesta from different parts of the murine GI of mice fed during a period up to 21 days with a control diet or a diet containing IMMP were analyzed by HPAEC-PAD, in order to monitor the degradation of these dietary fiber polymers from the diets and the formation of oligosaccharides. α-1-4-linked maltodextrin peaks were present in the stomach and small intestine digesta of animals from both dietary treatment groups (Fig. 1). These maltodextrin peaks were products of starch digestion by murine digestive enzymes. Differences were also noticed in the small intestine between the two groups of mice: the isomaltose peak (elution at ~6 min) and a broad peak (17-24 min) were only present in the mice receiving IMMP supplemented diets. The broad peak corresponded to the unseparated IMMP polymer fraction, whereas the isomaltose peak indicated ongoing microbial fermentation at a very low level. When comparing the digesta of cecum and large intestine between the two groups, a series of separated α-1-6-linked isomalto-oligosaccharide peaks were clearly seen in the IMMP mice, whereas hardly any carbohydrate peak was detected in the control mice’s digesta. Some remaining isomalto-oligosaccharides were still present in the fecal samples (result not shown). The release of the isomalto-oligosaccharides indicated the degradation of the polysaccharides during microbial fermentation of IMMP, and this fermentation took mainly place in the cecum and large intestine, although low level fermentation activity was also already detected in the small intestine.
Interplay between dietary fibers and gut microbiota for promoting metabolic health

Figure 1: High performance anion exchange chromatography (HPAEC) elution patterns of digesta from mice fed with (A) control and (B) IMMP supplemented diets. The digesta are taken from different parts of the gastrointestinal tract: (a) stomach, (b) small intestine, (c) cecum, (d) large intestine. IMMP peaks (2-11) in a box and maltodextrin peaks (①-⑥) are annotated, with the number indicating the degree of polymerization (DP).

Changes in GI microbiota composition

The total number of sequencing reads obtained for the 60 samples was 11440993 (min=1643, max=564991, median=173066.5, mean=190683.2, SD=130379.2). Samples were rarefied at 5000 reads per sample depth prior to alpha diversity analyses. Alpha diversity estimates included Shannon diversity, Chao1, and PD Whole Tree; however, no significant differences were detected in any of the measures between control and treatment groups (Supplementary figure 1).

The RDA analysis of fecal microbiota on day two showed that diet could explain 10.6% of the variation in the relative abundance of genus level taxa, however, the difference between the control and IMMP groups was not significant (FDR=0.252, data not shown). At day 21, contents from different parts of the murine GI tract were used for detailed microbiota analyses. In small intestine digesta, diet explained 12.1% of the microbiota variation, but the difference was not statistically significant (FDR=0.208, Fig. 2A). In cecum, however, diet explained 20.3% of the variation and this effect was significant (FDR=0.014, Fig. 2B). Finally, diet significantly explained 16.8% of the microbiota variation at genus level classification in large intestine samples (FDR=0.04, Fig. 2C).

In the small intestine 22 genus level taxa were detected in the IMMP group, and additional 19 taxa were found in the control group. The 19 taxa had low relative
abundance and together contributed to 1.7% the total bacteria detected in the small intestine of the control animals. ANOSIM analysis showed no significant difference between treatment groups when weighted or unweighted unifrac distances were used (p=0.47, and p=0.11, respectively). Kruskal–Wallis analysis showed no statistically significant differences in the relative abundance of genus level taxa between the two treatment groups (FDR adjusted p>0.05). When unadjusted p-values were used, a borderline significance was detected for Peptostreptococcaceae_Incertae_Sedis (p=0.049), which was 114 times more abundant in the IMMP group compared to the control (Fig. 2D). In addition, animals receiving IMMP diet had on average an 11 times lower relative abundance of genus Enterococcus, 19 times lower Akkermansia, 24 times lower Bacteroides, and 50 times less Turicibacter compared to the control group (Supplementary Table S1). Overall, these four taxa accounted for a cumulative relative abundance of 15.7% in the control group, and only 0.7% in the IMMP group. There were no differences in the average relative abundance of Bifidobacterium and Lactobacillus between the IMMP and the control groups.

In cecum, 37 genus level groups were detected with 4 groups found only in the IMMP fed animals and four other taxa detected only in the control group. ANOSIM analysis showed significant differences between treatment groups when weighted or unweighted unifrac distances were used (p=0.01, for both). Kruskal–Wallis analysis showed no statistically significant differences between relative abundances of individual genus level taxa between the two treatment groups (FDR>0.05, Fig. 2E), but based on the unadjusted p-values we could identify differences in the relative abundance of genera Alistipes, Prevotella, Roseburia, Pseudobutyribrio, Parabacteroides and Incertae Sedis in families Peptostreptococcaceae, Ruminococcaceae and Lachnospiraceae (each p<0.05). Compared to the control group, the IMMP group had a 5-fold higher average relative abundance for Roseburia, and a 26-fold lower relative abundance of Prevotella, 13-fold lower Akkermansia, 9-fold lower Alistipes and Parabacteroides. In addition, the IMMP treated animals had on average a 2-fold lower relative abundance of Bifidobacterium, and 3-fold higher relative abundance of Lactobacillus as compared to the control group, however, these differences were not statistically significant (Supplemental Table S1).

In the large intestinal samples, 40 genus level taxa were detected, of which four were only found in the control animals and eight were only found in the IMMP group. ANOSIM analysis showed no significant difference between treatment groups when weighted unifrac distances were used (p=0.1), however, the difference was significant when unweighted distances were compared (p=0.01).
Figure 2: Multivariate redundancy analysis of microbiota composition in different parts of the gastrointestinal tract of control and IMMP fed mice. The samples were collected from (A) small intestine, (B) cecum, (C) large intestine. Global composition at genus level in (D) small intestine, (E) cecum, (F) large intestine. When the taxonomic assignment could not be made at genus level, the lowest classifiable taxonomy assignment is used instead, and unidentified genus is indicated with “g_g”.
Kruskal–Wallis analysis showed no statistically significant differences between the two treatment groups (FDR>0.05, Fig. 2F), however unadjusted p values indicated differences in the relative abundance of *Odoribacter, Parabacteroides, Prevotella, Alistipes*, family Peptostreptococcaceae genus *Incertae Sedis*, and uncultured genus within the order Clostridiales. Compared to the control group, the IMMP animals showed a 11-fold lower relative abundance of *Parabacteroides* and *Turicibacter*, 8-fold lower *Akkermansia*, a 7-fold lower level of unidentified genus within the order Bacteroidiales and a 5-fold higher relative abundance of *Odoribacter*. The IMMP animals also had on average a two times lower relative abundance of *Bifidobacterium*, and a three times higher relative abundance of *Lactobacillus*, however, these differences were not statistically significant (p=0.87 for both taxa).

**Figure 3:** Short-chain fatty acid profiles in murine feces from day 0 to day 21. In fecal samples (A) lactic acid, (B) succinic acid, (C) acetic acid. (D) propionic acid and (E) butyric acid were measured. Solid line: control group; dashed line: IMMP group.
Interplay between dietary fibers and gut microbiota for promoting metabolic health

Short-chain fatty acid production in IMMP fed mice

SCFA are among the major products of bacterial fermentation. Our analyses of SCFA and other acids in feces showed that average succinic and lactic acid production increased in both groups at day two and three, and decreased thereafter, except on day 14 when an increase in lactic acid in feces from the IMMP group was noted (Fig. 3A & B). Propionic acid concentrations decreased throughout the duration of the study, while the levels of butyric and acetic acid remained stable (Fig. 3C, D & E). A significantly lower levels of propionic acid was seen in IMMP fed mice on day 21 (-65%, p<0.03).

![Fecal bile acids](image)

**Figure 4:** Bile acid composition in feces of control and IMMP-fed mice. At day 21 fecal samples were collected and processed for bile acid analysis as detailed in methods. Abbreviations: ω-MCA, ω-muricholic acid; β-MCA, β-muricholic acid; α-MCA, α-muricholic acid; CA, cholic acid; CDCA, chenodeoxy-cholic acid; DCA, deoxycholic acid; UDCA, ursodeoxy-cholic acid.

Metabolic and physiological responses to IMMP

To study the potential physiological effects of the IMMP-derived SCFA we investigated the impact of the different diets on lipid metabolism (Table 1). First, body weight and food intake in both groups remained stable throughout the dietary intervention period (Table 1). Plasma non-esterified fatty acids (NEFA) were higher (+8%, p<0.05) in IMMP fed mice compared with controls. Liver weight was unaffected and also liver cholesterol and triglyceride contents remained unchanged in either group. Also the biliary excretion of bile acids and cholesterol did not change upon IMMP feeding. At the end of the dietary intervention the IMMP supplemented group had a significantly higher fecal bulk mass (+35%, p<0.05) and lower levels of fecal dihydrocholesterol.
Dietary isomalto/malto-polysaccharides increase fecal bulk and microbial fermentation (-50%, p<0.05). Fecal total neutral sterol and bile acid excretion remained unchanged. Throughout the duration of the experiment the fecal water content did not vary between the treatment groups, except on days three and 14, when it was significantly (p<0.05) higher in the control group (d=3: control=14.0±2.6%; IMMP=10.5±3.3% and d=14: control=20.9±10.2%; IMMP=10.2±2.7%). In terms of bile acid species in feces, control and IMMP fed mice had a similar composition (Fig. 4).

**Table 1:** Animal characteristics, plasma, liver and fecal parameters of lipid metabolism in mice fed control or IMMP diets. Values are shown as means per group ± standard deviations.

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>IMMP Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (d=0)</td>
<td>19.48±1.38</td>
<td>20.28±0.66</td>
</tr>
<tr>
<td>Body weight at termination (d=21)</td>
<td>19.93±1.07</td>
<td>20.15±1.00</td>
</tr>
<tr>
<td>Food intake, g·day⁻¹</td>
<td>3.18±0.22</td>
<td>3.26±0.21</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mmol·L⁻¹</td>
<td>2.32±0.67</td>
<td>2.47±0.17</td>
</tr>
<tr>
<td>Triglycerides, mmol·L⁻¹</td>
<td>0.31±0.16</td>
<td>0.36±0.14</td>
</tr>
<tr>
<td>NEFA⁴, mmol·L⁻¹</td>
<td>1.01±0.05</td>
<td>1.10±0.03*</td>
</tr>
<tr>
<td>Total plasma bile acids, µmol·L⁻¹</td>
<td>3.05±1.25</td>
<td>5.49±3.23</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver weight (d=21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-absolute, g</td>
<td>0.80±0.11</td>
<td>0.87±0.07</td>
</tr>
<tr>
<td>-relative, % of body wt</td>
<td>4.02±0.47</td>
<td>4.32±0.44</td>
</tr>
<tr>
<td>Triglycerides, nmol·mg⁻¹ liver</td>
<td>21.31±4.24</td>
<td>20.26±7.23</td>
</tr>
<tr>
<td>Cholesterol, nmol·mg⁻¹ liver</td>
<td>10.24±3.31</td>
<td>9.30±1.30</td>
</tr>
<tr>
<td>Bile flow, µl·min⁻¹·100g body wt⁻¹</td>
<td>9.17±2.21</td>
<td>9.34±2.31</td>
</tr>
<tr>
<td>Biliary BA secretion, µmol·day⁻¹·100g body wt⁻¹</td>
<td>34.06±9.75</td>
<td>40.04±5.46</td>
</tr>
<tr>
<td><strong>Feces</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feces (dry), mg·day⁻¹·1 g body wt⁻¹</td>
<td>5.94±0.49</td>
<td>8.05±1.03*</td>
</tr>
<tr>
<td>Fecal Coprostanol, µmol·day⁻¹</td>
<td>0.95±0.46</td>
<td>0.76±0.58</td>
</tr>
<tr>
<td>Fecal Cholesterol, µmol·day⁻¹</td>
<td>1.55±0.13</td>
<td>1.65±0.34</td>
</tr>
<tr>
<td>Fecal DiH-Chol⁵, µmol·day⁻¹</td>
<td>0.23±0.03</td>
<td>0.18±0.03*</td>
</tr>
<tr>
<td>Total fecal neutral sterols, µmol·day⁻¹</td>
<td>2.72±1.69</td>
<td>2.60±0.45</td>
</tr>
<tr>
<td>Total fecal bile acids, µmol·day⁻¹</td>
<td>2.67±0.53</td>
<td>1.17±1.40</td>
</tr>
</tbody>
</table>

* P < 0.05; ⁴DiH-Chol – dihydrocholesterol; ⁵NEFA - nonesterified fatty acids
Discussion

This study is to the best of our knowledge the first to investigate in vivo properties and related physiological effects of IMMP supplementation in a murine model. Our data demonstrate that in mice fed IMMP-supplemented diets, the fermentation of IMMP occurred mostly in the cecum and large intestine and only at a very low level in the small intestine. This observation is in line with the changes observed in the microbiota composition in these regions of the GI tract. Beta diversity analysis with both, weighted and unweighted unifrac distances, as well as genus level relative abundance based RDA analysis (Fig. 2B) all showed that IMMP supplementation had a significant effect on microbiota composition in cecum and in large intestine, but not in ileum. In the large intestine, however, the significant effect was not detected when using weighted unifrac distances, suggesting that the significance was mostly due to changes in the composition of low abundance taxa.(19) In cecum, IMMP diet resulted in significantly higher levels of Lachnospiraceae Incertae Sedis (p<0.05), and, although not statistically significant, increases in a highly abundant related genus within the Lachnospiraceae family (unidentified) and in Bacteroides. The increase in Bacteroides was also observed in the large intestine and this finding was in line with an earlier in vitro study showing that IMMP fermentation can be linked with an increase in both, the relative abundance and activity of Bacteroides.(8) An increased abundance of Bacteroides is largely regarded as a beneficial effect, based on data demonstrating a strong association between decreased Bacteroides and obesity as well as metabolic disease.(20) In the cecum, IMMP was further associated with a significant increase in Roseburia, and in both, cecum and large intestine, we detected a higher relative abundance of Odoribacter. Lachnospiraceae and Roseburia are known to be saccharolytic groups associated with high fiber diets, while Odoribacter is largely asaccharolytic.(21,22) Lachnospiraceae, Roseburia and Odoribacter are important producers of butyrate. Butyrate is a relevant metabolite produced by the gut microbiota that has been implicated in improving metabolic control, as well as having inhibiting effects on cancer cell growth, largely via inhibition of histone deacetylases (HDAC). (23,24) Further, butyrate upregulates the expression of endogenous host defense peptides in the gut and increases energy expenditure by activating brown adipose tissue (25,26). Fecal analysis, though, did not reveal a significant increase in butyrate excretion in IMMP fed mice, a finding likely attributable to the highly efficient uptake of this SCFA into colonocytes.(27,28)

In stomach and small intestine we observed α-1-4-linked maltodextrins which were the main products of starch digestion by murine digestive enzymes. This was in line with previous in vitro studies in which small intestine extracts from rats were
Dietary isomalto/malto-polysaccharides increase fecal bulk and microbial fermentation incubated with IMMP (7). Given that IMMP represents a novel class of dietary fiber, it is essential to identify groups of bacteria that are promoted by dietary IMMP in vivo. Our study is the first to use the next generation sequencing of PCR-amplified 16S rRNA genes to systematically and comprehensively explore the relative abundance of various groups of bacteria in different parts of the intestine of IMMP-fed mice. Previous in vitro studies with human fecal inoculum incubated with IMMP have shown increases in *Bifidobacterium* and *Lactobacillus*.(7,8) These strains are considered probiotic microorganisms as they confer health benefits on the host via generation of key metabolites such as SCFA. However, in the current study it appeared that while the relative abundance of *Lactobacillus* increased upon IMMP feeding, bifidobacteria were relatively reduced in their relative abundance, although it should be noted that none of these changes in the relative abundances were statistically significant. Interpreting these findings one needs to take into account though that the murine gut ecosystem is different from that of the human GI tract. Therefore, further human studies seem warranted to corroborate these results.

Humans lack several digestive enzymes required for degrading dietary fibers. In rodents, even though some microbial communities are present in stomach and small intestine, measurements of microbial metabolites have revealed that large intestine and, most importantly, cecum are active fermentation sites.(29) Thus, most dietary fibers pass through the upper gastrointestinal tract and are fermented in cecum and large intestine. Fermentation results in generation of multiple groups of metabolites such as intermediate acids as lactic acid and succinic acid and final metabolites as SCFA. SCFA can regulate several pathways related to lipid and glucose metabolism. (30–32) The present study followed the production of specific SCFA over several time periods. Subtle differences between both groups were observed for most SCFA and their precursors, such as lactic and succinic acids. At the end of the dietary intervention a significantly lower propionic acid was detected in IMMP fed animals. Propionic acid has been shown to attenuate lipid biosynthesis in the liver.(33,34) However, the unchanged hepatic lipid content observed in our study argues against a physiological significance of this result. On the other hand, lactic acid which is normally produced endogenously in high concentrations upon exercise in host muscles but only in low concentration in the intestine, is an intermediate of bacterial fermentation and can be used by some bacteria, together with acetic acid, to synthesize butyric acid. In adipose tissue lactic acid has been shown to have a signaling function, being a natural ligand for GPR81 thereby inhibiting lipolysis.(35) At the end of the dietary intervention, higher lactic acid levels were detected in IMMP fed mice compared to the control group. However, it is also important to point out that an increase in luminal SCFA does not always reflect uptake of SCFA by the host and the subsequent induction of
metabolic effects. Thus, it is possible that the uptake of SCFA is different in both groups which needs to be further investigated, ideally using animal models with the capacity to resemble human metabolic disease states.

Cholesterol homeostasis is maintained by a balance between cholesterol intake, cholesterol synthesis in the host, absorption of cholesterol in the intestine, and the removal of cholesterol via the feces. Cholesterol is also a key biomarker of cardiovascular disease. Gut bacteria can reduce cholesterol to its derivatives, such as dihydrocholesterol and coprostanol, which are not absorbed by the host, but largely excreted into the feces. In the present study a small but significant decrease in fecal dihydrocholesterol output was noted after feeding the IMMP diet. However, since dihydrocholesterol represents only a minor fraction of total fecal neutral sterol output, overall fecal neutral sterol excretion upon IMMP administration remained unaltered. The intestinal microbiota also plays an important role in modulating bile acids and thereby subsequently cholesterol turnover. However, the present study shows that IMMP fed mice have no substantial alterations in the respective composition of plasma, biliary and fecal bile acids. Fecal bile acid profiles integrate endogenous synthesis and modifications by bacterial enzymes suggesting that based on our results no major effects either in the host or in microbial communities involved in bile acid metabolism are discernible.

A significant increase was seen in the total fecal output of IMMP fed mice, indicating improved fecal bulk in these animals. Bulk in the large intestine is associated with several beneficial effects such as stimulating defecation, diluting toxins and distributing intracolonic pressure, while lower fecal weight is associated with constipation and colorectal cancer. Since the total fecal neutral sterol and bile acid excretion remained comparable between the control and the IMMP fed mice groups, we believe that the changes in fecal bulk have no major impact on the (chole-) sterol balance. The overall weak metabolic response to IMMP supplementation might be explained by the fact that this dietary fiber is designed to function in the lower parts of the GI tract where it can be fermented by the microbiota. In mice cecum and large intestine are the main fermentation sites, while the proximal intestine is metabolically more active.

In summary, IMMP supplementation increased fecal bulk and microbial fermentation in the intestine resulting in potentially beneficial alterations in microbiota composition without adversely impacting host metabolism. Subsequently, studies in disease models and humans are needed to investigate whether the intriguing changes observed here translate into actual health benefits.
References
