Fecal Metabolomics of Healthy Breast-Fed versus Formula-Fed Infants before and during in Vitro Batch Culture Fermentation

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ABSTRACT: Nontargeted metabolomics analyses were used (1) to compare fecal metabolite profiles of healthy breast-fed (BF) and formula-fed (FF) infants before and during in vitro fermentation in batch culture and (2) to evaluate fecal metabolomics in infant diet. Samples from healthy BF (n = 4) or FF (n = 4) infants were individually incubated at 37 °C in anaerobic media containing 1% (wt/vol) galactooligosaccharides, 6'-sialyllactose, 2'-fucosyllactose, lacto-N-neotetraose, inulin, and gum arabic for up to 6 h, and supernatants were analyzed using GC/MS and LC/MS/MS to assess changes in various compounds. Comparison of over 250 metabolites prior to incubation showed that BF samples contained higher relative concentrations (P < 0.05) of 14 compounds including but not limited to human milk oligosaccharides and other metabolites presumably transferred through breast feeding (linoleaidate, myo-inositol) (P < 0.05). Conversely, feces from FF infants contained 41 identified metabolites at higher levels (P < 0.05). Our data are consistent with the notion that carbon-limited cultures catabolize protein and amino acids to obtain energy, whereas the provision of fermentable carbohydrate creates anabolic conditions relying on amino acids for bacterial growth. Results also suggest that fecal metabolomics can be a useful tool for studying interactions among diet, microbes, and host.

KEYWORDS: infant, feces, metabolomics, metabonomics, oligosaccharides, fermentation

INTRODUCTION

The human gut microbiota play a crucial role in shaping infant health and development. Abnormal patterns of gut microbiota have been clinically linked to late onset sepsis1 and necrotizing enterocolitis,2 two important causes of morbidity and mortality in preterm infants. A growing body of evidence also implicates the intestinal microbiota in the development of colic,3 allergic disease,4 and obesity later in life5 as well as the formation of a key metabolite involved in melanoma-induced kidney stone formation.6 Indeed, the gut microbiota modulate brain development and subsequent adult behavior in animal models.7

The composition of the infant gut microbiome is profoundly affected by diet. In general, studies using molecular techniques indicate that a majority of breast- and formula-fed infants harbor significant numbers of bifidobacteria.8 Peaces from exclusively breast-fed infants, however, are dominated by bifidobacteria, whereas those from formula-fed infants also contain other bacterial species, including E. coli, Clostridium difficile, bacteroides, and lactobacillus.8,9 Despite the abundance of studies regarding the effect of diet on infant fecal microbiota and health10 relatively little attention has been paid to the influence of diet on the infant fecal metabolome.11-15 The metabolomics approach, which can sometimes involve the analysis and identification of thousands of small metabolites, offers the potential to gain insight into the complex interactions among gut microbes, diet, and host. In turn, these data may be used to gain a clearer understanding of how diet affects both the gut microbiota and the host and to identify potential areas for future research. Overall, the main objectives of the current study were to use nontargeted metabolomics analysis of fecal samples to (1) compare metabolite profiles of healthy breast-fed to healthy formula-fed infants before and during in vitro fermentation in batch culture and (2) to evaluate the usefulness of metabolomics in assessing the infant diet.

MATERIALS AND METHODS

Donors

The infant fecal samples used were collected for use in a previous study. Briefly, eight infants were enrolled for participation in this study between March and June of 2010 from the Champaign-Urbana area, and infants consumed their normal diet of exclusive breast milk (n = 4) or exclusive infant formula (n = 4) for at least 2 months immediately prior to fecal collection. Formula-fed infants were fed one of three lactose-based formulas: Similac Advance (Abbott Laboratories Columbus, Ohio).
In Vitro Experiment

Table 1. Composition of Microbiological Medium Used in the in Vitro Experiment

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration in Medium (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>5.4</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>2.7</td>
</tr>
<tr>
<td>CaCl2·H2O</td>
<td>0.16</td>
</tr>
<tr>
<td>MgCl2·6H2O</td>
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</tr>
<tr>
<td>MnCl2·4H2O</td>
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</tr>
<tr>
<td>CoCl2·6H2O</td>
<td>0.06</td>
</tr>
<tr>
<td>NH4H2PO4</td>
<td>5.4</td>
</tr>
<tr>
<td>K2HPO4</td>
<td>2.7</td>
</tr>
<tr>
<td>MgCl2·H2O</td>
<td>0.12</td>
</tr>
<tr>
<td>FeCl2·4H2O</td>
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<tr>
<td>CuCl2·2H2O</td>
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</tr>
<tr>
<td>H2NCO3</td>
<td>20</td>
</tr>
<tr>
<td>ZnSO4·7H2O</td>
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<tr>
<td>MnCl2·4H2O</td>
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<tr>
<td>NiCl2·6H2O</td>
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<tr>
<td>Na2MoO4·2H2O</td>
<td>3</td>
</tr>
<tr>
<td>H3PO4</td>
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<td>COCl2-6H2O</td>
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<td>FeCl3·6H2O</td>
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<tr>
<td>H3PO4</td>
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<tr>
<td>CuCl2·2H2O</td>
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</tr>
<tr>
<td>NaCl</td>
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<tr>
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<td>Na2MoO4·2H2O</td>
<td>3</td>
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<tr>
<td>H3PO4</td>
<td>30</td>
</tr>
<tr>
<td>CuCl2·2H2O</td>
<td>1</td>
</tr>
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</table>

Optimized for positive ionization and one optimized for negative ionization. Peak extraction, data curation, and QC procedures were performed using proprietary software. At the time of analysis, samples were thawed and prepared according to the described standard method extraction protocol, which was designed to remove proteins, dislodge small molecules bound to protein or physically trapped in the precipitated protein matrix, and recover a wide range of chemically diverse metabolites, and split into aliquots for analysis on the three platforms.

Data Normalization

Data were collected over multiple platform run days and were adjusted by scaling to the median values for each group-balanced run-day block for each individual component. This minimizes any interday instrument gain or drift but does not interfere with intraday sample variability. Data were not otherwise adjusted or normalized.

Statistical Analysis

Repeated measures ANOVA was performed to leverage the data from the multiple time points within the study. There were 165 tests embedded within the repeated measures ANOVA: (1) DIA 'Main', (2) TIME Main, and (3) Interaction (DIA × TIME). Essentially, the "DIA" Main effect tested whether the means of the three groups were different from the BLANK when averaged across all time points. The "TIME" Main effect examines whether the means at each time point were different from the BLANK when averaged across the groups. Finally, the "Interaction" tests whether the time profiles are nonparallel between the groups (nonparallel profiles signify a difference during the time-course between the groups). The calculations also took into account the repeated measure inherent in sampling at each time point of the cultures from individual infants.

Fecal Collection

On the day of the in vitro experiments, fecal samples were collected in diapers within 15 min of defecation. Diapers were double-sealed in plastic bags and transported to the laboratory in a cooler containing tepid water. Fecal samples were diluted 1:10 (wt/vol) in anaerobic diluent, homogenized for 15 s in a Waring blender, filtered through four layers of cheesecloth, and sealed in serum bottles under CO2. Serum bottles containing the inocula were stored at 4 °C for approximately 12 h to enable hydration of the substrates before initiating fermentation. These tubes were placed in a 37 °C water bath approximately 30 min before inoculation. Due to the limited supply of substrates and unpredictability associated with obtaining fecal samples from infants, tubes containing GOS, 6'SL, 2'FL, and LNNT were hydrated upon obtaining fecal samples. Fecal samples from the individual infants were also incubated in media without added carbohydrate, and these cultures are referred to as "blank" tubes. Supernatant samples were collected at 0, 3, and 6 h of fermentation and were immediately frozen at -80 °C until further analysis.

Fecal Metabolomic Profiling

Frozen samples were shipped under dry ice to a commercial laboratory (Metabolon, Durham, NC) for metabolite analysis. Procedures for metabolic profiling have been described previously for the three platforms used in combination for the analysis, including GC/MS and two LC/MS systems, one optimized for positive ionization and one optimized for negative ionization. Peak extraction, data curation, and QC procedures were performed using proprietary software. At the time of analysis, samples were thawed and prepared according to the described standard method extraction protocol, which was designed to remove proteins, dislodge small molecules bound to protein or physically trapped in the precipitated protein matrix, and recover a wide range of chemically diverse metabolites, and split into aliquots for analysis on the three platforms.

Data Normalization

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Statistical Analysis

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Table 2. Metabolic Pathway Classification and Fold-Change Values for Compounds Significantly Higher in Blank BF Samples at Time = 0 h

<table>
<thead>
<tr>
<th>super-pathway</th>
<th>sub-pathway</th>
<th>biochemical name</th>
<th>BF/FF $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>amino acid</td>
<td>phenylalanine and tyrosine metabolism</td>
<td>3-(4-hydroxyphenyl)lactate</td>
<td>2.56</td>
</tr>
<tr>
<td>carbohydrate</td>
<td>fructose, mannose, galactose, starch, and sucrose metabolism</td>
<td>fructose</td>
<td>2.50</td>
</tr>
<tr>
<td>glycolysis, gluconeogenesis, pyruvate metabolism</td>
<td>2-fucosyllactose</td>
<td>&gt;30.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2-propanediol</td>
<td>lacto-N-fucopentaose</td>
<td>&gt;30.00</td>
</tr>
<tr>
<td>energy</td>
<td>oxidative phosphorylation</td>
<td>lactate</td>
<td>1.96</td>
</tr>
<tr>
<td>lipid</td>
<td>long-chain fatty acid</td>
<td>1,3-propanediol</td>
<td>25.00</td>
</tr>
<tr>
<td></td>
<td>bile acid metabolism</td>
<td>phosphate</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>glycerolipid metabolism</td>
<td>linoleate (tr 18:2:n6)</td>
<td>&gt;30.00</td>
</tr>
<tr>
<td></td>
<td>inositol metabolism</td>
<td>taurine</td>
<td>2.33</td>
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<tr>
<td></td>
<td>lysolipid</td>
<td>taurine choline sulfate $^b$</td>
<td>9.09</td>
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<tr>
<td>nucleotide</td>
<td>purine metabolism, guanine containing</td>
<td>uridine</td>
<td>3.45</td>
</tr>
<tr>
<td></td>
<td>pyrimidine metabolism, uracil containing</td>
<td>uridine</td>
<td>3.45</td>
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</table>

$^a$BF/FF = breast-fed to formula-fed. $^b$Indicates compound that has not been officially "plexed" (based on a standard), but we are confident in its identity.

**RESULTS**

Comparison of Initial Conditions

Figure 1 displays fold differences of identified metabolites that were present at higher levels (P ≤ 0.05) in either the BF or in the
Table 3. Metabolic Pathway Classification and Fold-Change Values for Compounds Significantly Higher in Blank FF Samples at Time = 0 h

<table>
<thead>
<tr>
<th>super-pathway</th>
<th>sub-pathway</th>
<th>biochemical name</th>
<th>FF/BBa</th>
</tr>
</thead>
<tbody>
<tr>
<td>amino acid</td>
<td>glutamate metabolism</td>
<td>N-acetylglutamate</td>
<td>2.08</td>
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<td>phenylalanine and tyrosine metabolism</td>
<td>phenylalanine</td>
<td>10.39</td>
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<td></td>
<td>tryptophan metabolism</td>
<td>tryptamine</td>
<td>3.85</td>
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<tr>
<td>carbohydrate</td>
<td>urea cycle, arginine and proline metabolism</td>
<td>5-aminovaleate</td>
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<tr>
<td>energy</td>
<td>pyruvate metabolism</td>
<td>3-aminobutyrate</td>
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<tr>
<td>lipid</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>short-chain fatty acid</td>
<td>2-hydroxypalmitate</td>
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<tr>
<td>medium-chain fatty acid</td>
<td>13-methylmyristic acid</td>
<td>2.76</td>
<td></td>
</tr>
<tr>
<td>fatty acid, branched</td>
<td>palmitoyl ethanoamidne</td>
<td>8.00</td>
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<tr>
<td>carotenoid</td>
<td>deoxyyarnitine</td>
<td>6.82</td>
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</tr>
<tr>
<td>bile acid metabolism</td>
<td>3-dehydroxycarnitine</td>
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<td>inositol</td>
<td>7-ketocholesterol</td>
<td>3.27</td>
<td></td>
</tr>
<tr>
<td>sterol/sterol</td>
<td>inositol 1-phosphate (1P)</td>
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<td></td>
</tr>
<tr>
<td>hemoglobin and porphyrin metabolism</td>
<td>betacarotene</td>
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<td>vitamin B6 metabolism</td>
<td>stigmastanol</td>
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<td>nicotinate and niacinamide metabolism</td>
<td>pregnen-30-diol diolate</td>
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<tr>
<td>tocopherol metabolism</td>
<td>urebilinogen</td>
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<tr>
<td></td>
<td>nicotinate ribonucleoside</td>
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<td>pantothenate</td>
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<td>alpha-tocopherol</td>
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<td>gamma-tocopherol</td>
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<tr>
<td></td>
<td>pyridoxate</td>
<td>4.19</td>
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</table>

*FF/BB = formula-fed to breast-fed. Indicates compound that has not been officially "pivoted" (based on a standard), but we are confident in its identity.

185 FF groups. These differences were calculated by comparing the levels of the various metabolites in the blank tubes at time = 0. Since the contents of the blank tubes were identical except for the fecal samples from the individual infants, any differences in the levels of the various metabolites between the groups presumably represent differences in the relative levels of fecal metabolites between the two diet groups. Samples from BF infants contained significantly greater concentrations of 14 compounds compared to FF infants. As expected, supernatants from BF infants had significantly lower levels of the human milk oligosaccharides (HMO), 2'-fucosyllactose and lacto-N-fucopentaose, and the HMO precursor, fucose, than those from FF infants. These two oligosaccharides were not detected in FF samples, but based on detection limits, these values must have been at least 25-fold higher in stool samples from BF infants (Table 2). Furthermore, time = 0 samples from BF infants were higher in a single amino acid super-pathway-related compound, [3-(4-hydroxyphenyl) lactate], a metabolite of tyrosine. Samples from the BF infants also were higher in five lipid super-pathway-related compounds (Table 2) and were particularly elevated in the C18:2, n-6 trans fatty acid, linolenate (>30-fold) and in the cyclic alcohol structural membrane component, myo-inositol (14.29-fold).

In contrast, supernatants from FF infants contained a total of 41 identified metabolites that were present at significantly higher concentrations compared to those from BF infants (Figure 1). More specifically, samples from FF infants contained seven 202 compounds related to the amino acid super-pathway, a total of 206 two compounds related to carbohydrate and/or energy super-pathways, 26 compounds related to the lipid super-pathway, 208 and seven compounds related to cofactor and/or vitamin 209
Addition of Exogenous Carbohydrates

A heat map displaying the compounds whose fermentation patterns were altered by the presence of exogenously added carbohydrate in one or both of the diet groups (BF or FF) is shown in Figure 5. Fermentation patterns for GOS, 2'-FL, and LNnT were highly similar to each other, with corresponding increases (red) and decreases (green) in various amino acids, SCFA, carbohydrate, and lipid-related metabolites over the 6 h fermentation. By comparison, the profiles for 6'SL and HP showed similarity to one another but differed substantially from those of GOS, 2'-FL, and LNnT. Lastly, the profile for GA was essentially identical to the blank (no carbohydrate added control) and did not resemble those of the other carbohydrate treatments. In Figure 3, it is evident that some metabolites responded differentially to oligos addition between the BF and FF groups (flagged as blue cells in the far right column), which included various metabolites related to amino acid metabolism, the urea cycle, pyruvate metabolism, and specific SCFA (e.g., valerate and isovalerate).

As an example of a differential response to oligos, Figure 4 displays in greater detail changes in the relative concentrations of the fatty acid, valerate, in FF versus BF samples during the course of fermentation. Valerate, a product of proline degradation, accumulated rapidly during the 6 h fermentation in the blank, 6'SL, HP, and GA treatments, but the increase was only observed in samples collected from FF infants. Relative levels of valerate changed very little for BF infants, regardless of carbohydrate substrate. Similar results were observed for isovalerate, a product of valine fermentation (data not shown).

The relative concentrations of selected biogenic amines during the course of fermentation are presented in Figure 5. With a few exceptions, the fold differences between FF and BF (BF/FF) infants changed very little upon addition of carbohydrate. However, the addition of either HP or GA resulted in significantly greater concentrations of putrescine after 6 h of fermentation from FF compared to BF infant inoculum (P < 0.05). Likewise, supplementation with LNaH led to higher relative concentrations of agmatine in FF compared to BF infants at 6 h (P < 0.05).

DISCUSSION

Fecal Metabolites: Breast-Fed versus Formula-Fed

A major objective of this study was to compare fecal metabolite profiles of FF and BF infants. To accomplish this objective, we identified compounds present in differing amounts in the time = 0 h blank FF and BF samples, because these compounds would presumably represent those residing in the fecal inocula. Inoculum from BF infants contained elevated levels of many amino acid metabolites such as phenyllactate from the degradation of phenylalanine, 4-hydroxyphenylacetate from the breakdown of tyrosine, and 5-aminovalerate from the metabolism of lysine. Samples from this group also contained higher levels of isovalerate and valerate, which are indicative of protein fermentation.

The predominance of protein fermentation, as previously noted by Heavy et al., may be due to a number of contributing factors. To compensate for differences in the amino acid composition between bovine and human milk, infant formulas have been formulated to contain greater concentrations of protein than human milk, so a greater quantity of protein could potentially escape digestion and absorption in the small intestine. Moreover, samples from FF infants presumably contain a more...
Figure 3. Compounds altered by the addition of exogenously added carbohydrate (relative to the blank) regardless of diet groups. Red cells indicate an increase in the compound, green cells indicate a decrease in the compound; light red cells indicate an initial increase at 3 h with a subsequent decrease at 6 h; blue cells indicate that the compound changed differentially depending on treatment group. (a) Compounds flagged as blue cells responded differentially between the breast-fed and formula-fed groups. (b) Indicates compound that has not been officially “plexed” (based on a standard), but we are confident in its identity.

Because bacteroides and clostridia possess proteolytic and/or amino-acid-fermenting capabilities, samples from FP infants 303 diverse fecal microbiome than those from BF infants with a greater proportion of bacteroides and clostridia than BF infants.
addition of fermentable carbohydrates, such as GOS, 2′-FL, and LNnT, to the cultures reduced the levels of various amino acids, decreased the accumulation of amino acid-related metabolites, and increased the levels of metabolites related to energy generation. These observations are consistent with the idea that cultures lacking a source of fermentable carbohydrate will 335 ferment amino acids to obtain energy, whereas the provision of carbon would obviate the need for metabolism of amino acids and 339 energy and create anabolic conditions that would require 338 utilization of amino acids for growth.

A more detailed examination of these data revealed that cultures from FF infants responded differently to carbon limitation than those of BF infants. Although both BF and FF samples generated amino acid super-pathway-related compounds in response to carbohydrate supplementation, only those from FF infants also fermented protein under carbon-limited conditions (e.g., 0 h blank or GA). Continuous degradation of amino nitrogen by samples from FF infants during carbon-limited conditions was likely due to the presence of bacteria that are able to exclusively utilize amino acids and/or peptides such as clostridia, Shigella, enterococci, bacteroides, Escherichia coli, or staphylococci.

Interestingly, fecal microbiota from all four FF infants quickly degraded 2′-FL in vitro within the first 3 h of incubation, but those from BF infants varied considerably in their ability to ferment this oligosaccharide (data not shown). The rapid disappearance of 2′-FL in cultures from FF infants is consistent with previous work showing that the introduction of foods other than breast milk to infants improves the ability of fecal bacteria to ferment complex carbohydrate including HMO.

More specifically, the rapid 2′-FL degradation of 2′-FL might possibly be attributed to the presence of a significant population of bacteria in FF infants, some strains of which have been documented to readily utilize HMO as a sole source of carbohydrate. In contrast, the variation in the ability of microbiota from BF infants to catabolize 2′-FL might have been caused by either the presence or absence of bifidobacteria species that are capable of utilizing HMO or differences in the levels of bifidobacteria species that are capable of utilizing HMO in the gut. Although we did not determine whether the mothers of the infants who participated in the study were secretors or nonsecretors, it is also possible that variability in the fecundated oligosaccharide content of breast milk could influence the ability of the infant colonic microbiota to degrade this particular HMO. Additional experiments are needed to verify the difference in the ability of fecal microbiota from BF and FF to degrade various HMO and to determine whether

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>BF Blank</th>
<th>GOS</th>
<th>2′-FL</th>
<th>LNnT</th>
<th>HP</th>
<th>GA</th>
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<tr>
<td>[gamma-amino butyrate (GABA)]</td>
<td>0.77</td>
<td>0.57</td>
<td>0.50</td>
<td>0.38</td>
<td>0.50</td>
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<td>2.73</td>
<td>0.94</td>
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<td>0.47</td>
<td>0.22</td>
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<td>0.82</td>
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<td>0.89</td>
</tr>
<tr>
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<tr>
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<td>0.68</td>
<td>1.32</td>
<td>1.35</td>
<td>1.92</td>
</tr>
</tbody>
</table>

Shaded cells indicate $P \leq 0.05$ (red indicates that the mean values are significantly higher for that comparison; green values significantly lower). Blue-bolded text indicates $0.05 < P \leq 0.10$. Noncolored text and cells indicate that mean values are not significantly different for that comparison.
secretor status influences the ability of the microbiota to degrade fucosylated oligosaccharides.

Biogenic Amines

Emerging research has linked the intestinal microbiome to both brain development and behavior via the brain-gut-enteric microbiota axis. This relationship is mediated in part through signaling molecules generated by the gut bacteria, such as the biogenic amine/neurotransmitter gamma-aminobutyric acid.

On the basis of previous work showing elevated levels of the biogenic amine, tyramine, in feces from healthy infants fed either cow's milk or cow milk formula compared to those who were breast fed, we originally hypothesized that (a) stool samples from the BF infants would contain higher relative levels of the various amines than those from FF infants and (b) the addition of fermentable carbohydrate would suppress accumulation of amines. However, our limited data suggest that this was not necessarily the case, at least with the biogenic amines that were identified in the current study. To illustrate, at time = 0 h, out of seven biogenic amines identified, only a single amine, tryptamine, was higher in FF than in BF infants (Table 3). Furthermore, provision of fermentable carbohydrate actually resulted in increases rather than decreases in several biogenic amines (e.g., GABA, cadaverine, and agmatine) regardless of diet (Figure 3).

Lastly, the excess production of amines by samples from FF infants depended on the specific amine analyzed and on the type of carbohydrate that was added to the cultures (Figure 5).

Although we did not anticipate that supplementation with fermentable carbohydrate would increase generation of amines, our data are consistent with the observation that the ability to produce amines is widespread among human intestinal bacteria.

Other Compounds of Dietary Origin

Direct comparison of the time = 0 h blank samples also showed that BF and FF infants contained elevated levels of several metabolites that presumably originated from breast milk and infant formula, respectively. To illustrate, samples from FF infants contained at least 8-fold more α- and γ-tocopherols than those of BF infants. Because tocopherols are considered an essential nutrient and cannot be synthesized endogenously, the presence of elevated levels of tocopherols in the stool samples of FF suggest two possible scenarios: (a) tocopherols are being provided in the levels at excess of infant need or (b) tocopherols provided in infant formula are not completely absorbed by the small intestine. If (a) were true, one might consider lowering the amount of tocopherols that are incorporated into infant formula. On the other hand, if (b) were true, further research would be needed to identify methods for improving tocopherol absorption by infants.

CONCLUSIONS

In this study, we used metabolomics analyses to compare the fermentation profiles generated by fecal inocula from BF and FF infants. Comparison of the samples at time = 0 h revealed significant differences in the carbon limitation and predominant protein fermentation in samples from FF infants versus the presence of HMO and less carbon restriction in the BF group. Furthermore, the comparison revealed differences in relative levels of some compounds that were most likely acquired through either breast milk (e.g., lacto-Neotetraose) or infant formula (e.g., tocopherols, soy-based compounds). Supplementation with fermentable carbohydrates led to the accumulation of compounds indicative of energy generation. Cultures from BF infants that were not supplemented with carbohydrate (blank) did not accumulate compounds indicative of protein fermentation, but those from FF infants continued to accumulate compounds involved in protein catabolism. Although our results suggest that supplementation with fermentable carbohydrates had little effect on the accumulation of biogenic amines, further investigations are needed to first determine the biological significance of amino acid catabolites, including biogenic amines, on infant gut health and, more broadly, on overall infant health. In light of these findings, fecal metabolomics appears to be a useful tool for assessing the quality of infant diets regardless of whether the source of nourishment comes from breast feeding or formula feeding.