Diet-Induced Metabolic Improvements in a Hamster Model of Hypercholesterolemia Are Strongly Linked to Alterations of the Gut Microbiota

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The mammalian gastrointestinal microbiota exerts a strong influence on host lipid and cholesterol metabolism. In this study, we have characterized the interplay among diet, gut microbial ecology, and cholesterol metabolism in a hamster model of hypercholesterolemia. Previous work in this model had shown that grain sorghum lipid extract (GSL) included in the diet significantly improved the high-density lipoprotein (HDL)/non-HDL cholesterol equilibrium (T. P. Carr, C. L. Weller, V. L. Schlegel, S. L. Cuppett, D. M. Guderian, Jr., and K. R. Johnson, J. Nutr. 135:2236-2240, 2005). Molecular analysis of the hamsters' fecal bacterial populations by pyrosequencing of 16S rRNA tags, PCR-denaturing gradient gel electrophoresis, and Bifidobacterium-specific quantitative real-time PCR revealed that the improvements in cholesterol homeostasis induced through feeding the hamsters GSL were strongly associated with alterations of the gut microbiota. Bifidobacteria, which significantly increased in abundance in hamsters fed GSL, showed a strong positive association with HDL plasma cholesterol levels ($r = 0.75; P = 0.001$). The proportion of members of the family *Coriobacteriaceae* decreased when the hamsters were fed GSL and showed a high positive association with non-HDL plasma cholesterol levels ($r = 0.84; P = 0.0002$). These correlations were more significant than those between daily GSL intake and animal metabolic markers, implying that the dietary effects on host cholesterol metabolism are conferred, at least in part, through an effect on the gut microbiota. This study provides evidence that modulation of the gut microbiota-host metabolic interrelationship by dietary intervention has the potential to improve mammalian cholesterol homeostasis, which has relevance for cardiovascular health.
through Fiaf (fasting-induced adipocyte factor), which is selectively suppressed in the intestinal epithelium by the gut microbiota (1, 2). Fiaf is an important regulator of lipid metabolism (e.g., through its inhibition of lipoprotein lipase) and has been shown to increase total cholesterol and high-density lipoprotein (HDL) cholesterol levels when overexpressed in transgenic mice (26).

There are several reasons why hamsters are an excellent model for studying the metabolic relationships among diet, cholesterol metabolism, and gut microbiota in relation to health. First, hamsters are omnivorous, and their blood lipid profiles respond to diets in a predictive manner similar to humans (5). Second, unlike mice and rats which lack cholesterol ester transfer protein, hamsters exhibit all of the enzymatic pathways in lipoprotein and bile metabolism that are also present in humans. They exhibit limited hepatic synthesis of cholesterol and bile acids, resulting in more relevant data when extrapolating to humans (23). Third, hamsters develop atherosclerosis in a predictive manner in response to dietary manipulation (31).

Using the Golden Syrian hamster model, Carr and coworkers have shown that the hexane-extractable lipid fraction of grain sorghum whole kernels (GSL), when included in the hamsters’ diet, leads to a significant reduction of plasma non-HDL and liver cholesterol levels while increasing HDL cholesterol levels (8). We extended this research and performed a comprehensive molecular characterization of the fecal microbiota of the hamsters by pyrosequencing of 16S rRNA tags, denaturing gradient gel electrophoresis (DGGE), and Bifidobacterium specific quantitative real-time PCR (qRT-PCR) in order to test whether metabolic effects of GSL were associated with specific modifications of the gut microbiota.

MATERIALS AND METHODS

Animal experiments. The fecal samples analyzed here were obtained during a previous study that determined the effect of GSL included in the diet on the cholesterol metabolism of hamsters, and the handling of animals, feed composition, GSL composition, sample collection, and preparation have been described previously (8). Briefly, groups of seven or eight male F3B Syrian hamsters (Biot Feeders, Watertown, MA) were housed in cages (each hamster in an individual cage) and kept at 25°C with a 12-h light–12-h dark cycle. Hamsters were fed a bar-coded universal primers 8F and 518R containing the A and B sequencing bar code. Sequences (1,000 to 2,000 per animal) were quality controlled and binned according to bar codes. Taxonomy-based analyses were performed by assigning taxonomic status to each sequence using the CLASSIFIER program of the Ribosomal Database Project (47). To estimate species richness and diversity, taxonomy-independent methods were used. Sequences were aligned using Infernal Aligner; sequences from different individual animals and a control sample sequence were clustered based on their alignment. A complete linkage clustering algorithm available through the Pyrosequencing pipeline of the Ribosomal Database Project (9). Clustering was done with a 97% cutoff for inclusion into an operational taxonomic unit (OTU) and was performed on alignments of sequences from individual animals. The number of species and species richness were estimated by further sampling-based (rarefaction) analyses of OTUs using the Mothur software (48). Rarefaction and Shannon diversity indexes were calculated (49). The fecal fingerprints from two or three animals were excised from the gel, purified, and amplified with the QIAprep spin miniprep kit (Qiagen), and inserts were sequenced by a commercial provider following the manual of the cloning kit. Closest relatives of the partial 16S RNA sequences were determined using the nucleotide blast web tool at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Seqmatch web tool provided through the Ribosomal Database Project (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp). A phylogenetic tree was generated from the consensus sequence of the F bands in three individual animals using the unweighted-pair group method using average linkages and neighbor-joining algorithms in the MEGA4 software package (42). There were a total of 175 peaks in the final DGGE gel. The condensation distances were computed by using the Kimura two-parameter method and are reported as the number of base substitutions per site.

Specific quantification of bifidobacteria by qRT-PCR. Quantification of total bifidobacteria was performed by quantitative real-time PCR using primers Bif-
For (5′-TGGCGTGYGGTGYAGAAG-3′- and BifRev (5′-CCACATCCAGCR TCCAC-3′) (39). PCRs were performed using a Mastercycler Realplex2 (Eppendorf AG, Hamburg, Germany). Each PCR was done in a 25 μL volume. The reaction mixture consisted 11.25 μL of the 20X SYBR solution and 2.5X RealMasterMix (Eppendorf AG, Hamburg, Germany), 0.5 μL of each primer, and 1 μL of DNA template. The amplification program consisted of an initial denaturation step of 5 min at 95°C, followed by 35 cycles, where 1 cycle consisted of 15 s at 95°C (denaturation), 20 s at 58°C (annealing), and 30 s at 68°C (extension), and fluorescence at each step was measured. To control the specificity of the amplifications, a melting curve was done consisting of a denaturation step of 15 s at 95°C, an increase from 58°C to 95°C over a 20-min period, and a final step of 15 s at 95°C. Cultures of B. animalis ATCC 25527T and B. infantis ATCC 15697T were used to generate standard curves for absolute quantification of bifidobacteria in the fecal samples. Bacterial counts of overnight cultures (12 h) were determined by plate counting, and a 10-fold dilution series was performed in phosphate-buffered saline buffer for each strain. DNA was isolated from individual samples of the dilution series using the method for fecal samples. Standard curves were made by plotting the threshold cycle values obtained from DNA of the dilution series as a linear function of the base 10 logarithm of the number of bifidobacteria. Two individual qRT-PCR runs of all fecal DNA templates in duplicate were performed, and means of all four values were used for the analysis. Despite the use of two different strains of bifidobacteria to generate one standard curve, its correlation coefficient r² was >0.96.

To quantify the Bifidobacterium animalis-like phylotype detected by DGGE, we used a specific primer (Bh1) based on a highly variable region of the sequence of fragment F in the DGGE gel (5′-GGCAAGGGGGTTTTCCTG-3′). This primer was used in combination with primer BifRev (39) used for the Bifidobacterium genus-specific qRT-PCR. BifRev was also used as a universal primer (described above). The efficiency of the PCR was tested using DNA isolated from fecal samples from 10 human subjects and DNA from B. animalis ATCC 25527T and Bifidobacterium infantis ATCC 15697T. The PCRs all gave negative results with the primer combination Bif1 and BifRev and positive results with primers BifFor and BifRev (data not shown). As we had no cultural representative of the phylotype represented by band F, we used a standard curve generated as described above with B. animalis ATCC 25527T and B. infantis ATCC 15697T and primers BifFor and BifRev. Although the standard curve was generated with a different forward primer, it can be assumed that no significant bias is introduced, as the efficiencies of the two PCR systems were virtually identical (0.51 for primers BifFor and BifRev and 0.56 for primers Bif1 and BifRev).

Correlation analysis of gut microbiota-host metabolic functional relationships. Correlation analysis between metabolic host parameters and bacterial populations at different taxonomic levels was performed by the method of Cani and coworkers (7). Metabolic parameters included in the correlation analysis were the levels of cholesterol absorption, fecal cholesterol, plasma total cholesterol, plasma HDL cholesterol, plasma non-HDL cholesterol, liver total cholesterol, liver-free cholesterol, liver triglycerides, liver phospholipids, and liver-esterified cholesterol. The determination of these metabolic parameters and the methods employed were reported previously (16).

Genome comparisons. The web-based Integrated Genomes Platform of the Joint Genome Institute (JGI) was used to identify functions enriched in bifidobacteria (27). The Abundance Profile Search was used to identify clusters of related sequences (7), and genome comparisons. The remaining bacteria belonged to the phyla Actinobacteria (94%), comprising 31%, 28%, 19%, 11%, and 5% of the total sequence tags) and Proteobacteria and candidate division TM7 (0.07% and 0.024% of sequences, respectively).

At the family level, the predominant groups in hamsters of the control group were the Erysipelotrichaceae, Eubacteriaceae, Ruminococcaceae, and Lactobacillaceae, represented by an average of 59%, 19%, 15% and 5% of the total fecal microbiota, respectively (Fig. 1A). Of the bacterial groups on the genus level, the most dominant were unclassified Erysipelotrichaceae, Allobaculum, unclassified Eubacteriaceae, Ruminococcus, and Lactobacillus, comprising 31%, 28%, 19%, 11%, and 5% of the total sequence pool on average in control animals, respectively (Fig. 1B). With the exception of Allobaculum, these genera are also shared with the gut microbiota reported for mice, humans, and primates (15, 24, 29, 44). As shown in Fig. 1S in the supplemental material, pyrosequencing revealed high animal-to-animal variability on both the family and genus levels.

Effects of GSL on specific taxa of the hamster gut microbiota. Sequences determined by pyrosequencing were used to establish the effects of the GSL on the gut microbiota composition. To identify specific taxa that were affected by the feeding treatments, the proportions of taxa in each rank of each animal were tested for treatment effects. As shown in Table 1, ANOVA identified one family, the Coriobacteriaceae (P = 0.042), and two bacterial groups at the genus level, unclassified members of the family Erysipelotrichaceae (P = 0.0016) and genus Pseudorabibacter (P = 0.017), as being significantly affected by the inclusion of GSL to the hamsters’ diet. Moreover, values for the genus Allobaculum (P = 0.096)
and unclassified members of the family Coriobacteriaceae ($P = 0.064$) approached statistical significance.

Taxonomy-independent analysis of the hamster gut microbiota from individual animals showed that with a conservative level of 97% identity as a cutoff for OTUs, nearly 200 OTUs were observed in the average of 1,600 sequences from each animal (Fig. 1C). Individual animals in the 5% GSL feeding group showed a trend toward fewer OTUs in the rarefaction curves. The Shannon diversity indices of the gut microbiota of individual hamsters fed 0% GSL (red), 1% GSL (green), and 5% GSL (blue) showed a trend of fewer OTUs in animals fed 5% GSL (blue lines) compared to animals fed 0% GSL (Fig. 1D). Grouping of the samples by GSL showed significant differences between 0% and 5% GSL ($P < 0.0001$, Mann-Whitney test). Thus, 5% GSL had the effect of reducing the diversity of the gut microbiota.

**DGGE analysis of fecal microbiota of hamsters fed GSL.** To validate the findings obtained with pyrosequencing, fecal bacterial populations of the hamsters were also analyzed by PCR-DGGE. The DGGE gel is shown in Fig. 2A, and the results of analysis of the gel are presented in Table 2A. Feeding the ham-
TABLE 1. Abundance of bacterial groups in the fecal microbiota of hamsters that changed by including GSL in the diet as determined by pyrosequencing of 16S rRNA tags

<table>
<thead>
<tr>
<th>Bacterial taxa</th>
<th>Abundance of bacterial group (%) of total sequences obtained with sample [mean ± SD] in hamsters fed:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% GSL</td>
</tr>
<tr>
<td>Family level</td>
<td></td>
</tr>
<tr>
<td>Coriobacteriaceae</td>
<td>1.22 ± 0.79</td>
</tr>
<tr>
<td>Genus level</td>
<td></td>
</tr>
<tr>
<td>Allobaculum</td>
<td>27.82 ± 15.9</td>
</tr>
<tr>
<td>Pseudoramibacter</td>
<td>0.11 ± 0.09</td>
</tr>
<tr>
<td>Unclassified members of the following families</td>
<td></td>
</tr>
<tr>
<td>Coriobacteriaceae</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>Erysipelotrichaceae</td>
<td>31.0 ± 7.4</td>
</tr>
</tbody>
</table>

* There were seven hamsters in each group.

b Values that were significantly different or approaching statistical significance are shown in boldface type.

c Statistically significantly different from the value for hamsters fed 0% GSL (P < 0.05) by ANOVA.

d This value was statistically significantly different from the value for hamsters fed 0% GSL (P < 0.01) and from the value for hamsters fed 1% GSL (P < 0.01) by ANOVA.

e Approaching statistical significance compared to the value for hamsters fed 0% GSL (P < 0.1) by ANOVA.

The analysis of 16S rRNA genes to study the human gut microbiota and using the primer 8F, which is one of the most commonly used primers for such approaches, resulted in a significant underrepresentation of Bifidobacterium species (15, 35, 41, 50).

Quantification of bifidobacteria using qRT-PCR. Since primer 8F resulted in an underrepresentation of bifidobacteria in pyrosequencing and to confirm and quantify the bifidogenic effect of the GSL detected by DGGE analysis, a specific qRT-PCR procedure was used to determine the numbers of total bifidobacteria and the Bifidobacterium animalis-like phylotype. As shown in Fig. 2C and D, qRT-PCR analysis showed a significant increase in cell numbers of total bifidobacteria (P = 0.012) and the Bifidobacterium animalis-like phylotype (P = 0.019). As shown in Fig. 2E, the numbers of bifidobacteria from individual hamsters were highly variable, but a significant correlation between cell numbers and daily GSL intake was observed.

Bifidobacteria and Coriobacteriaceae showed high correlations with important markers of host cholesterol metabolism. In a previous study using the animals studied here, dietary GSL reduced cholesterol absorption, plasma non-HDL cholesterol concentrations, and liver esterified cholesterol levels, while raising plasma HDL cholesterol levels (8). To determine whether alterations of the gut microbiota in hamsters fed GSL were associated with an improvement in cholesterol metabolism, a correlation analysis was used to determine correlations between all bacterial taxa at different taxonomic levels and host metabolic phenotypes. The analysis revealed highly positive correlations between HDL plasma concentrations and total bifidobacteria (r = 0.75; P = 0.0011), between HDL plasma concentrations and Bifidobacterium animalis-like phylotype (r = 0.77; P = 0.0009), among total Coriobacteriaceae and non-HDL plasma concentrations (r = 0.84; P = 0.0002), and between unclassified Coriobacteriaceae and both non-HDL plasma concentration (r = 0.82; P = 0.0004) and cholesterol absorption (r = 0.71; P = 0.0042). These high correlations were observed only in animals fed 1% and 5% GSL, and inclusion of the values from control animals significantly reduced correlations (Table S3). Graphs showing the highest correlations between bacterial taxa and metabolic phenotypes are shown in Fig. 3, and a metabolic network diagram linking GSL, bacterial phylotypes, and host cholesterol metabolism is shown in Fig. 4. Interestingly, the correlations between bifidobacteria and HDL cholesterol concentration and between Coriobacteriaceae and non-HDL concentration showed higher significance than correlations achieved between GSL intake and the respective host metabolic phenotypes.

Genome comparisons of bifidobacteria and other gut organisms. By comparing the relative abundance of different functional categories in 47 genomes of gut bacteria, we observed that proteins belonging to the COG clusters COG0400 (predicted esterase), COG0657 (esterase/lipase), and COG2272 (carboxylesterase type B) are enriched in six Bifidobacterium genomes (see Table S1 in the supplemental material). Carboxylesterases represented by COG0400 and COG2272 belong to enzymes that hydrolyze a wide variety of substrates, ranging from methyloclyrate to p-nitrobenzyl (21, 49).
DISCUSSION

In humans, CHD is associated with high levels of low-density lipoprotein and low levels of high-density lipoprotein. The characterization of the gut microbiota in a hamster model of hypercholesterolemia showed that dietary intervention with GSL had a major impact on the composition of the gut microbiota and that these modulations were highly associated with improvements in the HDL and non-HDL cholesterol equilibrium. With consumption of GSL, population levels of bifidobacteria increased and showed a strong positive association with increases in HDL cholesterol levels. In contrast, relative abundance of members of the family Coriobacteriaceae decreased with feeding the hamsters GSL, and a high positive correlation with non-HDL cholesterol and cholesterol absorption was discovered. The findings indicate that GSL intake influences the HDL/non-HDL equilibrium, at least in part, through an alteration of the gut microbiota. We infer this because correlation coefficients between bifidobacterial and Coriobacteriaceae populations and plasma cholesterol concentrations were higher than associations among GSL intake, host phenotypes, and cholesterol absorption (Fig. 4). In addition, if bacterial phylotype/host phenotype correlations were merely a result of GSL affecting both bacterial taxa and cholesterol metabolism independently, one would assume that all bacterial taxa whose abundance correlated with GSL intake would show an association with host phenotypes. However, much lower correlation coefficients with non-HDL and HDL plasma concentrations were observed between relative abundance of unclassified members of the family Erysipelotrichaceae and the genus Allobaculum, although these taxa showed significant as-

FIG. 2. Impact of GSL on the gut microbiota composition of hamsters fed 0% GSL (n = 7), 1% GSL (n = 7), and 5% GSL (n = 8) as determined by DGGE and qRT-PCR. (A) DGGE showing fingerprints of DNA isolated from the fecal samples of hamsters. Lanes 1 to 32 contain DNA from individual hamsters. Lane M contains markers from reference strains. Bands C and F showed significant increases in staining intensity in fecal fingerprints of hamsters fed 5% GSL. The bands A, C, and F marked by an arrow were excised, purified, and sequenced (Table 2). (B) Phylogenetic tree of DGGE band F with sequences that revealed highest similarities in GenBank. The tree was inferred using the unweighted-pair group method using average linkages, and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. A neighbor-joining tree resulted in essentially the same phylogeny (data not shown). (C) Cell numbers of total bifidobacteria in hamster fecal samples as determined by qRT-PCR. (D) Quantification of the Bifidobacterium animalis-like phenotype detected by DGGE in hamster fecal samples by qRT-PCR. (E) Correlation of cell numbers of bifidobacteria with daily GSL intake.
associations with GSL intake (Table 3). However, it should be considered that bifidobacteria and Coriobacteriaceae are just two of hundreds of groups, and other bacteria, independent of GSL administration, are likely to interact with host cholesterol metabolism.

Changes in the composition of the hamsters’ gut microbiota induced by GSL consumption were limited to a relatively small number of bacterial groups (Table 1 and 2). These compositional adjustments had the net effect of reducing the overall richness of individual species per unit population. However, the overall composition of the microbiota at the phylum level was not affected by GSL. The reason for this finding was that increases of dominant bacterial taxa were “balanced” by a reduction of related bacterial taxa, leaving the relative proportions of higher taxonomic taxa unaffected. Allobaculum belongs to the family Erysipelotrichaceae, and uncharacterized bacteria of this family declined as Coriobacteriaceae increased with feeding the hamsters GSL. Thus, the overall proportions within the family Erysipelotrichaceae and the phylum Firmicutes were not changed. Similar findings were obtained for the phylum Actinobacteria, where numbers of bifidobacteria increased while the abundance of members of the family Coriobacteriaceae declined. As shown in Fig. S3 in the supplemental material, significant inverse correlations were obtained between these related bacterial groups in individual animals. Similar diet-induced compositional adjustments of the gut microbiota that maintain the overall composition at higher taxonomic levels have also been observed in humans. For example, the decline of bacteria belonging to the Roseburia and Eubacterium rectale groups induced through reduced carbohydrate intake was balanced by an increase in the number of related members of the Clostridium coccoidei cluster in human fecal samples (14). Furthermore, the diet of human infants appears to influence the Bifidobacterium/Coriobacteriaceae ratio, with higher numbers of bifidobacteria in breast-fed infants while there were higher numbers of coriobacteria when the infants were fed formula (19). Collectively, these findings indicate that homeostatic reactions that restore the overall equi-

Table 2. Ratio of staining intensities of major bands as a proportion of total fingerprint intensity and results of sequence analysis of selected bands

<table>
<thead>
<tr>
<th>Band</th>
<th>Mean band intensitya (SD) in DNA from hamsters fed:</th>
<th>Closest GenBank hitd</th>
<th>Closest type straine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% GSL</td>
<td>1% GSL</td>
<td>5% GSL</td>
</tr>
<tr>
<td>A</td>
<td>20.0 (17.5)</td>
<td>**0.06 (0.16)**a</td>
<td>14.2 (14.3)</td>
</tr>
<tr>
<td>B</td>
<td>5.6 (4.5)</td>
<td>7.8 (8.1)</td>
<td>6.2 (6.9)</td>
</tr>
<tr>
<td>C</td>
<td>9.2 (7.9)</td>
<td>7.9 (4.3)</td>
<td>**17.4 (8.2)**a</td>
</tr>
<tr>
<td>D</td>
<td>11.4 (11.0)</td>
<td>13.1 (7.1)</td>
<td>10.3 (7.6)</td>
</tr>
<tr>
<td>E</td>
<td>3.4 (3.2)</td>
<td>7.5 (10.3)</td>
<td>4.3 (5.1)</td>
</tr>
<tr>
<td>F</td>
<td>5.7 (1.0)</td>
<td>6.2 (3.4)</td>
<td>**11.1 (3.7)**a,e</td>
</tr>
<tr>
<td>G</td>
<td>4.0 (1.6)</td>
<td>3.2 (2.2)</td>
<td>3.2 (1.3)</td>
</tr>
<tr>
<td>H</td>
<td>3.0 (2.2)</td>
<td>8.0 (7.6)</td>
<td>1.7 (2.0)</td>
</tr>
</tbody>
</table>

**a** Ratio of staining intensities of major bands as a proportion of total fingerprint intensity (shown as a percentage). Values that were significantly different are shown in boldface type.

**b** Statistically significantly different from the value for hamsters fed 0% GSL (P < 0.05) by ANOVA.

**c** Statistically significantly different from the value for hamsters fed 1% GSL (P < 0.05) by ANOVA.

**d** The GenBank accession number and species or clone is shown. The values in parentheses are the percentages of similarity.

**e** The closest type strain is shown first. The GenBank accession number is shown in brackets. The values in parentheses are the percentages of similarity.

**f** ND, not determined.

Table 3. Correlations between abundance of bacterial taxa and markers of cholesterol metabolisma

<table>
<thead>
<tr>
<th>Bacterial taxa</th>
<th>Correlationb (r value) between abundance of bacterial taxa and the following marker of cholesterol metabolism:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSL intake</td>
</tr>
<tr>
<td>Family level Coriobacteriaceae</td>
<td>−0.54 (−0.50)</td>
</tr>
<tr>
<td>Genus level Bifidobacterium</td>
<td>0.56 (0.61)</td>
</tr>
<tr>
<td>Allobaculum</td>
<td>0.50 (0.48)</td>
</tr>
<tr>
<td>Pseudoramibacter</td>
<td>−0.24 (0.23)</td>
</tr>
<tr>
<td>Unclassified members of the following families Coriobacteriaceae</td>
<td>−0.48 (−0.52)</td>
</tr>
<tr>
<td>Erysipelotrichaceae</td>
<td><strong>−0.74 (−0.70)</strong></td>
</tr>
</tbody>
</table>

**a** Values for animals fed 1% and 5% GSL are presented.

**b** Values for all animals, including control animals, are presented in parentheses. Correlation coefficients with an r of >0.7 are shown in boldface type.
Equilibrium of the gut microbiota are often a natural consequence of compositional changes induced through diet. Nevertheless, as indicated by the correlation analysis in our study, an alteration of the gut microbiota at lower taxonomic levels is still likely to have important functional consequences for the host.

The mechanisms by which bifidobacteria and coriobacteria affect cholesterol metabolism remain an important field of future research. Including GSL in the diet reduced cholesterol absorption efficiency, which was directly correlated with non-HDL cholesterol concentration (8). The high correlations of unclassified members of the family Coriobacteriaceae with both non-HDL cholesterol and cholesterol absorption suggest that these bacteria could have a negative impact on cholesterol homeostasis through increasing cholesterol absorption. Bifidobacteria, on the other hand, showed high positive correlation with HDL cholesterol levels and no association with cho-

**FIG. 3.** Specific bacterial populations in the guts of hamsters show high associations with both cholesterol metabolic phenotypes and GSL intake. (A and B) Correlations between cell numbers of total bifidobacteria (A) and the *Bifidobacterium animalis*-like phenotype (B) with HDL cholesterol. (C) Correlation between proportion of *Coriobacteriaceae* and non-HDL cholesterol. (D) Correlation between unclassified members of the family *Coriobacteriaceae* and cholesterol absorption. Data from control animals (0% GSL) were excluded from the analysis.

**FIG. 4.** Metabolic network showing the associations between daily GSL intake, gut microbiota composition, and host cholesterol metabolism in hamsters fed 0%, 1%, and 5% GSL. Results of the correlations of cell numbers of bifidobacteria and proportions of *Coriobacteriaceae* and phenotypic markers were obtained with data from animals fed 1% and 5% GSL. Red connections indicate a positive correlation, while blue connections show correlations that are inverse. Green connections show associations with no statistical significance. Metabolic data were obtained by Carr and coworkers in a previous study (8).
lesterol absorption. Bifidobacteria have been shown to affect cholesterol and lipid metabolism in animal models when administered as probiotics or when stimulated by prebiotics (11, 13). The mechanism by which bifidobacteria achieve these effects remain speculative, but they might impact cholesterol metabolism indirectly by suppressing numbers of Coriobacteri-aceae. For both bacterial groups, the capability to transform bile acids has been reported (38), and this phenotypic trait might influence host cholesterol metabolism through an impact on enterohepatic circulation. The strong correlations between bacterial taxa and cholesterol metabolism were observed only in animals fed GSL and not in control animals, suggesting that GSL influences the relative abundance of these organisms as well as metabolic characteristics.

The consumption of lipids has not yet been associated with increases in numbers of intestinal bifidobacteria. In contrast, Cani and coworkers (7) showed that a high-fat diet significantly lowered the number of bifidobacteria in mice. The composition of the GSL administered to the hamsters in our study contained not only mono-, di-, and triglycerides but also esters, alcohols, and other lipophilic compounds, such as waxes, sterols, and polycosanols (8). Carbohydrates or fiber are an unlikely explanation for the bifidogenic effect of GSL, as the amounts of fiber in hexane extracts of grains are negligible (8). Interestingly, genome comparisons revealed that bifidobacteria possess metabolic capacities that could allow them to utilize complex lipids, including lipids that may not be utilized by other members of the gut microbiota or the host. Schell and coworkers (40) detected four genes encoding long-chain fatty acyl-coenzyme A synthetases in the genome of Bifidobacterium longum, more than any other prokaryote genome available at that time, except for Streptomyces coelicolor and another gastro-intestinal tract inhabitant, Bacteroides fragilis. These findings together with the enrichment of putative esterases in bifidobacterial genomes detected above indicate that bifidobacteria are likely to utilize specific components of GSL leading to increases in numbers in the gut.

The correlation analysis identified the Bifidobacterium/Coriobacteriaceae equilibrium to be important for the plasma cholesterol levels in hamsters, with bifidobacteria being beneficial and coriobacteria being detrimental. While extrapolation of our observations to humans is still speculative, our findings suggest that bifidobacteria and coriobacteria could be potential targets for the prevention of metabolic aberrations that play a role in CHD. Clearly, it will be essential to first identify the exact bacterial taxa within the human gut microbiota that have strong correlations to cholesterol metabolism, which in itself is a challenging task. Unlike the inbred population of hamsters used in our study, human subjects have significant genetic diversity, and genetic factors that affect cholesterol metabolism play a more important role in humans than in the animal model. Furthermore, human subjects follow individual lifestyles and consume different diets, and they harbor variable and individual communities of the gut bacteria. All these factors will hamper the identification of bacterial contributors to human cholesterol metabolism. Nevertheless, it is tempting to speculate that the positive impact of breast-feeding on the Bifidobacterium/Coriobacteriaceae ratio in human infants (19) could be responsible for the higher HDL cholesterol levels observed in adults that were breast-fed in infancy (36). This study provides new and important perspectives on dietary modulation of the mammalian gut microbiota and its effects on the host. The findings indicate that a complex mixture of lipids can exert a “prebiotic” effect that leads to improvements in host cholesterol metabolism. In conclusion, this study provided evidence that modulation of bacterial populations in the gut has the potential to improve mammalian cholesterol homeostasis, which has relevance in the prevention of CHD.

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