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

Inés Martínez  
*University of Nebraska-Lincoln*

Chaomei Zhang  
*University of Nebraska - Lincoln*

Ryan Legge  
*University of Nebraska-Lincoln*

Andrew K. Benson  
*University of Nebraska - Lincoln, abenson1@unl.edu*

Timothy P. Carr  
*University of Nebraska - Lincoln, tcarr2@unl.edu*

*See next page for additional authors*

Follow this and additional works at: [http://digitalcommons.unl.edu/foodsciefacpub](http://digitalcommons.unl.edu/foodsciefacpub)

Part of the [Food Science Commons](http://digitalcommons.unl.edu/foodsciefacpub)

Martínez, Inés; Zhang, Chaomei; Legge, Ryan; Benson, Andrew K.; Carr, Timothy P.; Moriyama, Etsuko; and Walter, Jens, "Diet-Induced Metabolic Improvements in a Hamster Model of Hypercholesterolemia Are Strongly Linked to Alterations of the Gut Microbiota" (2009). *Faculty Publications in Food Science and Technology*. Paper 82.  
[http://digitalcommons.unl.edu/foodsciefacpub/82](http://digitalcommons.unl.edu/foodsciefacpub/82)

This Article is brought to you for free and open access by the Food Science and Technology Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications in Food Science and Technology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Diet-Induced Metabolic Improvements in a Hamster Model of Hypercholesterolemia Are Strongly Linked to Alterations of the Gut Microbiota

Inés Martínez, Grant Wallace, Chaomei Zhang, Ryan Legge, Andrew K. Benson, Timothy P. Carr, Etsuko N. Moriyama, and Jens Walter

Department of Food Science and Technology and Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, Nebraska 68583-0919, and School of Biological Sciences and Center for Plant Science Innovation, University of Nebraska, Lincoln, Nebraska 68588-0118

Received 16 February 2009/ Accepted 21 April 2009

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 F1B Syrian hamsters (Bio-Industries of America) 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 modified AIN-93 M diet (37) supplemented with 0%, 1%, and 5% grain sorghum on a mass basis. Daily feed intake was determined for each hamster was collected over 7 days. The fecal samples were ground, the hamsters were on their respective diets for 3 weeks, the complete fecal output of the entire molecular fingerprint of the sample. To determine the effects of feeding hamsters GSL, normalized fragment intensities of all bands in DGGE fingerprints were determined and compared for the feeding groups.

In order to identify species represented by bands detected by DGGE, bands from two or three animals were excised from the gel, purified, and reamplified by the method of Ben Omar and Ampe (3), and cloned using the Topo TA Cloning kit for sequencing (Invitrogen). Plasmids were isolated from three transformants per band using the QiAprep spin miniprep kit (Qiagen), and inserts were sequenced by a commercial provider following the manual of the cloning kit. Clone equivalents of the partial 16S rRNA sequences were determined using the nucleotide blast web tool at the NCBI website (http://www.ncbi.nlm.nih.gov/Blast.cgi) and the Seqmatch web tool provided through the Ribosomal Database Project (http://rdp.vbi.vt.edu/seqmatch/seqmatch_intro.jsp). A phylogenetic tree was generated from the consensus sequence of the F bands in individual animal samples by using the Clost program (42).

Analysis of the gut microbiota of hamsters by pyrosequencing of 16S rDNA tags. The V1-V3 region of the 16S rDNA gene was amplified by PCR using bar-coded universal primers 8F and 518R containing the A and B sequencing tags.

**PCR-DGGE analysis.** PCR was performed using universal primers PRB1236fGC (5′-CGCCCGGCGCGCGCGGCGGGCGGGGCGGGGGCACG-3′) and PRUN518r (5′-ATTACCGGCGGTGTCTCAG-3′) (34), which amplify the V3 region of the 16S rDNA gene. DGGE was performed by the method of Walter and coworkers (46) using a DCode universal mutation detection system (Bio-Rad, Hercules, CA). DNA bands in the DGGE gel were visualized by standard ethidium bromide staining and photographed using the ImageQuant densitometry system (Syngene, Frederick, MD). DGGE images were analyzed using BioNumerics software version 5.0 (Applied Maths, Kortrijk, Belgium). Bands were manually assigned, and the normalized banding patterns were used to estimate distance matrices by calculating the Pearson product moment correlation coefficients for all pair-wise combinations of patterns. This method compares profiles in a pairwise manner based on the entire densitometric curve, therefore accounting for both band position and intensity. DGGE fingerprints were transformed to peak profiles using the BioNumerics software, and the intensities of individual bands were determined as a percentage of the peak surface area relative to the surface area of the entire molecular fingerprint of the sample. To determine the effects of feeding hamsters GSL, normalized fragment intensities of all bands in DGGE fingerprints were determined and compared for the feeding groups.

Specific quantification of bifidobacteria by qRT-PCR. Quantification of total bifidobacteria was performed by quantitative real-time PCR using primers Bif-
For (5' - TCGCGTGGGGTGGAAG-3' and BifRev (5'- CCATCCAGGCR 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 μM 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 25527 and B. infantis ATCC 15697 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 $r^2$ 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'GCGAGGTTTGTCTCCT3'). This primer was used in combination with primer BifRev (39) used for the Bifidobacterium generic-specific qRT-PCR. The PCR was performed as 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 25527 and Bifidobacterium infantis ATCC 15697. The PCRs all gave negative results with the primer combination Bh1 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 25527 and B. infantis ATCC 15697 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 Bh1 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 phenotypes and the methods used are reported previously (8).

**Genome comparisons.** The web-based Integrated Genomics Platform of the Joint Genome Institute (JGI) was used to identify functions enriched in bifidobacteria. To identify specific taxa that were affected by the feeding treatments, the proportions of taxa in the control group were the diversity indices of gut populations. Correlations between metabolic parameters and bacterial populations were assessed by Pearson’s correlation test using GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA).

**RESULTS**

**Characterization of the hamster gut microbiota by pyrosequencing of 16S rRNA tags.** To determine whether proportional changes of the gut microbiota were associated with the effects of GSL on cholesterol metabolism in hamsters, we analyzed the fecal microbiota of hamsters fed 0% ( $n = 7$, 1% ( $n = 7$), and 5% ( $n = 7$) GSL by pyrosequencing of the V3 region of the 16S rRNA gene. A total of 34,424 sequences were studied; the average sequence length was around 250 bp, and an average of 1,639 sequences per animal were studied. Taxonomy-based analysis showed that the composition of the hamster gut microbiota at the phylum level is similar to that of humans and mice, being dominated by *Firmicutes* and *Bacteroidetes*. An unusual feature of the hamster gut microbiota, however, was that *Firmicutes* comprised the vast majority of the taxa (94%) with *Bacteroidetes* making up only 4% of the population. The remaining bacteria belonged to the phyla *Fusobacteria* and *Actinobacteria* (each representing around 1% 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*, *Eubacte riaeaceae*, *Ruminococcaceae*, and *Lactobacillaceae*, represented by an average of 59%, 19%, 13% 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 *Eubacte riaeaceae*, *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. S1 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.** Sequence proportions 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 *Coriobacte riaeaceae* ( $P = 0.042$), 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* ( $P = 0.0096$).
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 from individual animals also 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 \((P < 0.0001, \text{ Mann-Whitney test})\).

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.

![Family level:](image1)

![Genus level:](image2)

**Fig. 1.** Characterization of the gut microbiota composition of hamsters fed different amounts of GSL as determined by pyrosequencing of 16S rRNA tags (V3 region). Composition of the gut microbiota of hamsters fed 0%, 1%, and 5% GSL \((n = 7\) per group) at the family level (A) and the genus level (B). (C) Rarefaction curves of OTUs from sequences of fecal samples from individual hamsters fed 0% GSL (red), 1% GSL (green), and 5% GSL (blue). (D) Shannon diversity indices of the gut microbiota of individual hamsters fed 0% GSL (red), 1% GSL (green), and 5% GSL (blue). OTUs were identified using 97% cutoffs for rarefaction and Shannon diversity indices.
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></td>
</tr>
<tr>
<td>Genus level</td>
<td></td>
</tr>
<tr>
<td>Allobaculum</td>
<td>12.22 ± 0.79</td>
</tr>
<tr>
<td>Pseudoramibacter</td>
<td>27.82 ± 15.9</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.
* Values that were significantly different or approaching statistical significance are shown in boldface type.
* Statistically significantly different from the value for hamsters fed 0% GSL (P < 0.05) by ANOVA.
* 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.
* Approaching statistical significance compared to the value for hamsters fed 0% GSL (P < 0.1) by ANOVA.

During 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 phyotype. 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 phyotype (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 phyotype (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 3). 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 COG2227 (carboxylesterase type B) are enriched in six Bifidobacterium genomes (see Table S1 in the supplemental material). Carboxylesterases represented by COG0400 and COG2227 belong to enzymes that hydrolyze a wide variety of substrates, ranging from methylycaprylate 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-
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 species richness (number 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 bacteria, leaving the relative proportions of higher taxonomic taxa unaffected. Allobaculum belongs to the family Erysipelotrichaceae, and uncharacterized bacteria of this family declined as Allobaculum 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 coccoides 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>
<thead>
<tr>
<th>Band</th>
<th>Mean band intensityc (SD) in DNA from hamsters fed:</th>
<th>Closest GenBank hiď</th>
<th>Closest type strainë</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><strong>0.06 (0.16)</strong></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><strong>17.4 (8.2)</strong></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><strong>11.1 (3.7)</strong></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>

<table>
<thead>
<tr>
<th>TABLE 3. Correlations between abundance of bacterial taxa and markers of cholesterol metabolismf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial taxa</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Family level Coriobacteriaceae</td>
</tr>
<tr>
<td>Genus level Bifidobacterium</td>
</tr>
<tr>
<td>Allobaculum</td>
</tr>
<tr>
<td>Pseudoramibacter</td>
</tr>
<tr>
<td>Unclassified members of the following families Coriobacteriaceae</td>
</tr>
<tr>
<td>Erysipelotrichaceae</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.
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 remains speculative, but they might impact cholesterol metabolism indirectly by suppressing numbers of Coriobacteriaceae. 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 polyols (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, more than any other prokaryote genome available at that time, except for Streptomyces coelicolor and another gastrointestinal 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 the increase 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 homoeostasis, which has relevance in the prevention of CHD.

ACKNOWLEDGMENTS
We thank the members of the University of Nebraska—Lincoln Nutraceutical Team and especially Curtis Weller, Vicki Schlegel, and Susan Cuppett for their contributions to the hamster feeding trial. We thank Ty Nguyen for programming the pyrosequencing data analysis pipeline.

Grant Wallace was supported by the UCARE Program of the University of Nebraska. This study was funded in part by the Nebraska Grain Sorghum Board.

REFERENCES


