

HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

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Background

- Bile acids (BA) and gut microbiomederived BA metabolites (secondary BA [SBA]) modulate immune functions and contribute to intestinal tumorigenesis.
- While diet is known to influence BA production, the effects of dietary fat, carbohydrate, and protein on BA levels remain inconsistent.
- We hypothesize that the impact of diet on BA, particularly SBA levels, may depend on the host's gut microbial features.

Objectives

• We hypothesize that the impact of diet on BA, particularly SBA levels, may depend on the host's gut microbial features.

Methods

• Leveraging the integrated stool metagenomes, stool and plasma metabolome, and long-term habitual dietary data from a subset of the Microbiome Among Nurses study (Micro-N), including Micro-Ndiverticulitis substudy (n = 225), Micro-N-polyp substudy (n = 400), and Mind-Body Study (MBS) (n = 220), we explored the interplay between gut microbial species and functions, stool and blood BA metabolites, and diet (41 food groups and 51 nutrients).

Summary

• The methanogenesis pathway encoded primarily by *M. smithii* and the resultant production of methane gas may influence the biotransformation of dehydro-LCA and isoallo-LCA in gut microbial communities and modulate the dietary effect on BA metabolism.

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Key Findings





Figure 2. Carriage of the microbial methanogenesis

25 individual stool BA metabolites explained by 41 food groups according to the carriage of M. smithii and the methanogenesis pathway in the gut, represented as mean R² from a 5-fold cross-validation Random Forest regression model. It suggests that explained variance in certain BA metabolites, especially the LCA and its derivatives, was much higher in carriers of the methanogenesis pathway than non-carriers. (B) Carriage of *M. smithii* substantially modifies the associations of food groups v LCA and its conjugates and derivatives. Several food groups, e.g., processed and red meats, high-energy drinks and fruit juice showed stronger positive correlations, while nuts, salad dressing, leafy vegetables, et al. showed stronger negative correlation with LCA and its conjugates and derivatives in the carriers of methanogenesis pathway, compared to non-carriers. (C) Similar intakes of nutrients also showed stronger associations with LCA and its conjugates and derivatives among carriers of the methanogenesis pathway, compared to non-carriers. Notably, the findings also showed that the associations of dietary fat with LCA and its conjugates and derivatives depended on the nutrient source. For example, animal fat and trans-fat showed strong incremental association, while vegetable fat and polyunsaturated fat showed strong decremental association with the metabolites, particularly among the methanogenesis carriers. (D) Prediction for dehydro-LCA level in stool using subject demographics characteristics (including age, body mass index, smoking status, physical activity level, antibiotic use, probiotic use, and Bristol stool scale), dietary information, and gut methanogenesis level.

Linking the Gut Microbiome to Stool and Plasma Metabolome and Proteome Uncovers the Diet-Microbial Interactions underlying Secondary Bile Acid Production and Function in the Colon

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Figure 1. Association of Bile Acid (BA) Metabolites with Species-Level Gut Microbial

Taxonomy. (A) Percentage of the variance in the levels of 25 individual BA metabolites explained by gut microbial species, represented as mean R² from a 5-fold cross-validation Random Forest regression model. Stool and plasma BA metabolites were modeled using 384 and 306 microbial species, respectively, after excluding species with a relative abundance of <0.01% in less than 10% of samples. It suggests that stool BA metabolites, especially microbial BA conjugates and structural derivatives detected only in stool, were more strongly associated with gut microbial taxonomy than plasma BA metabolites. (B) Associations between logtransformed intensities of the 25 individual stool BA metabolites and the relative abundances of gut microbial species. Among the metabolites, LCA and its derivatives, particularly isoallo-LCA and dehydro-LCA, exhibited distinct microbial associations compared to other BA metabolites. The strongest associations were observed between isoallo-LCA, dehydro-LCA, and Methanobrevibacter smithii. (C) The microbial species with a mean importance coefficient of >10 in the 5-fold cross-validation Random Forest analysis of the top 5 microbial-associated BA metabolites (dehydro-LCA, isoallo-LCA, alpha-MCA, leuco-CA, and UDCA). It shows that the *M. smithii*dehydro-LCA was the pair with the highest importance.





alcium without supplement nin D without supplement refined grain Heme iron Animal protein Sugars Added suga Sucrose Sucralose sweetener Carbohydrates Energy intake Calcium Starch Protein Iron without supplement Fiber from cereal Glucose Fructose Vitamin D Vitamin B12 Choline Alcohol Fiber from cruciferous Fiber from fruit Monounsaturated fat Caffeine Folic acid Vitamin C Phylloquinone Vitamin K1 iber from vegetable DHA, DPA, and EPA

Omega 3 Polyunsaturated fat Fiber from leaume Vegetable fat BA category