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Diet and Gut Microbial Function in Metabolic and Cardiovascular Disease Risk

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Abstract

Over the past decade, the gut microbiome has emerged as a novel and largely untapped source of variability for metabolic and cardiovascular disease risk, including diabetes. Animal and human studies support several possible pathways through which the gut microbiome may impact health, including the production of health-related metabolites from dietary sources. Diet is considered important to shaping the gut microbiota; in addition, gut microbiota influence the metabolism of many dietary components. In the present paper, we address the distinction between compositional and functional analysis of the gut microbiota. We focus on literature that highlights the value of moving beyond surveys of microbial composition to measuring gut microbial functioning to delineate mechanisms related to the interplay between diet and gut microbiota in cardiometabolic health.

Keywords

Gut microbiota; metabolites; nutrition; diabetes; cardiovascular disease

Introduction

There is an intimate relation between diet and the gut flora. Gut microbial communities have co-evolved with host species to provide unique metabolic functions, as reflected in broad patterns of food consumption, with distinct microbiota among carnivores, omnivores, and

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Compliance with Ethics Guidelines

Conflicts of Interest

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herbivores[1, 2]. Geographic surveys in humans reveal differences in microbiota corresponding to the extent of plant and animal consumption or Westernization of diet[3–5]. On an individual level, dietary composition contributes, at least in part, to shaping the gut microbiota through the delivery of energy for microbial growth[6–8]. In turn, gut microbiota influence the production of health-related metabolites from dietary components[9, 10], with the potential for personal variability in metabolite production based on differences in gut microbiota[11, 12]. A growing literature supports a role for gut microbiota in the development and progression of metabolic and cardiovascular disease risk[13–16], including type 2 diabetes[17–19], obesity[20–22], insulin resistance[23], inflammation[24], atherosclerosis[12, 25], hypertension[26, 27], dyslipidemia[28], and cardiovascular disease events[29, 30]. The purpose of the current review is to frame a discussion around the complex interplay between diet and and available assessments of the gut microbiota in pathways to metabolic disease. Additionally, we will review evidence supporting the growing emphasis in the field to move from surveys of taxonomic composition of the microbial community to measuring microbiota functional potential and activity for improved understanding of health-related pathways through microbiota.

Studying the gut microbiota, diet, and cardiometabolic health: From microbiota composition to function

Measuring the gut microbiota: a brief introduction

Historically, study of the gut microbiota focused on distinct organisms or a set of specific organisms using culture-dependent approaches[31–33]. However, time- and labor-intensive culture-based methods require the development of appropriate media for the laboratory cultivation of specific microorganisms, limiting the scope of the gut community that can be studied. The introduction of culture-independent methods allowed the direct isolation of microbial DNA from collected samples, such as fecal samples for study of the gut microbiota, and enabled researchers to begin to characterize the larger gut microbial community[34]. This work provided key insights into taxonomic composition and functionality of the gut microbiota as a whole. Past-decade advances in high-throughput (next-generation) sequencing technology have dramatically improved the ease and cost of sequencing, contributing to a rapid acceleration of research in the area[35, 36]. Culture-independent DNA-based approaches have revealed a vast and more diverse community than previously appreciated based on culture-dependent approaches, since many gut microbiota identified through culture-independent methods had not previously been cultivated.

Gut microbiota composition: Challenges for studies of diet and health

To date, microbiome research has largely focused on measures of microbial composition based on sequencing one or more regions of the 16S rRNA marker gene[37–40]. 16S rRNA is an evolutionarily well-conserved prokaryotic gene found in all bacteria and archaea, with sufficient variability in gene regions to allow taxonomic assignment of microbiota to the genus level[41]. Characterization of the gut microbiota based on 16S rRNA include the relative abundance of specific taxonomic groups, as well as ecologic measures of the microbiome as a whole, such as diversity and richness[38].

16S rRNA remains easiest, fastest, and most affordable approach for characterizing the microbial community with respect to taxonomic composition, but it is limited in its ability to provide detailed understanding of gut microbiota functioning. High-throughput sequencing technology has made it economically feasible for researchers to further examine the role of the gut microbiota in health by obtaining a comprehensive survey of genes in the microbiota, using whole genome sequencing (WGS), termed metagenomics[40]. WGS data allows taxonomic assignment at a finer level than 16S rRNA and profiles the genes present in the microbiota, which can be mapped to metabolic pathways, providing a measure of functional potential[42].

The NIH-funded Human Microbiome Project (HMP) generated 16S rRNA and WGS data for microbial communities from multiple body locations, including gut, in roughly 300 U.S. adults[43]. A primary goal of the HMP was to establish the taxonomic entities and composition that constitute a healthy gut microbiota in humans[44]. However, a clearly-defined core microbiota has defied easy identification; rather, the HMP and other studies have found large variation in the taxonomic composition of the gut microbiota among healthy individuals[43]. For example, initial animal and human studies supported associations between obesity and the ratio of Firmicutes to Bacteroidetes, the two most abundant phyla in the human gut microbiota. In one study, ob/ob mice had a greater abundance of Firmicutes relative to Bacteroidetes (F/B ratio) than lean mice[45]. A study of 12 human subjects also found that obese individuals had a higher F/B ratio[20]. In an intervention of calorie-restricted diets, weight loss was associated with decreases in F/B, irrespective of macronutrient composition (low-fat or low-carbohydrate)[20]. Although several subsequent studies reported similar findings, however, other studies showed no F/B difference between obese and lean individuals, the reverse association (lower F/B in obese vs. lean), or no change in F/B with weight loss[46].

Other research has revealed large within-phyla variability that may help explain inconsistent findings. In a study of obese mice fed a Western-style diet, weight gain was accompanied by increases in specific members of Firmicutes from the Mollicutes class[47]. In another animal study, a high-fat diet was associated with increases in Mollicutes, as well as Firmicutes classes Clostridiales and Delta-Proteobacteria[48]. In a dietary intervention among 14 overweight men, carbohydrate subtype did not reveal a change in F/B, but did show changes at lower taxonomic levels[49]. Additional evidence of the importance of more refined taxonomic analysis can be illustrated by findings related to Prevotella, one of the two primary genera in the phylum Bacteroidetes. Prevotella has been associated with plant-based diets[50, 51] and improved glucose metabolism[52], but also with chronic inflammation[53]. Such apparent discordance in health associations may reflect within-genus variability. Indeed, gene-level diversity has been observed among genomes of strains within a single Prevotella species[52]. These data support the need for finer compositional analysis than has been standard, as well as motivate increased focus on measures of microbial function.

In contrast to the large compositional variability, functional potential measured through WGS is relatively stable among healthy individuals[21, 43]. These findings support a model of redundancy in the presence of genes for core metabolic functions across a diverse gut microbial composition. Additional work is needed to improve our understanding of how

gene sets align with specific microorganisms, particularly to inform our assessment of the functional impact of microorganism-based targeted interventions. Redundancy in gene presence across microbiota may underlie the finding that greater microbial diversity is generally positively associated with health status[21], with some exceptions, as a diverse microbiota may be more likely to capture the core set of necessary genes.

Metagenomics studies have revealed significant differences in bacterial genes and metabolic pathways in obese and lean twins[21] and among individuals with and without T2D[17, 18]. In a study of 345 Chinese adults with and without T2D, WGS was used to derive species-level compositional information as well as to functionally annotate the gut community[17]. Although specific microbiota were differentially enriched in the T2D and control samples, there were even greater differences in functional potential, most notably with respect to decreased butyrate-producing bacteria among individuals with T2D[17]. Other metagenomics studies of T2D also support a role for butyrate production in distinguishing T2D from controls[18, 54].

There is growing consensus that to truly delineate the role of the gut microbiota in human physiology, we must pursue measures of microbial functional activity. This shift is reflected in Phase 2 of the HMP, the integrated Human Microbiome Project (iHMP), which includes a collection of multiple functional measures, including transcriptomics (RNA-seq), metabolomics, and proteomics[55] The trend towards functional measures in studies of the gut microbiota mirrors the current emphasis on integrating host-level functional measures into animal models and human studies[56, 57]. Functional measures reflect the biologic activity of ultimate physiologic interest; with respect to the gut community, functional redundancy across taxonomic groups limit the ability to infer functional activity from community composition. Thus, functional analysis will be increasingly important for defining microbial pathways to metabolic disease, and for determining targets for intervention.

Production of dietary metabolites as a functional measure of gut microbiota

The gut microbiota has evolved complementary metabolic capacity to its human host, and a substantial proportion of circulating metabolites have microbial origin[9, 10]. A subset of microbiota-related metabolites derive from dietary components[58], several of which have been studied with respect to cardiometabolic outcomes. For example, short-chain fatty acids generated from microbial metabolism of non-digestible polysaccharides, and in particular butyrate, have been shown to have positive effects on glucose and lipid metabolism[59]. Phenolic compounds undergo extensive gut microbiota metabolism, and these metabolites may help explain the health benefits associated with a plant-based diet[60]. Microbiota-dependent metabolites may also have adverse effects on cardiometabolic health, such as trimethylamine N-oxide (discussed below)[12, 29, 30].

The extent to which metabolites from dietary components are dependent on the gut microbiota for production can be estimated in germ-free (gnotobiotic) animal models, which lack gut microbiota. Comparing germ-free and conventional mice, Wikoff and colleagues reported variable production of metabolites[10]. For example, hippuric acid, produced from

gut microbial metabolism of phenolic compounds such as in tea, wine, and fruit juices, was 17.4 times higher in conventional as compared to germ-free mice. Equol sulfate and p-cresol sulfate, produced from isoflavones and amino acids, respectively, were only observed in conventional mice.

Based on this and related work, circulating concentrations of microbiota-dependent metabolites in host urine or blood have been proposed as relatively efficient measures of microbial function, particularly at this stage of the science where metabolic pathways through the gut microbiota remain to be defined. Personalized metabolite profiles have been identified under controlled dietary delivery of precursors, and this measure of microbial function is maintained upon transfer of host microbiota into germ-free mice (termed, humanized germ-free model)[61]. That is, the production of metabolites varied among humanized mice in a way that reflected the human host, even when mice were maintained on the same diet[61]. Humanized germ-free animals provide an experimental model for illustrating the role of human gut microbiota in metabolic of dietary components, and have been used to demonstrate rapid shifts in the expression of microbial genes in response to a dietary change[62].

Integrated approaches to functional understanding of the interplay of diet and gut microbiota in cardiometabolic health

We present two examples that we consider illustrative of integrative approaches to the study of diet, gut microbiota function, and cardiometabolic health. These examples highlight what will likely be the translational and transdisciplinary nature of future work to define targeted interventions related to the gut microbiota.

Example 1: Trimethylamine N-oxide (TMAO)

The potential for nutrient metabolite trimethylamine N-oxide (TMAO) to increase CVD risk was identified in a metabolomics screen and confirmed in an independent sample[30]. Subsequent work has supported associations between TMAO and cardiovascular disease[29], chronic kidney disease[63, 64], and type 2 diabetes[65–67], though significant findings have not been consistently observed[68, 69]. Proposed mechanisms for TMAO in cardiometabolic risk include platelet activation[70], cholesterol efflux[12], uremic toxicity[63], and glucose metabolism[67].

The production of TMA from nutrient precursors, choline[29] and L-carnitine[12], is dependent on gut microbiota, with conversion of TMA to TMAO in the liver through flavin monooxygenase 3 (FMO3)[71]. Variability in circulating TMAO will thus reflect: 1) consumption of dietary precursors, 2) gut microbiota metabolic capability for production of TMA, and 3) FMO3 genotype for conversion of TMA to TMAO. A GWAS of mouse and human studies revealed that variability in TMAO production is likely more reflective of differences in diet and gut microbiota, rather than genetic variation in FMO3[72]. Several distinct gut microbial pathways for TMAO production have been identified[73–75] or proposed[76].

The production of TMAO varies among individuals exposed to a dietary challenge of TMAO precursors[12, 29, 74, 77]. In a well-controlled egg challenge study, individual-level dynamics of plasma TMAO was highly variable over a 24-hour period across 6 study participants[77]. Larger choline doses yielded a greater spike in TMAO production over the 24-hour observation period, but considerable variation in TMAO production was observed even at high doses (4–6 eggs, or 476–714 mg choline). Participants were provided standardized meals throughout the testing periods to maintain equal precursor contribution through diet.

Many sources of choline and L-carnitine are animal products, including fish, eggs, and red meat, and it is not surprising that TMAO measured in fasting samples is significantly lower among vegans and vegetarians as compared to omnivores[12] and among individuals with high, as compared to low, adherence to the Mediterranean diet[51]. Interestingly, long-term diet may influence the capacity for TMAO to be produced, perhaps through selection of gut microbiota based on nutrient availability. In an L-carnitine challenge in 10 individuals, TMAO was produced among omnivores, but not among vegans and vegetarians[12]. In addition to microbiota pathways related specifically to TMAO precursors, it is intriguing to consider the possibility that consumption of dietary components that are not direct precursors of TMAO may impact metabolite production through effects on the gut microbiota. For example, recent data indicate that TMAO production may be decreased with increased resveratrol[78], a phenol found in red wine, or allicin[79], an organosulfur compound found in garlic. These data may be consistent with findings from another dietary challenge showing that TMAO production was lower in individuals with less gut microbiota richness[80]. Another possibility is that other nutritional components may serve to offset an adverse impact of TMAO. For example, fish oil appeared to lessen the adverse effect of TMAO on glucose tolerance in mice on a high-fat diet[67], providing a possible explanation for fish being both cardioprotective as well as a major contributor of TMA directly and of TMA precursors.

This example illustrates the combination of discovery and targeted metabolite analysis in observational cohorts, controlled mechanistic work in animal models, and nutrient challenge studies in small human samples to elucidate health effects of the gut microbiota-dependent metabolite TMAO and pathways to TMAO production from dietary precursors through the gut microbiota.

Example 2: Polyphenols: Isoflavones and lignans

Isoflavones and lignans are polyphenols, which are a large class of compounds from plant sources[60]. It has been estimated that 90% of polyphenols have incomplete absorption in the small intestine and undergo metabolism by microbiota in the lower intestine[81, 82]. There is large individual variability in the production of metabolites following consumption of dietary polyphenolic precursors, which may reflect differences in gut microbiota. Plant consumption and many polyphenols, including isoflavones and lignans, have been proposed to be related to a range of health outcomes, including cardiovascular disease and diabetes[83, 84]. It is hypothesized that polyphenol metabolites may explain, at least in part, the health benefits of plant-based diets. Soy is a rich source of the isoflavone daidzein, from

which metabolites equol and O-desmethylangolensin are generated; lignans are found in a variety of foods, including rye, berries, and flaxseed, and yield metabolites enterodiol and enterolactone. In two cohorts of U.S. women, higher urinary levels of isoflavones and lignans metabolites have been inversely associated with T2D[85, 86].

The gut microbiota-dependence of isoflavone and lignan metabolite production was demonstrated in a humanized germ-free rat model. Human subjects were classified as high or low producers of equol based on their response following consumption of a soy meal[87]. Equol, O-desmethylangolensin, and enterolactone were not produced in germ-free rats, but were detectable upon introduction of human fecal microbiota. The equol production phenotype was transferrable to germ-free rats: rats produced equol when they received a fecal microbiota transplant from individuals with high equol production, but not when they received a transplant from individuals with low equol production. There is a lack of data on microbial pathways for isoflavone and lignan metabolite production, but one recent study reported a positive association between gut microbiota diversity, based on 16S rRNA analysis, and enterolignan production in 115 premenopausal U.S. women[11].

Equol production is higher in Asian populations and in vegetarians, potentially supporting a role for long-term diet in gut microbiota-dependent metabolite production. In one study, equol production was 51% in Korean-American women as compared to 36% in non-Korean-American women[88]. In a U.S. sample of 41 healthy individuals, equol production varied from 25% among non-vegetarians (n=12) to 59% among vegetarians (n=29)[89]. However, studies of long-term dietary intake and equol production have yielded inconsistent results[90, 91]. In addition, research is needed into the extent to which these phenotypes are inducible with dietary change. A 1-month soy intervention did not alter ability to produce isoflavone metabolites[92]. It is possible that diet is important, but that effects of diet on microbiota are not restricted to the direct metabolite precursor.

Metabolite production studies have generally been small-scale highly controlled challenge studies. Designs that would allow the measurement of metabolite-producer phenotypes in large-scale population-based studies would provide data on the distribution of metabolite production in the population, and allow improved study of covariates that influence metabolite production, including diet and other lifestyle factors. In addition, larger samples are needed to power discovery analysis of microbial components related to metabolite production. In a recent study, Melby and Watanabe addressed some of the necessary pharmacokinetic considerations for integrating a challenge component into an epidemiologic study[93], including the optimal time window for post-challenge sample collection and the dose required to distinguish producers from non-producers.

Challenges for delineating the interplay among diet, gut microbiota, and cardiometabolic health

The rapid growth of microbiome research has significantly advanced our appreciation of the potential for the gut microbiota to impact metabolic and cardiovascular disease risk, but there remain several key challenges for future work.

First, despite the enormous progress in microbiome research, there is a paucity of data that establish causality. Alongside broad enthusiasm for the untapped potential of gut microbiota for understanding human physiology, there are calls for cautious interpretation of findings until data are available that delineate mechanistic pathways through the gut microbiota to health[94]. Findings that microbiota functional measures change upon changes in diet are compelling, but we do not know whether gut microbiota function is altered through targeted dietary interventions or whether changes in metabolite production and gene expression reflect existing functional potential that is activated through the delivery of dietary precursors. TMAO and isoflavones/lignans case studies are useful illustrations of an integrated approach to understanding pathways through diet, but there are few such examples in the literature. With respect to health outcomes, data indicate the potential for complex inter-connections; for example, obesity may be both influenced by the gut microbiota[22] as well as influence the gut microbiota[45]. Most observational studies of the microbiome have included a limited set of covariates to control for potential confounders. A recent analysis of T2D demonstrates the value of adjustment for medication use to clarifying discordant findings in observational studies[54].

Second, there is need for richer microbiota data from well-designed human studies. Model systems are exceptional experimental resources, but they may not reflect human biology. Germ-free animal models have illustrated the relevance of gut microbiota for health, including through the production of certain dietary metabolites[10]. Humanized microbiota-associated mice have been proposed as an efficient method to determine causality by incorporating human-derived gut microbiota in a controlled model organism, but there are many questions about the extent to which these models will reflect the active microbiota of the source human hosts[95]. Human studies are needed to address the potential lack of representation in animal models, as well as to capture the breadth of microbiota existing in human populations. For example, the HMP covered a relatively small sample (n~300) with little racial/ethnic diversity. To date, most observational studies have been cross-sectional comparisons of individuals with and without a health condition, and there is need to incorporate microbiota sample collection in ongoing cohort studies. Human experiments have been informative, but may not always reflect typical behavior, such as a study comparing plant- and animal-based diets[96], which are rare extremes in most societies.

Third, there is increasing recognition of the need for standards in the collection and processing of samples, and the analysis of microbiome data. Using a set of generated communities with known composition, the extent of possible bias was demonstrated in a recent study of vaginal microbiome. This work revealed the potential for large and differential-by-organism errors, largely attributable to DNA extraction and PCR amplification steps of sample processing[97]. The newly-established Microbiome Quality Control project will contribute to elucidating differences stemming from variability in methods in the field, the laboratory, and bioinformatics; and providing guidance in the future for microbiome researchers[98]. In the interim, protocols from the HMP have been published and their use allows comparison with HMP data and other data from researchers using the HMP protocols[39].

Summary and Conclusions

A growing body of literature supports a role of gut microbiota in the development and progression of metabolic and cardiovascular disease risk, but precise causal mechanisms remain to be elucidated. The microbiota-dependent production of health-related nutrient metabolites may be an important pathway through which the microbiota mediates the impact of diet on disease risk; however, the complex interplay between diet and the gut microbiota is only beginning to be understood. Diet appears to be important to shaping the gut microbiota, though the extent to which gut microbiota can be changed for improved health through targeted dietary interventions is not known. Multiple dietary components rely on gut microbiota for metabolism, and there is large individual variability in the gut microbiota-dependent production of health-related metabolites. A direction for future research is integrating gut microbiota measures into approaches for personalized nutrition[99]. Future advances in the field will be aided by shifting to a focus on microbiota functional activity, as well as deeper compositional measures to capture important within-species variability, along with increased attention to study designs for causal inference, a greater number of well-designed human studies, and standards for the conduct of microbiome studies.

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