Gut microbiota and cardiometabolic outcomes: influence of dietary patterns and their associated components[1–4]

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ABSTRACT

Many dietary patterns have been associated with cardiometabolic risk reduction. A commonality between these dietary patterns is the emphasis on plant-based foods. Studies in individuals who consume vegetarian and vegan diets have shown a reduced risk of cardiovascular events and incidence of diabetes. Plant-based dietary patterns may promote a more favorable gut microbial profile. Such diets are high in dietary fiber and fermentable substrate (ie, nondigestible or undigested carbohydrates), which are sources of metabolic fuel for gut microbial fermentation and, in turn, result in end products that may be used by the host (eg, short-chain fatty acids). These end products may have direct or indirect effects on modulating the health of their host. Modulation of the gut microbiota is an area of growing interest, and it has been suggested to have the potential to reduce risk factors associated with chronic diseases. Examples of dietary components that alter the gut microbial composition include prebiotics and resistant starches. Emerging evidence also suggests a potential link between interindividual differences in the gut microbiota and variations in physiology or predisposition to certain chronic disease risk factors. Alterations in the gut microbiota may also stimulate certain populations and may assist in biotransformation of bioactive components found in plant foods. Strategies to modify microbial communities may therefore provide a novel approach in the treatment and management of chronic diseases.

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INTRODUCTION

A shift toward emphasizing dietary patterns has occurred in recent years for chronic disease risk reduction rather than the traditional approach that focuses on specific nutrients or foods, which has occupied much of the history of dietary guidelines that originate from a model for the prevention of nutrient deficiencies (1, 2). The human gut is home to trillions of microbes, and recent advances in sequencing techniques for characterizing the human gut microbial communities have accelerated our ability to understand how dietary patterns may modulate the gut microbiota and, most importantly, its effect on human health. The purpose of this presentation at the Sixth International Congress on Vegetarian Nutrition was to provide an overview of the link between dietary patterns, cardiometabolic health, and the gut microbiota. A comprehensive review of this topic area is beyond the scope of this article.

DIETARY PATTERNS AND CARDIOMETABOLIC HEALTH

Many dietary patterns have been associated with a reduction in cardiometabolic risk. A commonality between these dietary patterns is the emphasis on plant-based foods including higher intakes of fruit, vegetables, and whole grains that are naturally high in fiber with minimal processing. Large prospective cohort studies, the Nurses' Health Study and the Health Professionals Follow-Up Study, identified the emergence of 2 major dietary patterns: "prudent" and "Western." The "prudent" dietary pattern (higher intakes of fruit, vegetables, whole grains, legumes, fish, and poultry) has been shown to be significantly associated with a reduced risk of cardiovascular disease (CVD)[5] (3–5), type 2 diabetes (T2D) (6, 7), and all-cause mortality (5). In contrast, the "Western" dietary pattern (higher intakes of red meats, processed meats, refined grains, sweets and desserts, and French fries) was significantly associated with increased risk of these diseases (3–7), in addition to increased cancer mortality (5). Intakes of low-carbohydrate diets that were higher in plant-based sources of proteins and oils rather than animal-based sources were also associated with reduced coronary heart disease (CHD) events and incidence of T2D (8–10) In an analysis of 3 large prospective cohort studies on the relation between changes in diet components and weight, the consumption of plant-based foods, such as vegetables, nuts, whole grains, and fruit, was inversely associated with weight gain (11). Similarly, greater disease risk reduction was observed in cohort studies of "traditional" dietary patterns around the world, including those of the Mediterranean, Asia, and Nordic populations, which have a greater emphasis on plant-based rather than animal-based foods (12–18).

Cohort studies in vegetarians and vegans provide further evidence of the benefits of dietary patterns that emphasize plant-based foods. A pooled analysis of 5 prospective cohort studies in vegetarians and nonvegetarians observed a 24% reduction in

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[5] Abbreviations used: CHD, coronary heart disease; CVD, cardiovascular disease; SCFA, short-chain fatty acid; TMAO, trimethylamine-N-oxide; T2D, type 2 diabetes.

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mortality from CHD in vegetarians compared with nonvegetarians (death rate ratio: 0.76; 95% CI: 0.62, 0.94) (19). Vegetarian dietary patterns were also associated with an increase in longevity and decreases in all-cause mortality, T2D, and obesity (20–22). In a cross-sectional analysis of the Adventist Health Study 2, BMI and risk of T2D were greatest among nonvegetarians and lowest among vegans, where an inverse gradient was observed according to the extent of animal products excluded (23).

Clinical trials in individuals consuming vegan or vegetarian diets were shown to reduce the progression of CHD and improve glycemic control. A low-fat vegetarian diet, along with other lifestyle modifications, significantly reduced serum lipids and the progression of coronary artery lesions after 1 y (assessed by coronary angiography) (24). In contrast, coronary artery lesions continued to increase in the control group. In a longer-term clinical trial of diabetes management with a low-fat vegan diet compared with a conventional therapeutic diet, both diets resulted in reductions in weight and plasma lipid concentrations (25). After medication changes were controlled for, the low-fat vegan diet improved glycemic control (as assessed by glycated hemoglobin) and plasma lipids in comparison to the conventional diet. In the recently published Prevención con Dieta Mediterránea (PREDIMED) trial on the primary prevention of CVD, the RR of cardiovascular events decreased by ∼30% in those adhering to Mediterranean diets (emphasizing plant-based foods along with increases in fish and decreases in red meat consumption) supplemented with either extra-virgin olive oil (HR: 0.70; 95% CI: 0.54, 0.92) or mixed nuts (HR: 0.72; 95% CI: 0.54, 0.96) compared with the control diet (advice to reduce dietary fat) (26). Other shorter-term studies that focused on reducing CHD risk factors also support the findings from epidemiologic and longer-term clinical studies. Diets that emphasize low-fat dairy (ie, approaching a lactoovovegetarian diet) or plant protein foods with higher intakes of fruit and vegetables (ie, approaching a vegetarian diet), such as the Dietary Approaches to Stop Hypertension (DASH) and Optimal Macronutrient Intake Trial to Prevent Heart Disease (OMNIHeart) diets, were shown to improve blood pressure (27) and blood lipids (28, 29). Those who followed a vegan diet using a dietary portfolio approach (based on fruit, leafy vegetables, plant sterols, soy, and nuts) in a metabolically controlled feeding study had reductions in LDL cholesterol of 29% and in C-reactive protein of 28% (30). This effect was equivalent to a first-generation statin when provided along with a diet very low in saturated fat in which the reductions in LDL cholesterol and C-reactive protein were 31% and 33%, respectively (30). A similar plant-based dietary approach following a calorie-reduced low-carbohydrate diet also showed significant improvements in serum lipids compared with a high-carbohydrate, low-fat lactoovovegetarian diet, despite similar weight loss on both diets (31). The observed beneficial effects of plant-based foods are likely attributed to the presence of an array of active components, which include vegetable proteins, vegetable oils, sterols, fibers, and other nondigestible carbohydrates, antioxidants, vitamins, minerals, and phytochemicals.

Despite the strong evidence from both epidemiologic and clinical studies of the benefits of plant-based dietary patterns, the current dietary intake of such foods by most Americans is far below the recommended servings based on national dietary guidelines. Data from NHANES identify that the majority of Americans do not meet the recommended intake for food group servings of fruit, vegetable, legumes, and dairy. Although >50% do meet the recommended servings for total grains, almost all do not meet the whole-grain serving recommendations, which indicates that the consumption is primarily from refined grains (32). This is clearly evident in the low estimated average fiber intake/d of ∼16 g (33), which is far from the recommendation of 25–38 g/d or 14 g/1000 kcal (34). This has a major impact on the gut microbiota because of the significant reduction in the availability of fermentable substrates used as metabolic fuel.

HUMAN GUT MICROBIOTA

Recent advances in both sequencing techniques (ie, culture-independent methods: 16S ribosomal DNA sequencing, whole-genome shotgun or metagenomic sequencing) and bioinformatics (ie, computational tools to analyze such data) have accelerated our understanding of the gut microbiota (microbial communities present) and the gut microbiome (genes of the microbiota). This has given us the ability to identify and characterize microbial species that were previously uncultured because of the shortcomings of traditional or conventional microbiological techniques. Significant strides have been made in unraveling the composition of the gut microbiota and its associated microbiome; more importantly, this opens the door to the next crucial step of understanding the symbiotic relation between the gut microbiota and its associated microbiome on metabolic and physiologic outcomes of the host (ie, metagenomics, metatranscriptomics, and metabolomics). This is evident in the emergence of large-scale multimillion-dollar projects around the world including the NIH-funded Human Microbiome Project Consortium and the European Commission's MetaHIT (Metagenomics of the Human Intestinal Tract) project (35, 36). The Human Microbiome Project Consortium has generated comprehensive details of the microbial communities in the human body (airways, skin, oral cavity, gut, and vagina) and their relations with their host (37) and has produced reference genomes (viral, bacterial, and eukaryotic) from "healthy" adults, which provide a framework for subsequent metagenomic analysis (38). The MetaHIT project focuses on understanding the associations between the human gut microbiome and a wide range of health statuses and physiologic conditions including inflammatory bowel disease and obesity (39). This research is still in its infancy, but it holds tremendous promise in unraveling complex relations because the gut is host to a diverse population of trillions of microbes.

The clustering of 16S ribosomal RNA sequencing into relatedness groups on the basis of the percentage of sequence identity is often used to characterize the gut composition by categorizing the microbes by taxonomic level (40). Five bacterial phyla dominate the human gut microbiota (Firmicutes, Bacteriodetes, Actinobacteria, Proteobacteria, and Verrucomicrobia) and one Archaea (Euryarchaeota) (41). Bacteroidetes and Firmicutes appear to dominate the adult gut microbiota, but it appears that their relative proportions and the species present within these phyla vary greatly between individuals (42–44). For example, the relative abundance of these 2 dominant phyla has been associated with phenotypical differences, as observed in lean compared with obese individuals (45). Relevant genera in the Firmicutes phylum include *Ruminoccoccus*, *Clostridum*, and

Lactobacillus (contains probiotic strains) and the butyrate producers *Eubacterium*, *Faecalibacterium*, and *Roseburia.* In the Bacteriodetes phylum, relevant genera include *Bacteriodes*, *Prevotella*, and *Xylanibacter* and in the Actinobacteria phylum these include *Bifidobacterium* (contains probiotic strains) (41).

The diversity of microbes within the gut microbiota can be defined as the number and abundance distribution of distinct types of organisms (36), where lower diversity has been observed in obesity, inflammatory bowel disease, and meat-based dietary patterns (44, 46, 47). Furthermore, interindividual variations in microbial diversity over time were consistently greater than intraindividual variations (36, 44, 46, 48). The uniqueness of an individual's microbial composition may therefore be a key contributor to variations in physiology or predisposition to certain risk factors associated with metabolic disorders, but this is yet to be confirmed. Emerging research supports such a potential link.

Evidence has shown that there is a lesser degree of functional diversity (metagenomics, which refers to the techniques and tools used to isolate and sequence genomes from an environment linking both functional and phylogenetic information) (49, 50) than microbial diversity, and there appears to be functional redundancy (ie, a number of microbes perform similar functions) (36, 43, 51). A suggested advantage of having greater microbial diversity may be to ensure that key metabolic functions are unaffected by changes in the microbial composition. In other words, different microbes with similar functions can substitute for one another when the microbial composition is more diverse, resulting in the host potentially being more resilient in response to stress or change. This further suggests that key metabolic functions (ie, functionality) carried out by the gut microbiome may be more important than the specific microbes' presence (ie, phylogeny).

DIETARY PATTERNS AND ASSOCIATIONS WITH GUT MICROBIOTA

Variations in dietary patterns appear to be a key contributing factor to the diversity of the human gut microbiota. This diversity is likely to have evolved in parallel with the dietary pattern of our evolutionary ancestors in which a symbiotic relation exists. Analyses of fecal microbial communities of 106 individual mammals showed a clustering by dietary pattern—herbivore, omnivore, and carnivore (44). Furthermore, the phylogenetic diversity was related in the following stepwise fashion: herbivore > omnivore > carnivore (44). Fecal samples from "healthy" humans show a clustering with other omnivores with respect to microbial composition (44). As mentioned previously, increased microbial diversity may be advantageous for maintaining functional diversity. This suggests that adhering to a plant-based dietary pattern may confer greater resilience or adaptability to change.

There is interest in the concept of classifying individuals on the basis of enterotypes—a classification based on the notion that the gut microbiome congregates in a relatively stable composition over time (52). In turn, responses to a specific intervention can then be predicted on the basis of an individual's enterotype. This can be seen as an extension of personalized medicine or nutrition but specific to an individual's gut microbiome. Analysis of the gut microbiome across different populations showed the emergence of distinct clustering (enterotypes) on the basis of the relative abundance of each of the genera: *Bacteroides*, *Prevotella*, and *Ruminocuccus* (52). Other studies showed that the clustering is driven primarily more by the ratio of 2 dominant genera: *Prevotella* and *Bacteroides* (43, 48, 53). It has been suggested that the ratio between *Bacteroides* and *Prevotella* is influenced by different dietary patterns (43, 48, 53, 54). The *Bacteroides* enterotype was associated with animal protein, a variety of amino acids, and saturated fats, whereas the *Prevotella* enterotype was associated with higher carbohydrates and simple sugars (48). Other studies support these findings showing the association between dietary patterns and these 2 enterotypes (54, 55).

The characterization of the gut microbial communities from stool samples was performed in 531 individuals (151 families) representing populations from diverse geographical regions and cultural traditions, which included individuals from the Guahibo Amerindians, Malawi, and the United States (53). The main contributors to gut microbial communities were age (early infancy compared with adulthood) and geography or cultural traditions. Interestingly, there were pronounced differences in the phylogenetic composition of the fecal microbiota, with a distinct separation between the gut communities of those from the United States and those from Malawi and Amerindians. Furthermore, the fecal microbiota of US adults was the least diverse of the 3 populations, which was already evident in children ≥3 y of age (53).

Many environmental, genetic, and cultural factors are likely to be responsible for the separation of Western and non-Western individuals' gut communities; however, differences in dietary patterns may play a significant role. Amounts of *Prevotella* were greater in those from Malawi and Amerindians, where the dietary pattern is dominated by corn and cassava (plant-based polysaccharides). In contrast, amounts of *Bacteroides* were greater in those from the United States, where the typical dietary pattern is rich in protein (mostly animal protein) (53). Analysis of the fecal microbiota, with the use of traditional cultural techniques, of self-reported vegetarians (n = 144) and vegans (n = 105) showed a significantly lower presence of *Bacteroides* in vegetarians and vegans compared with matched controls who consumed an omnivore diet (55). Similarly, children living in a village of rural Africa following their traditional dietary pattern showed a higher abundance of *Prevotella*, whereas children from Western Europe adhering to a typical Western dietary pattern had a higher abundance of *Bacteroides* (54). The traditional rural African diet was primarily vegetarian (mainly made up of cereals, legumes, and vegetables), which was low in fat and animal protein and rich in starch, fiber, and plant polysaccharides. In contrast, the Western dietary pattern of the children from Western Europe was high in animal protein, sugar, starch, and fat and low in fiber (54). Furthermore, analyses showed that the fecal samples of the children in rural Africa were significantly higher in short-chain fatty acids (SCFAs), primarily from propionic and butyric acids, with greater microbial diversity, suggesting a strong influence of dietary patterns that are rich in fermentable substrates. What still needs further investigation is the understanding of the health implication of these enterotypes (ie, causal relation or a marker of dietary patterns), whether individuals can switch between enterotypes through modulation of the dietary pattern, and if this

can be altered in the long term [not shown in short-term feeding (48)] and ultimately influence health outcomes of the host.

GUT MICROBIOTA AND CARDIOMETABOLIC HEALTH

Modulation of the gut microbiota has gained tremendous interest in recent years in part attributable to the observation that the human gut microbial communities may play a much greater role in human health than previously thought: for example, the emerging evidence for a link between the gut microbiota and obesity. Both human and animal data support the notion that obesity is associated with a shift in the relative abundance of the 2 main phyla: Bacteroidetes and Firmicutes. Genetically obese mice have significantly less Bacteroidetes and more Firmicutes relative to their lean counterparts (56). Furthermore, there also appears to be a greater capacity of the genetically obese mice to harvest energy from the diet, as assessed by bomb calorimetry of fecal matter (57). Interestingly, germ-free mice are protected against diet-induced obesity, and the lean and obese phenotypic traits are transmissible by transplantation of cecal microbiotas into these mice (57). Suggested mechanisms include AMP-activated protein kinase– and fasting-induced adipose factor–associated pathways (58, 59). More recently, successful transplantation of human fresh or frozen fecal microbial communities into germ-free mice resulted in microbial diversity in the humanized gnotobiotic mice that closely resembled that of the host (60). This gnotobiotic mouse model holds tremendous promise as an approach to understanding the role of microbial and functional diversity and its effect on human health. There are many advantages to such a model: mice that are raised in germ-free environments, without any exposure to microbes, can be subsequently colonized with different microbial communities from an array of individuals who are healthy or have a particular disease of interest. Furthermore, this model also allows for control of host phenotype, microbial community composition, and housing conditions (60). Most recently, the gut microbiota from a mouse model of gastric bypass surgery was transferred to gnotobiotic mice, resulting in weight loss and decreased fat mass (61). The authors suggested that this may be a consequence of altered microbial production of SCFAs. This is an example of the valuable insights that can be generated from gnotobotic mice in exploring such relations to generate preliminary data before launching into costly epidemiologic and clinical trials.

The observed difference in the abundance of Bacteroidetes and Firmicutes in animal studies was also observed in a year-long weight-loss study in 12 obese individuals (45). Participants were randomly assigned to follow 1 of 2 low-calorie diets that differed in macronutrient composition (ie, fat- or carbohydrate-restricted). Irrespective of which of the 2 diets individuals were randomly assigned to receive, both diets were associated with an increase in the abundance of Bacteroidetes and a decrease in the abundance in Firmicutes. Furthermore, the increased abundance of Bacteroidetes correlated with the percentage loss of body weight and not with the caloric content of the diet. The greater abundance of Bacteroidetes and lower abundance of Firmicutes was also observed in children of rural Africa consuming a traditional plant-based dietary pattern compared with children in Western Europe consuming a typical Western diet (54). There is also evidence from gnotobiotic mice that links the influence of dietary patterns to gut microbial communities where the obesity phenotype can be induced by switching mice to a Western-type diet compared with a low-fat plant polysaccharide–rich diet (60, 62). As mentioned previously, there is also decreased phylogenetic diversity in obese individuals compared with lean individuals (46). This again suggests that obese individuals may be less resilient to change in their microbial communities with regard to preserving functional diversity.

Recent studies suggested a potential role of the gut microbiome in the pathogenesis of atherosclerosis. Trimethylamine-containing compounds (eg, choline, phosphatidylcholine, and carnitine) are metabolized by the gut microbiota to trimethylamine, which is then converted by hepatic flavin-containing monooxygenases to trimethylamine-N-oxide (TMAO), a proatherogenic metabolite (63, 64). Major dietary sources of phosphatidylcholine include many animal-based foods such as liver, eggs, and red meat (63, 65). Both animal and human studies provide evidence to support the role of gut microbial biotransformation in the formation of TMAO. After a carnitine or choline challenge, germ-free mice showed no detectable amounts of TMAO (63, 64). However, after conventionalization (germ-free mice are placed in conventional cages to allow for microbial colonization), the germ-free mice acquired the ability to produce TMAO (63, 64). In humans, short-term use of oral broad-spectrum antibiotics (for 1 wk) resulted in the suppression of TMAO production after a phosphatidylcholine or L-carnitine challenge (64, 65). TMAO was detectable after a subsequent rechallenge several weeks after the discontinuation of antibiotics. Long-term dietary patterns appear to influence the capacity to produce TMAO. Individuals adhering to vegan and vegetarian diets have lower fasting TMAO concentrations and produce significantly less TMAO after a carnitine challenge compared with omnivores (64). Individuals were further classified by enterotype, which showed that those with a relative abundance of *Prevotella* had higher plasma TMAO than those with a relative abundance of *Bacteriodes* (64). This contrasts with previous studies in which dietary patterns rich in animal protein had a greater abundance of *Bacteroides* (48, 54, 55). This observation suggests that different species within these genera may be responsible for the formation of TMAO and that classification at the genus level may not reflect these differences. In a cohort of >4000 individuals who underwent elective diagnostic cardiac catheterization, those who had major cardiovascular events (defined as death, myocardial infarction, or stroke) during a 3-y follow-up period had higher baseline concentrations of TMAO than those who did not have cardiovascular events, even after traditional risk factors were adjusted for (65). Furthermore, those individuals in the highest quartile of TMAO concentrations were at greater risk of a major cardiovascular event compared with those in the lowest quartile (HR: 2.54; 95% CI: 1.96, 3.28) (65). The proposed mechanisms of action of TMAO in promoting atherosclerosis include the suppression of reverse cholesterol transport (64) and upregulation of proatherogenic scavenger receptors (63). Collectively, these data may provide a further clue to explain the positive association between red meat consumption and CVD risk.

The gut microbiome has also been suggested to play a role in T2D. A small study in men with or without T2D showed a lower abundance of Firmicutes and the class Clostridia as well as a nonsignificant increase in Bacteroidetes and Proteobacteria in

those with T2D (66). Furthermore, the ratio of Bacteroidetes to Firmicutes was positively associated with plasma glucose concentrations. A larger metagenome-wide association study in Chinese individuals with T2D reported differences in the gut microbiota relative to controls, with a decrease in the number of butyrate-producing bacteria and an increase in the number of opportunistic pathogens (47) Similarly, this shift in the gut microbiota was also observed in a European cohort with T2D (67).

Future studies are needed to differentiate whether changes in the gut microbiome are related to cause or effect and how the associated changes and end products (eg, SCFAs, TMAO) affect metabolic functions of the host within different populations. Results of such studies may pave the way for strategies to modify the gut microbial profile and thereby provide novel approaches in the treatment and management of metabolic diseases.

EXAMPLES OF DIETARY COMPONENTS TARGETING THE GUT MICROBIOTA

Various dietary interventions have been examined in clinical trials that showed modulation or association with the gut microbiota. A few examples will be briefly discussed including dietary prebiotics, resistant starch, and interindividual differences in the metabolism of polyphenols (eg, soy isoflavones).

Undigested or nondigestible carbohydrates, such as dietary prebiotics and resistant starches, are primary substrates that escape digestion in appreciable amounts. These substrates subsequently become available for the gut microbiota for colonic fermentation. Many factors may influence the digestion of carbohydrates in the small intestine, including rate of digestion (68, 69), the food form (physical form and particle size) (70), type of preparation (cooking method and processing) (70–73), type of starch (amylose or amylopectin) (70, 74), presence of antinutrients such as α-amylase inhibitors (75, 76), transit time (77), and amount of fiber, fat, and proteins (78, 79). Gut microbial fermentation of undigested or nondigestible carbohydrates results in major end products including SCFAs (acetic, butyric, and propionic acids), gases (CO_2, CH_4, and H_2), heat, and bacterial cell mass (80, 81). Specific SCFAs have been associated with reducing the risk of developing gastrointestinal disorders, cancer, and CVD; now more recently, evidence is emerging that suggests an obesogenic role as discussed previously (82).

The term "prebiotics" was originally coined by Gibson and Roberfroid (83) who have since expanded their original definition. The prebiotic concept ("prebiotic effect") is defined as "the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host" (84). To date, much of the interest in prebiotics has been focused on nondigestible oligosaccharides, specifically inulin-type fructans (eg, inulin and oligofructose) and galactans (eg, galactooligosaccharides, *trans* galacto-oligosaccharides), which have been consistently shown to selectively stimulate the growth of bifidobacteria and in some cases lactobacilli, resulting in a significant change in the gut microbiota composition and, in turn, metabolic effects in the host (84–88). Inulin-type fructans are oligo- or polymers of D-fructose joined by $\beta(2\rightarrow1)$ bonds with an α-$(1\rightarrow2)$–linked D-glucose at the terminal end. Oligofructose refers to those fructans with degrees of polymerization between 3 and 10, and inulin are those with a degree of polymerization between 10 and 65 (89).

Gibson et al (90) showed that intakes of 15 g of oligofructose or inulin/d by participants for 15 d resulted in a significant increase in bifidobacteria from 8.8 to 9.5 $\log_{10}$/g stool and from 9.2 to 10.1 $\log_{10}$/g stool, respectively. The total bacterial counts remained unchanged, indicating that the increase in bifidobacteria resulted in a shift in the balance of microflora in the large intestine, where decreases in bacteriodes, clostridia, and fusobacteria were observed. Numerous other human studies with varying dose, substrate, duration, and subject population also resulted in similar outcomes of increased fermentation and bifidobacteria (90–97). Furthermore, increases in breath hydrogen excretion, as an indirect marker of colonic fermentation, were also observed with intakes of oligofructose and inulin (90, 98, 99). It has been suggested that a prebiotic intake of ~5–20 g/d is sufficient to induce a significant increase in colonic microbiota (100–102).

Resistant starches are a major source of fermentable carbohydrates (81, 103). Studies using resistant starch have been consistent in showing increased fecal butyrate amounts (104–107). Studies have shown that the *Ruminococcus bromii* species of Firmicutes is the key primary degrader ("keystone species") of resistant starch within the microbial community of the human colon (108, 109). Butyrate has been suggested to be an important factor in maintaining gut health, with particular relevance to colon cancer and inflammatory bowel disease (82). Butyrate is not only the preferred fuel of the colonic epithelial cells, where up to 70–90% of butyrate is metabolized by colonocytes (110), it also plays a role in regulation of epithelial cell proliferation and differentiation (80, 102, 111, 112).

The gut microbiota has the ability to biotransform an array of phytochemicals to more bioactive derivatives (113). Soy isoflavones are a family of plant-derived polyphenolic compounds with similar structure and properties to mammalian estrogen and they possess weak estrogenic activity; hence, they are commonly known as phytoestrogens. On ingestion of soy isoflavones, which are primarily found as β-glucosides, there is a need for biotransformation by the gut microbiota into bioactive metabolites (114, 115). Specifically, colonic biotransformation of soy isoflavones into more bioactive cholesterol-lowering selective estrogen receptor modulators was suggested as a mechanism by which the gut microbiota may enhance the hypocholesterolemic effect of soy (116). In a study in hyperlipidemic individuals, the consumption of soy alone resulted in a nonsignificant (~3%) reduction in LDL cholesterol, whereas when soy was taken with a prebiotic, a significant (~5%) reduction in LDL cholesterol was observed (117). The observed cholesterol-lowering may have been a result of a combination of both increased colonic biotransformation of soy isoflavones into more bioactive metabolites and/or increased colonic production of the SCFA propionate. However, other studies that examined the combination of soy with prebiotic or probiotic on lipid profile yielded mixed results (118, 119).

The emergence of the "equol hypothesis" has renewed interest in the cholesterol-lowering potential of soy, suggesting that those individuals who have a gut microbiota capable of biotransforming daidzein to equol (ie, daidzein-metabolizing

phenotype) have greater health benefits with consumption of soy foods than those who produce little or very low concentrations of equol (120). Interestingly, not all individuals appear to have the capacity to produce equol, which may be related to interindividual differences in the gut microbial communities. It is estimated that only 30–40% of adults in Western populations are "equol producers" when soy is consumed (121–126). However, adults living in the Orient, who consume soy as part of their traditional diet, have a higher prevalence of equol producers at 50–60% (127–129). Two more recent clinical trials documented a higher prevalence of equol producers as seen in the Orient, one in a Western population of vegetarians (59%) (130) and the other trial in an Italian population (69%) (131). A number of other studies assessed the effect of equol production on the cholesterol-lowering potential of soy, with mixed results (119, 121, 123, 126, 131, 132). In a series of soy food studies conducted by our group (133), equol producers (n = 30) showed a relative increase in HDL cholesterol of 5% after soy compared with nonproducers (n = 55), but no significant differences were observed for LDL cholesterol or other lipids. This study was one of the first to show that soy consumption resulted in a similar reduction in LDL cholesterol in equol producers and nonproducers, but that only in equol producers had their concentrations of HDL cholesterol and apolipoprotein A-1 preserved.

The "equol hypothesis" has generated research interest not only in determining differences between producers and nonproducers but also in the quest to identify the specific gut microbial profile responsible for the biotransformation of daidzein to equol. It was previously shown that germ-free rats colonized with a human fecal sample from an equol producer and fed a soy isoflavone–containing diet acquired the ability to produce equol (134). The same did not occur on colonization with a fecal sample from an equol nonproducer. This suggests that the daidzein-metabolizing phenotype may be transmissible. Future studies are needed with the aim of developing strategies to identify and target these microbes with the goal of increasing equol production, and more relevant, to convert an equol nonproducer to an equol producer if the benefits of equol production can be clearly defined.

CONCLUSIONS

Dietary patterns that favorably alter the gut microbiota, specifically those that emphasize plant-based foods, may have significant implications to human health. To further understand the link between such dietary patterns and the gut microbiota, future studies are needed to examine the relation between the changes in both the microbial composition and the end products on metabolic functions in the host. In turn, such studies will provide evidence to support effective strategies for modulating the gut microbial profile, thereby providing novel approaches in the treatment and management of metabolic disorders.

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