Dietary Interventions to Modulate the Gut Microbiome—How Far Away Are We From Precision Medicine

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The importance of the gut microbiome in human health and disease is fully acknowledged. A perturbation in the equilibrium among the different microbial populations living in the gut (dysbiosis) has been associated with the development of several types of diseases. Modulation of the gut microbiome through dietary intervention is an emerging therapeutic and preventive strategy for many conditions. Nevertheless, interpersonal differences in response to therapeutic treatments or dietary regimens are often observed during clinical trials, and recent research has suggested that subject-specific features of the gut microbiota may be responsible. In this review, we summarize recent findings in personalized nutrition, highlighting how individualized characterization of the microbiome may assist in designing ad hoc tailored dietary intervention for disease treatment and prevention. Moreover, we discuss the limitations and challenges encountered in integrating patient-specific microbial data into clinical practice.

Key Words: personalized nutrition, gut microbiome, dietary intervention, clinical trials

INTRODUCTION

The human body is home to at least 100 trillion (10^{14}) microorganisms, most of them inhabiting the human gut. This community includes taxa from the 3 domains of life (Bacteria, Eukarya, and Archaea) and viruses, whose combined genome harbors at least 100 times as many genes as our own genome.[1] The consortium of microbial symbionts that resides in and on the human body collectively constitutes our "microbiota"; when also considering their pool of genes and the functions for which they encode, we refer to it as the "microbiome." The human body can be considered the result of human and microbial cells, with our genetic and metabolic potential representing the arrangement of what comes from our genome and microbiome. For these reasons, our gut microbiome has earned the appellative of our "other genome."[2]

Several studies have focused on the exploration of the composition and functionality of the gut microbiome, with an emphasis on its bacterial members.[3] In healthy adults, 2 bacterial *phyla* dominate, namely Firmicutes and Bacteroidetes, which vary in their proportions across the population. Proteobacteria, Actinobacteria, and Verrucomicrobia are present at lower levels.[4] A categorization of individuals in 3 groups ("enterotypes") based on the prevalence of *Prevotella*, *Bacteroides*, or *Ruminococcus* in the gut microbiota was proposed in an attempt to simplify the complexity of the gut microbiome.[5] This classification, although appealing for understanding microbial changes in health and disease, has recently been criticized, as it may lead to an oversimplified vision of the gut microbiome, whereas the existence of smooth gradients in the abundance of dominant taxa is now considered to be more plausible.[6, 7]

In this review, we focus on the role of diet in influencing the composition and functions of the microbiome with a particular emphasis on inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). We also discuss the most recent evidence for the role of microbiome-targeted dietary interventions for promoting host health.

MICROBIAL METABOLITES IN THE GUT

Microbial symbionts interact among themselves and with the host, impacting human physiology and health. They relate to the host immune system, promoting the maturation of immune cells and driving the development of immune functions.[8, 9] Moreover, gut microbes perform a wide range of useful activities, such as fermenting and absorbing undigested compounds and synthesizing vitamins.[10, 11] They can influence host health through the production of beneficial or detrimental metabolites. Such compounds can be derived from both metabolic intermediates of the host and dietary precursors. Therefore, diet plays a major role in affecting the metabolic

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potential of gut microbes. Some specific gut microbiota members can produce short-chain fatty acids (SCFAs) from degradation of complex plant-derived polysaccharides. SCFAs, mainly butyrate, propionate, and acetate, exert recognized health-promoting functions, such as anti-inflammatory, anticarcinogenic, and immune-regulatory functions.[12, 13] Nevertheless, gut microbiota can also be responsible for the production of harmful molecules associated with detrimental effects for the host and related to the development of several diseases.[12, 13] A high-protein diet leads to the accumulation of several amino acid–derived products in the colon, such as branched-chain fatty acids, phenols, *p*-cresol, and phenylacetic acid, previously reported to be associated with pro-inflammatory and carcinogenic effects.[13, 14] Sulfate-reducing bacteria (eg,, *Desulfovibrio* spp.) produce sulfides through the catabolism of sulfur amino acids and taurine, which are toxic to colonocytes.[13] *N*-Nitroso compounds (NOCs) produced by nitration of amines derived from microbial fermentation of proteins may exert carcinogenic and mutagenic effects and are correlated with the incidence of colorectal cancer.[13] In addition, a diet rich in fat attracts more bile in the colon, where bile acids may be converted by microbial enzymes into secondary bile acids, mainly deoxycholic and lithocholic acids. These can be involved in processes linked to colorectal carcinogenesis, such as apoptosis, cell proliferation, and DNA damage induction.[14]

MICROBIOME-RELATED DISORDERS

In recent years, the "1 microbe–1 disease" model has been regarded as extremely simplistic and obsolete, whereas an increasing number of studies of the human microbiome have highlighted that many diseases may be a consequence of an overall dysbiosis status of the gut microbiome. Several gut commensal microbes may be considered pathobionts; that is, they normally inhabit the gut but may act as pathogens and cause disease when the normal gut microbial community is altered.[15] Indeed, perturbation of the equilibrium in the gut microbiome has been linked to the development of several disorders. As they are extensively reviewed elsewhere,[16–18] we provide a short overview below, with particular emphasis on IBD and IBS.

- Obesity: Gut microbiota dysbiosis in obesity is often associated with increased Firmicutes-to-Bacteroidetes ratio[19] and persistent inflammation triggered by increased systemic levels of lipopolysaccharides arising from Gram-negative bacterial cells (metabolic endotoxemia).[20, 21] Indeed, the perturbation in the gut microbiota composition caused by the consumption of a high-fat diet leads to amplified gut permeability, resulting in endotoxemia.[21]
- Type 2 diabetes and metabolic syndrome: The gut microbiome plays an important role in modulating the glycemic response, and thus in the development of type 2 diabetes (T2D). The gut microbiota of T2D patients is usually depleted of fiber-degrading and SCFA-producing bacteria, such as *Roseburia*, *Eubacterium*, and *Faecalibacterium.*[22]
- Cardiovascular diseases: Gut microbiota metabolism of choline, phosphatidylcholine, and carnitine leads to trimethylamine (TMA), which is oxidized in the liver to trimethylamine oxide (TMAO) and seems to be associated with the development of atherosclerotic plaques.[23, 24]
- Immune-mediated adverse food reactions: food allergy and celiac disease. These conditions are characterized by an abnormal response of the immune system to specific food antigens that do not affect the normal population. A genetic predisposition is involved in the development of these conditions, but environmental factors acting at least in part on gut microbiota could be also implicated.[25] Current evidence suggests that the gut microbiota and its metabolites, together with exposure to dietary factors in early life, critically influence the immunology of T-cells.[26]
- Colorectal cancer (CRC): Some bacterial species are suspected to be involved in CRC development.[27] Although the exact mechanisms remain unknown, the production of pro-inflammatory or pro-oxidative metabolites and/or toxins, such as *Bacteroides fragilis* BFT toxin,[27] and the transformation of primary bile acids into secondary bile acids[13] may be involved.
- Neurological disorders: Gut microbiota may interact with the nervous system through the production of several neuro-active molecules and modulate brain development, functions, human mood, and behavior.[28, 29] The complexity of these interactions is implied by the term "gut-brain axis."[30] Moreover, recent evidence suggests that the gut microbiome is implicated in the etiology of autism spectrum disorder, Parkinson's, Alzheimer's, and other neurodegenerative diseases, although the mechanisms underlying these diseases remain unclear.[31–33]

GUT MICROBIOME IN IBD AND IBS

Inflammatory Bowel Diseases

Inflammatory bowel diseases are chronic intestinal diseases characterized by inflammation of the bowel, including Crohn's disease (CD) and ulcerative colitis (UC). UC is manifested exclusively in colonic mucosa, whereas CD affects all areas of the gastrointestinal (GI) tract, with features such as granulomas and intestinal fibrosis. IBD affects up to 0.5% of the population, with the highest incidence in North America and Europe.[34] The incidence is increasing, especially in developing countries, with the rate of CD rising faster than that of UC.[35] This phenomenon seems to be related to changes in the Western lifestyle, suggesting that environmental factors, particularly diet, play a role in the development and progression of IBD.[36]

Furthermore, eating habits also seem to be a key factor in the clinical care of patients with IBD. Indeed, acute CD

treatment with an elemental diet resulted in a remission comparable to treatment with corticosteroids.[37] As dietary and bacterial antigens are the most common types of luminal antigen, it is reasonable to suppose that dietary factors may play an important role in the pathogenesis of IBD, possibly interacting with the gut microbiota and the mucosal immune system.[38] Gut microbiota dysbiosis was often observed in IBD patients, although a causal relationship has not been established.[39] Some studies reported a decrease of Firmicutes in IBD patients, in particular the butyrate-producing *Roseburia hominis* and *Faecalibacterium prausnitzii*.[40, 41] Conversely, Proteobacteria, particularly *Escherichia coli*,[40, 42] are commonly increased in patients with IBD, whereas the implication of some pathogenic bacteria, such as *Mycobacterium avium paratuberculosis* (MAP), adherent invasive *Escherichia coli* (AIEC), *Clostridium difficile*, *Campylobacter*, and *Salmonella*, is still controversial.[39] In addition, CD patients also showed a higher proportion of fungi compared with bacteria, with an increased *Basidiomycota:Ascomycota* ratio, a decreased proportion of *Saccharomyces cerevisiae*, and an increased abundance of *Candida albicans*.[43, 44] However, whether the dysbiosis has a causative role in the onset of the inflammation that drives IBD development or inflammation arises independently and leads to dysbiosis is a matter of ongoing debate.[39, 45]

Irritable Bowel Syndrome

IBS is a chronic disorder characterized by abdominal pain related to defecation or changes in bowel habits.[46] Several analyses reported a global prevalence of 14% among females and 9% among males.[47] IBS is commonly categorized into subtypes according to the predominant bowel habit: diarrhea-predominant, constipation-predominant, or mixed/alternating.[46] Several factors seem to be involved in the development of IBS: visceral hypersensitivity, altered brain-gut signaling, immune dysregulation, psychosocial factors, and microbiota modification.[48] However, most IBS patients report symptoms being triggered by specific foods; this causes them to limit or exclude these food items, with consequent changes in dietary habits for the management of IBS.[49] IBS patients often show an increase in Firmicutes, particularly unclassified *Clostridium* cluster IV and XIV, known SCFA producers.[50] Abnormal levels of butyrate can promote visceral hypersensitivity and atypical intestinal contractions, which are the primary clinical manifestations of IBS.[50, 51] Moreover, higher levels of mucin-degrading bacteria, such as *Ruminococcus torques* and *Akkermansia muciniphila*, were found. These species can degrade the intestinal mucus barrier and therefore may explain the gut inflammatory status in these subjects.[50]

Microbiome and diet

The gut microbiome may be influenced by several factors, among which diet may be considered the most important. The long-term habitual diet seems to be the primary factor influencing the gut microbiota. Several recent studies focused on the co-evolution between humans and their gut microbiota to understand to what extent the Westernization of diet and lifestyle have impacted our microbial symbionts and how this has affected human health. To this end, it is fundamental to study rural and traditional African or South American populations, whose lifestyles likely resemble those of Paleolithic or Neolithic humans.[14, 52–54] Consistent differences in the gut microbiome have been found. Westernization induced a loss of microbial diversity and the disappearance of specific taxa, with consequent reduction in the capability to degrade complex polysaccharides and to produce beneficial metabolites from fiber utilization.[55] Agrarian populations around the world, habitually consuming a diet rich in fruit, vegetables, and fibrous tubers and lacking in animal products, often show an enrichment in fiber-degrading bacteria, such as *Prevotella*, *Lachnospira*, *Treponema*, and *Xylanibacter*.[14, 52, 54] These changes in gut microbiota composition are reflected in its functionality. Indeed, agrarians also showed a different metabolome compared with Western subjects, with higher levels of beneficial metabolites of microbial origin, for example, SCFA.[14, 52, 54] Consistently, the consumption of a diet rich in fiber in Western subjects (vegetarian/vegan or Mediterranean-style diet) promotes higher levels of *Prevotella* and *Lachnospira* in the gut microbiota, boosting the production of beneficial SCFA.[56] Nevertheless, studies also highlighted the possibility of inducing changes in the gut microbiome through a dietary intervention, although these changes are often transitory, and the gut microbiota tends to revert to the original condition.[57] The possibility of modulation of gut microbiota composition and activity through ad hoc dietary interventions is not only fascinating but also a promising research avenue in the prevention and treatment of disease.

DIET, GUT MICROBIOTA, AND IBD-IBS TREATMENT

Several studies confirmed an association between high animal protein and fat intake and increased risk of developing IBD, both in children and in adults,[45, 58] whereas a diet rich in olive oil, fish, vegetables, fruits, grains, and nuts has been inversely associated with CD in children.[59] Moreover, several recent studies showed a protective role of breastfeeding against the risk of IBD in pediatric patients, although the mechanism is largely unclear.[60]

Based on the dietary recommendations of the National Institute for Health and Care Excellence (NICE) and the British Dietetic Association,[61, 62] the "traditional" IBS diet is based on "healthy eating," reducing fats, caffeine, and excessive fiber intake, and avoiding soft drinks and gas-producing foods. Furthermore, patients are advised to eat slowly and to chew meticulously.[61, 62]

Several dietary therapies have been proposed for the treatment of IBD/IBS patients or symptom relief; these have been extensively and recently reviewed.[45, 63] However, their efficacy is still controversial, and little is known about the effects

of these dietary treatments on the gut microbiome and thus on human health.

Specific Carbohydrate Diet

SCD is based on the restriction of complex carbohydrates and the exclusion of refined sugars from the diet, based on the rationale that the sugars and complex carbohydrates are badly absorbed and could promote intestinal inflammation.[64] SCD may contain almond, nut, and coconut flours and exclude grains (wheat, rice, corn); most dairy products are avoided, except for homemade yogurt fermented for 24 hours to deplete lactose. Two retrospective studies that assessed SCD as the sole method of treatment in CD children showed that the use of SCD for 5 to 30 months had positive effects on inflammatory markers and clinical presentation of the disease. The direct reason for the improvement was not known, but modification of the intestinal microbiome was proposed as one of the reasons.[65, 66]

Exclusive Enteral Nutrition

Exclusive enteral nutrition (EEN), is likely to have substantial effects on gut microbiota, in part due to carbohydrate composition. EEN therapy with elemental, semi-elemental, or polymeric formula diets has been widely studied; it is the only dietary intervention that induced remission of Crohn's disease and is therefore the firstline therapy in many parts of the world. In addition to reducing symptoms, EEN has been associated with mucosal healing, which may be a superior predictor of long-term outcome.[67] The results about the effect of EEN on gut microbiota are still preliminary and conflicting. Lewis and coworkers[44] showed that EEN induces changes in the gut microbiome of CD children, but these changes did not lead to a microbiome resembling that of healthy controls, whereas Shiga and collaborators[68] reported a reduction of *Bacteroides fragilis* group, commonly found in inflammatory lesions of CD patients. Markers commonly associated with health, such as microbial diversity or abundance of butyrate-producing microbes (eg,, *Faecalibacterium prausnitzii*) and butyrate levels were decreased during EEN intervention.[69, 70] Another interesting observation was the higher efficacy of EEN in ileal CD compared with colonic CD or UC.[71] However, identifying the exact role of the diet is hampered by interindividual variability in the microbial community and response to intervention. The role of specific bacteria in disease improvement is yet to be confirmed, as conflicting data have been reported on the protective effect of *Faecalibacterium prausnitzii* and other microbes in pediatric patients.[72, 73]

Low-FODMAPs Diet

A recent dietary strategy proposed for IBD/IBS management is the low–fermentable oligo-, di- and monosaccharides and polyols (FODMAPs) diet.[45, 63, 74] Diets rich in highly fermentable but poorly absorbed short-chain carbohydrates and polyols are hypothesized to trigger symptoms in patients with IBS.[75] The digestibility of carbohydrates varies according to the absence, or reduced production, of hydrolase enzymes needed for their digestion. When these sugars arrive undigested in the colon, they cause an osmotic effect that attracts water in the small intestine. Moreover, they can be fermented by the colonic microbiota, with accumulation of gases, including hydrogen and methane. Both water and gas increase intestinal volume, which, together with visceral hypersensitivity, causes pain. However, other mechanisms are evoked in the injurious effect of FODMAPs. Indeed, these molecules seem to increase GI motility, which further reduces absorption and increases potential colonic fermentation.[76] A low-FODMAPs diet can reduce the production of SCFAs as a result of limited availability of fermentable substrates and decreased levels of taxa involved in SCFA production.[77] Because SCFAs induce visceral hypersensitivity, the decrease in their luminal levels may represent another mechanism accounting for the efficacy of a low-FODMAPs diet.[78, 79]

FODMAPs might be considered "fast food" for bacteria and can lead to the expansion of specific bacterial populations in the distal small intestine. This bacterial overgrowth is similar in patients with IBS, celiac disease, and Crohn's disease.[80, 81] Bacterial overgrowth in the small intestine has been associated with increased small intestinal permeability. Fructo-oligosaccharides increase intestinal permeability also in the colon, and in rats experimentally infected with *Salmonella*, FODMAPs predispose to severe colitis, in comparison with a mild colonic inflammation occurring in controls.[82] It is worth emphasizing that rapidly fermentable fiber compromises the proximal large bowel, whereas concurrent ingestion of slowly fermentable or nonfermentable substrates decreases the rate of fermentation, shifting it more distally in the colon.[83] This mechanism could also be explained by the different localization of the inflammatory processes. Hence, the pathogenic hypothesis of inflammation development in IBD considers an initial increase in fermentable sugar (eg,, FODMAPs) consumption, which determines bacterial overgrowth and a consequent increase in intestinal permeability. Bacterial or antigen translocation triggers an immunologic response and activation of the inflammatory cascade. Data from the literature suggest that a low-FODMAPs diet decreases gut symptoms in IBD and IBS patients, also promoting a shift in the gut microbiota.[45, 48, 83] In a single-blind randomized clinical trial (RCT) in patients with IBS, a low-FODMAPs diet promoted an increased richness of Actinobacteria, Firmicutes, and Clostridiales and a decreased abundance of bifidobacteria compared with a high-FODMAPs diet.[83] In the same study, a higher abundance of the hydrogen-consuming genus *Adlercreutzia* was found in the low-FODMAPs group, possibly contributing to a reduction of symptoms, due to the consumption of the accumulated gas in the intestine.[83] Consistently, another study

reported a decreased proportion of bifidobacteria in patients with IBS after a low-FODMAPs diet.[75] In patients with Crohn's disease, FODMAPs intake is also associated with changes in fecal microbiota. Indeed, an Australian diet rich in FODMAPs boosted the levels of butyrate-producing *Clostridium* cluster XIVa and mucus-associated *Akkermansia muciniphila*. This diet seems to increase IBS-like symptoms.[75] Moreover, Chumpitazi and coworkers[84] observed that children with IBS showing a positive response to a 2-day low-FODMAP intervention had higher baseline abundance of *Sporobacter* and *Subdoligranum* and decreased *Bacteroides* compared with nonresponders. Modulation of FODMAPs intake seems to be a promising strategy for treating abdominal symptoms in patients with IBD and IBS. However, potential hazards do exist, such as the decrease in bifidobacteria and other fiber-degrading bacteria, recognized for their health-promoting benefits, along with the consequent reduction of beneficial SCFA levels.[85] Therefore, the routine utilization of this strategy for IBD/IBS treatment should be applied carefully, ideally coupled with gut microbiota monitoring, to avoid induction of dysbiosis. Considering these factors, the addition of probiotics during a low-FODMAPs diet may be a possible solution to optimize clinical management and prevent the detrimental effects of a low-FODMAPs diet on the gut microbiome.

PERSONALIZED NUTRITION AND GUT MICROBIOME

Key Factors in a Dietary Intervention for Personalized Clinical Applications

Clinical interventions cannot be based solely on clinical experience and observational studies but need to be based on evidence provided by RCTs.[86, 87] Despite their cost and labor-intensiveness, RCTs are the most valid research design for evidence-based medicine; nutrition is a field in need of better clinical research.[88] This gap is the focus of the European Clinical Research Infrastructures Network (ECRIN) Integrating Activity (IA; http://www.ecrin.org/activities/projects), which has identified barriers to good clinical research and offered solutions to improve evidence-based clinical practice.[89–91]

Critical Factors in Nutritional Studies

The main difficulty in identifying a causal relationship between the consumption of a specific diet, food, or nutrient and a health outcome is the coexistence of several factors that interact and may possibly lead to biased results if not appropriately considered. These factors include population characteristics and lifestyle; the bioavailability of the nutrient (which is in turn influenced by aspects related to the technology of production and method of consumption); the timing, frequency, and duration of nutrient exposition; and contextual factors (eg,, foods, supplements, medications, and diseases that can aid or hinder absorption).

Therefore, to demonstrate a causal relationship of a specific nutrient/diet on a health outcome, a multidisciplinary and multisystem approach is necessary. This approach requires simultaneously considering nutrition, genetics, the microbiome, and environment (exposome) in the implementation of nutritional intervention studies, as was recently suggested in an epidemiological study.[92] This strategy could have multiple spillover effects, including being able to explain and overcome the discrepancies present in the scientific literature for the efficacy of some nutrients/diets, achieving stronger evidence and consensus in the scientific community, and producing practical and valuable applications in the fields of personalized nutrition, preventive and precision medicine, and functional food development.

Critical factors in the implementation of nutritional intervention studies and opportunities beyond nutrition are summarized in Figure 1; a systems-based approach could work in nutritional interventions and specifically in nutritional RCTs. The scheme highlights 4 critical points in RCTs, namely, study design, subjects, biomarkers, and data analysis. Each of them must be carefully considered during the implementation of the trial tailored to nutrient/diet and targeted health outcomes.

Study design

The type and form of nutrient, whether provided as a supplement or included in a food, can considerably influence the study design. Indeed, in the case of a supplement, a placebo (a pill/powder/tablet/liquid similar to the experimental one but without the putative bioactive compound) can be provided and a double-blind trial (which is the gold standard design because it guarantees the blinding of both subjects and investigators) can be performed. Conversely, in the case of a nutrient included in a food or a diet, finding the correct control intervention can be a remarkably critical point. This affects the study design and the data analysis. In the frame of study design, blinding can be compromised because, without a placebo, a double-blind trial is impossible and the dose/frequency of food consumption and the duration of the intervention must be carefully considered in view of the bioavailability of the putative bioactive nutrient in the food.

Sample size calculation and study power

Power analysis for sample size calculation is usually carried out based on variations in clinical parameters. Calculating sample size based on microbiome features, although possible, may often be impracticable, as we do not know the effect that a specific dietary intervention may have on the complex consortium of gut microorganisms, with diverse taxa responding in different ways to the treatment. Focusing on the abundance of only a few taxa may be also unsuccessful. Moreover, due to the high interpersonal variability in gut microbiome composition, the resulting sample size would be excessively high, making the study unfeasible. As many of the studies found

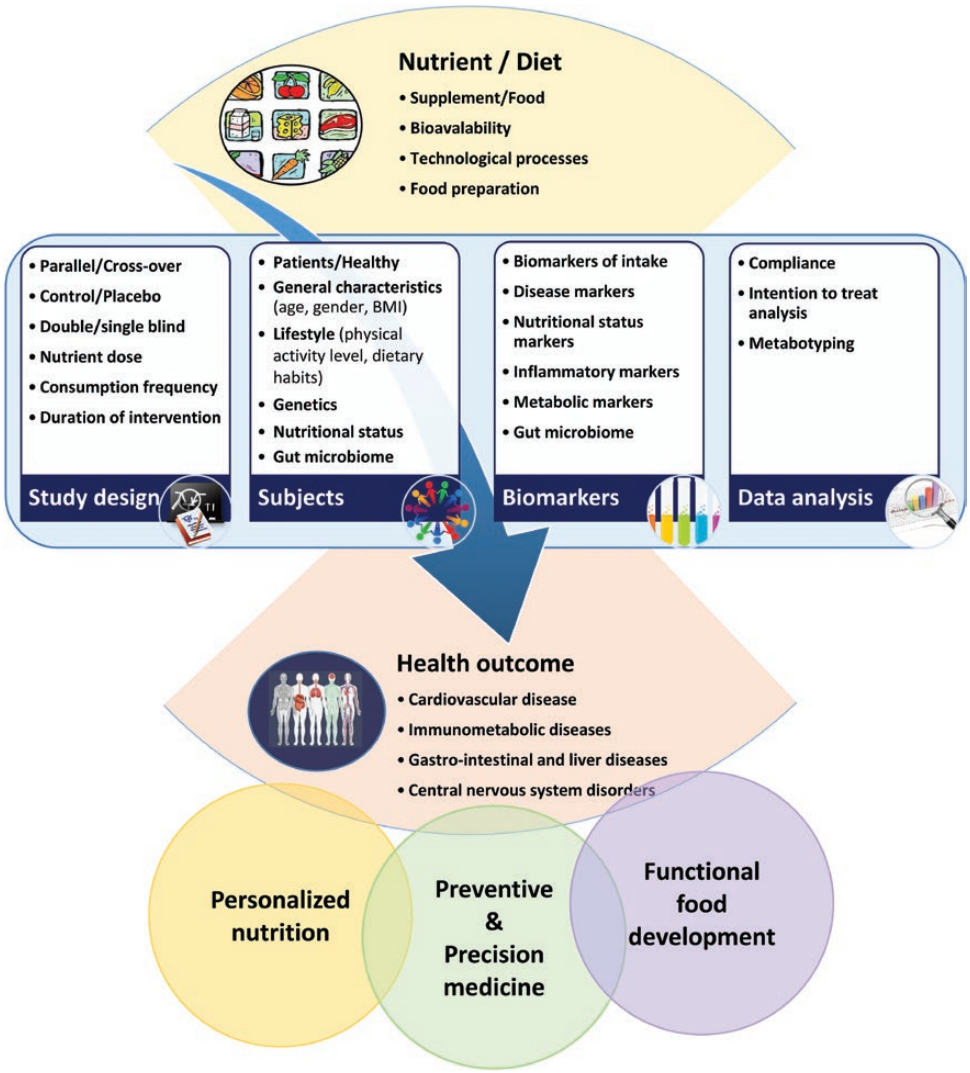

FIGURE 1. Critical factors in the implementation of nutritional intervention studies and opportunities beyond nutrition. Randomized clinical trials represent the gold standard to achieve evidence-based medicine. Effective nutritional RCTs must be tailored to the specific nutrient/diet and health outcome of interest. The scientific validity of evidence achieved by RCTs depends on the critical management of 4 main points, namely study design, subjects, biomarkers of responsiveness, and data analysis. Numerous factors coexist and interact between each other to influence the effect of nutrient/diet on a specific health outcome. These factors mainly include population characteristics and lifestyle, dose and bioavailability of the nutrient, timing, frequency, and duration of nutrient exposition, and contextual factors. Due to the involvement of the gut microbiome in the fine interplay between nutrient/diet-subjects-health, its consideration at multiple steps of RCTs (ie, at selection of subjects, biomarkers, and data analysis) may be crucial. The proper consideration and management of all the critical factors allows scientists to achieve evidence that can find application in the fields of personalized nutrition, preventive and precision medicine, and functional food development.

in the literature are based on a small number of subjects, the effects on the microbiome may be hidden by high interpersonal variability, and this may explain the often inconclusive results obtained in terms of the modulatory effect on the microbiome. Tools for sample size calculation in microbiome studies have been recently developed; however, their use remains limited.[93, 94]

Subjects selection

Subjects' characteristics, such as age, nutritional and health status, genetics, the gut microbiome, and behavioral and lifestyle factors may enormously influence the metabolic response and the health effect of a nutrient/diet. Thus, having clear and strict inclusion and exclusion criteria in RCTs may allow investigators to have interventions with smaller numbers of subjects while maintaining a good power of the study to reduce costs and time of the trials and to enhance the probability of clarifying the mechanisms underlying the health effect. The latter aspect is also influenced by biomarkers of effectiveness monitored during the study. In the case of clinical trials involving patients, the evaluation of markers of the specific

disease under investigation is necessary. In addition, other biomarkers that are usually considered in subjects at a predisease stage may also be monitored. These include inflammatory, nutritional, and metabolic biomarkers, and gut microbiota composition. Most belong to the fine net of signaling mediators involving the metabolic, neuroendocrine, and immune systems; orchestrating the response to a nutrient/diet; and the organ-by-organ communication and homeostasis processes within the body.[11, 95, 96] Finally, to monitor changes in the gut microbiome, antibiotic or probiotic assumption should be considered as an exclusion criterion, or, when this is not possible (eg, when intervention is addressed to patients), investigators should consider it a possible confounding factor.

Biomarker evaluation and data analysis

In the frame of data analysis, the evaluation of individual compliance is the critical factor. Indeed, in the presence of a supplement/placebo, the count of not-consumed doses may be sufficient, whereas in the case of a food/diet-based intervention, the evaluation of individual dietary intake (through food frequency questionnaires, 24-hour recall, weighed food record, etc.) or biomarkers of food/diet intake in biological fluids (through metabolomics analysis) is necessary. Although univocal biomarkers of intake have not been recognized for many foods, metabolomics of biological fluids is inestimably valuable for RCTs because it can show the impact of the intervention on individual nutrient metabolism in relation to the subjects' characteristics being considered in the study. This aspect is fundamental in targeted nutrition and medicine. Mounting evidence shows that through metabotyping—that is, grouping metabolically similar individuals—personalized nutritional and pharmacological strategies may be achieved.[92, 97] On the other hand, the approach of grouping similar individuals for some features can be applied in RCTs both at the stage of subject selection and during data analysis.

Overall, a multisystem approach in clinical trials is the most valuable way to achieve evidence-based medicine and to reduce the gap currently existing between research and clinical findings with possible practical applications in the fields of personalized nutrition, preventive and precision medicine, and functional food development.

Dietary Interventions for the Modulation of the Gut Microbiome

Several studies highlighted the effect of a dietary intervention on the gut microbiome, focusing on the addition of a specific supplement to the diet (mainly different types of dietary fiber) or administering diets enriched in carbohydrates, proteins, or fats (Table 1). Adding prebiotic fiber to the diet may lead to the enrichment of fiber-degrading bacteria in the gut and improved metabolic health, but results reported in the current literature have been conflicting. Studies are often limited to a small number of subjects (20 per treatment group or fewer in many cases) (Table 1).

Moreover, comparison of results across studies is not always possible, as many confounding factors exist, making it necessary to have standardized procedures for sample collection, storage, and subsequent analyses,[115] such as those proposed by the International Human Microbiome Standard Consortium (http://www.microbiome-standards.org).

Although host genomics may also be implicated, intersubject variation in gut microbiota composition may explain the different metabolic responses observed with the same treatment. Interpersonal differences in response to the same type of drug have been often observed in therapeutic routines. The gut microbiome may be involved in this process through bio-transformation of bioactive compounds contained in administered drugs, reducing or sometimes enhancing their effects.[10, 116] Statins, commonly used to reduce plasma low-density lipoprotein (LDL) levels, are an example of microbiome-driven personalized response to drugs. Indeed, subjects positively responding to statin treatment showed increased levels of specific secondary bile acids of microbial origin.[10] Therefore, the design of therapeutic treatments should consider personalized microbiome features and their effects on drug metabolism, toxicity, and efficacy. Accordingly, recent studies highlighted that subject-specific gut microbiome traits cannot be disregarded if diet must be used to beneficially modulate microbiome activities for therapeutic approaches (Fig. 2). Indeed, the gut microbiota may be responsible for unpredictable results in intervention studies. Features of the gut microbiome before starting the intervention (eg, overall microbial diversity or abundance of specific taxa, such as *Prevotella copri* or *Akkermansia muciniphila*) were previously suggested to be discriminant factors between subjects showing a metabolic improvement (eg, decrease in LDL cholesterol, inflammatory markers, or insulin resistance) to a dietary intervention and those who were not beneficially impacted (Fig. 2). For example, 3-day consumption of barley kernel fiber led to improved glucose metabolism, reducing postprandial glycemic response (PPGR) and insulin, but only in a subset of the cohort studied.[108] Separating responders from nonresponders, the authors observed higher *Prevotella copri* concentrations in responders. Moreover, gavaging mice with live *P. copri* cells, they confirmed the positive effect exerted on glucose metabolism, possibly due to a promotion of hepatic glycogen storage.[108] In contrast, Pedersen and coworkers[117] suggested that *P. copri* may be responsible for branched-chain amino acid production and induce insulin resistance, demonstrating that the association of a whole microbial genus or species with a specific metabolic outcome may be an oversimplification.[118, 119]

Indeed, it must be noted that high variability at the strain level exists in the gut microbiome, and in any other complex environment, although this point was largely overlooked until recently. Every species may be represented by several different strains, with intersubject variability.[120] Strains belonging to

TABLE 1: Main Clinical Trials Assessing the Gut Microbiome and Metabolic Responses After Dietary Intervention

	Intervention	Population		Outcomes	
Study	Description	No.	Description	Microbiota	Bioclinical Variables
Bennet et al., 2017[98]	4 weeks of low-FODMAPs or traditional IBS	67	IBS patients BMI 24.0 ± 6.0 kg/m^2 17% male	No change in the microbiota after traditional IBS diet both in responders and nonresponders; >*Bacteroides stercoris*, *Ruminococcus gnavus*, *Dorea*, and several *Enterobacteriaceae* at baseline in nonresponders compared with responders to low-FODMAPs diet	Decrease in IBS symptoms Severity score ≥50 in responders
Candela et al., 2016[99]	3 weeks of macrobiotic diet (Ma-Pi; enriched in fiber, hypocaloric) or control diet with the same energy intake	40	Type 2 diabetes BMI 34.3 ± 6.5 kg/m^2 50% male	>*Akkermansia*, *Ruminococcus*, *Faecalibacterium*, *Blautia* after both the dietary intervention; <*Collinsella*, *Streptococcus*, and >*Akkermansia* after Ma-Pi compared with control diet	Decrease of postprandial glucose, LDL cholesterol, insulin resistance, inflammatory markers after Ma-Pi diet
Chumpitazi et al., 2015[100]	2 days of low-FODMAPs or typical American diet	33	IBS pediatric patients (age 7–17 years) BMI and sex not provided	>*Bacteroides*, *Ruminococcaceae*, *Dorea*, *Faecalibacterium prausnitzii* at baseline in responders	Decrease in abdominal pain and gastrointestinal symptoms in responders
Costabile et al., 2008[101]	3 weeks of supplement with 48 g of whole grain (WG) or wheat bran	31	BMI 25.0 ± 5.0 kg/m^2 48% male	>*Bifidobacterium*, *Lactobacillus* after WG	Increase of phenolic acids in WG, no changes in blood lipids, cholesterol, glucose, insulin
Cotillard et al., 2013[102]	6 weeks of hypocaloric, high-protein diet (1200 and 1500 Kcal/d for men and women, respectively); 6 weeks of stabilization diet (20% caloric increase)	49	BMI 33.2 ± 0.5 kg/m^2 16% male Divided into low (LGC; n = 20) and high (HGC; n = 29) gene count	>diversity in LGC after the dietary intervention	Decrease of insulin resistance, triglycerides, inflammatory markers in HGC after intervention
Dao et al., 2016[103]	6 weeks of hypocaloric, high-protein, low-carbohydrate diet enriched in fiber; 6 weeks of weight maintenance diet	49	BMI 32.5 ± 1.0 kg/m^2 17% male Divided into high (HAk; n = 25) and low (LAk; n = 24) *Akkermansia muciniphila* abundance	<*Akkermansia muciniphila* after the intervention in HAk but still higher than LAk	Higher decrease of LDL cholesterol, plasma glucose, tryglycerides, insulin resistance in subjects with higher baseline levels of *Akkermansia* (HAk)
Hald et al., 2016[104]	4 weeks of Western-style diet or high-carbohydrate diet (enriched in arabinoxylan and resistant starch)	19	BMI 30.0 ± 2.0 kg/m^2 Sex not provided	>*Bifidobacterium* and <*Bacteroides*, *Odoribacter*, *Desulfovibrionaceae*, *Ruminococcus* (*Lachnospiraceae*)	-
Haro et al., 2016[105]	12 months of Mediterranean-style diet (MD; 35 fat—22% monounsaturated; 6% polyunsaturated and 7% saturated) or low-fat, high-carbohydrate diet (LFHCD; 28% fat—12% monounsaturated; 8% polyunsaturated and 8% saturated)	20	BMI 32.2 ± 0.5 kg/m^2 100% male	>*Roseburia*, *Oscillospira*, and <*Prevotella* in MD >*Prevotella*, *Faecalibacterium prausnitzii*, and <*Roseburia* in LFHCD	Increase of insulin sensitivity in both MD and LFHCD

TABLE 1: Continued

	Intervention	Population		Outcomes	
Study	Description	No.	Description	Microbiota	Bioclinical Variables
Hjorth et al., 2017[106]	6 months of ad libitum New Nordic Diet (NDD; rich in fruit, vegetables, and whole grains) or Average Danish Diet	62	BMI 30.0 ± 1.0 kg/m^2 33% male Divided into high (n = 28) and low (n = 34) *Prevotella*-to-*Bacteroides* ratio groups	-	Higher body fat and waist circumference loss after NDD in high *Prevotella*-to-*Bacteroides* group
Korem et al., 2017[107]	1 week of supplement with 145g/d of sourdough-leavened whole grain bread or 110 g/d of industrial white bread, separated by 2 weeks of washout	20	BMI 27.9 ± 4.0 kg/m^2 45% male	No effect detected	Interpersonal variation in the glycemic response after bread consumption is dependent on baseline microbiome features
Kovatcheva-Datchary et al., 2015[108]	3 days of supplement with barley kernel bread (BKB) or white bread	20	BMI 25 ± 3.0 kg/m^2 10% male Divided into responders (n = 10) and non-responders (n = 10) based on metabolic response to BKB intervention	>*Prevotella* in responders compared with nonresponders; >*Prevotella copri* and methanogenic Archaea after BKB intervention only in responders	Decrease of postprandial blood glucose and insulin after BKB intervention only in responders
Louis et al., 2016[109]	3 months of very low-calorie diet (800 Kcal/d, enriched in inulin); 9 months of gradual reintroduction of a normal diet; 12 months of weight maintenance diet	16	BMI 43.0 ± 7.0 kg/m^2 44% male Persistent success group (PS; >10% weight loss after 24 months, n = 9); no persistent success (NS; <10% weight loss after 24 months, n = 7)	Different microbiota at baseline in PS and NS groups >*Akkermansia*, *Alistipes*, *Clostridium leptum*, and <*Bacteroides* in PS at baseline Only *Akkermansia* stable after 2 years	Decrease of insulin resistance
Pedersen et al., 2016[110]	12 weeks of supplement with galacto-oligosaccharyde mixture or placebo	29	Type 2 diabetes BMI 28.0 ± 6.5 kg/m^2 100% male	No effect detected	No effect detected
Roager et al., 2017[111]	8 weeks of intervention with a whole grain–enriched diet and 8 weeks of intervention with a refined grain diet, separated by a washout period of 6 weeks	60	High risk of metabolic syndrome BMI 25–35 kg/m^2 Sex not provided	>*Faecalibacterium prausnitzii* and *Prevotella copri* after whole grain diet *Bacteroides thetaiotaomicron* increased after refined grain diet	Decrease of inflammatory markers Weight loss
Vanegas et al., 2017[112]	2 weeks of run-in consuming a Western-style diet, followed by 6 weeks of weight-maintaining diet supplemented with 8 g/1000 Kcal of refined grains (RG) or 16 g/1000 Kcal WG	81	BMI 26 ± 0.47 kg/m^2 63% and 59% male in RG and WG, respectively	>*Lachnospira*, *Roseburia*, and <*Enterobacteriaceae* after WG intervention compared with RG	No effect on inflammatory and immune markers
Vitaglione et al., 2015[113]	8 weeks of WG (70 g/d) or refined grain (RF; 60 g/d)	68	BMI 30 ± 0.9 kg/m^2 31% and 37% male in WG and RF groups, respectively	>*Prevotella* and <*Dialister*, *Bifidobacterium*, *Blautia*, *Collinsella* in WG	Decrease of inflammatory markers, increase of phenolic acids
Walker et al., 2011[114]	1 week of weight maintenance, 3 weeks of non-starch polysaccharides, followed by 3 weeks of resistant starch (RS) and 3 weeks of high-protein, hypocaloric diet	14	BMI 39.4 ± 1.5 kg/m^2 100% male	>*Eubacterium rectale*, *Ruminococcus bromii* in RS	-

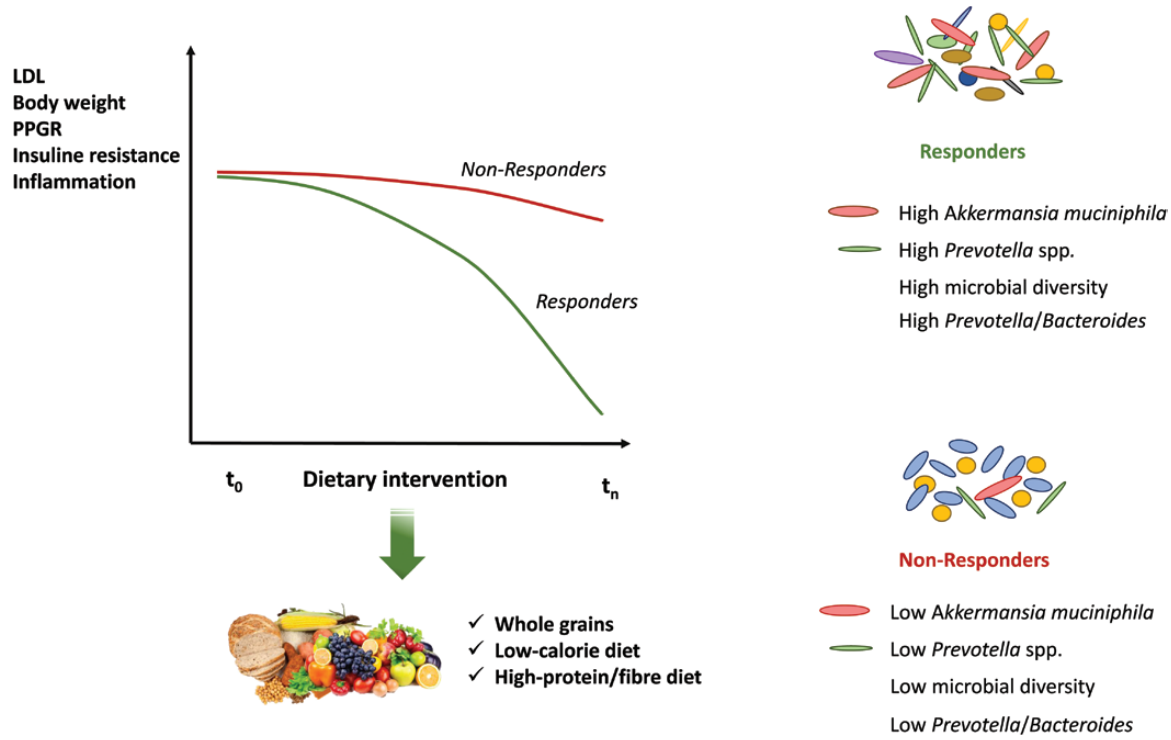

FIGURE 2. Subject-specific gut microbiota features may affect the response to a dietary intervention. The metabolic response to a dietary intervention is person-specific, and the same type of food or dietary pattern may produce different effects. Dividing responders and nonresponders, recent studies showed differences in gut microbiota composition and that this may be the cause of the personalized response to a dietary intervention, thus highlighting the necessity of personally tailored nutrition based on gut microbiota composition for therapeutic and preventive purposes.

the same species may harbor a significantly different genomic repertoire and may respond in different ways to dietary components,[118, 119] making it even more difficult to demonstrate a causative role of diet in the modulation of the gut microbiome.

Different subjects may have distinctive metabolic responses to the same food. Subjects with a higher *Prevotella*-to-*Bacteroides* ratio (P/B) had higher weight loss after consuming a 6-month high-fiber diet compared with the low P/B group,[106] whereas Dao and coworkers[103] observed that obese subjects with higher levels of *Akkermansia muciniphila* showed better metabolic outcomes (lower insulin resistance, LDL cholesterol) compared with those with a lower baseline concentration of this microbe, when treated with a hypocaloric, high-protein and -fiber diet (Table 1). In addition, it was demonstrated that a meal cannot be considered "good" for everybody.[121] The authors integrated gut microbiota features with anthropometric and metabolic measures, dietary habits, physical activity, and lifestyle in a machine-learning algorithm that accurately predicted personalized PPGR to real-life meals. This predictive strategy was then used to personalize dietary intervention and modify postprandial glucose response. They demonstrated that baseline microbiota composition may be implicated in the subject-specific response to a dietary intervention and that considering these differences may help in designing tailored meals for improving metabolic health.[121] Accordingly, the same authors suggested that the glycemic index of a food alone cannot always be useful in predicting the glycemic response. They found the PPGR to white or whole grain bread to be person-specific and used microbiome composition to predict which type of bread resulted in the best glycemic response.[107] Indeed, subject-specific signatures in the gut microbiome may be responsible for weight regain after a dietary intervention, and the extent of such regain may be predicted by integrating gut microbiome features in a machine-learning algorithm.[122]

Although many studies focused on the administration of specific food supplements, the habitual consumption of a healthy and diverse diet, such as that based on the Mediterranean model, is recognized to shape the gut microbiome and to promote the production of beneficial metabolites.[56] Although with a limited number of subjects, Haro and coworkers[105] demonstrated that dietary treatment for 1 year with a Mediterranean-style diet improved insulin sensitivity and increased the abundance of SCFA-producing microbes (Table 1). These examples suggest that following a healthy and diverse dietary pattern and lifestyle can contribute to maintaining a "healthy" microbiome without necessarily adopting microbiome-targeted nutritional interventions.

Most of the studies available on targeted dietary modulation of the gut microbiome have focused on metabolic disorders (Table 1). However, the same approach may be useful for

other pathologies, such as IBD and IBS. Indeed, Bennet and coworkers[98] found different baseline microbiota composition in adult IBS patients responding or not to a low-FODMAP diet (ie, showing a decrease of the IBS Symptoms Severity Score). Nonresponders had higher baseline abundance of several taxa, eg, *Bacteroides stercoris*, *Pseudomonas*, *Acinetobacter*, and *Ruminococcus gnavus*, compared with responders. Baseline microbiota features were implemented in a Random Forest model, which was used to predict the probability of a positive response to the dietary treatment. This pioneer study first highlighted the possibility to choose the most appropriate dietary treatment for IBS patients, based on their gut microbiota composition.

Based on these observations, it is plausible that in the near future, subjects will be stratified based on their gut microbiome features and study enrollment will be performed considering their baseline microbiome. As we know some associations of specific microbial genera/species with diet and/or diseases, we can speculate on the possibility of stratifying subjects according to the abundance of microbial taxa recognized for certain functional activities (eg, fiber-degrading) or for the production of beneficial/detrimental metabolites. Although considering the microbiota as a stratification factor for subject enrollment is promising, several issues arise, including increased enrollment costs and the intrasubject variability of the gut microbiota even in a short time frame. The possibility of stratifying the study population a posteriori for the similarity of the microbiome or microbial metabolite profile during data analysis is also an alternative. Nevertheless, it must be pointed out that these secondary analyses do not preserve the benefits of randomization. In addition, such stratifications are less likely to be reproducible due to the risk of chance findings related to multiple testing. Despite the abovementioned limitations, modulating and manipulating the gut microbiome with a personally designed dietary intervention to induce changes in its composition and functions is surely a promising application for both therapeutic and preventive clinical strategies.

CONCLUSIONS

Microbiome-targeted dietary interventions constitute a powerful and tantalizing tool for the prevention and treatment of different diseases. We are still quite far from microbiome-targeted precision medicine, but we are surely on the right scientific path to developing an exhaustive set of tools and clinical knowledge to fill the current gaps. Personalized nutrition based on microbiome features is currently being attempted, although its real impact and benefits suffer from the aforementioned limitations. Most of the studies available are observational, and controlled clinical trials targeting the microbiome are still too scarce to draw definitive conclusions or to propose a standardized protocol for modulating the gut microbiome through the diet for therapeutic purposes. Future intervention studies will surely provide new knowledge and will help in overcoming the current issues associated with such types of interventions. Indeed, interindividual variations in gut microbiome composition and functions, along with the need to study the effect of dietary changes on microbiome functions at the strain level, make the use of such interventions still only exploratory in clinical practice.

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