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Over-feeding the gut microbiome: A scoping review on health implications and therapeutic perspectives

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Abstract

The human gut microbiome has gained increasing attention over the past two decades. Several findings have shown that this complex and dynamic microbial ecosystem can contribute to the maintenance of host health or, when subject to imbalances, to the pathogenesis of various enteric and non-enteric diseases. This scoping review summarizes the current knowledge on how the gut microbiota and microbially-derived compounds affect host metabolism, especially in the context of obesity and related disorders. Examples of microbiome-based targeted intervention strategies that aim to restore and maintain an eubiotic layout are then discussed. Adjuvant therapeutic interventions to alleviate obesity and associated comorbidities are traditionally based on diet modulation and the supplementation of prebiotics, probiotics and synbiotics. However, these approaches have shown only moderate ability to induce sustained changes in the gut microbial ecosystem, making the development of innovative and tailored microbiome-based intervention strategies of utmost importance in clinical practice. In this regard, the administration of next-generation probiotics and engineered microbiomes has shown promising results, together with more radical intervention strategies based on the replacement of the dysbiotic ecosystem by means of fecal microbiota transplantation from healthy donors or with the introduction of synthetic communities specifically designed to achieve the desired therapeutic outcome. Finally, we provide a perspective for future translational investigations through the implementation of bioinformatics approaches, including machine and deep learning, to predict health risks and therapeutic outcomes.

Key Words: Gut microbiome; Microbial metabolites; Obesity; Next-generation probiotics; Fecal microbiota transplantation; Deep learning

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Core Tip: The gut microbiome (GM) has gained increasing attention in recent years due to its key role in contributing to host health, potentially serving as a target for personalized precision medicine. This review summarizes the current evidence for the involvement of the GM in the regulation of various pathophysiological aspects, particularly in obesity and related comorbidities. The influence of diet and the molecules produced by commensal microorganisms is discussed, together with traditional and innovative microbiome-based strategies in the prevention and treatment of obesity, up to the development of machine and deep learning bioinformatics tools for the prediction of health risks and therapeutic outcomes.

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INTRODUCTION

Over the past two decades, the trillion-member community that resides in the human gastrointestinal tract (*i.e.*, the gut microbiome-GM) has emerged as a key regulator of host physiology, supporting overall host health or, vice versa, contributing to triggering and sustaining pathological conditions when altered. The wide range of metabolic activities and the multiple levels of bidirectional interaction with the host strongly support GM as a strategic therapeutic target, laying the foundations for the development of innovative microbiome-tailored intervention strategies aimed at restoring an eubiotic layout. In parallel, advances in sequencing techniques and bioinformatics tools are proving crucial to deepen our understanding of the complex interactions established by the GM with the host, as well as to rationally fine-tune and successfully translate personalized microbiome-based interventions into clinical practice.

Our scoping review aims to discuss the state of the art on research and application aspects related to the role of GM in obesity, a complex and multifactorial disease that represents a major health risk factor. In particular, we first discuss the influence of GM on human physiology and its contribution to the pathogenesis of numerous enteric and non-enteric diseases when imbalances occur. Next, we focus on evidence for a link between GM and obesity and discuss the growing literature on the impact of diet on GM structure, and the key role played by GM-produced or derived bioactive compounds [*e.g.*, fatty acids, amines, bile acids (BAs) and neuroactive metabolites] in affecting host physiology and metabolism, at both local and systemic level. We then review the adjuvant interventions currently available to manipulate GM and alleviate the obesity phenotype, which are based on diet modulation and the supplementation of prebiotics, probiotics and synbiotics. Since these traditional approaches have shown only moderate ability to induce sustained change in the complex and dynamic GM ecosystem, we stress the need to develop innovative and tailored microbiome-based intervention strategies, such as the administration of next-generation probiotics (NGPs) and engineered microbiomes, to be organically integrated into clinical practice. More radical approaches involving replacement of the dysbiotic microbial ecosystem through fecal microbiota transplantation (FMT) or the infusion of synthetic microbial communities, rationally designed to meet the desired therapeutic outcome and patient needs, are also discussed as promising alternatives for personalized clinical applications. Finally, we provide a perspective for future translational investigations through the implementation of bioinformatics approaches, including machine and deep learning, to predict health risks and therapeutic outcomes. Current clinical practice is increasingly leveraging new artificial intelligence technologies and refined bioinformatics approaches, but still little has been done in relation to GM. Therefore, we conclude by discussing the possibilities offered by machine and deep learning for the development of microbiome-based strategies, especially in the context of obesity and related morbidities. All articles included in this review were identified through

the PubMed platform, the full-text archive of biomedical and life sciences journal literature at the United States National Institutes of Health's National Library of Medicine. The most pertinent and relevant articles for each aforementioned topic were selected and commented on.

HUMAN GM: WHERE DO WE STAND?

From the development of next-generation sequencing approaches and their application to the microbiome field, the analysis of the intestinal microbial community has had a strong burst, as evidenced by > 3 million hits returned by typing "gut microbiota" on the NCBI database (as accessed on April 26, 2021). Of course, this is not surprising when one considers the plethora of actors and functions involved in the whole GM field[1,2]. Indeed, GM is composed of a multitude (over trillions) of microbial entities from all domains of life (*i.e.*, bacteria, archaeabacteria and micro-eukaryotes), which encode a number of genes probably more than 500 times greater than the human genome. This genetic heritage, still largely underestimated, allows them to perform various functions recognized as instrumental to maintaining host homeostasis[3,4]. With specific regard to the bacterial counterpart, indisputably the most explored to date, despite the thousands of different species identified so far, most of them belong to 2 phyla, Firmicutes and Bacteroidetes, which together account for approximately 90% of an adult-like community, with Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia representing the most commonly found subdominant taxa[3]. This ecosystem is characterized by essential ecological features, such as stability, resistance and resilience, associated with high diversity and functional redundancy, the loss of which can lead to unhealthy microbe-microbe and microbe-host interactions[5]. Established at birth, GM develops structurally and functionally over time, in relation to the personal exposome (*i.e.*, the totality of exposures that individuals experience in their lives), while always providing the host with a series of fundamental immunological and metabolic ecosystem services for a mutualistic GM-host relationship[6,7]. In particular, GM is known to act as a protective barrier against infectious threats (the so-called "colonization resistance" by occupying niches, taking up resources and producing antimicrobials) and play an active role in the development and modulation of immune responses[8,9]. On the other hand, GM is also called "metabolic organ" as it provides numerous bioactive molecules from the degradation of dietary compounds, which are the main actors of the well-known local and systemic functions attributed to GM, just to name a few: (1) Nutritional support for the intestinal epithelium; (2) Synthesis of vitamins and balance of energy intake; (3) Lipid and carbohydrate metabolism-related effects; (4) Immune system modulation; and (5) Enteric and central nervous system regulation through the gut-brain axis[10]. Moreover, recent findings on GM-drug interactions have indicated its role in influencing the response to treatments, including the occurrence of side effects, with potentially groundbreaking implications in precision medicine[11].

That said, it is not hard to imagine how strategic it is for our health to maintain this intricate and complex balance with our microbial inhabitants. Indeed, when this balance fails, the so-called dysbiosis is established, *i.e.*, a disease-promoting or associated GM alteration[12]. Several endogenous (*e.g.*, immune dysregulation and inflammation) and exogenous (unbalanced diet, antibiotic intake, pathogen infection, *etc.*) factors are capable of promoting GM dysbiosis, and several studies have tried to explain the mechanisms underlying these events[13-15]. Generally speaking, dysbiosis may present with one or more of the following characteristics: (1) Loss of biodiversity (a recognized hallmark of healthy gut); (2) Depletion of beneficial, health-associated taxa, typically short-chain fatty acid (SCFA) producers from the *Lachnospiraceae* and *Ruminococcaceae* families; and (3) Enrichment in pathogens or pathobionts[16]. To date, dysbiotic profiles have been associated with a plethora of enteric and non-enteric disorders, including metabolic, hepatic, respiratory, cardiovascular, immunological and oncological disorders, and are supposed to cause some of these[12]. However, it is of the utmost importance to corroborate claims on GM-related causality in human diseases, as only a few of them have been validated[17]. Regardless, a very hot topic in the field now is the development of GM modulatory strategies, to increase the resilience of healthy states (prevention) or overcome that of unhealthy states (treatment) to alleviate the disease phenotype and restore eubiosis.

EVIDENCE FOR A LINK BETWEEN GUT MICROBIOTA AND OBESITY

The prevalence of overweight and obesity has dramatically risen over the past four decades[18]. Combined with polygenic host susceptibility, increased food consumption and sedentary lifestyles lay the foundation for a widespread obesity epidemic[19]. Recognized as a multifactorial disease, obesity represents a major risk factor for health, with dramatic consequences on quality of life and healthcare costs [20-22]. Comorbidities linked to obesity (*e.g.*, diabetes, cardiovascular disease, cancer) are indeed among the leading causes of premature death, and researchers are striving to find more effective treatments for these conditions[23]. Over the past 15 years, pioneering studies have proposed GM as a key factor involved in energy storage and fat mass gain. Among the first, Bäckhed *et al*[24,25] demonstrated that germ-free mice gained less body weight and fat mass than conventional mice harboring a GM[24], as well as providing proof of concept by showing that the lack of a microbial ecosystem confers resistance to obesity induced by a high-fat diet[25]. In addition, a pivotal study by Turnbaugh *et al*[26] showed that the obese phenotype can be induced in lean mice by transferring the GM of obese animals[26], thus suggesting a causality between unbalanced GM and the development of obesity. Subsequently, the GM of obese individuals was shown to have reduced microbial richness and biodiversity, combined with an altered representation of the two main phyla, namely Bacteroidetes and Firmicutes, compared to lean subjects[27]. These findings have paved the way for several epidemiological studies focused on showing differences in the GM of obese and lean individuals, which have led to accumulating evidence of the GM role in mediating the impact of environmental factors on obesity pathogenesis[28-31]. As expected, given the high taxonomic level, not all studies have been successful in validating the association between the Firmicutes/Bacteroidetes ratio and obesity, and the biological relevance of this ratio is now highly controversial. On the other hand, greater resolution was gradually provided in the description of the bacterial composition, as well as in the understanding of the underlying mechanisms through which a dysbiotic layout might contribute to triggering host metabolic imbalances. For instance, species-level characterization of GM in twins highlighted a positive association of SCFA producers *Eubacterium ventriosum* and *Roseburia intestinalis* with obesity[32]. Similar correlations were observed for *Collinsella* spp.[33], which were also found to be overrepresented in type 2 diabetes (T2D) and atherosclerosis[34,35]. The hypothesized mechanisms include reduced expression of tight junction proteins, possibly leading to gut leakage and metabolic endotoxemia, as well as impaired cholesterol absorption, decreased hepatic glycogenesis and increased triglyceride synthesis[36,37]. It is therefore not surprising that *Collinsella* has been proposed as a target in future GM intervention studies for the improvement of metabolic parameters [38]. On the other hand, the most common methanogenic archaeon found in GM, *Methanobrevibacter smithii*, as well as butyrate producers, such as potentially heritable *Oscillospira* spp., were found to be more represented in lean individuals[39]. *Bacteroides thetaiotaomicron* was depleted as well in obesity-related GM configurations and inversely correlated with serum concentration of glutamate, a common food additive able to induce obesity and insulin resistance[40]. *In vivo* studies have highlighted the ability of this glutamate-fermenting commensal to protect against adiposity[40], suggesting its potential application in probiotic-based intervention strategies in obese individuals. Similarly, *Parabacteroides goldsteinii*, whose levels are reduced in high-fat diet-fed mice, has been proposed as an anti-obesogenic probiotic, capable of promoting adipose tissue thermogenesis and intestinal integrity, and reducing inflammation and insulin resistance[41]. Finally yet importantly, the mucin-degrading bacterium *Akkermansia muciniphila* has been repeatedly and consistently found to be inversely correlated with body fat mass, fasting blood glucose levels and subcutaneous adiposity in mice and humans[42,43], potentially representing a NGP or live biotherapeutic candidate for obesity treatment. In human studies, *A. muciniphila* has shown protective effects against gut permeability and endotoxemia, as well as improving glucose homeostasis and promoting better overall health[43]. Very recently, in a proof-of-concept exploratory study, Depommier *et al*[44] demonstrated that its daily oral administration, as live or pasteurized bacteria, for three months to overweight/obese insulin-resistant volunteers, was safe and well tolerated, and led to the improvement of multiple metabolic parameters[44]. However, it should also be remembered that microorganisms interact with each other in complex syntrophic networks, the understanding of which could help guide more rational preventive and therapeutic strategies. In an attempt to take a step forward in this direction, Tavella *et al*[45] recently identified a distinct GM compositional structure (with elevated proportions of *Christensenellaceae*, *Porphyromonadaceae* and *Rikenellaceae*) associated with reduced

visceral adipose tissue and healthier metabolic profile in elderly Italians[45]. It has also been hypothesized that peculiar “steady states” of GM, combined with long-term dietary habits, may predict the development of childhood obesity[31].

INFLUENCE OF DIET ON THE GUT MICROBIOTA

Diet is a major driver of GM variation and undoubtedly plays a central role in promoting and maintaining GM diversity, a strategic element to ensure eubiosis and resilience. Indeed, GM can rapidly shift its composition and functionality in response to dietary changes, contributing to the generation of health-relevant metabolic outputs [46]. In recent years, interest in understanding the relationship between dietary habits, GM and host physiology has grown remarkably. Different dietary patterns, such as Western-type diets, vegetarian or vegan diets and Mediterranean-style diets have been explored and each has been found to be associated with quite distinct GM profiles, which obviously affect host metabolism in a distinct way[47]. In particular, the advent of a Westernized lifestyle, with the fast-paced globalization of food, excessive sanitation and modern medicines, has led to the introduction of a dietary pattern mostly based on saturated fat, sugar and salt, and dramatically low in fiber, otherwise known as “microbiota-accessible carbohydrates”[13]. Several studies have shown that this type of diet is associated with alterations in the GM structure and functionality, in particular enrichment in mucus degraders, bile-tolerant and antibiotic-resistant species (“BloSSUM”, *i.e.*, bloom or selected in societies of urbanization/modernization) and the loss of diversity, ancestral fiber-degrading taxa and related functions (“VANISH”, *i.e.*, volatile and/or associated negatively with industrialized societies of humans)[48]. This has collectively been referred to as the “microbiota insufficiency syndrome” and is supposed to result in broad dysfunctions, including obesity and chronic inflammation, thus contributing to the emergence of non-communicable chronic diseases. In contrast, high-fiber dietary patterns are typically associated with highly diverse GMs, enriched in fibrolytic SCFA-producing bacteria, *e.g.*, *Ruminococcus*, *Faecalibacterium*, *Eubacterium* and *Roseburia*, along with high fecal levels of SCFAs[49,50], to which multiple beneficial effects are attributed, as detailed below. Moreover, a microbial footprint of this dietary habit is the greater abundance of *Prevotella*, as also found in hunting-gathering and rural populations who consume a plant-based diet with unprocessed foods[51-53]. It is also worth noting that these dietary habits involve increased intake of polyphenols, which are known to have important GM-mediated health benefits[54]. In a recent study in Italian individuals habitually following omnivore, vegetarian or vegan diets[55], we found that high-level adherence to a Mediterranean diet (rich in fruit, legumes and vegetables) was associated with beneficial GM and metabolome profiles, *i.e.*, increased proportions of fiber-degrading bacteria, higher levels of fecal SCFAs and lower urinary levels of trimethylamine N-oxide (TMAO), a risk factor for cardiovascular disease, potentially helping to explain the effectiveness of this diet against obesity, T2D and other inflammatory disorders. Consistently, a 1-year Mediterranean diet intervention was found to positively modulate GM and metabolome (including lower production of secondary BAs and p-cresols) and reduce frailty in elderly subjects, thus paving the way for novel intervention strategies possibly based on Mediterranean diet-responsive taxa and/or metabolites[56]. All this considered, it is not surprising that in recent years, the Paleolithic diet, with a high intake of plant foods while totally excluding industrially processed products and refined sugars, has received a lot attention. Despite some concerns about its long-term adherence, especially due to the consumption of fat and meat[57], it appears to lead to improved metabolic parameters in obese and T2D patients[58] and high levels of GM diversity, similar to those found in traditional rural populations[59].

MICROBIOME-DERIVED COMPOUNDS

SCFAs, protein metabolites, TMAO and BAs

SCFAs, mainly acetate, propionate and butyrate, are the best-known examples of diet-derived microbial metabolites with several local and systemic functions. Indeed, the human genome encodes only a limited number of carbohydrate-active enzymes (CAZymes), thus requiring complementation by the GM for the degradation of otherwise indigestible dietary fibers (*e.g.*, glycans, xylans, *etc.*)[60]. SCFAs are the end-

products of fermentation of these complex polysaccharides. These metabolites can be variously beneficial to health, as local (butyrate) and peripheral (acetate and propionate) energy sources, inflammation modulators, regulators of gut motility, vasodilators and even wound healing promoters[4]. SCFAs also affect the proliferation and differentiation of colonic epithelial cells, modulate their gene expression, reinforce the epithelial barrier (through increased mucus production and strengthening tight junctions), and influence the expansion and function of other cell lineages, including hematopoietic lineages[61]. With specific regard to metabolic health, they control the expression and secretion of appetite and glucose regulatory peptides, such as peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), by enteroendocrine L-cells, and activate intestinal gluconeogenesis mainly by the regulation of gene expression[10,61]. They have also been attributed functions involved in lipid metabolism, with acetate exerting an anti-lipolytic effect, which could be beneficial in the long term by reducing systemic lipid spillover[62]. Their immunomodulatory and anti-inflammatory activity is also extremely important, for maintaining the delicate balance between tolerogenic and immunogenic signals[61].

On the other hand, branched-chain fatty acids, such as isobutyrate, 2-methylbutyrate and isovalerate, resulting from protein fermentation, have been associated with insulin resistance[63], probably through activation of mammalian target of rapamycin complex 1 (mTORC1)[64]. Protein metabolism by GM may also lead to other potentially harmful compounds, including: (1) P-cresyl and indoxyl sulfate, both of which are associated with cardiovascular morbidity and mortality; (2) 4-ethylphenyl-sulfate, implicated in promoting autism-like behavior in animal models; and (3) Phenylacetate, which has been shown to contribute to the development of fibrosis and non-alcoholic fatty liver disease (NAFLD)[65].

An admirable but unfortunate example of GM-host co-metabolism of dietary compounds is TMAO. Several gut microbes, *e.g.*, *Campylobacter*, *Shigella* and *Ruminococcus gnavus*, can in fact convert choline and L-carnitine present in seafood, cheese, eggs and red meat, into trimethylamine (TMA) that, once absorbed, circulates to the liver where it is oxidized by host enzymes of the flavin monooxygenase family to TMAO[66-68]. TMAO has recently emerged as a candidate risk factor for cardiovascular disease, as it is proatherogenic, increases platelet hyperactivity and therefore the risk of thrombosis[69]. In particular, TMAO can reduce reverse cholesterol transport and BA synthesis, interfering with the normal pathway of cholesterol metabolism and elimination[70]. However, it is worth noting that some condiments, such as cold-pressed extra virgin olive oil, grape seed oil and balsamic vinegar, along with some red wines contain a structural analogue of choline, 3,3-dimethyl-1-butanol, which inhibits TMA lyase (*i.e.*, the microbial enzyme involved in TMA formation), thus paving the way for the use of selective enzyme inhibitors for the prevention and treatment of cardiometabolic diseases.

The metabolism of BAs is another example of GM-host co-metabolism, through which the GM can modify the composition of the pool of primary and secondary BAs available to the host and therefore modulate their signaling, meaning not only the traditional role in fat absorption but various effects related to glucose, lipid and energy homeostasis, thermogenesis, insulin signaling, immune responses and inflammation [71].

Finally, it is worth mentioning that GM is increasingly recognized as a major, still largely underestimated, player in determining the toxicity of environmental pollutants [72]. With specific regard to diet, for example it can mediate the adverse metabolic effects of non-calorie artificial sweeteners, whose chronic consumption increases the risk of glucose intolerance[73]. In contrast, some GM components have been shown to be involved in the bioremediation of common food processing products, including Maillard reaction products and advanced glycation end-products, which have been implicated in a wide variety of civilization disorders, *e.g.*, atherosclerosis and diabetes [74].

Neuroactive metabolites

GM is well known to interact with the enteric and central nervous systems *via* the bidirectional gut-brain axis[75]. On the one hand, GM can be directly influenced by mental health, through the luminal secretion of endocrine mediators capable of interacting with microbial receptors, thus having direct effects on microbial gene expression and signaling mechanisms. At the same time, the microbial community can be indirectly modulated as a result of induced changes in the gut environment. On the other hand, as anticipated above, the GM is capable of producing neuroactive metabolites in a diet-dependent manner, *e.g.*, SCFAs and conjugated fatty acids, which besides exerting peripheral effects can modulate the central nervous system through

direct or indirect mechanisms, involving a complex network of neuroendocrine factors and their receptors. Interestingly, these interactions have been shown to affect central appetite, food reward signaling and energy balance[76-79]. GM has also been found to influence eating behaviors through vagal nerve stimulation and immune activation [80]. Perturbations of the gut-microbiome-brain axis could therefore compromise the inhibitory mechanisms normally involved in the regulation of food intake, resulting in unbalanced eating patterns towards cravings, overeating and hedonic-driven eating behavior[79,81]. Regarding neuroactive GM metabolites, it is worth noting that propionate modulates reward pathways by reducing anticipatory reward responses to high-energy foods *via* striatal pathways[82]. Moreover, tryptophan metabolites have been closely implicated in the modulation of gut-microbiome-brain interactions, and indole propionate has recently been associated with increased food addiction behaviors in obese individuals[83,84]. GM metabolites may also interact with the endocannabinoid system, affecting the homeostatic and hedonic control of appetite and food intake[85], while the dopaminergic mesolimbic system, involved in reward mechanisms and hypothesized to play an important role in the development of obesity, is influenced by GM through the modulation of gut hormone secretion[86]. Not least, it should be remembered that microbes can even produce neurotransmitters, *e.g.*, serotonin and GABA, potentially affecting our mood and feeding[87,88].

In conclusion, particular GM layouts with distinct metabolic activities might have pleiotropic effects on host physiology, including eating behaviors, thus strongly contributing to the development of obesity and eating disorders. In this context, GM modulation or its replacement could be valuable tools to implement and increase the success of current preventive or therapeutic interventions against obesity and related comorbidities, as detailed below.

MICROBIOME-BASED STRATEGIES FOR PREVENTION AND TREATMENT OF OBESITY

The main microbiome-based strategies that are or could be effective for the prevention and treatment of obesity are discussed below and summarized in [Figure 1](#). In short, traditional (prebiotics, probiotics and synbiotics) and innovative (NGPs and engineered microbes) interventions were considered, along with microbiome replacement strategies, based on FMT and synthetic ecology approaches. Finally, in the next paragraph, the potential of bioinformatics tools, such as machine learning and deep learning, for health risk or outcome prediction is discussed.

Prebiotics, probiotics and synbiotics

Prebiotics are typically referred to as “a substrate that is selectively utilized by host microorganisms conferring a health benefit”[89]. Prebiotic supplementation has been proposed as a means of driving changes in GM while benefitting the host in the context of various disorders, including obesity, for improved glucose homeostasis and enteroendocrine L-cell activity. For instance, dietary supplementation with whole-grain barley and brown rice improved GM diversity, increasing the Firmicutes/Bacteroidetes ratio and the relative abundance of *Blautia* (an acetate producer from the *Lachnospiraceae* family), as well as attenuating postprandial blood glucose levels and decreasing plasma interleukin (IL)-6 levels in healthy individuals[90]. Oligosaccharide supplementation has shown promising anti-obesity effects *via* the SCFA and BA pathways. In particular, several animal studies have confirmed that SCFAs produced following oligosaccharide fermentation stimulate the secretion of PYY and GLP-1 *via* the G-protein-coupled receptors (GPRs) GPR41 and GPR43 expressed on enteroendocrine L cells[91-93]. These appetite-decreasing intestinal hormones help reduce food intake[94], increase satiety and energy expenditure[95] and improve glucose metabolism and insulin secretion[96]. Microbial fiber metabolism has additional, SCFA-independent effects, mediated by ferulic acid, a plant cell wall component with antioxidant, anti-inflammatory and anti-diabetic effects, and by alteration of the intestinal BA pool, with downstream implications in terms of energy and glucose homeostasis[97]. Not least, adding fermentable fiber to a high-fat diet in mice has been shown to result in IL-22 induction, increased enterocyte proliferation, reduced microbiota encroachment into the mucosa and pro-inflammatory gene expression, and increased antimicrobial gene expression, thereby protecting against high-fat diet-induced metabolic syndrome[97]. Although current prebiotics are mainly carbohydrate-based, other substances, such as polyphenols and polyunsaturated fatty acids (PUFAs), could exert prebiotic effects as well, as tested in both mice and humans

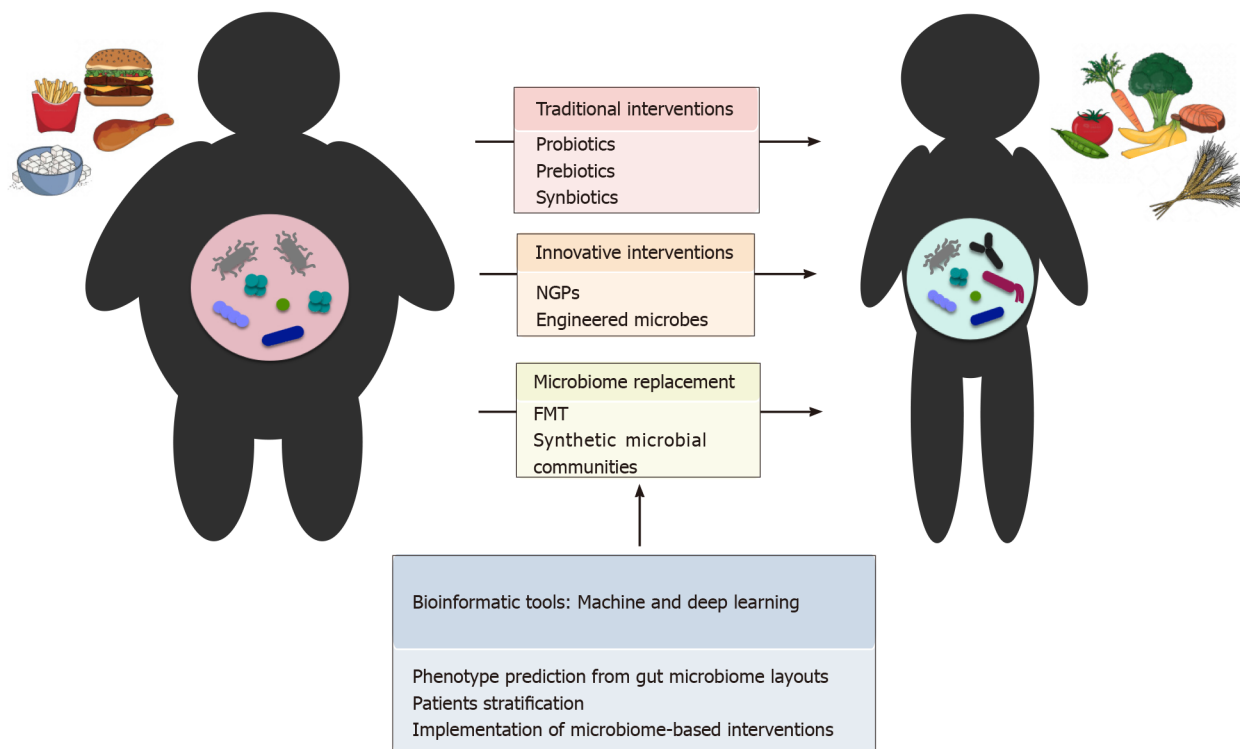


Figure 1 Overview of the main microbiome-based strategies currently in use or potentially effective in the prevention and treatment of obesity.

Traditional intervention strategies include dietary supplementation with prebiotics, probiotics and synbiotics, which have generally been shown to be moderately effective for the prevention and amelioration of obesity. Innovative strategies have therefore been implemented for improved treatment efficacy, using next-generation probiotics and non-pathogenic engineered microorganisms designed for the *in-situ* delivery of specific modulators. More recently, more direct modulation strategies based on gut microbiome replacement by means of fecal microbiota transplantation or synthetic communities, are being considered. In this scenario, bioinformatic tools, including machine and deep learning, could be crucial not only for the rational design of synthetic communities, but also for stratifying patients based on disease-associated phenotypes and thus predicting their health risks and outcomes. In the near future, all the accumulating knowledge about the gut microbiome and technological advances should lead to a rational implementation of innovative microbiome-based interventions geared towards personalized precision medicine. Food items were obtained from the Mind the Graph platform (<https://mindthegraph.com/>). NGPs: Next-generation probiotics; FMT: Fecal microbiota transplantation.

[89]. Polyphenols, *i.e.*, secondary metabolites derived from plant sources, are extensively metabolized in the intestine and converted into phytoestrogens with multiple beneficial effects[50,98]. Following regular consumption of polyphenols, a reduced risk of cardiometabolic diseases has been observed, together with antioxidant, anti-inflammatory and anti-obesogenic effects[99,100]. However, the prebiotic effects of polyphenols can be affected by the food source and although these compounds are generally recognized as GM compositional modulators, further research is needed to validate their prebiotic potential[101]. Multiple health benefits have also been reported for nutritional supplementation with PUFAs, such as eicosapentaenoic acid and docosahexaenoic acid, including anticancer activity[102,103], secondary prevention of ischemic heart disease[104] and prevention of cardiovascular diseases[105], as well as a reduction of mucosal inflammation and modulation of the GM composition in patients with ulcerative colitis[106]. Dietary supplementation with PUFAs may therefore be useful not only for obesity mitigation but also for the treatment of obesity-associated comorbidities[107,108]. In addition, PUFA-derived mediators, such as resolvins and protectins, have shown the potential to counteract inflammation in the context of obesity[109]. In this regard, a recent study in obese diabetic *db/db* mice demonstrated a marked improvement in insulin sensitivity following the administration of protectin D1[110]. In light of these findings, prebiotic supplementation should be considered a potential integrative therapy for the prevention and treatment of obesity.

Probiotics, *i.e.*, “live microorganisms that, when administered in adequate amounts, confer a health benefit to the host”[111], represent one of the most widely used GM manipulation tools, for which an increasing number of clinical studies have been carried out in subjects with various pathological conditions, including obesity[112, 113]. However, it should be noted that conflicting results have emerged in relation to the ability of probiotics to counterbalance weight loss and obesity-related features. In particular, two meta-analyses of randomized controlled clinical trials[114,115] found

almost no efficiency in terms of weight and body mass index (BMI) reduction in obese individuals, especially in short-term interventions. On the other hand, the meta-analysis carried out by Zhang *et al*[116] on a large number of clinical trials reported substantially different results and a significant reduction in body weight and BMI, with consistent maximum outcomes with multi-strain product supplementation for at least 8 wk. Similar conclusions were drawn by John *et al*[117] in a similar meta-analysis, showing that probiotic administration was associated with a reduction in all considered parameters (*i.e.*, body weight, BMI and fat mass). As expected, several studies have found strain-specific probiotic effects on body weight and metabolism, with only a few species belonging to *Lactobacillus* (*e.g.*, *L. acidophilus*, *L. casei*, *L. rhamnosus*, *L. reuteri*) and *Bifidobacterium* (*e.g.*, *B. bifidum*, *B. lactis*, *B. longum*) proven effective in overweight/obese individuals[104,118,119]. The greatest decreasing effect on BMI and body weight was reported with high doses of single-strain probiotics[117, 120], although John *et al*[117] observed a considerable reduction in both parameters even at lower doses when interventions continued for more than 12 wk[117]. More recently, a study by our group showed that administering a multi-strain probiotic mixture along with a hypocaloric Mediterranean diet led to weight loss, improvement in oxidative stress markers and an increase in *Akkermansia* in elderly obese women, even in 15 d[33]. Regardless of the strain, the beneficial effects of probiotics in the treatment of obesity are also attributable to the following general mechanisms of action: (1) Antimicrobial activity, by inhibiting the growth of pathogenic microorganisms and exerting antagonistic effects against colonization of the intestinal mucosa and epithelium adherence; (2) Improvement of the barrier function, reducing intestinal permeability and increasing mucus production; and (3) Immunomodulation, through interaction with innate and adaptive components of the immune system[120]. Taken together, these mechanisms contribute to positively modulate the GM composition towards restoring a health-associated layout, which in turn can help alleviate host metabolic imbalances.

Synbiotics refer to “a mixture comprising live microorganisms and substrates selectively used by host microorganisms that confers a health benefit on the host” [121]. Accumulating evidence has reported the stronger effect of synbiotics in terms of GM modulation than either probiotics or prebiotics alone[122,123], with an overall improvement in lipid metabolism, glycemic status and inflammatory mediators[124, 125]. Although the appropriate dose, duration of administration and the composition of a synbiotic product necessary to confer a health benefit are influenced by several factors (*e.g.*, baseline GM layout, medications, habitual diet and lifestyle), the randomized clinical trial conducted by Dao *et al*[42] on 225 overweight and obese adults resulted in a 4.5% reduction in body fat mass when administering a combination of *B. animalis* subsp. *Lactis* 420 and polydextrose[42]. The control of body fat mass in overweight or obese individuals by the aforementioned synbiotic was also confirmed in a second clinical trial, along with a peculiar rearrangement in the GM composition that included an increased abundance of *Akkermansia*, *Christensenellaceae* and *Methanobrevibacter*, all taxa related to improved metabolic health and leanness [126]. GM modulation with increased proportions of potentially beneficial microbial groups (*e.g.*, *Lactobacillus*) was also observed in a clinical trial in obese individuals following administration of a synbiotic consisting of a multi-strain probiotic formulation (*i.e.*, *B. lactis*, *B. longum*, *B. bifidum* and *L. acidophilus*) and galactooligosaccharides as a prebiotic component[127].

In conclusion, prebiotics, probiotics and synbiotics are promising dietary agents for the modulation of human GM also in the context of obesity and metabolic disorders. However, given the still conflicting data in the scientific literature on probiotics, further studies are needed to rationally include their prescription as a preventive or therapeutic supplement for obesity.

NGPs and engineered microbes

Moving from the “one-size-fits-all” concept related to traditional probiotics, researchers are striving to thoroughly elucidate the role of each commensal member within the complex ecosystem of the human gastrointestinal tract in influencing host health. Compared to the modest ameliorative effects generally shown by traditional probiotics in obese individuals, emerging NGPs are beginning to reveal their great potential as novel preventive and therapeutic tools[128]. In this perspective, several studies have shown differences in the GM composition between obese and lean individuals, pointing out that an increased abundance of *A. muciniphila* could lead to an improvement in obesity and metabolic disorders[129,130]. Subsequently, the mechanism underlying the beneficial effect of *A. muciniphila* was investigated and the involvement of an immunomodulatory membrane protein “Amuc_1100” was

proposed, which showed the same beneficial effects as live bacteria[131]. Cani *et al* [132] demonstrated the ability of this promising NGP to modulate the endocannabinoid system[132], a crucial regulatory system involved in controlling glucose metabolism in obesity, T2D and inflammatory conditions[132]. More recently, as anticipated above, the daily oral administration of live or pasteurized *A. muciniphila*, for three months to overweight/obese insulin-resistant volunteers, has been shown to be safe and well tolerated, while leading to improved metabolic parameters[44]. However, since some animal studies have reported an increase in *A. muciniphila* in multiple sclerosis[133] and Parkinson's disease[134], further studies are needed to fully unravel its effects on host health.

Christensenella minuta also showed potential probiotic effects by ameliorating obesity and associated metabolic disorders through modulation of dysbiotic GM layouts[135], and its abundance was greater in lean individuals with low BMI[136]. However, Yang *et al*[137] recently highlighted potential pathogenic features (*e.g.*, LPS-mediated triggering of a mild inflammatory response *via* NF- κ B pathway) of *C. minuta*, suggesting that its application should be limited to therapeutic interventions focused on obesity control[137]. As mentioned above, *P. goldsteinii* is also a promising anti-obesity and anti-inflammatory NGP candidate. Being selectively enriched in the GM of mice fed a high-fat diet supplemented with oriental medicinal fungi, this commensal bacterium has been associated with increased adipose tissue thermogenesis, reduced levels of inflammation, enhanced intestinal integrity and amelioration of insulin resistance[41]. While promising, these *in vivo* results have not yet been translated into clinical trials. On the other hand, *Faecalibacterium prausnitzii*, one of the most promising NGPs due to its well characterized anti-inflammatory activity[138,139], showed a lower clade diversity in intestinal diseases and obese individuals[140]. In light of this finding, caution must be taken in selecting the most suitable strain for the development of therapeutic interventions. Despite the growing amount of NGP candidates so far isolated and characterized, further strain-level functional analyses are required to fully assess the underlying mechanisms by which they could confer health benefits to the host, before pushing them for clinical application.

Synthetic biology approaches have recently been exploited to address disease-specific mechanisms and meet medical needs by designing non-pathogenic and commensal bacteria to deliver therapeutic effectors[141,142]. Regarding the feasibility of using engineered probiotics to alleviate obesity-related characteristics, Long *et al* [143] demonstrated a decrease in body weight gain in overweight mice given an engineered *Bifidobacterium* strain secreting oxyntomodulin, an anorexigenic hormone that reduces appetite and food intake[143]. In a similar study, Chen *et al*[144] developed an engineered probiotic strain of *Escherichia coli* with increased secretion of N-acyl-phosphatidylethanolamine, the immediate precursor of the anorexigenic metabolite N-acyl-ethanolamide, resulting in reduced weight gain and less accumulation of fat mass in mice fed a high-fat diet[144]. Another potential strategy to alleviate obesity and metabolic disorders has recently been proposed by Bai *et al*[145]. Administration of *Bacillus subtilis* SCK6 strain BsS-RS066550 engineered to increase butyric acid production resulted in decreased body weight and food intake in high-fat diet-fed mice, along with beneficial effects on insulin resistance, blood glucose and hepatic biochemistry[145]. In addition, Wang *et al*[146] showed that administration of genetically engineered *Lactococcus lactis* expressing GLP-1 significantly reduced body weight and blood glucose of obese mice fed a high-fat diet[146]. Anti-obesity mechanisms included the promotion of fatty acid oxidation and the restoration of GM biodiversity[146]. To date, numerous therapeutic interventions based on engineered live bacteria have entered the early or mid-stage of clinical development[141]. If successfully completed, such studies will be crucial in providing the missing proof of concept required to pave the way for a new class of precision therapeutics. Once their efficacy and risk ratio have been verified and approved for use in humans, engineered bacterial therapeutics will enable specific disease mechanisms and unmet medical needs to be addressed, even in obese patients.

FMT

Consisting of the transfer of microbes from healthy individuals to recipients hosting a dysbiotic GM layout with the aim of restoring eubiosis[147,148], FMT has attracted considerable attention in clinical practice, particularly for the treatment of recurrent infections by antibiotic-refractory *Clostridioides difficile*[149,150]. Recently, the potential of FMT in treating a large number of conditions, including obesity, has been explored. In a pivotal study, Ridaura *et al*[63] showed that mice receiving GM from obese individuals developed obesity, while those receiving GM from healthy individuals remained lean[63]. Sequencing analysis of post-treatment stools confirmed the

successful engraftment of the donor microbiota, along with the transfer of functions associated with either “lean” or “obese” microbial communities. Shortly before, a clinical study on diabetic and obese adult males by Vrieze *et al*[151] demonstrated improved microbial diversity and insulin sensitivity, along with the expansion of Bacteroidetes and butyrate-producing taxa, following GM transplantation from lean donors[151].

As of April 2021, there were 15 registered clinical trials (ClinicalTrials.gov search terms: “gut microbiota”, “obesity” and “fecal microbiota transplantation”) on obese individuals undergoing FMT for the replacement of the obesogenic microbial community (Table 1). Of these, four have been completed and only two have made the results publicly available. Yu *et al*[152] performed FMT on 24 obese individuals with the aim of improving metabolic outcomes[152]. Administration of FMT capsules ensured engraftment, persisting for at least 12 wk after treatment, although no clinically significant metabolic effects were observed. Allegretti *et al*[153] performed FMT on 22 obese, metabolically uncompromised patients, and although no significant changes in BMI occurred, a sustained shift of obesity-associated microbiomes towards the donor microbiome layout was observed, along with improved BA profiles and decreased fecal levels of taurocholic acid[153]. In a secondary analysis focused on the prevention of metabolic syndrome within the same patient group, the authors found a significant change in glucose and insulin levels after FMT[154]. Similar to other microbiome-based therapeutics, while promising, FMT is still at an early stage for the treatment of obesity and associated comorbidities. Therefore, it should be recognized as a separate pharmacological category, consisting of an entirely novel class of agents and requiring systematic research to fill knowledge gaps, thereby facilitating the development of standardized next-generation microbiota therapeutic interventions with improved safety and efficacy.

Microbial replacement therapy through synthetic ecology approaches

It is generally believed that the administration of multi-species microbial consortia is more useful than a single probiotic organism[155], as it probably retains some properties of the community structure, with community members continuously interacting and communicating with each other. Furthermore, a consortium of bacteria possesses a larger gene pool than monocultures, resulting in greater diversity in metabolic pathways. This richness is reflected in a greater ability of the consortium to perform more complex tasks than single organisms, thus exploiting the resources available in the surroundings more efficiently and better adapting to the environment, also through self-organization to form spatial patterns in response to substrates and metabolite gradients[156-160].

Synthetic ecology indicates the rational design of ecosystems, where two or more defined microbial populations are assembled in a well-characterized and controlled environment[161]. This approach requires *in vitro* controlled environments, biologically relevant bacterial strains and mathematical models of ecological interactions [162] to simulate community behavior in response to several factors. The idea is to shape a complex microbial community in order to obtain a desired compositional and functional profile that meets the needs of specific industrial production processes or pharmacological interventions.

Synthetic microbial communities are systems of known and trimmed-down complexity that can undergo experimental treatments and mathematical modelling, enabling a system-level understanding of the consortium[163,164]. These communities are not only a way to study how the microbial consortium structure emerges and the conditions necessary to generate specific interaction networks among its components, but they can also elucidate the overall function, resistance and resilience of microbial systems[10]. By the so-called microbial resource management[165,166], *i.e.*, the management of consortium parameters such as richness, evenness, predation, abiotic factors, quorum sensing and spatial disposition, the community can be steered to the desired functionality, in order to obtain novel products and processes or potentially improve human health by transplanting the desired synthetic community into a recipient patient.

To date, many studies[167-169] have reported the *in vitro* assembly of synthetic GM communities with at least two bacterial strains, but clinical applications are still limited. Petrof *et al*[170] obtained a synthetic consortium consisting of 33 individual microbial species derived from a stool sample from a healthy donor and demonstrated the potential of such synthetic microbiota in the treatment of *C. difficile* infection. Furthermore, they reported that some of the administered bacteria that were forming the synthetic community stably colonized the recipient’s colon, as opposed to most commercially available probiotics, which only transiently colonize the intestine.

Table 1 Registered clinical trials on ClinicalTrials.gov (as accessed on April 2021) focused on fecal microbiota transplantation for the replacement of obesogenic microbial communities. Search terms included “gut microbiota”, “obesity” and “fecal microbiota transplantation”

Rank	Title	Status	Results	Condition	Intervention	Location	URL
1	Fecal Microbiota Transplantation for the Treatment of Obesity	Completed	Available	Obesity	FMT <i>vs</i> placebo	United States	https://ClinicalTrials.gov/show/NCT02741518
2	Faecal Microbiota Transplantation in Obesity	Recruiting	Not available	Obesity	FMT <i>vs</i> placebo	Finland	https://ClinicalTrials.gov/show/NCT03391817
3	Randomized Controlled Trial of Fecal Microbiota Transplantation in Severe Obesity	Enrolling by invitation	Not available	Obesity	FMT <i>vs</i> placebo	Norway	https://ClinicalTrials.gov/show/NCT03273855
4	Fecal Microbiota Transplant (FMT) to Induce Weight Loss in Obese Subjects	Active, not recruiting	Not available	Obesity	FMT and mucosal microbiota assessment	China	https://ClinicalTrials.gov/show/NCT03789461
5	FMT and Fiber in Patients With Metabolic Syndrome	Completed	Not available	Obesity, Metabolic Syndrome	FMT and dietary supplement with fiber (cellulose) <i>vs</i> placebo	Canada	https://ClinicalTrials.gov/show/NCT03727321
6	Assessment of the Health Improvement of Obese Patients After Fecal Microbiota Transplantation (FMT)	Completed	Not available	Obesity, Type 1 and 2 Diabetes	FMT	Russian Federation	https://ClinicalTrials.gov/show/NCT04579263
7	Fecal Microbiota Transplantation for Diabetes Mellitus Type II in Obese Patients	Unknown status	Not available	T2DM, Obesity	FMT and dietary intervention (high-fat low-fiber diet, sham diet or low-fat high-fiber diet)	Israel	https://ClinicalTrials.gov/show/NCT02346669
8	Fecal Microbial Transplantation and Fiber Supplementation in Participants With Obesity and Metabolic Syndrome	Active, not recruiting	Not available	Obesity, Metabolic Syndrome	FMT and dietary supplement with fiber (cellulose) or FMT only <i>vs</i> placebo	Canada	https://ClinicalTrials.gov/show/NCT03477916
9	Randomised Placebo-controlled Study of FMT to Impact Body Weight and Glycemic Control in Obese Subjects With T2DM	Active, not recruiting	Not available	T2DM, Obesity	FMT and lifestyle modification program or FMT only <i>vs</i> placebo	China	https://ClinicalTrials.gov/show/NCT03127696
10	Fecal Microbiota Transplant for Improvement of Metabolism	Completed	Available	Obesity	FMT <i>vs</i> placebo	United States	https://ClinicalTrials.gov/show/NCT02530385
11	The Role of Microbiome in Recurrent Obesity	Not yet recruiting	Not available	Obesity	FMT <i>vs</i> placebo	Israel	https://ClinicalTrials.gov/show/NCT04697550
12	Effects of Fecal Microbiota Transplantation on Weight in Obese Patients With Non-alcoholic Fatty Liver Disease	Recruiting	Not available	NAFLD	FMT, dietary intervention and physical activity <i>vs</i> placebo	India	https://ClinicalTrials.gov/show/NCT04594954
13	Proposal to Examine the Effect of Fecal Transplantation on Obesity	Unknown status	Not available	Obesity	FMT <i>vs</i> placebo	Israel	https://ClinicalTrials.gov/show/NCT02336789
14	Safety and Efficacy of Fecal Microbiota	Recruiting	Not available	IBD, IBS, Obesity,	FMT	China	https://ClinicalTrials.gov/show/NCT04014413

Transplantation				Metabolic Syndrome, Infections, Others			
15	Transplantation of Microbes for Treatment of Metabolic Syndrome & NAFLD	Completed	Not available	Type 1 and 2 Diabetes, NAFLD, Obesity	FMT	Canada	https://ClinicalTrials.gov/show/NCT02496390

FMT: Fecal Microbiota transplantation; IBD: Inflammatory bowel disease; IBS: Inflammatory bowel syndrome; NAFLD: Non-alcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus.

Despite its proven efficacy for the treatment of *C. difficile* infection, FMT hides some uncertainties that may be resolved by a synthetic stool replacement strategy. Indeed, the synthetic ecology approach has multiple advances over the canonical use of fecal matter from a donor: (1) The exact composition of the administered bacteria is known and can be reproduced; (2) The bacterial composition can be virtually tailored to the specific patient's needs; (3) The absence of pathogens and viruses can be more reliably guaranteed, improving safety; and (4) The administered microorganisms forming the consortia can be selected based on their sensitivity to antimicrobials, resulting in further improvement of the safety profile.

Applications of synthetic communities have been reported in other fields, such as bioremediation[157] and chemical production[171,172]. Notwithstanding the potential of the synthetic ecology approach in GM replacement therapy, there is still some way to go before synthetic ecology can be translated into the clinic. One major limitation is the lack of a truly representative *in vitro* system to mimic the *in vivo* microbiota, but steps in this direction have been achieved by the gut-on-a-chip model[173] and the HuMiX system[173]. In addition, a large and well-documented collection of cultures of human microbiota members will be extremely useful for improving ecology experiments and building mathematical models. In this regard, since 2015, culturomics has led to the discovery of 232 novel human gut species[174] and it is expected that this number will increase in the coming years. The technique is based on the multiplexing of bacterial isolation conditions through serial addition of specific growth promoters and/or inhibitors, coupled with high-throughput identification with MALDI-TOF mass spectrometry, and is capable of overcoming the limitations of conventional single-medium strategies[175-177]. The resulting knowledge could then be used to improve our understanding of the complex gut ecosystem and rationally design efficient and sophisticated synthetic communities, tailored for the treatment of the disease of interest.

THE FUTURE OF MICROBIOME-BASED PRECISION MEDICINE: HEALTH RISK OR OUTCOME PREDICTION THROUGH DEEP LEARNING

Machine learning and deep learning application to microbiome data

Machine learning and, in particular, deep learning have been the subject of intense media hype in recent years. Thanks to the explosion of available data and the rapid growth in the number and size of databases, they have accomplished nothing short of a revolution in the field of modern artificial intelligence, with notable progress in perceptual problems, such as facial and speech recognition[178,179], and have the potential to do the same in medical disciplines[180,181]. However, we are still exploring the full extent of what machine learning and deep learning can do and have just begun to apply them to a wide variety of problems outside of classical algorithms.

In light of the rapid increase in data from microbiome studies induced by the concomitant decrease in sequencing costs (< 10000 times in the past 10 years)[182], there are requirements to apply machine learning and deep learning algorithms to host-microbiome data, exploiting their associations in various diseases. Improved data analytical tools are needed to explore all the information contained in those datasets and identify key features that represent different aspects of the microbiome and that can be linked to host phenotypes. In particular, the possibility of predicting the patient's phenotype from one's GM is an integral part of personalized medicine, as it represents not only a way to overcome individual variability, but also a potential therapeutic target for pharmacological interventions. In this field, machine learning

might provide new insights, by developing models capable of stratifying individual patients into therapeutic classes, thus paving the way for the fine-tuning of interventions based on the GM structure. An example of machine learning algorithms are Support Vector Machines (SVMs), which were implemented by Cui and Zhang [183] to classify metagenomic samples into inflammatory bowel disease (IBD) and non-IBD classes. More recently, Pasolli *et al* [184] used SVMs to predict diseases such as liver cirrhosis, colorectal cancer and IBD from fecal metagenomes. To date, deep learning is the most advanced machine learning technique for a variety of applications [185] and has already achieved several results in the microbiome field [186-189], including predicting the microbiome structure in terms of bacterial relative abundance and metabolic layout. Other machine learning methods include Ensemble Methods, which combine multiple classifiers for better performance. The best known ensemble method is Random Forest [190], which has been widely used in microbiome studies for patient stratification [191,192] and biomarker search [193,194].

Machine learning, GM and obesity

In a prospective study on obesity in European children, Rampelli *et al* [31] underscored the importance of the microbiome–host–diet configuration as a possible predictor of obesity. In particular, GM was found to mediate dietary impact on individual metabolic and immunological homeostasis, which, in the context of other individual lifestyle and genetic variables, may be involved in the development of the multifactorial obese phenotype. Such results were based on experimental observations and no machine learning/deep learning algorithms were applied or developed. However, the study suggested that applying a machine learning algorithm to microbiome data could be a feasible method of predicting obesity, when other variables concerning host physiology, diet and lifestyle (*e.g.*, physical activity) are included.

Some attempts have been made in recent years, with poor results, mainly due to a lack of data (often lifestyle and dietary information is not collected or collected in a non-systematic and non-standardized way). Specifically, Pasolli *et al* [184] developed a Random Forest-based approach that exclusively utilized metagenome data without success in predicting obesity and T2D. A few years later, Fernández-Navarro *et al* [195] have implemented several machine learning methods, such as decision tree-based methods, ensemble methods and SVMs, to identify predictors of obesity. Starting from serum free fatty acid levels, microbial quantitative polymerase chain reaction information and dietary intake interviews, their model revealed a non-obese profile related to serum eicosapentaenoic acid levels and *Bacteroides* amount in feces.

Nevertheless, this represents just the tip of the iceberg, as the full deployment of machine learning methods in the human GM sphere for full integration into the field of personalized precision medicine requires additional efforts. Indeed, machine learning often runs like black boxes, which makes it difficult to conduct feature selection. In addition, a large amount of data and computational power are required to train powerful and reliable machine learning-based algorithms. In general, novel technologies have dramatically increased our ability to characterize the human GM, but the way to effectively harness that information is uncertain and presents several key challenges. For example, there is a high need for dimensionality reduction to handle the information of hundreds of thousands of gene markers for just a few hundred samples. In this regard, neural encoder-decoder networks [196] based on a deep learning architecture have proved effective [187], but further efforts are still needed to fully exploit microbiome data. What is certain is that machine learning has already shown its great potential when applied to the microbiome field. In the next years, cutting-edge machine learning-based models might enable a further step towards the microbiome implementation in personalized precision medicine.

LIMITATIONS OF CURRENT EVIDENCE AND NEXT STEPS

As discussed in the respective paragraphs, there are several limitations to the design and application of microbiome-based strategies in clinical routine, with particular reference to the prevention/treatment of obesity and related comorbidities. Although promising data come from the field of NGPs/engineered microbes, synthetic ecology and even FMT, it should be remembered that the evidence is still too little to support the clinical benefit of these novel microbiome modulation tools. Added to this are the sometimes inconsistent results on GM compositional and functional changes in the pathological context, and the lack of a full understanding of the underlying mecha-

nisms. Of course, several steps forward have been made, especially methodological ones, but there is still no standardization of study designs and the way of reporting the results, which makes it difficult to compare different studies. In parallel with the implementation of internationally recognized standard operating procedures for GM analysis, it is expected that in the future: (1) -Omics approaches, including metagenomics, metatranscriptomics, metaproteomics and metabolomics are combined to provide mechanistic insights; (2) The mechanisms are possibly validated in animal models; (3) Culturomics approaches are increasingly exploited to unravel the dynamics and ecological rules that govern the establishment of microbial networks; (4) The accumulating microbiome knowledge allows to fine-tune the design of precision strategies to achieve specific objectives, whether based on traditional or next-generation tools; and (5) Progressively generated datasets, including host and microbiome data, enable machine and deep learning technologies to maximize translational impact, through accurate prediction of health outcomes and thus provision of high-quality personalized care. As expected, the same limitations and implications also apply in other pathological contexts, where the modulation of GM can be impactful as well.

CONCLUSION

The emerging role of GM as a contributor to various pathological conditions is fascinating even if not yet easy to untangle. Diseases resulting from multiple factors, such as obesity and metabolic diseases in general, may be difficult to prevent or treat effectively solely relying on currently available therapies. In this scenario, gut microbes and their influence on the host constitute a piece of an intricate puzzle, to be exploited for novel integrated intervention strategies. In a systems biology approach, the advent of -omics technologies and the development of bioinformatics tools are pushing microbiome research to the next level, allowing to extrapolate general principles on community structure and translate the results into rationally designed, personalized microbiome-based interventions aimed at restructuring dysbiotic layouts, thereby contributing to the restoration and maintenance of host health.

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