You are what you eat: diet, health and the gut microbiota

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Abstract | Since the renaissance of microbiome research in the past decade, much insight has accumulated in comprehending forces shaping the architecture and functionality of resident microorganisms in the human gut. Of the multiple host-endogenous and host-exogenous factors involved, diet emerges as a pivotal determinant of gut microbiota community structure and function. By introducing dietary signals into the nexus between the host and its microbiota, nutrition sustains homeostasis or contributes to disease susceptibility. Herein, we summarize major concepts related to the effect of dietary constituents on the gut microbiota, highlighting chief principles in the diet–microbiota crosstalk. We then discuss the health benefits and detrimental consequences that the interactions between dietary and microbial factors elicit in the host. Finally, we present the promises and challenges that arise when seeking to incorporate microbiome data in dietary planning and portray the anticipated revolution that the field of nutrition is facing upon adopting these novel concepts.

The past decade has marked an explosion of research focusing on the trillions of indigenous microorganisms residing within and throughout the human body, collectively termed the microbiota, and their interactions with the eukaryotic host. These previously ignored prokaryotic members of the 'human holobiont' have been recognized to provide essential functions for host physiology, including its metabolism, immunity and neuronal development, whereas aberrations in their configuration or function have been suggested to contribute to disease states[1,2]. Notably, unlike the host genome, the microbiome exhibits a great deal of plasticity and can readily adjust to a large variety of environmental and host-derived stimuli. Of these environmental factors, diet constitutes a pivotal determinant of gut bacterial assembly and genes, thereby rendering it a potentially compelling target of manipulation.

Human nutrition bears profound influences on both individual and population-wide health. As such, nutritional research stands at the centre of medical, economic, cultural and social focus. The concept of "let food be thy medicine" was coined by Hippocrates over 2,000 years ago, and health organizations worldwide have been striving to set standards for a 'healthy diet' that define the recommended intake of micronutrients, macronutrients and total calories. The WHO has issued dietary guidelines for healthy weight management, yet obesity and its comorbidities continue to constitute a pandemic, with increasing incidence in both adults and children[3]. Although many weight-reducing strategies are efficient in the short term, the majority of dieters regain most or all of their previous weight over an intermediate to long-term period[4,5]. Furthermore, dietary recommendations designed to tackle IBD[6,7], IBS[8], autoimmune diseases[9] and cancer[10,11] are often based on inconclusive, conflicting or non-existing medical evidence. The conspicuous gap between the large body of research and the lack of efficacious or conclusive guidelines thereof is a major source of confusion and frustration among dieters, which have given rise to potentially problematic nutritional trends and unsupported practices.

The evident interrelationships between diet and the microbiota and their collective effect on the host, only now beginning to be deciphered, might reconcile some of the discrepancies that have been troubling nutrition researchers and could explain some of the previously unintelligible variability encountered in the response to diet, at times observed in apparently similar conditions. In this Review, we attempt to untangle some aspects of this tripartite diet–microbiota–host crosstalk by discussing each aspect separately and consequently attempt to assemble meaningful and applicable conclusions, which could have direct translational implications. Owing to the vast body of literature, the main focus of this Review is the bacterial component of the microbiota; the role of the virome, mycome and protozoa is illustrated briefly (BOXES 1,2).

Dietary modulation of the microbiota

The contribution of diet to modulating the microbiota and its crucial role in orchestrating the host–microbiota crosstalk is evident from the beginning of life, when human milk oligosaccharides (HMOs) participate in the

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https://doi.org/10.1038/s41575-018-0061-2

Key points

- Common multifactorial diseases in both industrialized and developing countries are often related to diet, yet current nutritional approaches aimed at their treatment and prevention are of limited efficacy.
- Diet contents and quantity have a major role in shaping the human microbiota composition and function.
- Complex interactions between nutrients and microorganisms dictate beneficial or detrimental outcomes to host health.
- Conflicting reports highlight several nutrients, metabolites and microorganisms as both beneficial and detrimental to host health, which could stem from methodological differences between studies and interindividual variations.
- Personalized nutrition is an emerging data-driven approach, potentially enabling diets tailored to the individual in various clinical contexts.

maturation of the microbiota in early infancy[12], followed by increased bacterial richness associated with the introduction of solid foods[13], and concludes with decreased richness observed in frail ageing populations in long-stay care, probably due to reduced food diversity[14]. Members of the gut microbiota are not only sensitive to proportions of certain dietary constituents[15] but also respond differently to nutrition in a myriad of temporal and geographical contexts. In this section, we aim to depict key concepts by which dietary factors influence the community structure and function of gut bacteria in states of homeostasis and nutritional imbalance.

Microbiome responses to foods

Direct mechanisms. Nutrients can directly interact with microorganisms to promote or inhibit their growth, and the capability to extract energy from specific dietary constituents bestows a direct competitive advantage to selected members of the gut microbial community, rendering them more capable of proliferating at the expense of less-adept members. This aspect is reflected by the observation that diet affects not only the relative and absolute abundance of gut bacteria but also their growth kinetics[16]. The central nutrients in this mechanism are indigestible carbohydrates termed glycans, which are mostly derived from plant but also animal, fungal and algal sources in the diet[17].

The human genome encodes a limited number of glycoside hydrolases and no polysaccharide lyases (collectively referred to as carbohydrate-active enzymes, or CAZymes)[17]. Thus, glycans such as resistant starch, inulin, lignin, pectin, cellulose and fructo-oligosaccharides (FOS) reach the large intestine in their undigested forms. In contrast to humans, the microbiome is estimated to encode tens of thousands of CAZymes[17]. Bacteria that can degrade glycans are termed primary degraders, including members of the *Bacteroides*, *Bifidobacterium* and *Ruminococcus* genera. Their competitive advantage is reflected by the ability to predict bacterial abundance according to glycan degradation patterns[18]. Within *Bacteroides*, the CAZyme genetic repertoire is predictive of a glycan-induced species-specific competitiveness that has an important role in establishing the in vivo fitness of members of this genus[19,20]. During food shortage, bacteria can switch between energy sources by employing sensing and regulatory mechanisms controlling gene expression. Taxa that can readily adjust to altering energy sources, such as members of the Bacteroidetes phylum that possess a fairly large number of genes encoding CAZymes, are therefore favoured[21–23]. Primary degradation of glycans liberates glucose and, coupled with fermentation by secondary degraders, results in formation of acetate, propionate, formate, butyrate, lactate and succinate and initiates a complex cross-feeding metabolic network. For example, fermentation often results in the production of hydrogen gas, which is consumed in the human gut by sulfate-reducing bacteria, methanogens and acetogens[24]. There is great interest in modelling these cross-feeding interactions, which might enable prediction of community structure on the basis of dietary variations[25,26]. In addition to direct interaction that promotes the growth of adept bacteria, nutrients can also inhibit bacterial growth. Plant nutrients such as quinones, flavonoids, terpenoids and alkaloids feature in vitro antimicrobial activity[27]. Others, such as the plant antimicrobial berberine[28], are associated with in vivo elimination of certain bacterial taxa and reduced gut microbiota. However, it is difficult to attribute direct inhibition in the latter setting.

Indirect effects. Diet-derived antigens and compounds can shape the gut microbiota in an indirect fashion by affecting host metabolism and its immune system. For example, activity of the aryl hydrocarbon receptor (AhR) is important for the maintenance of intraepithelial lymphocytes in the intestine, and in its absence, there is an increase in bacterial load attributed to members of the Bacteroidetes phylum[29]. Indole-derived and tryptophan-derived AhR ligands can be obtained from the diet (for example, from cruciferous vegetables)[29]. Furthermore, acute vitamin A deficiency leads to a bloom of *Bacteroides vulgatus* in mice due to inhibitory effects of retinol on the bacterium that can be direct or potentially mediated by a decrease in bile acids that inhibit its growth, such as deoxycholic acid, in the deficient-diet-fed mouse[30]. Vitamin D is required for gut mucosal immune defence against pathogens and the sustenance of beneficial commensals, as vitamin-D-deficient mice exhibited: diminished expression of Paneth cell defensins, tight junction genes and mucin 2 (MUC2)[31]; a decline in epithelial cadherin (E-cadherin) on the gut epithelium and immune cells; and a reduction in the proportion of tolerogenic dendritic cells and an increase in T cell receptor (TCR) αβ cells in the lamina propria[32]. Additionally, vitamin D intake in humans was associated with decreased levels of circulatory lipopolysaccharide (LPS; a component of the Gram-negative bacterial cell wall), decreased abundance of *Coprococcus* and *Bifidobacterium* and increased abundance of *Prevotella*[33]. Moreover, mice harbouring a balanced tissue omega-6:omega-3 polyunsaturated fatty acid (PUFA) ratio showed heightened production and secretion of intestinal alkaline phosphatase, which suppresses LPS-producing members of the microbiome, such as Proteobacteria[34]. Regulatory T (T_{reg}) cells have an important role in maintaining homeostasis in the gut, with deficiencies leading to intestinal inflammation and diseases as well as dysbiosis[35]. Bacterial fermentation of dietary fibre results in the production of short-chain fatty acids (SCFAs), which play an important part in maintaining T_{reg} cell homeostasis[36]. Bile acids can also indirectly

Box 1 | **Diet and fungi, viruses and archaea**

A fascinating yet largely unexplored facet of diet–microbiome–host interactions relates to its non-bacterial members — viruses, fungi, archaea, protozoa and multicellular parasites — and the complex network of interdependencies between kingdoms within the gut microbiota. Although most data have accumulated in livestock and other animals[271,272], several associations have been made between long-term and short-term dietary patterns and the fungi or archaea in the human gut[273]. Cross-kingdom communication might occur through the host by means of malabsorption, inflammation or bleeding, or through syntrophism, whereby waste products of one microorganism nourish another; for instance, yeast mannan can be utilized by the bacterium *Bacteroides thetaiotaomicron*[274].

The human virome displays a high degree of intrapersonal stability over time[275]. Small-scale studies in humans revealed that divergence from the typical developmental programme of the virome could be linked to malnutrition in neonatal life[65] and that the human virome can change following alterations in dietary fat, sugar and fibre content[276]. Another study in mice suggested that these changes are more pronounced in the mucosa-associated virome than the luminal virome[277]. Nutritional insufficiency can exert selective pressure on members of the virome to directly affect the host; for instance, selenium deficiency triggered genomic evolution in an avirulent strain of *Coxsackievirus*, which enabled it to cause myocarditis in mice[278]. Moreover, dietary modulation of the viral repertoire can influence the host through integration of bacteriophage chromosomes into bacterial genomes, thereby altering the composition[276] and functionality[279,280] of the bacterial microbiota. Thus far, this mechanism has been shown to affect bacterial virulence factors; however, its capacity to alter bacterial metabolism and its downstream effects on the host merit further research.

inhibit bacterial growth through the nuclear farnesoid X-activated receptor (FXR; also known as NR1H4)[37].

Dietary constituents might also disrupt protective functions of the intestinal barrier in ways that could affect the host–microbiome interface and prompt dysbiosis, contributing to inflammatory processes and conferring downstream implications on the host. For instance, the use of selected emulsifiers in processed foods can erode the host's protective epithelial mucous layer and lead to dysbiosis-mediated low-grade inflammation and the promotion of the metabolic syndrome in experimental models[38]. Additionally, diets rich in fat[39], Western-style diets[40] or diets low in fibre[41] were also suggested to disrupt barrier function in mice, which might be improved by fibre supplementation[40,42]; these diets will be further discussed later.

Passive transfer. Some members of the microbiota, including lactic-acid producing bacteria, *Candida* and *Penicillium* fungi and plant viruses[43], can be foodborne and therefore passively transferred and introduced into the indigenous gut microbial ecosystem by the diet. It has been proposed that the colonization of food-derived gut microbiota was dependent on the pre-existing composition of the microbiota, in both rats and humans, as some bacterial communities were more 'permissive' to allochthonous bacteria colonization whereas others were more 'resistant'[44], although additional work is required to generalize the microbial factors that mediate permissiveness and resistance.

Dietary contents as modulators

A major aspect by which diet influences the microbiota is its contents — namely, the macronutrients and micronutrients that make up consumed meals. This aspect of nutrition has been broadly investigated as it is believed that the striking surge in metabolic diseases and other sequelae in modernized societies can be attributed to changing dietary trends in the past century[2].

Dissimilarities in microbiomes of populations consuming disparate diets can be robustly inferred from studies in modern-urban versus agrarian cohorts and in herbivores versus carnivores. Various mammalian lineages have co-evolved with their microbiome assemblages that discriminate them by their dietary preferences, rather than host phylogeny: bacterial communities decrease in diversity from herbivores to omnivores to carnivores and harbour typical microbial configurations[45,46]. The gut microbiome of hunter-gatherers, as well as of rural and agricultural populations around the world, showed increased bacterial richness compared with those of modernized societies, suggesting that the former requires a greater functional repertoire to maximize their energy intake from dietary fibres than the latter, who consume mostly processed food, although such causality needs to be formally validated[47–53]. However, microbiome obtained from non-industrialized agricultural populations tended to be uniform in composition, whereas microbiome obtained from urban populations was more diverse[52], an observation that could be attributed to increased dispersal of faecal material in the rural population or to a larger variety of food products in the menus in the urban population.

Microbiota assemblages are highly plastic and responsive to some, but not all, dietary interventions. In humans, consumption of a diet composed entirely of animal products triggers enrichment in bile-tolerant bacteria (*Alistipes*, *Bilophila* and *Bacteroides*) and depletion in Firmicutes that metabolize plant polysaccharides (*Roseburia*, *Eubacterium rectale* and *Ruminococcus bromii*)[43]. Metagenomic and metabolomic analyses confirmed the observed trade-off between protein fermentation and degradation in protein-rich, animal-based diets as opposed to carbohydrate fermentation and amino acid biosynthesis in plant-based diets[43,46]. Additionally, microbiome gene richness has been reported to be positively correlated with the consumption of fruits, vegetables and fish in humans with overweight or obesity[54]. In mice, high-fat diet (HFD) or high-fat, high-sugar 'Western' diet (HFHSD) consumption has been associated with a decrease in Bacteroidetes levels and an increase in Firmicutes and Proteobacteria in a dose-dependent manner, regardless of the genotype studied[55–57]. The compositional change was accompanied by a functional change, as an HFHSD prompted increased sucrose metabolism, urea metabolism, membrane transport systems, metabolism of cofactors and vitamins and protein folding, sorting and degradation[58,59]. Conversely, less drastic and short-term dietary interventions failed to induce major microbiome alterations, some contrary to popular beliefs. For instance, only minute differences were observed in human gut bacteria composition after short-term (two 1-week intervention periods; $n = 20$) consumption of industrial white bread versus artisanal sour-dough-leavened bread[60]. Larger cohorts exposed to this intervention for longer periods of time are merited to exclude more subtle or chronic microbial effects. Likewise, 6–12 g of psyllium fibre did not alter the gut microbiota in children with

IBS (6-week intervention periods; $n = 33$)[61], fructans did not prompt changes in the microbiome composition in wild-type mice[62] and a high-cholesterol diet did not trigger dysbiosis in LDL-receptor-deficient mice[63]. These results are important to improve understanding of the true and range of effects of nutritional constituents on the microbiota[61].

The absence of nutrients has a profound effect on the microbiota and host. The study of populations in developing countries has suggested that malnutrition is often a 'two-hit process', which requires both perturbed microbiome and dietary inadequacy[64]. Stunted growth in a paediatric population in Malawi was associated with reduced levels of HMOs in maternal breast milk. When faeces from infants with stunted growth were transplanted into germ-free (GF) mice fed a Malawian diet, the growth impairment was replicated in various organs. Dietary supplementation with sialylated bovine milk oligosaccharides rescued the growth-restricted phenotype in mice and piglets[12]. Similarly, Malawian twin pairs discordant for kwashiorkor harboured different microbiome[64] (including virome[65]) consortia, and their transplantation into GF mice fed a Malawian diet resulted in greater weight loss in the group receiving a 'kwashiorkor' microbiome than in the group receiving microbiomes from the healthy siblings. Administration of a therapeutic food to the conventionalized mice, composed of peanut paste, sugar, vegetable oil and milk fortified with vitamins and minerals, attenuated this phenotype and altered faecal microbiota assembly, although it was still distinct from the healthy configuration[64]. By using a machine-learning algorithm, severe acute malnutrition could be predicted in Bangladeshi children by calculating the degree of microbiota immaturity or the diversion from a healthy microbiota composition, and the same measure could be used to evaluate the efficacy of nutritional intervention[66]. Likewise, it has been suggested that the idiopathic entity 'environmental enteropathy' (or tropical sprue), which is prevalent in developing countries, also results from dysbiosis occurring in a susceptible host[67]. Furthermore, specific nutritional deficiencies were also reported to influence the microbiome (discussed later).

On the other end of the spectrum, populations in developed countries tend to consume diets that are low in fibre. Low fibre intake in mice induced an increase in Firmicutes and a decrease in Bacteroidetes[15]. Similarly, in humans, microbiota obtained from African children, who consumed high amounts of plant polysaccharides, exhibited a low abundance of Firmicutes and a high abundance of Bacteroidetes, predominantly *Prevotella*, compared with Italian children, whose diet was characterized by a paucity of dietary fibre and who harboured increased levels of Enterobacteriaceae, predominantly *Shigella* and *Escherichia*[47]. Furthermore, gnotobiotic mice transplanted with synthetic microbiota, which included 14 human commensals, showed that switching between fibre-rich to fibre-free diets resulted in striking alterations in the gut microbial composition[41]. In the absence of dietary fibres, mucus-degrading bacteria (*Akkermansia muciniphila* and *Bacteroides caccae*) increased in abundance at the expense of fibre-degrading species (*Bacteroides ovatus* and *Eubacterium rectale*). These taxonomical changes corresponded to transcriptional changes, as upon dietary fibre deficiency mucin-degrading bacteria exhibited increased expression of mucin-degrading CAZymes[41]. Furthermore, as mentioned earlier, the absence of fibre in the diet can selectively adapt the transcriptional responses of some members of the gut microbiota, such as *Bacteroides thetaiotaomicron*, to forage on the host mucus glycans[23], thereby extending the consequences of this nutritional deficiency from the microbiota to the host.

Diet quantity as a microbial modulator

The quantities of food consumed can affect the gut microbiota. Calorie restriction — a dietary regimen based on reduced food intake in the absence of malnutrition — can trigger changes to the microbiota composition and to serum and urinary metabolic profiles in mice, both on HFDs and low-fat diets[68,69]. In humans, short-term carbohydrate restriction (24–164 g per day for 4 weeks) resulted in a decrease of butyrate-producing bacteria and consequently butyrate[70], and a calorie-restrictive regimen (10–40% reduction in energy intake for 10 weeks) led to alterations in microbiome composition,

Box 2 | **Diet and parasitic infections**

Diet might aid in the containment of parasitic infections[281,282] or in modulating their severity. For instance, combinations of an elemental diet and infection by the nematode *Nippostrongylus brasiliensis* or the protozoa *Giardia muris*, but not each of them separately, resulted in deleterious clinical outcomes and increased histological changes to the mouse gut mucosa[283], and a high-protein diet improved the course of nematode infection in ruminants[284]. Dietary constituents have been proposed to evoke host immune and metabolic transcriptional responses, such as for cinnamaldehyde and jejunal infection with *Ascaris suum* in pigs[285]. However, a more intriguing relationship between protozoa or multicellular eukaryotes and the host is mediated by the bacterial microbiota. This association was reported long before the microbiome field entered the genomic revolution and encompasses various members of the parasitome, including protozoa such as *Entamoeba*[286] and *Blastocystis*[287] and worms such as *Schistosoma*[288,289] and helminths[290–292]. This bidirectional interaction extends beyond the gastrointestinal tract, as parasites residing in the biliary tree have been shown to trigger intestinal bacterial dysbiosis[293]; conversely, gut bacterial assembly has been associated with protection against the acquisition of malaria infection[294], possibly by triggering a protective immune response through molecular mimicry[295]. Similarly, the gut microbiota can confer resistance or susceptibility to malaria infection in mice, and this phenotype can be transferred to GF mice by faecal microbiota transplantation or by probiotic treatment with *Lactobacillus* and *Bifidobacterium* spp.[296]. Preliminary attempts to utilize this parasite–bacteria crosstalk to the benefit of the host have already been proposed; for instance, prebiotic inulin supplementation in malnourished mice with giardiasis triggered microbiota alterations, increased antibody production against the protozoa and attenuated the disease phenotype[297].

Evidence suggests that helminth infections can drive gut bacterial compositional and functional shifts, especially at the gastrointestinal site of infection[298], and hence modulate the metabolism of nutrients, such as carbohydrates, amino acids and vitamin D[299,300]. Furthermore, through dysbiosis, parasites can dampen inflammatory responses in the host[300–302]. Indeed, infection of mice with the helminth *Heligmosomoides polygyrus bakeri* mediated an immunomodulatory effect by altering the microbiome and increasing short-chain fatty acid production. Transfer of the aforementioned bacterial microbiome assembly into antibiotic-treated or GF mice protected them against allergic asthma[303]. These findings warrant additional research to uncover whether cross-kingdom immunomodulatory interactions can be harnessed to modulate other systemic inflammatory responses, such as the metabolic syndrome. This new avenue of research is exceptionally engaging in light of inverse associations, which have been found between *Schistosoma* infection and diabetes in Chinese populations[304] and lymphatic filariasis and diabetes in Indian populations[305].

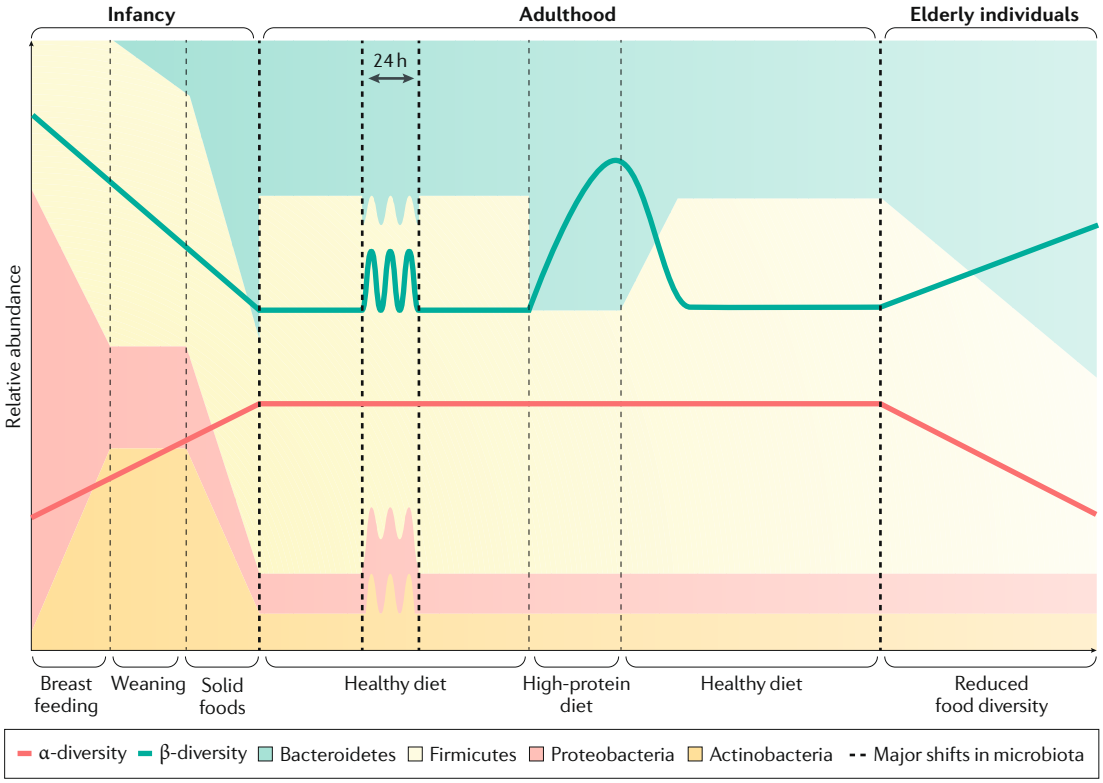

Fig. 1 | **Temporal dietary modulation of the gut microbiota.** Diet influences the gut bacterial structure and function throughout the human lifespan. Microbiota alterations mirror adaptations to nutritional shifts at different time frames: diurnal oscillations correspond to sleep–wake and feeding–fasting cycles; major alterations in food composition and quantities (in this case low-fibre, high-fat or high-protein diet) trigger transient shifts to the microbiota, which persist longer than the duration of the dietary perturbation; and long-standing dietary practices drive indolent changes to the gut microbiota. The blue line indicates the degree of resemblance of the microbiota configuration at a certain time point to an arbitrary homeostatic configuration during adulthood (β-diversity). The red line indicates the faecal microbial richness (α-diversity). Background colours indicate typical taxa abundances during each phase. Note that owing to high variability in the microbiota assembly among humans and discrepancies between studies, these microbiota patterns are only conceptual and do not aim to provide a precise representation at a personalized level.

including a decrease in *Blautia coccoides* and an increase in *Bacteroides*[71]. A longer 1-year intervention led to an increase in faecal Bacteroidetes and a decrease in Actinobacteria relative abundances, which were not initially apparent at early time points[72]. Although not fully elucidated, it is plausible that these particular changes modulate the numerous health-promoting and lifespan-promoting effects associated with calorie-restricted diets[73]. As one example, faecal *A. muciniphila* abundance was correlated with improved metabolic outcomes upon calorie restriction intervention in humans with overweight or obesity[74]. As limiting the quantity of nutrients in the diet, and more specifically energy intake, is a popular weight-loss strategy, taking into account microbial features, such as gene richness[54,75] or a 'post-obesity microbiome signature'[76], might complement the current nutritional toolbox to better contend with the obesity epidemic.

Temporal diet effects

Temporal effects of diet on microbiome composition and function can take place on multiple timescales, ranging from the diet inducing daily microbiome fluctuations through nutrition-related effects observed within days of exposure to chronic changes noted after longer exposure periods (FIG. 1). At the highest resolution, host daily circadian rhythms of sleep–wakefulness and feeding–fasting cycles are accompanied by marked compositional and functional gut microbiome changes, with absolute abundance oscillations observed in members of the three major phyla, Bacteroidetes, Firmicutes and Proteobacteria, and in levels of bacterial metabolites in the stool and the circulation[77–81]. The microbiome diurnal rhythmicity is dictated by host transcriptional oscillations and feeding times in both mice and humans[77,81]. A series of experiments in mice, nocturnal animals that normally feed during night hours, showcased that time-restricted feeding during the light phase provoked a phase shift of ~12 h in microbiota rhythms. Conversely, circadian clock knockout mutations and diet-induced obesity attenuated these microbial circadian rhythms, which were partially remedied by imposing time-restricted feeding.

Some dietary shifts have the potential to modify the gut microbiota composition and function within the course of days, although the exact time frame might be person-specific, such as the case of dietary fibre supplementation: in some individuals, microbiome alterations were observed as early as 1 day[82], 2 days[83],

or 3–4 days[84,85] following supplementation, whereas in others no effects could be noted 3 days[84], 1 week[60], 3 weeks[85] or even 12 weeks[86] after such consumption. Likewise, David et al. reported no statistically significant compositional alterations after participants switched to a fibre-rich, plant-based diet for 5 days. By contrast, switching to an animal-based diet rapidly altered the microbiome composition and function, which was reversible upon cessation and might have been attributed to very low intake of fibre or elevated intake of dietary fat and animal protein[43]. This observation was also replicated in mice colonized with a human microbiota, which displayed shifts in microbiota composition, metabolic pathways and gene expression just 1 day after switching from a plant-based polysaccharide diet to an HFHSD[15]. Interestingly, although some of the changes in mice are reversible upon dietary switch, other taxa and microbial functions were more persistent[58], thereby playing a part in exacerbated weight regain upon repeating cycles of diet-induced weight loss and gain[76]. Energy-restricted weight-loss diets can affect the microbiome composition in a time frame ranging from a few days[87] to several weeks following initiation[54], depending on the individual's microbiome gene richness. Importantly, in the absence of dietary perturbations, the human microbiota composition is considered stable[88–90]. The rural Hadza community in Tanzania is characterized by seasonal and cyclic shifts in microbiome composition, reflective of differential nutrient availability and dietary patterns in the dry versus wet seasons[50]. Microbiomes of individuals living in industrialized societies do not exhibit such variations and, interestingly, they have very low representation for taxa that fluctuate in the Hadza microbiome[50].

Long-term alterations in microbiome configurations are noted with respect to maturation and ageing and can evolve over years[14,91]. The drastic shifts in nutrition during infancy drive corresponding structural and functional adaptation to infants' indwelling gut bacteria, as the neonate microbiome harbours lactose, galactose and sucrose uptake and utilization pathways, whereas carbohydrate fermentation and vitamin biosynthesis pathways, which characterize the adult microbiome, appear only upon the introduction of solid food by the end of the first year of life[92,93]. Later in life, microbiome alterations are both substantially driven by and have a causative role in age-associated systemic inflammatory processes in old (18–22 months of age) mice[94], including increased levels of circulating pro-inflammatory cytokines and macrophage dysfunction. These alterations are highly modifiable by diet; therefore, the microbiota in elderly humans shows a great degree of interindividual variation and could serve as a marker of frailty[14,95]. Interestingly, dietary regimens can also have cross-generational consequences, as the lack of dietary fibre reduced gut bacterial diversity in mice, which could be restored over a single generation, whereas shortage in dietary fibre over several generations resulted in permanent reduction of bacterial richness, rendering some microbial taxa irreversibly extinct[96]. Similar cross-generational dysbiosis was also observed in primates[97] and mice[98–100] consuming an HFD (further discussed below).

Complex dietary interactions

Diet is inseparable from a plethora of host and environmental settings in which it is consumed. As such, it is often difficult to separate physiological effects that are caused by a diet-altered microbiota from those that are directly caused by the diet and from those in which microbiota alterations are merely a bystander or secondary effect. Unlike in vivo animal experiments, which are performed in genetically similar settings and involve normalization of diet in a well-controlled environment, humans vary considerably in their genetic makeup, are exposed to numerous exogenous factors and their diets often consist of a large diversity of nutrients. This multitude of variables can have synergistic or opposing outcomes on the gut microbiota, thereby making it difficult to anticipate the net effect of dietary interventions on the gut microbiota and downstream on the human host.

Some micronutrients or their deficiencies were found to trigger distinct patterns of microbiota structural alterations in humans, mice, rats and piglets. Noteworthy examples include iron[101–104], magnesium[105], zinc[106,107], selenium[108], nitrite or nitrate[109], vitamin A[30], vitamin D[31,32,110] and flavonoids[111,112]. Other compounds manifested properties counteracting those of modern diets, emerging as potential candidates for the prophylaxis, diagnosis and treatment of diet-induced obesity and metabolic syndrome. For example, cranberry extract increased the abundance of *A. muciniphila* in mice consuming an HFHSD and ameliorated the metabolic syndrome phenotype[113].

Geographical variations have been speculated to mask or modulate dietary influences. One study suggested that the aforementioned variability between herbivores and carnivores did not stem from dietary but from global environmental influences, as healthy human vegans and omnivores sampled in an urban environment in the USA did not show marked differences in their microbiota configuration and host metabolome[114]. By contrast, the diet of African Americans is characterized by a high content of animal fat and protein and low fibre content compared with that of South Africans and is associated with increased colon cancer risk. Performing a dietary switch between these geographically distinct groups induced shifts in the microbiome composition, function, secreted metabolites and proliferative and inflammatory markers[115]. In line with this observation, the absence of distinction between vegans and omnivores in the USA might stem from these self-reported categories being too general and insufficiently informative of diet contents; an analysis of samples in the American Gut Project published in 2018 indicated that the diversity of plants consumed in the diet enables better microbiome separation than reductive dietary categories such as veganism[116].

Nonetheless, in the geographical context, it is still important to consider that dietary recommendations beneficial in modern populations can sometimes be detrimental in developing ones. A prominent example of

this discrepancy is iron and folic acid supplementation, which resulted in increased malaria and other infection-related mortality in children residing in Zanzibar[117], presumably owing to enrichment in enteric pathogens, such as *Escherichia*, *Shigella* and *Clostridium* species and augmented inflammation[118].

The meta-community in which the host dwells can influence its microbiome, especially in co-housed rodents practising coprophagia but also in cohabitating primates[119] and humans[120], prompting horizontal bacterial dispersion among the community members[121]. Moreover, the bacterial milieu of the consumed diet can also have a role in shaping the gut microbiota, as bacteria residing in the same environment can dynamically evolve through interspecies genetic rearrangements, gene duplications and lateral gene transfers[122]. These genetic modifications broaden the gut bacterial metabolic capacity and enrich the repertoire of digestible substrates[123]. For instance, consumption of seaweed by Japanese populations contributes to gene transfer from marine microorganisms to the gut microbiome, enabling the latter to digest algal species[124], a feature that could be utilized for diet-based niche modulation for engraftment of beneficial bacteria[125]. Furthermore, preliminary data point to noteworthy interactions between diet and the host virome, mycome, protozoa and other eukaryotes, adding an additional facet to diet–microbiome–host interactions (BOXES 1,2).

Finally, the host genetic makeup can influence digestion. For example, human populations that consume starch-rich diets possess a higher number of copies of the salivary amylase gene than those consuming low-starch diets[126]. Moreover, mice harbouring mutations in signal transduction pathways or steroidogenesis manifest dysbiosis and downstream metabolic consequences affecting obesity, adipose tissue inflammation and insulin resistance[127,128]. However, the true extent of genetic contribution to microbiome structure in humans seems minor according to evidence from twin studies[129], and diet seems to be dominant over genotype in multiple genetically distinct inbred and outbred mice[58,130]. In humans, diet is not only dominant over genetics in affecting the microbiome composition but also superior in prediction of multiple host traits, such as blood glucose levels and obesity measures[131].

Diet–microbiota interactions and health

Modulation of the gut microbiota composition and function by the diet could result in beneficial or detrimental consequences on host health. This could be due to immunomodulatory effects of the modified microbiota, downstream effects on host gene expression or alterations in the landscape of microbiota-produced metabolites, which might act locally in the gut or systemically in other organs. Importantly, microbiota-mediated effects of diet on health do not necessarily require alteration of the global community configuration but could result in dietary input differentially interacting with distinct microbial populations (for example, distinct microbiota communities might have a role in the outcome of a therapeutic dietary intervention for malnutrition[64]). Here, we discuss how major food components interact with the microbiota to affect host health through multiple mechanisms.

Fibre

Fermentation of dietary fibre is one of the dominant functions of the caecal and colonic microbiota and a major source for SCFAs, which are the fermentation end products (FIG. 2). SCFAs serve as signalling molecules, either by inhibiting histone deacetylases (HDACs) or by acting as ligands for several G protein-coupled receptors (GPRs; including GPR41 (also known as FFAR3), GPR43 (also known as FFAR2) and GPR109A (also known as HCAR2)) and peroxisome proliferator-activated receptor-γ (PPARγ)[132,133]. Supplementing the HFD of mice with butyrate prevented diet-induced obesity and insulin resistance, and increased energy expenditure[134,135]. In humans treated with propionate, weight gain was prevented in individuals who were overweight (24-week supplementation of 10 g per day inulin-propionate ester; $n = 60$)[136] and glucose tolerance was improved in healthy women (7-week supplementation of 7.5 g per day sodium propionate; $n = 10$)[137]. Colonic infusions with acetate, propionate or butyrate in levels matched to those derived from fibre intake improved energy metabolism in men who were overweight or obese (two rectal administrations of 40 mmol acetate, propionate or butyrate repeated four times; $n = 12$)[138]. De Vadder et al.[139] suggested a mechanistic link in which butyrate and propionate derived from microbiome fermentation of fibre promoted gene expression related to intestinal gluconeogenesis by cAMP-dependent activation or via an FFAR3-dependent gut–brain neural circuit. Frost et al. also reported a beneficial role for fibre-derived acetate mediated by a central appetite-modulating mechanism, as HFD-fed mice supplemented with fermentable fibre were leaner, consumed less food and expressed an anorectic neuropeptide expression profile in the hypothalamus[140]. By administering labelled carbohydrates, they showed that colonic acetate accumulated in the hypothalamus and confirmed changes in hypothalamic neuronal activation by functional brain imaging after intravenous acetate infusion[140].

SCFAs, and especially butyrate, have an important role in maintaining intestinal immune homeostasis and protecting against inflammation and carcinogenesis[141,142]. This process could be achieved by regulation of the inflammasome[143] or by promoting and regulating T_{reg} cells[36,144,145]. SCFAs can also act outside the gut; a fibre-rich diet can suppress allergic airway disease by enhancing T_{reg} cell number and function through HDAC9 inhibition[143,146] or by FFAR3-dependent haematopoiesis of dendritic cells that reduce T helper 2 (T_H2) cell effector function[147]. Fermentation of dietary fibre to SCFAs can also help the host defend against pathogens such as *Clostridium difficile*[148] and *Salmonella enterica* subsp. *enterica* serovar Typhimurium[149] in mice and piglets, respectively.

In addition to production of SCFAs, the gut microbiota can mediate the health effects of fibre through additional mechanisms. Supplementing the diet with barley-kernel-based bread was associated with improved glucose tolerance that was more apparent in individuals with high levels of *Prevotella*, which protected against *Bacteroides*-mediated glucose intolerance and promoted hepatic glycogen storage in mice[84].

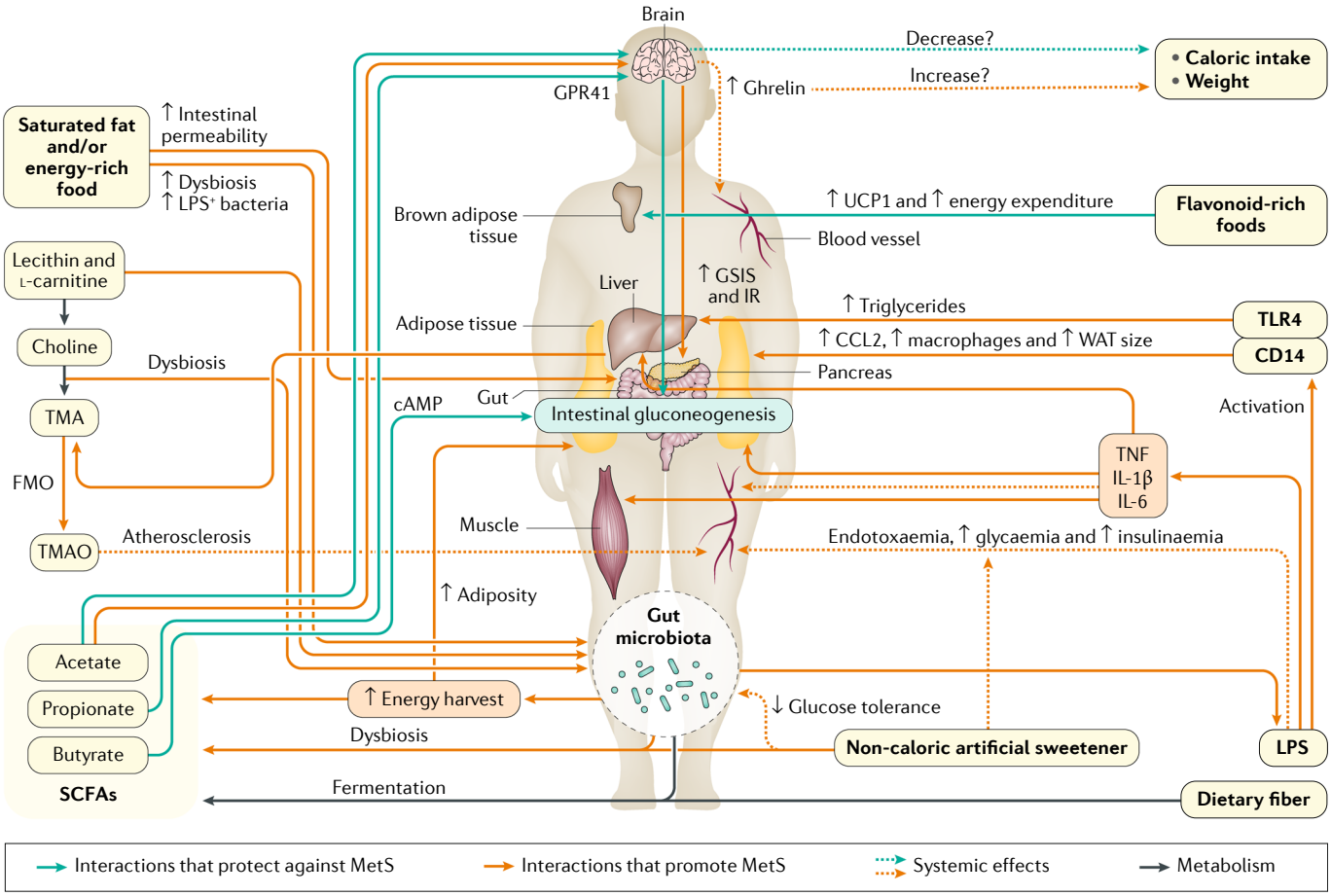

Fig. 2 | **Microbiota–diet interactions in the metabolic syndrome.** Common dietary components are metabolized by the gut microbiota to produce metabolites (for example, dietary choline and trimethylamine (TMA)) that modulate host metabolism (e.g. in atherosclerosis). In parallel, diet modifies the composition of the microbiota and consequently the landscape of microbial-associated products, of which some are linked to a beneficial or detrimental effect on the host (for example, fat, lipopolysaccharide (LPS) and endotoxaemia). Some of the interactions are localized to the gut (for example, fibre, short-chain fatty acids (SCFAs) and intestinal gluconeogenesis), whereas others have a systemic effect (for example, fat, acetate and insulin resistance (IR)). Orange lines indicate interactions that promote the metabolic syndrome (MetS), and green lines indicate interactions that protect against the MetS. Dashed lines indicate systemic effects. CCL2, CC-chemokine ligand 2; FMO, flavin-containing mono-oxygenase; GPR41, G protein-coupled receptor 41; GSIS, glucose-stimulated insulin secretion; TLR4, Toll-like receptor 4; TMAO, trimethylamine *N*-oxide; UCP1, mitochondrial brown fat uncoupling protein 1; WAT, white adipose tissue.

Several studies from the past few years point to an important role of fibre in promoting intestinal barrier function. Protection against pathogens is impaired when animals are fed a low-fibre diet owing to a switch of the gut microbiota nutrient source from fibre to the host mucus. This process leads to erosion of the mucous layer, which disrupts barrier function and enables lethal colitis when mice are infected with the mucosal pathogen *Citrobacter rodentium*[41]. Although supplementing the low-fibre diet with purified fibres (such as inulin) did not abrogate *Citrobacter* susceptibility, purified fibres might mitigate the detrimental effects of a diet rich in fat on the gut barrier and consequently on host health in a mechanism that involves either fibre-mediated promotion of bacteria critical for mucus function[40] or IL-22 induction[42].

Interestingly, the interaction between fibre and the gut microbiota might not always be beneficial to the host. In contrast to several aforementioned beneficial reports[142,150], in at least one example fibre-derived butyrate was associated with tumorigenesis in a genetically susceptible mouse model of colorectal cancer deficient in both the *Apc* gene and the mismatch repair gene *Msh2*. In this setting, butyrate promoted tumorigenesis by inducing stem-cell-like characteristics in the intestinal crypts, potentially leading to stem cell generation and self-renewal[151]. This observation remains to be validated in humans.

Fat

For decades, high intake of dietary fat was discouraged owing to a presumed association with cardiovascular diseases (CVDs) and obesity. A meta-analysis of prospective cohort studies published between 1981 and 2007 did not supported such an association[152]; consequently, the latest version of dietary guidelines issued

in 2015 by the US Departments of Agriculture and Health no longer call for a reduction in total fat intake but rather for optimization of fat types in the diet, and specifically reduced intake of saturated and trans fats[153]. This recommendation is supported by mechanistic studies demonstrating that the quantity and the source of fat can have differential effects on the host and that some of these fat-mediated effects are transmitted through changes induced in the gut microbiome. A gut microbiota modified by a diet rich in fat is characterized by over-representation of LPS-expressing bacteria, leading to elevated levels of LPS in the circulation of both mice[57] and humans[154], a pro-inflammatory state that is termed 'metabolic endotoxaemia'. LPS then signals through Toll-like receptor 4 (TLR4)[155] and CD14 (REF.[57]) in haematopoietic cells to promote weight gain and adiposity, elevation of inflammatory markers in white adipose tissue (WAT) macrophages and insulin resistance. In parallel, metabolic endotoxaemia is also associated with increased gut permeability, and reduced expression of genes encoding for tight junction proteins could be the cause[156]; all the aforementioned alterations were reversible upon antibiotic treatment[156]. Interestingly, these adverse effects seem to be specific to saturated fat; mice fed a lard-rich diet are characterized by blooms of *Bacteroides*, *Turicibacter* and *Bilophila* spp., which promote WAT inflammation as well as adiposity and insulin insensitivity in a manner dependent on myeloid differentiation primary response protein MyD88 (MYD88), TIR domain-containing adapter molecule 1 (TRIF; also known as TICAM1) and CC-chemokine ligand 2 (CCL2)[157]. By contrast, mice fed unsaturated fish oil were characterized by expansion of *Bifidobacterium*, *Akkermansia* and *Lactobacillus* spp. and did not demonstrate metabolic impairments. Replication of the metabolic phenotype in GF mice transplanted with these distinct microbial compositions suggested a role for the gut microbiota in mediating the differential effects of fat type on the host health[157]. In human individuals at risk of the metabolic syndrome ($n = 22$ in a 24-week trial), switching from a diet rich in saturated fat to an isocaloric diet rich in unsaturated fat did not affect microbiota composition but did reduce total bacteria counts[158]. More direct comparisons are needed to understand the differential effect of fat type on the human microbiota.

In addition to metabolic implications, the gut microbiota could also link fat consumption to an increased propensity for intestinal inflammation in the host. This aspect was noted in wild-type HFD-fed mice but not in TLR4-deficient HFD-fed mice, suggesting a role for Gram-negative commensal microorganisms and associated LPS in mediating this dietary-metabolic phenotype[39]. In addition to over-representation of LPS-expressing bacteria, the HFD-associated microbiome is sometimes associated with decreased levels of the SCFAs butyrate and retinoic acid (RA)[159], which both contribute to gut homeostasis and regulating the homing and differentiation of dendritic cells and T_{reg} cells[144,160]. Thus, the depletion of butyrate and RA by HFD results in exacerbation of chemically induced colitis in mice[159]. In addition to promoting colitis by repression of T_{reg} cells, fat-altered microbiota can also activate dendritic cells to promote T_H1-mediated colitis in genetically susceptible mice[161]. Saturated fat can also contribute to colitis by promoting taurine conjugation of bile acids by the host and thereby expanding the abundance of *Bilophila wadsworthia*, which utilizes them as terminal electron receptors and produces hydrogen sulfide or secondary bile acids, potentially leading to intestinal barrier disruption and consequently immune cell infiltration[161].

Reports on the interaction between dietary fat, obesity and SCFAs contradict the aforementioned positive effects of SCFAs in the context of fibre intake. In their seminal study, Turnbaugh et al. have reported an increased capacity for energy harvest from food by the microbiome of obese mice. In this proposed mechanism, fermentation of indigestible carbohydrates results in the production of the SCFAs acetate, propionate and butyrate[162], a process also demonstrated in humans with obesity[163]. These SCFAs can serve as energy sources in the colon (butyrate) or peripheral tissues (acetate and propionate), among multiple other metabolic and immune modulatory roles[2], and it is hypothesized that this process also leads to more available energy for the host and therefore weight gain and adiposity. Coincidently, in humans consuming a diet rich in saturated fat and in HFD-fed mice, elevated levels of faecal SCFAs[158] were accompanied by reduced faecal energy content, suggesting that dietary fat can contribute to obesity through increased energy harvest[164]. However, it is important to note that the lower faecal energy content could also be a result of increased energy expenditure or decreased food intake, which is not always reported, although there is currently no direct evidence implicating SCFA-induced increased energy harvest with weight gain. Furthermore, a high-fibre diet, which also increases the levels of SCFAs, is associated with reduced weight gain in humans[165], and SCFA supplementation protects mice from HFD-induced obesity[166].

In addition to their proposed association with increased energy harvest, the SCFA acetate can contribute to metabolic syndrome through effects on the gut–brain axis. Perry et al.[167] reported that HFD-fed rats have elevated plasma and faecal levels of microbiota-derived acetate, which activates the parasympathetic nervous system to overproduce insulin in response to glucose and elevates the levels of the hunger-associated hormone ghrelin, resulting in a vicious cycle in which fat promotes overfeeding and in parallel disrupts glucose homeostasis. This finding is in contrast to the aforementioned report by Frost et al., in which acetate activity in the mouse hypothalamus repressed appetite[140]. The multiple mechanisms by which dietary fat interacts with the microbiota to promote metabolic outcomes are summarized in FIG. 2. Notably, additional works are required to resolve multiple conflicts regarding the role of SCFAs in the metabolic syndrome and their interaction with fibre versus dietary fat.

Interestingly, the disruptive effect of fat on the microbiome crosses generations, as the offspring of HFD-fed primates[97] or mice[98,99] also harbour a dysbiotic gut microbiome. In mice, this inherited microbiome was associated with reduced gut immunity, increased susceptibility to infections, and development of allergies and autoimmunity in an LPS-dependent mechanism[98],

as well as with non-alcoholic fatty liver disease and steatohepatitis[99]. In both primates and mice, feeding the offspring with a low-fat diet did not completely reverse these effects. Likewise, maternal HFD feeding was suggested to be associated with increased susceptibility to dextran sodium sulfate (DSS)-induced colitis in mouse offspring[100]. Nevertheless, parental HFD feeding is also associated with altered epigenetic signatures[99]. As some of the studies did not discuss this aspect and others have not demonstrated an uncoupling of epigenetic-related consequences from microbiota-related consequences, the extent to which the inherited microbiome has a causative and epigenetic-independent role in the detrimental effects observed in the offspring remains to be determined. Future studies with antibiotic treatment of the offspring might be insightful for the role of the microbiome in these cross-generational phenotypes.

The dietary saturated long-chain fatty acid (LCFA) palmitate was also associated with aggravated central nervous system autoimmunity in a mouse model of multiple sclerosis, in part owing to a reduction in microbiota-produced SCFA levels (specifically propionate), which are protective in this model[168]. Importantly, in a different mouse model of autoimmune osteomyelitis, saturated fat had a protective role owing to HFD-mediated microbiome modulations, repressing microbial groups that were shown to promote inflammasome-mediated and caspase-8-mediated maturation of IL-1β and osteomyelitis[169], an effect attributed by the authors to *Prevotella*.

To conclude, available evidence suggests that saturated fat modifies the microbiome to promote detrimental effects that are partially inheritable, resulting in context-specific risk of the metabolic syndrome, colitis or central nervous system autoimmunity, by altering the immune landscape in the gut or systemically, increasing energy harvest from food and modifying levels of SCFAs. Additional studies, especially in humans, are required to resolve conflicting reports regarding the ability of dietary fat to increase or decrease SCFA levels and how these changes might affect satiety. Importantly, current data indicate that the type of fat[157], and multiple additional factors such as disease susceptibility[161] and presence of specific commensals that interact with fat[169], should be considered.

Animal protein and processed meat

Red and processed meat are commonly associated with an increased risk of developing CVD, with the suspected culprits often cited as saturated fat and cholesterol owing to an established link between hyperlipidaemia and hypercholesterolaemia and CVD[152]. Nevertheless, insufficient evidence is available supporting a role for dietary intake of fat in this link to CVD[152], suggesting that other factors or nutrients could be involved (FIG. 2). Red meat is specifically rich in L-carnitine, which is metabolized by the gut microbiota to trimethylamine (TMA)[170]. TMA is in turn transported by the portal circulation to the liver and converted into trimethylamine *N*-oxide (TMAO) by flavin mono-oxygenases. TMAO is associated with promoting atherosclerosis and, indeed, mice chronically fed with L-carnitine had an altered gut microbiota composition, elevated synthesis of TMA and TMAO and increased atherosclerosis, which were inhibited by antibiotic treatment. Omnivore humans challenged with L-carnitine had higher TMAO levels than vegans or vegetarians, which was also blocked by antibiotic treatment. In both mice and humans, specific members of the gut microbiota were associated with the ability to transform L-carnitine to TMA or TMAO, with a common association with *Prevotella* in both organisms[170]. In addition to atherosclerosis, microbial production of TMAO was also associated in humans with platelet hyper-reactivity and associated risk of thrombosis[171].

Processed meat has also been associated with colorectal cancer risk in humans owing to the production of carcinogenic heterocyclic amines in the process of charring[172,173]. Lactic-acid-producing bacteria (such as *Lactobacillus*) can directly bind heterocyclic amines and therefore potentially protect the host from the induction of DNA damage and neoplasia according to experimental evidence[174]. Red meat is also rich in haem, which is associated with colonic cytotoxicity and hyperproliferation[175]. Interestingly, a haem-rich diet in mice leads to a bloom of mucin-degrading bacteria such as *A. muciniphila*, leading to impaired intestinal barrier function due to degradation of the mucous layer[175]. Consumption of red meat has also been linked with colon and gastric cancers owing to its association with elevated endogenous production of carcinogenic *N*-nitroso compounds[176]. Comparison of *N*-nitroso compounds in GF versus conventionalized rats consuming nitrate suggested that the gut microbiota is responsible for *N*-nitroso compound production[177], potentially through enzymatic activity of nitrate reductase.

Thus, specific members of the gut microbiota might protect against or mediate the health consequences of metabolites associated with red and processed meat consumption, although many of these associations lack a proof of causation and merit further studies.

Food additives

One of the major alterations to human diet during the past decades is the consumption of processed foods, which often contain synthetically produced or natural additives, such as preservatives, sweeteners, emulsifiers and fortifying agents. These additives are usually considered by food regulators as safe on the basis of published scientific evidence at the time of approval[178]. With advances in our ability to study the microbiome and its interactions with diet and disease, it will also be important to determine whether any of these compounds interact with the resident microorganisms and what the consequences of such interactions would be.

Dietary emulsifiers are added to many foods (such as industrially produced ketchup) to maintain an emulsion of oil and water. Chassaing et al. reported that low quantities of two common emulsifiers, carboxymethylcellulose and polysorbate-80, promote a dysbiotic microbiota, which induces low-grade inflammation, metabolic syndrome and colitis in mice[38]. When the responses to these compounds were analysed in culture with a human gut microbiota, elevated levels of bioactive flagellin were measured, stemming from either dysbiosis or altered

bacterial gene expression[179]. Moreover, transplant of these modified human microbiota into GF mice recapitulated many of the phenotypes observed in mice fed with emulsifiers[179]. Another emulsifier that could interact with the microbiota to affect human health is phosphatidylcholine (a type of lecithin). As with L-carnitine and other choline moieties, lecithin is transformed by the gut microbiota to TMA and consequently increases the levels of TMAO and the risk of CVD[180].

Another commonly consumed group of food additives are non-caloric artificial sweeteners (NAS), which are promoted as a common weight-loss strategy to limit the number of calories consumed in the diet by switching foods and drinks containing calorie-rich sugars with non-caloric sweet substitutes. Studies on the efficacy of this approach demonstrate mixed and conflicting results, in both observational studies in humans and interventions in rodents: some demonstrate a beneficial role for NAS in weight loss, whereas others report the counterintuitive effect of NAS in promoting weight gain and other associated metabolic derangements. These opposing findings are reviewed elsewhere[181] and could be reconciled, at least in part, by considering a role for some microbiome configurations in mediating the effects of NAS on metabolism.

Several studies have reported both dysbiosis and disruption of metabolic homeostasis in rodents consuming NAS such as saccharin[182–184], sucralose[185,186], aspartame[187,188], cyclamate[189], neotame[190] and acesulfame-potassium[191] (FIG. 2). Functional analyses performed either on the gene content of the altered microbiome or its secreted metabolites suggest that the NAS-induced dysbiosis led to the metabolic phenotypes, and for saccharin, a direct link was established by replicating glucose intolerance in naive GF mice transplanted with faecal microbiota from saccharin-drinking mice or naive microbiota modified in vitro by saccharin[182].

Interestingly, in two rodent studies[182,187] on different NAS (saccharin and aspartame), consumption was associated with increased levels of acetate and propionate, suggesting an increased energy harvest capacity of the NAS-altered gut microbiota. In a small-scale intervention trial in humans, disrupted glucose homeostasis after saccharin consumption was evident in some, but not all, of the participants, pertaining both to their pre-exposure and saccharin-induced alterations in their microbiome composition (6-day supplementation of 120 mg saccharin per day, $n=7$)[182]. Although large-scale replication of these findings in prospective randomized trials is mandated, it suggests that opposing outcomes regarding the health consequences of NAS consumption stem from differences in the microbiomes of the participants and that by identifying the microbiome susceptibility signature, we can distinguish between individuals who might benefit from substituting caloric sweeteners with NAS and those who should avoid them.

Minerals

Supplementing the diet with iron is a common approach to prevent and treat anaemia, particularly in infants. However, bacteria and especially some pathogens are efficient iron scavengers[192]. Iron supplementation could therefore result in dysbiosis and bloom of pathogens[103,118]. Similarly, supplementing the diet with manganese increased bacterial colonization of the heart and the lethality of *Staphylococcus aureus* infection in mice, potentially owing to utilization of manganese by the bacterium to protect from reactive oxygen species and neutrophil killing[193].

Plant-derived bioactive nutrients

In addition to fibre, plants contribute many bioactive compounds to the human diet. The polyphenols are a large and diverse group of compounds, several of which have been associated with beneficial health claims. For example, supplementation of HFD-fed mice with polyphenols derived from either grapes[194] or cranberries[113] reduced the inflammatory and obesogenic effects of the diet, which was associated with a bloom in *A. muciniphila*. Despite these and multiple other associations, it is difficult to dissect the health effects of polyphenols in humans, and especially flavonoids, owing to considerable interindividual variation in the response to the compounds, which could stem from differences in the gut microbiota[195]. Identifying the bacteria that interact with polyphenols and the mechanisms is therefore an important step in understanding their effect on the host. An important role for flavonoids, in close association with microbiota alterations, was described in mice undergoing repeated dieting cycles[76]. HFD-fed mice had a marked depletion in gut levels of the flavonoids apigenin and naringenin due to low dietary availability and an expansion of flavonoid-degrading commensals. Switching HFD-fed mice to a normal polysaccharide diet normalizes their metabolic parameters, but not their gut microbiota composition, which persistently degraded these flavonoids, resulting in low levels. As the successfully dieted mice were re-fed an HFD, the low flavonoid levels served as a 'microbiome memory' to further aggravate the metabolic effects of HFD by affecting brown adipose tissue heat production. Supplementing dieting mice with dietary apigenin and naringenin prevented the exacerbated weight regain by replenishing their ability to regulate energy expenditure. Thus, an interaction between the microbiota and a diet low in flavonoids or flavonoid supplementation can exacerbate or protect against the detrimental health effects of an HFD. As weight loss-and-gain cycles are common in humans, it will be important to determine whether this mechanism is shared across mammals.

Examples of other plant compounds modified by the gut microbiota to a form that is associated with health benefits include the hydroxycinnamates caffeic, coumaric and ferulic acids, present as ester conjugates in plants and considered in their free chemical form to be anti-inflammatory and antioxidative compound[196]. Members of the *Bifidobacterium*, *Lactobacillus* and *Escherichia* genera are able to liberate these compounds from their conjugated plant form[197], influencing individualized levels of these bioactive compounds[197]. At the same time, the gut microbiota degrades otherwise toxic plant-derived compounds such as oxalate, which is abundant in several greens, nuts, berries and tea and forms calcium oxalate crystals that might lead to renal stone formation[198]. Of the bacteria that catabolize

oxalate, *Oxalobacter formigenes* is a key player, and low abundances of this taxon are associated with elevated concentrations of urinary oxalate and increased risk of urinary tract stones in humans[198].

Dietary-based microbiota therapies

The numerous studies associating dietary regimens, gut microbiota changes and health led to a plethora of interventions aimed at promoting a 'healthy microbiota' and pursuing a 'healthy diet'. Although several dietary approaches might be universally beneficial or detrimental, diet–microbiota–host crosstalk is emerging to be highly complex, with multiple components presenting both beneficial and detrimental effects in different clinical contexts (TABLE 1). Thus, the search for a 'magic bullet' beneficial dietary intervention strategy could be limited and confounded by the many factors affecting dietary responses at the individual level. For example, evidence from mouse models suggests that limiting saturated fat in the diet improves the metabolic syndrome[56,57,157,167], IBD[39,159,161] and multiple sclerosis[168] but could adversely affect the features of osteomyelitis by promoting blooms of *Prevotella* and associated inflammatory responses[169]. Possible beneficial effects mediated by dietary compounds such as polyphenols or NAS on prevention of the metabolic syndrome[113,181] might depend on an individual's gut microbiota composition[195] and in some instances could even be associated with elevated risk of the metabolic syndrome[182]. Consuming fibre has been shown to be beneficial for combating the metabolic syndrome in humans by multiple potential mechanisms, including preventing weight gain and improving insulin sensitivity[199], but in at least one mouse model ($Apc^{Min/+}Msh2^{-/-}$ animals) fibre aggravated colorectal cancer[151]. The abundance of *Prevotella* has been associated with IBD (in mice[200] and humans[201]), osteomyelitis (in susceptible $Pstpip2^{cmo}$ mice[169]) and rheumatoid arthritis (in humans[202]) but can be beneficial for glucose tolerance in humans and mice[84]. Experimental evidence has shown that supplementation of *A. muciniphila*[203] or its associated molecules[204] could be beneficial in preventing features of the metabolic syndrome, but its elevated abundance might promote colitis[205] or colorectal cancer[175]. The Firmicutes:Bacteroidetes ratio has been shown to increase[206–208], decrease[163] or have no change[209–211] in individuals with obesity versus those who are lean. SCFAs are associated with beneficial effects on the host in a range of conditions[136,140,147,148,212], but some detrimental effects were also noted[162,167,187].

Given this complexity, several layers of precision should be considered when aiming to promote health by altering the diet or gut microbiota (FIG. 3). One consideration is the desired health benefit: is the goal to prevent a specific disease or to treat an active one? Does the individual have a genetic or congenital predisposition to this disease[213]? Equally important are dietary considerations: how will the supplemented or subtracted nutrient interact with the rest of the diet? Might the dietary intervention introduce exogenous bacteria that could have a detrimental interaction with the current diet? These questions are coupled with microbiota considerations: will the interaction of the microbiota with the selected nutrient be beneficial or detrimental? Will exogenous bacteria be able to colonize the niche? Although the complexity of these questions might seem demotivating, we will discuss how these can be resolved to benefit from the promise of microbiota-modifying dietary approaches.

Prebiotics

Prebiotic dietary interventions — typically referred to as non-digestible food ingredients or substances that stimulate the growth or activity of health-promoting bacteria colonizing the large intestine[214] — have been proposed as a means of driving gut microbiota shifts to benefit the host. The administration of fermentable dietary fibre in the form of inulin, oligofructose, FOS or galacto-oligosaccharide has been extensively studied and generally suggested to increase the abundance of *Bifidobacterium* and *Lactobacillus* spp. in human stool (with *Bifidobacterium* spp. being associated with an increase in SCFAs) across several age groups and medical conditions[215,216]. However, it is important to consider the limitations of the available evidence, as study populations and methodologies varied greatly, and the aforementioned effects were not always reproducible and only occasionally translated into clear clinical outcomes in humans, such as immunomodulatory effects[217], metabolic effects[218] or protection against enteropathogenic infections[219,220]. Notably, the response to prebiotics in humans has been suggested to be person-specific[221] and dependent on the initial gut microbiota composition[222,223]. Moreover, easily accessible stool sampling might recapitulate, at least to some extent, the large intestinal lumen while under-representing the mucosal microbiota, an ecosystem at the intersection between the microbiota and the host[224].

Other prebiotic agents have been identified and tested in both mice and humans for their capacity to modulate the microbiota and benefit the host. For instance, wholegrain barley and brown rice (60 g per day of either or a mixture of both) improved faecal bacterial diversity, increased the Firmicutes:Bacteroidetes ratio and the abundance of *Blautia*, attenuated postprandial peak blood glucose levels and decreased plasma IL-6 levels in healthy individuals ($n = 28$)[225]. A diet based on vegetable and fruit juice (6 bottles daily for 3 days) decreased the abundance of faecal Firmicutes and Proteobacteria, increased Bacteroidetes and Cyanobacteria and induced functional changes suggestive of beneficial metabolic properties in healthy volunteers ($n = 20$)[226]. Nopal, a cactus used in Mexican traditional medicine, and berberine, a component of a Chinese herb, have been suggested to modulate gut microbiota composition, promote SCFA production and lead to an improved metabolic phenotype in rats[28,227]. Other microbiota-modifying prebiotics include oligosaccharides[203,228–230], conjugated linoleic acid[231] and milk sphingomyelin[232], which have been suggested to enhance metabolism in HFD-consuming mice. Surprisingly, some commonly prescribed medications might also serve as prebiotics (for instance, the antidiabetic drug metformin increased the proportion of *A. muciniphila* in diet-induced obese mice[233] and individuals with type 2 diabetes[234]), potentially owing to

an increase in the number of mucin-producing goblet cells[233] and alluding to a microbiota-dependent mechanism for its anti-diabetic properties. Notably, the definition for 'prebiotics' has been revised, emphasizing their implication on microbial ecology and functional features relevant to the host physiology rather than focusing on the specific activity of selective bacteria[235].

Probiotics

Dietary supplementation with probiotic bacterial strains aims at replenishing the gut with advantageous commensal bacteria, which grant favourable metabolic properties to the host. This multibillion dollar industry has been adopted worldwide by food manufacturers and suggested to confer health benefits for various conditions, including the metabolic syndrome[236], gastrointestinal infections[237,238] and IBD[239]. However, many aspects of probiotic therapy remain controversial, and in most cases probiotics have not been reproducibly shown to induce health benefits in humans compared with placebo in randomized controlled trials (RCTs) and meta-analyses on antibiotic-associated diarrhoea[240], asthma[241] and Crohn's disease[242]. Moreover, many of the findings related to probiotics are associative, lack insights into the underlying mechanism and have been performed in animal models or in vitro conditions with limited human studies. As such, no single probiotic has been approved by the FDA for medical purposes[243].

One limitation in the utilization of probiotics is that strains used by the industry and approved by regulatory agencies are often characterized by low virulence, which are chosen based on their lack of effect on the taste of food and their capability of surviving in dairy products or pills and are universally provided as a 'one-size-fits-all' intervention[244]. Hence, albeit less popular, commensal-based interventions might also be considered as probiotics and can potentially surpass the commonly used strains with regard to some health benefits. For example, treatment with *A. muciniphila*[203] or *B. thetaiotaomicron*[245] has successfully reversed several components of the metabolic syndrome in HFD-consuming mice. *A. muciniphila* could also serve as a prognostic and diagnostic tool for the assessment of dietary interventions, as individuals who were overweight or obese with a higher abundance of this taxon showed greater improvement in insulin sensitivity and other features of the metabolic syndrome in response to a calorie restriction intervention ($n = 49$, 1,200–1,500 kcal per day for 6 weeks)[74]. An alternative or a complementary approach could be strain mixtures, which might be more effective than some single-strain preparations[246]. In light of the great variations in microbiome configurations among humans, the current universal probiotics approach seems debatable and an individualized approach is warranted[247].

Personalized nutrition

Given the multiple variables affecting the intricate interrelationships between the host, its resident microbiota and their responses to diet, it is apparent that one diet cannot fit all, and the commonly used notion of personalized medicine should also be practised when devising individualized menus[2]. These diets should not only be personalized in terms of constituents and their quantities, but also ideally take other considerations, such as the temporal, geographical and medical context, into account. The evolution of precision diets started with the identification of a single or a few microbiota-related variables that modify the outcomes of dietary interventions. For instance, reduced microbial gene richness was found to be inversely correlated with the efficacy of diet-induced weight loss and weight stabilization interventions in individuals who were overweight or obese ($n = 49$)[54]; the initial assembly of the gut microbiota predicted enrichment of specific taxa in response to dietary interventions in men who were overweight ($n = 14$)[85]. Healthy individuals ($n = 20$) who improved their glucose metabolism following the consumption of barley-kernel-based bread harboured a high *Prevotella:Bacteroides* ratio in their faecal microbiota before supplementation[84], and healthy individuals who exhibited impaired glucose tolerance following the consumption of artificial sweeteners harboured a distinct microbiota composition before the initiation of the intervention and developed more pronounced dysbiosis than non-responders ($n = 7$)[182]. With the advent of advanced big data analytical methods, it is now possible to decipher multivariate interactions and propose precision interventions. As such, a statistical model based on mice harbouring a ten-member bacterial community and exposed to perturbations in four defined ingredients (protein, fat, polysaccharide and simple sugar) could predict more than half of the variation in microbiota species abundance attributed to diet[248]. Similarly, a simple model based on specific faecal taxa abundances and the host genotype could reliably predict susceptibility to choline deficiency-induced fatty liver in healthy women ($n = 15$)[249].

Collectively, precision diets should be constructed according to personalized parameters such as age, gender, location, metabolic status, initial gut bacterial assembly and function and food preferences, among many others. Indeed, the glycaemic response to bread in healthy humans was found to be dependent on individual parameters to a greater extent than on the type of bread consumed ($n = 20$)[60], rebutting the prevailing axiom that 'healthiness' is an inherent property of the food consumed and therefore some foods are universally 'healthier' than others[60]. A study in 800 healthy individuals[250] proposed to incorporate similar individual parameters in dietary planning by implementing a machine-learning algorithm, which was fundamentally based on structural and functional microbiome features, and demonstrated that it could accurately predict postprandial glucose responses to various types of food, surpassing the widely used current gold standard models of carbohydrate counting or calorie counting. Moreover, a short-term dietary intervention based on personally predicted postprandial glucose responses could successfully maintain normoglycaemia in healthy individuals. Notably, applying personally tailored diets was associated with shifts in the gut microbiota composition following 1 week of intervention, thus meriting periodic reassessments of the

Table 1 | **Complexity of diet–microbiome–health crosstalk**

Dietary component	Bacteria	Metabolites or mediators	Disease risk
Red meat (L-carnitine)	*Prevotella*[a]	↑ TMAO	↑ CVD
Red meat (L-carnitine)	*Bacteroides*[b]	↓ TMAO	↓ CVD
Emulsifiers (lecithin)	?	↑ TMAO	↑ CVD
Emulsifiers (P80 and CMC)	↑ Proteobacteria[a]	↑ LPS and flagellin	↑ Colitis and metabolic syndrome
Emulsifiers (P80 and CMC)	↑ *Akkermansia*[a]	↑ LPS and flagellin	↑ Colitis and metabolic syndrome
Red meat (heterocyclic amines)	*Bacteroides*[a]	↑ 7-OHIQ	↑ Carcinogenesis
Red meat (heterocyclic amines)	*Clostridium*[a]	↑ 7-OHIQ	↑ Carcinogenesis
Red meat (heterocyclic amines)	*Eubacterium*[a]	↑ 7-OHIQ	↑ Carcinogenesis
Red meat (heterocyclic amines)	*Lactobacillus*[b]	↑ IQ and PhIP	↓ Carcinogenesis
Red meat (haem)	↑ *Bacteroides*[a]	↑ LPS?	↑ Colon cancer
Red meat (haem)	↑ Sulfate-reducing bacteria[a]	↑ Hydrogen sulfide	↑ Colon cancer
Red meat (haem)	↑ *Prevotella*[a]	↑ LPS?	↑ Colon cancer
Red meat (haem)	↑ *Akkermansia*[a]	↓ Mucus	↑ Colon cancer and IBD
Polyphenols (caffeic acid)	↑ *Akkermansia*[b]	?	↓ IBD
Polyphenols (resveratrol)	↓ *Prevotella*[a]	↓ TMAO	↓ CVD
Polyphenols (grape and/or cranberry extract)	↑ *Akkermansia*[b]	?	↓ Metabolic syndrome
NAS (saccharin)	↑ *Bacteroides*[a]	↑ Acetate, propionate and LPS[a]	↑ Metabolic syndrome
NAS (saccharin)	↓ *Akkermansia*[b]	↑ Acetate and propionate[a]	↑ Metabolic syndrome
NAS (saccharin)	↑ *Turicibacter*[a]	↑ LPS?	↑ Metabolic syndrome
NAS (aspartame)	↑ *Clostridium leptum*[a]	↑ Acetate, propionate and butyrate[a]	↑ Metabolic syndrome
NAS (acesulfame-potassium)	↑ *Bacteroides*[a]	↑ LPS, pyruvate and cholate	↑ Metabolic syndrome
High-fat and high-sugar diet	↑ Firmicutes, Mollicutes and *Eubacterium*[a]	↑ Lactate, acetate and butyrate[a]	↑ Metabolic syndrome
High-fat and high-sugar diet	↓ Bacteroidetes[b]	?	↑ Metabolic syndrome
Saturated fat	↑ *Bacteroides* and *Turicibacter*[a]	↑ LPS	↑ Metabolic syndrome
Saturated fat	Supplemented *Bacteroides uniformis*[b]	?	↓ Metabolic syndrome
Saturated fat	↑ *Bilophila*[a]	↑ LPS	↑ IBD
Saturated fat	↓ S24-7 (Bacteroidetes) and Lachnospiraceae[b]	↓ Butyrate and retinoic acid[b]	↑ IBD
Saturated fat	↑ *Bacteroides*, Mollicutes and *Lactobacillus*[a]	↓ Flavonoids and UCP1	↑ Metabolic syndrome
Saturated fat (palmitate)	↓ S24-7 (Bacteroidetes) and Prevotellaceae[b]	↓ Propionate?[b]	↑ Multiple sclerosis
Unsaturated fat	↑ *Akkermansia*, Mollicutes and *Lactobacillus*[b]	↓ LPS?	↓ Metabolic syndrome
High-fat (saturated and unsaturated)	↓ *Prevotella*, *Bacteroides* and *Turicibacter*[a]	↓ Pro-IL-1β	↓ Osteomyelitis
Fibre	Clostridiales[a]	↑ Butyrate[a]	↑ Colon cancer
Fibre	?	↑ Butyrate, IL-10 and IL-18[b]	↓ Colon cancer
Fibre	↑ Actinobacteria and Bacteroidetes[b]	↑ Propionate, butyrate and IGN[b]	↓ Metabolic syndrome

Table 1 (cont.) | **Complexity of diet–microbiome–health crosstalk**

Dietary component	Bacteria	Metabolites or mediators	Disease risk
Fibre	*Prevotella*[b]	↑ Glycogen storage	↓ Metabolic syndrome
Fermentable fibre (inulin)	↑ *Bifidobacterium* and *Akkermansia*[b]	↑ IL-22	↓ Metabolic syndrome
Fermentable fibre (inulin)	*Bifidobacterium*[b]	↑ Mucus growth	↓ IBD
Fermentable fibre (inulin)	?	↑ Acetate[b]	↓ Metabolic syndrome
		↓ Appetite	
High-fat	?	↑ Acetate[a], GSIS and hyperphagia	↑ Metabolic syndrome
Low-fibre diet	↑ *Akkermansia* and *Bacteroides caccae*[a]	↓ Mucus	↑ *Citrobacter* susceptibility

Macronutrients, micronutrients and food additives interact with the microbiota to modify the abundance of specific genera or the microbial metabolite landscape, resulting in considerable effects on host health. Within this complex network, the majority of food components and microorganisms are multifaceted, displaying both beneficial and detrimental effects on the host. Arrows on bacteria and mediators indicate that an increase or decrease in abundance is observed following consumption of the nutrient. Absence of an arrow before the bacterium indicates that when the nutrient is fed in the presence of this bacterium, the following metabolites, mediators or diseases risks are observed. Question marks indicate no description of the relevant bacterium or mediator. CMC, carboxymethyl cellulose; CVD, cardiovascular disease; GSIS, glucose-stimulated insulin secretion; IGN, intestinal gluconeogenesis; LPS, lipopolysaccharide; NAS, non-caloric artificial sweetener; P80, polysorbate-80; IQ and 7-OHIQ, 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline and its 7-keto derivative, respectively; PhIP, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine; TMAO, trimethylamine *N*-oxide; UCP1, mitochondrial brown fat uncoupling protein 1. [a]Associations detrimental to host health. [b]Associations beneficial to host health.

individualized parameters and adjustment of the dietary regimen accordingly ($n = 26$)[250].

Challenges in research

Given the complexity and the myriad of personalized factors affecting dietary–microbiome–host interactions, it is crucial to consider the factors that complicate interpretation of knowledge and present challenges in its integration into public health policies and dietary recommendations.

Association versus causation

A dietary intervention that is associated with microbiome alterations and any kind of downstream phenotype in the host does not necessarily imply that the diet altered the microbiome and that the microbiome is the cause for the phenotype. For example, diet might have a direct effect on the host and a discrete effect on the microbiome that does not contribute to the host phenotype. Alternatively, microbiome alterations can result from changes in the host physiology rather than being the cause of such change. Although earlier descriptive works serve as an important starting point for future research, they are limited in their contribution for understanding complex interactions, especially when conducted in heterogeneous human populations.

Several approaches can shed light on a direct or positive link. Albeit still descriptive, complementing compositional analyses with functional approaches such as shotgun sequencing and metabolomics can help in deciphering potential mechanisms by which the microbiome is contributing to the phenotype. Abolishing a phenotype by antibiotic treatment suggests a role for the gut microbiota; however, the effect on the microbiota is often crude and does not enable pointing out the specific bacteria that contribute to the phenotype, and antibiotics can have unexpected effects on the host that are unrelated to the microbiota (for example, dysglycaemia, immunomodulation and increased gastrointestinal motility)[251]. Demonstrating a direct effect of a nutrient on the gut microbiota might be achieved by co-culturing in vitro in a complete host-free environment, which can be controlled for multiple environmental factors to mimic the conditions in the various regions of the gut and their luminal and mucosal microbial assemblages using biofilm reactors and chemostats[252]. One such approach, termed M-SHIME, was used to demonstrate a direct effect of dietary emulsifiers on the human microbiome[179]. Although these host-free systems can demonstrate direct interaction between a nutrient and the microbiota, they cannot demonstrate causality in a given phenotype by themselves. Transplanting the in vitro modulated cultures into GF mice might therefore complement these approaches and substantiate causality by recapitulating the phenotype observed in animals exposed to the nutrient itself[179,182]. Gastrointestinal organoids[253] or more elaborate gut organ cultures that preserve tissue architecture[254] provide an opportunity to study mechanistic interactions between environmental stimuli, microorganisms and the host in a more tightly controlled and variable-limited system. GF mice serve as a gold standard for determination of causality, either by failing to replicate a diet-related phenotype in the absence of microorganisms or by reproducing a phenotype in a GF mouse transplanted with microbiota from a diet-fed donor. By feeding the recipient mice with a control diet or the same diet fed to the donors, one can potentially identify direct effects of the microbiota on the host versus those requiring an interaction between the microbiome and the diet, as in the case of malnutrition[64]. Administering single species or even microorganism-associated metabolites can further refine experiments in GF mice. However, GF experiments have their own limitations, as is discussed later. Thus, an integrated microbiota-centred approach

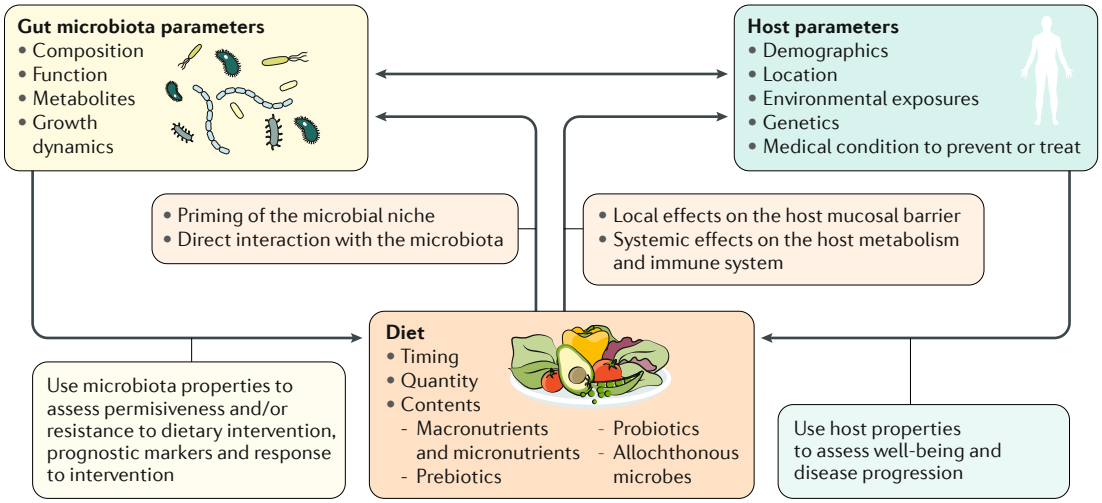

Fig. 3 | **Therapeutic principles in utilizing the food–microbiota axis.** Diet interacts with the human 'holobiont' in a person-specific way. Obtaining multiple parameters from the host and its resident microbiota can assist in devising precision dietary interventions, which encompass food quantities, contents and timing. These interventions might be used for prophylactic or treatment purposes in a variety of medical conditions, as well as assessing prognosis, predicting the likelihood of the dietary intervention to succeed and monitoring the clinical response to the intervention. This paradigm shift in nutrition from 'generalized' to 'personalized' merits periodic reassessments of host and microbiota parameters, as they are susceptible to constant change following the dietary intervention itself or due to other environmental factors.

aiming at achieving a mechanistic understanding of a dietary and microbiota-mediated effect on the host would optimally combine several complementary computational, experimental, in vitro and in vivo systems. Table 1 in the Supplementary Information highlights findings in which a causative role for the microbiota was experimentally demonstrated and those that show an association that requires further validation.

Designing the dietary intervention

One of the biggest challenges when comparing nutritional interventions is the disparity between the applied protocols. In animal models, standardization is becoming more common, as researchers utilize commercial, reproducible and open-source diets, enabling comparison of both the macronutrients and micronutrients between studies. However, earlier works utilized non-standardized protocols, in which complete information regarding the diet contents is often unavailable, and these should be interpreted with caution until their validation using more uniform dietary interventions. Nevertheless, even the standardized diets in animal studies do not necessarily represent an ideal model, as often overabundance of a nutrient comes at the expense of another; for example, HFDs typically contain less carbohydrates and fibre[255]. Thus, some of the effects attributed to the fat moiety in HFD might in fact be due to a paucity of fibre. Although these diets could serve as a convenient tool for screening, it would be advisable to follow up with experiments focused on the specific nutrient of interest.

Moreover, differential intake of nutrients between groups can also stem from differential chow consumption by the animals due to palatability or the effect of the diet on satiety regulation[255,256]. This caveat is important in many studies demonstrating an HFD-counteracting phenotype without reporting whether the treatment affected HFD consumption, as HFD is a strong determinant of gut microbiota composition even independently of obesity[55]. Monitoring such differences can be achieved using metabolic cages, which can control for additional important parameters such as liquid intake (especially if the drinking water is laced with antibiotics or nutrients) and energy expenditure. Furthermore, in both model animals and human trials, dietary interventions are often extreme and do not reflect common human lifestyle and intake. Although such protocols enable a convenient and often quick route for establishing a proof of concept, their findings should be replicated in realistic settings so that applicable conclusions to human health can be drawn.

Nutrition research in humans is naturally further complicated. Case–control studies, such as some of those that suggested a link between dietary fat and CVD, are prone to both recall and selection bias, and should only provide the basis for further research and not used as definitive answers to nutritional questions as they indicate association and not causation. RCTs are preferable but likewise can feature important limitations. In RCTs, the dietary intervention is often added (or omitted) to the standard diet of the individual, which might vary considerably, thereby affecting the outcome of the intervention. Designing a complete diet is ideal but rarely feasible for extended periods of time owing to non-compliance and the inability to control the entire diet of individuals outside institutional settings. Thus, researchers should control for the intake of calories, macronutrients and micronutrients, preferably using real-time food diaries that are less prone to recall bias than food frequency questionnaires. As compliance to the dietary regimen can be suboptimal, when possible, it is advisable to monitor the levels of a signature metabolite in biological samples from treatment and control

groups. Blinding is often a challenge in human dietary interventions and might lead to lifestyle differences between groups during intervention. This aspect can be partially addressed by the use of activity logs (for example, physical activity). These limitations and the need to control for many parameters often result in smaller cohorts and shorter exposures, which should be taken into account when interpreting results[43,84,85,182,226,249].

Human versus animal models

Experiments in rodents enable controlled nutritional settings in overcoming the aforementioned challenges encountered in humans. However, mice are distinct from humans in several important diet–microbiome aspects[257]. The first relates to the structure and function of the intestine and to the anatomical sites where some nutrients are metabolized. In mice, fermentation of indigestible food components occurs in the caecum, whereas in humans, the caecum is much smaller and fermentation occurs in the colon, which, unlike that of the mouse, is subcompartmentalized[257]. This discrepancy also highlights a difference in the colonic microbial communities and the region in which SCFAs are produced. Goblet and Paneth cells, which have a role in maintaining host–microbiota equilibrium, are distributed differently between the two organisms. Paneth cells are exclusively found in the small intestine in mice but are also found in the caecum and proximal colon in humans. Goblet cells are abundant in the mouse proximal colon and their numbers decrease at the base of the crypt distally, whereas in humans they are abundant throughout the large intestine[257].

Although many bacterial genera are shared between the two organisms, they differ in their relative abundance. One of the strategies used to address this discrepancy is the humanized gnotobiotic mouse model[15], in which GF mice are transplanted with human microbiota; however, even in this model some members of the human microbiota do not colonize the transplanted mouse[15]. These limitations notwithstanding, the mouse does constitute an important dietary model relevant to human physiology in many aspects. For example, microbiota from both humans with obesity[258] and obese mice[162] can promote weight gain in a recipient GF mouse, and obesity is associated with reduced bacterial diversity in both organisms[56,207]. However, validation of any strain-specific effects, when noted in mice, is merited in human studies. In Table 1 in the Supplementary Information, we list observations that were demonstrated in humans versus those that were shown only in an animal model.

Even when comparing studies performed on mice, one should be cautious when different genotypes were involved, as even different genotypes of wild-type mice harbour distinct microbiome configurations, and this is even more apparent when experimenting with genetically altered mouse models[259]. Although diet has been shown to be dominant over genotype in terms of its effect on microbiota composition[58], only a limited number of diets have been studied in depth in this context, and it is possible that some diets might interact differently with distinct microbiota configurations.

GF mice serve as the best available model to study causal effects of the microbiota on the host health, yet this comes at the price of several important distinctions between GF and colonized specific pathogen-free mice. To name a few, GF mice require dietary supplementation with vitamins B and K; have less body fat but higher cholesterol levels; and feature increased food intake, decreased basal metabolic rate, longer intestinal transit time, altered intestinal morphology and function and considerably enlarged caeca[260]. In addition, they feature defects in the development of gut-associated lymphoid tissues and in antibody production and fewer and smaller Peyer's patches and mesenteric lymph nodes. Additional differences between GF and colonized mice are reviewed elsewhere[260,261]. One useful approach in that context involves comparison between GF mice and conventionalized GF mice rather than specific pathogen-free mice as a better-controlled comparison that might limit the bias stemming from congenital GF defects.

Microbiome characterization protocols

The interest in microbiome, diet and health interactions predates next-generation sequencing (NGS), and as such, many reports utilized gel-based methods, PCR, fluorescence in situ hybridization or cultures to characterize the microbial population. The limitations of these methods should be considered when comparing between studies. Currently, researchers setting up an NGS pipeline for microbiome characterization face a line of decisions that can introduce biases and different results for the same sample, including: sample collection, handling and storage[262]; microbial DNA purification protocol[263]; 16S ribosomal DNA amplicon sequencing versus shotgun metagenomics[264]; the 16S variable region to amplify[265]; the polymerase and PCR conditions[266]; and multiple decisions during in silico sequence processing and data mining[267,268]. When comparing publications, one should be aware of the potential biases introduced by these choices. Costea et al. have reported that, in shotgun sequencing pipelines, differences due to the DNA purification protocols had the largest effect on variations in results stemming from the same samples (compared with library preparation and sample storage) and have therefore compared multiple protocols to suggest those that are the most reproducible[263]. Similar standardization is encouraged for other steps of the microbial DNA analysis pipeline.

Relative versus absolute abundance

Diet-related microbiota alterations are often reported to induce changes in relative abundance, whereas the absolute abundance of seemingly involved bacterial strains is rarely reported. Care must be taken when interpreting such results, as an increase in the relative abundance of a bacterial group might signify no change in its absolute abundance but rather a decrease in other members of the microbiota. This constraint can be overcome using statistical algorithms, such as a log-ratio analysis[269], or using workflows that combine sequencing-based relative abundances with microbial quantities derived from methods such as flow cytometry[270]. Alternatively, once a potential bacterium of interest has been identified through relative abundance analysis, directly

quantifying absolute abundance (for example, using selective culture conditions where applicable or using strain-specific quantitative PCR primers) could address this issue. In addition, if secreted bacterial metabolites are suspected to mediate the phenotype, their quantification can bypass the need to determine absolute abundance changes.

Conclusions

Taken together, the field of nutrition is currently plagued with many non-evidence-based practices and recommendations — some gleaned from misinterpreted or insufficient scientific research and others stemming from commercial interests or as the result of arbitrary statements. General dieting schemes often result in failure and disappointment at the personal level and a constant increase in the incidence of obesity and the metabolic pandemic at the population level, urging the public to waver between short-lived trends. The advent of microbiota research and the increasing body of evidence pointing to its tight interactions with dietary habits and interventions and its salient role in food metabolism have introduced a potentially attractive new target for dietary manipulation. Nevertheless, the number of conflicting reports substantially hampers the translation of diet–microbiome–host research into clinical use. Focusing on only studies that have demonstrated causation, or those studied in humans rather than only in animal models, eliminates some of these conflicts, although some nutrients or bacteria are still reported as both beneficial and detrimental (see TABLE 1 and Supplementary Table 1). With the shift in the microbiota field towards more mechanistic works, one can expect that standardization of both microbiome analysis and dietary intervention protocols will resolve some of the conflicts to facilitate identification of nutrients that can be recommended for the general public or of bacteria that can be utilized as probiotics. In parallel, some of these conflicts could arise from actual biological variation. Although the need for precision and personalization when applying dietary therapeutics for distinct disease conditions might seem intuitive, interindividual variation in the response to the same nutrient is only just being appreciated. This emerging field bears the potential to revolutionize the perception of nutrition from uniform food-intrinsic guidelines to flexible person-specific and context-specific recommendations, which are designed to prevent or correct metabolic derangements and even ameliorate inflammatory and neoplastic processes. Such conceptual change might shift the standard modus operandi from the traditional universal approaches to ones involving the integration of numerous individual parameters by utilizing an array of advanced bioinformatic tools capable of processing big data, enabling planning of therapeutic strategies while taking the patient's preferences into account (FIG. 3). This individualized approach might pose new challenges to dietary planning, as some nutritional programmes devised to address specific maladies could hinder or conflict with other health considerations. Additionally, as the gut microbiome is amenable to change, dietary interventions could trigger structural and functional alterations in the gut bacteria, which might merit periodic reassessments of the individual parameters and adjustment of the dietary regimen accordingly. Nevertheless, this uncharted territory could create an exciting opportunity to harness our endogenous gut microbial members in rationalizing and optimizing the health benefit conferred by human nutrition.

Published online 27 September 2018

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Acknowledgements
The authors thank the members of the Elinav laboratory for discussions and apologize to authors whose work was not cited because of space constraints. N.Z. is supported by the Gilead Sciences International Research Scholars Program in Liver Disease. J.S. is the recipient of the Strauss Institute Research Fellowship. E.E. is supported by Y. and R. Ungar, the Abisch Frenkel Foundation for the Promotion of Life Sciences, the Gurwin Family Fund for Scientific Research, the Leona M. and Harry B. Helmsley Charitable Trust, the Crown Endowment Fund for Immunological Research, the estate of J. Gitlitz, the estate of L. Hershkovich, the Benoziyo Endowment Fund for the Advancement of Science, the Adelis Foundation, J. L. and V. Schwartz, A. and G. Markovitz, A. and C. Adelson, the French National Centre for Scientific Research (CNRS), D. L. Schwarz, the V. R. Schwartz Research Fellow Chair, L. Steinberg, J. N. Halpern, A. Edelheit, grants funded by the European Research Council, a Marie Curie Integration grant, the German-Israeli Foundation for Scientific Research and Development, the Israel Science Foundation, the Minerva Foundation, the Rising Tide Foundation, the Helmholtz Foundation, and the European Foundation for the Study of Diabetes. E.E. is the incumbent of the Rina Gudinski Career Development Chair, a senior fellow of the Canadian Institute For Advanced Research (CIFAR) and a research scholar. E. E. is supported by the Bill and Melinda Gates Foundation and the Howard Hughes Medical Institute (HHMI).

Author contributions
All authors researched data for the article, made substantial contribution to discussion of content, and wrote, reviewed and edited the manuscript before submission.

Competing interests
E.E. is a paid scientific consultant for DayTwo. The other authors declare no competing interests.

Supplementary information
Supplementary information is available for this paper at https://doi.org/10.1038/s41575-018-0061-2.