Published in final edited form as:

Nat Med. 2023 March ; 29(3): 551–561. doi:10.1038/s41591-023-02260-4.

Cardiometabolic health, diet and the gut microbiome: a meta-omics perspective

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Abstract

Cardiometabolic diseases have become a leading cause of morbidity and mortality globally. They have been tightly linked to microbiome taxonomic and functional composition, with diet possibly mediating some of the associations described. Both the microbiome and diet are modifiable, which opens the way for novel therapeutic strategies. High-throughput omics techniques applied on microbiome samples (meta-omics) hold the unprecedented potential to shed light on the intricate links between diet, the microbiome, the metabolome and cardiometabolic health, with a top-down approach. However, effective integration of complementary meta-omic techniques is an open challenge and their application on large cohorts is still limited. Here we review meta-omics techniques and discuss their potential in this context, highlighting recent large-scale efforts and the novel insights they provided. Finally, we look to the next decade of meta-omics research and discuss various translational and clinical pathways to improving cardiometabolic health.

Cardiometabolic diseases (CMDs), including diabetes, insulin resistance, heart attack, stroke and nonalcoholic fatty liver disease, are on the rise in aging societies and are the principal

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Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-023-02260-4.

Peer review information *Nature Medicine* thanks the anonymous reviewers for their contribution to the peer review of this work. Primary Handling Editor: Karen O'Leary, in collaboration with the *Nature Medicine* team.

cause of morbidity and mortality in Western countries[1,2]. There are several well-established genetic and environmental risk factors associated with CMD, including smoking, abdominal obesity, insulin resistance, high blood pressure, high cholesterol and unhealthy diets[3]. In addition, links between the composition of the human microbiome and the development and progression of CMD are being noted[4–6]. As the gut microbiome is modifiable with dietary and therapeutic interventions[7], understanding the interplay between the underlying dietary and microbial factors that promote or inhibit the transition from a healthy state to CMD opens new avenues for prevention and treatment.

The relationship between an individual's diet, their gut microbiome and their cardiometabolic phenotype is, however, inextricably multidirectional, multifactorial and complex (Fig. 1a) and thus very challenging to understand. Translating our current knowledge into the implementation of targeted microbiome-dependent modulation strategies such as personalized dietary guidelines is thus far from straightforward[8–11], necessitating a systems-level understanding of the microbial and host systems in cardiometabolic health (CMH) and disease[12]. Targeted, mechanistic, bottom-up investigations of specific microbial organisms[13], metabolites[14] or foods[15] will continue to be helpful in building such systems-level modeling, but it is only via top-down, untargeted, cultivation-free, meta-omics approaches that a comprehensive and dynamic picture can be reconstructed. So far, a small number of pioneering studies have begun to identify potential microbial biomarkers for CMH, building an evidence base and informing further studies (Table 1).

In this article, we review untargeted, high-throughput genomic and molecular approaches applied to microbial communities (meta-omic approaches) and discuss their potential to disentangle the intricate and complex interactions between the human microbiome, host metabolism and diet in CMD. We highlight the recent large-scale efforts to better understand the interaction between the diet–microbiome–metabolome axis and CMH, and finally we discuss how this knowledge can be integrated to develop precision-based nutritional strategies with the potential to lower the risk and severity of CMDs. We refer the reader interested in complementary omics technologies applied to the human host in connection with dietary patterns and systemic response to other reviews[16,17].

Meta-omics to disentangle the diet–microbiome–metabolome axis in CMD

Recent studies have rapidly accelerated our understanding of the role of the human microbiome in CMDs. New approaches have expanded beyond simply profiling the high-level taxonomic composition of the microbiome to characterizing microbiome members at the resolution of single genomes; moreover, recent studies are also surveying microbial gene expression[18,19] and the metabolites that are produced by these microbes[20].

Metagenomics and metatranscriptomics

Shotgun metagenomics involves high-throughput sequencing of the DNA in a microbial community (typically including bacteria, archaea, viruses and microeukaryotes). Computational analysis of the sequencing output allows for characterization of the taxonomic composition of the sample (that is, the presence of microbial taxa and their relative abundances) and its functional metabolic potential — ranging from antibiotic

resistance profiles or virulence factors to the genes encoding enzymes that break down specific nutritional compounds[21] and the identification of known and novel microbial genetic material[22]. Amplicon-based 16S ribosomal RNA (rRNA) gene sequencing, which involves PCR-based amplification of hypervariable regions of the ribosomal 16S gene followed by sequencing, is a technique that was more cost effective than metagenomics in the past but can only detect bacteria and archaea, and provides limited taxonomic resolution. In addition, as it does not consider genes other than the 16S rRNA gene, it does not inform on the functional potential of the microbiome, which can then only be predicted[23]. Therefore, while 16S rRNA gene sequencing was used in many of the pioneering microbiome CMD studies, as sequencing costs continue to decrease it is being replaced by shotgun metagenomics for the study of the human microbiome.

Metatranscriptomics, in turn, performs high-throughput sequencing of the RNA transcript pool expressed by a microbial community (usually by DNA sequencing of the retro-transcribed RNA). It provides microbial gene expression levels, informing on the actively expressed gene functions, and enables monitoring of changes in microbial gene expression over time when used within longitudinal study designs[24]. Metatranscriptomics still presents major technical challenges (for example, maintaining RNA integrity and enriching for messenger RNA), especially compared with metagenomic analyses[22], but it is becoming of increasing relevance in the field.

Metaproteomics and metabolomics

Metaproteomic and metabolomic approaches further characterize the molecular cascade of the microbiome by directly surveying the proteins and other molecules that are produced[25]. Metaproteomics provides large-scale determination of the functional product (whole protein repertoire) encoded by a microbial community in a given sample, revealing which metabolic processes are ongoing. Metaproteomics uses high-resolution mass spectrometry optionally coupled with liquid chromatography to separate peptide mixtures and identify them. Peptide sequences, when available, can then be combined with genomic databases to link the proteins with the microorganisms that encode them. While currently only a fraction of the protein material is reliably identified and consists of a mixture of host and microbial material, standardization and cataloging efforts are increasing its use in meta-omics studies[26].

Metabolomics targets the low-molecular-weight molecules (metabolites) produced by a microbial community, by the host and by a combination of microbial and host pathways, informing on the overall metabolic states and interactions. Identification and quantification of the whole metabolite pool remains challenging due to diversity in size, polarity and abundance. Using the different technologies available (NMR and mass spectrometry), metabolomics approaches can either measure defined sets of characterized small metabolites (targeted metabolomics) or perform a more exploratory and comprehensive analysis of the metabolome (untargeted metabolomics)[27]. The former provides higher sensitivity and (semi)-absolute quantifications (by using internal standards and normalizing across batches) and can reduce bias by using specific sample preparation protocols depending on the array of metabolites of interest. Untargeted approaches, in contrast, have the advantage

of detecting a much larger number of metabolites and potentially as-yet uncharacterized metabolites that can be difficult to interpret but enable the generation of novel hypotheses on pathways involved in CMH[28]. Untargeted and targeted metabolomics can also be combined to exploit the advantages of both approaches[29]. Although processing and interpreting high-throughput metabolomic outputs remains challenging, metabolomics provides an effective, direct functional readout of the physiological state of the host and the host–microbiome interface[30].

Single-cell genomics and culturomics

Single-cell genomic approaches can provide higher-resolution meta-omics data by sorting and targeting specific microbes before sequencing to reach the resolution of single cells[31]. This enables the study of microbiome heterogeneity and analysis of low-abundance and uncultured taxa, and although sorting biases prevent precise quantitative estimations, this technique holds great promise for unraveling the diversity of microbes within species and strains[32–34].

Cultivating the human microbiome remains challenging, but novel approaches to isolate and cultivate microbial taxa from environmental samples at high throughput are rapidly developing[35]. Omics techniques can then be applied on single colonies to complement the meta-omic findings, with the advantage that high-throughput isolation and cultivation approaches (culturomics) allow single components of the microbiome to be further characterized as part of in vitro or in vivo experiments and translational initiatives[34,35]. Attempts at cultivating the microbiome as a whole are also ongoing, with commercial versions of pioneering multi-vessel bioreactors simulating the human gastrointestinal tract gaining popularity[36]. In such systems, the microbiome inoculum reaches an ecological equilibrium that is intended to resemble the original composition of the community, and can be more easily investigated with meta-omics approaches also in response to well-defined external stimuli.

Virome sequencing

Meta-omics techniques are also being used to survey the composition of the much understudied virome, which has been shown to be modified upon dietary interventions, paralleling changes in the bacterial fraction of the microbiome[37,38]. Bacteriophages (viruses that only infect bacteria) are even more abundant than bacteria and can dramatically change the population of the target bacterial host and the ecology of the whole microbiome. Virome studies, if performed in appropriate experimental settings, can thus open up new possibilities for targeted therapeutic interventions to modulate — via bacteriophages — specific components of the microbiome, with the advantage of avoiding side effects of broad-spectrum drugs and the spread of antibiotic resistance[39].

Other approaches and outlook

Finally, although not the focus of this Review, animal models of CMD have been developed and widely adopted[40]. Meta-omics can be applied on these to survey the microbiome with fewer challenges in terms of collection, storage and processing biases. Meta-omics approaches are thus very versatile and can be applied on a wide set of scenarios with

essentially the same methodological principles; however, independent of the application domain, what remains challenging is the integration and interpretation of the different layers of information they produce. With the continuous improvement and increased availability of all meta-omics, we see their integration as the current main obstacle toward elucidating microbiome–host–diet interaction[41].

Large-scale meta-omics efforts in CMD

Given the intrinsic interindividual variability of the human microbiome (both within and across populations)[42,43] and the high dimensionality of omic readouts, meta-omic studies necessarily require large sample sizes and relatively complex study designs. As such, only a few studies have so far been able to provide preliminary systems-level understanding of the microbiome–diet–host interplay. Most of these studies used well-established shotgun metagenomics approaches (Supplementary Table 1), but those also coupled with metabolomics and other technologies (Table 1) showcase the added value of the multi-omic approach.

The Metagenomics in Cardiometabolic Diseases (MetaCardis) project showcased the potential of large-scale multiple meta-omics applied to CMD (Table 1). Employing metagenomics and metabolomics at a large scale (n = 1,241 individuals)[44], it compared patients with ischemic heart disease, metabolically impaired controls (for example, individuals with diabetes or obesity) and a random subset of healthy controls. Individuals with obesity and type 2 diabetes and those with both early and late clinical manifestations of heart disease presented multiple microbiome and serum and urinary metabolome alterations. Such alterations reflected distinct metabolic pathways that were also linked to nutrient composition of their diets, overall energy intake and lifestyle[44]. This suggests that major alterations of the gut microbiome and metabolome might begin long before clinical onset of ischemic heart disease. In another large-scale effort, the Personalised Responses to Dietary Composition Trial (PREDICT 1), metagenomics was performed in combination with blood metabolomics under fasting conditions and at multiple time points postprandially after a standardized meal in 1,102 individuals. The study also collected short- and long-term dietary information to detect multiple associations between gut microorganisms and specific nutrients and food groups, especially plant-based foods. In addition, the authors identified microbial stool biomarkers of more and less favorable glycemic, lipemic and inflammatory postprandial responses (all proxies of cardiovascular health status) and of obesity[45].

In contrast with metagenomics and metabolomics, metatranscriptomics and metaproteomics have so far only been employed in very few and relatively small-scale meta-omic studies to decipher the complex diet–microbiome–CMH interplay (Table 1). They arguably have not unlocked their full potential, but as they evolve they may complement the more commonly used meta-omics techniques. The metaproteome remains particularly understudied, but large-scale studies rapidly advance our knowledge of the composition of the microbial dark matter[46].

It will also be important to integrate these and other meta-omics with host omics to gain insight into the host–microbiome relationship and links with CMD. One of the studies

that integrated microbiome and host omics is the second phase of the Human Microbiome Project (HMP2), which coupled metagenomics and metabolomics with host transcriptomics and proteomics[47,48]. In one of the HMP2 studies, the gut and nasal microbiomes of 106 healthy individuals and individuals with prediabetes were sampled longitudinally for 4 years, to assess host–microbiome dynamics and identify signatures of insulin resistance. Multi-omics data integration by integrated canonical pathway analysis revealed coordinated changes in the host immune system and in microbiome composition in healthy participants upon viral infections, while those with prediabetes had both impaired immune responses and microbiome alterations at the taxonomic and functional levels upon exposure to viruses[48]. In another HMP2 study involving patients with inflammatory bowel disease (IBD), integrative meta-omic analyses, involving metatranscriptomics, metaproteomics and metabolomics together with metagenomics, were used to identify the characteristics of dysbiosis during IBD at the functional level[49], which could also be applied in the CMD context. Other emerging meta-omics such as meta-epigenomics (analysis of the DNA methylation patterns in a microbial community; for example, using single-molecule real-time and circular consensus sequencing techniques)[50,51] could also in the future be used to complement the most commonly used meta-omics approaches.

The tight association between diet and gut microbiome composition

Diet is a strong determinant of CMH, but it also shapes the composition and characteristics of the gut microbiome[52] — more so than host genetics[53]. Gut microbes display specific nutritional preferences[54]. While some bacterial species (mostly in the Firmicutes phylum) are generalists in exploiting the three major sources of nutrients in the intestine — namely (poly)saccharides, proteins and lipids[55] — many others are specialized toward specific nutrients. *Bifidobacterium* species (in the Actinobacteria phylum) are predominantly saccharolytic (meaning that they mostly metabolize carbohydrates), whereas *Alistipes* predominantly metabolize proteins[56] and *Prevotella* species target complex carbohydrates and vegetary fibers[57,58]. Dietary regimens influence the microbiome in the long term[58–60], but short-term dietary interventions have also been shown to rapidly alter microbiome composition[61]. David et al.[61] assessed microbiome taxonomic composition (metagenomics) and expression (metatranscriptomics) after providing either a plant- or an animal-based diet to ten study participants for five consecutive days. The animal-based diet decreased the levels of species that metabolize plant polysaccharides while increasing those of bile-tolerant bacteria — a signal that was mirrored on metatranscriptomic data, showing a trade-off between carbohydrate and protein metabolism[61]. Another study including daily fecal sampling, metagenomics and 24-h food records in 34 individuals for 17 d found that diet diversity was linked to microbiome stability[62].

Meta-omics techniques are quickly improving our ability to capture the effects of such interventions, but due to the high dimensionality of the data these require dense longitudinal sampling together with large sample sizes, which are only now starting to be attainable. In addition, improved methods for individual diet profiling are warranted, as collecting and analyzing the information in food frequency questionnaires and nutritional diaries is not straightforward. After solving these limitations, dietary interventions seem a promising

therapeutic strategy to modulate the microbiome toward more favorable compositions linked to decreased CMD risk[63].

Meta-omics in dietary intervention studies

The Mediterranean diet (also known as MedDiet), characterized by a high intake of plant-based, minimally processed foods and a low intake of animal-derived and highly processed foods[64], has been investigated for its positive influence on gut microbiome composition in well-powered longitudinal observational and interventional studies[65,66]. A substudy of the long-running observational Health Professionals Follow-Up Study analyzed 925 shotgun metagenomes and 340 shotgun metatranscriptomes over 6 months[63]. In this study, the MedDiet index (a measure of adherence to the diet) accounted for the third largest proportion of variation in microbiome composition (only preceded by triglyceride levels and proton pump inhibitor use), thus even more so than antibiotic use. A higher adherence to MedDiet was positively associated with the abundance of short-chain fatty acid (SCFA) producers in the gut microbiome. Conversely, a lower adherence to MedDiet was associated with enrichment of secondary bile acid biosynthesis potential. The protective association between MedDiet and cardiometabolic risk was notably stronger in participants with gut microbiomes depleted of *Prevotella copri*[63]. Interestingly, *P. copri* has a dramatically decreased prevalence in societies adopting a typical Westernized lifestyle compared with those adopting less industrialized and urbanized lifestyles[32,67], possibly due to the divergent dietary intake of complex vegetable fibers[68]. *P. copri* was also shown to mediate improvements in glucose metabolism[69] and to be negatively associated with fasting and postprandial cardiometabolic and inflammation markers such as visceral fat, very low-density lipoprotein cholesterol and GlycA[45]. *P. copri* remains an elusive bacterium with recently expanded species diversity and a context-dependent role in health[68,70], and is a clear example that the presence of a species alone is insufficient to drive strong associations with host health and diet as the complete multi-taxa potential of the microbiome should be studied.

The beneficial aspects of the MedDiet and their association with the microbiome have been confirmed in several interventional studies. Meslier and colleagues[71] enrolled healthy overweight and obese participants in a randomized controlled trial and found an increased abundance of *Faecalibacterium prausnitzii* and *Roseburia* species and a lower abundance of *Ruminococcus gnavus* and *Ruminococcus torques* in the MedDiet group relative to the control (regular diet) group. Consistent with independent results[63], the observed improvement in insulin resistance was linked to specific bacteria including lower relative abundances of *P. copri* together with increased *Bacteroides uniformis* and *Bacteroides vulgatus* at baseline. Thus, mounting evidence supports a beneficial effect of fiber-rich diets such as the MedDiet on CMH via reproducible changes in the gut microbiome[72,73]. Other diets that have been explored to improve CMH are time-restrictive (that is, intermittent fasting)[74,75] and ketogenic diets (reduced carbohydrate intake)[76], but their positive effects on the microbiome are less clear. With knowledge on the diet–microbiome–CMH link expanding, more specific diets may be designed to modulate the microbiome toward an optimal CMH-supporting composition.

Metabolite trafficking resulting from diet–host–microbiome interactions

Besides influencing microbiome composition, the highly complex human diet (containing thousands of so far uncharacterized dietary compounds[77]) results in intricate molecular trafficking when digested by the host and microbial metabolism. Metabolites are highly dynamic and thus informative for diagnosis, prognosis and monitoring treatment efficacy[28]. Given that metabolomic approaches typically only identify a limited fraction of metabolites with acceptable confidence, the ability to accurately distinguish between the different types of metabolites of importance in CMH will be crucial. These different types of metabolites (see Fig. 2 and sections below) include dietary metabolites, numerous metabolites of microbial origin produced from dietary substrates or host-derived compounds, drug compounds and microbiome-modified drugs, and host metabolites (although host metabolites are not the focus of this Review). Metabolites detected via metabolomics are thus typically a mixture of molecules resulting from dietary and drug intake, metabolites produced by our body, metabolites resulting from microbial pathways and metabolites that can be produced by both us and our microbiome. These broad categories of metabolites are present in varying proportions depending on the nature of the sample (Fig. 1b). Indeed, as metabolomics can be conducted on a wide variety of biological samples, including urine, blood, stool and saliva, tracking metabolites across organs will be highly important to unravel systemic mechanisms.

Dietary compounds

Diet is an incredibly large source of diverse compounds, with simple dietary items such as coffee containing thousands of distinct molecules, many of which are uncharacterized or dependent on the specific type of coffee or preparation method[78]. While discussing the diversity of dietary compounds is daunting and outside the scope of the present Review, one class of dietary metabolites that are of particular interest for their potential benefits in CMH[79] are polyphenols. These are a complex group of thousands of molecules (phenolic acids, flavonoids, lignans, lignins, coumarins and stilbenes) of dietary origin present in berries and vegetables, tea, coffee, wine, cocoa, olive oil, nuts and seeds as defense chemicals for the plants. Many polyphenols are detected by existing targeted metabolomic panels and have been associated with decreased microbiome-mediated cardiometabolic risk[80]. Only 10% of dietary polyphenols are estimated to be metabolized and absorbed in the small intestine, while the rest pass to the colon where they are metabolized by the gut microbiome[81]. In line with this, polyphenol intake has been linked to increased relative abundances of specific taxa[81] and microbiome diversity[82].

Conjugated linoleic acids (CLAs) are another group of metabolites of dietary origin (found mostly in the meat and dairy products derived from ruminants) of particular relevance for their reported link to improved CMH markers, including reduced body weight and fat mass[83] and improved mucosal barrier integrity[84]. The fact that they can also be synthesized by members of the gut microbiome highlights the difficulty of identifying the origin and types of CLAs and of metabolites in general. Oral administration of a CLA-producing *Bifidobacterium breve* strain resulted in modulation of the fatty acid composition of adipose tissue in mice[85]. However, while the determinants of a positive response to interventions

involving CLA-rich foods or CLA-producing bacteria are not fully understood, baseline levels of certain metabolites (identified by untargeted metabolomics) were predictive of a positive response to CLA supplementation, allowing mechanistic hypotheses as to their function[86].

Microbial metabolites

Gut microbes produce hundreds to thousands of metabolites that can have systemic effects on the host upon entering the bloodstream, and even cross the blood–brain barrier[87,88]. The nature of the metabolites produced depends on the substrates provided by diet and the host that reach the large intestine and that are further converted by the microbiome[89], together with the composition of the microbiome itself, which determines the specific enzymatic pathways they encode[90,91].

For example, trimethylamine (TMA) is produced by diverse members of the microbiome, including Clostridia, Enterobacteriaceae and Eubacteriaceae species, upon degradation of nutrients found in foods of animal origin, including carnitine, choline and lecithin[92,93]. When absorbed into the liver, TMA is oxidized by hepatic enzymes to trimethylamine-*N*-oxide, a uremic toxin linked to increased cardiovascular risk[92]. Three alternative pathways for TMA synthesis have been described and metagenomics detailed their phylogenetic distribution in the bacterial kingdom[92,94]. In addition, metagenomic data mining uncovered variants of the choline TMA-lyase (CutC) and carnitine oxygenase (CntA) synthesis pathways, which were validated by metabolomics (using liquid chromatography–mass spectrometry)[95]. Unlike antibiotics, which nonspecifically affect gut bacteria and can lead to adverse side effects and resistance, compounds targeting specific microbial gene products (for example, CutC inhibition[96]) hold therapeutic potential without harming the gut microbes associated with healthy phenotypes.

Other microbial metabolites with well-known effects on CMH are SCFAs (particularly acetate, butyrate and propionate), which are produced through fermentation of complex resistant carbohydrates and amino acids that escape digestion and absorption in the proximal gut[77,92]. Butyrate and propionate are the main SCFAs that exert CMH-promoting functions[97]. Butyrate accounts for 70–80% of the energy source for colonocytes (epithelial cells of the colon)[98] and helps to maintain the anaerobic environment that favors a healthy gut microbiome[98], while propionate contributes to gluconeogenesis in the liver[99]. Butyrate and propionate biosynthetic pathways are well known and their distribution in gut microorganisms can be tracked with metagenomics[56,100,101]. Many other classes of potentially relevant microbial metabolites exist, but they are underinvestigated or still to be discovered, and so far integrated stool metagenomics and metabolomics analysis is the most promising approach for trying to fill this important gap.

Finally, bile acids are metabolites produced by the host that are then modified by the microbiome. Primary bile acids are synthesized in the liver from cholesterol[102] and released upon food ingestion into the small intestine, where they assist in the digestion and absorption of dietary fat[102]. Although most primary bile acids are reabsorbed in the ileum and return to the liver, a small fraction reach the colon[103]. These bile acids are then modified by bacteria into secondary bile acids (such as deoxycholic acid and lithocholic

acid), which are reabsorbed passively into the circulation or excreted in the stool[104]. Microbial metabolism of bile acids alters their bioavailability and consequently the impact of the metabolic responses in which they are involved[105]. A recent study in centenarians suggested that by generating unique secondary bile acids[106], gut microbiome profiles may partially account for these individuals' decreased susceptibility to age-associated illnesses, chronic inflammation and infectious diseases[106]. The range of metabolites of interest that are produced by the microbiome but were previously thought to only be produced by the host is ever-increasing[107], thereby expanding the space for potential therapeutic discovery and development.

Products of drug metabolism by the microbiome

Besides antimicrobials, many other drugs affect the microbiome. In a high-throughput culturomics study, Maier et al.[108] found that up to 24% of nonantibiotic marketed drugs inhibit the growth of at least one bacterial strain. Moreover, members of the microbiome also play a critical role in drug metabolism[109]. For example, metformin impacts the relative abundances of certain microbial taxa and in turn the microbiome seems to mediate some of this drug's therapeutic effects in individuals with type 2 diabetes (metformin promotes the production of SCFAs, regulates bile acid metabolism and improves glucose homeostasis)[110,111]. While the mechanism of action of metformin remains debated, the drug was shown to suppress an intestinal bile acid receptor by increasing levels of the bile acid glycoursodeoxycholic acid (an endogenous antagonist of the receptor) via a decrease in abundance (and consequently the bile salt hydrolase activity) of *Bacteroides fragilis*[112]. Statins have also been identified as an important covariate of microbiome composition, with their intake being linked to a less dysbiotic microbiome[113]. In turn, baseline microbiome composition is linked to response to statins[114].

Another mechanism by which the microbiome alters the availability of therapeutic drugs is bioaccumulation (that is, storing drug compounds intracellularly without altering their structure). Bioaccumulating bacteria were found to limit the response to the antidepressant duloxetine[115]. Besides bioaccumulation, certain members of the microbiome can also directly metabolize drugs. A common member of the gut microbiome, *Eggerthella lenta*, can inactivate the cardiac drug digoxin, except when the drug is coadministered with the amino acid arginine[116]. Also L-DOPA — a commonly used drug for Parkinson's disease — is metabolized by *Enterococcus faecalis* and *E. lenta*, and inhibition of the metabolic pathway results in increased bioavailability[117]. Therefore, the gut microbiome might at least partly explain the wide range of drug responses that are observed in different individuals, and refining therapies to account for microbiome composition or environment could improve outcomes. A better dissection of drug–microbiome interactions could ultimately improve drug efficacy; therefore, drug development requires careful consideration of the microbiome[118], and high-throughput approaches are needed to survey this in a systematic way[119].

The next 10 years of meta-omics and CMD

The meta-omics approaches described in this Review, together with the increasingly large scale of clinical studies and the development of CMH monitoring devices (for example, continuous glucose monitoring devices and food logging applications), all enable multidisciplinary teams of scientists and clinicians to identify novel signatures of CMD — with the potential for translational applications that improve CMH.

To enable clinical translation, it will be crucial to advance the effective analysis of meta-omics data and to better integrate these within and across studies. Indeed, most studies so far have focused on a single meta-omic approach (mostly metagenomics or metaproteomics; Supplementary Table 1), or performed several of them but with little integration among the different layers of information. Commercial initiatives have started providing microbiome testing to consumers using less common meta-omics (for example, metatranscriptomics to provide individualized microbiome-based health scores), but multi-omic and integrative approaches are still lacking. As the single meta-omics are evolving, their integration and interpretation will probably become easier. Meta-omics data integration can benefit from applying the same statistical methods and tools that are commonly used to integrate multiple host omics datasets, including supervized methods such as network, multi-kernel and multi-step-based methods[120]. With several completed studies involving thousands of participants already successful, we expect more will follow, with increased sample sizes and meta-omic depth and resolution[12,44,45,121].

Population health

The cumulative knowledge gained from over a decade of meta-omics research has demonstrated that only with large cohort sizes (at least in the order of thousands of individuals) is it possible to start identifying strong microbiome signatures of CMH. However, an increase of another order of magnitude in sample size (reaching tens of thousands of individuals, similar to the numbers typically included in genome-wide association studies) is probably needed to obtain microbiome signatures that are reproducible and generalizable to subpopulations. Indeed, some follow-up studies of the initiatives discussed in this Review (Supplementary Table 1) are starting to attain these numbers, including PREDICT 3, which now includes over 50,000 individuals. In addition to the increase in scale, carefully designed studies with dense longitudinal sampling and extensive metadata to account for the many covariates and potential confounders of microbiome composition are required, to allow the issuing of general recommendations aimed at decreasing the microbiome-mediated and steeply rising CMD risk.

Precision nutrition

Large cohorts have shown high variability in metabolic responses to identical meals, even between identical twins[45,122,123]. This highlighted the possibility of providing dietary advice that takes into account the foods that minimize postprandial metabolic readouts of CMD risk, in a personalized way. While this is a very promising venue with commercial initiatives already underway to exploit microbiome-informed precision nutrition strategies, there are important limiting factors such as the high intraindividual variability of diet–microbiome–

CMH markers, the difficulty of identifying the effect of a single food or compound in our complex dietary intake and the large number of unknown interaction factors at the host–microbiome interface. Public scientific and translational agencies are, for the most part, unable to economically support the research infrastructure needed to overcome these limitations. It is very likely that consolidation of public initiatives with commercial ones will be needed to advance the field — similar to what happened with variable success for commercial initiatives exploring human genetics[124].

Novel pre-, pro- and postbiotics

Nondietary approaches to modulating the microbiome and the host–microbiome interface to improve CMH are an area of very rapid expansion. The therapeutic interventions of this type include prebiotics (selected compounds or mixtures of compounds that aim to alter the composition and/or activity of the microbiota[125]), probiotics (live microorganisms that supposedly confer health benefits on the host by transient or stable colonization[126]), synbiotics (combinations of pre- and probiotics[127]) and postbiotics (microbial metabolites generated ex vivo, inanimate microorganisms and/or their components[128]). These interventions were originally aimed at decreasing intestinal inflammation but are increasingly being evaluated for their potential to modulate host CMH. For example, probiotics are evolving from a narrow group of well-studied taxa (*Lactobacillus* and *Bifidobacterium* species) that are common in fermented foods and easily delivered in commercial products, to next-generation probiotics based on more diverse taxa identified via meta-omics studies[129]. A recent example is *Akkermansia muciniphila*, which has been found to be negatively correlated with obesity[130]. It was also tested successfully in a pilot trial as a postbiotic intervention, in which a pasteurized form of the bacteria was shown to improve insulin sensitivity and decrease insulinemia and plasma total cholesterol[131]. Meta-omics approaches are consistently identifying candidates and fueling the design of ever more effective, targeted, next-generation probiotics for CMD[129], with several next-generation probiotics moving toward pilot clinical trials (such as NCT05114018 and NCT04797442 for *A. muciniphila*). Besides *A. muciniphila*, other candidates include *F. prausnitzii*, *Eubacterium hallii*, *P. copri* and *Bacteroides* species[132].

Fecal microbiota transplantation

A successful strategy to dramatically modulate the gut microbiome of a patient toward a healthier state is through administration of stool from a healthy donor, by means of fecal microbiota transplantation (FMT). FMT is now a well-established treatment for recurrent *Clostridium difficile* infection (approved by European and US guidelines)[133–135] and it is also being investigated to counterbalance gut dysbiosis in ulcerative colitis[136] and to improve responses to immunotherapy for cancer (specifically advanced melanoma)[137,138]. In the context of CMD, studies have shown that using lean donors for FMT leads to increased insulin sensitivity in obese individuals with metabolic syndrome[139]. However, the beneficial effects of lean donor FMT reported so far are transient and driven by baseline gut microbiome composition, while the improvement in insulin sensitivity is linked to changes in plasma metabolites. Indeed, the results of FMT in CMD have to date been of limited success and the potential side effects of FMT should be carefully evaluated in light of currently limited therapeutic or protective effects. Personalized donor selection and

protocols developed by collaboration between clinicians and experts in meta-omics, as well as cultivated microbial mixture alternatives to donor stool samples, all hold the potential to exploit whole-microbiome modulation as a support for CMH[140]. As it is now understood that microbiome transmission is massive, even as a consequence of individuals sharing the same house for a few years[141], and that the microbiome-associated risk factors for CMDs are thus partially transmissible[142], non-FMT-based novel microbiome-modulating strategies are likely to appear in the future.

Conclusions

Meta-omics approaches hold great potential for deciphering the intricate crosstalk between diet, the gut microbiome, the metabolome and their role in CMH and disease. While still only a few large-scale studies have integrated multiple meta-omics in the context of studying CMD (in contrast with studies in the general population[143,144] or in other conditions such as IBD[47,49]; Table 1), these have already identified promising signatures that, after tackling the current challenges and using larger sample sizes, hold great promise for designing effective next-generation therapeutic strategies in the near future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the European Research Council (ERC-STG project MetaPG-716575 and ERC-CoG microTOUCH-101045015) to N.S. and by EMBO ALTF 593–2020 to M.V.-C. The work was also partially supported by the European Union's Horizon 2020 program (ONCOBIOME-825410 project, MASTER-818368 project and IHMCSA-964590) to N.S., the European Union NextGenerationEU (Interconnected Nord-Est Innovation program, INEST) to N.S., the National Cancer Institute of the National Institutes of Health (1U01CA230551) to N.S. and the Premio Internazionale Lombardia e Ricerca 2019 to N.S. C.M. is funded by the Chronic Disease Research Foundation.

Competing interests

S.E.B., A.M.V., T.D.S. and N.S. are consultants to Zoe Global. N.S. reports consultancy and/or Scientific Advisory Board contracts with Roche, YSOPIA Bioscience, Freya Biosciences and Alia Therapeutics and speaker fees from Illumina and is cofounder of PreBiomics. The other authors declare no competing interests.

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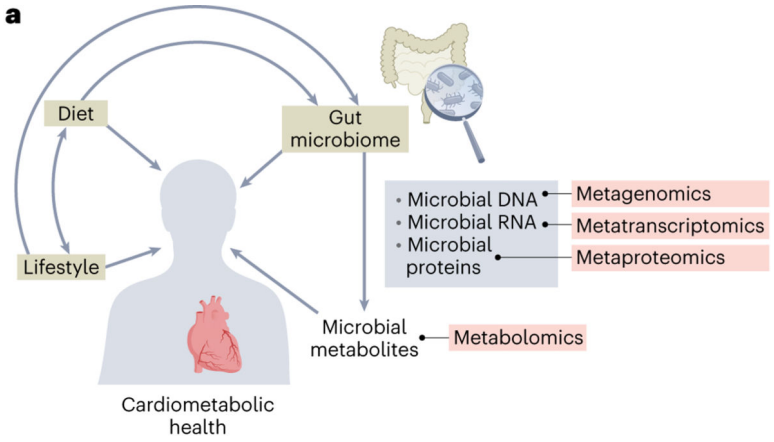

b

	Stool	Saliva	Urine	Blood	Food
Metagenomics	X	X			X
Metatranscriptomics	X	X			X
Metaproteomics	X	X	X	X	X
Metabolomics	X	X	X	X	X
Host-omics		X		X	
Single-cell genomics	X	X			X

Fig. 1 |. The intricate, multidirectional relationship between diet, lifestyle, the gut microbiome and the metabolome and their influence on CMH.

a, Effectors of host CMH meta-omics approaches to studying the microbiome layer of the interaction. Note that metabolomics targets the whole metabolite pool; thus, microbial, host and shared metabolites can be detected. **b**, Meta-omics techniques and the sample types to which they are typically applied.

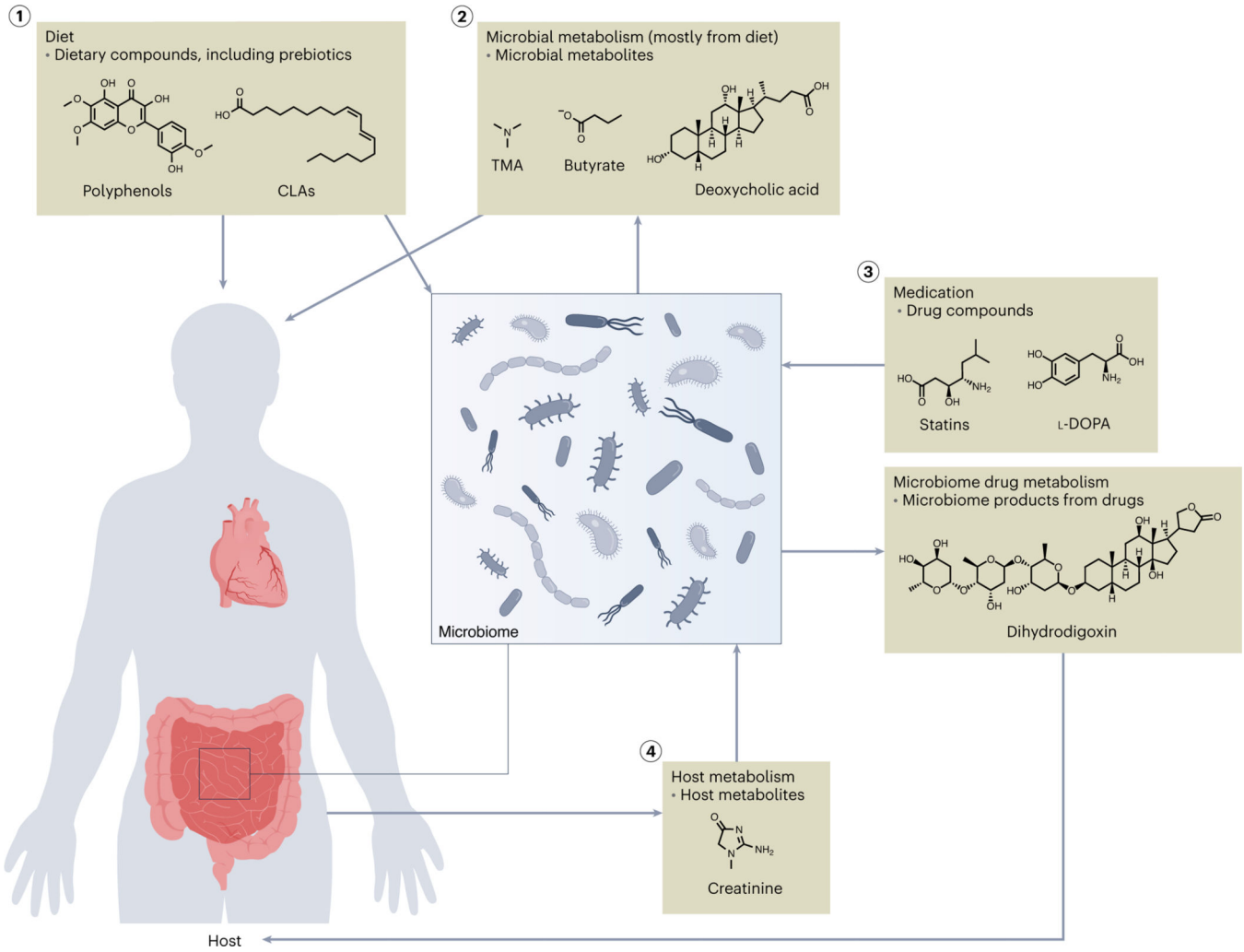

Fig. 2 |. Metabolite trafficking and their detection with metabolomics.

(1) Dietary metabolites are digested by the host and/or its microbiome. (2) Members of the microbiome produce a pool of metabolites from dietary substrates or other metabolites produced by the host. (3) Drug compounds can also be modified (for example, inactivated or bioaccumulated) by the microbiome. (4) Metabolomic technologies detect metabolites produced by the host, as well as those produced by the microbiome. All types of metabolites can affect CMH status.

Table 1 |

Examples of large efforts integrating multiple meta-omics techniques to decipher the diet–microbiome–metabolome crosstalk

Study/cohort	Design	Cohort size (*n*)	Meta-omics	Key findings
Zhang et al.[49]	Cross-sectional cohort	1,595	Metagenomics, metatranscriptomics and metaproteomics	Identification of microbial proteins that potentially interact with the host immunesystem in IBD
MetaCardis[44]	Cross-sectional cohort	1,241	Metagenomics and metabolomics	Identification of microbiome and metabolome features of ischemic heart disease
PREDICT 1 (refs. [45,123])	Cross-sectional cohort	1,102	Metagenomics and metabolomics	Large interpersonal variation, identification of a panel of gut microbiome species linked to a healthy diet/CMH, and postprandial response prediction with microbiome components
Health Professionals Follow-Up Study[63]	Longitudinal cohort	307	Metagenomics and metatranscriptomics	Protective associations between Mediterranean diet and CMH depend on microbiome composition
Talmor-Barkan et al.[12]	Cross-sectional cohort	199	Metagenomics and metatranscriptomics	Microbiome alterations linked to coronary artery disease are also associated with diet and the serum metabolome
HMP2 (refs. [47,48])	Longitudinal cohort	106 and132	Metagenomics, metabolomics, metatranscriptomics and metaproteomics	Host–microbe interactions linked to insulin resistance, and potential early signatures of type 2 diabetes identified
Meslier et al.[71]	Dietary intervention	82	Metagenomics and metabolomics	Mediterranean diet associated with specific microbial taxa and metabolites

Other studies using single meta-omics are reported in Supplementary Table 1.