GUT MICROBIOTA IN 2018

Microbiota and metabolites in metabolic diseases

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In 2018, more than 4,000 publications were dedicated to the study of the gut microbiota, and an important proportion investigated cardiometabolic disorders associated with overweight and obesity. Novel mechanisms and strategies have emerged, some of which were focused not only on specific bacteria or nutrients, but also on new metabolites.

Over the past 15 years, a large number of studies have associated obesity, type 2 diabetes mellitus (T2DM) and cardiovascular diseases with specific changes of the gut microbiota composition and function[1]. The majority of the investigations have focused their attention on the modulation of nutrients, such as fatty acids, but also on the composition of sugars and dietary fibres[2,3]. Hence, various researchers have studied the effect of specific bacterial metabolites, such as the short-chain fatty acids (SCFAs; which result from the fermentation of non-digestible carbohydrates), on host metabolism. Some dietary fibres and prebiotics, which are defined as substrates that are selectively used by host microorganisms and confer health benefits, have also been well studied[3]. Over the past 5 years, papers have demonstrated that the microbial transformation of food components, such as carnitine, choline or lecithin, to produce trimethylamine *N*-oxide (TMAO), contributed to the onset of atherosclerosis in rodent models and strongly correlated with cardiovascular disease risk factors in humans[4] (FIG. 1).

Based on these data, microbiota-derived molecules are considered key metabolic regulators that can act on different organs throughout the body and, ultimately, control a range of functions in a beneficial or harmful way (FIG. 1). In addition to the example of TMAO as a potential key player, other microbiota-derived metabolites, such as those produced from aromatic amino acids, have been linked with anti-inflammatory processes in the liver[5] (FIG. 1); however, only a handful of studies have clearly dissected the molecular mechanisms by which specific microbiota-derived metabolites of amino acids contribute to the onset of diseases.

A key example of a study that has described how microbiota-derived metabolites can contribute to the onset of diseases is the 2018 study by Ara Koh and colleagues. The authors identified imidazole propionate, produced from histidine, as an important microbial metabolite that contributes to the development of insulin resistance and, eventually, T2DM[6]. Starting from untargeted metabolomic analysis comparing the portal vein plasma of individuals with obesity and T2DM with plasma from BMI-matched participants who did not have T2DM, the investigators found that levels of four metabolites derived from amino acids were higher in patients with T2DM than in the controls. The team then cross-validated the link with microbiota by showing that imidazole propionate was the only metabolite present at increased levels in conventionalized mice versus germ-free mice or antibiotic-treated mice. In a second cohort of 649 individuals, Koh and colleagues[6] demonstrated that treatment-naive patients with T2DM had higher levels of imidazole propionate than the participants who had normal glucose tolerance.

By combining in vitro models and animal models, the authors of this study have dissected the molecular mechanism by which imidazole propionate contributes to the onset of glucose disorders. This compound impairs the ability of cells to respond correctly to insulin, thereby inducing insulin resistance, which can eventually lead to T2DM. They identified imidazole propionate as an inhibitor of the intracellular insulin receptor signalling cascade. Imidazole was shown to act as an activator of the p38γ–p62–mTORC1 pathway, which inhibits the formation of the insulin receptor substrate protein and mTORC1 complex. mTORC1 is part of the intracellular cascade and regulates various pathways, including the insulin receptor cascade (FIG. 1). This outstanding paper shows how starting from a simple correlation detected between a bacterial metabolite and T2DM has led to the discovery of a novel mechanism that explains how a specific molecule contributes to the disruption of an essential metabolic pathway and to metabolic disorders (FIG. 1).

In addition to the classic nutrients discussed above, high salt intake has been associated with cardiometabolic risk factors and metabolic disorders. Until now, most of the studies on this topic have investigated the role of this specific mineral and its assumed implication on cardiac diseases and obesity. Knowing that salt is a mineral that has been used for millennia to circumvent microbial proliferation, and thereby improve food preservation, in a 2018 paper, El Hadji Seck and colleagues[7] investigated the link between salt intake, gut microbiota composition and obesity. In a cohort of >1,300 individuals from different geographical regions, they discovered a potential unexpected link between salt and microbiota.

First, they found that stratified by country, faecal salinity was increased among individuals who were obese, and high faecal salinity was even a predictor of obesity independently of age, sex and country. Moreover, they found a dose-dependent relationship between faecal salinity and body weight, suggesting a link between salt levels in the gut and obesity.

Interestingly, the authors did not simply use the classic 16S metagenomic sequencing method, they also used culturomics with salted media to study the halophilic bacteria. This work, which involved the analysis of 572 samples and the development of >85,000 microbial colonies, led to the discovery of new bacterial and archaeal species[7]. In addition, metagenomic

Key advances

- Faecal salinity shapes the microbiota by reducing the abundance of *Bifidobacterium* and *Akkermansia*[7].
- Imidazole propionate is increased in patients with type 2 diabetes mellitus (T2DM); this compound directly contributes to the development of insulin resistance[6].
- *Bifidobacterium* and *Akkermansia muciniphila* have been inversely associated with low-grade inflammation, insulin resistance and T2DM[8].

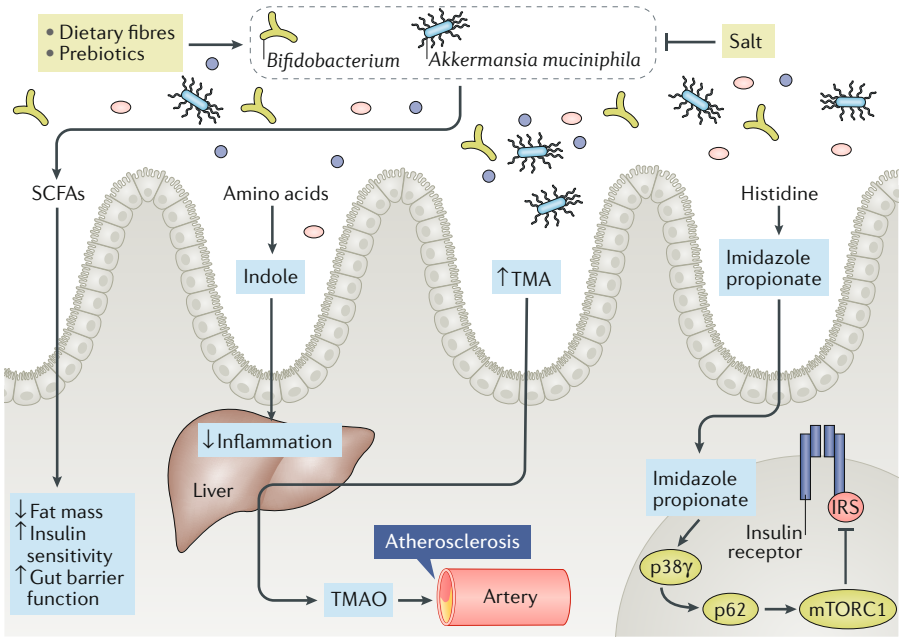

Fig. 1 | **Novel mechanisms linking microbiota-derived molecules and metabolism.** *Bifidobacterium* and *Akkermansia* are known to produce specific short-chain fatty acids (SCFAs) that are linked to a lower fat mass, increased insulin sensitivity and improved gut barrier function. Depending on the source of amino acids, the gut microbiota can improve or alter metabolism. For example, indole decreases hepatic inflammation, whereas imidazole propionate contributes to the onset of insulin resistance by blocking the insulin receptor signalling pathway following the inhibition of the function of insulin receptor substrate (IRS), which is induced by the activation of p38γ–p62–mTORC1. TMA, trimethylamine; TMAO, trimethylamine *N*-oxide.

analysis of 164 samples showed an inverse relationship between salinity and microbial diversity. Thus, both approaches (sequencing and culturomics) have contributed to the demonstration that the composition of the gut microbiota is linked not only with obesity, but also with salinity.

The authors also found that in addition to the enrichment of halophiles, the salinity of stools was a key factor associated with the depletion of specific bacteria, namely *Bifidobacterium* and *Akkermansia muciniphila*. Both of these bacteria have been inversely associated with metabolic disorders, as well as low-grade inflammation, insulin resistance and T2DM[8,9]. Moreover, these bacteria are well-known producers of SCFAs. These microbial metabolites are involved in the regulation of a variety of targets such as glucose, lipid and energy metabolism but also immunity and cancer[1]. In addition, the bifidobacteria[8] are described as probiotic, that is, a live microorganism that when administered in adequate amounts, confers a health benefit to the host[10], and *A. muciniphila* is considered as a next-generation beneficial bacteria[9]. This elegant study strongly suggests that excessive salt intake can have an instrumental role in the equilibrium of the gut microbiota by reducing levels of specific bacteria considered as potentially beneficial for the host health. The authors also identified a novel mechanism by which a micronutrient, such as salt, might contribute to the onset of metabolic diseases associated with obesity.

In connection with *Bifidobacterium* and *A. muciniphila*, another 2018 study has demonstrated that individuals with obesity who are treated with a specific strain of *Bifidobacterium* exhibited improved adiposity biomarkers[8]. In this randomized, double-blind, placebo-controlled trial, Anna Pedret and colleagues found that following treatment with 10^{10} colony forming units (CFUs) of either live or heat-killed *Bifidobacterium animalis* subsp. *lactis* CECT 8145 for 3 months, individuals with obesity had improved anthropometric adiposity biomarkers (including decreased BMI and visceral adiposity) and the results were particularly pronounced in women. Interestingly, the authors also highlighted that the ingestion of the live bacteria was linked with an increased abundance of *A. muciniphila*. Based on the results, however, it is difficult to ascertain whether the increase in *A. muciniphila* contributed to the phenotype observed as the authors only observed a beneficial effect of the heat-killed form of *B. animalis* on visceral adiposity, and in these experiments the abundance of *A. muciniphila* was unchanged. In addition, microbial metabolites were not investigated; therefore, whether the supplementation with the live versus the heat-killed bacteria differentially affected host metabolism remains to be determined. Indeed, a greater understanding of the link between the presence of specific bacteria, metabolites and host metabolism would be of great interest to the scientific community.

In conclusion, this series of interesting studies from 2018 has led to the discovery of novel mechanisms linking either the presence of specific bacteria or the production of metabolites with the initiation of metabolic disorders. In addition, the human data discussed here are of utmost interest as they support previous preclinical work. More importantly, these data demonstrate how specific microbial-derived metabolites or bacteria might be linked to nutrients such as amino acids or salt, thereby offering future perspectives for the development of preventive interventions or even therapeutic options.

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Competing interests
P.D.C. is the inventor on patent applications dealing with the use of *A. muciniphila* and its components in the treatment of obesity and related disorders. P.D.C. is co-founder of A-Mansia biotech SA.