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Gut microbiome and cardiometabolic risk

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Abstract
The last decade has been characterized by an intense research on the composition of the gut microbiome and the links with human health. While previous work was focused on the effects of prebiotics and probiotics, nowadays several laboratories are describing the gut microbiome and its metabolic functions. Gut microbiome interaction with nutrients allows the gut microbiome to survive and at the same time determines the production of metabolites that are either adsorbed by intestinal cell in a mutual relationship or promote detrimental effect. Metabolomics, a new method to approach identification of biomarkers has been used to identify small metabolites in blood and other biofluids. The study of metabolome revealed several microbial derived metabolites that are circulating in blood and potentially affect human health. In this review we describe the links between regulation of metabolism and microbial derived metabolites.

Keywords Gut microbiome · Insulin resistance · Obesity · Diabetes · Atherosclerosis · Inflammation

1 The intestinal microbiome - a new player in cardiometabolic risk

In the past three decades life-style, especially energy imbalance due to high calorie food intake, has led to marked derangements in the metabolic pathways of lipid and carbohydrates and contributed to setting the stage for the onset and progression of metabolic and cardiovascular disorders [1]. Recently, it has been pointed out that the intestinal microbiome might contribute to the pathogenesis of metabolic and cardiovascular diseases as well as many other chronic disorders [2]. In this review, we expand previous discussion on how the microbial products and metabolites may affect human physiology [3–5].

While considered relevant for disease in the gut-liver system the role intestinal bacteria in cardiometabolic risk received little attention, but the research of the last decade has fundamentally changed this view. A human subject hosts an enormous amount of bacteria in different tissues such skin, lung and intestine. Due to improved sequencing techniques more than than 22 million different microbial genes have been identified in the human gut, therefore largely exceeding the number of human genomes by a factor of almost 10^3 [6]. Thanks to this huge amount of genes it is clear that bacteria have metabolic functions more vast than the host, potentially contributing to the metabolism of the host organism through the generation of small metabolites, synthesis of essential amino acids and vitamins [7, 8]. Therefore, the intestinal microbiome is considered a tissue with wide metabolic potential with the ability to influence host metabolism thanks to the possibility to elaborate the nutrients contained in the diet and allow their components to be adsorbed and re-used by human cells (Figure 1).

The metabolic potential of the gut microbiome became clear after studies from Gordon's group presenting the connection between intestinal bacteria and metabolic diseases. In these studies, the intestinal bacterial flora from overweight mice was transplanted into germ-free recipient mice. Despite a constant diet, these mice developed obesity and insulin resistance suggesting that the obese phenotype, at least in part, is transferred by the microbiome [9]. A similar relationship emerged from studies of the microbiome structure in human monozygotic twins [10].

The architecture of the individual intestinal microbiome is variable and influenced by factors such as diet, genetic background, age, stress, physical excercise, coexistent disorders, use of antibiotics as well as other drugs such as metformin [11–14]. The term *dysbiosis* refers *to* a disproportion of certain

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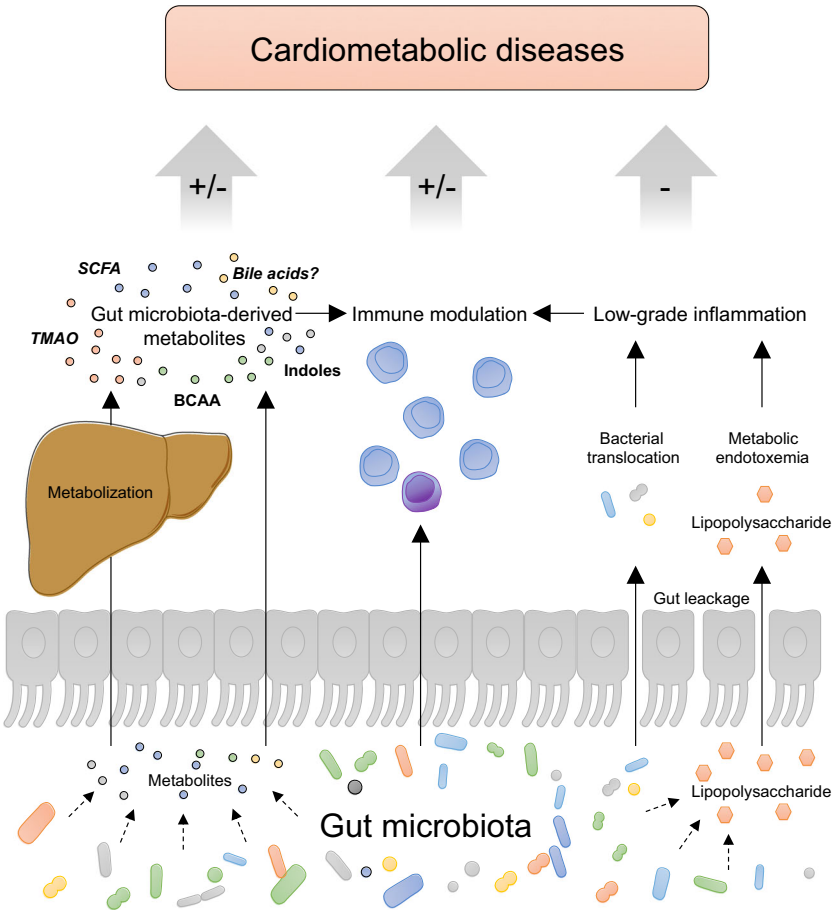

Fig. 1 Gut microbiota-associated mechanism in cardiometabolic diseases including atherosclerosis, heart failure, diabetes, obesity and NAFLD. BCAA: branched-chain amino acids, SCFA: short chain fatty acids, TMAO: Trimethylaminoxide, "±" indicates either a positive or negative effect, "-"indicates a negative effect on cardiometabolic diseases

components in the intestinal flora of a subject respect to physiology and it has been associated with metabolic diseases such as obesity, type 2 diabetes, non-alcoholic steatohepatitis [NASH] and cardiovascular diseases such as atherosclerosis and heart failure as well as many other chronic diseases [15, 16]

Actually, in both cardiovascular diseases and type 2 diabetes mellitus [T2DM] a change in Firmicutes/Bacteroidetes ratio has been detected; the causes of this alteration are still unclear although they maybe consequent to an interplay among dietary and other lifestyle habits resulting in an effect on obesity and insulin resistance depending from different metabolic functions in Firmicutes with respect to Bacteroidetes [15]. Intestinal bacterial species, such as *Roseburia intestinalis*, *Eubacterium halii* and *Faecalibacterium prausnitzii*, are generally decreased while *Lactobacilli gasseri*, *Streptococcus mutans* and *Escherichia coli* are increased in subjects with type 2 diabetes and CVD [3, 4]. It is known that gut-microbiota-based T2DM index might be used to identify subsets of the population that are at high risk for progressing to clinically defined T2DM [17, 18].We and other groups have suggested that both intestinal dysbiosis and bacterial translocation are involved in the development of cardiometabolic disorders such as type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) and atherosclerosis [17, 19–24]. Patients with obesity, insulin resistance and steatosis show signs of reduced microbial gene richness and increased genetic potential for processing of dietary lipids and endotoxin biosynthesis [notably from *Proteobacteria*], hepatic inflammation and dysregulation of aromatic and branched-chain amino acid [AAA and BCAA] metabolism [25]. In patients with symptomatic atherosclerosis, the genus *Collinsella* was enriched whereas *Roseburia* and *Eubacterium* were enriched in healthy controls [26].

2 Gut microbiome-associated mechanisms in cardiovascular diseases

Due to the close association between the intestinal microbiome and cardiometabolic diseases, the issue arises whether a dysbiotic gut microbiome, through activation of specific functions or loss of other activities, may swift cardiovascular risk factors such as diabetes, hypertension and dyslipidemia thus contributing to the onset and the progression of atherosclerosis [27].

There are several mechanisms that have been proposed to explain how intestinal bacteria could influence cardiometabolic diseases.

Dysbiosis could prompt an inflammatory status in the intestine that result in the production and absorption of factors triggering *low-grade* inflammation such as lipopolysaccharides (LPS) [28]. In particular, *low-grade* inflammation in the adipose tissue may pave the onset for insulin resistance [29].

Since several studies underlined that in cardiovascular diseases the immune system has a role, it is conceivable that deviation in the integrity of the immunomodulatory functions of the gut microbiome may determine inflammation [30]. For instance it is known that interleukin-23-22 axis regulates diet-induced atherosclerosis by repressing pro-atherogenic microbiota. Loss of IL-23-IL-22 signaling causes a deterioration of the intestinal barrier, dysbiosis, and expansion of pathogenic bacteria with distinct biosynthetic and metabolic properties, causing systemic increase in pro-atherogenic metabolites such as LPS [31]. This study perfectly emphasizes mechanisms explaining the pro-atherogenic effect of dysbiosis.

Segmented filamentous gut bacteria have been shown to regulate Th17 cells, which are able to produce IL-22, but depending on the circumstances also the pro-inflammatory cytokine IL-17 [32]. Gut microbiota-facilitated Th17/IL-17 response has been linked to vascular inflammation as well as salt-sensitive hypertension [33, 34].

However, which factors instigate the divergence from a physiological microbiome to a dysbiotic one linked to immunomodulation is still debated [35].

Another mechanism is intrinsic to gut microbiome functions and its metabolic activity. The gut microbiome produces several metabolites as the result of its interaction with nutrients. Once these are adsorbed by the intestine wall they can enter the circulation and exert a direct influence on the host phenotype [8].

The massive number of enzymatic pathways that constitute the core of bacteria metabolism is a feature of the diversity of the gut microbiome allowing the symbiont to take advantage to adsorb substances that otherwise could be neither produced nor extracted from the digestive process [36].

Thanks to the improvement in analytical methods for small metabolites (metabolomics) we now appreciate that thousands of molecules are potentially detectable in the blood [37]. Recent analysis suggested that many of these metabolites have their source in the metabolism of intestinal bacteria. Nutrients enter the intestine, where they are metabolized by various bacteria and absorbed into the circulation. Some of these metabolites are moreover modified or conjugated in the liver, resulting in microbiome-host co-metabolites [8]. Microbial metabolites or co-metabolites can in turn achieve numerous pleiotropic effects, for example via specific receptors, as has already been described for short-chain fatty acids, indoles or secondary bile acids [38].

3 Cardiometabolic inflammation by endotoxinemia

Bacterial endotoxins, so-called lipopolysaccharides [LPS], are structurally conserved components of cell membranes of gram-negative bacteria [39]. As such, they activated the innate immune system and trigger an inflammatory reaction in picomolar concentrations [40, 41]. A gram-negative sepsis leads to a dramatic increase in LPS concentrations. On the contrary, the concept of "metabolic endotoxemia" was coined to refer to slightly elevated LPS levels in the blood without a manifest infection [28]. Numerous studies show that serum measured LPS levels correlate with the incidence or prevalence of cardiovascular disease [40–44]. The hypothesis that endotoxins are among the causal mediators of these diseases is supported by the observation that experimentally induced *low-grade* endotoxinemia accelerates the development of atherosclerosis in rodents [45, 46]. The *toll-like receptor* (TLR) 4 acts as a receptor for LPS. Accordingly, TLR4-deficient mice are more resistant to atherosclerotic vascular disease [47, 48].

A question arises what the source of circulating endotoxin in the absence of infection is. One hypothesis, supported by experimental data, suggest that LPS translocates from the intestinal lumen, where it is either produced by intestinal bacteria or ingested through food, into the circulation [28]. Metabolic endotoxinemia can be promoted by dietary fat intake chylomicron-mediated [49, 50] and is elevated postprandially in both rodents and humans [28, 51]. Since levels of endotoxins in the intestinal lumen are massively elevated in relation to the serum, intestinal permeability seems to play a decisive role. For example, elevated levels of LPS could be detected in the blood of patients with acutely decompensated heart failure and consequently intestinal edema with impaired permeability [52]. The gut microbial flora is also able to regulate intestinal permeability. It could be shown that intestinal bacteria regulate intestinal permeability via a GLP-2-dependent mechanism and thus influence systemic *low-grade* inflammation [53].

4 Bacterial translocation from the gut into the host tissues

In addition to endotoxinemia, increased intestinal permeability may also lead to translocation of bacteria into blood and tissue, maintaining chronic *low-grade* inflammation [20, 54]. Burcelin and coworkers showed in mice fed a high-fat diet and showing insulin resistance, a large number of DNA of Gram-negative bacteria were detected in blood and fatty tissue. Probiotic treatment with a *Bifidobacterium strain* could improve both translocation and systemic inflammation and insulin resistance [55].

The same group also investigated bacterial DNA in the blood of 3936 participants with a cardiovascular risk profile. In a multivariate model, bacterial DNA from *Proteobacteria was* shown to be a sign of dysbiosis predictive for cardiovascular [56].

5 Metabolites with direct evidence to affect cardiovascular diseases

Gut microbiota produces numerous metabolites, some of which are absorbed into the systemic circulation and are biologically active, whereas others are further metabolized by host enzymes, and then serve as a mediator of microbial influence on the host. Particularly, these metabolites may favour an inflammatory status, and then to be associated to the pathogenesis of acute coronary syndrome [ACS], and involved in atherosclerosis onset and progression [57].

5.1 Trimethylamine oxide

The Hazen group was able to demonstrate the importance of the gut flora-dependent metabolite *trimethylamine oxide* (TMAO) in the development of atherosclerosis, thrombosis and ischemic heart failure [57–61]. Food-supplied phosphatidylcholine, a component of red meat, is converted by intestinal bacteria to trimethylamine, which is metabolized in the liver via the enzyme flavin-containing monooxygenase 3 (FMO3) to TMAO [57]. TMAO is therefore a microbiome-host co-metabolite. The mechanism underlying TMAO effects are still under investigation. However, it has been pointed out that dietary supplementation of mice with choline or TMAO promoted upregulation of multiple macrophage scavenger receptors linked to atherosclerosis, and supplementation with TMAO promoted atherosclerosis [60]. TMAO is also considered to influence the reverse cholesterol transport (RCT) in macrophages as suggested from studies with FMO3 knockdown mice, in which basal and liver X receptor (LXR)-stimulated macrophage RCT is increased leading to improved cholesterol balance [61]. TMAO, directly contribute to platelet hyperreactivity and enhanced thrombosis potential via enhanced sub-maximal stimulus-dependent platelet activation from multiple agonists through augmented Ca[2+] release from intracellular stores [62]. Interestingly, 3,3-dimethyl-1-butanol (DMB, a structural analog of choline) was shown to inhibit TMA formation and inhibited endogenous macrophage foam cell formation and atherosclerotic lesion development in experimental models without alterations in circulating cholesterol levels [63]. Overall, we may comment that the TMAO through effects on lipid metabolism, platelet and immune functions deteriorates cardiovascular health.

5.2 Short chain fatty acids

Short chain fatty acids (SCFA) such as butyrate, acetate and propionate are known to affect several human diseases [38]. SCFA are the prototypical examples of the holobiont metabolism. In fact, starch and non-starch polysaccharides that cannot be degraded by human enzymes are broken downto SCFA by various bacterial species such as *Roseburia* species and *Faecalibacterium prausnitzii* thanks to primary bacterial butyrate synthesis pathways, butyryl-CoA:acetate CoA-transferase (but) and butyratekinase (buk) [64]. SCFA mediate some of their effects via G protein coupled receptors (GPR41 and GPR43) and play a role in the regulation of immune cells, the release of cytokines, and the expression of adhesion molecules in vascular cells. These in turn are all processes that are directly related to cardiovascular diseases [65].

A recent paper suggested that *Roseburia intestinalis* cooperates with dietary plant polysaccharides to produce butyrate. This interaction has an impact on gene expression in the gut, exerts modulatory effects on metabolism shifting it from glycolysis to fatty acid utilization (an effect often associated to anti-inflammatoy cytokines and lower systemic inflammation) and finally ameliorate atherosclerosis in experimental models [66].

Interestingly, in hypertensive mice a diet rich in fiber improved blood pressure control as well as heart failure progression in part through acetate. Acetate was shown to reduce the burden of fibrosis in heart and kidney acting on the transcription factor Egr1 [67].

5.3 Branched chain amino acids

Branched-chain amino acids (BCAA) metabolism is in part dependent on the intestinal flora. Bacteria have a wide range of enzymatic functions to trigger BCAA biosynthesis and BCAA are also adsorbed to enter the human circulation. In the host BCAA catabolism is especially active in adipose and hepatic tissues. A recent analysis in non-diabetic subjects showed that elevated BCAA levels and insulin resistance were associated with an gut microbiome characterized by increased biosynthesis of BCAA and reduced bacterial inward transporters for these amino acids, suggesting increased production and availability for absorbance by intestinal wall. This effect was primarily mediated by *Prevotellacopri* and *Bacteroides vulgatus.* In the mouse model, treatment with *Prevotella copri* increased serum levels of BCAA and led to insulin resistance [68]. Although circulating BCAAs come from nutrients and microbial metabolism, it is unclear how they induce insulin resistance despite the strong evidence that BCAA are predictive for the development of type 2 diabetes as shown in the Framingham Heart Study and other cohorts [69]. A potential mechanism recently described suggests that 3-hydroxyisobutyrate (3-HIB), a catabolic intermediate of the

BCAA valine, is a new paracrine regulator of trans-endothelial fatty acid transport. Arany and coworkers found in mice that 3-HIB after being secreted from muscle cells stimulates muscle fatty acid uptake *in vivo*. As consequence of this, it promotes lipid accumulation in the muscle leading eventuallyto insulin resistance [70]. It is unclear whether the same mechanism is active in the myocardium. However,increased BCAA levels also correlate with the incidence of cardiovascular disease [71].

5.4 Microbial metabolites derived from aromatic amino acids

A recent study in obese women revealed that a disturbed aromatic amino acid (AAA) metabolism was associated with intestinal dysbiosis and hepatic steatosis. This effect was in part mediated by phenylacetate (PAA), an intestinal flora-dependent metabolite from the AAA metabolism. PAA was shown to affect insulin-mediated Akt phosphorylation in human primary hepatocytes and this effect can possibly also be transferred to other cardiometabolic diseases [25]. The microbial source of PAA is still undefined but a recent study observed that was negatively associated with the *Bacteroides* group and *Clostridium cluster XIVa* and positively with *Lactobacillus* [72]. Whether these microbes possess the oxidative pathway required to transform phenylalanine in PAA is unclear.

Clostridium sporogenes may be the source of another microbial metabolite derived from the AAA tryptophan with a potential effect on cardiovascular health: indole-3 propionic acid (IPA) produced exclusively by the microbiota from dietary tryptophan that accumulates in host serum [73]. Recent studies have shown that IPA can exert anti-inflammatory effects by directly engaging the pregnane X receptor (PXR) [74]. Interestingly, IPA was found negatively associated with type 2 diabetes, low-grade inflammation and advanced atherosclerosis [75, 76, 77].

Recently, it was shown that *Lactobacillus* species were able to modulate Th17 response and to protect against salt-sensitive hypertension. This effect was mediated by another gut microbiota-derived tryptophan metabolite, indole-3-acetic acid (IAA), which dose dependently reduced Th17 polarization [34]. IAA has previously been identified as a ligand of aryl hydrocarbon receptor exerting positive effects on inflammation by modulation of IL-22 response [78]. An inbalance between gut microbiota-derived tryptophan metabolites ("indoles") and endogenous tryptophan derivatives (kynurenine pathway) may play an important role in cardiometabolic disease modulation. The human enzyme indoleamine 2,3-dioxygenase, which is inducible in several different immune cells,is the rate-limiting first step in tryptophan degradation via kynurenine pathway [79]. High activity has been linked to cardiovascular events [80]. Laurans and colleagues revealed that an obese phenotype induces indoleamine 2,3-dioxygenase and consequently diminishes IL-22 response by bacterial tryptophan derivatives with impact on insulin sensitivity, gut mucosal barrier and inflammation as well as lipid metabolism [79].

Other microbial AAA-derived host co-metabolites such as phenylpropionic, indoxyl sulfate (IS) and p-cresyl sulfate (PCS) may exert negative functions in particular incresing the cardiometabolic risk in subjects with kidney failure [81, 82]. In fact, both IS and PCS are protein-bound uremic toxins that increase in the sera of patients with chronic kidney disease (CKD), and are not effectively removed by dialysis. Some studies suggested that both elevated levels of PCS and IS are associated with increased mortality in patients with CKD, while PCS, but not IS, is associated with an increased risk of cardiovascular events [82]. Both metabolites promote vascular calcification and associate with glucose intolerance through mechanisms involving LXR signaling pathway and reduced GLUT1 expression [83].

5.5 Other amino acid derived metabolites

Recently, a study based on the Lifelines DEEP cohort identified bacterial L-methionine biosynthesis and a *Ruminococcus* species were associated to cardiovascular phenotypes in obese individuals, in particular atherosclerosis and liver fat content. This study also found that several microbial pathways involved in amino acid metabolism are associated with metabolic risk score of cardiovascular disease independent of diet and inflammation [84].

5.6 Bile acids

Primary bile acids arise from the oxidation of cholesterol in the liver and are excreted into the gut to make lipids soluble for absorption. There they are deconjugated by microbial bile salt hydrolase, mainly from *Bifidobacteria*, *Clostridia* and *Bacteroidetes*, to secondary bile acids, which are reabsorbed into the circulation [85]. Secondary bile acids can achieve various pleiotropic effects via the farnesoid X (FXR) receptor and the Takeda G protein-coupled receptor 5 (TGR5). Their activation leads to transcriptional inhibition of pro-inflammatory cytokines, regulation of GLP-1 secretion in L-cells, glucose-dependent insulin secretion in the pancreas, influence on hepatic lipoprotein and glucose metabolism and control of bile acid synthesis and secretion in the liver via the FXR-*fibroblast growth factor* 15/19 signaling pathway [86]. However, a direct mechanism of these metabolites on atherosclerosis onset and progression is still unclear. Nevertheless, INT-767, a specific co-agonist for both FXR and TGR5 bile acid receptors could reduce atherosclerosis in the mouse model [87]. Furthermore, a recent analysis showed that 2 circulating secondary bile acids (glycocholenate and glycolithocolate sulfate) associated with atrial fibrillation

(AF) risk [88]. Another study found increased levels of secondary bile acids in patients with heart failure [89].

6 Conclusions

The intestinal microbiome is an essential factor in human health and is closely linked to physiologicaland pathological processes that go far beyond local processes in the intestine. Cardiometabolic diseases in particular appear to be closely linked to the metabolic pathways triggered by the interaction of nutrients with the intestinal flora. Several potential targets for diagnostic or therapeutic use have been identified including microbial species and their metabolic products. However, we should be cautious in the translation of experimental data to clinical practice given that our knowledge of which bacteria are involved and which metabolic pathways is still incomplete. It is conceivable that in few years the scenario linking gut microbiota to microbial metabolites will be completed and potential applications will enter the clinical practice.

In fact, despite promising data, probiotic therapy and fecal matter transplantation remain limited at the moment to very specific applications and in the cardiometabolic field we are still at the level of experimental approaches. A specific therapy for the modulation of the intestinal microbiome in cardiometabolic diseases must therefore still be established and above all subsequently validated in large patient cohorts.

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Compliance with ethical standards

Conflict of interest M.F. is co-inventor on pending patents held by INSERM Transfert, INSERM, University of Rome Tor Vergata, University of Girona and Imperial College on NAFLD diagnostics and has the right to receive royalty payments for inventions or discoveries related to NAFLD diagnostics.

References

1. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, et al. Type 2 diabetes mellitus. Nat Rev Dis Primers. 2015;1:15019.
2. Tang WHW, Bäckhed F, Landmesser U, Hazen SL. Intestinal microbiota in cardiovascular health and disease: JACC state-of-the-art review. J Am Coll Cardiol. 2019;73(16):2089–105.
3. Federici M. Our second genome and the impact on metabolic disorders: why gut microbiome is an important player in diabetes and associated abnormalities. Acta Diabetol. 2019;56(5):491–2.
4. Federici M. Gut microbiome and microbial metabolites: a new system affecting metabolic disorders. J Endocrinol Investig. 2019;42(9):1011–8.
5. Kappel BA, Lehrke M. Microbiome, diabetes and heart: a novel link? Herz. 2019;44(3):223–30.
6. Tierney BT, Yang Z, Luber JM, Beaudin M, Wibowo MC, Baek C, et al. The landscape of genetic content in the gut and Oral human microbiome. Cell Host Microbe. 2019. https://doi.org/10.1016/j.chom.2019.07.008.
7. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. Science. 2012;336(6086):1262–7.
8. Holmes E, Li JV, Marchesi JR, Nicholson JK. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. Cell Metab. 2012;16(5):559–64.
9. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–31.
10. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341(6150):1241214.
11. Schroeder BO, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. Nat Med. 2016;22(10):1079–89.
12. Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota*Nat*. Hepatol: Rev. Gastroenterol; 2019. https://doi.org/10.1038/s41575-018-0061-2. You are what you eat: diet, health and the gut microbiota
13. Quigley EMM. Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet? Nat Rev Gastroenterol Hepatol. 2017. https://doi.org/10.1038/nrgastro.2017.29 Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet?
14. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature. 2015;528:262–6.
15. Tilg H, Zmora N, Adolph TE, Elinav E. The intestinal microbiota fuelling metabolic inflammation. Nat Rev Immunol. 2019:1–15. https://doi.org/10.1038/s41577-019-0198-4.
16. Canfora EE, Meex RCR, Venema K, Blaak EE. Gut microbial metabolites in obesity, NAFLD and T2DM. Nat Rev Endocrinol. 2019;15(5):261–73.
17. Schüssler-Fiorenza Rose SM, Contrepois K, Moneghetti KJ, Zhou W, Mishra T, Mataraso S, et al. A longitudinal big data approach for precision health. Nat Med. 2019;25(5):792–804.
18. Wilmanski T, Rappaport N, Earls JC, Magis AT, Manor O, Lovejoy J, et al. Blood metabolome predicts gut microbiome α-diversity in humans. Nat Biotechnol. 2019;37:1217–28. https://doi.org/10.1038/s41587-019-0233-9.
19. Serino M, Fernández-Real JM, García-Fuentes E, Queipo-Ortuño M, Moreno-Navarrete JM, Sánchez A, et al. The gut microbiota profile is associated with insulin action in humans. Acta Diabetol. 2013;50(5):753–61. https://doi.org/10.1007/s00592-012-0410-5 Epub 2012 Jun 19.
20. Amar J, Serino M, Lange C, Chabo C, Iacovoni J, Mondot S, et al. Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept. Diabetologia. 2011;54(12):3055–61.
21. Lelouvier B, Servant F, Païssé S, Brunet AC, Benyahya S, Serino M, et al. Changes in blood microbiota profiles associated with liver fibrosis in obese patients: A pilot analysis. Hepatology. 2016;64(6):2015–27.
22. Koren O, Spor A, Felin J, Fåk F, Stombaugh J, Tremaroli V, et al. Human oral, gut, and plaque microbiota in patients with atherosclerosis. Proc Natl Acad Sci U S A. 2011;108(Suppl 1):4592–8.
23. Lindskog Jonsson A, Hållenius FF, Akrami R, Johansson E, Wester P, Arnerlöv C, et al. Bacterial profile in human atherosclerotic plaques. Atherosclerosis. 2017;263:177–83.
24. Fåk F, Tremaroli V, Bergström G, Bäckhed F. Oral microbiota in patients with atherosclerosis. Atherosclerosis. 2015;243(2):573–8.

25. Hoyles L, Fernández-Real JM, Federici M, Serino M, Abbott J, Charpentier J, et al. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. Nat Med. 2018;24(7):1070–80.
26. Karlsson FH, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun. 2012;3:1245.
27. Wilkins LJ, Monga M, Miller AW. Defining Dysbiosis for a cluster of chronic diseases. Sci Rep. 2019;9(1):12918.
28. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761–72 Epub 2007 Apr 24.
29. Moreno-Navarrete JM, Escoté X, Ortega F, Serino M, Campbell M, Michalski MC, et al. A role for adipocyte-derived lipopolysaccharide-binding protein in inflammation- and obesity-associated adipose tissue dysfunction. Diabetologia. 2013;56(11):2524–37. https://doi.org/10.1007/s00125-013-3015-9.
30. Burcelin R. Gut microbiota and immune crosstalk in metabolic disease. Mol Metab. 2016;5(9):771–81.
31. Fatkhullina AR, Peshkova IO, Dzutsev A, Aghayev T, McCulloch JA, Thovarai V, et al. An Interleukin-23-Interleukin-22 Axis RegulatesIntestinal microbial homeostasis to protect from diet-induced atherosclerosis. Immunity. 2018;49(5):943–57.
32. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous Bacteria. Cell. 2009. https://doi.org/10.1016/j.cell.2009.09.033.
33. Karbach SH, Schönfelder T, Brandão I, Wilms E, Hörmann N, Jäckel S, et al. Gut Microbiota Promote Angiotensin II-Induced Arterial Hypertension and Vascular Dysfunction. J Am Heart Assoc. 2016;5. https://doi.org/10.1161/JAHA.116.003698.
34. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, et al. Salt-responsive gut commensal modulates TH17 axis and disease. Nature. 2017;551:585–9.
35. Weiss GA, Hennet T. Mechanisms and consequences of intestinal dysbiosis. Cell Mol Life Sci. 2017;74(16):2959–77.
36. Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, et al. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr. 2018;57(1):1–24.
37. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, et al. HMDB 4.0: the human metabolome database for 2018. Nucleic Acids Res. 2018;46(D1):D608–17.
38. Abdul Rahim MBH, Chilloux J, Martinez-Gili L, Neves AL, Myridakis A, Gooderham N, et al. Diet-induced metabolic changes of the human gut microbiome: importance of short-chain fatty acids, methylamines and indoles. Acta Diabetol. 2019;56(5):493–500.
39. Lepper PM, Kleber ME, Grammer TB, Hoffmann K, Dietz S, Winkelmann BR, et al. Lipopolysaccharide-binding protein (LBP) is associated with total and cardiovascular mortality in individuals with or without stable coronary artery disease–results from the Ludwigshafen risk and cardiovascular health study (LURIC). Atherosclerosis. 2011;219(1):291–7.
40. Krogh-Madsen R, Plomgaard P, Akerstrom T, Møller K, Schmitz O, Pedersen BK. Effect of short-term intralipid infusion on the immune response during low-dosebendotoxemia in humans. Am J Physiol Endocrinol Metab. 2008;294(2):E371–9.
41. Gnauck A, Lentle RG, Kruger MC. The characteristics and function of bacterial lipopolysaccharides and their Endotoxic potential in humans. Int Rev Immunol. 2016;35(3):189–218.
42. Wiedermann CJ, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F, et al. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck study. J Am Coll Cardiol. 1999;34(7):1975–81.
43. Pussinen PJ, Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, et al. Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident cardiovascular disease events. Arterioscler Thromb Vasc Biol. 2007;27(6):1433–9.
44. Szeto CC, Szeto CC, Kwan BC, Chow KM, Lai KB, Chung KY, et al. Endotoxemia is related to systemic inflammation and atherosclerosis in peritoneal dialysis patients. Clin J Am Soc Nephrol. 2008;3(2):431–6.
45. Cuaz-Pérolin C, Billiet L, Baugé E, Copin C, Scott-Algara D, Genze F, et al. Antiinflammatory and antiatherogenic effects of the NF-kappaB inhibitor acetyl-11-keto-beta-boswellic acid in LPS-challenged. Arterioscler Thromb Vasc Biol. 2008;28(2):272–7.
46. Malik TH, Cortini A, Carassiti D, Boyle JJ, Haskard DO, Botto M. The alternative pathway is critical for pathogenic complement activation in endotoxin- and diet-induced atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation. 2010;122(19):1948–56.
47. Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, et al. Lack of toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. Proc Natl Acad Sci U S A. 2004;101(29):10679–84.
48. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006;116(11):3015–25.
49. Herieka M, Faraj TA, Erridge C. Reduced dietary intake of pro-inflammatory toll-like receptor stimulants favourably modifies markers of cardiometabolic risk in healthy men. Nutr Metab Cardiovasc Dis. 2016;26(3):194–200.
50. Ghoshal S, Witta J, Zhong J, de Villiers W, Eckhardt E. Chylomicrons promote intestinal absorption of lipopolysaccharides. J Lipid Res. 2009;50(1):90–7.
51. Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr. 2007;86(5):1286–92.
52. Niebauer J, Volk HD, Kemp M, Dominguez M, Schumann RR, Rauchhaus M, et al. Endotoxin and immune activation in chronic heart failure: a prospective cohort study. Lancet. 1999;353(9167):1838–42.
53. CCani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58(8):1091–103.
54. Burcelin R, Serino M, Chabo C, Garidou L, Pomié C, Courtney M, et al. Metagenome and metabolism: the tissue microbiota hypothesis. Diabetes Obes Metab. 2013;15(Suppl 3):61–70.
55. Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermúdez-Humarán LG, et al. Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO Mol Med. 2011;3(9):559–72.
56. Amar J, Lange C, Payros G et al (2013) Blood microbiota dysbiosis is associated with the onset of cardiovascular events in a large general populati- on: the D.E.S.I.R. study. PLoS ONE 8:e54461. https://doi.org/10.1371/journal.pone.0054461.
57. Brown JM, Hazen SL. Microbial modulation of cardiovascular disease. Nat Rev Microbiol. 2018;16(3):171–81.
58. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013;19(5):576–85.
59. Organ CL, Otsuka H, Bhushan S, Wang Z, Bradley J, Trivedi R, et al. Choline diet and its gut microbe-derived metabolite, Trimethylamine N-oxide, exacerbate pressure overload-induced heart failure. Circ Heart Fail. 2016;9(1):e002314.
60. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472(7341):57–63.

61. Warrier M, Shih DM, Burrows AC, Ferguson D, Gromovsky AD, Brown AL, et al. The TMAO-generating enzyme Flavin Monooxygenase 3 is a central regulator of cholesterol balance. Cell Rep. 2015;10(3):326–38.
62. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, et al. Gut microbial metabolite TMAO enhances platelet Hyperreactivity and thrombosis risk. Cell. 2016;165(1):111–24.
63. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, et al. Non-lethal inhibition of gut microbial Trimethylamine production for the treatment of atherosclerosis. Cell. 2015;163(7):1585–95.
64. Vital M, Penton CR, Wang Q, Young VB, Antonopoulos DA, Sogin ML, et al. A gene-targeted approach to investigate the intestinal butyrate-producing bacterial community. Microbiome. 2013;1(1):8.
65. Bolognini D, Tobin AB, Milligan G, Moss CE. The pharmacology and function of receptors for short-chain fatty acids. Mol Pharmacol. 2016;89(3):388–98.
66. Kasahara K, Krautkramer KA, Org E, Romano KA, Kerby RL, Vivas EI, et al. Interactions between Roseburia intestinalis and diet modulate atherogenesis in a murine model. Nat Microbiol. 2018;3(12):1461–71.
67. Marques FZ, Nelson E, Chu PY, Horlock D, Fiedler A, Ziemann M, et al. High-Fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice. Circulation. 2017;135(10):964–77.
68. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016;535(7612):376–81.
69. Cheng S, Rhee EP, Larson MG, Lewis GD, McCabe EL, Shen D, et al. Metabolite profiling identifies pathways associated with metabolic risk in humans. Circulation. 2012;125(18):2222–31.
70. Jang C, Oh SF, Wada S, Rowe GC, Liu L, Chan MC, Rhee J, Hoshino A, Kim B, Ibrahim A, Baca LG, Kim E, Ghosh CC, Parikh SM, Jiang A, Chu Q, Forman DE, Lecker SH, Krishnaiah S, Rabinowitz JD, Weljie AM, Baur JA, Kasper DL, Arany Z. A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. Nat Med. 2016;22(4):421–6. https://doi.org/10.1038/nm.4057.
71. Tobias DK, Lawler PR, Harada PH, Demler OV, Ridker PM, Manson JE, et al. Circulating branched-chain amino acids and incident cardiovascular disease in a prospective cohort of US women. Circ Genom Precis Med. 2018;11(4):e002157.
72. Jang C, Oh SF, Wada S, Rowe GC, Liu L, Chan MC, et al. A branched-chain amino acid metabolite drives vascular fatty acid transport andcauses insulin resistance. Nat Med. 2016;22(4):421–6.
73. Dodd D, Spitzer MH, Van Treuren W, Merrill BD, Hryckowian AJ, Higginbottom SK, et al. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. Nature. 2017;551(7682):648–52.
74. Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and toll-like receptor 4. Immunity. 2014;41(2):296–310.
75. Tuomainen M, Lindström J, Lehtonen M, Auriola S, Pihlajamäki J, Peltonen M, et al. Associations of serum indolepropionic acid, a gut microbiota metabolite, with type 2 diabetes and low-grade inflammation in high-risk individuals. Nutr Diabetes. 2018 May;8(1):35.
76. de Mello VD, Paananen J, Lindström J, Lankinen MA, Shi L, Kuusisto J, et al. Indolepropionic acid and novel lipid metabolites are associated with a lower risk of type 2 diabetes in the Finnish diabetes prevention study. Sci Rep. 2017;7:46337.
77. Cason CA, Dolan KT, Sharma G, Tao M, Kulkarni R, Helenowski IB, et al. Plasma microbiome-modulated indole- and phenyl-derived metabolites associate with advanced atherosclerosis and postoperative outcomes. J Vasc Surg. 2018;68(5):1552–1562.e7. https://doi.org/10.1016/j.jvs.2017.09.029.
78. Heath-Pagliuso S, Rogers WJ, Tullis K, Seidel SD, Cenijn PH, Brouwer A, Denison MS. Activation of the Ah receptor by tryptophan and tryptophan metabolites. Biochemistry. 1998;37(33):11508–15.
79. Laurans L, Venteclef N, Haddad Y, Chajadine M, Alzaid F, Metghalchi S, et al. Genetic deficiency of indoleamine 2,3-dioxygenase promotes gutmicrobiota-mediated metabolic health.Nat Med. 2018;24:1113–20. https://doi.org/10.1038/s41591-018-0060-4.
80. Eussen SJPM, Ueland PM, Vollset SE, Nygård O, Midttun Ø, Sulo G, et al. Kynurenines as predictors of acute coronary events in the Hordaland health study. Int J Cardiol. 2015. https://doi.org/10.1016/j.ijcard.2015.03.413.
81. Gutiérrez-Díaz I, Fernández-Navarro T, Salazar N, Bartolomé B, Moreno-Arribas MV, López P, et al. Could fecal Phenylacetic and Phenylpropionic acids be used as indicators of health status? J Agric Food Chem. 2018;66(40):10438–46.
82. Lin CJ, Wu V, Wu PC, Wu CJ. Meta-analysis of the associations of p-Cresyl sulfate (PCS) and Indoxyl sulfate (IS) with cardiovascular events and all-cause mortality in patients.
83. Opdebeeck B, Maudsley S, Azmi A, De Maré A, De Leger W, Meijers B, et al. Indoxyl sulfate and p-Cresyl sulfate promote vascular calcification and associate with glucose intolerance. J Am Soc Nephrol. 2019;30(5):751–66.
84. Kurilshikov A, van den Munckhof ICL, Chen L, Bonder MJ, Schraa K, Rutten JHW, et al. Van Faassen M; LifeLines DEEP cohort study, BBMRI metabolomics consortium, Slagboom PE, Xavier RJ, Kuipers F, Hofker MH, Wijmenga C, Netea MG, Zhernakova A, Fu J. gut microbial associations to plasma metabolites linked to cardiovascular phenotypes and risk. Circ Res. 2019;124(12):1808–20.
85. Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res. 2006;47(2):241–59.
86. Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. Gastroenterology. 2017;152(7):1679–94.
87. Jadhav K, Xu Y, Xu Y, Li Y, Xu J, Zhu Y, et al. Reversal of metabolic disorders by pharmacological activation of bile acid receptors TGR5 and FXR. Mol Metab. 2018;9:131–40.
88. Alonso A, Yu B, Sun YV, Chen LY, Loehr LR, O'Neal WT, et al. Serum metabolomics and incidence of atrial fibrillation (from the atherosclerosis risk in communities study). Am J Cardiol. 2019;123(12):1955–61.
89. Mayerhofer CCK, Ueland T, Broch K, Vincent RP, Cross GF, Dahl CP, et al. Increased secondary/primary bile acid ratio in chronic heart failure. J Card Fail. 2017;23(9):666–71.