Review

The gut microbiome in coronary artery disease and heart failure: Current knowledge and future directions

Marius Trøseid[a,b,c,*], Geir Øystein Andersen[d], Kaspar Broch[e], Johannes Roksund Hov[a,c,f,g]

[a] Research Institute of Internal Medicine, Sognsvannsveien 20, 0027 Oslo, Norway
[b] Section of Clinical Immunology and Infectious diseases, Norway
[c] Institute of Clinical Medicine, University of Oslo, Norway
[d] Department of Cardiology, Oslo University Hospital Ullevål, Norway
[e] Department of Cardiology, Oslo University Hospital Rikshospitalet, Norway
[f] Norwegian PSC Research Center, Norway
[g] Section of Gastroenterology, Oslo University Hospital Rikshospitalet, Norway

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ABSTRACT

Host-microbiota interactions involving inflammatory and metabolic pathways have been linked to the pathogenesis of multiple immune-mediated diseases and metabolic conditions like diabetes and obesity. Accumulating evidence suggests that alterations in the gut microbiome could play a role in cardiovascular disease. This review focuses on recent advances in our understanding of the interplay between diet, gut microbiota and cardiovascular disease, with emphasis on heart failure and coronary artery disease. Whereas much of the literature has focused on the circulating levels of the diet- and microbiota-dependent metabolite trimethylamine-N-oxide (TMAO), several recent sequencing-based studies have demonstrated compositional and functional alterations in the gut microbiomes in both diseases. Some microbiota characteristics are consistent across several study cohorts, such as a decreased abundance of microbes with capacity for producing butyrate. However, the published gut microbiota studies generally lack essential covariates like diet and clinical data, are too small to capture the substantial variation in the gut microbiome, and lack parallel plasma samples, limiting the ability to translate the functional capacity of the gut microbiomes to actual function reflected by circulating microbiota-related metabolites. This review attempts to give directions for future studies in order to demonstrate clinical utility of the gut-heart axis.

1. Introduction

The gut microbiota, comprising the trillions of bacteria in the gastrointestinal tract, is a complex community whose metabolic activities and interactions with the immune system extend beyond the gut itself [1]. Host-microbiota interactions involving inflammatory and metabolic pathways have been proposed to contribute to the pathogenesis of multiple immune-mediated diseases and metabolic conditions like diabetes and obesity [1].

Cardiovascular disease is the leading cause of death worldwide [2]. Atherosclerosis, the most common cause of cardiovascular disease, is the result of a complex series of events occurring within the arterial wall involving rheology, lipid metabolism, and inflammation [3]. The resulting stenoses in coronary, renal, precerebral and peripheral arteries precipitate end-organ ischemia, thromboembolic infarction, and necrosis.

Heart failure (HF) is a syndrome caused by the impaired ability of the heart to fill or eject blood [4]. Any disorder affecting the structural and/or functional integrity of the heart, such as valvular, coronary or myocardial disease, can commence HF. Hemodynamic stress [5], neurocrine activation [6], and inflammation [7] all contribute to the structural changes observed in advanced HF.

Accumulating evidence suggests that alterations in the gut microbial community could play a role in cardiovascular disease. This review focuses on recent advances in our understanding of the interplay between the gut microbiota and cardiovascular disease, with emphasis on HF and coronary artery disease (CAD). Some microbiota characteristics have consistently been identified in both diseases, such as a decreased abundance of microbes with capacity for producing butyrate and increased circulating levels of the diet- and microbiota-dependent metabolites trimethylamine-N-oxide (TMAO) (Fig. 1). However, most published studies lack essential covariates like diet, and are too small to capture the substantial variation in the gut microbiome. This review attempts to give directions for future studies in order to demonstrate a clinically useful gut-heart axis.

* Corresponding author at: Research Institute of Internal Medicine, Sognsvannsveien 20, 0027 Oslo, Norway.
E-mail address: marius.troseid@medisin.uio.no (M. Trøseid).
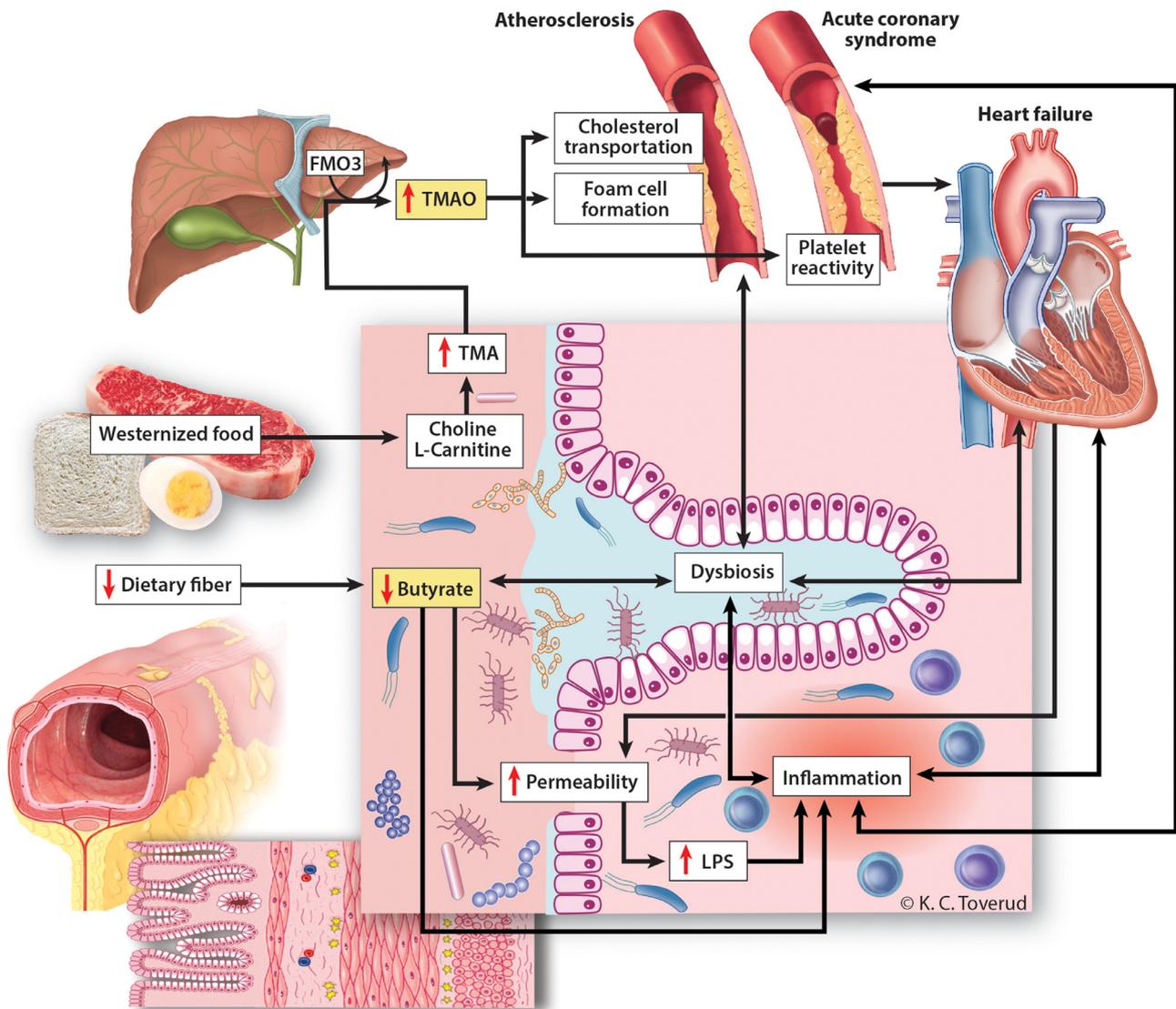
Fig. 1. Diet-gut-heart interactions: proposed mechanisms. Interactions between diet and the gut microbiome could contribute to atherosclerosis, acute coronary syndromes and heart failure through common and separate mechanisms. Westernized food rich in red meat promotes bacterial production of TMA, which is oxidized in the liver to the pro-atherogenic metabolite TMAO. TMAO may contribute to atherosclerosis by interference with cholesterol transportation, foam cell formation and platelet aggregation, the latter playing a potential role in acute coronary syndromes. Reduced dietary fiber is associated with reduced bacterial production of the short chain fatty acid butyrate, which has immune modulatory effects in the gut mucosa, and also serves as the main energy substrate for colonocytes. Reduction of butyrate levels in the gut could promote local inflammation, aggravate dysbiosis and contribute to impaired gut barrier function, the latter resulting in leakage of bacterial toxins such as LPS, further fueling local and systemic inflammation. FMO3; flavin-containing monooxygenase 3, LPS; lipopolysaccharide, TMA; trimetylamine, TMAO; trimethylamine-N-oxide. Printed with permission from Kari Toverud ©.

2. A brief introduction to microbiota-related laboratory methods

The gut microbiome can be considered an endocrine organ, and each microbe has the capacity to produce hundreds of different known and unknown metabolites which act beyond gut itself [1]. The composition of the microbiota is typically analyzed by high-throughput sequencing and bioinformatic analyses of extracted microbial deoxyribonucleic acid (DNA). The methodologies are extensively reviewed in the literature [8,9], but as basis for this review, we provide a brief overview of the most relevant methods.

This review addresses the bacterial component of the gut microbiome, for which segments of the 16S ribosomal ribonucleic acid (rRNA) marker gene are amplified and submitted to next-generation sequencing [10−12]. A given bacterial species (e.g. *Faecalibacterium prausnitzii*) can be described on different taxonomic levels; as part of a phylum (e.g. Firmicutes), class (Clostridia), order (Clostridiales), family (Ruminococcaceae) or genus (*Faecalibacterium*). With 16S sequencing, bacteria can typically be identified at genus level resolution, more rarely at species level.

When using the full metagenomic (shotgun) sequencing, all the DNA in the sample is sequenced, providing resolution on species level [9]. This method is more expensive and computationally demanding, but provides an overview of potential microbial functions. Similar data may be generated (imperfectly) by predicting the functional gene content from 16S rRNA-based data [13]. Methods for characterizing microbial functions include metatranscriptomics, metaproteomics and metabolomics [14]. Typically, metabolomic methods may be applied to stool or peripheral bloods samples and provide direct measures of microbial activity.

3. Coronary artery disease

3.1. Altered gut microbiome with inflammatory properties and reduced capacity for short chain fatty acid (SCFA) production

Three recent studies have investigated the gut microbiota composition in patients with CAD, using different sequencing methodologies (Table 1). Cui et al. reported differences on phylum level, with

Table 1
Contemporary gut microbiota sequencing studies in patients with coronary artery disease (CAD).

Study	Cui et al. [15]	Jie et al. [16]	Zhu et al. [17]
Patients	CAD verified by coronary angiography	CAD verified by coronary angiography	CAD verified by coronary angiography
Patient age	68.3 ± 9.5 years	40−80 years	63.6
Gender% (f/m)	85/15	−	58/42
Sample size	n = 29 CAD	n = 218 CAD	n = 70 CAD
	n = 35 controls	n = 187 controls	n = 98 controls
Methods	16 s rRNA	Metagenomics	16 s rRNA
Parallel plasma/serum	No	No	No
Dietary data	No	No	No
Increased relative abundance in patients	-Firmicutes phylum	Enterobacteriaceae family and *Streptococcus* species	*Escherichia-Shigella* and *Enterococcus*
Decreased relative abundance in patients	-Bacteroidetes phylum	*Roseburia Intestinalis* and *Faecalibacterium prausnitzii*	*Faecalibacterium, Roseburia, Subdoligranulum* and *Eubacterium rectale*
Functional findings	−	-Less fermentative capacity and more inflammatory properties in CAD microbiomes	-Several predicted functions, including lipopolysaccharide biosynthesis and propanoate metabolism enhanced in CAD microbiomes

decreased proportion of Bacteroidetes and increased proportion of Firmicutes in patients with coronary heart disease [15]. In a much more comprehensive study, Jie et al. reported increased levels of several *Streptococcus* species and genera of the Enterobacteriaceae family, and reduced abundance of *Roseburia Intestinalis* and *Faecalibacterium prausnitzii,* known producers of the SCFA butyrate [16]. Zhu and colleagues reported increased abundance of *Escherichia-Shigella* and *Enterococcus* and lower abundance of the butyrate producers *Faecalibacterium, Roseburia* and *Eubacterium rectale* [17]. The studies by Jie and Zhu are in line with a previous study on symptomatic carotid atherosclerosis by Karlsson et al. reporting decreased relative abundance of *Roseburia* and *Eubacterium*, known producers of butyrate [18]. Butyrate and other SCFAs are end products of fermentation of dietary fibers, and the main energy source for colonocytes maintaining the gut mucosal barrier [19]. A reduction in the overall genetic potential for butyrate production could also be observed in the metagenome data in the study by Jie et al. [16]. Furthermore, Karlsson et al. observed a strong negative correlation between genes encoding butyrate production (butyrate-acetoacetate CoA-transferase) and C-reactive protein (CRP) levels in the metagenomes of patients with symptomatic carotid atherosclerosis [18]. Other SCFAs could also be relevant, including acetate, which was shown to attenuate cardiac fibrosis and improve cardiac function in experimental mouse models [20].

3.2. Dysfunctional gut barrier, lipopolysaccharide (LPS) and inflammation

The gut microbial changes affecting butyrate may also influence inflammatory pathways, as butyrate exerts local anti-inflammatory effects in the intestinal mucosa by inducing colonic regulatory T cells [21]. Loss of butyrate producing bacteria may result in a dysfunctional gut mucosal barrier, facilitating passive leakage of microbial toxins such as LPS that binds to Toll-like receptors and other receptors of the innate immune system, thereby triggering inflammation [1,22−24]. Of interest, an increased potential for LPS biosynthesis in the microbiome has been reported among patients with CAD, [17] and previous studies have linked circulating levels of LPS to insulin resistance [22], glycemic control and abdominal obesity [23]. We recently reported that increased plasma levels of LPS-binding protein and soluble CD14 predicted cardiovascular events in a high-risk population [25].

Recent work has demonstrated different bioactivity of LPS, with hexa-acylated, but no penta-acylated LPS triggering inflammation [26,27]. Jie et al. reported that genes required for synthesis of the LPS O-antigen were enriched in CAD, whereas the lipid A module was depleted, most likely due to depletion of *Bacteroides*, which produce non-inflammatory penta-acylated lipid A [16].

3.3. The microbiota and the diet-dependent metabolite TMAO

The most compelling evidence of a link between the gut microbiome and CAD is related to microbial metabolism of dietary factors like carnitine and choline [28−30]. In a landmark paper from the Hazen group, the metabolite TMAO was identified as a strong predictor of CAD [28]. More than just a marker of the disease, TMAO is potentially a causative agent in atherosclerosis [29]. The source of TMAO is trimethylamine, which is produced by the gut microbiota from nutrients containing L-carnitine or phosphatidylcholine, and subsequently oxidized by hepatic flavin-containing monooxygenases to TMAO [28,29]. Precursors of TMAO promote foam cell formation and atherosclerosis in animal models, but not when adding antibiotics to the drinking water, suggesting a microbiota dependent mechanism [29]. In several independent cohorts from USA and Europe, plasma levels of TMAO predicted myocardial infarction, stroke and all-cause mortality [28−32].

In a recent study, TMAO levels increased in healthy individuals after dietary intake of red meat as compared with a non-meat or a white meat enriched diet [33]. The effect of diet on TMAO levels was reversible. Additionally, isotope techniques demonstrated that the increased TMAO production was from carnitine, not choline [33].

Although TMAO is the most studied microbiota-related metabolite in relation to cardiovascular risk, other metabolites along the TMAO pathway are of potential interest. Gammabutyrobetaine (γBB) is a partly microbiota-related metabolite on the pathway from L-carnitine to TMAO, which has also been linked to CAD [34]. Trimethyllysine (TML) is a partly endogenous, partly diet-derived precursor of both γBB and TMAO. It has been linked to increased CAD risk, alone, or in combination with TMAO [35,36]. In recent studies, circulating levels of γBB and TML, but not TMAO, predicted cardiovascular mortality in patients with carotid artery atherosclerosis [37]. Hence, there is a need to include also TMAO precursors in future studies to delineate which pathways are involved in atherosclerosis and CAD.

3.4. From the chronic atherosclerotic process to acute cardiovascular events

The atherosclerotic process starts with the fatty streak and culminates in plaque rupture and acute atherothrombosis, causing acute clinical events such as stroke or myocardial infarction [3,38]. Whereas inflammation is involved in all phases of atherosclerosis, most literature on the microbiome in CAD has not separated clearly between chronic CAD and acute events.

The published studies regarding the role of the microbiome in CAD all investigated patients with mostly stable CAD using cross-sectional designs [16−18]. Hence, prospective studies powered for

Table 2
Contemporary gut microbiota sequencing studies in patients with heart failure (HF).

Study	Luedde et al. [42]	Kamo et al. [43]	Cui et al. [44]	Kummen et al. [45] Mayerhofer et al. [66]
Patients	Chronic HF: 70% exacerbation, 30% stable	Acute HF or exacerbation of chronic HF	Stable chronic HF: Ischaemic or dilated cardiomyopathy	Stable systolic HF
Age patients	65 ± 3.2 years	Two strata: 47.4 ± 2.8 years 73.8 ± 2.8 years	58.1 ± 13.3 years	58.9 (39−74) years
Gender% (f/m)	45/55	18/82	17/83	59/41
Sample size	n = 20 HF n = 20 controls	n = 12 HF <60years n = 10 HF >60years n = 12 controls	n = 53 HF n = 41 controls	n = 84 HF (discovery- validation) n = 266 controls
Methods	16 s rRNA	16 s rRNA	16 s rRNA	16 s rRNA
Parallel plasma/serum	No	No	Yes	Yes
Dietary data	No	No	No	Yes
Increased relative abundance in patients	–	–	*Ruminococcus gnavus*	*Prevotella, Hungatella, Succinclasticum*
Decreased relative abundance in patients	Coriobacteriaceae, Erysipelotri-chaceae, Ruminococcaceae (family level) *Blautia* (genus level)	-*Eubacterium rectale, Dorea longicatena* -Depletion of *Faecalibacterium* in older patients	*Faecalibacterium prausnitzii*	-Lachnospiracea family: 9 differ-ent genera, including *Blautia* and *Eubacterium halli* - Ruminococcaceae: *Faecalibacterium* -Bifidobacteriaceae: *Bifidobacterium*
Functional findings	–	–	-Increased capacity for lipopoly-saccharide biosynthesis and TMA production and reduced capacity for butyrate production in HF microbiomes	-Lower genetic potential for butyrate production in HF microbiomes -*Eubacterium halli* associated with soluble CD25 and mortality -Dysbiosis related to dietary fiber intake

clinical events, as well as studies of the microbiome during acute coronary syndromes, should be a priority. Whereas direct analyses of the content of the microbiome could be difficult to perform in patients with acute disease, studies of microbiota-related metabolites could be more feasible in this setting. Interestingly, increased bacterial translocation has been reported in patients with acute myocardial infarction, with LPS and D-lactate blood levels being associated with adverse outcomes [39]. Furthermore, elevated levels of TMAO [32] and TML, alone or in combination with TMAO [36], have been shown to be associated with major adverse cardiac events 30 days after acute coronary syndrome, independent of troponin T levels. TMAO has been shown to interfere with platelet reactivity, which could be relevant for acute thromboembolic events [40]. Hence, although microbiota analyses are not yet ready for clinical use in the emergency room [41], microbiota-dependent biomarkers including LPS and TMA are potential therapeutic targets in patients with CAD.

4. Heart failure

4.1. Altered gut microbiome in HF with reduced capacity for butyrate production

The last two years, several sequencing-based studies have reported that the gut microbiota composition and functions differ between patients with HF and healthy subjects, with some common findings, but also considerable variation between studies (Table 2). Luedde and colleagues observed a reduced abundance of Ruminococcaceae on the family level and reduced abundance of *Blautia* from the Lachnospiracea family on the genus level in 20 patients with HF [42]. In a similar-sized study, Kamo et al. found a reduced relative abundance of *Eubacterium rectale* and *Dorea longicatena* from the Lachnospiracea family, and levels of *Faecalibacterium* from the Ruminococcaceae family were lower in older patients [43]. Furthermore, Cui et al. reported reduced levels of *Faecalibacterium prausnitzii* in patients with HF [44]. A common finding in these studies is the relative reduction in taxa from the Lachnospiracea or Ruminococcacea families, known for their capacity for butyrate production.

In order to define a more robust HF-related gut microbiota signature, we investigated two independent cross-sectional cohorts, finding that patients with HF had reduced biodiversity in the gut microbiome, as well as altered abundance of 15 core taxa. Most of the microbes that were depleted in HF belonged to the Lachnospiracea family, in addition to *Faecalibacterium* from the Ruminococcacea family [45], again pointing to reduced capacity for butyrate production as a key element, supported by a lower predicted genetic potential for butyrate production (genes encoding butyrate-acetoacetate CoA-transferase). Of relevance, the abundance of several members of the Lachnospiracea family correlated with soluble CD25, a marker of T cell activation, and depletion of the known butyrate producer *Eubacterium Halli* and increased plasma levels of soluble CD25 were associated with death or heart transplantation [45].

4.2. Gut mucosal biofilm, pathogens and leaky gut

The recent studies mentioned above are partly contrasting older data. In a study by Sandek et al. using fluorescence in situ hybridization, an enrichment of *Eubacterium rectale* and *Faecalibacterium* in gut mucosal biofilms were observed in patients with HF [46]. Pasini and colleagues, using traditional culture techniques, found an increased abundance of several pathogenic bacteria in HF, including *Campylobacter, Shigella, Salmonella, Yersinia Enterolytica* and *Candida* species [47]. The methodological differences are probably the key to explain these contradictory findings, although the different sampling site is a relevant factor in the former study, as mucosa-adherent microbes might differ from luminal fecal samples [48].

Of interest, the patients in the studies by Sandek and Pasini had increased gut permeability as measured by the lactulose-mannitol test and the cellobiose sugar test [47,49] which fits well with reduced capacity for production of butyrate, the main energy source for colonocytes and critical for maintaining the gut barrier. Finally, the downregulation of a healthy core microbiome in HF patients could facilitate outgrowth of pathogenic microbes as reported by Pasini et al. [47].

4.3. TMAO: of prognostic value in HF?

Inspired by the role of TMAO in CAD, several independent studies have investigated the role of TMAO in chronic HF. It turns out that TMAO is a strong predictor of clinical outcomes in patients with HF, regardless of the underlying etiology [50]. In a study from our hospital, TMAO was elevated in patients with ischemic HF but not with dilated cardiomyopathy, and TMAO levels were associated with increased pulmonary artery pressure and wedge pressure, which are indices of left atrial stress [51]. Hence, the TMAO pathway could be related to decompensated HF and congestion, as TMAO levels were associated with prognosis in patients with acute decompensated HF [52]. An experimental study also supports this concept, since feeding animals with TMAO or its dietary precursors aggravated hemodynamic parameters [53].

Heart transplantation could represent a human "model" to provide additional information on the potential impact of the gut microbiota in HF. Recently, we found increased plasma levels of TMAO and TMAO-precursors in *de novo* heart transplant recipients, whereas the partly microbiota-dependent metabolite γ-BB increased steadily after transplant. This metabolite was associated with acute rejection and cardiac allograft vasculopathy [54]. Although alloimmunity, treatment with immunosuppressive drugs and other factors could be relevant in these disease processes, there is a major knowledge gap related to the role of the gut microbiota in the post-transplant setting [55].

4.4. Primary and secondary bile acids

We recently analyzed the circulating bile acid pool in patients with HF and healthy controls and found an increased ratio of secondary to primary bile acids in HF, which was associated with reduced overall survival in unadjusted, but not adjusted analyses [56]. Whereas bile acids are traditionally regarded as emulsifiers to facilitate the absorption of dietary fat and fat-soluble vitamins, bile acids are now recognized as signaling molecules that interact with plasma membranes as well as nuclear receptors, exerting regulatory effects on glucose and lipid metabolism [57], energy homeostasis [58] and other physiological processes [59]. In fact, several bile acid receptors are expressed in cardiomyocytes, and it has been proposed that bile acids influence cardiovascular function [60]. In the gut, primary bile acids undergo metabolism to *secondary* bile acids [61] before reabsorption as a part of the enterohepatic cycle. These microbial bile acid modifications have a major impact on the agonist activity on the bile acid receptors such as the farnesoid X receptor, which has several pleiotropic effects, and could represent a link between the gut microbiome and cardiovascular health [61]. Interestingly, a pilot study targeting the bile acid pool by ursodeoxycholic acid, reported improved peripheral blood flow as well as improved markers of liver function in patients with chronic HF [62].

4.5. Uremic toxins

Although reviewed only briefly here, the role of microbiota-derived uremic toxins could be of particular relevance for targeting cardiovascular risk in patients with chronic kidney disease (CKD) [63], including patients with HF as part of the cardiorenal syndrome.

In CKD, the loss of urinary excretion results in retention of various substances known as uremic retention solutes, many of which have toxic properties, and certain uremic toxins are synthesized by gut microbes [63,64]. Indoles are bacterial metabolites of tryptophan, a semi-essential amino acid found in various food sources such as red meat, egg and fish. Indoles are metabolized into indole derivatives, such as indoxyl sulfate (IS) and indole-3-acetic acid (IAA), which act as endogenous ligands of transcription factors interacting with various regulatory and signaling pathways, thereby mediating cardiotoxicity and vascular inflammation [63].

Another microbiota-generated uremic toxin is P-Cresyl Sulfate (PCS), which is derived from bacterial metabolism of aromatic amino acids that are subsequently sulfonated into PCS in the liver. In several studies, elevated levels of IS, IAA and PCS have been associated with increased mortality and increased risk of cardiovascular events [63]. TMAO is dependent on renal elimination, resulting in elevated plasma levels in CKD. The TMAO pathway has been implicated in the development of renal insufficiency and increased mortality in patients with CKD [31].

5. Is dysbiosis of the gut microbiota linked to plasma metabolites?

Circulating microbial metabolites are potential disease-modifying mediators of bacterial functions, provided that the disease-associated dysbiosis correlates with metabolite concentrations. The majority of microbiota analyses in CAD and HF (Tables 1 and 2) have not been accompanied by parallel plasma samples. Even though TMAO is elevated in CAD, Karlsson et al. found no upregulation of the metabolic pathway from phosphatidylcholine to TMA in the gut microbiomes of patients with CAD [18]. In contrast, the larger study by Jie et al. reported enrichment of gut microbial enzymes involved in trimethylamine formation in microbiomes from patients with CAD [16], although none of these studies reported circulating TMAO levels. In HF, Cui et al. found increased genetic potential for TMA production in the gut microbiome, but no association with circulating TMAO [44]. In a study comprising 22 patients with HF and 11 matched controls, elevated TMAO levels in HF correlated with the abundance of Escherichia and Shigella, although the abundance of these genera did not differ between HF patients and controls [65]. In an animal study of TMAO formation and platelet reactivity, taxa of the Lachnospiracea family were negatively associated with circulating TMAO levels [40]. In contrast, in our own HF cohort, in which several members of the Lachnospiracea family were depleted, we found no association between the dysbiosis in HF and circulating TMAO. TMAO generation is determined by a complex interplay between dietary factors, microbiota-dependent activity and hepatic oxidation, and expecting the gut microbiota alterations observed in CAD and HF to correlate directly with circulating TMAO may be too simplistic.

We recently measured circulating butyrate in plasma from patients with HF, but found no association with gut dysbiosis, possibly due to low circulating levels of butyrate [66]. Butyrate and other SCFAs can be measured in fecal samples, providing a more direct measure of microbial activity, but this requires snap frozen samples without preservatives.

6. Search for novel microbiota-related pathways in CAD and HF

With the combined genes of the microbiome outnumbering the human genome by two orders of magnitude, and each microbe having the potential to turn on and off the production of hundreds of metabolites, several undiscovered microbiota-related metabolites are likely to be relevant in human disease. The seminal TMAO-report by Tang et al. reported on several metabolites predictive of CAD, the nature of which are presumably still unknown [29].

There is a need to apply more extensively full metagenomic sequencing to better define changes in the functional genetic alterations in the gut microbiome in patients with HF. Such methods are expensive and resource demanding, but provide species level resolution, as well as the *functional potential* of the microbes and the host in the gut compartment, as assessed in coronary heart disease in the studies by Karlsson et al. and Jie et al. [16,18]. Ultimately, combined analyses of the actual byproducts of microbial activity (unbiased metabolomics and/or proteomics analyses of parallel plasma samples) and metagenomics analyses must be performed in multi-level bioinformatics controlling for relevant confounders, in order to

identify functional alterations influencing the clinical phenotype of interest.

For translation to a clinical setting, biomarkers that are easily measurable in a reproducible way in plasma or urine will probably be of more value. Of relevance, Feng et al. measured metabolites in parallel plasma and urine samples in CAD patients and controls. The results were integrated with metagenomic analyses, identifying several metabolites, including GlcNAc-6-P, mannitol and 15 plasma cholines, as novel candidate biomarkers potentially derived from the gut microbes [67]. In order to make such a biomarker useful, it should preferably provide prognostic information beyond that of established biomarkers or point to novel therapeutic principles. Furthermore, whereas the above-mentioned studied are based upon known proteins and metabolites, a recent study identified thousands of uncharacterized microbiota-generated small molecules, probably small proteins coded from open reading frames [68]. This approach could open up other avenues in microbiota-related studies.

7. Controlling for diet, drugs and comorbidities

Host genetic factors have a small but significant impact on the composition of the gut microbiota [69,70]. However, environmental factors probably play a greater role [71]. One limitation of the studies published so far is the lack of dietary data. In an attempt to address this, we gathered food frequency questionnaires from patients with HF, finding that several characteristics of the dysbiosis observed in HF, including the low diversity and the reduction in butyrate-producing microbes, correlated with a low dietary fiber intake [66]. Importantly, diet has a major impact on the gut microbiota and related metabolites (Fig. 1), and dietary data should preferably be registered among other relevant co-variates in microbiota studies.

Other important confounders include concurrent medication and comorbidities. Recent large-scale studies have identified several commonly used drugs and their metabolism to be associated with microbiota alterations [72]. An interesting in vitro study examined nearly 1000 different drugs, finding that an estimated 24% of the drugs, most of which were not antibiotics, had the ability to suppress the growth of at least one commensal microbe in cultures [73]. Some drugs are even believed to mediate part of their therapeutic effects trough their influence on the gut microbiome, as shown for metformin [74]

The studies summarized in Tables 1 and 2 all have limitations, regarding sample size, methodology, lack of parallel fecal and plasma samples or relevant covariates. All these factors should be considered when planning future microbiota-related studies.

8. Targeting the gut microbiome

Almost 20 years after Ross defined atherosclerosis as an inflammatory disease [38], Paul Ridker published the CANTOS trial [75], showing that inhibition of IL-1β by the monoclonal antibody canakinumab, can reduce cardiovascular events. Clinical translation will hopefully take shorter time in microbiota medicine, but there are several obstacles, including safety issues and substantial inter-individual variation in gut microbiota composition and function.

8.1. Probiotics and prebiotics

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [76]. Probiotics in clinical use comprise bacterial and fungal microorganisms, including the genera Lactobacillus and Bifidobacterium and the fungus Saccharomyces boulardii [77]. Results from animal models suggest that certain strains of Lactobacilli could have cardioprotective effects. Rats treated with a supplement containing Lactobacillus plantarum 299v before coronary artery ligation had reduced infarct size and improved

left ventricular function [78]. Another study showed similar cardioprotective results in a rat model of myocardial ischemia after supplement with Lactobacillus rhamnosus GR-1 [79].

In humans, a pilot study reported not only reduced systemic inflammation, but also improved left ventricular ejection fraction after an intervention with the probiotic yeast Saccharomyces Boulardii in patients with chronic HF [80]. The number of participants was low (n = 20), and the results should be interpreted with caution. We are currently performing a randomized controlled trial (RCT) including 150 patients with systolic HF, powered to detect an increase in left ventricular ejection fraction of 5% [81]. Results are expected during 2020. Given the potential clinical impact of microbiota modulation, as well as the high morbidity and mortality in HF, microbiota modulation is not without risk [82], and close clinical monitoring and predefined safety measures should follow the same standards as in other clinical trials [83]. Notably, genomic and epidemiological evidence of bacterial transmission from probiotic capsules to blood was recently reported in patients in intensive care units [84].

Prebiotics are substrates that are selectively utilized by host microorganisms and confer a potential health benefit, e.g. non-digestible dietary fibers and oligosaccharides [85]. Most contemporary deep sequencing studies of patients with cardiovascular disease report depletion of microbes with the capacity of producing SCFAs such as butyrate (Tables 1 and 2). Prebiotics promoting microbial fermentation of dietary fibers to SCFAs may therefore be of potential benefit in the gut, as well as in splanchnic and peripheral tissues, which in total may result in improved metabolic regulation [86]. Some prebiotics, such as inulin, have the potential to counteract harmful effects of antibiotics by promoting the diversity and functional capacity of the gut microbiota [87]. A recent RCT showed that dietary supplementation with inulin or inulin-proprionate ester improved insulin sensitivity and reduced markers of systemic inflammation by raising colonic delivery of the SCFA proprionate [88]. Hence, targeting microbial SCFAs production by supplement of inulin or other prebiotics are attractive strategies for future trials in cardiovascular disease, although current scientific evidence does not support the use of probiotics or prebiotics as supplemental therapy in patients with HF or CAD.

8.2. Antibiotics for gut decontamination

Early studies targeting the gut in patients with HF have focused on gut decontamination with broad-spectrum antibiotics to reduce bacterial translocation and inflammation. Although this approach has been successful in reducing markers of systemic inflammation, a clinical effect has not been demonstrated [89,90].

In a recent study, it was shown that a broad-spectrum cocktail of oral antibiotics markedly increased post- infarction rupture and death in a murine model of ligation of the left anterior descending artery [91], suggesting that an intact microbial community is required around the time of myocardial injury for proper myocardial repair [92]. This study contrasts with previous animal models, reporting that oral vancomycin decreased infarct size and improved post-infarct cardiac function in rats [78], as well as a subsequent study, reporting that a mixture of streptomycin, neomycin, polymyxin B and bacitracin reduced infarct size along with alterations in microbiota-related metabolites [93]. Furthermore, antibiotics reduced bacterial translocation, inflammation and myocardial injury in an experimental mouse model [39]. Taken together, these diverging animal studies strongly suggest a role of the gut microbiota composition in acute myocardial infarction, but the direction of microbiota alterations and the potential metabolic or inflammatory pathways are poorly understood.

Targeting cardiovascular disease with antibiotic therapy is not a new idea. Between 1995 and 2005 > 19 000 patients were included in RCTs aimed to target chlamydia pneumonia in patients with CAD

Table 3
Strengths, limitations and future possibilities of potential microbiota-related biomarkers in cardiovascular disease.

Biomarker	Relevance and main findings	Limitations	Future directions
TMAO	-Predicts clinical end points in numerous studies on HF, stable CAD and acute CAD	-Circulating TMAO weakly linked to disease-specific dysbiosis.	-Potential therapeutic target in dietary interventions and pharmacological products interfering with TMA production
	-Reproducible measurements with mass spectrometry	-TMAO levels influenced by diet, renal and liver function	-Microbiota-derived precursors such as TML should be studied further
Butyrate	-Low microbial butyrate producing potential linked to dysbiosis in several cohorts of HF and CAD.	-Low circulating levels, not suitable as soluble biomarker	-Potential therapeutic target in high fiber dietary interventions
		-Measurable in snap frozen fecal samples without preservatives, but rapidly degraded.	
		-Confounded by fiber intake	
Gut leakage markers	-Increase in LPS-producing microbes linked to dysbiosis in several cohorts of HF and CAD.	-Direct measurement of gut permeability is so far not feasible in the clinic	-Need of better standardization of LPS measurements
	-Increased plasma LPS in HF	-Large variability in LPS LAL-assay.	-Other markers of bacterial translocation such as LBP, I-FABP, zonulin, as well as functional measurements of gut leakage should be further studied
	-Increased gut permeability measured by lactulose-mannitol test and cellobiose sugar test in HF	-LAL assay does not separate between hexa- and penta-acylated LPS variants.	
Bile acids	-Increased conversion from primary to secondary bile acids in HF	-Large variability and technically difficult to measure	-Circulating bile acid pool should be investigated in relation to disease-specific dysbiosis
			-Pleiotropic effects of bile acid receptor FXR should be further studied in CVD
Uremic toxins	-Microbiota-generated toxins such as PCS and IS accumulate as a result of reduced urinary excretion and predict clinical end points in CKD patients	-Mostly relevant for CKD populations	-Interventions targeting uremic toxins, such as oral absorbants and synbiotics, should inspire research also in non-CKD populations

CAD: coronaray artery disease; HF: heart failure; CKD: chronic kidney disease; LPS: lipopolysaccharide; LBP: LPS-binding protein; LAL-assay: limulus amebocyte lysate assay; I-FABP: intestinal fatty acid binding protein; FXR: farnesoid X receptor; PCS: P-Cresyl Sulfate; IS: indoxyl sulfate TMA: Trimethylamine; TMAO: Trimethylamine-N-Oxide; TML: Trimethyllysine.

[94]. Positive experimental studies and small RCTs were followed by large trials with adequate sample size, which demonstrated no clinical benefit of antibiotic therapy.

In addition to the obvious risk of antimicrobial resistance, other safety concerns of antibiotics have recently emerged with potential relevance for future trials. Recently, 10 years of follow-up data from the CLARICOR trial demonstrated increased cardiovascular death in clarithromycin-treated patients with stable CAD, [95] leading to an FDA alert in 2018 on the use of clarithromycin in patients with CAD (https://www.fda.gov/Drugs/DrugSafety/ucm597289.htm). In December 2018, another FDA alert on the use of fluoroquinolones warned about increased risk of aortic ruptures and aortic dissection in patients at increased risk, such as elderly patients with hypertension or peripheral atherosclerotic vascular disease (https://www.fda.gov/Drugs/DrugSafety/ucm628753.htm). Finally, a recent study reported increased risk of cardiovascular events in elderly women with increased cumulative exposure to antibiotics in adulthood [96]. The explanation for this increased risk in unknown, but could involve prolongation of the QT-interval and Torsade de Pointes arrhythmia, pro-inflammatory activities mediated by translocation of commensal gut microbes [97], or other effects mediated by the gut microbiota. Given these safety concerns and the lack of clinical effect in numerous trials, antibiotics should be used with caution in future studies targeting the gut microbiota in cardiovascular disease.

8.3. Fecal microbiota transplant (FMT)

FMT is the most radical current intervention for targeting the gut microbiome and an established treatment for recurrent *Clostridioides difficile* infection. FMT from lean donors was previously shown to normalize insulin sensitivity in obese subjects with the metabolic syndrome [98,99], although the effect was only temporary. In a subsequent study on 20 subjects with the metabolic syndrome, FMT from vegan donors changed the gut microbiota composition toward a vegan profile in some patients; however, without altering TMAO production capacity or parameters related to vascular inflammation [100]. FMT is now tested in several clinical settings, but with the current mode of endoscopic delivery, its use in acute and high-risk settings such as acute coronary syndrome and decompensated HF is limited. FMT is not without risk and was recently shown to transmit drug-resistant *E. Coli* resulting in bacteremia in two patients, one of whom died [101]. It is critical to standardize and optimize procedures for FMT, including screening for suitable donors, development of non-invasive delivery modes such as capsules, as well as determination of the active components, in order to develop personalized treatment strategies [102].

8.4. Precision medicine targeting enzymatic pathways

Whereas probiotics, antibiotics and FMT are all potential candidate interventions in proof of principle studies, next generation probiotics need to be more goal-directed, targeting specific enzymatic pathways. Interestingly, inhibitors of TMA production that target distinct microbial TMA lyases have been developed. These drugs reduce TMAO levels and reverse atherosclerosis in animal models [103]. TMA lyase has become a current potential therapeutic target of TMAO modulation. An appealing side of this "drug the bug" approach is that microbial pathways may be targeted, apparently without having bactericidal effects.

For now, dietary interventions, including adherence to a Mediterranean diet [104] or discontinuation of dietary red meat [33], are more accessible ways of reducing TMAO levels and possibly, cardiovascular risk. In light of our recent findings of dietary fiber intake being negatively associated with dysbiosis in HF, dietary intervention with a high-fiber diet, alone or in combination with a Mediterranean diet, could be a logical next step for targeting the gut heart axis.

8.5. Personalized approach

One aspect with particular relevance to microbiota research is the tremendous inter-individual variation in the gut microbiota composition [105]. In an elegant study, Zeevi et al. all showed that integration of microbiota profiles and metadata in a machine learning model made it possible to precisely predict individual glycemic responses in order to personalize nutritional advice [106]. These findings have been independently validated [107]. Hence, although certain

microbiota-metabolite traits, such as increased TMAO or reduced butyrate production, may be identified in groups of patients, different microbiota-related pathways may be relevant in different individuals. In addition to the requirement of demonstrated effect in RCTs, a personalized approach is probably necessary if gut microbiota interventions should be of clinical significance.

9. Conclusion

Recent studies of the gut microbiome have identified some common traits in CAD and HF, in particular a decreased abundance of gut microbes with capacity for producing butyrate and elevated circulating levels of TMAO. However, a link between gut microbiota alterations and circulating metabolites in cardiovascular disease is yet to be defined, and most published studies lack information about essential covariates like drugs, diet and comorbidities and are too small to capture the substantial variation in the gut microbiome. Given the complexity and magnitude of the gut microbiota and its metabolites, as well as the potential benefits and risks of targeting the gut microbiota in high-risk clinical settings, a multidisciplinary approach is necessary. Close collaboration between dedicated clinicians and microbiota-focused groups with capacity for metagenomics and multi-level bioinformatics will be necessary to demonstrate a clinically relevant gut-heart axis. (Table 3).

10. Outstanding questions

Adequately powered studies; including well-designed randomized trials with parallel microbiota and plasma samples, controlled for essential covariates like diet; are needed to move the field from associative studies to possible causation.

11. Search and selection criteria

This review is based on a systematic search in PubMed using the term (microbiota OR microbiome) AND (heart failure OR coronary artery disease OR atherosclerosis OR cardiovascular) as of December 10th 2019. We limited our search to articles on adult patients, written in English and published over the last three years.

Declaration of Competing Interest

Dr. Trøseid, Dr Broch and Dr Andersen declare no conflict of interest. Dr. Hov has received funding from Biogen, personal fees from Novartis, and personal fees from Orkla Health.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2020.102649.

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