How strong is the evidence that gut microbiota composition can be influenced by lifestyle interventions in a cardio-protective way?

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ABSTRACT

Alterations in composition and function of the gut microbiota have been demonstrated in diseases involving the cardiovascular system, particularly coronary heart disease and atherosclerosis. The data are still limited but the typical altered genera include *Roseburia* and *Faecalibacterium*. Plausible mechanisms by which microbiota may mediate cardio-protective effects have been postulated, including the production of metabolites like TMA as well as immunomodulatory functions. This raises the question of whether it is possible to modify the gut microbiota by lifestyle interventions and thereby improve cardiovascular health. Nevertheless, lifestyle intervention studies that have involved modification of dietary intake and/or physical activity, as well as investigating changes in the gut microbiota and subsequent modifications of the cardioprotective markers are still scarce, and furthermore the results have been inconclusive. Current evidence points to benefits of consuming high-fibre foods, nuts and an overall healthy dietary pattern in order to achieve beneficial effects on both gut microbiota and serum cardiovascular markers, primarily lipids. The relationship between physical exercise and gut microbiota is probably complex and may be dependent on the intensity of exercise. In this article we review the available evidence on lifestyle, specifically diet, physical activity and smoking as modifiers of the gut microbiota, and subsequently as modifiers of serum cardiovascular health markers, we have attempted to elucidate the plausible mechanisms and further critically appraise the caveats and gaps in the research.

INTRODUCTION (Figure 1)

The WHO estimates that cardiovascular diseases (CVD) were responsible for 17.9 million deaths worldwide in 2016 [1]. During the last decades, the incidence of these diseases has increased and they have become the principal cause of death all around the world. Ischemic heart disease, responsible for almost 13% of all global deaths is the leading culprit. This is especially true in high- and intermediate-income countries although there is extensive variability between countries. The higher mortality observed in certain countries may reflect a higher exposure to risk factors and/or inadequate preventive policies. However, the complex etiologies of these diseases and the incomplete understanding of the underlying mechanisms have hampered the development of prevention strategies.

Diet has long been recognized as a major critical factor for cardiovascular health. However, it was not until recently that the complex interactions between dietary components and the intestinal microbiota, and from their food produced metabolites have been acknowledged to play a role in cardiovascular health. This increasing awareness of the role of the microbiota has attracted the attention of researchers interested in the potential role of the intestinal microbiota as a study target e.g. in the prevention of atherosclerosis and other forms of CVD [2-5].

Moreover, recent evidence has underlined the potential role of metabolomics as a tool to analyze the effects of the diet in modifying the concentrations of substances with cardiometabolic implications. A strong link between diet and cardiovascular health has been established through the determinants of metabolic stress and overweight, i.e. adiposity and the presence of visceral fat representing one of the main risks for cardiovascular events. Genetic variation has an important influence on

BMI and the distribution of body fat; this linkage has been revealed not only in genome wide association studies (GWAS) but also in studies on twins and adopted children. However, the single nucleotide polymorphisms (SNPs) and genes related with monogenic obesity can only partially explain the phenotypical variation. Environmental factors are believed to make a major but still-to-be elucidated contribution to the variation in obesity between different individuals. For example, the intestinal microbiota seems to play a critical role in mediating the diet-health relationship in the context of cardiovascular health [6].

The intestinal microbiota has been the topic of several recent large-scale projects (i.e. Metagenomics of the human intestinal tract [MetaHIT] or the International Human Microbiome Consorptium [HMC] projects). At the high taxonomic level, it is dominated by only two phyla, Bacteroidetes and Firmicutes, although a wide individual variability is often reported on the composition of the intestinal microbiota. [7-10] Different authors have proposed the existence of enterotypes [8] and although the number of such enterotypes is still being debated, it is clear that the long-term diet is the main determinant for the microbiota type [11]. Today we know that gut microbiota is affected not only by different factors such as diet, exposure to drugs and antibiotics, or the age of the individual, but it has also been shown that the composition of the microbiota is affected by different diseases. According to the Disbiome database [12, 13] , studies on the microbiota-disease relationship have been carried out in over 300 diseases, with gastrointestinal diseases being the most frequently studied followed by those related with the metabolic syndrome. In this regard, CVDs are not an exception, e.g. there are some published studies relating these diseases with an altered intestinal microbiota structure and function (Table 1).

Most of the available evidence on the association of the intestinal microbiota with CVD originates from Asia. Interestingly, in spite of geographical and study design differences, there are some results, such as reduced levels of genera like *Roseburia* and *Faecalibacterium*, that have been repeatedly reported in the different studies (Table 1). Although the knowledge on the changes in the composition of the microbiota associated with coronary heart disease or atherosclerosis is still limited there is a growing body of evidence supporting this relationship [14].

There seem to be multifactorial mechanisms by which the microbiota can influence cardiovascular health [15]. The immunomodulatory functions of the microbiota have been extensively studied. Similarly, the influence of the intestinal microbiota on the endocrine system and the metabolic control of the host have also been investigated. Moreover, mechanistic information is also becoming available, making it possible to elucidate the role of the diet-microbiota interaction in these diseases. It is now evident that the gut microbiota is able to produce diet-derived metabolic products capable of influencing the host's cardiovascular status. For example, circulating levels of branched-chain amino acid metabolites, tryptophan or histidine, have been associated with insulin resistance and vascular disease [16]. In recent years, research has focused attention on certain molecules potentially mediating the microbiota-cardiovascular health interaction [17]. One of these compounds, trimethylamine (TMA), synthesized by intestinal bacteria from dietary components such as L-carnitine, lecithin, choline and betaine has attracted special attention. TMA is oxidized in the liver to trimethylamine-N-oxide (TMAO) which has been associated with CVD in several studies [18].

In this paper we will review firstly, the available evidence of lifestyle, specifically diet, physical activity and smoking as modifiers of gut microbiota and subsequently as modifiers of cardiovascular health. We consider primarily the established metabolic markers, i.e. serum markers of lipid and glucose metabolism, but also less established surrogate markers, namely inflammatory markers and metabolomics profiles. Secondly, we will strive to identify plausible mechanisms in the diet-gut microbiota-cardiovascular pathway and thirdly critically appraise the caveats and gaps in the research.

IMPACTS OF LIFESTYLE INTERVENTIONS ON MICROBIOTA AND SERUM MARKERS OF CARDIOVASCULAR DISEASE

Dietary interventions (Table 2)

In addition to the direct effects of diet on cardiovascular health, it is plausible that diet can exert impacts through the gut microbiota. This had become evident in a range of observational and intervention studies demonstrating that various nutrients and other dietary compounds can modify either the composition or function of the gut microbiota including the production of microbial metabolites. Subsequently, the gut microbiota has been linked with cardiovascular health and credible mechanisms have been presented. The best-known properties of diet in modulation of the gut microbiota relate to dietary fibre [19], but more and more evidence is emerging for other compounds including fats [20].

Table 2 presents intervention studies which have examined in the same trial, the impacts of dietary modification on microbiota and metabolic markers considered as being potentially cardioprotective, i.e. lipid and glucose metabolism as well as surrogate markers, namely inflammatory markers. The most widely used approach has been to increase the fibre content of the diet by providing high-fibre products for consumption e.g. whole grain and bran of different crops, beta-glucan, polydextrose or arabinoglycan, and also ingredients with prebiotic properties including fructo and galacto oligosaccharides, inulin and β2-1-fructans. These approaches have resulted in variable results. Some trial have reported beneficial changes in gut microbiota, mainly an increase in bifidobacteria and lactobacilli and diversity, and subsequently in improved lipid and glucose metabolism [21-25]. Nevertheless, in other trials, even though gut microbiota changes have been induced by dietary modification, no changes in serum markers of lipid or glucose metabolism have been evident [26-34]. Similarly, even though the dietary intervention did not have any apparent effect on the microbiota, changes in serum markers have been observed by other authors [35, 36]. There are also studies with no impact on either microbiota or serum markers exist [32, 37, 38].

Similarly, studies investigating the impacts of single foods, e.g. those rich in antioxidant vitamins or polyphenols like berries, apples, orange, kiwi fruits, herbs or red wine have reported variable results with regard to the impact on either gut microbiota or serum markers of lipid and glucose metabolism [39-44]. An interesting approach was taken in studies in which several dietary components were combined with the intent of improving cardiovascular health. A diet high in fibre, polyphenols

and vegetable proteins induced an increase in 1) gut microbiota diversity and 2) specific bacteria and 3) in the lipid profile in type 2 diabetic patients [45].

There may be several reasons for the inconsistent results emerging from dietary intervention studies on gut microbiota and subsequently on serum lipid and glucose metabolism e.g. related to study design and analytical choices as well as compliance with the planned intervention. Surprisingly, many of the intervention studies have been performed with relatively small numbers of participants, typically ranging from 10 to 50 participants per study, although some studies have applied a crossover design that lowers the number of participants needed in the trial (Table 2). An alternative reason for the variable results may be that other mechanisms than those through gut microbiota mediate the impact on cardiovascular health. For example, considering dietary fibre, these mechanisms include gel formation from the soluble fibres in the small intestine and subsequent attenuation of the postprandial glucose concentration and altered lipid metabolism [46]. Further, the individual variation in responses to diet, as well as in the baseline composition of the microbiota may impact on the outcomes of the intervention. This was demonstrated in the study where higher abundances of *Bifidobacterium* spp. and *Akkermansia municiphila* were detected in those individuals who displayed lowered cholesterol levels (i.e. cholesterol responsive individuals) as a response to the dietary beta-glucan intervention [23]. In the study of Dao and coworkers [47], a higher abundance of *A. municiphila* at baseline was associated with greater improvements in glucose and lipid metabolism after an energy restricted diet. In a study examining 893 subjects, it was estimated that the gut microbiota explained only 6% of the variance in triglycerides and 4% in HDL, independent of age, sex and genetic risk factors [48].

Correlations between gut microbial composition, particularly genera such as *Blautia* and *Lachnosphira*, with serum lipidomics have also been reported [49]. It is noteworthy that weight loss per se induces changes in both microbiota and metabolic markers [47, 50], thus complicating the interpretation of the potential causal relations of dietary changes. Studies investigating microbial function might prove enlightening. Indeed, Haro and co-workers [51] demonstrated the impacts of their dietary intervention not only on the composition of the microbiota but also on their functions by applying faecal metabolomics analytics. A more individualized approach was utilized in a study where personalized dietary interventions were executed based on a machine-learning algorithm that integrated lifestyle measures like dietary habits as well as incorporating knowledge on blood parameters and gut microbiota [52]. This personalized approach subsequently resulted in lower postprandial glucose responses and also alterations in gut microbiota.

Probably the best evidence with regard to dietary intake in the primary prevention of cardiovascular disease has originated from the PREDIMED study in which the risk of major cardiovascular events was lower in the intervention groups assigned to a Mediterranean diet with either nuts or extra-virgin olive oil compared to a reduced-fat diet [53], but unfortunately faecal samples were not collected in that trial [54]. The role of nuts on gut microbiota has been studied elsewhere; in a cross-over study involving 18 healthy women, daily consumption of 42 g of walnuts was shown to modify the gut microbiota composition and to lower LDL cholesterol levels [55]. In another cross-over study involving 45 men and women at risk for CVD, walnuts (57-99 g/d), compared to a diet with the same amount of fatty acids but from different sources other than walnuts differentially influenced the gut microbiota. [56, 57]. Thus,

it may be that the benefits of nut consumption are partially mediated through the gut microbiota. The same concept applies to modifying dietary fat. In a 1-year-intervention study with a Mediterranean diet in obese men, the authors reported changes in the microbiota composition, but not in serum variables [51]. A good adherence to a Mediterranean diet or a healthy diet, as defined in dietary recommendations, in general has been associated with a beneficial composition of the gut microbiota [20, 58, 59].

It is well-known that blood LDL cholesterol levels may be lowered by plant derived phytosterols which interfere with the intestinal absorption of cholesterol [60], but this impact is not related to the gut microbiota [61, 62], although a recent study indicated that these microorganisms may use plant sterols as a substrate, i.e. these phytosterol can influence the metabolic activity of the gut microbiota [63]. Probiotics are one group of dietary ingredients or food supplements that are widely used to modify the gut microbiota. In several recent meta-analyses including subjects with mild to moderate hypercholesterolemia, these agents have been demonstrated to improve lipid metabolism, particularly to lower the total and LDL-cholesterol [64-67]. Another group of nutraceuticals that has been of interest with regard to cardiovascular health is fish oil. The effects of fish oil on gut microbiota are not clearly elucidated and human studies are scarce, but some studies indicate that fish oil or other supplements containing long-chain polyunsaturated n-3 fatty acids, docosahexaenoic acid (DHA) and/or eicosapentaenoic acid (EPA), induce changes in gut microbiota. These include increase in diversity or short chain fatty acid (SCFA) producing bacteria or modification of particular bacteria or bacterial groups [68, 69], although also null results have been reported [70].

Exercise interventions (Table 3)

The beneficial effects of physical activity and exercise on glucose metabolism, lipids and cardiovascular events have been demonstrated in numerous studies [71].These effects may be at least partially mediated by alterations in the gut microbiota and their metabolites. Exercise has been associated with an increased diversity of gut microbiota not only in experimental animals but also in cross-sectional human studies [72]. However, the relationship between exercise and the microbiota is complex; it is dependent on the intensity of exercise, i.e. both beneficial and harmful effects have been observed. Exercise exerts metabolic effects via increased energy expenditure, but has also an effect on food intake, food choices and furthermore exercise usually decreases intestinal transit time [71]. In cases of high intensity exercise, ischemic effects on intestinal mucosa and increased permeability for bacteria and toxins can lead to an inflammatory response which is not present after regular exercise [73].

We evaluated the exercise intervention studies in humans on gut microbiota and metabolites and these are depicted in Table 3. We found that the trials were heterogeneous in terms of both design and study population. For example, the study populations have been healthy adults (sedentary, active, obese or non-obese, elderly), healthy athletes, but also individuals with an underlying illness (inflammatory bowel disease, diabetes) have been investigated. Several, but not all, studies have detected influences on microbiota but only a few have assayed plasma and fecal metabolites. Three studies in athletes focused on higher intensity training e.g. one involving trans-Atlantic rowing; these interventions increased the diversity of

microbiota [74-76]. Two other studies investigated the effects of (half)marathon running; directly after running a half marathon there was no increased diversity whereas in the days after the marathon, the microbiota changed with *Veillonella* being the most abundant [77, 78]. The duration of the exercise intervention in healthy adults was between 5 and 12 weeks. Some of the studies also examined diversity; they did detect some effect on diversity, although in some cases it was dependent on the presence of obesity, or alternatively there were increases or decreases in specific microbiota [79-82]. However, in one study examining elderly men, the exercise program did not change the taxonomic composition or metabolic pathways as compared to baseline [83]. Finally, among the patients with inflammatory bowel disease, no effect was seen on diversity after 8 weeks of resistance training [84], whereas in the patients with diabetes a reduction of mycetes was seen after a mixed exercise intervention lasting 6 months [85]. Taken together, these studies only suggest, but do not prove, that the beneficial effects of physical exercise are mediated by changes in gut microbiota.

Smoking

Smoking is one of the main risk factors of atherosclerosis acting via several mechanisms. Not only nicotine, but also the inhalation of aromatic hydrocarbons, oxidizing agents including oxygen radicals and particulate matter potentially exert a direct effect on atherogenesis. Smoking also contributes to the progression of atherosclerosis by inducing catecholamine release, increasing both blood pressure and the tendency for platelet aggregation [86].

Cross-sectional human studies have revealed that smoking does influence the gut microbiota composition and diversity. In a population based study from Korea, the gut microbiota of current smokers was found to contain more Bacteroidetes and less Firmicutes and Proteobacteria as compared to never smokers [87]. Another epidemiological study from the Netherlands examining 1135 persons investigated the association of current smoking status, smoking history, parental smoking, and maternal smoking during pregnancy with gut microbiota [88]. Although these parameters were associated, they showed only a modest effect and the study did not detect significant associations with individual species or pathways. Some mechanisms have been suggested to explain how smoking influences gut microbiota such as alterations of intestinal tight junctions, mucin composition, oxidative stress and biofilm formation [89]. Furthermore, indirect effects of smoking via alterations of the oral microbiota are possible since smoking has a strong influence on the microbes in the mouth and is a major risk factor for periodontitis. Periodontitis itself has been repeatedly associated with atherosclerotic vascular events. In periodontitis, both local and systemic inflammation is caused by a dysbiotic biofilm rather than by specific pathogenic bacteria (such as *Porphyromonas gingivalis*). It is interesting that bacterial diversity is more enriched in patients with periodontitis and that health-associated bacteria are not lost [90]. The periodontitis associated taxa in biofilm differ between patients with mild periodontitis (enriched with *Campylobacter, Corynebacterium, Fusobacterium, Leptotrichia, Prevotella, Tannerell*a, and *Saccharibacteria*) and those with severe periodontitis (enriched with *Filifactor alocis*, *Treponema* species and *Fretibacterium* species) [91].

Rather few studies have evaluated the effect of smoking cessation on the microbiota. In a study with 22 smokers, eleven of whom managed to quit, it was found that the subgingival prevalence of microbiota decreased for *Porphyromonas endodontalis* and *Dialister pneumosintes*, there was also a decrease in the level of *Parvimonas micra, Filifactor alocis*, and *Treponema denticola*, while levels of *Veillonella parvula* increased [92]. Biedermann et al investigated the gut microbiota in 10 smokers who quit smoking, 5 non-smokers and 5 continuing smokers. These investigators applied 16S rRNA gene sequencing and observed that in addition to large shifts in the microbial composition after smoking cessation that there was also an increase in microbial diversity [93]. Subsequently fluorescence *in situ* hybridization was applied which showed that smoking cessation led to an increase in the Firmicutes *Clostridium coccoides, Eubacterium rectale,* and *Clostridium leptum* subgroup as well as the Actinobacteria HGC bacteria and bifidobacterial. There was also a decrease in Bacteroidetes *Prevotella* spp. and *Bacteroides* spp. and beta- and gamma-subgroup of Proteobacteria [94].

Microbiota and atherosclerosis: mechanisms (Figure 2)

Atherosclerosis and metabolites: TMA and TMAO

TMAO is one of the more extensively studies metabolites formed by the gut microbiota with a potential role in atherosclerosis. TMA is formed by gut microbiota after meals containing choline, phosphatidylcholine or carnitine, which are present in food with high levels of saturated or unsaturated fat [18]. Humans do not possess TMA lyases, so that all of the TMA is formed by the gut microbiota. After absorption,

TMA is transported to the liver where the hepatic enzyme flavin-monooxygenase-3 (FMO3) oxidizes TMA to TMAO [95].

A role for TMAO in atherosclerosis has been shown in several mice models; in addition, in a number of studies in humans, an association was demonstrated between the TMAO level in plasma and the risk of atherosclerosis or future cardiovascular events. Some prospective cohort studies have also concluded that increased in serum TMAO levels predict an increased risk for cardiovascular events [96]. In this way, serum TMAO levels are affected by the intestinal microbiota and, therefore, the microbiota would be able to influence the cardiovascular risk [97]. Other studies, however, could not confirm this association. Although changes in diet, exercise and weight loss interventions were reported to reduce TMAO plasma levels, whereas a single faecal microbial transplantation (FMT) of vegan donors failed to have the same effect [98-100].

One of the mechanisms by which TMAO may increase the risk of cardiovascular events is mediated via platelet activation. Zhu et al. showed that the direct exposure of platelets to TMAO enhanced sub-maximal stimulus-dependent platelet activation by multiple agonists through augmented Ca2+ release from intracellular stores [101]. Enhanced platelet aggregation has been shown both in animal models employing dietary choline or TMAO and microbial transplantation as well as after choline supplementation in humans. TMAO also contribute to endoplasmic reticulum stress, hyperglycemia and metabolic syndrome; it binds to the endoplasmic reticulum stress kinase PERK and induces the production of the FoxO1 transcription factor, an important driver of metabolic disease [102]. Thus a lowering of TMAO production reduced PERK activation and FoxO1 levels in the liver.

TMAO also seems to exert effects on lipid metabolism. Koeth et al. detected a relevant reduction of reverse cholesterol transport in mice after dietary supplementation with TMAO or either carnitine [103]. While, they could not detect relevant alterations in cholesterol production or breakdown via the LDL receptor, TMAO supplementation suppressed the levels of bile acid synthetase and bile acid transporters [103]. This synthetic pathway for bile acids is important in the elimination of cholesterol. A direct effect of TMAO on endothelial cells was observed in experiments conducted in human umbilical vein endothelial cells (HUVECs). A suppressed migration of HUVECs, upregulation of vascular cell adhesion molecule-1 expression and increased monocyte adherence was observed; these effects were mediated by protein kinase C [104].

Atherosclerosis and metabolites: imidazole-propionate

Microbial metabolites of amino acids in food may exert metabolic effects relevant for atherosclerosis. An important example is imidazole-propionate which is formed after the metabolism of histidine, one of the essential amino acids. Imidazole-propionate appeared to be one of these histidine metabolites; there was a clearly higher concentration of imidazole-propionate in the portal blood of obese diabetes patients as compared to obese patients without diabetes [105]. Subsequent studies showed that imidazole-propionate can impair glucose tolerance in mice; it interferes with insulin signaling through the activation of p38γ MAPK, which promotes p62 phosphorylation and the activation of mTORC1. Whether imidazole-propionate has

other effects relevant for atherosclerosis has yet to be determined. In addition, the role of other amino acid metabolites needs to be elucidated.

Atherosclerosis and metabolites: bile acids

Bile acids are primarily formed in the liver from cholesterol via two pathways: the classical pathway and the alternative pathway. In the classical pathway, cholesterol is converted into primary bile acids cholic acid and chenoxycholic acid by the enzymes CYP7A1, CYP8B1 and CYP27A1 [106]. It has been suggested that the classical pathway accounts for 75% of primary bile acid production. Fibroblast growth factor 19 is involved in the regulation of the classical pathway; it is produced in enterocytes and activated by bile acid receptor FXR signaling. FGF19 downregulates CYP7A1 in the classical pathway in hepatocytes leading to a reduction of bile acid production. In the alternative pathway, initially 27-hydroxy-cholestesterol is formed by CYP27A1 and subsequently chenodeoxycholic acid by CYP7B1. There is a considerable difference in bile acid production and regulation between rodents and humans, a factor that is important to be taken into account when the effects of bile acids are reported in animal studies [107].

Bile acids are conjugated with glycine and taurine to form glycocholic acid, taurocholic acid, glycochenoxycholic acid and taurochnoxycholic acid; these compounds lower pH and the solubility of many nutrients is improved. These bile acids are released into the duodenum, especially after the intake of food, to facilitate digestion and to improve the uptake of lipids and lipophilic vitamins [107]. The vast majority of these bile acids are reabsorbed in the distal ileum via the sodium-

dependent bile acid transporter SLC10A2 and returned to the liver via the portal system [108]. The amount of primary bile acids reaching the colon is dependent on intestinal motility and the activity of microbiota in the small intestines. The colonic microbiota is able to convert primary bile acids which are not reabsorbed to secondary bile acids i.e. deoxycholic acid and lithocholic acid and other secondary bile acids. Secondary bile acids are toxic for gut microbiota at higher concentrations. This may be one of the explanations why the small intestine is less colonized by microorganisms than the colon. The composition of the colonic microbiota has a strong influence on the amount of secondary bile acids bein formed [109]. Lifestyle is known to be able to influence bile acid levels, for example directly after exercise, bile acid concentrations are reduced as compared to before exercise. Conversely, adherence to a Mediterranean diet has elevated the amounts of faecal bile acids [110, 111]. In obese persons, a higher intake of fibre and protein also increased the levels of bile acids in serum [112].

In addition to their function in promoting the absorption of lipids and vitamins, bile acids are involved in metabolic processes, intestinal motility, inflammatory processes and liver regeneration. Bile acids exert these effects via bile acid receptors, of which FXR and TGR5 are the most important [107]. In particular, secondary bile acids bind to these receptors. FXR is present in many cell types and tissues: hepatocytes, enterocytes, renal tubular cells, pancreatic beta cells, white adipose tissue, and this receptor has also been demonstrated in arterial walls. Binding of bile acids to FXR reduces lipid levels, improves insulin sensitivity and suppresses hepatic gluconeogenesis. Nonetheless, experiments in either LDL-R or apo E deficient mice have shown contradictory results with respect to the relevance of FXR for

atherosclerosis [113, 114] i.e. both increased and reduced plaque formation was observed. However, stimulation by FXR agonists appeared to reduced plaque formation in mice [115]. Unfortunately, the use of the FXR agonist, obeticholic acid, in humans led to an increase in the levels of LDL cholesterol accompanied by a reduction in that of HDL cholesterol [116].

TGR5 is also present in many cell types such as intestinal enteroendocrine cells, enteric neurons, endothelial cells, sinusoidal cells, smooth muscle cells, epithelial cells of the gall bladder, brown adipose tissue, Kupffer cells and nonparenchymal cells of the liver. It has been found that stimulation of TGR5 reduces the production of cytokines. Treatment of endothelial cells with taurolithocholic acid which binds to TGR5 induces NO production via Akt activation followed by an increase in the intracellular Ca2+ level, which inhibits monocyte adhesion in response to inflammatory stimuli [117]. Administration of bile acids in a murine model of obesity and insulin resistance increased energy expenditure in brown adipose tissue and improved insulin sensitivity [107].This TGR5-mediated effect appeared to be dependent on thyroid hormone which activated deiodinase type 2, both in brown adipose tissue and skeletal muscle cells.

Two other bile acid receptors have been implicated in the pathogenesis of atherosclerosis i.e. sphingosine-1-phosphate receptor 2 (S1PR2) and the pregnane X receptor (PXR). Several bile acids can activate S1PR2. In contrast to binding to TGR5, in mice, this has been associated with increased cytokine production and more plaque formation, whereas S1RP2 knock-out in apo E deficient mice has a reduced risk of atherosclerosis [118, 119]. It is believed that PXR can be activated by

the secondary bile acids, i.e. the bile acids produced by gut microbes. Deletion of PXR in apo E knock-out mice attenuated plaque formation and reduced lipid uptake by macrophages [120]. On the other hand, activation of PXR caused increases in the LDL concentration and plaque formation.

Atherosclerosis and metabolites: short chain fatty acids (SCFA)

Non-digestible fibers from food are fermented in the colon by gut microbiota, which leads to the production of SFCAs, mainly butyrate, propionate and acetate. The concentrations of SFCAs are lower in patients with atherosclerotic vascular disease or hypertension [121]. In apo E knock-out mice, elevated levels of butyrate due to inoculation with the butyrate producing microorganism *Roseburia intestinalis* led to reduced plaque formation [122]. It has been proposed that SCFA have a beneficial effect on atherosclerotic plaque formation by improving intestinal barrier function. A diet enriched with polyphenols was reported to increase the SFCA concentration whereas a diet leading to weight loss led to decreased levels [35, 123, 124]. Exercise has also been associated with increased SFCA levels [80].

A mechanism to explain SCFA's effects on host immune homeostasis has also been proposed. In mouse models of hypertensive cardiovascular disease, propionate attenuated hypertension and its cardiovascular sequelae, reduced the atherosclerotic plaque area and there were also reductions in the frequencies of splenic effector memory T cell and splenic T helper 17 cells [125]. The cardioprotective effects of propionate were abrogated by depletion of regulatory T cells, indicating that propionate exerted a T cell-dependent effect. SCFAs modulate immune and

inflammatory responses via many receptors i.e. activation of free fatty acid (FFA) receptors type 2 and 3 (FFA2 (GPR43) and FFA3 (GPR41) receptors), G protein-coupled receptor 109A (GPR109A) and inhibition of histone deacetylases (HDACs) [126]. Since, FFA2 and FFA3 are present in endothelial cells, binding of SFCAs to receptors may evoke not only the stimulation and dampening of the production of inflammatory cytokines, but also influence migration and recruitment of immune cells to the atherosclerotic plaque. SCFAs also have metabolic effects mediated via FFA2 and FFA3.

SCFAs have also direct effects on endothelial cells via HDACs. Butyrate inhibits the actions of the adhesion molecule VCAM-1 in ox-LDL pretreated endothelial cells and reduces the migration and adhesion of monocytes [127]. VCAM-1 expression and adhesion of monocytes was also decreased in HUVECs stimulated with TNF-alpha and pretreated with either butyrate or propionate [128, 129].

In humans, interventions have hinted at beneficial metabolic effects of SFCAs. In overweight or obese men, colonic infusions of SCFA mixtures either enriched with acetate, butyrate or propionate in concentrations which are reached with fiber intake were able to exert metabolic effects. However, oral supplementation with butyrate induced differential metabolic effects in lean and metabolic syndrome subjects suggesting that they have differential regulatory effects [130]. Whether acetate is infused into the proximal or in the distal colon appeared to be of importance. Distal colonic acetate infusions affected whole-body substrate metabolism, with a pronounced increase in fasting fat oxidation and plasma PYY, whereas this was not observed after proximal infusions [131].

Atherosclerosis and microbiota: inflammation

Low grade inflammation has been proposed as being the cornerstone for different chronic diseases including metabolic syndrome [132], osteoarthritis [133], type-2 diabetes [134] and fibromyalgia [135]. It is also a well-known factor in CVD [136]. Moreover, this low-grade inflammation increases with age, being common in people of advanced age, so-called “inflammageing”, which is known to be a risk factor for CVD [137]. In all of these conditions, higher plasma levels of pro-inflammatory mediators such as TNF□, IL1 or IL6, among others, are frequently found. Very often, this inflammation has been linked to an increased intestinal permeability, with elevated intestinal translocation of pro-inflammatory mediators of bacterial origin such as LPS, causing the so-called “metabolic endotoxemia” [138].The role of this endotoxemia on CVD risk has been recently addressed in a large epidemiological study carried out in Japan [139]. In this prospective study, over 2500 community-dwelling individuals, older than 40 years of age, were followed for 10 years. The authors observed an increased cumulative incidence of CVD with increased serum levels of LPS-binding protein. This underlines the contribution that endotoxemia, and the concomitant low-grade inflammation, may have on to the pathogenesis of atherosclerosis and CVD. LPS and other bacterial cell membrane constituents are recognized by several receptors (Toll-like receptors, NOD-like receptors and RIG-1-like receptors) on endothelial cells [140]. Binding of LPS directly induces adhesion molecules such as ICAM-1 and P-selectin on endothelial cells which are important for the interaction with leukocytes [141]. Other bacterial membrane constituents

stimulate cultured endothelial cells to form and secrete Weibel Pallade bodies which leads to an increase in von Willebrand Factor [142].

The above-mentioned data highlight the potential role of the gut microbiota in controlling intestinal permeability and endotoxemia [138] and therefore on the development of chronic low-grade inflammation and the risk for CVD. This makes inflammation an attractive target for lowering the risk of CVD and opens the door to the modulation of the gut microbiota to this end [143]. Moreover, animal models of CVD have further underlined the association between the microbiota and CVD severity [144] and demonstrated the potential of microbiota modulation, for instance by means of FMT in treating ischemic stroke [145]. Recently, animal studies have also provided insights into the role of the gut microbiota in provoking autoimmune inflammatory cardiomyopathy due to antigenic mimicry between a peptide formed by the gut commensal *Bacteroides thetaiotaomicron* and myosin [146].

All these data explain why there is increasing interest in developing intervention strategies targeting the microbiota to achieve down-regulation of low-grade inflammation as a way of preventing CVD. Interestingly, different foods and nutrients have demonstrated their capability to modulate the inflammatory status of the host [147]. Therefore, these foods and ingredients, such as polyphenols or probiotics, represent promising tools for the dietary management of CVD risk.

DISCUSSION: CAVEATS IN RESEARCH AND FUTURE PROSPECTS

Thus far, there is limited evidence that lifestyle effects on cardiovascular health are mediated by actions of gut microbiota. Several studies have revealed the effects of lifestyle interventions on the microbiota and their metabolites, whereas other trials have detected associations between atherosclerosis or other cardiovascular events and microbiota. In addition, the effects of lifestyle on diabetes, metabolic syndrome or low-grade inflammation, which are important in the pathogenesis of atherosclerosis, may also be mediated by the microbiota and their metabolites [143, 148-152].

Since lifestyle changes do also have direct effects on atherosclerosis via metabolic effects and blood pressure, it will be difficult to establish which proportion of the beneficial effects is mediated by changes in the microbiota. The complexity of the human metabolic network, together with the convoluted nature of the composition of both diet and microbiota makes it almost impossible to evaluate the impacts of specific components. Atherosclerosis is a slow process, in which changes in wall thickness and plaque formation take several years to evolve and ischemic events only occur after decades in the late stages of the disease.

Optimally, in dietary interventions only one dietary component is modified in a randomized placebo-controlled setting but in practice this is seldom possible. For example, a change in one of the energy-yielding nutrients, e.g. carbohydrate intake, in an isocaloric diet, will automatically result in a change in either protein or fat intake. Studies on smoking cessation and physical exercise have the same problem as these interventions often have also effects on food intake and/or energy expenditure. Future studies on lifestyle interventions and microbiota information should register diet, physical exercise, smoking behaviour and medication use on prespecified time points in attempts to address this complexity.

For the interpretation of studies on gut microbiota and atherosclerosis it is important to know that several medications used in patients with atherosclerosis also appear to have an influence on gut microbiota. Several studies indicate that statins have a beneficial effect. Recently it was shown in several cohorts that use of statins was associated with a lower prevalence of the unfavorable Bact2 enterotype. Bact2 is characterized by a high proportion of Bacteroides, a low proportion of Faecalibacterium and low microbial cell densities [153]. The administration of ezetimibe resulted in an increase in Lactobacillus species in mice [154]. Whether other lipid lowering drugs like fibrates or PCSK9 inhibitors have an influence on microbiome is uncertain. Also other medication used in patients with atherosclerosis, such as metformin and proton pump inhibitors have a clear influence. In addition, it is important to realize that gut microbiota have the ability to metabolize medications, which can results in altered drug pharmacokinetics and pharmacodynamics or formation of toxic metabolites which can interfere with drug response. Some studies indicate that reponse to statins and antihypertensive medication is modulated by microbiota [155].

Animal studies, especially murine models, can be used to focus on one specific element in a metabolic pathway, or to establish the effects of some controlled diet or exercise intervention. However, in order to establish atherosclerosis in mice, extreme diets are used or knock-out mice with an extreme phenotype are exploited. Since, food patterns, microbiota and bile acids also differ between mice and humans, the findings emerging from these murine models should always be confirmed in humans.

The field of microbiota research is relatively new and complex and the methods used are far from standardized and harmonized. In many clinical studies, sample sizes have been relatively small, with control groups often lacking. Several studies have gathered information on the use of antibiotics or drugs such as metformin which has an important influence on microbiota. Another issue is that different methods have been applied in how faecal samples are collected, processed and stored since it has been shown that these methological differences e.g. in sample processing protocols, the oligonucleotides used for 16S rRNA gene amplification (in 16S rRNA gene-based analyses), the sequencing technology utilized, all pose a risk for introducing artefacts [156-158]. The enormous datasets generated when microbiota, metabolome, genome and transcriptome are evaluated in cohorts or in intervention studies are challenging and different complex bioinformatics methods have been applied. Standardisation and harmonisation and data sharing in this field would clearly help in the interpretation and comparison of studies.

In general, the studies reviewed have not differentiated between responders and non-responders. A lifestyle intervention may not have the same effect on microbiota and metabolites in all persons; it is likely that there are high and low responders. This may be a key factor that explains the inconsistent study results. Some studies have applied algorithms to create more personalized dietary and microbial approaches [52]. There is clearly a need to develop this area of research both in observational and randomized intervention trials, such that stratification of subjects should be done according to their expected response to the intervention. This approach has been recently applied when examining the impacts of dietary fatty acids as modifiers of

lipid and glucose metabolism, i.e. the randomization was conducted according to the polymorphisms in the trial subject's fatty acid desaturase (FADS) gene cluster [159].

As already mentioned, the pathological processes leading to clinically manifest CVD takes decades. Within this context it is perhaps over-optimistic to design and execute clinical trials whereby relatively subtle lifestyle changes would result in meaningful benefits in both microbiota and cardiovascular risk markers. On the other hand, a change of several elements of lifestyle might accumulate and thus provide an overall detectable cardioprotective effect within an acceptable follow-up time. An optimal study would need to involve stratification of the study participants into the intervention groups according to their existing microbial composition, and preferably also according to relevant polymorphisms. Thus, to enhance the current level of understanding, we call for well-designed clinical trials involving all the aspects of lifestyle, gut microbiota and metabolites and genetic background. Since clinical relevance is being sought, the main outcome should be cardiovascular events. It is possible that the on-going PredimedPlus study in Spain [160], will provide these kinds of insights.

Although there are several observational and epidemiological studies pointing to a possible causal relationship between gut microbiota and its metabolites and CVD, as yet, there is no formal proof of causality. A logical therapeutic intervention to prove causality and to reduce cardiometabolic risk would be the use of FMT. In this regard, more than 240 randomized controlled trials are ongoing around the world to dissect the role of donor FMT on human disease and the interrelationship between donor microbiota composition and host immunesystem. Although still in its infancy, we and

others have shown that FMT by introducing donor feces in small and large intestine can dissect causality of gut microbiota in human cardiometabolism [161] with lower baseline intestinal microbiota diversity being the driver of FMT metabolic efficacy [162, 163] as well as metabolic characteristics of the FMT donor [164]. The drivers of these beneficial effects are thought to be alterations in bileacids, plasma metabolites (including TMAO and serotonin) and gut microbiota-diet-medication interactions. Other interventions to prove causality in humans could be administration of capsules containing microbiota or use of prebiotics. It is clear that there are several hurdles to be overcome before this kind of evidence becomes available.

Summary and conclusions

There is no doubt that a healthy lifestyle comprising of a dietary intake in accordance with the recommendations, for example, a diet rich in fruits and vegetables, fibre and unsaturated fats, a moderate or high level of physical activity and abstinence from smoking are beneficial in advancing health and lowering the risk of many diseases, including CVD. All of these lifestyle factors, particularly diet, but also physical activity, have been found to act as modifiers of the composition and function of the gut microbiota. Studies linking these two aspects in a clinical trial setting, i.e. an evaluation of how a diet modification or physical activity can alter gut microbiota composition and exert a subsequent modification of serum cardiovascular risk markers have however yielded equivocal results, but the most of the evidence points to the benefits of high-fibre diet, consumption of good quality of dietary fat (e.g. nuts), adherence to an overall healthy diet and regular physical activity.

In conclusion, with regard to the published research evidence, and in the absence of more detailed studies linking diet and other lifestyle factors together with gut microbiota to the cardiovascular health, it may be stated that the lifestyle recommended for cardiovascular health [165] is likely to be also beneficial for gut microbiota. These interactions introduce gut microbiota as one important component in the diet-gut microbiota-cardiovascular health pathway. Indeed, an aberrant microbiota composition has been reported in coronary heart disease and atherosclerosis. In addition, we have examined the potential mechanisms by which microbiota may mediate the benefits on cardiovascular health, these include production of metabolites like TMAO as well as immunomodulatory functions. Thus, the modulation of gut microbiota by lifestyle may offer opportunities for the non-pharmacological prevention and management of CVD. Nevertheless, we emphasize that each dimension of the lifestyle may also influence the cardiovascular risk markers, regardless of its impact on the gut microbiota. These preliminary findings and acknowledgement of research gaps (Table 4), should stimulate further research to clarify the lifestyle-gut microbiota-cardiovascular health triad in order to identify specific non-pharmacological means to improve cardiovascular health.

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CONFLICTS OF INTEREST

MN is on the Scientific Advisory Board of Caelus Pharmaceuticals, the Netherlands and of Kaleido Biosciences, USA. None of these companies are directly relevant to the current paper. There are no patents, products in development or marketed products to declare. The other authors declare no competing financial interests.

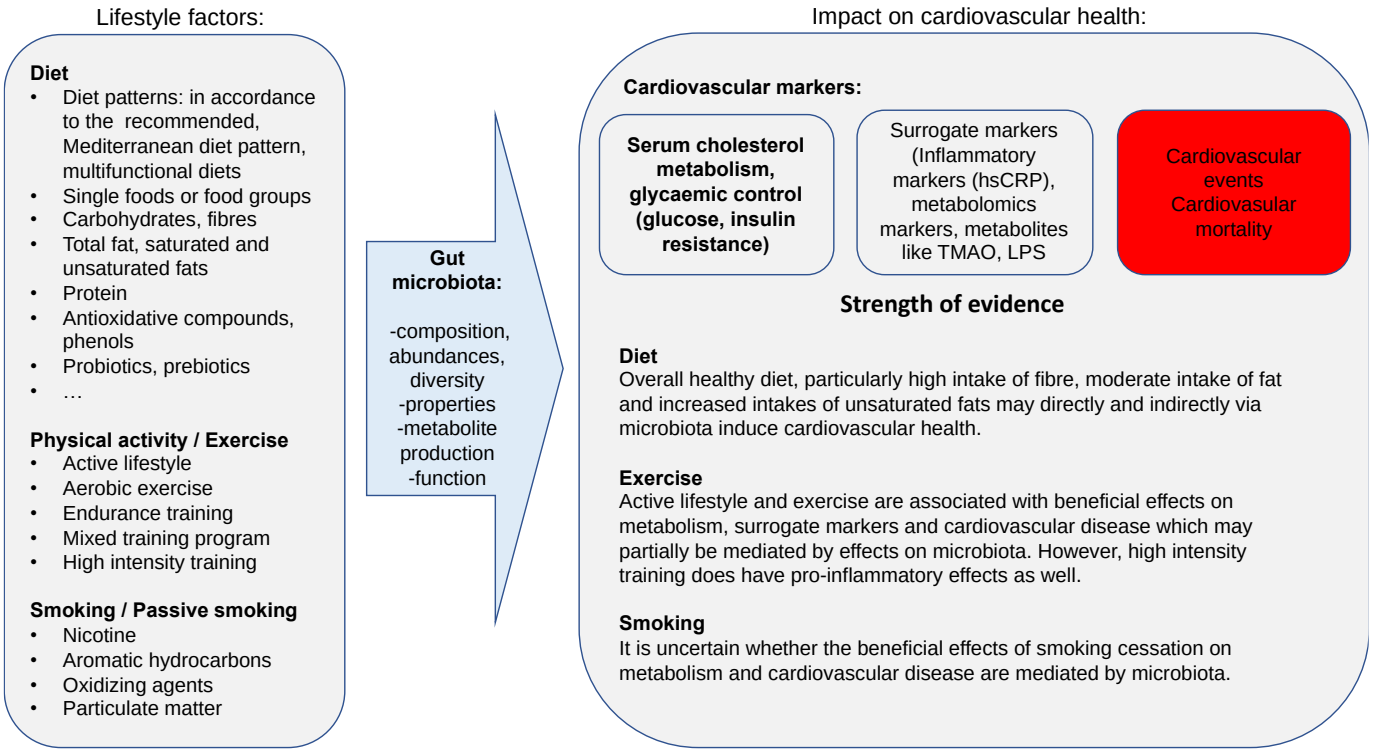
AUTHOR CONTRIBUTIONS

VG, MG, MN and KL designed the review. VG, MG and KL wrote and MN provided critical revision of the article. LP contributed to data collection and compiled the table 2. All authors commented the article and accepted the final version.

FIGURE LEGENDS

Figure 1: Strength of evidence from the lifestyle interventions for lifestyle-microbiota-cardiovascular health triad. We have utilized traffic light colours from green, i.e. good evidence to red, i.e. no evidence.

Figure 2. Potential mechanisms to explain the contributions of gut microbiota to the development of atherosclerosis. SCFA=short chain fatty acids, BA=bile acids, TMA= trimethylamine, TMAO= trimethylamine-N-oxide. IMP=imidazole propionate, LPS=lipopolysaccharides.



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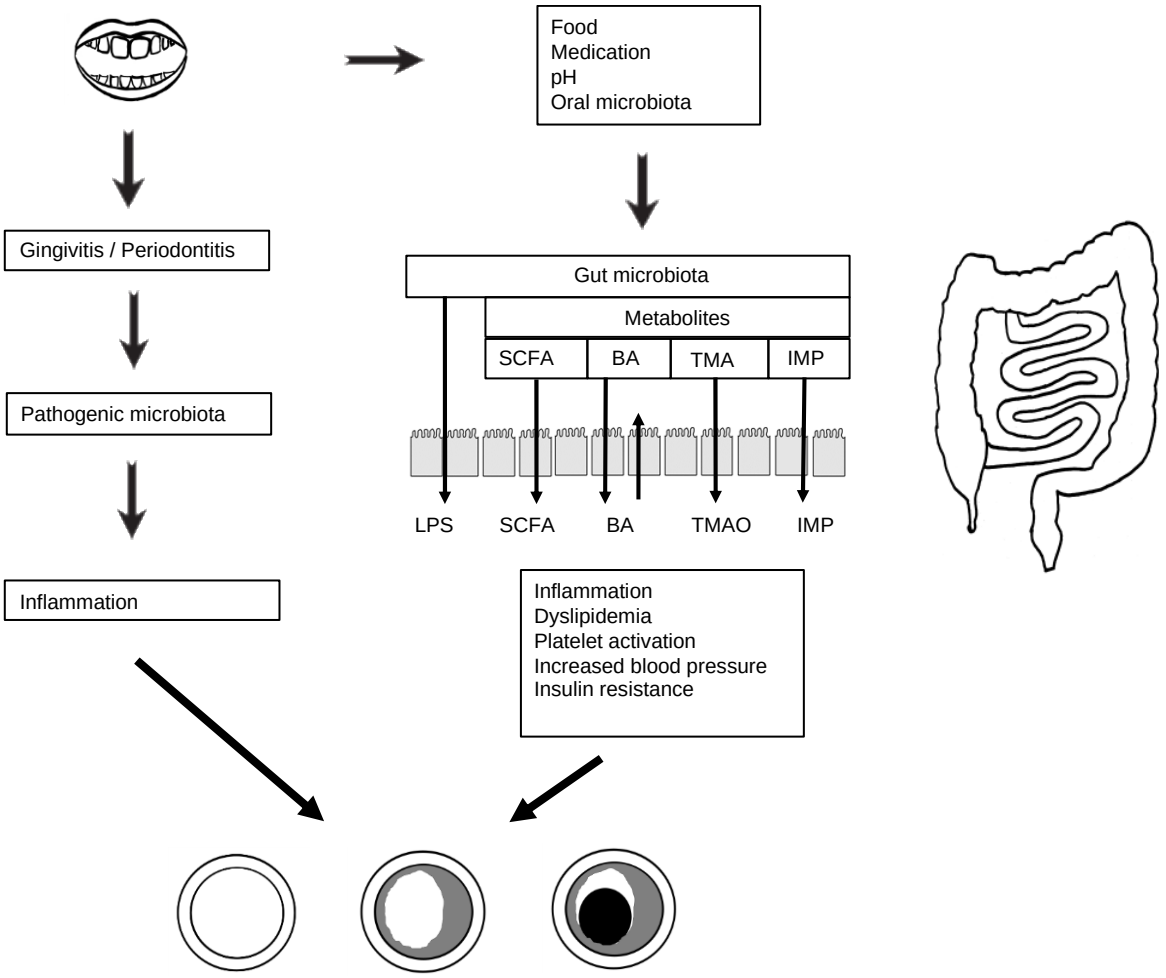

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Table 1. Human studies showing microbiota dysbiosis in cardiovascular diseases and cardiovascular disease risk[a].

-**Author** -**Year** -**Country**	**N**	**Condition**	**Study groups**	**Main microbiota results (vs control group)**
-Koren et al. -2011 -Sweden [166]	15 (vs 15 Controls)	ATCD	Patients with atherosclerotic plaque undergoing endarterectomy for minor ischemic stroke, transient ischemic attack or amaurosis fugax	No differences
-Karlsson et al. -2013 -Sweden [151]	12 (vs 13 Controls)	ATCD	Patients with atherosclerotic plaque undergoing endarterectomy for minor ischemic stroke, transient ischemic attack or amaurosis fugax	↑ *Collinsella* ↓ *Roseburia*, *Eubacterium, s*ome *Bacteroides* sp.
-Yin et al. -2015 -China [167]	141 (vs 94 Controls)	ATCD	Patients with atherosclerotic plaque with clinical presentations of ischemic stroke or transient ischemic attack	↑ *Proteobacteria* ↓ *Bacteroides, Prevotella, Faecalibacterium*
-Jie et al. -2017 -China [2]	218 (vs 187 Controls)	ATCD	Patients with atherosclerotic plaque with clinical presentations of stable or unstable angina or acute myocardial infarction	↑ *Enterobacteriaceae*, *Streptococcus, Lactobacillus salivarius, Atopobium parvulum*, *Ruminococcus gnavus*, *Eggerthella lenta* ↓ *Roseburia*, *Faecalibacterium*
-Emoto et al. -2017 -Japan [168]	39 (vs 30 controls)	ATCD	Patients with stable angina and old myocardial infarction who underwent percutaneous coronary intervention or by-pass.	↑ *Lactobacillales* ↓ *Bacteroides, Clostridium*
-Zhu et al. -2018 -China [169]	70 (vs 98 Controls)	ATCD	Patients with coronary artery disease	↑ *Escherichia-Shigella, Lactobacillus, Enterococcus, Streptococcus* ↓ *Faecalibacterium, Roseburia, Eubacterium, Subdoligranulum*

-Li et al. -2017 -China [170]	56+99 (vs. 41 controls)	Hypertension	Pre-hypertensive patients (125/80 – 139/90 mmHg) and patients with hypertension (≥ 140/90 mmHg)	↑ *Prevotella, Klebsiella, Porphyromonas* ↓ *Faecalibacterium, Roseburia, Bifidobacterium, Oscillibacter, Coprococcus, Butyrivibrio*
-Yan et al. -2017 -China [171]	60 (vs 60 Controls)	Hypertension	Patients with hypertension (≥ 140/90 mmHg)	↑ *Klebsiella, Salmonella, Streptococcus, Clostridium, Parabacteroides, Eggerthella* ↓ Faecalibacterium, *Roseburia*, *Synergistetes*
-Cui et al. -2018 -China [172]	53 (vs 40 controls)	Heart failure	Patients with ischemic or dilated cardiomyopathy	↑ *Ruminococcus, Acinetobacter, Veillonella* ↓ Faecalibacterium, *Alistipes, Oscilibacter*
-Luedde et al. -2017 -Germany [173]	20 (vs 20 controls)	Heart failure	Stable systolic HF	↑ *Escherichia-Shigella* ↓ *Blautia, Collinsella, Ruminococcaceae, Erysipelotrichaceaem Faecalibacterium*
-Kummen et al. -2018 -Norway [174]	84 (vs 266 controls)	Heart failure	Patients with ischemic or dilated cardiomyopathy	↑ *Prevotella, Hungatella* (Lacnospiraceae*), Succiniclasticum* ↓ *Blautia, Anaerostipes, Faecalibacterium, Lachnospiraceae, Bifidobacterium, Eubacterium, Coprococcus*
-Katsimichas et al. -2018 -Japan	28 (vs 19 controls)	Heart failure	Non ischemic HF with reduced ejection fraction	↑ *Streptococcus, Veillonella, Eggerthela* ↓ *Prevotella, SMB53 (Clostridiaceae)*

[175]				

[a] ATCD=atherosclerotic cardiovascular disease.

Table 2. Intervention studies examining the impacts of dietary modification on microbiota and serum metabolic markers considered cardioprotective[a].

-Author **-Year** **-Country**	**-Population** - N (intervention) - N (control)	**-Design** **-Duration** **-Intervention diet/food** **-Control diet/food**	**Results (vs. controls)**	
			Gut microbiota	**Blood markers**
Interventions with whole grains or fibers				
-Ampatzoglou et al. -2015 -Sweden [37]	-Healthy middle-aged men and women 40-65 yrs, BMI 20-35 kg/m^2 -33 --	-An intervention, cross-over -6 wks -Diet high in whole grains -Diet low in whole grains	-↑↓ SCFA, composition of microbiota	-↑↓ Total cholesterol, HDL, LDL, triglycerides, glucose
-Brighenti et al. -1999 -Italy [21]	-Healthy men, 23.3±0.5 yrs, BMI 25.7±1.2 kg/m^2 -12 --	-An intervention, placebo-controlled, cross-over -3x4 wks -50 g of a rice-based ready-to-eat cereal containing 18% inulin (test) as a substitution for their habitual breakfast -50 g of the same cereal without inulin (placebo) →	-↑↓SCFA -↓ Total facultative anaerobes -↑ *Bifidobacteria* (adjusted for total anaerobe counts)	-↓ Total cholesterol and triacylglycerol -↑↓ LDL, HDL, total-HDL ratio

-Canfora et al. -2017 -The Netherlands [26]	-Overweight or obese (BMI 28-40 kg/m^2) prediabetic men and postmenopausal women (45-70 yrs) -21 -23	-A double-blind, placebo-controlled, randomized, parallel -12 wks -Galacto-oligosaccharides 15 g daily -Isocaloric placebo 15 g (maltodextrin) daily	-↑ Abundance of Bifidobacterium species -↑↓ Richness and diversity -↑↓ Acetate, propionate, and butyrate concentrations	-↑↓ Insulin sensitivity, glucose, insulin, glycerol, free fatty acids triglycerides, leptin, PYY, GLP-1, IL6, IL8, TNF-α, and LBP -↑↓ Acetate, propionate, and butyrate concentrations
-Carvalho-Wells et al. -2010 -UK [27]	-Healthy women and men, 20-51 yrs (BMI 20-30 kg/m^2) -16 -16 (tot 32 cross-over)	-A double-blind placebo-controlled cross-over -21 d -48 g/d whole grain cereal -placebo cereal	-↑ *Bifidobacteria* -↓ The numbers of lactobacilli in both groups -↑ Atopobium levels in both groups -↑↓ The number of *Bacteroides spp*., *C. histolyticum subgroup*, *C. perfringens/histolyticum* subgroup and total bacterial numbers -↑↓ SCFA	- ↑↓ Lipid and glucose
-Clarke et al. -2016 -Canada [28]	-Healthy women and men, 18-50 yrs (BMI 18-30 kg/m^2) -30 --	-A double-blinded, placebo-controlled, randomized, cross-over -2x 28-d periods -Beta-1 fructan 3x5 g/day -Placebo 3x5 g/day (maltodextrin)	-↑ *Bifidobacteria* -↑ SCFA	-↑↓ Lipid and cholesterol concentrations, circulating lymphocytes and macrophages -↑ Liposaccharide -↑ IL-4 -↓ IL-10

-Cooper et al. -2017 -USA [176]	-Healthy men and female (19-46 yrs), BMI 20-28 kg/m^2 -35 -11	-A placebo-controlled, randomized intervention -6 wks -Whole grain products -Refined grain products (placebo)	-↑↓ Relative proportion of bacteria at the phylum level, relative abundance of bacteria at the specific level	- ↓ Total cholesterol, LDL, and non-HDL -↑↓ HDL, triglycerides -↑↓ Glucose
-Costabile et al. -2008 -UK [29]	-Healthy men and women, 20-42 yrs (BMI 20-30 kg/m^2) -16 -15	-A double-blind, randomized, crossover -2x3 wks -Daily 48 g breakfast cereals, either whole grains -or wheat bran	-After whole grain intervention: ↑ numbers of fecal *bifidobacteria* and *lactobacilli* -↑↓ SCFA -↑↓ Ferulic acid concentration	-Both breakfast cereals: ↑ ferulic acid concentrations -↑↓ Glucose, insulin, total cholesterol, triglycerides or HDL
-Dewulf et al. -2013 -Belgium [30]	-Women with obesity (BMI>30 kg/m^2), 18-65 yrs -15 -15	-A double-blind, placebo controlled, intervention -3 mo -ITF (inulin/oligofructose 50/50 mix) -placebo (maltodextrin) (16 g/day)	-↑ *Bifidobacterium* and *Faecalibacterium prausnitzii*; both bacteria negatively correlated with serum lipopolysaccharide levels -↓ *Bacteroides intestinalis, Bacteroides vulgatus* and *Propionibacterium*	-↑↓ HbA1c, glycaemia and insulinaemia, post-OGTT insulinaemia, HOMA-IR or adiponectinaemia, total, HDL, LDL, and triglycerides, CRP -↓ Post-OGTT glycaemia
-Foerster et al. -2014 -Germany [22]	-Healthy men and women, 20-60 yrs -20 --	-An intervention cross-over -3 wks -Diet rich in whole grain products (WG), 40g/d -Diet high in red meat (RM), 200g/d	-↑↓ SCFA concentrations and calprotectin -8 bands changed in at least 4 participants during the RM and during the WG diets -RM: ↑ 5 bands and ↓ 3 bands -WG: ↓ 3 bands and ↑ 5 bands	-WG: ↑↓ lipids and other blood parameters -RM: ↑ creatinine and uric acid

			-Band appearing after WG and increasing after RM *Collinsella aerofaciens* and *Clostridium sp*	
-Jenkins et al. -1999 -Canada [38]	-Healthy men and women, 22-53 yrs -24 --	-A four-phase randomized crossover -2 wks /phase -Four wheat (low-fibre or high fibre) or corn starch (high-amylose or retrograded) supplements as muffins and breakfast cereal	-↑↓ Starch excretion (used to reflect metabolic activity of colonic microbiota) between the treatment groups -↑↓ Total bacteria, bifidobacteria, fusobacteria, bacteroidetes -Starch excretion was related positively to fecal fusobacteria and bacteroides	-Mean starch excretion was related positively to pretreatment HDL and negatively to LDL apolipoprotein B:AI
-Jie et al. -2000 -China [31]	-Healthy men and women -Group A (control):30 -Group B: 30 -Group C: 30 -Group D: 30	-A double-blind, randomized, placebo-controlled -28 d -Ingestion of 0 (A), 4 (B), 8 (C), and 12 (D) g polydextrose/d	- Groups C and D: ↑ SCFA -All groups: -↓ *Bacteroides* species -↑ *Lactobacillus and Bifidobacterium*	- ↑↓ Liver and renal function, glucose, triacylglycerol, cholesterol, and serum Hb A1c -Groups C and D: ↓ incremental AUC and glucose -Group D: ↓ glycemic index
-Martínez et al. -2013 -USA [177]	-Healthy men and female, 25.9±5.5 yrs -28 --	-A randomized cross-over -4 wks -60 g of whole-grain barley (WGB), brown rice (BR), or an equal mixture of the two (BR+WGB)	-All treatments: ↑ microbial diversity, the *Firmicutes/Bacteroidetes* ratio, and the fecal abundance of the genus *Blautia*	-Whole grains and the BR+WGB treatment: ↓ IL-6 and peak postprandial glucose -↓ Glucose level in women and overweigh subjects -↓ Total cholesterol on women

-Roager et al. -2019 -Denmark [32]	-Men and women at risk of developing metabolic syndrome, 20-65 yrs (BMI 25–35 kg/m^2 and/or increased waist circumference) -50 --	-A randomized, controlled cross-over trial -8 wks x2 -75 g/day of whole grains during the whole grain intervention - <10 g/day of whole grains during the refined grain intervention	-↑↓ Bacterial species diversity or richness	-↑↓ HOMA-IR, HbA1c, serum C-peptide, fasting glucose and insulin -↑↓ Triacylglycerol, total cholesterol, HDL and LDL and free fatty acids -↓ CRP, IL-6, IL-beta -↑↓ TNF-α
-Rebello et al. -2015 -USA [35]	-Overweight or obese men and women (18-70 yrs) -14 -14	-A randomized, controlled -4 wks -A test product (powder containing 3.8 g inulin, 2 g β-glucan, 0.16 g blueberry anthocyanins, and 0.72 g blueberry polyphenols, total fiber 8.8 g) -Placebo (0 g β-glucan, 8.7 g fiber)	-↑↓ Bacterial composition -↑ SCFA	-↑ Glucose tolerance, PYY, plasma satiety hormones -↓ Ghrelin -↑↓ Lipids, hsCRP, HOMA-IR, and HbA1C, insulin sensitivity
-Robinson et al. -2001 -USA [33]	-Healthy female and male subjects -20 -	-A randomized cross-over intervention -2x3 wks -Usual diet in addition to 15 or 30 g arabinogalactan (AG) in a beverage sweetened with aspartame	-↑ Total anaerobes and *Lactobacillus spp.* after 6 wks with both doses - ↑↓ *Bifidobacterium spp, Clostridium spp*., Enterobacteriaceae, fecal enzyme activity, SCFA -↓ Fecal ammonia levels	-↑↓ Total cholesterol, HDH, LDL, triglycerides, Apo-A1, Apo-B, insulin -↑ Glucose level after 30 g of AG compared to baseline

		-Usual diet with the control beverage		
-Sandberg et al. -2019 -Sweden [178]	-Healthy men and women, 50-70 yrs (BMI <28 kg/m^2) -33 --	-A randomized crossover intervention -3 d -Barley kernel bread -White wheat bread	-Subgroups based on baseline microbiota composition: *Prevotella* and *Bacteroides* and the ratio of *Prevotella/Bacteroides* -HP: high *Prevotella*, low *bacterioides* (n=12) -LP: low *prevotella*, high *bacterioides* (n=13) -HBP: high *Prevotella* and *high Bacterioides* (n=8)	-↓ Glucose responses, postprandial s-insulin responses - ↑↓ b-glucose and s-insulin concentrations -HP: ↓ insulin response and lower IL-6 compared to LP -LP: ↑ b-glucose concentrations - ↑↓ PYY, GLP-1 or GLP-2 -HBP: ↑ GLP-1 and GLP-2 -HP: ↑ p-PYY -↑↓ IL-6 and CRP -HP: ↓ IL-6, CRP -↑↓ Free fatty acids (treatment and subgroups)
-Schutte et al. -2018 -The Netherlands [34]	- Overweight men and postmenopausal women, 45-70 yrs -25 -25	-A randomized controlled, double-blind, parallel -12 wks -Whole grain wheat products (98 g/d) -Refined wheat products (98 g/d)	-↓ Diversity decreased in the refined wheat group when compared with the whole grain wheat group	-↑↓ Total cholesterol, HDL, triglycerides, nonesterified fatty acids -↑↓ Glucose, insulin, HOMA-IR -↑ Intrahepatic triglycerides (refined grains group) -↑ Postprandial triglycerides

-Vitaglione et al. -2015 -Italy [36]	-Healthy overweight/obese subjects, >18 yrs -36 -32	-A placebo-controlled, parallel-group randomized -8 wks -Replacement of precise portions of refined wheat with a fixed amount of selected whole grain wheat or refined wheat	-↑ Fecal ferulic acid -↑↓ The microbial community structure	-↑ Serum dihydroferulic acid after 4-8 wks, IL-10 after 4 wks -↓ TNF-α after 8 wks -↑↓ Plasma metabolic disease markers, anthropometric measurements and body composition
-Vulevic et al. -2013 -UK [24]	-Overweight men and women who had ≥3 risk factors associated with metabolic syndrome, 18-65 yrs, BMI>25 kg/m2 -45 --	-A double-blind, randomized, placebo controlled, crossover -12 wks - Galactooligosaccharide mixture [Bi2muno (B-GOS)] -Placebo (maltotrexin)	-↑↓ Total bacteria, *Lactobacillus/Enterococcus spp*., *Clostridium coccoides/Eubacterium rectale group, Atopobium cluster, E. cylindroides, E. hallii, b-Proteobacteria, Clostridium cluster IX,* and *F. prausnitzii* -↑ Number of *bifidobacteria* -↓ *Bacteroides spp. and C. histolyticum* group, number of *Desulfovibrio spp., b-Proteobacteria*	-↑↓ G-CSF, IL-6, IL-10, IL-8, and TNF-α, CRP, glucose, HDL and LDL -↓ Insulin, total cholesterol, triglycerides, triglycerides:HDL ratio, CRP
-Xiao et al. -2014 -China [25]	-Obese men and women, 25-55 yrs, (BMI≥28 kg m^2) -93 --	-A self-controlled clinical trial -9 wks + 14 wks - Whole grains, Chinese traditional medicinal foods, and prebiotics (WTP diet) -Maintenance period	-↓ *Enterobacteriaceae and Desulfovibrionaceae* -↑ Related to gut barrier-protecting bacteria of Bifidobacteriaceae -↓ Gut permeability	-↑ Insulin sensitivity, lipid profiles, adiponectin -↓ Plasma endotoxin load as lipopolysaccharide-binding protein, TNF-α, IL-6

Interventions with single food or food group				
-Balfegó et al. -2016 -Spain [179]	-Overweight or obese men and women (BMI 26-35 kg/m2) with type 2 diabetes, (60.6±1.4 yrs) -19 -16	-A randomized intervention -6 mo -Type 2 diabetes standard diet with 100 g of sardines 5 d/wk -Type 2 diabetes standard diet	-↑↓ Abundance of the bacterial groups	-↑↓ Glucose, HbA1c, insulin and HOMA-IR -↑↓ Adiponectin, IL-6, IL-8, IL-10
-Chambers et al. -2019 -UK [180]	-Men and women with overweight or obesity, 18-65 yrs -12 --	-A randomized, double-blind, placebo-controlled, cross-over -42 d -Inulin-propionate ester (IPE) -Low-fermentable fibre control (cellulose) -High-fermentable fibre control (inulin)	-Inulin: 1) class level ↑Actinobacteria and ↓ Clostridia 2) order level ↓ Clostridiale 3) ↑*Anaerostipes hadrus*, *Bifidobacterium* faecale and *Bacteroides caccae* and *Blautia obeum, Blautia luti, Oscillibacter spp, Blautia faecis* and ↓ *Ruminococcus faecis* compared to cellulose -IPE: ↑ *Bacteroides uniformis* and *Bacteroides xylanisolvens* and ↓ *B. obeum and Eubacterium ruminantium* compared to cellulose -IPE: ↑ *Fusicatenibacter saccharivorans* and ↓ *A. hadrus, B. faecal* and *Prevotella copri* compared to inulin	-IPE and Inulin: ↑ Insulin resistance and ↓ insulin compared to cellulose -IPE: ↓ IL-8 compared to cellulose -IPE: ↑ propionate compared to cellulose -↑↓ Th17, Treg, CD19+ B-cells -IPE: ↑ IgG, ↓ IL-8

			-↑↓ SCFA	
-Han et al. -2015 -Korea [181]	-Obese women (BMI>25), 30-60 yrs -12 -11	-A randomized controlled parallel -8 wks -Fermented kimchi 180 g/d -Fresh kimchi 180 g/d	-↑ Relative abundance of *Bacteroides* and *Prevotella* -↓ Relative abundance of *Blautia* after fermented kimchi	-↑↓ Glucose, triglycerides, HDL, total cholesterol, insulin, CRP, HOMA-IR
-Holscher et al. -2018 -USA [55]	-Healthy women and men, 25-75 yrs - 18 - -	-A controlled-eating randomized cross-over -3 wks -42 g walnuts/d -0 g walnuts/d	-↑↓ α -diversity -↑ Relative abundance of *Firmicutes* -↓ Relative abundance of *Actinobacteria* -↑ Bacterial genera: *Faecalibacterium, Clostridium, Roseburia, and Dialister*	-↓ LDL and total cholesterol -↑↓ HDL, triglycerides, glucose, IL-6, CRP
-Lear et al. -2019 -UK [182]	-Non-obese men and women, 40-60 yrs -13 -15	-A randomized, placebo controlled -4 wk -Montmorency cherry supplementation (296 mg total anthocyanins and 1040 mg total polyphenols per day) -Cherry flavoured placebo	-↑↓ *Bacteroides and Faecalibacterium* abundance, species richness and diversity	-↓ Matsuda index (↑ insulin concentration) -↑ Oral glucose insulin sensitivity after placebo -↑↓ IL-6 and CRP
-Lima et al. -2019 -Brazil [39]	-Healthy young female subjects, 28.5±8.4 yrs -10	-A controlled non-randomized, intergroup -30 d basal period, 60 d, 30 d washout	-↑ *Bifidobacterium spp*., *lactobacillus spp,* and total anaerobic bacteria after 60 d -↓ NH4+ (after 60 d)	-After 60 d: ↓ glucose, insulin, triglycerides, total cholesterol, LDL-C, HOMA-IR -After 30 d: ↓ LDL-C

	- - 	-Orange juice (300ml/d) - -	-↑ SCFAs production for acetic acid but ↓ for propionic acid (after 60 d)	
-Medina-Vera et al. -2019 -Mexico [45]	-Men and women with type 2 diabetes and healthy controls, 30-60 yrs -81 -30	-A double-blinded, randomized, placebo-controlled -1 mo -Reduced energy diet + dietary portfolio (dehydrated nopal, chia seeds, soy protein and inulin) -Reduced energy diet + placebo (caseinate and maltodextrin)	-↑ α-diversity -↓ *P. copri* -↑ *Faecalibacterium prausnitzii and Akkermansia muciniphila*, abundance of *Bifidobacterium longum,* and *B. fragilis*	-↓ Glucose, total cholesterol, LDL, free fatty acids, HbA1c, triglycerides and CRP -↑ Antioxidant activity
-Moreno-Indias et al. -2016 -Spain [40]	-Healthy and obese (metabolic syndrome, MetS) men, 48±2 yrs -10 (healthy) -10 (MetS)	-A randomized, crossover, controlled, intervention ->30-d -Red wine -De-alcoholized red wine	-↑↓ Phyla *Bacteroidetes, Firmicutes, Actinobacteria, Proteobaceria* and respective genera after red wine or de-alcoholized red wine -MetS patients: ↑ number of *Fusobacteria* and *Bacteroidetes* and ↓ *Firmicutes* after red wine and de-alcoholized red wine intake -Healthy subjects: ↑ *Bacteroidetes* red wine and the de-alcoholized red wine intake	-↑ Triglycerides, gamma-glutamyl transferase in MetS group after both interventions -MetS: ↓ glucose, triglycerides, total cholesterol, CRP and LPS, and ↑serum level of HDL compared to baseline after both interventions -Healthy: ↓ total cholesterol compared to baseline after both interventions

			.	
-Ni et al. -2018 -China [41]	-Elderly men and women with metabolic syndrome (BMI ≥ 25.0 kg/m^2) and healthy elderly men and women (60-90 yrs) -12 -11	-A controlled, pre- and post-treatment comparison -4 wks -Traditional Chinese Herbal Formula 200 mLx2/d (Polygonatum sibiricum, Lycium barbarum Rehmannia glutinosa Libosch Rhodiola rosea Panax notoginseng, Ligusticum chuanxiong Hort , Lumbricus, Radix puerariae and Folium nelumbinis)	-↑ Relative abundance of *Moraxellaceae, Acinetobacter,* species *Acinetobacter Incertae Sedis* and *Erysipelotrichaceae Incertae Sedis* -↓ Relative abundance of *Aproteobacteria, Rhizobiales, genus Bacteroidales Incertae Sedis*, and species *Enterobacteriaceae Incertae Sedis*	-↓ Lipoprotein (a) -↑↓ Glucose, cholesterol, triglycerides, LDL, HDL, and uric acid
-Puupponen-Pimiä et al. -2013 -Finland [42]	-Men and women with metabolic syndrome -20 -12	-A randomized, controlled intervention -16 wks (8 wks intervention) -300 g/d fresh berries -No berries	-↑↓ Similarity values or diversity of predominant bacterial populations	-↑ Leptin -↑↓ Cholesterol, HDH, LDL
-Ravn-Haren et al. -2013 -Denmark [43]	-Healthy men and women, 18-69 yrs (BMI ≥ 25.0 kg/m^2) -23	-A randomized, single-blinded, crossover -5x4 wks -Control period, whole apples (550 g/day),	-↑↓ Composition of the gut microbiota	-↓ LDL after whole apple, pomace and cloudy juice intake -↑ LDL with clear juice compared to whole apple and pomace

	--	apple pomace (22 g/day), clear and cloudy apple juices (500 ml/day)		-↑↓ HDL-cholesterol, triglycerides, inflammation (hs-CRP), insulin, IGF1 and IGFBP3
-Redondo et al. -2019 -Spain [183]	-Healthy women and men (25-64 yrs) with BMI 19-28 kg/m^2 -30 --	-A randomized cross-over -3x5 wks -250g/yogurt per day (cow's milk yogurt 3 % fat, CW, ewe's milk yogurt, semi-skimmed 2.8 % fat, ES and ewe's milk yogurt, 5.8 % fat, EW) --	-↑↓ Gut microbiota by the intervention -Women in Cho1-group A: ↓ *Blautia coccoides - Eubacterium rectale* during EW compared to ES	-↑↓ Insulin, leptin, IL-8, TNF-α, VCAM, MCP, ICAM-1, P-selectin, IL-10 by the intervention -Cho1-group A: ↓ ICAM-1 after EW compared to CW and ↓ P-selectin after EW compared to ES -Women in Cho1-group A: ↓ MCP after EW compared to ES and CW (Cho1-group A= subjects with highest total cholesterol/HDL-cholesterol index, n=10)
-Sheflin et al. -2015 -USA [184]	-Healthy men and women -4 -3	-A randomized, single-blinded, controlled, pilot -4 wks -Study meals or snacks (meals, e.g., casseroles, soups and snacks e.g., smoothies, granola, crackers) containing 30 g/d heat-stabilized rice bran -Meals/snacks without stabilized rice bran	-↑ Eight operational taxonomic units, including three from *Bifidobacterium* and *Ruminococcus genera* after 2 wks -↑ Branched chain fatty acids, secondary bile acids and eleven other microbial metabolite	-↑ Cholesterol and bile acid metabolites, -↓ Fatty acids: sebacic acid, 2-hexendioic acid and pentadecanoic acid

-Wilson et al. -2018 -New Zealand [44]	-Men and women 44-85 yrs with prediabetes (BMI 29.4±7.3) -24 - -	-A pilot intervention -12 wks -Two kiwifruits per d --	-↑ Uncharacterized bacterial family Coriobacteriaceae members -↓↑ α-diversity	-↑ Plasma vitamin C level -↓ HbA1c and ↑ fasting glucose (not clinically significant)
Interventions with an overall healthy diet, a functional diet or a multifunctional diet				
-Dao et al. -2016 -France [47]	-Obese or overweight men and women -49 --	-Follow-up with two subsequent intervention periods -2x6 wks -Calorie restriction period followed by -Weight stabilisation diet -Participants divided to high (abundance≥median) and low (<median) Akkermansia abundance muciniphila abundance groups (Akk HI, Akk LO)	-Akk HI: ↓ *A. muciniphila* abundance after calorie restriction and total intervention period compared to Akk LO -*A. muciniphila* was associated with microbial species known to be related to health	-Akk HI: improvements in insulin sensitivity markers, total cholesterol and LDL after calorie restriction and total intervention period

-Fava et al. -2013 -UK [185]	-Men and women at high risk of MetS, 30-65 yrs -HS (high saturated fatty acids): 11 -HM/HGI (high MUFA/high GI): 17 -HM/LGI (high MUFA/low GI): 22 -HC/HGI (high CHO/high GI): 21 -HC/LGI (high CHO/low GI): 17 (GI=glycemic index)	-A randomized controlled -24 wks - Run-in diet HS → one of the experimental diets or HS diet -HM/HGI -HM/LGI -HC/HGI -HC/LGI	-MUFA diets: ↑↓ individual bacterial population numbers, ↓ total bacteria -HC diets: ↑ *Bifidobacterium* compared to baseline -HC/HGI: ↑ *Bacteroides* -HC/LGI and HS ↑ *Faecalibacterium prausnitzii* -HM/HGI and HM/LGI: ↓ total bacteria -HS: ↑ SCFA	-MUFA diets: ↓ total cholesterol and LDL -HC diets: ↓ glucose and cholesterol levels compared to baseline
-Haro et al. -2016 -Spain [51]	-Obese men with coronary heart disease, 20-75 yrs -10 -10	-A randomized intervention -One year -Mediterranean diet (Med diet) - Low fat, high complex carbohydrate diet (LFHCC)	-LFHCC: ↑ *Prevotella,* ↓ *Roseburia genera* -Med diet: ↓ *Prevotella*, ↑ *Roseburia* and *Oscillospira* genera -Both diets after long-term usage: ↑ abundance of *Parabacteroides distasonis* and *Faecalibacterium prausnitzii*	-↑↓ Glucose, HbA1c, HDL, LDL, triglycerides, total cholesterol -↑ Insulin sensitivity (both diets)

			-The changes in the abundance of 7 of 572 metabolites found in feces	
-Karusheva et al. -2019 -Germany [186]	-Men and women with type 2 diabetes (54±4 yrs) -12 --	-A randomized controlled crossover trial -4 wk -Isocaloric diet with branched-chain amino acids (BCAA) -Diet without BCAA (BCAA-)	-↑ *Bacteroidetes* -↓ *Firmicutes*	-↑↓ Insulin sensitivity, blood glucose, free fatty acids -↑ Postprandial insulin sensitivity, circulating fibroblast-growth factor 21 after BCAA-diet -↓ BCAAs, insulin secretion, CRP after BCAA-diet
-Kong et al. -2013 -France [50]	-Obese or overweight men and women, 26-65 yrs -Cluster A: 17 (continued to lost weight) -Cluster B: 15 (weight remained stable) -Cluster C: 17 (weight regain)	-Clinical -12 wks -Energy restriction diet and after -Weight maintenance	-Weight regain positively association with number of the *Lactobacillus, Leuconostoc, Pediococcus* group in feces	-Weight regain positive association with insulin, insulin resistant indices, and inflammatory markers (such as leukocytes, neutrophils, and IL-6) -Weight regain negative association with insulin sensitivity indices and the AUC of free fatty acids -Predictors for clusters: plasma insulin, IL-6, leukocyte number, and adipose tissue at baseline

-Marungruang et al. -2018 -Sweden [187]	-Healthy men and women with obesity or overweight, (BMI 25-33), 51-72 yrs -23 -24	-A randomized, controlled, parallel -8 wks -Multifunctional diet -Control diet (lacking the functional "active" components)	-↑↓ Phylum or genus taxonomic levels -↑↓ α-diversity -↑ Abundance of *Prevotella copri*	-↓ Total cholesterol, LDL, LDL-to-HDL ratio, Apo B-to-Apo A1 ratio, and triglycerides -↓ Reynold's cardiovascular risk score (based on age, systolic blood pressure, type 2 diabetes, smoking, HDL, total cholesterol, CRP, parental myocardial infarction <60y)
-Ostan et al. -2015 -Italy [188]	-Healthy elderly men and women, 65-85 yrs (BMI 22-30 kg/m2) -Arm A: 29 -Arm B: 30 -Arm C: 30 -Arm D: 31	-A randomized, four-arm, intervention -56 d -RISTOMED alone (A) -RISTOMED diet supplementation +probiotics (B), +fruit peel extracted monoterpene d-Limonene (C), +Argan oil (D) RISTOMED= healthy, personalized and balanced dietary intervention, enriched or not with specific nutraceutical compounds, to decrease and prevent inflammageing, oxidative	-Arm C: ↑ CL/B ratio Arm B: ↓ CL/B ratio (*Clostridium cluster IV and Bifidobacteria*) -Arm B: ↑ *Bifidobacteria*	-↑↓ hsCRP variations -RISTOMED: ↓ erythrocytes sedimentation rate -Arm C: ↓ fibrinogen, HOMAIR, insulin -Arms A and C: ↓ glucose -All arms: ↓ erythrocytes sedimentation rate -Arm D: ↑ fibrinogen

		stress and gut microbiota alteration		
-Salonen et al. -2014 -Finland -Lobley et el. -2013 -UK [189, 190]	-Obese males with metabolic syndrome, 27-73 yrs -14 --	-A randomized cross-over -10 wks (1 wk weight maintenance diet (M) followed by 2 diets for 3 wks each in randomized cross-over design (RS or NSP), followed by 3 wks weight-loss diet, WL) -Diet supplemented with resistant starch (RS) -Diet including non-starch polysaccharides (NSP) -Weight-loss (WL) diet (high protein, medium carbohydrate levels)	-After RS: ↓ α-diversity, richness and evenness compared to NSP and WL diets -Overall microbiota of the subjects differed between the diets -↑ Multiple *Ruminococcaceae phylotypes* (RS) -↑ *Lachnospiraceae phylotypes* (NSP) -↓ *Bifidobacteria* (WL) - RS and WL: ↓ SCFA acetate, propionate and butyrate, succinate compared to WL and M	-WL: ↓ Insulin, C-peptide, HOMA2-IR -↑↓ Glucose -WL: improvements in pancreatic function and insulin sensitivity compared to other diets -RS: ↓ HOMA2-IR compared to M and NSP diets.
-Tindall et al. -2019 and 2020 -the USA [56, 57]	-Men and women at risk for cardiovascular disease, 30-65 yrs , BMI 25-40 kg/m2 -45	-A randomized, controlled, 3-period, crossover, feeding trial -2 wks run-in diet, 3x6 wks isocaloric diets -Western diet (run-in), 3 isocaloric weight-maintenance diets:	-WD: most abundant relative taxa: *Roseburia, Eubacterium eligensgroup, Lachnospiraceae UCG004, Leuconostocaceae* -WD: *Gordonibacter* relative to WFMD	-WD: ↓ brachial and central mean arterial pressure -All diets: ↓ total cholesterol, LDL, HDL, and non-HDL cholesterol

	--	walnut diet WD), walnut FA-matched diet (WFMD), oleic acid–replaced-ALA diet (ORAD)	-WFMD : *Roseburia* (3.6%, LDA = 4) and *Eubacterium* eligensgroup -ORAD: *Clostridialesvadin* BB60 group and gut metagenome	
-Zeevi et al. -2015 -Israel [52]	-Healthy men and female -14 (expert-based intervention -12 (predictor-based intervention) --	-A blinded randomized personalized two-arm controlled dietary intervention based on the algorithm (prediction) including blood parameters, dietary habits, anthropometrics, physical activity, and gut microbiota measured in 800-person cohort and validated in 100-person cohort -1 wk -Predictors for good and bad diets -Without predictors	-Predictor-based diets: Consistent gut microbiota alterations Good week: -↑ *Roseburia inulinivorans, Eubacterium eligens, Alistipes putredinis* -↓*Bifidobacterium adolescentis, Anaerostipes* Bad week: -↑ *Bifidobacterium adolescentis, Anaerostipes* -↓ *Roseburia inulinivorans, Eubacterium eligens, Bacteroides vulgatus*	-↑ Glucose metabolism, such as postprandial responses, fluctuations in blood glucose levels after 1-week intervention

[a] Apo=Apolipoprotein, AUC=Area under curve, CRP=C-reactive protein, G-CSF=Granulocyte-colony stimulating factor, GLP-1=Glucagon-like peptide 1, GLP-2=Glucagon-like peptide 2, HbA1c=Hemoglobin-A1c, HDL=High density lipoprotein, HOMA-IR= Homeostatic Model Assessment for Insulin Resistance, ICAM-1= Intercellular Adhesion Molecule 1, IgG=Immunoglobulin G, IGF1= Insulin-like growth factor 1, IGFBP3= Insulin-like growth factor-binding protein 3, IL=Interleukin, LBP=Lipopolysaccharide binding protein, LDL=Low density lipoprotein, LPS=Lipopolysaccharides, MCP= Blood monocyte chemotactic protein-1, OGTT=Oral

glucose tolerance test, PYY=Gut hormone peptide YY, SCFA=Short-chain fatty acid, Th17=T helper 17 cell, TNF-α=Tumor necrosis factor α, Treg=Regulatory T cell, VCAM= Vascular cell adhesion protein.

Table 3. Intervention studies examining the impacts of exercise on microbiota and serum metabolic markers considered to be cardioprotective.

-Author **-Year** **-Country**	**-Population** -N (intervention) -N (control)	**-Design** **-Duration** **-Intervention** **-Control**	**Results**	
			Serum markers	**Microbiota and faecal metabolites**
Athletes				
-Murtaza -2019 -Australia [74]	-race walkers -21	-intervention study -3 weeks -intensified training program -no control group -also assignment to high carbohydrate, periodised carbohydrate or ketogenic low carbohydrate diets	-not reported	Distinct enterotypes, with either a Prevotella or Bacteroides dominated enterotype, were relatively stable and remained evident after training
-Zhao -2018 -China [77]	-marathon runners -20	-intervention study -24 hours -half marathon -no control group	-not reported	-no change in alpha diversity -↑ Coriobacteriaceae -↑ organic acids -↓ nucleic acid components
-Keohane -2019 -Ireland [75]	-rowers -4	-intervention study -33 days -transoceanic rowing race	-not reported	During race: -↑ microbial diversity

		-no control group		-↑ abundance of butyrate producing species -↑ Dorea longicatena, Roseburia hominis and members of genus subdoligranum -↓ Bacteroides finegoldii
-Scheiman -2019 -USA [78]	-marathon runners -15 -sedentary control -10 -rowers and ultramarathoners -87	-intervention study and control population -days before and after marathon -marathon -control: no marathon -replication: rowers and ultramarathoners -duration not reported -exercise	-not reported	Veillonella most abundant microbiota feature days after marathon Results reproduced in 87 athletes
-Hampton-Marcell -2020 -USA [76]	-swimmers -16 -no control group	-intervention study -months -tapering of training volume (32.6 km/wk to 11.3 km/wk) -no control group	-not reported	-↓overall microbial diversity -in microbial community structural similarity -↓ faecalibacterium and coprococcus
Healthy subjects				

-Cronin -2018 -Ireland [83]	-healthy sedentary adults -90	-intervention study -8 weeks -exercise program -no control group	-not reported	-no significant modulation in taxonomic composition or metabolic pathways compared to baseline
-Allen -2018 -USA [80]	-lean subjects -18 -obese subjects -14	-intervention study -6 weeks -supervised, endurance based exercise training -6 weeks sedentary washout period	-lean: increase of SCFA after 6 weeks exercise training	Exercise-induced changes beta diversity dependent on obesity status Exercise-induced shifts in metabolic output of the microbiota paralleled changes in bacterial genes and taxa capable of short-chain fatty acid production
-Munukka -2018 -Finland [82]	-sedentary obese women -17	-intervention -6 weeks -endurance exercise intervention -no control group	-decrease in VLDL -no change in CRP	-↑ Akkermansia -↓ Proteobacteria
-Taniguchi -2018 -Japan [81]	-elderly men -33	-intervention study -5 weeks -endurance exercise program -no control group	-not reported	-effect on gut microbiota diversity was not greater than interindividual differences -changes in α-diversity indices during

				intervention were negatively correlated with changes in systolic and diastolic blood pressure -↓ relative abundance of Clostridium difficile -↑relative abundance of Oscillospira
-Erickson -2019 -USA [98]	-obese adults -16	-intervention study -12 weeks -exercise 5 days/week with either hypocaloric or eucaloric diet -no control group	-hypocaloric diet decreased change in TMAO after 12 weeks -eucaloric diet increased change -absolute TMAO levels not altered	-not reported
-Morita -2019 -Japan [79]	-elderly wonen -32	-intervention study with non-randomized allocation -12 weeks -trunk muscle training or aerobic exercise -no control group	-not reported	-↑ Bacteroides in aerobic exercise group
Patients				
-Pasini -2019 -Italy [85]	-type 2 diabetes patients -30	-intervention study -6 months -endurance, resistance and flexibility training -no control group	-↓ glucose and markers of systemic inflammation	-↓ Mycetes

-Cronin -2019 -Ireland [83]	-inflammatory bowel disease patients -20	-randomized cross over trial -8 weeks -combined aerobic and resistance training -control situation: no exercise training	-not reported	-no clinically significant alterations in the alpha- and beta-diversity of gut microbiota

Table 4. Research gaps in intervention studies attempting to reveal lifestyle-gut microbiota-cardiovascular health pathway.

Area of research	Identified gaps
Lifestyle interventions	• Absence of proof of causality necessitating well-designed and powered intervention trials • Lack of definition of the overall lifestyle behavior (diet, physical exercise, smoking behavior and medication) that induces beneficial effects in gut microbiota composition and function and subsequently in cardiovascular health markers and end-point diseases • Limited knowledge on potential benefits of specific nutraceuticals, in the context of general diet, in modifying gut microbiota and cardiovascular health
Mechanisms	• Limited understanding on the specific molecular mechanisms mediating the effect of known microbial metabolites such as TMAO, bile acids and SFCA in the diet-microbiota-cardiovascular health interaction. • Presence of many 'unknown' metabolites which need to be characterized, especially their capacity to bind to specific receptors • Lack of knowledge on the specific dietary compounds modulating the microbiota-health interaction.
Methods	• Absence of standardized/normalized gut microbiota analytics and bioinformatics tools • Differences in metabolism between animals and humans • Lack of automatized tools for evaluation of food consumption and nutrient intakes • Paucity of novel risk markers for cardiovascular health (e.g. involving metabolomics) allowing evaluation of the intervention effects on complex metabolic network • Lack of personalized approaches, including consideration of gene polymorphism or baseline diet or gut microbiota in the allocation to the intervention groups

References

[1] WHO | Disease burden and mortality estimates, **2020**.

[2] Z. Jie, H. Xia, S. Zhong, Q. Feng, S. Li, S. Liang*, et al.*, The gut microbiome in atherosclerotic cardiovascular disease, *Nat Commun***8**(2017) pp. 845.

[3] A.L. Jonsson, F. Bäckhed, Role of gut microbiota in atherosclerosis, *Nat Rev Cardiol***14**(2017) pp. 79-87.

[4] W.H.W. Tang, T. Kitai, S.L. Hazen, Gut Microbiota in Cardiovascular Health and Disease, *Circ Res***120**(2017) pp. 1183-96.

[5] T. Katsimichas, A.S. Antonopoulos, A. Katsimichas, T. Ohtani, Y. Sakata, D. Tousoulis, The intestinal microbiota and cardiovascular disease, *Cardiovasc Res***115**(2019) pp. 1471-86.

[6] I. Attaye, S. Pinto-Sietsma, H. Herrema, M. Nieuwdorp, A Crucial Role for Diet in the Relationship Between Gut Microbiota and Cardiometabolic Disease, *Annu Rev Med***71**(2020) pp. 149-61.

[7] J. Qin, R. Li, J. Raes, M. Arumugam, K.S. Burgdorf, C. Manichanh*, et al.*, A human gut microbial gene catalogue established by metagenomic sequencing, *Nature***464**(2010) pp. 59-65.

[8] M. Arumugam, J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D.R. Mende*, et al.*, Enterotypes of the human gut microbiome, *Nature***473**(2011) pp. 174-80.

[9] J.C. Clemente, L.K. Ursell, L.W. Parfrey, R. Knight, The impact of the gut microbiota on human health: an integrative view, *Cell***148**(2012) pp. 1258-70.

[10] J. Li, H. Jia, X. Cai, H. Zhong, Q. Feng, S. Sunagawa*, et al.*, An integrated catalog of reference genes in the human gut microbiome, *Nat Biotechnol***32**(2014) pp. 834-41.

[11] G.D. Wu, J. Chen, C. Hoffmann, K. Bittinger, Y. Chen, S.A. Keilbaugh*, et al.*, Linking long-term dietary patterns with gut microbial enterotypes, *Science***334**(2011) pp. 105-8.

[12] Y. Janssens, J. Nielandt, A. Bronselaer, N. Debunne, F. Verbeke, E. Wynendaele*, et al.*, Disbiome database: linking the microbiome to disease, *BMC Microbiol***18**(2018) pp. 50.

[13] Disbiome database, https://disbiome.ugent.be/home.

[14] W.H.W. Tang, F. Bäckhed, U. Landmesser, S.L. Hazen, Intestinal Microbiota in Cardiovascular Health and Disease: JACC State-of-the-Art Review, *J Am Coll Cardiol***73**(2019) pp. 2089-105.

[15] M. Busnelli, S. Manzini, G. Chiesa, The Gut Microbiota Affects Host Pathophysiology as an Endocrine Organ: A Focus on Cardiovascular Disease, *Nutrients***12**(2019) .

[16] H.K. Pedersen, V. Gudmundsdottir, H.B. Nielsen, T. Hyotylainen, T. Nielsen, B.A.H. Jensen*, et al.*, Human gut microbes impact host serum metabolome and insulin sensitivity, *Nature***535**(2016) pp. 376-81.

[17] M. Onyszkiewicz, K. Jaworska, M. Ufnal, Short chain fatty acids and methylamines produced by gut microbiota as mediators and markers in the circulatory system, *Exp Biol Med (Maywood)***245**(2020) pp. 166-75.

[18] S.H. Zeisel, M. Warrier, Trimethylamine N-Oxide, the Microbiome, and Heart and Kidney Disease, *Annu Rev Nutr***37**(2017) pp. 157-81.

[19] C. Shortt, O. Hasselwander, A. Meynier, A. Nauta, E.N. Fernández, P. Putz*, et al.*, Systematic review of the effects of the intestinal microbiota on selected nutrients and non-nutrients, *Eur J Nutr***57**(2018) pp. 25-49.

[20] K. Mokkala, N. Houttu, T. Cansev, K. Laitinen, Interactions of dietary fat with the gut microbiota: Evaluation of mechanisms and metabolic consequences, *Clin Nutr***39**(2020) pp. 994-1018.

[21] F. Brighenti, M.C. Casiraghi, E. Canzi, A. Ferrari, Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers, *Eur J Clin Nutr***53**(1999) pp. 726-33.

[22] J. Foerster, G. Maskarinec, N. Reichardt, A. Tett, A. Narbad, M. Blaut*, et al.*, The influence of whole grain products and red meat on intestinal microbiota composition in normal weight adults: a randomized crossover intervention trial, *PLoS ONE***9**(2014) pp. e109606.

[23] A. Velikonja, L. Lipoglavšek, M. Zorec, R. Orel, G. Avguštin, Alterations in gut microbiota composition and metabolic parameters after dietary intervention with barley beta glucans in patients with high risk for metabolic syndrome development, *Anaerobe***55**(2019) pp. 67-77.

[24] J. Vulevic, A. Juric, G. Tzortzis, G.R. Gibson, A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults, *J Nutr***143**(2013) pp. 324-31.

[25] S. Xiao, N. Fei, X. Pang, J. Shen, L. Wang, B. Zhang*, et al.*, A gut microbiota-targeted dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome, *FEMS Microbiol Ecol***87**(2014) pp. 357-67.

[26] E.E. Canfora, van der Beek, Christina M., G.D.A. Hermes, G.H. Goossens, J.W.E. Jocken, J.J. Holst*, et al.*, Supplementation of Diet With Galacto-oligosaccharides Increases Bifidobacteria, but Not Insulin Sensitivity, in Obese Prediabetic Individuals, *Gastroenterology***153**(2017) pp. 87,97.e3.

[27] A.L. Carvalho-Wells, K. Helmolz, C. Nodet, C. Molzer, C. Leonard, B. McKevith*, et al.*, Determination of the in vivo prebiotic potential of a maize-based whole grain breakfast cereal: a human feeding study, *Br J Nutr***104**(2010) pp. 1353-6.

[28] S.T. Clarke, J.M. Green-Johnson, S.P.J. Brooks, D.D. Ramdath, P. Bercik, C. Avila*, et al.*, β2-1 Fructan supplementation alters host immune responses in a manner consistent with increased exposure to microbial components: results from a double-blinded, randomised, cross-over study in healthy adults, *Br J Nutr***115**(2016) pp. 1748-59.

[29] A. Costabile, A. Klinder, F. Fava, A. Napolitano, V. Fogliano, C. Leonard*, et al.*, Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study, *Br J Nutr***99**(2008) pp. 110-20.

[30] E.M. Dewulf, P.D. Cani, S.P. Claus, S. Fuentes, P.G.B. Puylaert, A.M. Neyrinck*, et al.*, Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women, *Gut***62**(2013) pp. 1112-21.

[31] Z. Jie, L. Bang-Yao, X. Ming-Jie, L. Hai-Wei, Z. Zu-Kang, W. Ting-Song*, et al.*, Studies on the effects of polydextrose intake on physiologic functions in Chinese people, *Am J Clin Nutr***72**(2000) pp. 1503-9.

[32] H.M. Roager, J.K. Vogt, M. Kristensen, L.B.S. Hansen, S. Ibrügger, R.B. Mærkedahl*, et al.*, Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial, *Gut***68**(2019) pp. 83-93.

[33] R.R. Robinson, J. Feirtag, J.L. Slavin, Effects of dietary arabinogalactan on gastrointestinal and blood parameters in healthy human subjects, *J Am Coll Nutr***20**(2001) pp. 279-85.

[34] S. Schutte, D. Esser, F.P.M. Hoevenaars, Hooiveld, Guido J. E. J., M.G. Priebe, R.J. Vonk*, et al.*, A 12-wk whole-grain wheat intervention protects against hepatic fat: the Graandioos study, a randomized trial in overweight subjects, *Am J Clin Nutr***108**(2018) pp. 1264-74.

[35] C.J. Rebello, J. Burton, M. Heiman, F.L. Greenway, Gastrointestinal microbiome modulator improves glucose tolerance in overweight and obese subjects: A randomized controlled pilot trial, *J Diabetes Complicat***29**(2015) pp. 1272-6.

[36] P. Vitaglione, I. Mennella, R. Ferracane, A.A. Rivellese, R. Giacco, D. Ercolini*, et al.*, Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber, *Am J Clin Nutr***101**(2015) pp. 251-61.

[37] A. Ampatzoglou, K.K. Atwal, C.M. Maidens, C.L. Williams, A.B. Ross, F. Thielecke*, et al.*, Increased whole grain consumption does not affect blood biochemistry, body composition, or gut microbiology in healthy, low-habitual whole grain consumers, *J Nutr***145**(2015) pp. 215-21.

[38] D.J. Jenkins, V. Vuksan, A.V. Rao, E. Vidgen, C.W. Kendall, N. Tariq*, et al.*, Colonic bacterial activity and serum lipid risk factors for cardiovascular disease, *Metab Clin Exp***48**(1999) pp. 264-8.

[39] A.C.D. Lima, C. Cecatti, M.P. Fidélix, M.A.T. Adorno, I.K. Sakamoto, T.B. Cesar*, et al.*, Effect of Daily Consumption of Orange Juice on the Levels of Blood Glucose, Lipids, and Gut Microbiota Metabolites: Controlled Clinical Trials, *J Med Food***22**(2019) pp. 202-10.

[40] I. Moreno-Indias, L. Sánchez-Alcoholado, P. Pérez-Martínez, C. Andrés-Lacueva, F. Cardona, F. Tinahones*, et al.*, Red wine polyphenols modulate fecal microbiota and reduce markers of the metabolic syndrome in obese patients, *Food Funct***7**(2016) pp. 1775-87.

[41] Y. Ni, C. Mu, X. He, K. Zheng, H. Guo, W. Zhu, Characteristics of gut microbiota and its response to a Chinese Herbal Formula in elder patients with metabolic syndrome, *Drug Discov Ther***12**(2018) pp. 161-9.

[42] R. Puupponen-Pimiä, T. Seppänen-Laakso, M. Kankainen, J. Maukonen, R. Törrönen, M. Kolehmainen*, et al.*, Effects of ellagitannin-rich berries on blood lipids, gut microbiota, and urolithin production in human subjects with symptoms of metabolic syndrome, *Mol Nutr Food Res***57**(2013) pp. 2258-63.

[43] G. Ravn-Haren, L.O. Dragsted, T. Buch-Andersen, E.N. Jensen, R.I. Jensen, M. Németh-Balogh*, et al.*, Intake of whole apples or clear apple juice has contrasting effects on plasma lipids in healthy volunteers, *Eur J Nutr***52**(2013) pp. 1875-89.

[44] R. Wilson, J. Willis, R.B. Gearry, A. Hughes, B. Lawley, P. Skidmore*, et al.*, SunGold Kiwifruit Supplementation of Individuals with Prediabetes Alters Gut Microbiota and Improves Vitamin C Status, Anthropometric and Clinical Markers, *Nutrients***10**(2018) .

[45] I. Medina-Vera, M. Sanchez-Tapia, L. Noriega-López, O. Granados-Portillo, M. Guevara-Cruz, A. Flores-López*, et al.*, A dietary intervention with functional foods reduces metabolic endotoxaemia and attenuates biochemical abnormalities by

modifying faecal microbiota in people with type 2 diabetes, *Diabetes Metab***45**(2019) pp. 122-31.

[46] J.L. Slavin, M.C. Martini, D.R. Jacobs, L. Marquart, Plausible mechanisms for the protectiveness of whole grains, *Am J Clin Nutr***70**(1999) pp. 459S-63S.

[47] M.C. Dao, A. Everard, J. Aron-Wisnewsky, N. Sokolovska, E. Prifti, E.O. Verger*, et al.*, Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology, *Gut***65**(2016) pp. 426-36.

[48] J. Fu, M.J. Bonder, M.C. Cenit, E.F. Tigchelaar, A. Maatman, J.A.M. Dekens*, et al.*, The Gut Microbiome Contributes to a Substantial Proportion of the Variation in Blood Lipids, *Circ Res***117**(2015) pp. 817-24.

[49] H. Röytiö, K. Mokkala, T. Vahlberg, K. Laitinen, Dietary intake of fat and fibre according to reference values relates to higher gut microbiota richness in overweight pregnant women, *Br J Nutr***118**(2017) pp. 343-52.

[50] L.C. Kong, P. Wuillemin, J. Bastard, N. Sokolovska, S. Gougis, S. Fellahi*, et al.*, Insulin resistance and inflammation predict kinetic body weight changes in response to dietary weight loss and maintenance in overweight and obese subjects by using a Bayesian network approach, *Am J Clin Nutr***98**(2013) pp. 1385-94.

[51] C. Haro, M. Montes-Borrego, O.A. Rangel-Zúñiga, J.F. Alcalá-Díaz, F. Gómez-Delgado, P. Pérez-Martínez*, et al.*, Two Healthy Diets Modulate Gut Microbial Community Improving Insulin Sensitivity in a Human Obese Population, *J Clin Endocrinol Metab***101**(2016) pp. 233-42.

[52] D. Zeevi, T. Korem, N. Zmora, D. Israeli, D. Rothschild, A. Weinberger*, et al.*, Personalized Nutrition by Prediction of Glycemic Responses, *Cell***163**(2015) pp. 1079-94.

[53] R. Estruch, E. Ros, J. Salas-Salvadó, M. Covas, D. Corella, F. Arós*, et al.*, Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts, *New England Journal of Medicine***378**(2018) pp. e34.

[54] M.Á Martínez-González, D. Corella, J. Salas-Salvadó, E. Ros, M.I. Covas, M. Fiol*, et al.*, Cohort profile: design and methods of the PREDIMED study, *Int J Epidemiol***41**(2012) pp. 377-85.

[55] H.D. Holscher, H.M. Guetterman, K.S. Swanson, R. An, N.R. Matthan, A.H. Lichtenstein*, et al.*, Walnut Consumption Alters the Gastrointestinal Microbiota, Microbially Derived Secondary Bile Acids, and Health Markers in Healthy Adults: A Randomized Controlled Trial, *J Nutr***148**(2018) pp. 861-7.

[56] A.M. Tindall, K.S. Petersen, A.C. Skulas-Ray, C.K. Richter, D.N. Proctor, P.M. Kris-Etherton, Replacing Saturated Fat With Walnuts or Vegetable Oils Improves Central

Blood Pressure and Serum Lipids in Adults at Risk for Cardiovascular Disease: A Randomized Controlled-Feeding Trial, *J Am Heart Assoc***8**(2019) pp. e011512.

[57] A.M. Tindall, C.J. McLimans, K.S. Petersen, P.M. Kris-Etherton, R. Lamendella, Walnuts and Vegetable Oils Containing Oleic Acid Differentially Affect the Gut Microbiota and Associations with Cardiovascular Risk Factors: Follow-up of a Randomized, Controlled, Feeding Trial in Adults at Risk for Cardiovascular Disease, *J Nutr***150**(2020) pp. 806-17.

[58] F. De Filippis, N. Pellegrini, L. Vannini, I.B. Jeffery, A. La Storia, L. Laghi*, et al.*, High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome, *Gut***65**(2016) pp. 1812-21.

[59] E.K. Mitsou, A. Kakali, S. Antonopoulou, K.C. Mountzouris, M. Yannakoulia, D.B. Panagiotakos*, et al.*, Adherence to the Mediterranean diet is associated with the gut microbiota pattern and gastrointestinal characteristics in an adult population, *Br J Nutr***117**(2017) pp. 1645-55.

[60] H. Gylling, J. Plat, S. Turley, H.N. Ginsberg, L. Ellegård, W. Jessup*, et al.*, Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease, *Atherosclerosis***232**(2014) pp. 346-60.

[61] R. Ayesh, J.A. Weststrate, P.N. Drewitt, P.A. Hepburn, Safety evaluation of phytosterol esters. Part 5. Faecal short-chain fatty acid and microflora content, faecal bacterial enzyme activity and serum female sex hormones in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margarine, *Food Chem Toxicol***37**(1999) pp. 1127-38.

[62] S. Baumgartner, R.P. Mensink, E.D. Smet, M. Konings, S. Fuentes, W.M. de Vos*, et al.*, Effects of plant stanol ester consumption on fasting plasma oxy(phyto)sterol concentrations as related to fecal microbiota characteristics, *J Steroid Biochem Mol Biol***169**(2017) pp. 46-53.

[63] M. Cuevas-Tena, J.D. Bermúdez, Silvestre, Ramona de Los Ángeles, A. Alegría, M.J. Lagarda, Impact of colonic fermentation on sterols after the intake of a plant sterol-enriched beverage: A randomized, double-blind crossover trial, *Clin Nutr***38**(2019) pp. 1549-60.

[64] X. Deng, J. Ma, M. Song, Y. Jin, C. Ji, W. Ge*, et al.*, Effects of products designed to modulate the gut microbiota on hyperlipidaemia, *Eur J Nutr***58**(2019) pp. 2713-29.

[65] Y. Dong, M. Xu, L. Chen, A. Bhochhibhoya, Probiotic Foods and Supplements Interventions for Metabolic Syndromes: A Systematic Review and Meta-Analysis of Recent Clinical Trials, *Ann Nutr Metab***74**(2019) pp. 224-41.

[66] S. Yan, Z. Tian, M. Li, B. Li, W. Cui, Effects of probiotic supplementation on the regulation of blood lipid levels in overweight or obese subjects: a meta-analysis, *Food Funct***10**(2019) pp. 1747-59.

[67] B. Pourrajab, S. Fatahi, A. Dehnad, H. Kord Varkaneh, F. Shidfar, The impact of probiotic yogurt consumption on lipid profiles in subjects with mild to moderate hypercholesterolemia: A systematic review and meta-analysis of randomized controlled trials, *Nutr Metab Cardiovasc Dis***30**(2020) pp. 11-22.

[68] S. Pu, H. Khazanehei, P.J. Jones, E. Khafipour, Interactions between Obesity Status and Dietary Intake of Monounsaturated and Polyunsaturated Oils on Human Gut Microbiome Profiles in the Canola Oil Multicenter Intervention Trial (COMIT), *Front Microbiol***7**(2016) pp. 1612.

[69] H. Watson, S. Mitra, F.C. Croden, M. Taylor, H.M. Wood, S.L. Perry*, et al.*, A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota, *Gut***67**(2018) pp. 1974-83.

[70] H. Rajkumar, N. Mahmood, M. Kumar, S.R. Varikuti, H.R. Challa, S.P. Myakala, Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial, *Mediators Inflamm***2014**(2014) pp. 348959.

[71] W.N. Hannah, S.A. Harrison, Lifestyle and Dietary Interventions in the Management of Nonalcoholic Fatty Liver Disease, *Dig Dis Sci***61**(2016) pp. 1365-74.

[72] J. Chen, Y. Guo, Y. Gui, D. Xu, Physical exercise, gut, gut microbiota, and atherosclerotic cardiovascular diseases, *Lipids Health Dis***17**(2018) pp. 17.

[73] J.P. Karl, L.M. Margolis, E.H. Madslien, N.E. Murphy, J.W. Castellani, Y. Gundersen*, et al.*, Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress, *Am J Physiol Gastrointest Liver Physiol***312**(2017) pp. G559-71.

[74] N. Murtaza, L.M. Burke, N. Vlahovich, B. Charlesson, H. O' Neill, M.L. Ross*, et al.*, The Effects of Dietary Pattern during Intensified Training on Stool Microbiota of Elite Race Walkers, *Nutrients***11**(2019) .

[75] D.M. Keohane, T. Woods, P. O'Connor, S. Underwood, O. Cronin, R. Whiston*, et al.*, Four men in a boat: Ultra-endurance exercise alters the gut microbiome, *J Sci Med Sport***22**(2019) pp. 1059-64.

[76] J.T. Hampton-Marcell, T.W. Eshoo, M.D. Cook, J.A. Gilbert, C.A. Horswill, R. Poretsky, Comparative Analysis of Gut Microbiota Following Changes in Training Volume Among Swimmers, *Int J Sports Med***41**(2020) pp. 292-9.

[77] X. Zhao, Z. Zhang, B. Hu, W. Huang, C. Yuan, L. Zou, Response of Gut Microbiota to Metabolite Changes Induced by Endurance Exercise, *Front Microbiol***9**(2018) pp. 765.

[78] J. Scheiman, J.M. Luber, T.A. Chavkin, T. MacDonald, A. Tung, L. Pham*, et al.*, Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism, *Nat Med***25**(2019) pp. 1104-9.

[79] E. Morita, H. Yokoyama, D. Imai, R. Takeda, A. Ota, E. Kawai*, et al.*, Aerobic Exercise Training with Brisk Walking Increases Intestinal Bacteroides in Healthy Elderly Women, *Nutrients***11**(2019) .

[80] J.M. Allen, L.J. Mailing, G.M. Niemiro, R. Moore, M.D. Cook, B.A. White*, et al.*, Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans, *Med Sci Sports Exerc***50**(2018) pp. 747-57.

[81] H. Taniguchi, K. Tanisawa, X. Sun, T. Kubo, Y. Hoshino, M. Hosokawa*, et al.*, Effects of short-term endurance exercise on gut microbiota in elderly men, *Physiol Rep***6**(2018) pp. e13935.

[82] E. Munukka, J.P. Ahtiainen, P. Puigbó, S. Jalkanen, K. Pahkala, A. Keskitalo*, et al.*, Six-Week Endurance Exercise Alters Gut Metagenome That Is not Reflected in Systemic Metabolism in Over-weight Women, *Front Microbiol***9**(2018) pp. 2323.

[83] O. Cronin, W. Barton, P. Skuse, N.C. Penney, I. Garcia-Perez, E.F. Murphy*, et al.*, A Prospective Metagenomic and Metabolomic Analysis of the Impact of Exercise and/or Whey Protein Supplementation on the Gut Microbiome of Sedentary Adults, *mSystems***3**(2018) .

[84] O. Cronin, W. Barton, C. Moran, D. Sheehan, R. Whiston, H. Nugent*, et al.*, Moderate-intensity aerobic and resistance exercise is safe and favorably influences body composition in patients with quiescent Inflammatory Bowel Disease: a randomized controlled cross-over trial, *BMC Gastroenterol***19**(2019) pp. 29.

[85] E. Pasini, G. Corsetti, D. Assanelli, C. Testa, C. Romano, F.S. Dioguardi*, et al.*, Effects of chronic exercise on gut microbiota and intestinal barrier in human with type 2 diabetes, *Minerva Med***110**(2019) pp. 3-11.

[86] J. Lee, J.P. Cooke, The role of nicotine in the pathogenesis of atherosclerosis, *Atherosclerosis***215**(2011) pp. 281-3.

[87] S.H. Lee, Y. Yun, S.J. Kim, E. Lee, Y. Chang, S. Ryu*, et al.*, Association between Cigarette Smoking Status and Composition of Gut Microbiota: Population-Based Cross-Sectional Study, *J Clin Med***7**(2018) .

[88] A. Zhernakova, A. Kurilshikov, M.J. Bonder, E.F. Tigchelaar, M. Schirmer, T. Vatanen*, et al.*, Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity, *Science***352**(2016) pp. 565-9.

[89] C. Huang, G. Shi, Smoking and microbiome in oral, airway, gut and some systemic diseases, *J Transl Med***17**(2019) pp. 225.

[90] A.L. Griffen, C.J. Beall, J.H. Campbell, N.D. Firestone, P.S. Kumar, Z.K. Yang*, et al.*, Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing, *ISME J***6**(2012) pp. 1176-85.

[91] B. Hong, M.V. Furtado Araujo, L.D. Strausbaugh, E. Terzi, E. Ioannidou, P.I. Diaz, Microbiome profiles in periodontitis in relation to host and disease characteristics, *PLoS ONE***10**(2015) pp. e0127077.

[92] S.L. Delima, R.K. McBride, P.M. Preshaw, P.A. Heasman, P.S. Kumar, Response of subgingival bacteria to smoking cessation, *J Clin Microbiol***48**(2010) pp. 2344-9.

[93] L. Biedermann, J. Zeitz, J. Mwinyi, E. Sutter-Minder, A. Rehman, S.J. Ott*, et al.*, Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans, *PLoS ONE***8**(2013) pp. e59260.

[94] L. Biedermann, K. Brülisauer, J. Zeitz, P. Frei, M. Scharl, S.R. Vavricka*, et al.*, Smoking cessation alters intestinal microbiota: insights from quantitative investigations on human fecal samples using FISH, *Inflamm Bowel Dis***20**(2014) pp. 1496-501.

[95] B.J. Bennett, de Aguiar Vallim, Thomas Q., Z. Wang, D.M. Shih, Y. Meng, J. Gregory*, et al.*, Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation, *Cell Metab***17**(2013) pp. 49-60.

[96] J.J. DiNicolantonio, M. McCarty, J. OKeefe, Association of moderately elevated trimethylamine N-oxide with cardiovascular risk: is TMAO serving as a marker for hepatic insulin resistance, *Open Heart***6**(2019) pp. e000890.

[97] S. Yang, X. Li, F. Yang, R. Zhao, X. Pan, J. Liang*, et al.*, Gut Microbiota-Dependent Marker TMAO in Promoting Cardiovascular Disease: Inflammation Mechanism, Clinical Prognostic, and Potential as a Therapeutic Target, *Front Pharmacol***10**(2019) pp. 1360.

[98] M.L. Erickson, S.K. Malin, Z. Wang, J.M. Brown, S.L. Hazen, J.P. Kirwan, Effects of Lifestyle Intervention on Plasma Trimethylamine N-Oxide in Obese Adults, *Nutrients***11**(2019) .

[99] Y. Heianza, D. Sun, X. Li, J.A. DiDonato, G.A. Bray, F.M. Sacks*, et al.*, Gut microbiota metabolites, amino acid metabolites and improvements in insulin sensitivity and glucose metabolism: the POUNDS Lost trial, *Gut***68**(2019) pp. 263-70.

[100] L.P. Smits, R.S. Kootte, E. Levin, A. Prodan, S. Fuentes, E.G. Zoetendal*, et al.*, Effect of Vegan Fecal Microbiota Transplantation on Carnitine- and Choline-Derived Trimethylamine-N-Oxide Production and Vascular Inflammation in Patients With Metabolic Syndrome, *J Am Heart Assoc***7**(2018) .

[101] W. Zhu, J.C. Gregory, E. Org, J.A. Buffa, N. Gupta, Z. Wang*, et al.*, Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk, *Cell***165**(2016) pp. 111-24.

[102] S. Chen, A. Henderson, M.C. Petriello, K.A. Romano, M. Gearing, J. Miao*, et al.*, Trimethylamine N-Oxide Binds and Activates PERK to Promote Metabolic Dysfunction, *Cell Metab***30**(2019) pp. 1141,1151.e5.

[103] R.A. Koeth, Z. Wang, B.S. Levison, J.A. Buffa, E. Org, B.T. Sheehy*, et al.*, Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis, *Nat Med***19**(2013) pp. 576-85.

[104] G. Ma, B. Pan, Y. Chen, C. Guo, M. Zhao, L. Zheng*, et al.*, Trimethylamine N-oxide in atherogenesis: impairing endothelial self-repair capacity and enhancing monocyte adhesion, *Biosci Rep***37**(2017) .

[105] A. Koh, A. Molinaro, M. Ståhlman, M.T. Khan, C. Schmidt, L. Mannerås-Holm*, et al.*, Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1, *Cell***175**(2018) pp. 947,961.e17.

[106] D.W. Russell, The enzymes, regulation, and genetics of bile acid synthesis, *Annu Rev Biochem***72**(2003) pp. 137-74.

[107] F. Kuipers, V.W. Bloks, A.K. Groen, Beyond intestinal soap--bile acids in metabolic control, *Nat Rev Endocrinol***10**(2014) pp. 488-98.

[108] B. Döring, T. Lütteke, J. Geyer, E. Petzinger, The SLC10 carrier family: transport functions and molecular structure, *Curr Top Membr***70**(2012) pp. 105-68.

[109] J.M. Ridlon, D. Kang, P.B. Hylemon, Bile salt biotransformations by human intestinal bacteria, *J Lipid Res***47**(2006) pp. 241-59.

[110] D. Schranner, G. Kastenmüller, M. Schönfelder, W. Römisch-Margl, H. Wackerhage, Metabolite Concentration Changes in Humans After a Bout of Exercise: a Systematic Review of Exercise Metabolomics Studies, *Sports Med Open***6**(2020) pp. 11.

[111] V. Meslier, M. Laiola, H.M. Roager, F. De Filippis, H. Roume, B. Quinquis*, et al.*, Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake, *Gut***69**(2020) pp. 1258-68.

[112] M.O. Weickert, J.G. Hattersley, I. Kyrou, A.M. Arafat, N. Rudovich, M. Roden*, et al.*, Effects of supplemented isoenergetic diets varying in cereal fiber and protein content on the bile acid metabolic signature and relation to insulin resistance, *Nutr Diabetes***8**(2018) pp. 11.

[113] E.A. Hanniman, G. Lambert, T.C. McCarthy, C.J. Sinal, Loss of functional farnesoid X receptor increases atherosclerotic lesions in apolipoprotein E-deficient mice, *J Lipid Res***46**(2005) pp. 2595-604.

[114] Y. Zhang, X. Wang, C. Vales, F.Y. Lee, H. Lee, A.J. Lusis*, et al.*, FXR deficiency causes reduced atherosclerosis in Ldlr-/- mice, *Arterioscler Thromb Vasc Biol***26**(2006) pp. 2316-21.

[115] E. Hambruch, S. Miyazaki-Anzai, U. Hahn, S. Matysik, A. Boettcher, S. Perović-Ottstadt*, et al.*, Synthetic farnesoid X receptor agonists induce high-density

lipoprotein-mediated transhepatic cholesterol efflux in mice and monkeys and prevent atherosclerosis in cholesteryl ester transfer protein transgenic low-density lipoprotein receptor (-/-) mice, *J Pharmacol Exp Ther***343**(2012) pp. 556-67.

[116] F. Nevens, P. Andreone, G. Mazzella, S.I. Strasser, C. Bowlus, P. Invernizzi*, et al.*, A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis, *N Engl J Med***375**(2016) pp. 631-43.

[117] T. Kida, Y. Tsubosaka, M. Hori, H. Ozaki, T. Murata, Bile acid receptor TGR5 agonism induces NO production and reduces monocyte adhesion in vascular endothelial cells, *Arterioscler Thromb Vasc Biol***33**(2013) pp. 1663-9.

[118] E. Studer, X. Zhou, R. Zhao, Y. Wang, K. Takabe, M. Nagahashi*, et al.*, Conjugated bile acids activate the sphingosine-1-phosphate receptor 2 in primary rodent hepatocytes, *Hepatology***55**(2012) pp. 267-76.

[119] A. Skoura, J. Michaud, D. Im, S. Thangada, Y. Xiong, J.D. Smith*, et al.*, Sphingosine-1-phosphate receptor-2 function in myeloid cells regulates vascular inflammation and atherosclerosis, *Arterioscler Thromb Vasc Biol***31**(2011) pp. 81-5.

[120] Y. Sui, J. Xu, J. Rios-Pilier, C. Zhou, Deficiency of PXR decreases atherosclerosis in apoE-deficient mice, *J Lipid Res***52**(2011) pp. 1652-9.

[121] S. Kim, R. Goel, A. Kumar, Y. Qi, G. Lobaton, K. Hosaka*, et al.*, Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure, *Clin Sci***132**(2018) pp. 701-18.

[122] K. Kasahara, K.A. Krautkramer, E. Org, K.A. Romano, R.L. Kerby, E.I. Vivas*, et al.*, Interactions between Roseburia intestinalis and diet modulate atherogenesis in a murine model, *Nat Microbiol***3**(2018) pp. 1461-71.

[123] G.D. Brinkworth, M. Noakes, P.M. Clifton, A.R. Bird, Comparative effects of very low-carbohydrate, high-fat and high-carbohydrate, low-fat weight-loss diets on bowel habit and faecal short-chain fatty acids and bacterial populations, *Br J Nutr***101**(2009) pp. 1493-502.

[124] S.A. Sowah, L. Riedl, A. Damms-Machado, T.S. Johnson, R. Schübel, M. Graf*, et al.*, Effects of Weight-Loss Interventions on Short-Chain Fatty Acid Concentrations in Blood and Feces of Adults: A Systematic Review, *Adv Nutr***10**(2019) pp. 673-84.

[125] H. Bartolomaeus, A. Balogh, M. Yakoub, S. Homann, L. Markó, S. Höges*, et al.*, Short-Chain Fatty Acid Propionate Protects From Hypertensive Cardiovascular Damage, *Circulation***139**(2019) pp. 1407-21.

[126] M. Li, van Esch, Betty C. A. M., G.T.M. Wagenaar, J. Garssen, G. Folkerts, P.A.J. Henricks, Pro- and anti-inflammatory effects of short chain fatty acids on immune and endothelial cells, *Eur J Pharmacol***831**(2018) pp. 52-9.

[127] E.C. Aguilar, A.J. Leonel, L.G. Teixeira, A.R. Silva, J.F. Silva, J.M.N. Pelaez*, et al.*, Butyrate impairs atherogenesis by reducing plaque inflammation and vulnerability and decreasing NFκB activation, *Nutr Metab Cardiovasc Dis***24**(2014) pp. 606-13.

[128] D. Zapolska-Downar, A. Siennicka, M. Kaczmarczyk, B. Kołodziej, M. Naruszewicz, Butyrate inhibits cytokine-induced VCAM-1 and ICAM-1 expression in cultured endothelial cells: the role of NF-kappaB and PPARalpha, *J Nutr Biochem***15**(2004) pp. 220-8.

[129] D. Zapolska-Downar, M. Naruszewicz, Propionate reduces the cytokine-induced VCAM-1 and ICAM-1 expression by inhibiting nuclear factor-kappa B (NF-kappaB) activation, *J Physiol Pharmacol***60**(2009) pp. 123-31.

[130] K. Bouter, G.J. Bakker, E. Levin, A.V. Hartstra, R.S. Kootte, S.D. Udayappan*, et al.*, Differential metabolic effects of oral butyrate treatment in lean versus metabolic syndrome subjects, *Clin Transl Gastroenterol***9**(2018) pp. 155.

[131] van der Beek, Christina M., E.E. Canfora, K. Lenaerts, F.J. Troost, Damink, Steven W. M. Olde, J.J. Holst*, et al.*, Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men, *Clin Sci***130**(2016) pp. 2073-82.

[132] K. Tamakoshi, H. Yatsuya, T. Kondo, Y. Hori, M. Ishikawa, H. Zhang*, et al.*, The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state, *Int J Obes Relat Metab Disord***27**(2003) pp. 443-9.

[133] W.H. Robinson, C.M. Lepus, Q. Wang, H. Raghu, R. Mao, T.M. Lindstrom*, et al.*, Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis, *Nat Rev Rheumatol***12**(2016) pp. 580-92.

[134] P. Conti, G. Ronconi, S.K. Kritas, A. Caraffa, T.C. Theoharides, Activated Mast Cells Mediate Low-Grade Inflammation in Type 2 Diabetes: Interleukin-37 Could Be Beneficial, *Can J Diabetes***42**(2018) pp. 568-73.

[135] F. Mastrangelo, I. Frydas, G. Ronconi, S.K. Kritas, L. Tettamanti, A. Caraffa*, et al.*, Low-grade chronic inflammation mediated by mast cells in fibromyalgia: role of IL-37, *J Biol Regul Homeost Agents***32**(2018) pp. 195-8.

[136] P. Libby, Inflammation in atherosclerosis, *Arterioscler Thromb Vasc Biol***32**(2012) pp. 2045-51.

[137] L. Ferrucci, E. Fabbri, Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty, *Nat Rev Cardiol***15**(2018) pp. 505-22.

[138] P.D. Cani, R. Bibiloni, C. Knauf, A. Waget, A.M. Neyrinck, N.M. Delzenne*, et al.*, Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice, *Diabetes***57**(2008) pp. 1470-81.

[139] M. Asada, E. Oishi, S. Sakata, J. Hata, D. Yoshida, T. Honda*, et al.*, Serum Lipopolysaccharide-Binding Protein Levels and the Incidence of Cardiovascular Disease in a General Japanese Population: The Hisayama Study, *J Am Heart Assoc***8**(2019) pp. e013628.

[140] H. Formes, C. Reinhardt, The gut microbiota - a modulator of endothelial cell function and a contributing environmental factor to arterial thrombosis, *Expert Rev Hematol***12**(2019) pp. 541-9.

[141] I.A. Hauser, D.R. Johnson, J.A. Madri, Differential induction of VCAM-1 on human iliac venous and arterial endothelial cells and its role in adhesion, *J Immunol***151**(1993) pp. 5172-85.

[142] T. Into, Y. Kanno, J. Dohkan, M. Nakashima, M. Inomata, K. Shibata*, et al.*, Pathogen recognition by Toll-like receptor 2 activates Weibel-Palade body exocytosis in human aortic endothelial cells, *J Biol Chem***282**(2007) pp. 8134-41.

[143] van den Munckhof, I. C. L., A. Kurilshikov, R. Ter Horst, N.P. Riksen, L.a.B. Joosten, A. Zhernakova*, et al.*, Role of gut microbiota in chronic low-grade inflammation as potential driver for atherosclerotic cardiovascular disease: a systematic review of human studies, *Obes Rev***19**(2018) pp. 1719-34.

[144] L. Sun, H. Jia, J. Li, M. Yu, Y. Yang, D. Tian*, et al.*, Cecal Gut Microbiota and Metabolites Might Contribute to the Severity of Acute Myocardial Ischemia by Impacting the Intestinal Permeability, Oxidative Stress, and Energy Metabolism, *Front Microbiol***10**(2019) pp. 1745.

[145] R. Chen, Y. Xu, P. Wu, H. Zhou, Y. Lasanajak, Y. Fang*, et al.*, Transplantation of fecal microbiota rich in short chain fatty acids and butyric acid treat cerebral ischemic stroke by regulating gut microbiota, *Pharmacol Res***148**(2019) pp. 104403.

[146] C. Gil-Cruz, C. Perez-Shibayama, A. De Martin, F. Ronchi, K. van der Borght, R. Niederer*, et al.*, Microbiota-derived peptide mimics drive lethal inflammatory cardiomyopathy, *Science***366**(2019) pp. 881-6.

[147] A.M. Minihane, S. Vinoy, W.R. Russell, A. Baka, H.M. Roche, K.M. Tuohy*, et al.*, Low-grade inflammation, diet composition and health: current research evidence and its translation, *Br J Nutr***114**(2015) pp. 999-1012.

[148] R.E. Ley, P.J. Turnbaugh, S. Klein, J.I. Gordon, Microbial ecology: human gut microbes associated with obesity, *Nature***444**(2006) pp. 1022-3.

[149] E. Le Chatelier, T. Nielsen, J. Qin, E. Prifti, F. Hildebrand, G. Falony*, et al.*, Richness of human gut microbiome correlates with metabolic markers, *Nature***500**(2013) pp. 541-6.

[150] J. Qin, Y. Li, Z. Cai, S. Li, J. Zhu, F. Zhang*, et al.*, A metagenome-wide association study of gut microbiota in type 2 diabetes, *Nature***490**(2012) pp. 55-60.

[151] F.H. Karlsson, V. Tremaroli, I. Nookaew, G. Bergström, C.J. Behre, B. Fagerberg*, et al.*, Gut metagenome in European women with normal, impaired and diabetic glucose control, *Nature***498**(2013) pp. 99-103.

[152] T. Vatanen, E.A. Franzosa, R. Schwager, S. Tripathi, T.D. Arthur, K. Vehik*, et al.*, The human gut microbiome in early-onset type 1 diabetes from the TEDDY study, *Nature***562**(2018) pp. 589-94.

[153] S. Vieira-Silva, G. Falony, E. Belda, T. Nielsen, J. Aron-Wisnewsky, R. Chakaroun*, et al.*, Statin therapy is associated with lower prevalence of gut microbiota dysbiosis, *Nature***581**(2020) pp. 310-5.

[154] E. Catry, B.D. Pachikian, N. Salazar, A.M. Neyrinck, P.D. Cani, N.M. Delzenne, Ezetimibe and simvastatin modulate gut microbiota and expression of genes related to cholesterol metabolism, *Life Sci***132**(2015) pp. 77-84.

[155] S. Tuteja, J.F. Ferguson, Gut Microbiome and Response to Cardiovascular Drugs, *Circ Genom Precis Med***12**(2019) pp. 421-9.

[156] C. Milani, A. Hevia, E. Foroni, S. Duranti, F. Turroni, G.A. Lugli*, et al.*, Assessing the Fecal Microbiota: An Optimized Ion Torrent 16S rRNA Gene-Based Analysis Protocol, *PloS one***8**(2013) pp. e68739.

[157] A. Hiergeist, U. Reischl, A. Gessner, Multicenter quality assessment of 16S ribosomal DNA-sequencing for microbiome analyses reveals high inter-center variability, *International Journal of Medical Microbiology***306**(2016) pp. 334-42.

[158] K. Gerasimidis, M. Bertz, C. Quince, K. Brunner, A. Bruce, E. Combet*, et al.*, The effect of DNA extraction methodology on gut microbiota research applications, *BMC Res Notes***9**(2016) .

[159] M.A. Lankinen, A. Fauland, B. Shimizu, J. Ågren, C.E. Wheelock, M. Laakso*, et al.*, Inflammatory response to dietary linoleic acid depends on FADS1 genotype, *Am J Clin Nutr***109**(2019) pp. 165-75.

[160] M.A. Martínez-González, P. Buil-Cosiales, D. Corella, M. Bulló, M. Fitó, J. Vioque*, et al.*, Cohort Profile: Design and methods of the PREDIMED-Plus randomized trial, *Int J Epidemiol***48**(2019) pp. 387-388o.

[161] A. Vrieze, E. Van Nood, F. Holleman, J. Salojärvi, R.S. Kootte, Bartelsman, Joep F. W. M.*, et al.*, Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome, *Gastroenterology***143**(2012) pp. 913,916.e7.

[162] R.S. Kootte, E. Levin, J. Salojärvi, L.P. Smits, A.V. Hartstra, S.D. Udayappan*, et al.*, Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition, *Cell Metab***26**(2017) pp. 611,619.e6.

[163] E.W. Yu, L. Gao, P. Stastka, M.C. Cheney, J. Mahabamunuge, M. Torres Soto*, et al.*, Fecal microbiota transplantation for the improvement of metabolism in obesity: The FMT-TRIM double-blind placebo-controlled pilot trial, *PLoS Med***17**(2020) pp. e1003051.

[164] P. de Groot, T. Scheithauer, G.J. Bakker, A. Prodan, E. Levin, M.T. Khan*, et al.*, Donor metabolic characteristics drive effects of faecal microbiota transplantation on recipient insulin sensitivity, energy expenditure and intestinal transit time, *Gut***69**(2020) pp. 502-12.

[165] L. Van Horn, J.A.S. Carson, L.J. Appel, L.E. Burke, C. Economos, W. Karmally*, et al.*, Recommended Dietary Pattern to Achieve Adherence to the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines: A Scientific Statement From the American Heart Association, *Circulation***134**(2016) pp. e505-29.

[166] O. Koren, A. Spor, J. Felin, F. Fåk, J. Stombaugh, V. Tremaroli*, et al.*, Human oral, gut, and plaque microbiota in patients with atherosclerosis, *Proc Natl Acad Sci U S A***108 Suppl 1**(2011) pp. 4592-8.

[167] J. Yin, S. Liao, Y. He, S. Wang, G. Xia, F. Liu*, et al.*, Dysbiosis of Gut Microbiota With Reduced Trimethylamine-N-Oxide Level in Patients With Large-Artery Atherosclerotic Stroke or Transient Ischemic Attack, *J Am Heart Assoc***4**(2015) .

[168] T. Emoto, T. Yamashita, T. Kobayashi, N. Sasaki, Y. Hirota, T. Hayashi*, et al.*, Characterization of gut microbiota profiles in coronary artery disease patients using data mining analysis of terminal restriction fragment length polymorphism: gut microbiota could be a diagnostic marker of coronary artery disease, *Heart Vessels***32**(2017) pp. 39-46.

[169] Q. Zhu, R. Gao, Y. Zhang, D. Pan, Y. Zhu, X. Zhang*, et al.*, Dysbiosis signatures of gut microbiota in coronary artery disease, *Physiol Genomics***50**(2018) pp. 893-903.

[170] J. Li, F. Zhao, Y. Wang, J. Chen, J. Tao, G. Tian*, et al.*, Gut microbiota dysbiosis contributes to the development of hypertension, *Microbiome***5**(2017) pp. 14.

[171] Q. Yan, Y. Gu, X. Li, W. Yang, L. Jia, C. Chen*, et al.*, Alterations of the Gut Microbiome in Hypertension, *Front Cell Infect Microbiol***7**(2017) pp. 381.

[172] X. Cui, L. Ye, J. Li, L. Jin, W. Wang, S. Li*, et al.*, Metagenomic and metabolomic analyses unveil dysbiosis of gut microbiota in chronic heart failure patients, *Sci Rep***8**(2018) pp. 635.

[173] M. Luedde, T. Winkler, F. Heinsen, M.C. Rühlemann, M.E. Spehlmann, A. Bajrovic*, et al.*, Heart failure is associated with depletion of core intestinal microbiota, *ESC Heart Fail***4**(2017) pp. 282-90.

[174] M. Kummen, C.C.K. Mayerhofer, B. Vestad, K. Broch, A. Awoyemi, C. Storm-Larsen*, et al.*, Gut Microbiota Signature in Heart Failure Defined From Profiling of 2 Independent Cohorts, *J Am Coll Cardiol***71**(2018) pp. 1184-6.

[175] T. Katsimichas, T. Ohtani, D. Motooka, Y. Tsukamoto, H. Kioka, K. Nakamoto*, et al.*, Non-Ischemic Heart Failure With Reduced Ejection Fraction Is Associated With Altered Intestinal Microbiota, *Circ J***82**(2018) pp. 1640-50.

[176] D.N. Cooper, M.E. Kable, M.L. Marco, A. De Leon, B. Rust, J.E. Baker*, et al.*, The Effects of Moderate Whole Grain Consumption on Fasting Glucose and Lipids, Gastrointestinal Symptoms, and Microbiota, *Nutrients***9**(2017) .

[177] I. Martínez, J.M. Lattimer, K.L. Hubach, J.A. Case, J. Yang, C.G. Weber*, et al.*, Gut microbiome composition is linked to whole grain-induced immunological improvements, *The ISME journal***7**(2013) pp. 269-80.

[178] J. Sandberg, P. Kovatcheva-Datchary, I. Björck, F. Bäckhed, A. Nilsson, Abundance of gut Prevotella at baseline and metabolic response to barley prebiotics, *Eur J Nutr***58**(2019) pp. 2365-76.

[179] M. Balfegó, S. Canivell, F.A. Hanzu, A. Sala-Vila, M. Martínez-Medina, S. Murillo*, et al.*, Effects of sardine-enriched diet on metabolic control, inflammation and gut microbiota in drug-naïve patients with type 2 diabetes: a pilot randomized trial, *Lipids Health Dis***15**(2016) pp. 78.

[180] E.S. Chambers, C.S. Byrne, D.J. Morrison, K.G. Murphy, T. Preston, C. Tedford*, et al.*, Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial, *Gut***68**(2019) pp. 1430-8.

[181] K. Han, S. Bose, J. Wang, B. Kim, M.J. Kim, E. Kim*, et al.*, Contrasting effects of fresh and fermented kimchi consumption on gut microbiota composition and gene expression related to metabolic syndrome in obese Korean women, *Mol Nutr Food Res***59**(2015) pp. 1004-8.

[182] R. Lear, M. O'Leary, L. O'Brien Andersen, C.C. Holt, C.R. Stensvold, M. van der Giezen*, et al.*, Tart Cherry Concentrate Does Not Alter the Gut Microbiome, Glycaemic Control or Systemic Inflammation in a Middle-Aged Population, *Nutrients***11**(2019) .

[183] N. Redondo, N. García-González, L.E. Diaz-Prieto, B. Olmedilla-Alonso, A.B. Martín-Diana, C. Asensio-Vegas*, et al.*, Effects of ewe's milk yogurt (whole and semi-skimmed) and cow's milk yogurt on inflammation markers and gut microbiota of subjects with borderline-high plasma cholesterol levels: a crossover study, *Eur J Nutr***58**(2019) pp. 1113-24.

[184] A.M. Sheflin, E.C. Borresen, M.J. Wdowik, S. Rao, R.J. Brown, A.L. Heuberger*, et al.*, Pilot dietary intervention with heat-stabilized rice bran modulates stool microbiota and metabolites in healthy adults, *Nutrients***7**(2015) pp. 1282-300.

[185] F. Fava, R. Gitau, B.A. Griffin, G.R. Gibson, K.M. Tuohy, J.A. Lovegrove, The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population, *Int J Obes (Lond)***37**(2013) pp. 216-23.

[186] Y. Karusheva, T. Koessler, K. Strassburger, D. Markgraf, L. Mastrototaro, T. Jelenik*, et al.*, Short-term dietary reduction of branched-chain amino acids reduces meal-induced insulin secretion and modifies microbiome composition in type 2 diabetes: a randomized controlled crossover trial, *Am J Clin Nutr***110**(2019) pp. 1098-107.

[187] N. Marungruang, J. Tovar, I. Björck, F.F. Hållenius, Improvement in cardiometabolic risk markers following a multifunctional diet is associated with gut microbial taxa in healthy overweight and obese subjects, *Eur J Nutr***57**(2018) pp. 2927-36.

[188] R. Ostan, M.C. Béné, L. Spazzafumo, A. Pinto, L.M. Donini, F. Pryen*, et al.*, Impact of diet and nutraceutical supplementation on inflammation in elderly people. Results from the RISTOMED study, an open-label randomized control trial, *Clin Nutr***35**(2016) pp. 812-8.

[189] A. Salonen, L. Lahti, J. Salojärvi, G. Holtrop, K. Korpela, S.H. Duncan*, et al.*, Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men, *ISME J***8**(2014) pp. 2218-30.

[190] G.E. Lobley, G. Holtrop, D.M. Bremner, A.G. Calder, E. Milne, A.M. Johnstone, Impact of short term consumption of diets high in either non-starch polysaccharides or resistant starch in comparison with moderate weight loss on indices of insulin sensitivity in subjects with metabolic syndrome, *Nutrients***5**(2013) pp. 2144-72.