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4	The gut microbiome and cardiovascular disease: current knowledge and
5	clinical potential
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31 32 33 34 35	Word Count: 7851

36 Abstract37

38	Cardiovascular disease (CVD) is the leading cause of death worldwide. The human body is
39	populated by a diverse community of microbes, dominated by bacteria, but also including
40	viruses and fungi. The largest and most complex of these communities is located in the
41	gastrointestinal system and with their associated genome, are known as the gut microbiome.
42	Gut microbiome perturbations and related dysbiosis have been implicated in the progression
43	and pathogenesis of CVD, including atherosclerosis, hypertension and heart failure. Although
44	there have been advances in the characterisation and analysis of the gut microbiota and
45	associated bacterial metabolites, the exact mechanisms through which they exert their action
46	is not well understood. This review will focus on the role of the gut microbiome and
47	associated functional components in the development and progression of atherosclerosis.
48	Potential treatments to alter the gut microbiome to prevent or treat atherosclerosis and CVD
49	are also discussed.
50	
51	
52	Keywords: Atherosclerosis; cardiovascular disease, dysbiosis, microbiome, short chain fatty
53	acids, TMAO
54	

56 57	List of abbreviations:
58	AAA: abdominal aortic aneurysm
59	ACS: acute coronary syndrome
60	AMPK: adenosine monophosphate-activated protein kinase
61	ApoE ^{-/-} : apolipoprotein E knockout
62	BA: bile acid
63	CAD: coronary artery disease
64	CLA: conjugated linoleic acids
65	CVD: cardiovascular disease
66	FOS: fructooligosaccharides
67	FXR: Farnesoid X receptor
68	GPR: G protein-coupled receptor
69	LDL-C: low density lipoprotein-c
70	PAD: peripheral arterial disease
71	SCFA: short chain fatty acid
72	TGR: Takeda G protein coupled receptor
73	TLR: toll-like receptors
74	TMA: trimethylamine
75	TMAO: trimethylamine-N- oxide

76 16S rRNA: 16S ribosomal ribonucleic acid

78 Introduction

79

80 Cardiovascular disease (CVD) is the leading cause of death and disability globally (60). The 81 human gut is populated by a diverse and complex community of bacteria, viruses, fungi and 82 protozoa, which along with their genome, are collectively known as the gut microbiome (90). 83 An alteration in gut microbiome composition and linked functionality is known as microbial 84 dysbiosis. Studies have shown that gut microbiota interact with the host, contributing to host 85 health and disease status. Although there is a large interindividual variation in terms of 86 microbial composition, healthy individuals share core functional capabilities (146). However, 87 several disease states, including CVD, are associated with microbial dysbiosis (78). There is 88 increasing evidence in CVD demonstrating an alteration in diversity and composition of the 89 gut microbiome and its associated metabolites which may influence the pathogenesis and 90 progression of diseases such as atherosclerosis [acute coronary syndrome (ACS) and stroke], 91 heart failure and hypertension. The complex host-microbiome interaction influences the 92 synthesis and release of several metabolites including trimethylamine-N-oxide (TMAO), bile 93 acids (BA) and short chain fatty acids (SCFA), which impact host homeostasis (139). While 94 recent advances in genomic and metabolomics have allowed the accurate characterisation and 95 quantification of these microbes and their metabolites, the exact mechanisms of their action 96 remain unclear. Interventions targeting the microbiome could provide new avenues for the 97 management and treatment of CVD patients (138). In this review we will discuss the 98 development and progression of atherosclerosis, the contribution of the microbiota, 99 specifically the gut bacteria, and the potential for targeting the microbiome and its associated 100 components as novel therapeutic options.

101

102 The gut microbiome

103

A complex and dynamic community of microbes live in the human gastrointestinal tract, a
nutrient rich microenvironment conducive for the growth of microorganisms. (146). There is
a dominance of bacteria in this environment belonging to five main phyla: Firmicutes,
Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia (Figure 1) (177). There is

108 large interindividual variation in the composition, diversity and abundance of bacteria and

- 109 microbes, predominantly due to host genome factors as well as environmental parameters like
- 110 lifestyle, diet, hygiene, health, medication such as antibiotics and the use of prebiotics and
- 111 probiotics (18). There is a symbiotic relationship between the host and the microbiota,

- developing from birth through to adulthood (76). The symbiotic relationship of the
- 113 microbiome and the host promote the growth of beneficial commensal bacteria while
- 114 inhibiting the overgrowth of pathogenic ones (102).
- 115
- 116 The normal physiological functions of the gut microbiome include fermentation of
- 117 indigestible dietary substances (87), synthesis of certain vitamins (122), energy metabolism
- via SCFA production (13) and the regulation of the intestinal epithelial mucosal barrier (73).
- 119 If disrupted, this barrier will result in 'leakage' allowing bacterial translocation and an
- 120 increase in exposure to bacterial derived products such as circulating endotoxins.
- 121

The gut microbiome has a very diverse role and can have distal effects in the body. There is a complex interplay between the gut microbiome and the health and function of the host via production and release of important metabolites including SCFA, BAs and TMAO as well as influencing various host metabolic pathways (139). These metabolites can be absorbed into the bloodstream via the intestinal epithelium, thus influencing the function of several distal organs and body systems (127).

128

129 Atherosclerosis

130

131 Atherosclerosis is a chronic inflammatory condition resulting from initial endothelial injury 132 followed by lipid build-up and plaque formation in the arteries. Risk factors for 133 atherosclerosis include hypercholesterolaemia, hypertension, diabetes mellitus, obesity, and a 134 sedentary lifestyle (113). Cellular events that contribute to the initiation and progression of 135 atherosclerosis involve; lipid migration to the inner layer of the arterial wall - the 136 endothelium, adhesion of circulating monocytes and lymphocytes to the endothelial cells, 137 migration of monocytes into the sub-endothelial space and acquiring macrophage 138 characteristics and converting into foam cells (113). Low density lipoprotein (LDL) particles 139 undergo modification, primarily through oxidation, becoming strong chemo attractants (113). 140 These processes, which involve cell adhesion, migration, differentiation, further proliferation 141 and interaction with the extra cellular matrix, are controlled and regulated by a cascade of 142 cytokines and growth regulatory peptides and ultimately lead to the accumulation of 143 cholesterol and the formation of fatty streaks (113). Vascular smooth muscle cells also 144 migrate to the endothelium and proliferate, with additional accumulation of macrophages,

145	modified LDL and inflammatory cells, together forming the atherosclerotic plaque (11). The
146	plaque consists of various cells (endothelial cells, foam cells, macrophages and leukocytes),
147	accumulated lipids (cholesterol, triglycerides), calcified regions and a necrotic core (113).
148	Vascular dysfunction and hypertension contribute to vessel damage via mechanisms
149	involving an increase in inflammation and LDL, causing plaque formation and build up in
150	arterial walls progressing to atherosclerosis (11). Atherosclerosis progresses to various
151	cardiovascular diseases such as ACS, stroke, AAA and ultimately heart failure.
152	
153	Microbiota and their metabolites profoundly modulate the progression of atherosclerosis, the
154	most common cause of ACS, stroke and peripheral vascular disease (Figure 2) (74).
155	However, despite this, the mechanisms of action as to how the gut microbiota contribute to
156	atherosclerosis remain to be elucidated. Recent reviews have highlighted the potential role of
157	dysbiosis in several disease states, including atherosclerosis, heart failure, hypertension and
158	diabetes mellitus (3, 145). In these states the gut bacteria diversity, composition and
159	associated metabolic functions can change, leading to disruption of vital physiological
160	processes, including inflammation, lipid metabolism, bacterial translocation and glucose
161	homeostasis which may all contribute to disease development and progression.
162	
163	Changes in the microbiome composition may also contribute to the development and
164	progression of CVD, with alterations in the ratio of the key phyla of Firmicutes to
165	Bacteroidetes proposed as a potential risk factor of CVD (20, 32, 135). Similarly, there is a
166	correlation between various gut microbiome metabolite levels, including TMAO, SCFA and
167	BA and the pathogenesis of CVD. Finally, risk factors and associated symptoms of CVD may
168	cause further microbial dysbiosis, including accumulation of toxic metabolites (such as
169	TMAO) and pathogenic bacterial species (73, 146, 177).

170

171 In the gut, symptomatic atherosclerotic patients had a greater abundance of *Collinsella*

172 compared to healthy controls (48, 68). However, several of these studies were limited by their

small sample sizes (<30) and thus further research is required. A recent metagenome wide

association study from 218 individuals with atherosclerosis and 187 healthy controls

175 observed that the gut microbiome deviates from a healthy status by an increase in the relative

- 176 abundance of Enterobacteriaceae (including Escherichia coli, Enterobacter aerogenes and
- 177 *Klebsiella* spp.) and *Streptococcus* spp (Table 1). Functionally, this impacts metabolism and

transport of molecules such as TMAO (64). These data suggest that the gut microbiome maybe more proinflammatory in patients with CVD (64).

180

181 *Carotid arteries*

182

183 Carotid atherosclerosis is linked to an increased risk of cardiovascular events such as stroke 184 and ACS (40). Stroke is divided into two categories; ischemic stroke which occurs as a result 185 of a blockage in a blood vessel supplying blood to the brain and haemorrhagic stroke, 186 occurring from the rupture of a weakened blood vessel in the brain (6). Ischemic stroke 187 accounts for 70 - 80% of all strokes (6). There is a bidirectional relationship between the 188 brain and gut microbiota via vagus nerve activity and the enteric nervous system (26). During 189 ischemic stroke, the brain produces molecules which signal damage, including damage 190 associated molecular proteins and cytokines. The damage associated molecular proteins and 191 cytokines produced in ischemic brain tissue are released into the circulation and 192 communicate with immune and lymphoid organs leading to systemic inflammatory and 193 immune responses (26). The gut will also release cytokines and gut inflammatory and

- immune cells will travel to the site of brain injury.
- 195

196 After ischemic or haemorrhagic stroke, 50% of patients experience gastro intestinal issues, 197 including gut microbiota dysbiosis, "leaky" gut, increased gut motility, disorganization and 198 gut haemorrhage (169) as well as a reduced diversity and composition of the gut microbiota 199 (173). As a result, intestinal inflammatory and immune responses are triggered (137). These 200 patients have increased mortality rates and worsening neurological function with poor 201 outcomes. This altered immune homeostasis largely occurs due to the gut microbiota's role in 202 communicating with intestinal epithelial cells, dendritic cells, B and T lymphocytes, 203 mononuclear phagocytes and innate lymphoid cells (67). Zeng and colleagues investigated 204 the association of the gut microbiome with high and low risk stroke and noted enrichment in 205 opportunistic pathogens (e.g. Enterobacteriaceae and Veillonellaceae) and depleted 206 abundance of butyrate producing bacteria (e.g. Lachnospiraceae and Ruminococcaceae) with 207 reduced faecal butyrate levels in patients with a high risk of stroke (179). Many aspects of gut 208 microbiota dysbiosis and modulation of inflammatory and immune responses have been 209 implicated in stroke. However, what these effects signify in reality for stroke therapy remains 210 to be investigated.

212

Coronary arteries 213 214 Coronary artery disease (CAD) involves a narrowing of the coronary arteries supplying blood 215 to the heart due to plaque build-up, thereby reducing blood flow. Atherosclerosis is the main 216 pathophysiological basis of CAD. A small study found CAD patients had alterations in the 217 gut microbiota, including an increase in Lactobacillales (from Firmicutes phylum and 218 consisting of the genera Lactobacillus, Streptococcus and Enterococcus) whereas the phylum 219 Bacteroidetes (composed of the genera Bacteroides and Prevotella) was reduced (42). 220 Bacteroides fragilis promotes regulatory T-cell function in mucosal T-cell homeostasis (99). 221 A recent study in CAD revealed a decreased microbial diversity and composition, as reflected 222 by enrichment in Esherichia/Shigella and Enterococcus, while Faecalibacterium, 223 Subdoligranulum, Roseburia and Eubacterium rectale were depleted (180). Faecalibacterium 224 has an important anti-inflammatory role (89, 93), while Roseburia are important for SCFA 225 production which potentially depletes energy supply for intestinal cells thus negatively 226 affecting the gut barrier (54, 76). In atheromatous plaques of patients with CAD, there was 227 an enhanced bacterial diversity of Staphylococcus species, Proteus vulgaris, Klebsiella 228 pneumoniae and Streptococcus sp. Although, there was a predominant bacterial signature in 229 these plaques, it is not proof that the gut microbiota plays a causal role in atherosclerosis. 230 Instead it has been suggested that the presence of the bacteria could contribute to 231 atherosclerosis development and/or progression (107). 232 233 ACS refers to a group of conditions including, unstable angina and acute myocardial 234 infarction, and is usually characterised by a reduced blood flow to the heart muscle usually 235 due to blockage (44). ACS is associated with inflammation, which plays a role in its 236 pathogenesis. Evidence suggests that microbial dysbiosis may have an impact on several 237 inflammatory or immune disorders via activating proinflammatory responses. Indeed, 238 bacterial DNA was present on epicardial adipose tissue obtained during coronary artery 239 bypass grafting in patients with ACS. This suggests that the epicardial adipose tissue 240 environment is susceptible to microbial colonization, stimulating proinflammatory responses 241 involved in vascular inflammation and plaque formation and instability. However, there is 242 agreement that further research is warranted to provide more clarity to this important area 243 (111).244

245 246	Abdominal aortic aneurysm
247	Abdominal aortic aneurysm (AAA) is a localised swelling of the walls of the abdominal
248	aorta, the rupture of which can be fatal. Pathogenesis of AAA includes complex
249	inflammatory pathways, extracellular matrix disruption, inflammation, thrombosis,
250	haemodynamic forces and associated signalling molecules (58). Atherosclerosis is an
251	important and independent risk factor for AAA (155) as patients with AAA frequently have
252	atherosclerosis and there is an association of coronary heart disease and peripheral artery
253	disease with AAA (57). An investigation of atherosclerotic plaques from vascular biopsies in
254	patients with vascular diseases (including AAA), with and without chronic periodontitis,
255	detected bacterial DNA in 95%, with most biopsies comprising DNA from multiple bacterial
256	species (5). This reinforces recent findings that suggest more than one bacterial species are
257	involved with CVD (41, 91). Although finding bacterial DNA in atherosclerotic plaques is
258	well established, it is not clear whether an infection initiates or promotes its formation.
259	Animal models have identified microorganisms such as
260	Aggregatibacter actinomycetemcomitans, Helicobacter pylori, Chlamydophila perfringens
261	and Porphyromonas gingivalis, which may contribute to increasing lesion areas in
262	atherosclerosis (120). However, further research and validation in humans is required.
263	In addition, gut microbiota function is affected by surgical abdominal aortic repair, which
264	involves aortic clamping and can result in negative changes in intestinal permeability, which
265	may also contribute to disease progression (109).
266	
267 268	Peripheral artery disease and mesenteric ischemia
269	Peripheral artery disease (PAD) is characterised by reduced blood flow to the periphery, is a
270	common consequence of systemic atherosclerosis and is associated with significantly higher
271	risks of cardiovascular mortality. In patients with stable PAD, elevated plasma TMAO, a
272	proatherogenic gut microbiota metabolite, was found to be a significant predictor of 5 year
273	all-cause mortality risk, independent of traditional risk factors (131).
274	
275	Mesenteric ischemia occurs as a result of reduced blood supply to the mesenteries supplying
276	the small intestine. The gut microbiome plays a key role in mesenteric ischemia via Toll-like
277	receptors (TLR) which recognise constituent molecules of bacteria and contribute to
278	maintenance of the intestinal environment by playing a key role in the innate immune system

279 (103). The TLR signalling pathway, specifically TLR2 and TLR4, activate inflammatory 280 responses, such as the release of tumour necrosis factor- α . Oxidative stress caused by reactive 281 oxygen species and free radicals, the source of which are neutrophils and macrophages, also 282 affect the intestine during ischemia (103). Neutrophils and macrophages release reactive 283 oxygen species and free radicals to sterilize invading bacteria, however, excess production 284 can cause local tissue injury (103). Prevention of oxidative stress is useful for treatment of 285 mesenteric ischemia. Previous studies showed heme oxygenase-1 plays a protective role in 286 mesenteric ischemia and reperfusion, by preventing oxidation (85, 168).

287

Gut microbiome metabolites

288 289

290

Trimethylamine-N-oxide

291 The gut microbiota is known to be involved in the synthesis of proatherogenic metabolites, 292 namely TMAO (Figure 3) (100). The breakdown of dietary substances rich in 293 phosphatidylcholine, choline or carnitine (from red meat, fish and eggs) forms 294 trimethylamine (TMA), the precursor to TMAO (84). TMA is absorbed in the intestine and 295 converted via the hepatic enzyme flavin monooxygenase 3 to TMAO, a plasma metabolite 296 released into the bloodstream and eventually cleared by the kidneys (76, 178). TMAO is a 297 biologically active molecule and has been shown to be a predictor of ACS, stroke and death, 298 likely through a proatherogenic pathway (74, 84, 178). When mice were supplemented with 299 chronic dietary L-carnitine, there was an alteration in caecal microbial composition and 300 increased synthesis of TMA and TMAO and enhanced atherosclerosis, however, this was not 301 seen when intestinal microbiota was concurrently suppressed (74). In fact, mice with intact 302 gut microbiota had a reduction in *in vivo* reverse cholesterol transport when supplemented 303 with either carnitine or choline (74), reinforcing the role of the gut microbiota in promoting 304 atherosclerosis via the metabolism of L-carnitine, found in red meat. Evidence shows that 305 individuals at higher risk for stroke and CVD have elevated TMAO levels as well as TMAO precursors (178). This was further reinforced when plaque progression and atherogenesis was 306 promoted in apolipoprotein E knockout (ApoE^{-/-}) mice who were administered TMAO 307 308 precursors or TMAO (55). Similarly, the administration of 3,3-dimethyl-1-butanol, a TMA 309 inhibitor prevented cardiac dysfunction caused by a high sugar and high fat western diet (30). 310 311 Studies have demonstrated a correlation between gut microbiota metabolism of TMAO and

risk of CVD (74, 84, 147, 148, 165), with a recent meta-analysis revealing a 67% increased

313 risk of developing CVD with high TMAO levels. Furthermore, for every 10 µmol/L increase 314 in TMAO, there was a 7.6% increase in all-cause mortality (127). TMAO enhances 315 atherosclerosis via vascular inflammation, monocyte endothelial cell interactions and 316 endothelial dysfunction as well as stimulating foam cell formation and atherosclerotic plaque 317 progression (92, 130, 142). TMAO has been shown to activate inflammatory pathways within 318 the vasculature, leading to leukocyte activation and atherosclerosis (130). TMAO levels were 319 also elevated in individuals presenting with ACS and showed a dose-dependent relationship 320 between increased risk of major adverse cardiac events and long-term mortality (84). TMAO 321 has also been shown to play a role in promoting platelet hyperresponsiveness and thrombosis 322 potential (181). TMAO acts to promote platelet reactivity through stimulus dependent calcium signalling, thereby increasing the risk of incident thrombotic events (181, 182). 323 ApoE^{-/-} mice with TMAO supplemented in their diet had enhanced aortic lesions (165). 324 325 Human studies have also reinforced the role of TMAO in CVD. In a prospective, 326 observational study of heart failure, stable CAD and healthy subjects, plasma TMAO, choline 327 and betaine were elevated in chronic HF patients compared to controls (156). Similarly, in 328 myocardial infarction patients, TMAO independently predicted death at 2 years [292 events, 329 hazard ratio 1.21 (95% CI, 1.03-1.43, P = 0.023)] (143). A study by Tang and colleagues 330 revealed a correlation between elevated TMAO levels and an increased risk of major adverse 331 cardiac events (148). 332

Bile Acid

333 334

BAs are synthesised from cholesterol in the liver in a multistep processes involving severalenzymes and are vital in the absorption of dietary fats in the intestine (78).

337 Primary BAs are secreted into the duodenum and play a role in emulsifying lipid-soluble

dietary substances and vitamins to facilitate digestion and absorption (176). Other features of

BAs include their strong microbial activity (176) and their function as signalling molecules,

340 acting as ligands for nuclear receptors, thus effecting metabolism. An example is the

activation of the 'Farnesoid-X-receptor' (FXR), which supresses the action of cholesterol 7

342 alpha-hydroxylase enzyme, which is important in the formation of primary BAs from its

343 cholesterol precursor (78).

344

345 In the intestine, gut microbiota modify primary BAs via bacterial salt hydrolase activity,

346 which remove the -OH groups, turning them into secondary BAs (167). Primary BAs are

toxic to bacteria, hence making the BA more insoluble provide bacteria with a mechanism to
reduce toxicity. Further modification of BAs by the gut microbiota may occur before being
returned to the liver for re-conjugation and return to circulation (122). BAs are a vital
pathway for cholesterol elimination via excretion in faeces, thereby decreasing circulating
cholesterol levels and risk of plaque build-up (78). The gut microbiome can decrease BA
synthesis rate, thus increasing the plasma levels of LDL and increasing the risk of
atherosclerosis (126).

- 354
- 355 356

Short chain fatty acid

357 Short chain fatty acids such as acetate, butyrate and propionate are produced as a result of the 358 fermentation of indigestible dietary fibres in the gut by the microbiota (47). They are straight 359 chain saturated fatty acids composed of less than six carbon atoms (13). SCFA can either be 360 absorbed via the gut epithelium to participate in various physiological processes or excreted 361 in faces (140). Butyrate is usually the primary source of energy for colonocytes (59), 362 however SCFA may also have an anti-inflammatory role as well as effecting various other 363 metabolic pathways including lipid metabolism and gluconeogenesis (13). Other SCFA such 364 as acetate and propionate enter the portal circulation and are metabolised by the liver, or 365 released into systemic circulation where they are able to bind to vascular endothelium SCFA 366 receptors to influence cardiometabolic health (35). To prevent high concentrations of SCFA 367 in blood, butyrate and propionate are cleared through the portal circulation (15). Increased 368 faecal SCFA levels have been shown to be associated with decreased gut microbiota 369 diversity, higher gut permeability, systemic inflammation, plasma glucose levels, 370 dyslipidaemia, obesity and hypertension even after adjustment for confounders including 371 diet, total calories and physical activity (33). Although they have very important regulatory 372 roles, they also have quantitative roles as catabolic or anabolic substrates for the formation of 373 glucose, cholesterol and lipid metabolism (35). 374 375 SCFAs have been shown to exert a positive effect on modulating appetite, obesity and 376 colonic inflammation (34, 49, 51). Despite this, elevated faecal SCFA have been associated

377 with the metabolic syndrome, a risk factor for CVD (46, 151). This was demonstrated in

- 378 TLR5 knock out mice, where microbiota dysbiosis generated uncontrolled prolonged
- 379 production of SCFA and promoted development of the metabolic syndrome (136). TLR5 is a
- 380 flagellin receptor required for gut microbiota homeostasis. In a study where rats were fed a

high fat diet, there was a positive correlation between obesity and the altered abundances of

382 Phascolarctobacterium, Proteus mirabilis and Veillonellaceae, producers of propionate and

acetate (79). Dysregulation of SCFA production has recently been suggested to also be a

trigger in the aetiology of diabetes in two independent human studies (125, 154). Since SCFA

- 385 energy is harvested from indigestible fibre, and they can enter the tricarboxylic acid cycle
- 386 (which also regulates lipogenesis), in hypercaloric diets, an overproduction of SCFAs could
- 387 result in an undesirable effect.

388 Gut dysbiosis – links to inflammation and dyslipidaemia

389

390 Atherosclerosis is considered a state of chronic inflammation coupled with lipid

accumulation in the vessel walls. Systemic activation of the innate immune response as well

as within and around the atherosclerotic plaque may enhance plaque progression and rupture.

393 The gut microbiome enhances low grade inflammation in the gut, allowing entry of bacteria

and their products into the circulation, triggering chronic inflammation (75). Associations

395 between markers of systemic inflammation including C-reactive protein, interleukin-6 and 8

and tumour necrosis factor- α have been described in humans previously (19). Immune cells

- 397 within coronary and carotid plaques of patients with ACS, have been shown to produce
- antibodies against gut microbial produced antigens (*Klebsiella* and *Proteus* strains) (22).

399 Atherosclerotic plaques have their own microbial communities, similar in makeup to the oral

400 cavity and gut (66, 77). However, a recent study has reported no association between

401 atherosclerotic plaque microbial composition and clinical characteristics, contrasting with

402 previous studies (86). These differences in results may be due to several reasons including

403 differing methodologies, differences in population studied as well as population size and diet.

404 As such, further research is highly warranted in this field. Hyperlipidemic ApoE^{-/-} knockout

405 mice exposed to nasal vaccination of outer membrane protein of *Porphyromonas gingivalis*, a

406 gram negative oral cavity bacteria, had reduced atherosclerotic plaques and lower circulating

407 levels of inflammatory cytokines (124).

408

409 Dyslipidaemia refers to defects in lipid metabolism, where there is an elevation of plasma

410 cholesterol, low-density lipoprotein-c, triglycerides or both, or a reduction of high-density

411 lipoprotein cholesterol-c levels (97). Lipid levels are in part influenced by genetics, however,

412 are also affected by dietary fat intake, exercise, smoking and alcohol consumption. There is

413 extensive evidence from animal studies, clinical trials and observational epidemiological

414 studies that outline the causal role of LDL-C in atherosclerosis. Reduction of LDL-C lowers 415 relative risk of major cardiovascular events (116) while HLD-C is inversely associated with 416 risk of coronary heart disease (37). Additionally, the gut microbiome has been positively 417 correlated with lipid and fatty acid metabolism and insulin resistance (14). The human gut is 418 incapable of digesting complex carbohydrates in the form of dietary fibre, thus, it is the 419 specific anaerobic gut bacteria that take on this role via fermentation, to produce SCFA. 420 SCFA are involved in host energy metabolism, regulate intestinal immunity as well as 421 influencing lipid and cholesterol metabolism (36). Host energy homeostasis is mediated by 422 ligand/SCFA interaction with G protein-coupled receptors such as GPR41, expressed 423 primarily in adipose tissue and GPR43, highest levels found in immune cells (17). GPR41 is 424 activated by propionate followed by butyrate and acetate while GPR43 is activated by all 425 three SCFAs at a similar rate (17). Both GPR41 and GPR43 proteins have been found in 426 human colonic tissue (150), white adipose tissue (4), liver and skeletal muscle (17), 427 suggesting that SCFAs may also have an effect on energy metabolism in peripheral tissues. 428 Peptide YY, a gut hormone released postprandially in proportion to the calorie content of a 429 meal and glucagon-like peptide-1 found on beta cells of the pancreas also maintain host 430 energy balance, mainly by appetite reduction and promoting insulin secretion respectively. 431 (9). Thus, it is expected that SCFA overproduction and/or different SCFA profiles can have 432 an impact in metabolic homeostasis in the host (129).

433

434 SCFA are also able to mediate fatty acid synthesis and oxidation and lipolysis via peroxisome 435 proliferator-activated receptors, which have roles in carbohydrate, protein and lipid 436 metabolism and tumorigenesis (23). As a result, there is an increase in lipolysis and beta 437 oxidation, thus reducing lipid levels and circulating free fatty acids (24, 34). Apart from 438 SCFA, the gut microbiome can influence lipid levels via the actions of conjugated linoleic 439 acids (CLAs) and BA. Conjugated linoleic acids are produced by Bifidobacteria, Roseburia 440 and Lactobacillus from omega-3 rich dietary sources (106). CLAs influence lipid metabolism 441 by promoting SCFA producing bacteria, creating a cycle of peroxisome proliferator-activated 442 receptor activation (29). BAs modulate hepatic and systemic lipid and glucose metabolism 443 via the FXR (78, 123) or TGR5 (Takeda G protein coupled receptor) (72) receptors. FXR is 444 an important BA receptor, primarily expressed in the liver and intestines, both it and TGR5 445 plays a major role in carbohydrate and lipid metabolism, specifically, promoting glycogen 446 synthesis and inhibiting gluconeogenesis (28). TGR5 is expressed in brown adipose tissue 447 and muscle, when activated by BAs it promotes glucagon-like peptide-1 release, improving

liver and pancreatic function and enhanced glucose tolerance (152) as well as attenuating dietinduced obesity (152).

450 Microbiota – targeted therapies

452 In recent years many studies have shown that the composition and diversity of gut microbiota
453 can be beneficially altered by diet, prebiotics, probiotics, and faecal transplantation, to confer
454 benefit to the host.

455

451

456 457

Dietary intervention

458 Protein, lipids and carbohydrates are macronutrients which compose the majority of the 459 human diet (117). Dietary habits can influence gut microbiota composition which in turn 460 modulates host health via digestion and absorption of nutrients (127). Microbiota bio-461 transform dietary substances and synthesise metabolites which travel in the bloodstream to 462 have distal effects on host metabolism and health (31). Reciprocally, diet composition can 463 modulate the composition and function of the gut microbiome (121). Diet provides one of the 464 simplest and easiest ways to modify the microbiome for therapeutic intervention (171). 465 Components such as macronutrients, fibre, polyphenols, prebiotics and probiotics play a 466 crucial role in shaping the gut microbiome, thereby influencing the production and release of 467 gut microbiome metabolites, such as SCFAs (31).

468

469 A study linking long term dietary patterns with gut microbial enterotypes demonstrated that 470 intake of animal protein, amino acids and saturated fats were associated with the *Bacteroides* 471 enterotype (171). In contrast, the *Prevotella* enterotype was low in these groups but high for 472 carbohydrates and simple sugars. Vegetarians had an enrichment in the *Prevotella* enterotype. 473 These dietary associations were similar in another study comparing the microbiome of 474 European and Burkina Faso children (32). The microbiome of the European children was 475 dominated by *Bacteroides* associated with the high protein and fat typical of a Western diet. 476 Conversely, the Burkina Faso children's microbiome was the *Prevotella* enterotype as 477 characterised by their typical high carbohydrate and low animal protein diet. 478 479 A diet high in vegetables, fruits, grains and legumes and low in red meats and processed

- 480 carbohydrates known as the Mediterranean diet, is beneficial in the prevention of CVD
- 481 (39). This is due to the presence of antioxidants, nitrate and fibre as well as low

482 saturated/trans fatty acids, sodium and phosphate in this diet. This is likely to cause a

- 483 reduction in oxidative stress and inflammation, enhanced antioxidant properties and nitric
- 484 oxide bioavailability and thus modulation of gut microbiota to improve vascular and cardiac

485 function (71). In contrast to the Mediterranean diet, the Western diet, high in saturated fat, is

- 486 known to increase CVD risk by decreasing gut microbiota diversity and commensal bacteria,
- 487 such as *Bifidobacterium* (10).
- 488

489 Currently, there is a clear link between the diet and gut microbiome, such that the gut

490 microbiota profile is different between various diets and geographic regions. However, there

491 is still a substantial gap in our understanding of exactly how our diet modulates the gut

492 microbiome and in turn the consequence of the gut microbiota in modulating host health.

- 493 Further investigations are required as diet provides a low cost and easily manageable strategy
- 494 in the potential prevention, management and treatment of diseases such as CVD.
- 495

Polyphenols

496 497

498 Polyphenols are a large class of aromatic compounds found in plant-based beverages and 499 foods such as apples, berries, citrus, cocoa, tea and coffee (170). There is substantial evidence 500 that a polyphenol rich diet has cardioprotective and anti-diabetic properties (2, 65, 80, 110, 501 174). Once ingested, polyphenols are broken down, metabolised and absorbed through the 502 role of the gut microbiota (170). Some polyphenols are affected by pH and change in 503 chemical structure thus affecting their bioactivity (45). In addition, many polyphenols 504 undergo hydrolysis via intestinal brush border or microbial enzymes. They may also undergo 505 glucuronidation, methylation and sulfation during intestinal absorption and liver passage 506 (94). There is extensive metabolism of polyphenols by the gut bacteria which will affect their 507 bioavailability and bioactivity (132).

508

509 Short term human studies, involving the consumption of tart cherry juice rich in polyphenols,

510 namely anthocyanins and flavonoids, demonstrated the ability of the gut microbiota to

- ferment polyphenols to influence polyphenol metabolites (98). In the group (n=5) of
- 512 individuals with a high *Bacteroides* group from low carbohydrate and fibre consumption,
- 513 drinking tart cherry juice decreased the levels of *Bacteroides* and increased fermentative
- 514 Firmicutes. In the low *Bacteroides* group (n=5), there was an increase in *Bacteroides* and
- 515 *Bifidobacterium* (due to polyphenol availability) and a decrease in Firmicutes. Anthocyanins

act as antiplatelet agents in atherosclerosis and CVD prevention, while also inducing nitric
oxide formation in the blood vessels, promoting vasodilation (53). Furthermore, individuals
consuming a more Western influenced diet, may have a reduced ability to metabolise
polyphenols, thus reducing their bioavailability, bioactivity and hence any potential health
benefits (98). Similarly, the intake of polyphenols via grape and red wine demonstrated the
gut microbiota's ability to promote beneficial microbial communities to enhance host health
(104).

523

524 Quercetin is a member of the flavonoid family, a subclass of polyphenols and is thought to 525 have antioxidant and anti-inflammatory properties (16, 153). Quercetin administered as a 526 treatment to mice fed a high fat sucrose diet, modified the gut microbiota and attenuated the 527 increase of the Firmicutes/Bacteroidetes ratio (43). Generally, gut microbiota associated with 528 obesity has been associated with a reduced Bacteroidetes to Firmicutes ratio (82, 115). 529 Similarly, quercetin reduced the abundance of *Erysiepelotrichaceae* on a family level and at 530 the genus level *Bacillus*, which have been related to the Western diet and weight gain (158). 531 Furthermore, it significantly altered the composition of several bacterial species, increasing 532 the relative abundances of Bacteroides vulgatus and Akkermansia muciniphila which have 533 been inversely associated with obesity, while reducing Eubacterium cylindroides and 534 Bilophilia wadsworthia, associated with diet-induced obesity (12, 43, 160). A metagenomic 535 study in mice that developed obesity via a high fat sucrose diet demonstrated that the 536 microbiome of high fat sucrose diet fed mice was rich in genes which are associated with 537 sugar uptake for intestinal bacteria (158). Moreover, quercetin improved basal insulin levels 538 (43). This suggests a modulating role of quercetin in altering bacterial profiles to attenuate or 539 improve obesity. 540

541 Specific dietary polyphenols have been shown to have beneficial effects on vascular function 542 and attenuation of atherosclerosis. Quercetin and its metabolites have been shown to 543 upregulate adenosine monophosphate-activated protein kinase (AMPK) expression (133). 544 AMPK is an enzyme that plays a role in cellular energy homeostasis and fatty acid oxidation. 545 Quercetin and its metabolites have been shown to protect blood vessels against endothelial 546 dysfunction in intact mouse aortic rings (133). Inhibition of AMPK diminished the positive 547 effects. In human aortic endothelial cells, guercetin and its metabolites induced AMPK and 548 endothelial nitric oxide synthase activation, leading to an increase in nitric oxide, a potent 549 vasodilator (133). In another study, quercetin and theaflavin significantly attenuated

- atherosclerotic lesion size in both the aortic sinus and thoracic aorta of ApoE^{-/-} mice through 550
- 551 reduction of inflammation, improving nitric oxide bioavailability and inducing heme
- 552 oxygenase-1, an enzyme that plays a crucial role in prevention of vascular inflammation (88).
- 553 In mice fed a high fat diet, quercetin protects against oxidant-induced endothelial dysfunction
- and ApoE^{-/-} mice against atherosclerosis (134). These results provide further reinforcement 554
- 555 and support for the cardioprotective effects of quercetin, a dietary polyphenol.
- 556

557 Dietary polyphenols are increasingly recognised as a novel strategy in the potential

558 prevention and treatment of several diseases including CVD and type 2 diabetes. This is

559 explained by the bidirectional relationship which exists between polyphenols and the gut

560 microbiota to influence factors such as blood pressure, endothelial function, insulin

561 sensitivity, thereby promoting cardioprotective and antidiabetic effects. Polyphenols

562 supplemented in the diet at high bioavailability, may improve gut microbiota modulation of

- 563 CVD and obesity. However, it is recognised that more targeted research activities are still
- 564 required to resolve the remaining unanswered issues.
- 565
- 566 567

Prebiotics, probiotics and synbiotics

568 Prebiotics are nondigestible food ingredients which promote gut microbiota composition and 569 activity with positive effects on the host (56). They are usually obtained in the diet from 570 fibre rich foods (63) and commonly screened for their ability to increase bifidobacterial 571 numbers. A high fibre diet has been reported to modify the gut microbiota to increase acetate 572 producing bacteria, which contributed to a reduction in gut microbiota dysbiosis and offered 573 cardioprotective effects (95). Acetate is associated with regulating many pathways and genes 574 involved in CVD, such as transcription factor *Egr1*, a regulator of CVD through 575 inflammation, cardiac fibrosis and hypertrophy (95). In contrast, mice fed an atherogenic 576 Paigen diet (mimics inflammation in lean subjects) high in saturated fat and low in fibre, led 577 to the development of the metabolic syndrome including hyperinsulinemia, steatohepatitis, 578 hyperglycaemia and inflammatory infiltration into the aorta, contributing to the development 579 of atherosclerosis via the gut microbiota (112). 580

- 581 Dietary supplementation with an inulin/fructooligosaccharides (FOS) mixture (10 g/day)
- 582 stimulated Bifidobacterium increase (114) while another study demonstrated the increase in
- 583 Bifidobacterium following FOS supplementation (149). Beta glucan is another prebiotic

584 shown to have an influence on lowering cholesterol levels and maintaining blood glucose 585 homeostasis. A study where beta glucan was administered as a dietary intervention over a 586 period of 2 months demonstrated a significant decrease in low density lipoprotein and total 587 cholesterol levels as well as improving endothelial function in healthy individuals thus 588 providing cardioprotective effects, mainly through beneficial SCFA production via the gut 589 microbiome (63). In a recent study involving C57BL6 mice, we demonstrated in a long term 590 dietary supplementation with a combination of isoquercetin, a polyphenol and inulin, a 591 prebiotic, that the development of metabolic syndrome was prevented (144). Compared to 592 mice fed a high fat diet, the mice supplemented with isoquercetin and inulin had attenuated 593 weight gain, improved glucose tolerance, insulin sensitivity, as well as improvements in the 594 gut microbiota composition and functionality and SCFA production (144). Inulin was found 595 to increase faecal SCFA levels of acetate, butyrate and propionate. Animal studies have also 596 demonstrated arabinoxylans as a potential prebiotic due to its role in promoting 597 bifidobacterial and propionate synthesis with beneficial effects on lipid and cholesterol 598 accumulation (105, 159). In humans arabinoxylanoligosaccharides supplemented in the diet 599 increased bacterial populations and butyrate concentrations in faeces (162). Taken together, 600 these findings reinforce the importance of diet in the prevention of CVD and the metabolic 601 syndrome.

602

603 Live bacteria, known as probiotics, can be ingested for maintaining or promoting gut 604 microbiota composition (146). Most probiotic products are likely to contain beneficial 605 Bifidobacteria, Lactobacilli, Lactococci and Streptococci (7). Probiotics depending on their 606 nature, may inhibit the overgrowth of pathogenic bacteria, stimulates the immune system, 607 modulates pH levels and prevent inflammation (177). Probiotics can improve host 608 metabolism by stimulating digestive enzyme activity and inhibiting bacterial enzyme activity 609 and production of ammonia. Lactobacillus and Bifidobacterium are beneficial as they 610 improve intestinal barrier function and play a protective role in inflammatory disease (163, 611 172) by modulating inflammatory and proinflammatory cytokines (such as tumor necrosis 612 factor- α), which may delay and/or improve CVD (62). Probiotics are designed to exert their 613 beneficial effects by restoring the normal composition of microbiota by producing SCFAs 614 (27). Akkermansia muciniphila is also known for its probiotic properties and has been 615 demonstrated to be linked with glucose, insulin and leptin which are involved in lipid and 616 glucose metabolism (128). It is also an important modulator of mucus thickness and gut 617 barrier integrity probably due to its close proximity in the mucus layer and closeness to

- 618 epithelial cells (108). In a randomized double-blind clinical trial, *Lactobacillus plantarum*, a
- 619 probiotic, significantly reduced LDL-C and total cholesterol levels and inhibited
- 620 atherosclerotic plaque formation in hypercholesterolaemic patients (50).
- 621
- 622 The combination of prebiotics with probiotics for use is known as synbiotics. Synbiotics may
- 623 further support the growth, survival and persistence of beneficial bacteria as a specific
- 624 substrate is provided for fermentation by the bacteria (141). Several studies have
- 625 demonstrated the potential benefits of synbiotics on the gut microbiota and immune functions
- 626 in chickens (38, 101). In high fat diet mice, symbiotic intervention significantly reduced body
- 627 weight gain and attenuated features of metabolic syndrome as well as restoring the gut
- 628 microbial ecosystem structure and function(69).
- 629

630 The studies discussed above have predominately investigated the effects of prebiotics,

631 probiotics and synbiotics on risk factors for cardiovascular disease, such as inflammation,

632 hypertension and effects on glucose and lipid metabolism, as opposed to investigating the

633 direct benefit in atherosclerosis. However, given their beneficial effects on various CVD risk

634 factors, further research investigating how these treatments affect development and

635 progression of atherosclerosis is warranted.

636 637

638

Selective TMAO inhibitors

639 A recent study was successful in developing a mechanism- based inhibitor drug (3,3-640 dimethyl-1-butanol), targeting major microbial TMA generating enzymes (CutC/D) where a 641 single oral dose caused a significant reduction in plasma TMAO levels for up to three days as 642 well as reducing enhanced platelet responsiveness and thrombus formation induced by diet 643 (118). This inhibitor is potent, time dependant, irreversible and did not affect other 644 commensals' normal functioning or observable toxicity. When dimethyl-1-butanol was given to atherosclerotic prone ApoE^{-/-} mice on a choline- supplemented diet, plasma TMAO levels 645 646 significantly reduced, macrophage cholesterol accumulation, foam cell formation and 647 atherosclerotic lesion development were also reduced (166). Microbial protein pairs (CutC 648 and CutD), encoded by genes of the choline utilisation gene cluster (*cut*) have been shown to 649 support choline TMA lyase enzyme activity (96, 119). Current antiplatelet drugs and 650 medication target mammalian enzymes and receptors and have the potential to cause bleeding 651 (52). In contrast, inhibiting microbial generation of TMAO would not supress platelet

652 function and therefore, increase the risks of bleeding. This study (118) suggests the selective 653 and nonlethal targeting of specific gut microbiome enzymes which are shown to be linked to 654 disease, thus, limiting systemic exposure of the inhibitor in the host. There is growing 655 evidence of the contributory role of the gut microbiota in health and disease, thus 'medicating 656 the microbiome' is at the forefront of clinical potential and could become a common therapy 657 in the future. The development of drugs which inhibit selective gut microbial catabolic 658 pathways mechanistically linked with pathogenesis of disease should be further investigated 659 as a potential therapy for CVD.

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- 661 662

Faecal microbiome transplant

663 Faecal microbiome transplantation refers to the transfer of one's own or a donor's faecal 664 sample to a receiver in order to restore gut microbiome dysbiosis and function (146). With 665 more than 70% of gut bacteria colonising the large intestine, they are commonly represented 666 in a stool sample (127). Currently, faecal transplantation is used for treating gastrointestinal 667 disease, such as *Clostridium difficile* infection and inflammatory bowel diseases such as 668 ulcerative colitis (146, 164). Several studies investigated faecal transplantation as a prospect 669 in altering the gut microbiome as a potential therapy for metabolic syndrome and obesity (8, 670 157). A study involving gut microbial infusion via a gastroduodenal tube in 9 obese and 9 671 healthy Caucasian males revealed peripheral insulin sensitivity improved after 6 weeks post 672 allogenic gut microbiota infusion (from lean donors) (161). In obese subjects there was a 673 lower microbial diversity in faecal microbiota, with high levels of *Bacteroidetes* and reduced 674 *Clostridium* levels compared to healthy controls. Upon allogenic gut microbial infusion, gut 675 microbiota diversity significantly increased in the obese group, including butyrate producing 676 bacteria Roseburia intestinalis which increased 2.5-fold. This suggests a regulating role of 677 butyrate derived from gut microbiota metabolism causing an improvement in insulin 678 sensitivity in obese Caucasian males. Butyrate has been suggested to prevent translocation of 679 endotoxins derived from the gut microbiota, by promoting intestinal mucosa barrier function 680 (25, 81), which has been associated with insulin resistance (25). Increased gut microbiota 681 diversity has also been linked with improved insulin resistance (25). Recently, faecal 682 transplantation from hypertensive mice to germ free mice have demonstrated that high blood 683 pressure is transferrable (1, 70).

685 There is currently limited evidence on the role of faecal microbiome transplantation in 686 relation to gut microbiota in human patients with CVD, prompting further research in this 687 area in the future. There are also many methodologies and variation in processing, such as, 688 donor choice and testing, faecal microbiome transplantation via upper gastrointestinal tract, 689 enema or colonoscopy, short and long-term monitoring of patients for adverse effects as well 690 as efficacy of treatment (21). These variations mean that faecal microbiome transplantation 691 needs to be regulated before being offered as a suitable treatment option. The clinical use and 692 consequence of faecal transplantation is further limited by news from a recent case involving 693 the severe sepsis and death of two immunocompromised adults after receiving faecal 694 transplantation containing multi-drug resistant organisms (as reported by the FDA). As such, 695 the future use of faecal microbiome transplantation remains challenging.

696

697 Future clinical impact of the gut microbiome on CVD

698

699 There is mounting evidence that the gut microbiome contributes to both normal health and 700 disease in the host. This provides a novel perspective with far reaching clinical impact. The 701 approach to disease diagnosis, management, treatment and prevention is expanding. 702 Metagenomic sequencing and analysis along with molecular and biochemical methodologies 703 has advanced, allowing identification and characterisation of specific microbiome taxa and 704 their associated metabolites in various samples. Although, on a mechanistic level, much 705 remains to be elucidated. In the future, microbiome profiling of patients through metabolomic 706 /biomarker analysis may be performed to determine the health of the individual, with possible 707 guidance on dietary and lifestyle changes as an approach to targeted intervention. 708 Furthermore, the use of faecal microbiome transplantation, prebiotics and probiotics to 709 manipulate and alter the gut microbial ecology to produce beneficial metabolites to improve

710 host health may provide an alternate adjunct therapy.

711 Conclusion

712

713 Microbiome-host interactions are now understood to be paramount in health and disease

- susceptibility. There is a mechanistic link between gut microbiota and physiological
- processes in the body that affect CVD risk. Furthermore, the gut microbiome is a key player
- at the intersection of diet and CVD via its role in metabolising dietary components and thus,
- the synthesis and release of important metabolites such as SCFA and TMAO. Current

718 literature provides evidence that there is a correlation between atherosclerosis and gut 719 microbiome dysbiosis (Figure 4). This change in gut microbiome may contribute to adverse 720 cardiac function resulting in an increase in morbidity and even death. However, it is not clear 721 whether gut microbiome dysbiosis is a contributor and/or consequence of disease. Further 722 research is required in order to characterise the microbiota in healthy individuals and those 723 with CVD. Research into the roles of various metabolites such as SCFA, TMAO and BA and 724 their mechanism of action is also highly warranted as they have been demonstrated to have a 725 vital role in the modulation and influence of host health. As we gain a more comprehensive 726 understanding of the role of the gut microbiota and their metabolites and exactly how they 727 cause the progression and pathogenesis of disease, we may be able to develop advanced 728 diagnostic strategies and potential therapeutic interventions to cater to a more personalised 729 clinical care of patients in the future. Novel therapies including manipulating the diet and 730 using newly designed prebiotic and probiotic options to treat CVD through modulating the 731 gut microbiota and associated metabolites are all gaining traction.

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1261 Tables, Figures and Figure Legends

1262

1263 Table 1: Summary of studies investigating associations between the gut microbiome and CVD.

Reference	Method	Number of	Disease status	Main findings
		subjects		
(121)	16S rRNA &	N =1046	Plasma lipids,	Microbiota predominantly shaped by
	metagenome		Glycaemic traits	non-genetic factors and explain \ge %
	sequencing			variance in HDL, fasting glucose,
				obesity
(64)	Metagenome	N =218 CVD	Atherosclerosis	In CVD, increased Enterobacteriaceae
	shotgun sequencing	N =187 controls		& Streptococcus
(83)	16S rRNA	N =56 pre-	Hypertension	Reduced bacterial diversity
		hypertensive		Overgrowth of Prevotella/Klebsiella in
		N =99 hypertensive		prehypertension and hypertension
		N =41 controls		compared to controls
(61)	qPCR	N =179 CAD	CAD	Aggregatibacter
		N=166 ACS		actinomycetemcomitans
		N =119 controls		levels linked with risk for CAD
(68)	MEDUSA	N =12 symptomatic	Atherosclerosis	Collinsella genus higher in symptomatic
		atherosclerosis		AS

		N=13 controls		Eubacterium & Roseburia higher in
				controls
(77)	qPCR	N =15 CVD	Atherosclerosis	Overgrowth of Veillonella and
	16S rRNA	N =15 controls		Streptococcus in atherosclerotic plaque
				samples

165 rRNA: 16S ribosomal ribonucleic acid, HDL: High density lipoprotein, CVD: cardiovascular disease, qPCR: quantitative polymerase chain reaction, CAD:

1265 coronary artery disease, ACS: acute coronary syndrome, MEDUSA: Metagenomic Data Utilization and Analysis.



1267

1268 Figure 1: Relative distribution of the five main bacterial phyla found in the gut obtained

1269 from the RDP (ribosomal database project) browser. Firmicutes and Bacteroidetes compose

1270 65% and 30% of the gut respectively. *Others* include the remaining phyla: Spirochaetes,

1271 Fusobacteria, Deferribacteres, Cyanobacteria, Planctomycetes, Lentisphaerae, TM7 and

1272 Tenericutes. Adapted from (175).

1273



- 1275 Figure 2: Cardiovascular disease manifestations in which gut microbiota and their associated
- 1276 metabolites are altered. BA: bile acid, SCFA: short chain fatty acid, TMAO: Trimethylamine-
- 1277 N- oxide.
- 1278
- 1279



1282 Figure 3: Interactions between diet and the gut microbiota to form metabolites such as SCFA

1283 and TMAO, which play a crucial role in host health and cardiovascular risk. CVD:

1284 cardiovascular disease, FMO3: Flavin monooxygenase 3, GPCR: G protein coupled receptor,

- 1285 PBA: primary bile acid, SBA: secondary bile acid, SCFA: short chain fatty acid, TMA:
- 1286 Trimethylamine, TMAO: Trimethylamine- N- oxide.





1312 Figure 4: The complex relationship between dietary components ingested and other factors,

1313 affecting the gut microbiota, whose composition then influences their functionality and

- 1314 metabolite production and release. A disruption to which leads to dysbiosis, thereby affecting
- 1315 host health and the progression and pathogenesis of various cardiovascular diseases in a
- 1316 vicious cycle. ATP: Adenosine triphosphate, BA: bile acid, CO₂: carbon dioxide, CH₄:
- 1317 methane, CVD: cardiovascular disease, H₂S: hydrogen sulphide, PBA: primary bile acid,
- 1318 ROS: reactive oxygen species, SBA: secondary bile acid, SCFA: short chain fatty acid,
- 1319 TMAO: Trimethylamine- N- oxide.
- 1320
- 1321
- 1322
- 1323

Tables, Figures and Figure Legends

Reference	Method	Number of	Disease status	Main findings
		subjects		
(117)	16S rRNA &	N=1046	Plasma lipids,	Microbiota predominantly shaped by
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16S rRNA: 16S ribosomal ribonucleic acid, HDL: High density lipoprotein, CVD: cardiovascular disease, qPCR: quantitative polymerase chain reaction, CAD: coronary artery disease, ACS: acute coronary syndrome, MEDUSA: Metagenomic Data Utilization and Analysis.



Figure 1: Relative distribution of the five main bacterial phyla found in the gut obtained from the RDP (ribosomal database project) browser. Firmicutes and Bacteroidetes compose 65% and 30% of the gut respectively. *Others* include the remaining phyla: Spirochaetes, Fusobacteria, Deferribacteres, Cyanobacteria, Planctomycetes, Lentisphaerae, TM7 and Tenericutes. Adapted from (170).



Figure 2: Cardiovascular disease manifestations in which gut microbiota and their associated metabolites are altered. BA: bile acid, SCFA: short chain fatty acid, TMAO: Trimethylamine-N- oxide.



Figure 3: Interactions between diet and the gut microbiota to form metabolites such as SCFA and TMAO, which play a crucial role in host health and cardiovascular risk. CVD: cardiovascular disease, FMO3: Flavin monooxygenase 3, GPCR: G protein coupled receptor, PBA: primary bile acid, SBA: secondary bile acid, SCFA: short chain fatty acid, TMA: Trimethylamine, TMAO: Trimethylamine- N- oxide.



Figure 4: The complex relationship between dietary components ingested and other factors, affecting the gut microbiota, whose composition then influences their functionality and metabolite production and release. A disruption to which leads to dysbiosis, thereby affecting host health and the progression and pathogenesis of various cardiovascular diseases in a vicious cycle. ATP: Adenosine triphosphate, BA: bile acid, CO₂: carbon dioxide, CH₄: methane, CVD: cardiovascular disease, H₂S: hydrogen sulphide, PBA: primary bile acid, ROS: reactive oxygen species, SBA: secondary bile acid, SCFA: short chain fatty acid, TMAO: Trimethylamine- N- oxide.