The role of gut microbiome in cardiometabolic disease.

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Abstract

The study of the human microbiota has recently emerged as an area of utmost importance. The human gut microbiome consists of trillions of microorganisms that outnumber human cells and play a vital role in host metabolism. Obesity is a risk factor for cardiometabolic disease (CMD), which includes type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension, cardiovascular disease and non-alcoholic fatty liver disease (NAFLD). Recent studies have shown a connotation between microbiota and CMD. Studies have established that the gut microbiota is one of the crucial factors, which influence the metabolic state of the host and acts as a propeller for the disease. In this review, we focus on the recent findings on the dysbiosis of gut microbiota and CMD pathogenesis and future therapeutic intervention involving gut microbiome manipulation.

Keywords: CMD, Gut microbiome, Metabolites, obesity, Type 2 diabetes, NAFLD.

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Introduction

Cardiometabolic Disease (CMD) is combinations of metabolic dysfunctions mainly includes obesity and related co-morbidities such as dyslipidemia, hypertension, Type 2 Diabetes Mellitus (T2DM), cardiovascular disease and Non-Alcoholic Fatty Liver Disease (NAFLD). With the increasing prevalence of obesity in both developing and developed countries, there has been an increase in CMD [1-7]. CMD is multifactorial disease, which plays crucial role including, genetic, and environment factors like diet, lifestyle, and also change in the gut microbiota [4,8]. The collection of microbes colonizing the human gastrointestinal (GI) tract is termed the 'gut microbiota'that has co-evolved with the host to form mutually beneficial relationship [9,10]. The human body has up to 100 trillion microorganisms [11]. The primary reservoir of microbes in a human body is the large intestine [12]. Although in utero it is thought that the human gut is not entirely sterile [13]. The pioneering colonization of the gastrointestinal tract in the child is due to transmission of bacteria from skin, anus and vagina of the mother [14].

As the child grows the gut microbial population upsurge in terms of quantity as well as diversity [15,16]. The specific bacterial taxa composition differs among children delivered vaginally and by cesarean section [17,18]. The bacterial communities *Lactobacillus, Prevotella*, or *Sneathia spp*. of mother's vaginal microbiota predominate in vaginally born babies whereas bacterial communities *Staphylococcus, Corynebacterium*, and *Propionibacterium* spp [19] which are similar to those on the skin surface predominates in babies born by cesarean-section. Differences in the microbiome by delivery mode persist into later childhood [20]. Besides, the mode of delivery of neonatal food is an important determining factor [21]. More adult-like microbiota dominated by *Clostridium* spp. and *Bacteroides* spp. by approximately 3 years of age as the introduction of more solid foods [16,22,23]. The gut microbiome alteration and the CMD association has been recently studied and established [24,25].

CMD and Host-Microbiota Interactions

The interactions of metabolites with organs and their impact have been summarized in Table 1 and Figure 1.Gut microbiota also takes part in food digestion through saccharolytic and proteolytic pathways [26]. Within the saccharolytic pathway, break down of sugars produces the bulk of Short-Chain Fatty Acid (SCFA). The proteolytic pathway is protein fermentation, which induces SCFA formation. In addition to digestion of food, the gut microbiota also interacts through different pathways with the host, like the Trimethylamine (TMA)/ Trimethylamine N-oxide (TMAO) pathway, SCFAs pathway, and primary and secondary bile acid (BAs) pathways [27-30], thereby influencing general systemic immunity and metabolism [31].

Human cannot hydrolyze complex carbohydrates such as cellulose, xylans, pectin, inulin, and resistant starch[32]. These indigestible plant polysaccharides are fermented by microbes in the colon to yield energy for their growth in the form of (SCFAs) such as butyrate, acetate, and propionate. Among SCFAs, butyrate is an important energy substrate for colonic epithelium [33]. SCFA interacts with G-protein-coupled receptor, GPR41, and GPR43, which stimulates the secretion of hormone Glucagon-Like-Peptide (GLP-1) lead to insulin sensitivity. GPR43-deficient mice develop obesity even if they are fed with a healthy diet, whereas mice that overexpress GPR43 specifically in the adipose tissue remain lean and thin [30]. GPR43 modulates metabolic effect after engaging with microbiota-derived SCFA. Butyrate works by different molecular mechanism in energy regulation like intake, storage and expenditure [34]. The other mechanisms by which SCFA induce their function is through regulating intestinal gluconeogenesis [35].

Table 1: *Summary of metabolites, their target organs and control of functions.*

Metabolites	Target organs	Function	References
Short-chain fatty acids (SCFAs)	Adipose tissue, brain, intestine, liver, muscle	i. Reduce ingestion behaviour. ii. Host insulin sensitivity iii. Improves intestinal barriers through mucous secretions and tight junction protein expression.	[51-54]
Salt	Adipose tissue,intestine, muscle	i. Low-grade inflammation, ii. Insulin resistance (T2DM)	[55,56]
Trimethylamine N-oxide (TMAO)	Adipose tissue, liver	i.Atherosclerosis ii.cardiovascular disease risk factors	[57-60]
Bile acid	Adipose tissue, intestine, liver	i.Hepatic metabolism, ii.lipid metabolism iii.Cholic acid decreases circulating triglycerides iv. Raising energy expenditure through fat oxidation.	[61-64]
Lipopolysaccharide (LPS)	Adipose tissue, liver, brain	i.Systemic inflammation, ii.hepatic glucose metabolism, iii. adipose tissue fibrosis	[65-67]
Branched-chain amino acids (BCAAs)	Adipose tissue, endothelium,skeletal muscle	i.Adipogenesis ii. lipid trafficking, lipogenesis, iii.Insulin resistance	[68-72]

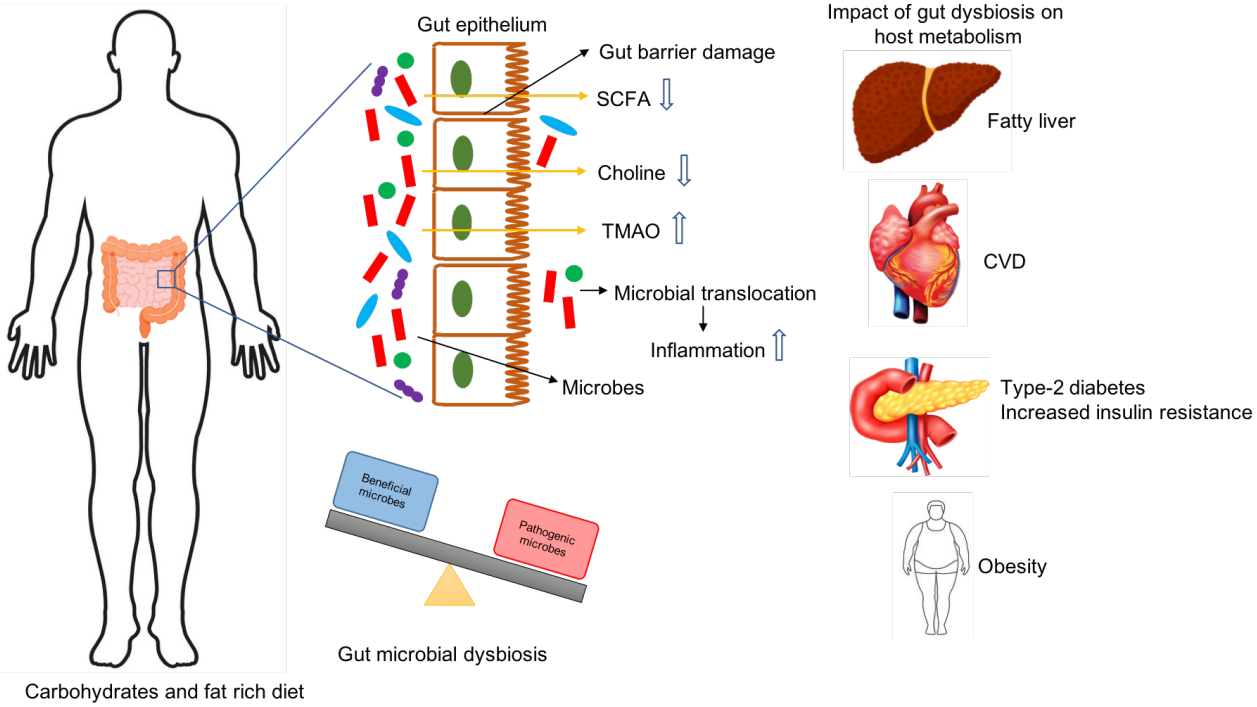

Figure 1. *Alternation of gut microbes and their impact on organs. Increased production of trimethylamine-N-oxide (TMAO) promotes risk of atherosclerosis and deficiency of choline to liver cells may promote Non Alcoholic Faty Liver Disease (NAFLD) due to accumulation of fatty acid in hepatocytes. High fibrous diet lead to production of Short Chain Fatty Acid (SCFA) such as propionate, butyrate and acetate production that act as signaling molecules to induce the insulin sensitivity to muscle cells and hepatocytes. Butyrate is essential for maintenances of low pH and integrity of GI epithelium to inhibit growth of pathogenic microbes and protect from endotoxemia.*

Butyrate has been shown to enhance the integrity of the intestinal barrier by promoting the assembly of tight junction proteins shown in Caco2 cell monolayer model [36] and also enhances intestinal barrier integrity by increasing mucus production and tight junction protein expression which has been proved in animal model [37].

Obesity-driven insulin resistance is a dominant pathophysiological factor of T2D [38]. The etiology of insulin resistance is complex and involves multiple pathways [39]. Inflammatory pathways play a vital role in the development of insulin resistance [40]. Overeating and specific diet could alter the gut microbiota, which may affect lipid metabolism leading to systemic inflammation [41]. An altered microbiota in metabolic disorder may initiate inflammatory processes via an impaired mucosal barrier. The impaired mucosal barrier facilitates microbial translocation resulting in elevated systemic inflammation as seen in the patients with T2D [42,43].

Process of inflammation, insulin resistance, bile acids, and choline metabolism promoted by gut microbiome alteration induces the development of NAFLD [44,45]. SCFAs, in particular, and also acetate and propionate, are transported to the liver via the portal vein where they serve as substrate for gluconeogenesis and lipogenesis. The absorption of intestinal monosaccharides, also promoted by gut microbiome which enhances hepatic lipogenesis and, the accumulation of triglycerides in adipocytes [46,47]. The fat accumulation and inflammation in hepatocytes in NAFLD is induced by insulin resistance [48].

The gut microbiota also influences the metabolic state through bile acid homeostasis [41]. Bile acids bind to nuclear Farnesoid X Receptor (FXR) and G-protein coupled TGR5 receptor, both these receptors regulate glucose metabolism in mice [49]. Primary bile acid activates FXR receptors which impairs hepatic lipogenesis and gluconeogenesis [41]. Unlike FXR, TGR5 binds to secondary bile acids and enhance glucose homeostasis. TGR5 promotes glucose tolerance in obese mice by stimulating GLP-1 hormone release, which boosts insulin secretion and has anti-diabetic effects in enteroendocrine L-cells [41]. Because bile acids are absorbed from the gut and distributed throughout the body, TGR5 and FXR activation in peripheral organs may aid overall host metabolism. TGR5 activation increases energy expenditure in muscle and adipose tissues, avoiding diet-induced obesity [50] (Table 1 and 2).

Gut microbiota also metabolizes choline and produce trimethylamine, which further metabolized in the liver to Trimethylamine N-oxide (TMAO) [30,43]. The association of plasma levels of TMAO with cardiovascular disease has been reported [43,81]. The relationship between atherosclerosis and the gastrointestinal microbiota has recently been focused on (TMA) and enzymes expressed by each bacteria as well as the host. The activity of a collection of microbial enzymes can convert dietary choline to TMA [82]. Trimethylamine N-oxide (TMAO), a TMA metabolite generated in plasma by mammalian flavin monooxygenases FMO1 and FMO3, has been associated to atherosclerosis [83]. A direct relationship between choline and phosphatidylcholine metabolism, the GI microbiota, and TMAO was recently examined in humans with and without antibiotics using deuterium-labeled eggs [84]. The GI microbiota also converts red meat to TMAO, and certain bacteria have recently been linked to TMAO synthesis [28].

Gut Microbiome and Cardiometabolic Disorders

The prevalence of the cardiometabolic disease has been increasing globally [75]. The study of metagenome derived from stool samples, which decipher the intestinal microbiota composition has been possible due to recent development in high throughput sequencing. It has been shown in different studies that dysbiosis causes cardiometabolic disease [65]. The correlation of dysbiosis and factors are summarized in Table 2.

Table 2: *Correlations between conditions and factors affecting the gut microbiome composition.*

Conditions	The abundance of specific bacteria	References
Carbohydrate- or fat-restricted	*Firmicutes* increases and *Bacteroidetes* decreases in obese.	[73]
Salt intake,	Bifidobacterium decreases and Akkermansiamuciniphila increases in obese compared to healthy.	[74]
Host Genotype and adiposity, environmental exposure	The decrease in *Bacteroidetes* proportion of Actinobacteria in obese.	[75]
	Reduced diversity with obesity.	
Caloric intake	The relative abundance of microbiota on the phylum *Bacteroidetes* versus *Firmicutes* changes in rich calorie diet.	[8]
The bacterial abundance in metabolic disease.	With rise in adiposity, insulin resistance, dyslipidemia, and more inflammatory phenotype shows decline in bacterial richness.	[75]
Gut microbiota composition and associated functions in terms of T2D	Decrease in Diversity. Decrease in *Firmicutes* with increases in abundance of Lactobacillus spp. Also, the abundance of *Clostridium* spp.	[76]
	The positive correlation between *Clostridium* spp. With fasting glucose and HbA1c, whereas a negative correlation between Lactobacillus spp. With fasting glucose, insulin, C-peptide.	
Atherosclerotic plaque,	No difference between CVD patients and healthy controls at the phylum or genus level.	[79]
Patients with CVD risk factors	Colinsella higher in patients, whereas Eubacterium and Roseburia higher in controls.	[65]
	Clostridiales, *Clostridium* and Peptostreptococcus negatively correlated with hsCRP.	
Role of gut microbiota in NAFLD	Increase of *Proteobacteria* and decrease of *Firmicutes*	[80]

Obesity

Several studies have found that genetically obese mice (ob/ob) had a greater *Firmicutes* count and a 50% reduced Bacteroidetes count [85]. Only a 20% increase in *Firmicutes* and a 20% drop in *Bacteroidetes* results in a 150 kcal/day increase in energy harvest. This suggests that the ratio of *Bacteroidetes* to Fermicutes is a predictor of obesity predisposition [8]. Apart from two phyla, *Firmicutes* and *Bacteroidetes*, dysbiosis in obesity is linked to several bacterial taxonomic levels (e.g., family, genus, and even species) [85]. When germ-free mice were colonised with the microbiota of obese littermates, total body fat increased considerably more than in control mice [47]. Microbiota from obese or lean humans implanted in germ-free mice achieved donor traits such as adipocity, according to studies [86]. In overweight/obese humans, reduced faecal bacterial diversity is linked to obesity, dyslipidemia, impaired glucose homeostasis, and low-grade inflammation [87]. When 12 obese people's faeces were compared to the faeces of five healthy people, metagenomic sequencing revealed that *Firmicutes* were greater and Bacteroidetes were approximately 90% lower [85]. In the Human Intestinal Tract (MetaHIT) study, a reduction in microbial gene richness and gene count by 40% was detected in 292 non-diabetic Danish individuals, 23 percent sample, and an increased risk of obesity-related comorbidities [88].

Type 2 Diabetes

Gut microbiota has a critical function in regulating metabolic pathways [88]. Obesity-driven insulin resistance is a dominant pathophysiological factor [89]. Studies reported that metabolic inflammation is reported to be the most crucial ones responsible for the evolution of insulin resistance [39]. Overeating and specific diet could alter the gut microbiota, which may affect lipid metabolism leading to systemic inflammation [41]. An altered microbiota in metabolic disorder may initiate inflammatory processes via an impaired mucosal barrier. The impaired mucosal barrier facilitates microbial translocation resulting in elevated systemic inflammation as seen in the patients with T2D [42,43].

Having observed a link between obesity and gut microbiota, it would be interesting to explore any association between gut microbiota and T2D, as obesity is considered as a precursor for insulin resistance and diabetes. The first study conducted by Larson et al. on patients with T2D studied that *Firmicutes* was convincingly higher in the control group, whereas the phylum *Bacteroidetes* and *Proteobacteria* were enriched in diabetic group [90]. A metagenome study on 344 Chinese T2D patients showed a significant dysbiosis with decrease in butyrate-producing bacteria belonging to the *phyla Firmicutes* comprising *Clostridiales* sp, however was no difference in diversity was observed between the T2D patient and control [90]. The faecal microbiota in Swedish women found that, out of 145 women, 53 had T2D, 49 had reduced glucose tolerance, and 43 had standard glucose tolerance found no compositional changes. It was observed an abundance of four Lactobacillus species and a decrease in the abundance of *Clostridium* species [91]. The difference in Chinese and Swedish study can distinguish few metagenomic changes. Both the reports indicate that still need to find and establish strong linkage (Figure 1).

Cardiovascular disease risk

The correlation of CVD and gut microbiome has been reported through various studies. A study of 15 atherosclerosis patients and healthy controls found that atherosclerotic plaques and gut bacteria shared operational taxonomic units (OTUs) [80]. A genome sequencing study revealed that atherosclerosis patients' faeces were enriched in the species Collinsella, whereas healthy people had high levels of Roseburia and Eubacterium [92]. *Clostridium* and Peptostreptococcus were found to have a negative relationship with high-sensitivity C-reactive protein, an inflammatory marker linked to an increased risk of cardiovascular disease [93].

The CAD and gut microbiome correlation has been reported in several studies [94-96]. A metagenomic analysis of feacal samples from 218 CAD patients and 187 healthy controls from China revealed that the CAD patients had a greater *Firmicutes/Bacteroidetes* ratio and a higher abundance of the order *Lactobacillales* than the controls [94]. *Bacteroides* spp. richness is reduced in patients with atherosclerotic ischemic stroke and transient ischemic attack [95]. Which may play a role in the development of atherosclerosis Antiinflammation marker Faecalibacterium prausnitzii was found dramatically reduced in CAD patients [97]? Japanese The researchers looked at 12 heart failure patients and 12 age-matched healthy people. The gut microbial diversity was not statistically different; however Dorea and *Clostridium* were significantly lower in HF patients compared to controls [98].

Nonalcoholic fatty liver disease

A chronic liver disease which includes steatosis without inflammation, nonalcoholic steatohepatitis (NASH), fibrosis, or NASH-induced cirrhosis is known as Nonalcoholic fatty liver disease (NAFLD) [97,99-103]. NAFLD has grown the most common chronic metabolic disease worldwide due to growing obesity [50,104]. The correlation of the gut microbiome with NAFLD in mice and humans has been reported in recent past [105]. Microbiome from mice that had hyperglycemia and insulinemia inoculated in germ-free mice, developed NAFLD [106]. Report suggested that some specific gut microbiota community may have a potential role in the pathogenesis of NAFLD [107]. The bacterial species, *Lachnospiraceae* and Barnesiella spp. were higher in NAFLD patient, compared to control that indicate these species may have role in development of NAFLD [106]. NAFLD patients had abundance of bacterial species, such as *Proteobacteria, Enterobacteria*, and *Escherichia* [108], or *Bacteroides* [109] as compared to healthy individuals. An increase in *Proteobacteria* and a decrease in *Firmicutes* were observed during the progression of NAFLD.

Microbiome Manipulation to Improve Health Outcomes

Convincing literature is available showing dysbiosis of the gut microbiota and development of the cardiometabolic disorder. It is now utmost interest in gut microbiota modulation as a therapeutic strategis as main target. The microbiome is a potential target to try and reduce disease burden as subject to modification unlike the human genome. Probiotics, targeted antibiotics, and dietary modifications may all represent ways to

change the microbiome. Moreover, specific processes to directly manipulate the microbiome may prove to be beneficial to mitigate some of the diseases. Fecal Microbiota Transplantation (FMT), is sufficient to treat recurrent *Clostridium* difficile infection, known to be associated with underlying microbiome dysbiosis [110-112]. Given the success of FMT in this one area, it is not surprising, that consideration of FMT for other conditions associated with disturbances of the microbiome is under active discussion [113]. Moreover, "vaginal seeding," the transfer of mother's vaginal microbiome to her cesarean delivered infant has been shown in a pilot study to partially restore the infant's microbiome to that closer of an infant born by vaginal delivery [114]. Additionally, vaginal seeding in murine models decreases the effect of cesarean section related excess weight gain [115]. Randomized controlled trials are ongoing to see if vaginal seeding can decrease the risk of chronic CMD associated with cesarean delivery. These methods are promising to manipulate the microbiome to improve health outcomes and learn more about the mechanisms by which the microbiome influences CMD development.

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AKR, RT, RC and Shalimar were, responsible for the conception and design of the manuscript. All authors edited and approved the version to be published. Ashwini Kumar Ray and Urvinder Kaur both are conrtibuted equally.

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