

The role of gut microbiome in cardiometabolic disease.

Ashwini Kumar Ray^{1,5#*}, Urvinder S. Kaur^{3#}, Jyoti Gupta¹, Alka Yadav¹, Tannu Bhagchandani³, Alok Kumar Singh⁴, Shalimar², Ravi Tandon^{3*}, Rupesh Chaturvedi^{1,6*}

¹Host-Pathogen Interaction Laboratory, School of Biotechnology, Jawaharlal Nehru University, New Delhi, India

²Department of Gastroenterology and Human Nutrition Unit, All India Institute of Medical Sciences, New Delhi, India

³Laboratory of AIDS Research and Immunology, School of Biotechnology, Jawaharlal Nehru University, New Delhi, India

⁴Department of Biochemistry, Shaheed Rajguru College of Applied Sciences for Women, University of Delhi, New Delhi, India

⁵Laboratory of Metabolic Disorder and Environmental Biotechnology, Department of Environmental Studies, Faculty of Science, University of Delhi, New Delhi, India

⁶Nanofludiks Research Pvt. Ltd. AIC-JNUFI, JNU New Delhi, New Delhi, India

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.	[51-54]
		ii. Host insulin sensitivity	
		iii. Improves intestinal barriers through mucous secretions and tight junction protein expression.	
Salt	Adipose tissue, intestine, muscle	i. Low-grade inflammation,	[55,56]
		ii. Insulin resistance (T2DM)	
Trimethylamine N-oxide (TMAO)	Adipose tissue, liver	i. Atherosclerosis	[57-60]
		ii. cardiovascular disease risk factors	
		ii. lipid metabolism	
Bile acid	Adipose tissue, intestine, liver	iii. Cholic acid decreases circulating triglycerides	[61-64]
		iv. Raising energy expenditure through fat oxidation.	
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	[68-72]
		ii. lipid trafficking, lipogenesis,	
		iii. Insulin resistance	

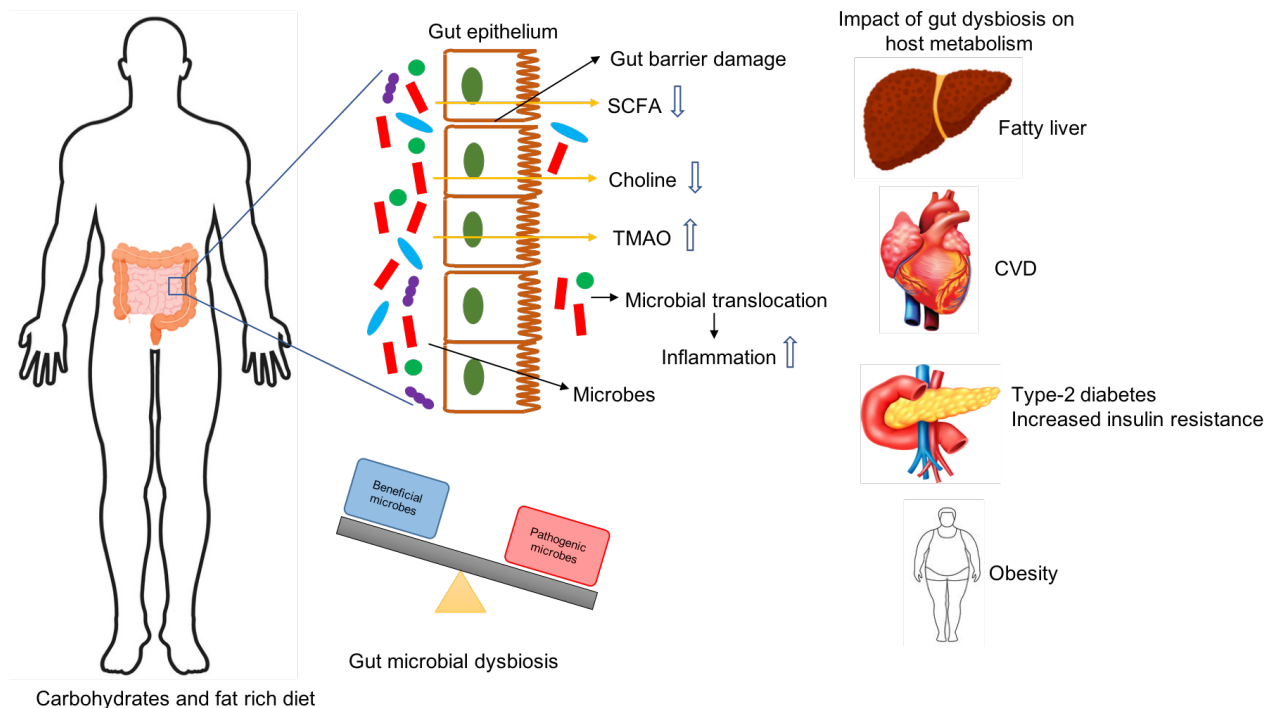


Figure 1. Alteration of gut microbes and their impact on organs. Increased production of trimethylamine-N-oxide (TMAO) promotes risk of atherosclerosis and deficiency of choline to liver cells may promote Non Alcoholic Fatty Liver Disease (NAFLD) due to accumulation of fatty acid in hepatocytes. High 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	<i>Firmicutes</i> increases and <i>Bacteroidetes</i> 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 <i>Bacteroidetes</i> proportion of Actinobacteria in obese.	[75]
Caloric intake	Reduced diversity with obesity. The relative abundance of microbiota on the phylum <i>Bacteroidetes</i> versus <i>Firmicutes</i> 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 <i>Firmicutes</i> with increases in abundance of <i>Lactobacillus</i> spp. Also, the abundance of <i>Clostridium</i> spp.	[76]
Atherosclerotic plaque,	The positive correlation between <i>Clostridium</i> spp. With fasting glucose and HbA1c, whereas a negative correlation between <i>Lactobacillus</i> spp. With fasting glucose, insulin, C-peptide.	[79]
Patients with CVD risk factors	No difference between CVD patients and healthy controls at the phylum or genus level. <i>Colinsella</i> higher in patients, whereas <i>Eubacterium</i> and <i>Roseburia</i> higher in controls.	[65]
Role of gut microbiota in NAFLD	<i>Clostridiales</i> , <i>Clostridium</i> and <i>Peptostreptococcus</i> negatively correlated with hsCRP.	[80]
	Increase of <i>Proteobacteria</i> and decrease of <i>Firmicutes</i>	

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 *Firmicutes* 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 adiposity, 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 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 faecal 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 strategies 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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Author's contributions:

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 contributed equally.

References

- Dumas ME, Barton RH, Toye A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci U S A*. 2006; 103: 12511-12516.
- Forslund K, Hildebrand F, Nielsen T, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*. 2015; 528: 262-266.
- Karlsson FH, Fak F, Nookaew I, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nat Commun*. 2012; 3: 1245.
- Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. *Nat rev. Endocrinol*. 2013; 9: 13-27.
- Han J, Lawlor D, Kimm. Childhood obesity. *Lancet*. 2010; 375: 1737-1748.
- Springer SC, Silverstein J, Copeland K, et al. Management of type 2 diabetes mellitus in children and adolescents. *Pediatrics*. 2013; 2012-3496.
- Kuklina EV, Tong X, George MG, et al. Epidemiology and prevention of stroke: a worldwide perspective. *Expert Rev Neurother*. 2012; 12: 199-208.
- Jumpertz R, Le DS, Turnbaugh PJ, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am J Clin Nutr*. 2011; 94: 58-65.
- Thursby E, Juge N. Introduction to the human gut microbiota. *Biochem J*. 2017; 474: 1823-1836.
- Koenig JE, Spor A, Scafone N, et al. Succession of microbial consortia in the developing infant gut microbiome. *PNAS*. 2011; 108: 4578-4585.
- Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. *Nature*. 2007; 449: 804-810.
- Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr*. 1999; 69: 1035s-1045s.
- Jiménez E, Marín ML, Martín R, et al. Is meconium from healthy newborns actually sterile?. *Res Microbiol*. 2008; 159: 187-193.
- Korpela K, de Vos WM. Early life colonization of the human gut: microbes matter everywhere. *Curr Opin Microbiol*. 2018; 44: 70-78.
- Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol*. 2016; 14: e1002533.
- Yatsunenkov T, Rey F.E, Manary M.J, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012; 486: 222-227.
- Biasucci G, Benenati B, Morelli L, et al. Cesarean delivery may affect the early biodiversity of intestinal bacteria. *J Nutr*. 2008; 138: 1796S-1800S.
- Azad MB, Konya T, Maughan H, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *Cmaj*. 2013; 185: 385-394.
- Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A*. 2010; 107: 11971-11975.
- Salminen S, Gibson G, McCartney A, et al. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut*. 2004; 53: 1388-1389.
- Fanaro S, Chierici R, Guerrini P, et al. Intestinal microflora in early infancy: composition and development. *Acta Paediatr Suppl*. 2003; 92: 48-55.
- Bergström A, Skov TH, Bahl MI, et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. *Appl Environ Microbiol*. 2014; 80: 2889-2900.
- Turnbaugh PJ, Hamady M, Yatsunenkov T, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009; 457: 480.
- Everard A, Cani PD. Diabetes, obesity and gut microbiota. *Best Pract Res Clin Gastroenterol*. 2013; 27: 73-83.

25. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology*. 2014; 146: 1513-1524.
26. Sekirov I, Russell SL, Antunes LCM, et al. Gut microbiota in health and disease. *Physiol Rev*. 2010; 90: 859-904.
27. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011; 472: 57.
28. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med*. 2013; 19: 576.
29. Zhu W, Gregory JC, Org E, et al. Gut microbial metabolite TMAO enhances platelet hyper reactivity and thrombosis risk. *Cell*. 2016; 165: 111-124.
30. Kimura I, Ozawa K, Inoue D, et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun*. 2013; 4: 1829.
31. Neish, AS. Microbes in gastrointestinal health and disease. *Gastroenterology*. 2009; 136: 65-80.
32. Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr*. 2002; 22: 283-307.
33. Hague A, Butt AJ, Paraskeva, C. The role of butyrate in human colonic epithelial cells: an energy source or inducer of differentiation and apoptosis?. *Proc. Nutr. Soc*. 1996; 55: 937-943.
34. Gao Z, Yin J, Zhang J, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes*. 2009; 58: 1509-1517.
35. De Vadder F, Kovatcheva-Datchary P, Goncalves D, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell*. 2014; 156: 84-96.
36. Peng L, Li Z-R, Green RS, et al. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr*. 2009; 139: 1619-1625.
37. Wang HB, Wang PY, Wang X, et al. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig Dis Sci*. 2012; 57: 3126-3135.
38. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest*. 2011; 121: 2126.
39. Moller DE, Kaufman KD. Metabolic syndrome: a clinical and molecular perspective. *Annu. Rev. Med*. 2005; 56: 45-62.
40. Johnson AM, Olefsky JM. The origins and drivers of insulin resistance. *Cell*. 2013; 152: 673-684.
41. Kau AL, Ahern PP, Griffin NW, et al. Human nutrition, the gut microbiome and the immune system. *Nature*. 2011; 474: 327.
42. Pussinen PJ, Havulinna AS, Lehto M, et al. Endotoxemia is associated with an increased risk of incident diabetes. *Diabetes care*. 2011; 34: 392-397.
43. Lassenius M.I, Pietiläinen K.H, Kaartinen K, et al. Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. *Diabetes care*. 2011; 34: 1809-1815.
44. He X, Ji G, Jia W, et al. Gut microbiota and nonalcoholic fatty liver disease: insights on mechanism and application of metabolomics. *Int. J. Mol. Sci*. 2016; 17: 300.
45. Mouzaki M, Bandsma R. Targeting the gut microbiota for the treatment of non-alcoholic fatty liver disease. *Curr Drug Targets*. 2015; 16: 1324-1331.
46. Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. *Curr Pharm Des*. 2009; 15: 1546-1558.
47. Turnbaugh PJ, Ley R.E, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006; 444: 1027-1031.
48. Tarantino G, Caputi, A. JNKs, insulin resistance and inflammation: A possible link between NAFLD and coronary artery disease. *World J Gastroenterol*. 2011; 17: 3785-3794.
49. Prawitt J, Abdelkarim M, Stroeve, JH, et al. Farnesoid X receptor deficiency improves glucose homeostasis in mouse models of obesity. *Diabetes*. 2011; 60: 1861-1871.
50. Wree A, Broderick L, Canbay A, et al. From NAFLD to NASH to cirrhosis—new insights into disease mechanisms. *Nat Rev Gastroenterol Hepatol*. 2013; 10: 627-636.
51. Inoue D, Kimura I, Wakabayashi M, et al. Short-chain fatty acid receptor GPR41-mediated activation of sympathetic neurons involves synapsin 2b phosphorylation. *FEBS Lett*. 2012; 586: 1547-1554.
52. Frost G, Sleeth ML, Sahuri-Arisoylu M, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun*. 2014; 5: 3611.
53. Perry RJ, Peng L, Barry NA, et al. Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. *Nature*. 2016; 534: 213-217.
54. Lin HV, Frassetto A, Kowalik EJ, et al. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One*. 2012; 7: e35240.
55. Pedret A, Valls RM, Calderon-Perez L, et al. Effects of daily consumption of the probiotic *Bifidobacterium animalis* subsp. *lactis* CECT 8145 on anthropometric adiposity biomarkers in abdominally obese subjects: a randomized controlled trial. *Int J Obes (Lond)*. 2018; 43: 1863-1868.
56. Cani PD, de Vos WM. Next-Generation Beneficial Microbes: The Case of *Akkermansia muciniphila*. *Front Microbiol*. 2017; 8: 1765.
57. Brown JM, Hazen SL. Microbial modulation of cardiovascular disease. *Nat Rev Microbiol*. 2018; 16: 171-181.
58. Bennett BJ, de Aguiar Vallim TQ, Wang Z. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab*. 2013; 17: 49-60.

59. Schugar RC, Shih DM, Warriar M, et al. The TMAO-Producing Enzyme Flavin-Containing Monooxygenase 3 Regulates Obesity and the Being of White Adipose Tissue. *Cell Rep.* 2017; 19: 2451-2461.
60. Koeth RA, Levison BS, Culley MK, et al. gamma-Butyrobetaine is a proatherogenic intermediate in gut microbial metabolism of L-carnitine to TMAO. *Cell Metab.* 2014; 20: 799-812.
61. Watanabe M, Houten SM, Wang L, et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest.* 2004; 113: 1408-1418.
62. Thomas C, Gioiello A, Noriega L, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab.* 2009; 10: 167-177.
63. Inagaki T, Moschetta A, Lee Y-K, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci U S A.* 2006; 103: 3920-3925.
64. Watanabe M, Houten SM, Matakai C, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature.* 2006; 439: 484-489.
65. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut.* 2009; 58: 1091-1103.
66. Okla M, Wang W, Kang I, et al. Activation of Toll-like receptor 4 (TLR4) attenuates adaptive thermogenesis via endoplasmic reticulum stress. *J Biol Chem.* 2015; 290: 26476-26490.
67. Vila IK, Badin P-M, Marques M-A, et al. Immune cell Toll-like receptor 4 mediates the development of obesity and endotoxemia-associated adipose tissue fibrosis. *Cell Rep.* 2014; 7: 1116-1129.
68. Mestdagh R, Dumas M-E, Rezzi S, et al. Gut microbiota modulate the metabolism of brown adipose tissue in mice. *J Proteome Res.* 2011; 11: 620-630.
69. Chevalier C, Stojanović O, Colin DJ, et al. Gut microbiota orchestrates energy homeostasis during cold. *Cell.* 2015; 163: 1360-1374.
70. Suarez-Zamorano N, Fabbiano S, Chevalier C, et al. Microbiota depletion promotes browning of white adipose tissue and reduces obesity. *Nat Med.* 2015; 21: 1497-1501.
71. Green CR, Wallace M, Divakaruni AS, et al. Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis. *Nat Chem Biol.* 2016; 12: 15-21.
72. Herman MA, She P, Peroni OD, et al. Adipose tissue branched chain amino acid (BCAA) metabolism modulates circulating BCAA levels. *J Biol Chem.* 2010; 285: 11348-11356.
73. Ley RE, Backhed F, Turnbaugh P, et al. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A.* 2005; 102: 11070-11075.
74. Seck EH, Senghor B, Merhej V, et al. Salt in stools is associated with obesity, gut halophilic microbiota and Akkermansia muciniphila depletion in humans. *Int J Obes (Lond).* 2019; 43: 862-871.
75. Mendis S, Davis S, Norrving B. Organizational update: the world health organization global status report on noncommunicable diseases 2014; one more landmark step in the combat against stroke and vascular disease. *Stroke.* 2015; 46: e121-122.
76. Larsen N, Vogensen FK, van den Berg FW, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One.* 2010; 5: e9085.
77. Karlsson FH, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature.* 2013; 498: 99-103.
78. Zhang X, Shen D, Fang Z, et al. Human gut microbiota changes reveal the progression of glucose intolerance. *PLoS One.* 2013; 8: e71108.
79. Greenland P, Alpert JS, Beller GA, et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines developed in collaboration with the American Society of Echocardiography, American Society of Nuclear Cardiology, Society of Atherosclerosis Imaging and Prevention, Society for Cardiovascular Angiography and Interventions, Society of Cardiovascular Computed Tomography, and Society for Cardiovascular Magnetic Resonance. *J Am Coll Cardiol.* 2010; 56: e50-e103.
80. Loomba R, Seguritan V, Li W, et al. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab.* 2017; 25: 1054-1062.e5.
81. Kimura A, Hara Y, Kimoto T, et al. Cloning and expression of a putative alcohol dehydrogenase gene of *Entamoeba histolytica* and its application to immunological examination. *Clin Diagn Lab Immunol.* 1996; 3: 270-274.
82. Craciun S, Balskus EP. Microbial conversion of choline to trimethylamine requires a glyceryl radical enzyme. *Proc Natl Acad Sci U S A.* 2012; 109: 21307-21312.
83. Bennett BJ, de Aguiar Vallim TQ, Wang, Z, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab.* 2013; 17: 49-60.
84. Tang WW, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med.* 2013; 368: 1575-1584.
85. Tseng CH, Wu CY. The gut microbiome in obesity. *J Formos Med Assoc.* 2019; 118 Suppl 1: S3-S9.
86. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science.* 2013; 341: 1241214.
87. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature.* 2013; 500: 541-546.
88. Tremaroli V, Backhed, F. Functional interactions between the gut microbiota and host metabolism. *Nature.* 2012; 489: 242-249.

89. Tilg H, Moschen AR. Inflammatory mechanisms in the regulation of insulin resistance. *Mol Med*. 2008; 14: 222-231.
90. Qin J, Li Y, Cai, Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012; 490: 55-60.
91. Karlsson, F.H, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*. 2013; 498: 99-103.
92. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev*. 2001; 81: 1031-1064.
93. Brahe LK, Astrup A, Larsen LH. Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases? *Obes Rev*. 2013; 14: 950-959.
94. Jie Z, Xia H, Zhong S-L, et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun*. 2017; 8: 845.
95. Yin J, Liao SX, He Y, 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*. 2015; 4: e002699.
96. Emoto T, Yamashita T, Sasaki N, et al. Analysis of gut microbiota in coronary artery disease patients: a possible link between gut microbiota and coronary artery disease. *J Atheroscler Thromb*. 2016; 23: 908-921.
97. Hena-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*. 2012; 482: 179-185.
98. Kamo T, Akazawa, H, Suda W, et al. Dysbiosis and compositional alterations with aging in the gut microbiota of patients with heart failure. *PloS one*. 2017; 12: e0174099.
99. DiBaise JK, Zhang H, Crowell MD, et al. Gut microbiota and its possible relationship with obesity. *Mayo clin proc*. 2008; 83: 460-469.
100. Chong-Nguyen C, Duboc H, Sokol H. The gut microbiota, a new cardiovascular risk factor?. *Presse med*. 2017; 46: 708-713.
101. Kitai T, Tang WW. The Role and Impact of gut microbiota in cardiovascular disease. *Rev Esp Cardiol*. 2017; 70: 799-800.
102. Tang WW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. *Circ Res*. 2017; 120: 1183-1196.
103. Wieland A, Frank D, Harnke B, et al. Systematic review: microbial dysbiosis and nonalcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2015; 42: 1051-1063.
104. Day CP, James OF. Steatohepatitis: a tale of two "hits"?. *Gastroenterology*. 1998; 114: 842-845.
105. Woodhouse CA, Patel VC, Singanayagam A, et al. Review article: the gut microbiome as a therapeutic target in the pathogenesis and treatment of chronic liver disease. *Aliment Pharmacol Ther*. 2018; 47: 192-202.
106. Le Roy T, Llopis M, Lepage P, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut*. 2013; 62: 1787-1794.
107. Da Silva HE, Teterina A, Comelli EM, et al. Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. *Sci Rep*. 2018; 8: 1466.
108. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology*. 2013; 57: 601-609.
109. Boursier J, Mueller O, Barret M, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology*. 2016; 63: 764-775.
110. Van Nood E, Dijkgraaf MG, Keller JJ. Duodenal infusion of feces for recurrent *Clostridium difficile*. *New Engl J Med*. 2013; 368: 407-415.
111. Cammarota G, Masucci L, Ianiro G, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection. *Aliment Pharmacol Ther*. 2015; 41: 835-843.
112. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal Microbiome in recurrent *Clostridium difficile*-associated diarrhea. *J Infect Dis*. 2008; 197: 435-438.
113. Sbahi H, Di Palma JA. Faecal microbiota transplantation: applications and limitations in treating gastrointestinal disorders. *BMJ Open Gastroenterol*. 2016; 3: e000087.
114. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, et al. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat Med*. 2016; 22: 250-253.
115. Martinez KA, Devlin JC, Lacher CR. Increased weight gain by C-section: Functional significance of the primordial microbiome. *Sci. Adv*. 2017; 3: eaao1874.

*Correspondence to:

Ashwini Kumar Ray
 Laboratory of Metabolic Disorder and Environmental Biotechnology
 Department of Environmental Studies
 University of Delhi
 New Delhi
 India
 E-mail: aray@es.du.ac.in

RRavi Tandon
 Laboratory of AIDS Research and Immunology, School of Biotechnology
 Jawaharlal Nehru University
 New Delhi
 India
 E-mail: ravitandon@jnu.ac.in

Rupesh Chaturvedi
 Host-Pathogen Interaction Laboratory, School of Biotechnology
 Jawaharlal Nehru University
 New Delhi
 India
 E-mail: rupesh@mail.jnu.ac.in