Heart Metab. (2014) 63:18–22

Gut microbes and cardiometabolic risk

Max Nieuwdorp[1,2]
[1]Department of Internal and Vascular Medicine, University of Amsterdam, Amsterdam, the Netherlands
[2]Wallenberg Laboratory, Sahlgrenska Center for Cardiovascular and Metabolic Research, University of Göteborg, Sweden

Correspondence: Max Nieuwdorp, MD PhD, Department of Internal and Vascular Medicine, Academic Medical Centre, Meibergdreef 9, room F4-159.2, 1105 AZ Amsterdam, Netherland.
E-mail: m.nieuwdorp@amc.uva.nl

Abstract

Obesity, type 2 diabetes mellitus, and consequent cardiovascular disease are major public health issues worldwide. There is growing evidence that the increasing prevalence of obesity cannot only be explained by a combination of genes, nutritional habits, and decreased physical activity. An additional factor influencing human metabolism and adiposity, which has recently been considered, is the intestinal microbiota. Obesity is associated with substantial changes in the composition and metabolic function of the commensal bacterial strains living within the human gut. However, the molecular mechanism(s) that mediate the effects of the gut microbiota on host metabolism and metabolic disease are still largely unknown. This review summarizes the latest results in this fascinating new area of research. ■ Heart Metab. 2014;63:18–22

Keywords: Cardiovascular disease; gut microbiota; insulin resistance; obesity.

We are in the midst of a worldwide obesity epidemic, a major factor in the development of common medical conditions such as type 2 diabetes, dyslipidemia, and cardiovascular disease. Obesity is a multifactorial disorder influenced by a mixture of genetic and environmental factors. During the last few years, a growing body of literature points toward a role for the gut microbiota and its function that is encoded by its genome (also called the microbiome) in various diseases ranging from gastrointestinal tract diseases, such as inflammatory bowel disease, to obesity.[1-3] This review discusses the most recent findings and insights into the relationship between the human microbiota, obesity, and insulin resistance.

The human gut microbiota

The adult human intestinal tract contains a large variety of microorganisms, of which bacteria are the most dominant and diverse. The size of this population, up to 100 trillion/g of intestinal material, far exceeds that of all other microbial communities associated with the body's surface. As a whole, the microbiome is more than 100 times larger than the human genome.[4,5] Accordingly, our microbiota can be viewed as a forgotten organ that contributes to overall metabolism and plays a role in converting food into nutrients and energy.[6]

Until recently, our understanding of human gut microbiota was limited, as the majority of the dominant (anaerobic) gut microbiota cannot yet be cultured. The development of 16S ribosomal RNA (rRNA) and genome sequence–based methods has improved the understanding of the gut microbial ecology in humans and mice.[7] The diversity of the gut microbiota in both mice and humans is low at the phylum level, where the majority of species (>90%) belong

Abbreviations

ANGPTL4: angiopoietin-like protein 4; **FIAF:** fasting-induced adipose factor; **LPL:** lipoprotein lipase; **LPS:** lipopolysaccharide; **rRNA:** ribosomal RNA; **SCFA:** short chain fatty acids; **TMAO:** trimethylamine-N-oxide

to Bacteroidetes, Firmicutes, and Actinobacteria. In contrast, the microbial diversity at the species level is very high,[8,9] which was confirmed in a recent metagenomic sequence analysis that revealed a reference set of over 3 million genes in over 100 subjects.

The intestinal microbiota of the newborn human was thought to be essentially sterile, but recent data suggest that modest bacterial translocation via placental circulation occurs antenatally and is likely to provide a primitive bacterial community to the meconium.[10] Although the new concept of fetal intestinal colonization remains controversial, ongoing studies using 16S ribosomal RNA (rRNA) gene pyrosequencing to characterize the bacterial population in the meconium of preterm infants suggest that the bacteria of the maternal intestine are able to cross the placental barrier and act as the initial inoculum for the fetal gut microbiota.[10,11] After transformation to the adult-type, the gut microbiota remains remarkably constant, fluctuating around an individual core of stable colonizers.[11-13] The composition of the microbiota is considered to be influenced by the host genotype, colonization history, the physiology of the host and environmental factors.[14]

Several studies showed that the genetic makeup of the individual influences the composition of the core microbiota as was confirmed in a recent analysis of obese and lean twins.[9] The human gut microbiota is shared among family members, but each person's gut microbial community varies in the specific bacterial lineages present, with a comparable degree of covariation between twin pairs.[9] However, there was a wide array of shared microbial genes among sampled individuals, comprising an extensive, identifiable 'core microbiome' at the gene, rather than at the organism lineage level, suggesting that certain functions are tolerated, or even transmissible, traits in host metabolism.[15]

Gut microbiota and obesity

The initial link between gut microbial ecology and obesity was made by Gordon et al.[8] They found that young conventionally reared mice have 42% more total body fat and 47% more gonadal fat than germ-free mice.[15] This was surprising since the control mice had a lower caloric intake than germ-free mice. The presence of microbiota per se apparently increased the energy yield from the host organism's diet. Following-up on this observation, the same group demonstrated that colonization of young germ-free mice with microbiota from conventionally reared mice produces a 60% increase in body fat mass associated with increased insulin resistance, despite lower energy intake. Moreover, they also demonstrated that fecal transplantation with microbiota from obese mice (ob/ob) results in a significantly greater increase in total body fat than colonization with microbiota from lean donors.[16] Again, these findings underscore the increased ability of microbiota, in obese animals, to extract energy from the diet and provide it to the host.[15,17]

The gut microbiota does not influence only host adiposity through energy extraction from the diet, but also by messenger molecules that influence metabolism throughout the body. For instance, the gut microbiota regulates an important gut-derived regulator of lipid metabolism; fasting-induced adipose factor (FIAF) also referred to as angiopoietin-like protein 4 (ANGPTL4). FIAF regulates fatty oxidation in both muscle and adipose tissue.[18] Microbial colonization of the gut suppresses FIAF expression, leading to suppression of lipoprotein lipase (LPL), and hence, to a greater proportion of triglycerides in adipose tissue. Furthermore, germ-free Fiaf-knockout mice are no longer protected against diet-induced obesity.[19] Bäckhed et al also demonstrated that germ-free mice have increased levels of phosphorylated AMP-activated protein kinase in the muscle and liver, which would stimulate free fatty acid oxidation.[19] Therefore, germ-free animals seem to be protected from diet-induced obesity by two complementary, but independent mechanisms, which results in decreased fatty acid storage, elevated levels of FIAF, and increased AMP-activated protein kinase activity.

Several studies in both mice and humans demonstrated that obesity is associated with an altered gut microbial ecology, hallmarked by lower microbial diversity and decreased levels of Bacteroidetes.[21-23] The shift in microbial composition is associated with alterations in the gut microbial metagenome, notably there is an enrichment of genes involved in energy

Heart Metab. (2014) 63:18–22

harvesting.[16] Ley et al analyzed 5088 bacterial 16S rRNA gene sequences from fat mice (ob/ob phenotype), lean mice (ob/+ phenotype), and wild-type mice and showed that obese animals have a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes.[8] A subsequent metagenomic analysis of these same microbial communities, which was based on shotgun sequencing of the microbial community DNA, showed enrichment in genes involved in energy extraction from food in the microbiome from ob/ob mice relative to the microbiome from ob/+ mice. A microbiota with a greater energy extraction efficiency resulted in less leftover energy in feces and higher levels of short-chain fatty acids (SFCAs) in the cecum.

Similar to these animal experiments, Bacteroidetes tend to decrease and Firmicutes increase in the feces of obese compared with lean humans.[2] Obese people harbor fewer Bacteroidetes and more Firmicutes than lean controls, and after following a carbohydrate- or fat-restricted low-energy diet, Bacteroidetes increased and Firmicutes decreased. These data suggest a relationship between obesity and diversity of intestinal microbiota. However, other studies were not able to support these specific findings,[3,21] which is most likely due to differences in formulation between (local) diets, as dietary composition has recently gained interest as one of the most important drivers of gut microbiota composition.[22]

Gut microbiota, low grade inflammation, and type 2 diabetes

Several studies provided evidence that gut microbial composition is associated with insulin resistance. As expected, the decreased adiposity in germ-free mice is associated with improved insulin sensitivity and glucose tolerance. However, gut microbiota may also have direct effects on host glucose metabolism.

One way for bacteria to affect insulin sensitivity is through metabolic inflammation caused by elevated endotoxin levels. Lipopolysaccharide (LPS) is continuously produced in the gut through lysis of gram-negative bacteria. LPS is a powerful trigger for secretion of a series of proinflammatory cytokines. Continuous subcutaneous low-rate infusion of LPS led to excessive weight gain and insulin resistance in mice, without

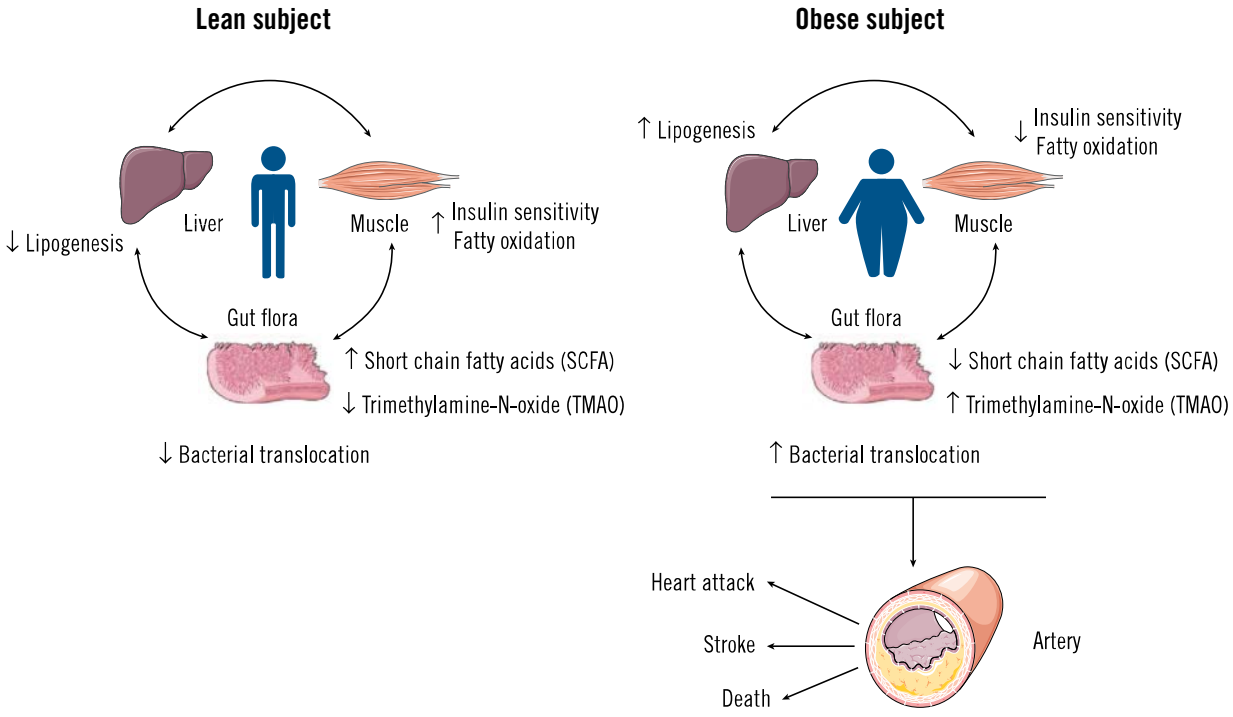

Fig. 1 *The major pathways by which intestinal microbiota can alter human cardiometabolism. Chronic bacterial translocation (due to increased intestinal permeability) can drive systemic inflammation leading to macrophage influx into (visceral) adipose tissue, activation of hepatic Kupffer cells resulting in NAFLD and insulin resistance. Moreover, SCFAs normalize intestinal permeability and alter de novo lipogenesis and gluconeogenesis via reduction of FFA production by visceral adipose tissue. Finally, TMAO can accelerate atherosclerosis and vascular inflammation via influx of macrophages and cholesterol accumulation by up-regulation of macrophage scavenger receptors and reduction in reverse cholesterol transport.*

Abbreviations: *FFA, free fatty acids; NAFLD, non-alcoholic fatty liver disease; SCFAs, short chain fatty acids; TMAO, trimethylamine-N-oxide.*

altering energy intake.[3] Le Chatelier et al showed that an altered diet affects gut microbiota composition, which was associated with an adverse cardiovascular risk profile including glucose intolerance, dyslipidemia, and a chronic low grade (LPS-driven) inflammatory state *(Figure 1)*.[23] Recent association studies by Qin et al (performed in China)[24] and Karlsson et al (performed in Europe)[25] reported differences in microbiota composition and diversity between a cohort of patients with type 2 diabetes and a group of healthy individuals. Independently, each study founds that the microbiota of subjects with type 2 diabetes had a lower proportion of butyrate-producing *Clostridia* (*Roseburia species* and *Faecalibacterium prausnitzii*), and a greater proportion of nonbutyrate producing *Clostridia* as well as pathogens such as *Clostridium clostridioforme*. Moreover, Karlsson et al found that an increased proportion of *Lactobacillus gasseri* and *Streptococcus mutans* (commensal bacteria in the mouth and upper intestinal tract) were predictive of developing type 2 diabetes in this cohort of obese but otherwise healthy postmenopausal females. Qin et al observed a greater proportion of *Escherichia coli*, which produces LPS to cause endotoxemia, in patients with type 2 diabetes. These studies raise interest in the association between intestinal bacterial composition, reduced butyrate production, and chronic low-grade inflammation leading to type 2 diabetes.

Gut microbiota and cardiovascular disease

Besides obesity, intestinal microbiota might also be involved in atherogenesis. Specific dietary nutrients characterized by trimethylamine groups (eg, choline, phosphatidylcholine, and carnitine) are metabolized into the atherogenic compound trimethylamine-N-oxide (TMAO) by bacteria *(Figure 1)*[26,27] and were found to be independent risk factors for cardiovascular events. Studies using germ-free mice or mice given broad-spectrum antibiotics demonstrated that the intestinal microbiota is required for the formation of TMAO. Bacterial colonization of germ-free mice increases their plasma levels of TMAO, indicating that the intestinal microbiota are required for generation of this compound from sources of dietary choline or carnitine (such as eggs, milk, and red meat).[30] For example, carnitine is an abundant nutrient in red meat, and the intestinal microbiota mediates production of TMAO from dietary L-carnitine.

Modulation of gut microbiota composition

Emerging data suggest that an imbalance in the composition of gut microbiota is related to obesity and metabolic disease. Taking a reductionist approach, directly interfering with gut microbiota may ameliorate obesity and the associated insulin resistance. Bile acids have been highlighted as crucial metabolic integrators and signaling molecules involved in the regulation of metabolic pathways including glucose, lipid, and energy metabolism.[3] Interestingly, short term administration of antibiotics in humans significantly altered the fecal bile acid with a reduction in secondary bile acids compared with primary bile acids as well as deterioration of insulin sensitivity.[28] Another intervention to support the causal role of intestinal microbiota in human metabolism and insulin resistance could be the use of fecal transplantation. We examined this hypothesis by transplantation of feces from a lean human donor in participants with a metabolic syndrome (body mass index ≥30 kg/m^2; fasting plasma glucose >5.6; triglycerides >1.6 mmol/L, with no medication use). In this double-blind randomized controlled trial we investigated the effect of transplantation of donor feces on glucose homeostasis and lipid metabolism.[29] Following poly-ethylene-glycol bowel lavage through the duodenal tube, subjects were randomized to either allogenic (from a lean male donor with body mass index <23 kg/m^2, n=9), or autologous (reinfusion of own collected feces, n=9) fecal transplantation. We studied changes in the gut microbiota composition, glucose metabolism (hepatic and peripheral insulin sensitivity as assessed by hyperinsulinemic euglycemic clamp with stable isotopes), and fasting lipid profiles. We found that bacteria producing short chain fatty acids (SCFA) were significantly upregulated in both small intestinal biopsies and fecal samples of metabolic syndrome patients that were treated with allogenic donor feces. However, the exact nature of this symbiotic relationship remains to be elucidated. With high-throughput approaches aimed at documenting diversity at the metagenome level, we might actually be able to unravel the role of the gut microbiota in human metabolism[3] and use this technique as a working tool to discover novel diagnostic and therapeutic capacities of the intestinal microbiota.[30]

Conclusion

Accumulating data from both patients and animal models relate imbalances in the composition of the

gut microbiota to obesity and its associated diseases. However, the exact role of the microbiota and the mechanism mediating its impact on metabolic functions are just beginning to be unraveled. The approaches used to characterize gut microbiota vary widely, which might explain, in part, why the specific alterations in the microbiota can also vary between studies. Comparisons between studies will require a uniform method for measuring the microbial composition. However, irrespective of the specific changes observed in microbial communities, evidence suggests that gut microbiota do indeed respond and contribute to the host's energy balance. They may do this by variable and possibly interactive signaling mechanisms. The major challenge will be to identify and modulate the gut microbiota (or its signaling to the host) to prevent disease and promote health. ■

REFERENCES

1. Tilg H, Moschen AR, Kaser A. Obesity and the microbiota. Gastroenterology. 2009;136:1476-1483.
2. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444:1022-1023.
3. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012;489:242-249.
4. Dethlefsen L, Fall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature. 2007;449:811-818.
5. Xu J, Gordon JI. Honor thy symbionts. Proc Natl Acad Sci U S A. 2003;100:10452-10459.
6. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep. 2006;7:688-693.
7. Zoetendal EG, Rajilic-Stojanovic M, de Vos WM. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut. 2008;57:1605-1615.
8. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005;102:11070-11075.
9. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457:480-484.
10. Jiménez E, Marín ML, Martín R, et al. Is meconium from healthy newborns actually sterile? Res Microbiol. 2008;159:187-193.
11. 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.
12. Zoetendal EG, Akkermans AD, de Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl Environ Microbiol. 1998;64:3854-3859.
13. Biagi E, Nylund L, Candela M, et al. Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. PLoS One. 2010;5:e10667.
14. Zoetendal EG, Antoon DL, Akkermans-van Vliet WM, de Visser AGM, de Vos WM. The host genotype affects the bacterial community in the Human Gastrointestinal Tract. Microb Ecol Health Dis. 2001;13:129-134.
15. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341:1241214.
16. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027-1031.
17. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307:1915-1920.
18. Mandard S, Zandbergen F, van Straten E, et al. The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity. J Biol Chem. 2006;281:934-944.
19. Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A. 2007;104:979-984.
20. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe. 2008;3:213-223.
21. Schwiertz A, Taras D, Schafer K, et al. Microbiota and SCFA in Lean and Overweight Healthy Subjects. Obesity. 2009;18:190-195.
22. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505:559-563.
23. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500:541-546.
24. 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.
25. 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.
26. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472:57-63.
27. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013;19:576-585.
28. Vrieze A, Out C, Fuentes S, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. J Hepatology. 2014;60:824-831.
29. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology. 2012;143:913-916.
30. Olle B. Medicines from microbiota. Nat Biotechnol. 2013;31:309-315.