CHAPTER 11.

The gut microbiota and obesity

Hervé M. Blottière

The human microbiota is composed of about as many microorganisms as there are cells in the human body. It is a very diverse ecosystem comprising more than 100 trillion microbes living in the intestines, the mouth, the skin, the vagina, and elsewhere in the body. Although it was previously called the gastrointestinal flora or microflora, the more pragmatic term "microbiota" is now preferred.

The microbiome, the "other genome" or "second genome" of the human body, is composed of about 10 million genes, compared with about 23 000 genes in the human genome, and thus provides a very rich functional potential. The colonic microbiome is the most diverse and also the best characterized microbial community. Although the human microbiome has fantastic potential, it has only been about 10 years since the scientific community first realized its importance outside the gut, especially after the pioneering work of Gordon and collaborators [1].

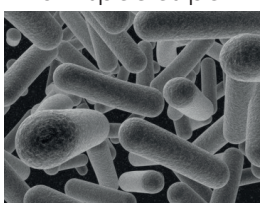
Recently, the development of molecular tools and subsequently of next-generation sequencing enabled the richness of the intestinal ecosystem to be revealed [2]. Each individual harbours hundreds of different species, most of which have not yet been cultured. Studies have revealed that 70–80% of the dominant species have no representative in culture collections. Only a few dozen species are conserved between individuals, representing a core that seems to be a stable community under healthy conditions. Although this view is controversial, some people consider the gut microbiota to be a true organ; as such, it could be transplanted. The recent success of faecal microbiota transplantation, especially in the context of *Clostridium difficile* infection, argues for such a definition [3]. In a healthy symbiotic state, the colonic microbiota is an important organ, interacting with food (in particular dietary fibre, enabling energy harvest from otherwise indigestible dietary compounds), interacting with cells (including immune cells, but also the metabolic and nervous systems), and protecting against pathogens by acting as a barrier to infection (Fig. 11.1).

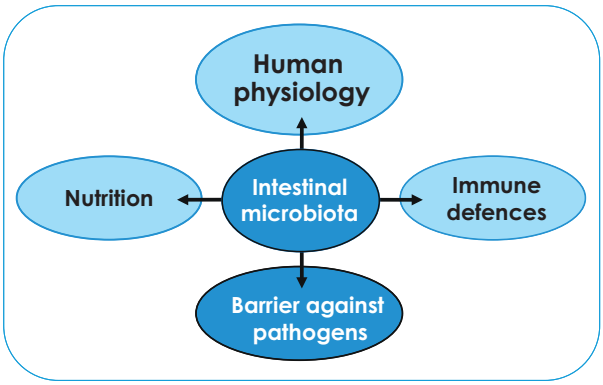
Gene catalogues of gut microbiota

The first draft of the human genome was published in 2000. In 2010, the Metagenomics of the Human Intestinal Tract (MetaHIT) consortium released the first catalogue of human gut microbial genes, obtained after sequencing whole faecal microbiota metagenomes from 124 European individuals [4]. Interestingly, the 3.3 million

Fig. 11.1. The gut microbiota.

- An average of 650 000 genes per microbiome
- About 25–30 times as many genes as the human genome
- About 500–1000 dominant species per individual
- A true organ





gut bacterial genes in the MetaHIT catalogue were also well represented in the other metagenomes that were available at the time, from faecal samples of individuals in the USA and Japan. In parallel, the Human Microbiome Project published a catalogue of 178 reference bacterial genomes distributed among different body sites and including 151 representative gastrointestinal species [5].

In 2014, the MetaHIT consortium published an integrated catalogue of 10 million bacterial genes derived from 1267 human gut metagenomes obtained from individuals on three continents, including 760 samples from Europe. As expected, the number of frequent genes stopped increasing, whereas the number of rare genes, present in not more than 1% of the cohort, continued to increase [6]. Analyses of this close-to-complete catalogue revealed country-specific signatures for xenobiotic metabolism and nutrient consumption for samples from individuals in China and Denmark.

More recently, a catalogue of the mouse gut metagenome was established, emphasizing the host specificity of the microbiota [7]. Only about 4.0% of the mouse gut microbial genes were shared with those of the human gut microbiome. It is important to take this into consideration when attempting to extrapolate results obtained in mouse models to the situation in humans.

Colonization

The colonization process starts at birth, and the delivery type is the first factor that has an impact. For infants that are vaginally delivered, the initial gut microbiota resembles the mother's vaginal microbiota, dominated by bacteria of the genera *Lactobacillus*, *Prevotella*, and *Sneathia*, whereas for infants delivered by caesarean section, the initial gut microbiota resembles the mother's skin microbial community, composed of *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* [8]. Colonization is also strongly affected by the administration of antibiotics in early life [9]. During the first 3 years of life, the infant's gut microbiota is highly unstable and is largely influenced by feeding habits. Key factors are the type of feeding (breastfeeding or formula feeding), the weaning time and process, and food composition, as well as the hygiene of the environment.

By the time an individual reaches adulthood, the intestinal microbiota is composed of several hundreds of different species, belonging only to a few phyla, predominantly Firmicutes, Bacteroidetes, and Actinobacteria [10], although Proteobacteria, Verrucomicrobia, and Fusobacteria are present to a lesser extent. About 50% of individuals harbour Archaea in their microbiota, especially *Methanobrevibacter smithii*, which is responsible for methane excretion. A core of species has been identified as being present in most individuals, but with different relative abundances. The number of species identified in the core depends on the analytical method used: 66 from 16S rDNA sequencing [11] or 57 from whole-metagenome sequencing [5]. Under healthy conditions, the intestinal microbiota is considered to be a stable community, influenced by dietary habits as well as by the physiology of its host.

Enterotypes

Further analysis of several metagenomes led to the discovery of three balanced ecological arrangements, termed enterotypes; the three enterotypes are dominated by *Bacteroides*, *Prevotella*, and *Ruminococcus*, respectively [12]. The third enterotype is also linked to the presence of *M. smithii*. This description of community types is not limited to the gut [13]. These enterotypes or community types emerged as being independent of sex and country of origin but probably associated with

long-term dietary habits [14]. Wu et al. [14] were able to associate consumption of protein and animal fat with the *Bacteroides* enterotype and consumption of carbohydrates with the *Prevotella* enterotype. Interestingly, by analysing samples from volunteers randomized to a high-fat, low-fibre diet or a low-fat, high-fibre diet for 10 days, this study revealed rapid changes in microbiome composition; however, the enterotype of an individual did not seem to be affected by this relatively short-term dietary intervention. Transit time of food through the gut has also been correlated with enterotypes [15].

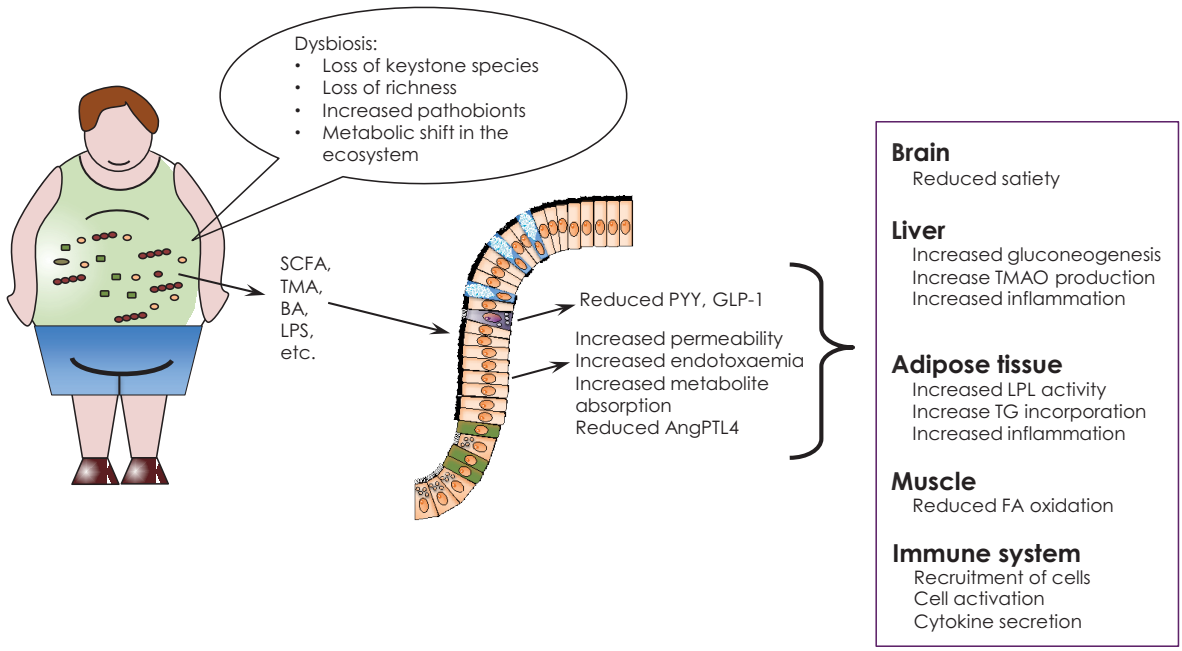
Dysbiosis

The human gut microbiota is very complex and diversified. The microbiome of an individual has more than 25 times as many genes as there are in the human genome. The fitness of this well-balanced symbiosis seems to be essential for the maintenance of a healthy state, and several reports have shown that a state of dysbiosis is often associated with diseases, including inflammatory bowel disease, allergies, colorectal cancer, and liver diseases, as well as obesity, diabetes, and cardiovascular diseases [2]. Dysbiosis may be defined as an imbalanced microbiota, including four types of imbalance: (i) loss of keystone species, (ii) reduced richness or diversity, (iii) increased pathogens or pathobionts, or (iv) modification or shift in metabolic capacities [9] (Fig. 11.2).

The link with obesity

The first link between gut microbiota and obesity came from studies in germ-free rodents. These animals eat more, move less, develop less fat content, and are resistant to diet-induced obesity. Conventionalization of germ-free mice resulted in a 60% increase in body fat mass, accompanied by increased leptin and insulin levels and linked to increased absorption of monosaccharides from the gut lumen, with resulting induction of hepatic de novo lipogenesis [16]. A comparison of the microbiota of lean and obese mice revealed that in obese mice (ob/ob animals), the relative abundance of Bacteroidetes was lower and that of Firmicutes was higher [17]. Moreover, transplanting microbiota from obese animal to germ-free mice resulted in a greater increase in total body fat compared with transplanting microbiota from lean animals, highlighting the contributory role of microbiota to obesity [18]. In a study comparing the microbiota from a dozen obese people with that of a few lean controls, the authors reported that the decreased proportion of Bacteroidetes and the increased proportion of Firmicutes observed in obese mice were also observed in obese people [19]. They

Fig. 11.2. Intestinal microbiota dysbiosis in obesity and physiological perturbation. AngPTL4, angiopoietin-like 4; BA, bile acids; FA, fatty acids; GLP-1, glucagon-like peptide 1; LPL, lipoprotein lipase; LPS, lipopolysaccharide; PYY, peptide YY; SCFA, short-chain fatty acids; TG, triglycerides; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide.



also reported that obese people losing weight on a low-calorie diet had a more balanced microbiota, with an increased proportion of Bacteroidetes and a decreased proportion of Firmicutes, more similar to the microbiota of lean controls.

After this pioneering work, other researchers developed approaches to better understand the mechanisms by which the microbiota can contribute to metabolic syndrome and obesity [20]. Large cohorts of patients were studied.

The MetaHIT consortium investigated the composition of the human gut microbiota in a population sample of 123 non-obese and 169 obese individuals from a Danish cohort study called Inter99 [21]. A quantitative metagenomic pipeline was applied, and the study found two groups of individuals that differed by the number of genes in their metagenome, and thus the gut bacterial richness. About a quarter of the population had low bacterial richness. Individuals with a low gene count had higher adiposity, reduced insulin sensitivity, higher dyslipidaemia, and higher inflammatory status compared with those with a high gene count. The obese individuals in the group with a low gene count gained more weight during the 10 years of follow-up before stool sampling [21].

Similar observations were made in a cohort of obese individuals in France who were recruited to follow a hyper-low-calorie diet with increased intake of protein and fibre [22]. Although the microbial gene richness of the participants increased by 25% after the 6-week diet, the obese individuals with low bacterial richness benefited the least from the diet, whereas those with higher bacterial richness at the start of the diet lost more weight and had a larger improvement in metabolic status.

Interestingly, only a few bacterial species are sufficient to distinguish between individuals with a low gene count and those with high bacterial richness [21]. Among the species that are more prevalent in individuals with high bacterial richness, the analysis highlighted two species: *Faecalibacterium prausnitzii*, a bacterium that was previously described as lacking in patients with inflammatory bowel disease and that has anti-inflammatory properties [23], and *Akkermansia muciniphila*, a bacterium that was found to be associated with body fat mass and glucose intolerance in mice and that was further confirmed to be linked with a healthier metabolic phenotype and better clinical outcomes after a hyper-low-calorie diet in overweight or obese adults [24]. Among the species that are more prevalent in individuals with low bacterial richness are *Bacteroides* strains and *Ruminococcus gnavus*, which are considered to be pro-inflammatory and are often found in patients with inflammatory bowel disease.

Such a phylogenetic shift has also been confirmed at the functional level. Low bacterial richness is associated with a reduction in butyrate-producing bacteria, reduced production of hydrogen and methane, increased sulfate reduction and mucin degradation, increased endotoxaemia, and a higher capacity to manage exposure to oxygen/oxidative stress [21].

Dietary habits seem to be associated with microbiota richness [25]. A dietary pattern with high consumption of potatoes, confectionery, and sugary drinks and low intake of fruits and yogurt was correlated with low microbiota richness, whereas a dietary pattern with low consumption of confectionery and sugary drinks and high intake of fruits, vegetables, soups, and yogurt was correlated with higher microbiota richness.

Mechanisms

The proposed mechanisms by which gut microbiota dysbiosis and loss of richness can promote obesity and insulin resistance are diverse. They are often derived from mouse models and still require complete validation in humans. Dysbiosis is linked to increased energy harvest from food, altered fermentation of fibres, and increased endotoxaemia. These changes in microbiota functions have an impact on different tissues, including the intestine, muscles, adipose tissues, the liver, and the brain [26].

In the intestine, the changes result in increased permeability of the epithelium, allowing translocation of bacteria as well as bacterial products, such as lipopolysaccharides. Moreover, secretion by enteroendocrine cells of hormones, including peptide YY (PYY), glucagon-like peptide 1 (GLP-1), and neurotensin, is impaired, with effects on the brain, resulting in reduced satiety, as well as on the liver and on gut motility. The short-chain fatty acids acetate and propionate are taken up by hepatocytes and serve as substrates for lipogenesis and gluconeogenesis. Thus, increased triglyceride production by the liver, associated with reduced expression of angiopoietin-like 4 (AngPTL4), an inhibitor of lipoprotein lipase, by the small intestine, leads to increased triglyceride incorporation in adipose tissues [26]. Increased inflammation is also observed in different tissues, including gut, liver, and adipose tissues. A reduction of fatty acid oxidation by muscles is also observed.

Finally, the metabolism of bile acids and choline is affected. Perturbation of choline metabolism results in increased production by intestinal microbes of trimethylamine, which is further metabolized by hepatocytes to trimethylamine *N*-oxide, a compound that is associated with liver and cardiovascular diseases [27]. Primary bile acids are transformed by the intestinal microbiota to secondary bile acids, which are potent signalling molecules through

the activation of FXR, a nuclear receptor, and TGR5, a G protein-coupled receptor; these receptors are expressed in intestinal enteroendocrine cells, resulting in the modification of glucose homeostasis [26].

Conclusions

Dysbiosis in intestinal microbiota has been associated with obesity. A loss of bacterial gene richness is linked to more severe metabolic syndrome and lower sensitivity to weight loss after caloric restriction. The role of the gut microbiota in the development and chronicity of obesity still needs to be clarified, and the mechanisms of action in humans remain to be deciphered. Strategies to transiently modulate the human intestinal microbiota and to potentially increase its richness need to be explored [22, 25]. Specific nutrition, prebiotics, and probiotics may be efficient avenues for the prevention of obesity. The recent success of a diet rich in non-digestible carbohydrates in children with Prader–Willi syndrome, resulting in weight loss and reduction of inflammation as well as structural changes of the intestinal microbiota, highlights the feasibility of dietary modulation of the gut microbiome to manage metabolic diseases [28].

Key points

- The human microbiota is a dense and diverse microbiome.
- It includes 100 trillion microorganisms, as many as the number of cells in the human body.
- Each individual harbours hundreds of different species, most of which (70–80% of the dominant species) have not yet been cultured.
- A few dozen species are conserved between individuals (a core), representing a stable community.
- The gut microbiota is a true organ, protecting health and well-being throughout all life stages.
- The colonic microbiota is a key organ, interacting with food (fermentation), interacting with cells (the immune and nervous systems), and protecting against pathogens (barrier function).
- Dysbiosis has been observed in several chronic diseases.
- Dysbiosis is observed in obesity, and a loss of microbiota richness and diversity is associated with inflammatory status.

Research needs

- Standardization of analysis tools and processes is required.
- Longitudinal studies are needed.
- The impact of medication/drugs should be considered.
- Mechanisms of action remain to be deciphered.
- Holistic studies should be designed, associating excellent phenotyping of patients and deep characterization using metabolomics, immunomics, transcriptomics, and metagenomics.
- An ecological understanding of the intestinal ecosystem is needed.

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