Gut microbiota and obesity: lessons from the microbiome

Patrice D. Cani

Abstract

The distal gut harbours microbial communities that outnumber our own eukaryotic cells. The contribution of the gut microbiota to the development of several diseases (e.g. obesity, type 2 diabetes, steatosis, cardiovascular diseases and inflammatory bowel diseases) is becoming clear, although the causality remains to be proven in humans. Global changes in the gut microbiota have been observed by a number of culture-dependent and culture-independent methods, and while the latter have mostly included 16S ribosomal RNA gene analyses, more recent studies have utilized DNA sequencing of whole-microbial communities. Altogether, these high-throughput methods have facilitated the identification of novel candidate bacteria and, most importantly, metabolic functions that might be associated with obesity and type 2 diabetes. This review discusses the association between specific taxa and obesity, together with the techniques that are used to characterize the gut microbiota in the context of obesity and type 2 diabetes. Recent results are discussed in the framework of the interactions between gut microbiota and host metabolism.

Keywords: gut microbiota; obesity; metagenomics; 16S rRNA; Akkermansia; type 2 diabetes

INTRODUCTION

The human gut microbiome has been continuously shaped by the co-evolution of host–microbe interactions. We humans are actually only 10% human; the remaining 90% of our cells are microbes [1]. The human gut is home to 10^{14} bacteria, which outnumber by 10-fold the total number of eukaryotic cells in the human body [2]. In the early 1900s, Robert Koch linked microbes to infectious diseases, and Ilya Mechnikov proposed the use of live microorganisms to maintain human health. Since their discoveries, progress in microbiology has relied on culture-dependent techniques. However, comprehensive knowledge of the gut microbiota has arisen in the last decade from the revolutionary development of culture-independent techniques.

Although not completely understood, this complex ensemble of microorganisms plays an essential role in host immune system development [3,4]; vitamin production and carbohydrate, lipid and amino acid metabolism [5]. Thus, microbial communities perform an extensive consortium of metabolic activities that humans cannot [6].

GUT MICROBIOTA AND DISEASES

Global obesity has more than doubled since 1980. Obesity is associated not only with metabolic disorders such as insulin resistance, type 2 diabetes, non–alcoholic fatty liver diseases and cardiovascular diseases but also with cancer, asthma, sleep apnoea, osteoarthritis, neurodegeneration and gall-bladder disease [7,8]. Although the major cause of obesity is unbalanced energy intake and expenditure coupled with genetic susceptibility, environmental factors contribute to the onset of obesity and its associated disorders. Among the 'external' factors impacting the host response to nutrients, the gut microbiota represents an important one. Changes in the composition and/or activity of the gut microbiota have been linked with numerous pathologies, such as atopic diseases [9], inflammatory bowel diseases [10],

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obesity, type 2 diabetes and cardiovascular diseases [5,11–15].

Due to the diversity of microbes present and the potential relationships between the gut microbiome and disease, the scientific community has new hopes for discovering and developing microbe-based therapeutic strategies. Comparative analyses of human and other animal gut microbiomes have revealed that specific bacterial phyla and species differ between healthy individuals and those diagnosed with obesity and/or type 2 diabetes [12].

Thus, comprehensive knowledge of the gut microbiota is required to understand the correlations between the resident microbial communities and the onset of metabolic disorders. In pursuit of this aim, several methodological approaches have been used. Both culture-dependent and culture-independent techniques are currently expanding our knowledge of these complex interactions.

TO CULTURE OR NOT TO CULTURE: THAT IS THE QUESTION

Until 20 years ago, the gut microbiota was investigated with culture-based techniques. However, culture alone does not provide a complete view of the resident microbes. Indeed, less than 30% of the gut bacteria have been cultured to date. This statistic does not mean that 70% of the gut microbiota is unculturable but rather that the optimal growth conditions of these organisms have not yet been identified [16].

Thus, the microbial diversity of the gut has been elucidated via molecular assays involving the 16S ribosomal RNA (rRNA) gene (Figure 1). This gene contains approximately 1500 nucleotide pairs and has been widely used as a taxonomic marker, providing in some cases resolution at the species level [17]. These conserved regions may be used as targets for assays that identify species. Although each technique has advantages and limits, the choice of approach depends on the key questions to be addressed. For example, the major advantages of quantitative polymerase chain reaction (qPCR) and fluorescence *in situ* hybridization (FISH) are that these techniques are highly sensitive, and they are suitable to quantify one or more bacterial groups that are targeted with specific primers or probes (Figure 1). For qPCR, specific primer design limits the number of taxa that can be analysed, and thus, only one or a few species can be detected in each experiment. However, FISH can be combined with
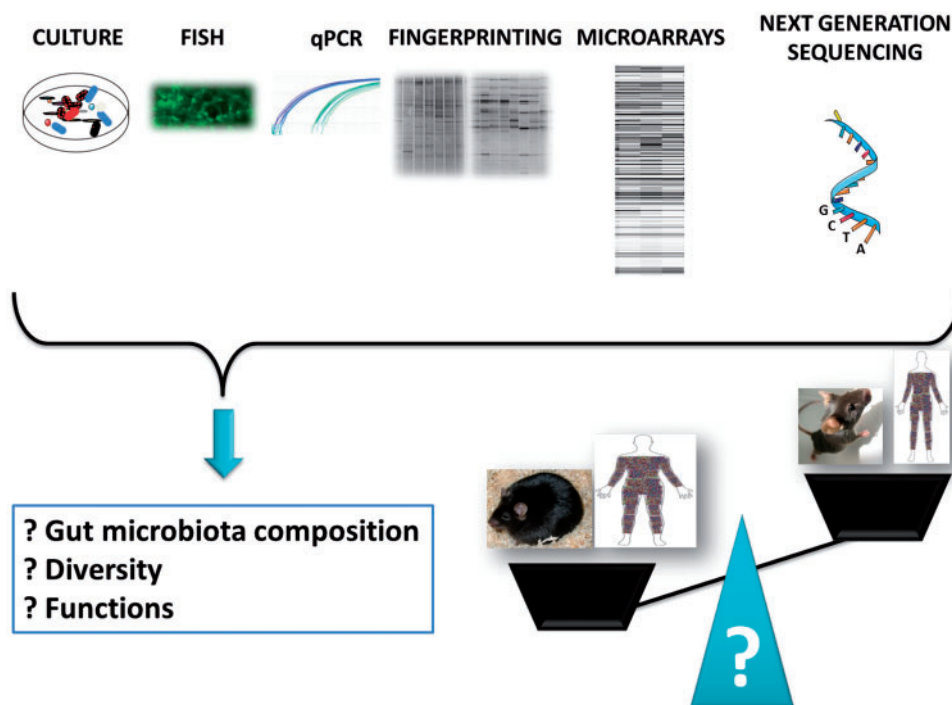
Figure 1: Techniques used to characterize the gut microbiota. FISH, fluorescence *in situ* hybridization; qPCR, quantitative polymerase chain reaction; fingerprinting: DGGE and TGGE; microarrays: MITChip and HITChip; Shotgun Sanger sequencing method, next-generation sequencing: shotgun, 454 pyrosequencing, Illumina and SOLiD.

flow cytometry for high-throughput screens [18,19]. Conversely, DNA fingerprinting techniques identify the most abundant phylotypes and allow rapid comparisons of profiles (e.g. within the same individual or between diseased and healthy individuals). When temperature gradient gel electrophoresis (TGGE) and denaturing gradient gel electrophoresis (DGGE) are used, specific bands can be excised for sequencing or hybridized to identify known or unknown probes (Figure 1). However, although these techniques are generally not quantitative, and only the most abundant groups are detected [16,20,21], in specific cases, DGGE can be adapted in order to be used as a semi-quantitative method.

DNA phylogenetic microarrays are high-throughput techniques that are fast and semi-quantitative. However, the detection of species depends on the inclusion of known reference sequences, so this technique may not be suitable for discovering novel phylotypes (Figure 1) [22]. Next-generation sequencing methods (454 Pyrosequencing, Illumina or SOLiD) generate gigabases of sequence data in a single run. These techniques allow to determine the relative abundance of both known and unknown bacteria (Figure 1). The processing of a large amount of data generated requires high-throughput bioinformatics analysis tools.

Although these techniques are widely used to decipher the links between gut microbiota composition (phylogenetic composition) and pathological situations, taxonomic profiles are not easily translated into metabolic functions. Shotgun sequencing of metagenomic DNA represents the most recent and powerful methods to capture functional differences between given gut microbiomes. This method involves sequencing DNA from the whole community simultaneously. Thus, both the genetic diversity (e.g. species profiles) and the potential metabolic function of the gut microbiota can be examined (Figures 1 and 2). The major limitations remain the cost and the amount of data generated, which are not easily managed.

OBESITY AND THE GUT MICROBIOME

The vertebrate gut is dominated by two phyla that constitute 80–90% of the resident bacteria, Bacteroidetes (e.g. genera *Bacteroides* and *Prevotella)* and Firmicutes (e.g. genera *Clostridium, Ruminococcus, Enterococcus* and *Lactobacillus*), and these phyla are followed in prevalence by Actinobacteria (e.g. genus *Bifidobacterium*) and Proteobacteria (e.g. genera *Helicobacter* and *Escherichia*) [23,24]. The total
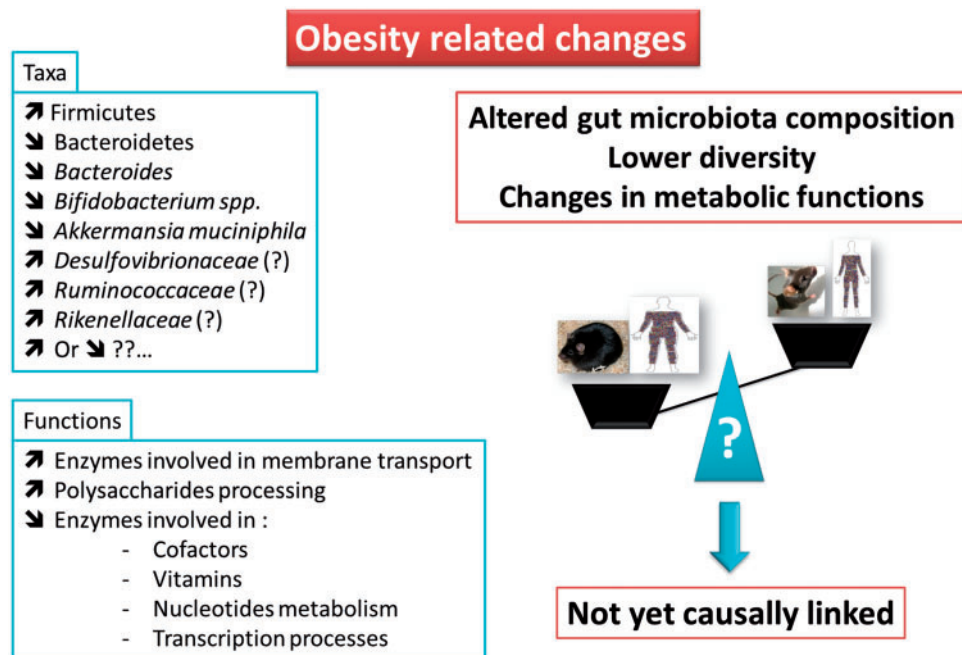
Figure 2: Obesity-related changes in the gut microbiota. Obesity is associated with changes in the composition of the gut microbiome, including lower species diversity and shifts in the abundance of genes involved in metabolism. This non-exhaustive list of the taxa and metabolic functions that differ between lean and obese individuals remains to be causally linked with the onset or the progression of obesity.

number of microbial genes is approximately 150 times that of the human genome [6], suggesting that there are many potential microbial genes with unknown functions.

The first demonstration of specific differences between the gut microbial communities of obese and lean phenotypes was made in leptin-deficient (*ob/ob*) mice. The guts of obese *ob/ob* mice contained fewer Bacteroidetes and more Firmicutes than their lean littermates (Figure 2) [25]. At that time, no causal relationships were demonstrated between these two phyla and the development of obesity. In a follow-up study, the proportional reduction in Bacteroidetes and increase in Firmicutes were correlated with the enrichment of genes that encode key enzymes involved in polysaccharide digestion, which consequently might increase the capacity to harvest energy from food [26]. Interestingly, transferring the gut microbiota into germ-free recipient mice reproduced the donor phenotype [26–28]. However, the exact role of one or more specific taxa remains unclear (Figure 2) since species from the same genus may respond in different ways following dietary intervention [29,30]. Additionally, germ-free mice are resistant to diet-induced obesity [31–33]. In parallel to these interesting observations linking the composition of gut microbiomes to energy homeostasis, the mechanistic basis for these phenomena have been postulated and reviewed elsewhere [31–34].

In 2007, we discovered that a high-fat diet profoundly affects gut microbiota. Using FISH, we found a reduced number of the newly recognized Gram-negative operating taxonomic unit, *Bacteroides*-like mouse intestinal bacteria, which reside within the Bacteroidetes phylum. The *Eubacterium rectale–Clostridium coccoides* group and *Bifidobacterium* spp. were also significantly decreased in obese mice, whereas *Lactobacilli/Enterococci* and *Bacteroides* were not affected [35]. Long-term ingestion of a high-fat diet (14 weeks) induced similar changes, with a significant decrease in the family *Enterobacteriaceae* and in *Bacteroides* spp. [36]. Interestingly, administration of *Bacteroides uniformis* CECT 7771 abolished the diet-induced immune and metabolic disorders associated with gut microbiota modifications in obese mice [37]. Proteobacteria and *Bifidobacterium* spp. may decrease [38] or Proteobacteria increase [39] during a high-fat diet. It is worth noting that a Proteobacteria bloom has been observed consistently after gastric bypass in both rodents and humans [40–44]. In a striking result,

we also found that obese mice treated with prebiotics (i.e. inulin-type fructans: oligofructose) had improved metabolic phenotypes (e.g. decreased metabolic endotoxaemia, glucose intolerance, improved leptin sensitivity and lipid metabolism) that were associated with a bloom in Proteobacteria [45]. It remains to be demonstrated whether specific bacteria belonging to this phylum are beneficial microbes.

Recent reports have confirmed by pyrosequencing and/or qPCR methods that a high-fat diet initiates the change in the Firmicutes/Bacteroidetes ratio (e.g. increase) and decreases *Bifidobacterium* spp. [37,46–49]. Together, these studies suggest that specific phyla and/or genera might be increased or decreased during high-fat diet-induced metabolic disorders.

With pyrosequencing and mouse intestinal phylogenetic microarrays (MITChip), we have observed a higher abundance of *Ruminococcaceae* and *Rikenellaceae* in leptin-resistant obese and diabetic mice (*db/db*) compared with their lean littermates [50]. Kim *et al.* [48] found that *Ruminococcaceae* and *Rikenellaceae* were also enriched in mice fed a high-fat diet, suggesting that specific changes in the gut microbiota are not solely dependent on the ingested diet but are closely linked with the phenotype (i.e. obesity and type 2 diabetes). We have recently discovered that *Akkermansia muciniphila* were dramatically decreased (100- to 1000-fold) in both genetically and diet-induced obese mice (P.D.Cani and A.Everard, personal communication). This species is a novel mucin-degrading bacterium living in the mucus layer [51] and represents 3–5% of the microbial community [51,52]. Moreover, the population size of this bacteria is inversely correlated with body weight [45,53–55], type 1 diabetes [56] and bowel diseases [57]. *Akkermansia muciniphila* increased by approximately 100-fold in prebiotic-treated obese mice, and this effect correlated with an improved metabolic profile [45]. Several less well-known bacteria, namely *Desulfovibrionaceae*, were positively associated with obesity and/or type 2 diabetes (Figure 2) [46,58].

In a recent study, Vrieze *et al.* [59] have shown that subjects with metabolic syndrome treated with fecal enema harvested from lean healthy donor exhibited an improved insulin sensitivity, which lasted for up to 6 weeks. More recently, Fei and Zhao [60] have demonstrated that mono-colonization of germ-free mice with the strain *Enterobacter cloacae* B29 (isolated from one obese subject) induces obesity

and glucose homeostasis disorders upon high-fat diet feeding but not upon normal chow diet.

Future investigations must determine whether one or more taxa are causally linked with the onset of or protection against metabolic disorders.

Although the previous paragraph described the differences that have been most consistently observed in the gut microbiota during nutritional and genetic obesity, it did not cover the overall changes reported in the literature. Few studies have investigated the role of the type and amount of dietary fat on gut microbiota composition [61,62]. Thus, it should be clarified whether the similar changes in both the gut microbiota and the fatty acid amount or composition (saturated versus unsaturated) are linked with the phenotype.

PHYLOGENY, DIVERSITY AND METABOLIC FUNCTIONS

Although there are apparent shifts in the microbial community profiles in obese and type 2 diabetic patients (taxonomic differences have been reported), the contribution of the microbiome to host metabolism is not completely understood. Several hundred microbial genes involved in metabolism are enriched or depleted in the gut of obese humans [63,64]. In a recent comparison of enzymatic gene abundance, the microbiomes of obese subjects and inflammatory bowel diseases patients were found to be similar [65]. They tended to have a higher proportion of genes encoding membrane transport functions, whereas the genes related to cofactor, vitamin and nucleotide metabolism or transcription were more frequently depleted. More recently, Ferrer *et al.* [64] have found that the genes involved in butyrate production were enriched in the gut microbiota from obese adolescents, whereas bacteria from lean adolescents seem to be more engaged in vitamin B(6) synthesis. Despite these specific changes in gene abundance, it appears that a core gut microbial metabolome exists [63]. Thus, there is most likely a degree of redundancy in the gut microbiome. Turnbaugh *et al.* [63] showed that no single bacterial phylotype was detectable at an abundant frequency in the guts of 154 human adults. These different studies provide evidence that variable combinations of species from different phyla could fulfil a partial functional redundancy required by the host, thereby suggesting that different metabolically active bacteria in both obese and lean microbiomes perform similar functions.

CONCLUSIONS

The gut microbiota is highly metabolically active. This consortium of microorganisms contains a subset of taxa that may share or capture functional differences in their metabolic potential. Taxonomic analysis of the gut microbiota improves the description of our most recently discovered 'external organ'. Metagenomics studies will further expand our understanding of the complex ecosystem that resides within the gut. By combining interventional studies, 'omics' and integrative physiological approaches, we will formulate a holistic view of our metabolism in both physiological and pathological situations.

Key Points

- Human are composed of eukaryotic cells, but the gut harbours 10-fold more bacteria and archaea than human cells.
- Numerous techniques examining 16S rRNA genes identify species and hint at the complexity of the gut microbiome.
- The metabolic functions of gut microbes should be investigated to better understand the gut microbiota–host interactions.
- Combining 16S rRNA-based approaches with metagenomics and integrative physiology will more effectively expand our knowledge.

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