The relationship between gut microbiota and weight gain in humans

Emmanouil Angelakis*, Fabrice Armougom, Matthieu Million & Didier Raoult
Unité des Rickettsies, URMITE -CNRS UMR 6236 IRD 198, IFR 48, Faculté de Médecine, Université de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France
*Author for correspondence: Tel.: + 33 491 38 55 17 ■ Fax: + 33 491 83 03 90 ■ angelotasmanos@msn.com

The human gut microbiota is a metabolic organ that is determined by a dynamic process of selection and competition. Age, dietary habits and geographical origin of people have an important impact on the intestinal microbiota. The role of the microbiota is still largely unknown, but the bacteria of the gut flora do contribute enzymes that are absent in humans and play an essential role in the catabolism of dietary fibers. Germ-free mice provide a complementary approach for characterizing the properties of the human gut microbiota. Recently, microbial changes in the human gut were proposed to be one of the possible causes of obesity. This review summarizes the latest research on the association between microbial ecology and host weight.

Obesity is a major, public health concern that affects at least 400 million individuals and is associated with severe disorders including diabetes and cancers [1]. The causes that drive obesity appear to be complex, and a consensus hypothesis is emerging that proposes that obesity is influenced by a mixture of environmental, genetic, neural and endocrine factors [1]. Infectious agents have also been proposed to be causes of obesity, and in human obesity, have been associated with small EDRK-rich factor 1A (SMAM-1), an avian adenovirus and adenovirus 36 [2]. Human genetics is believed to play a part in determining body weight [3]. In total, 32 genes were linked to BMI, but their total variance contribution to BMI in the population was less than 2% [4]. It is believed that other factors also play a role in obesity, such as the availability of inexpensive, calorically dense foods or the reduction in physical activity in our daily lives. Recently, microbial changes in the human gut was proposed to be another possible cause of obesity [5] and it was found that the gut microbes from fecal samples contained 3.3 million nonredundant microbial genes [6]. However, it is still poorly understood how the dynamics and composition of the intestinal microbiota are affected by diet or other lifestyle factors. Moreover it has been difficult to characterize the composition of the human gut microbiota due to large variations between individuals.

The human gut microbiota has been also associated with a number of disease states that include allergy, inflammatory bowel disease, cancer and diabetes [7]. Allergy, for example, has been associated with perturbations in the gastrointestinal microbiota [8]. In addition, evidence implicating the role of microbiota in inflammatory bowel disease was supported by a certain degree of effectiveness of antibiotics in the prevention and treatment of colonic inflammation in both human patients and animal models, as well as by the presence of microbes and microbial components in inflammation-induced colonic lesions [9]. The association of the gut microbiota with cancer is most commonly observed with gastrointestinal tumors, although there are examples of these microbiota modifying the cancer risk to other systems, such as in breast tumors [7]. Moreover, the notion that gut microbiota is important in the onset and development of diabetes, cardiovascular dyslipidemia and metabolic endotoxemia is becoming more widely accepted as the evidence base grows [7,10], and the beneficial effect of bariatric surgery in decreasing cardiovascular risk and cancer was associated with the increase of *Enterobacter hormaechei* in the gut microbiota [11].

The role of the digestive microbiota in the human body is still largely unknown, but the bacteria of the gut flora do contribute enzymes that are absent in humans for food digestion [12]. Moreover, the link between obesity and the microbiota is likely to be more sophisticated than the simple phylum-level *Bacteroidetes*:*Firmicutes* ratio that was initially identified [13], and it is likely to involve a microbiota–diet interaction [14]. Phages have also been proposed to play a possible role in driving the biodiversity of the gut flora by their influence on their bacterial hosts [15] and, recently, a novel pathway that involves dietary lipid phosphatidylcholine and choline

Review
Future Microbiology

Keywords
■ gut flora ■ microbiota ■ obesity

Future Medicine part of fsg

metabolism, an obligate role for the intestinal microbial community, and regulation of surface expression levels of macrophage scavenger receptors that were known to participate in the atherosclerotic process was proposed [16]. More subtle alterations in the levels of other bacteria in the gut may also impact human health. In the last few years, new technologies have been developed that have allowed researchers to attempt more systematic studies on intestinal bacterial flora and have given more realistic information about its composition (by way of detecting non-cultivable species). As a result, an increasing number of studies have related imbalances in the composition of the gut microbiota to obesity and its associated diseases. The approaches used to characterize the human gut flora vary widely, and this might explain, in part, why specific alterations in the microbiota that are associated with excess body fat or weight loss, can also vary between studies. This review summarizes the latest research on the association between the microbial ecology and host weight.

Human gut microbiota

The gut microbiota harbors large bacterial populations in the intestine and colon, approximately $10^{11–12}$ microorganisms per gram of content, and are comprised of mainly anaerobes (95% of the total organisms). The initial overview of the composition of the gut microbiota was culture based, and the predominant cultivable species that were identified included *Bacteroides* sp., *Eubacterium* sp., *Bifidobacterium* sp., *Peptostreptoccocus* sp., *Fusobacterium* sp., *Ruminococcus* sp., *Clostridium* sp. and *Lactobacillus* spp. [17]. The first, large-scale, 16S rDNA sequencing analysis of the gut microbiota by Eckburg *et al.* [18] revealed a high inter-individual variability at the species taxonomic level that was not recovered at the phylum level, as only nine phyla out of 70 were represented [1]. The overall and individual microbiota structures were dominated by the *Bacteroidetes* and *Firmicutes* phyla [18]. Finally, three gut microbiota studies [19] assigned 98% of 16S rRNA sequences to only four bacterial phyla: *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%) and *Actinobacteria* (3%). *Verrucomicrobia*, *Fusobacteria* and the *TM7* phylum together accounted for the remaining 2%.

The earliest large-scale, 16S rRNA or metagenomic studies identified *Methanobrevibacter smithii* as the dominant, methanogenic archaeon species in the human gut microbiota [18]. *M. smithii* in three healthy individuals comprised up to 11.5% of the gut microorganisms [18], and in a study of 650 individuals, the prevalence of *M. smithii* was 95.5%, whereas the prevalence of *Methanosphaera stadtmanae* was 29.4% in the human gut [20]. Moreover, molecular analyses provided various degrees of evidence for the presence of groups of archaea, including *Methanosarcina*, *Thermoplasma*, *Crenarchaeota* and halophilic archaea in the human gastrointestinal tract, but isolates have not been obtained [21].

Age & gut flora modification

During the first days to months of life, the microbiota of the infant gut and the temporal pattern in which it evolves is remarkably variable from individual to individual [22]. At birth, humans are essentially free of bacteria and over time, in a process of colonization that begins shortly after delivery and continues through to adulthood, the body becomes a host to complex microbial communities. The initial infant gut microbiota is usually dominated by *Bifidobacteria*, and through a series of successions and replacements, it migrates to a more complex, adult pattern [22]. Vael *et al.* found that the population of *Bacteroides fragilis* in the microbiota increased in infants from the age of 3 weeks until the age of 1 year, whereas the populations of *Staphylococcus*, *Lactobacillus*, *Bifidobacterium*, *Clostridium* and total anaerobes decreased starting at the age of 3 weeks and remained stable until 52 weeks [23].

Traditionally, it has been thought that between 1 and 2 years of age, the human gut microbiota start to resemble that of an adult [22]. Young children between 1 and 7 years of age presented higher numbers of enterobacteria than adults [24]. Moreover, a large-scale study by Enck *et al.* found significant shifts in relative genus abundances during the first 2 years of life and no noticeable changes in children between 2 and 18 years of age, including stable levels of *Bifidobacterium* and *Lactobacillus* [25]. In a recent study, the comparison of intestinal microbiota composition between adolescents and adults revealed a statistically significantly higher abundance of genera *Bifidobacterium* and *Clostridium* among adolescent samples [26].

The adult intestinal microbiota has been shown to be relatively stable over time [27] and is sufficiently similar between individuals. This observation allowed for identification of a core microbiome that was comprised of 66 dominant, operational, taxonomic units that corresponded to 38% of the sequence reads from 17 individuals [28]. Turroni *et al.* found that *Bifidobacterium pseudolongum* and *Bifidobacterium bifidum*, are

exclusively dominant in the adult bifidobacterial population, whereas *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium pseudocatenulatum* and *Bifidobacterium adolescentis*, were found to be widely distributed, irrespective of host age [29].

In the elderly, both *Bacteroides* numbers and species diversity is declined [30,31]. The analyses of fecal samples collected from subjects from four European study groups indicated higher proportions of enterobacteria in all elderly volunteers [32]. Zwielehner *et al.*, found that the proportion of *Bacteroidetes* in the fecal microbiota of 17 institutionalized, elderly subjects was significantly higher than in younger adults, but these patients had lower proportions of *Bifidobacterium* and *Clostridium* cluster IV [33]. Analysis of the core microbiota in the elderly showed a clear shift to a more *Clostridium* cluster IV-dominated community [34].

Several host factors have been correlated with methanogenic archaea carriage, and it has been proposed that the acquisition of methanogenic archaea occurs by environmental contamination. Additionally, it has been hypothesized that once methanogenic archaea find favorable physicochemical conditions and available substrates in the gut, stable colonization is established [21]. archaea were not detected in children who were younger than 27 months, but it has been shown that carriage increases with age, up to 60% in 5-year-old children. Moreover, it is possible that an adult diet may create an intestinal microbiota that is favorable for the implantation of methanogenic archaea [35]. A possible direct, mother-to-child route of transmission has also been proposed because archaea have been detected in the vaginal flora of pregnant women [21].

Gut flora variations among different populations

It is not yet completely understood how the different environments and wide range of diets that modern humans around the world experience has affected the microbial ecology of the human gut. Certain lifestyles of a person may have an impact on the composition of his/her gut microbiota (Figure 1), but these impacts are currently poorly understood. Qin *et al.*, in the largest study to date, found that only one-third of the bacterial gene clusters that were conserved across individuals of all 124 European (Nordic and Mediterranean) origins could be associated with a broad functional assignment [6]. Nearly 40% of the genes from each individual were shared with at least half of the individuals of the cohort. Of these, 99.1% of the genes had bacterial origin, and the remainder was mostly archaeal, with only 0.1% of eukaryotic or viral origins [6]. Therefore, it seems that important variations in the gut flora between close countries do not exist. As a result, Dicksved *et al.* did not observe differences between fecal samples collected from children from Germany, Switzerland and Sweden by the use of terminal restriction fragment length polymorphism [36]. Lay *et al.,* when testing the composition of the fecal microbiota assessed by FISH combined with flow cytometry, also did not find a significant correlation between the microbial compositions, with regard to age, geographical origin, or gender, among subjects from France, Denmark, Germany, the Netherlands and the UK [37]. However, 16S rDNA pyrosequencing analysis revealed that geographical origin has an important impact on the intestinal microbiota. As a result, differences in the gut microbiota have been observed between people living in northern and southern European countries. For instance, Fallani *et al.* observed that human infants from northern European countries were associated with higher *Bifidobacteria* in their gut microbiota, whereas infants with higher *Bacteroides* and *lactobacilli* were characteristic of southern countries [38]. Mueller *et al.* found that the proportion of *Bifidobacteria* was two- to three-fold higher in Italians than in the French, Germans or Swedes [32]. A bigger difference has been observed between European and Africans, and De Filippo *et al.* found that children from a rural African village presented more *Actinobacteria* and *Bacteroidetes* but less *Firmicutes* and *Proteobacteria* in their gut flora than European children [39]. Moreover, African children presented significantly more short-chain fatty acids in their gut flora than European children [39]. Li *et al.* found that there were distinct microbiota profiles at the species level between a Chinese family and American volunteers. Moreover, they identified a higher proportion of *Bacteroidetes thetaiotaomicron* in males than in females [40]. Finally, Arumugam *et al.*, by combining 22 sequenced, fecal metagenomes of individuals from four countries, identified three enterotype clusters that were not nation- or continent-specific [41]. Enterotype 1 was enriched in *Bacteroides* and seemed to derive energy primarily from carbohydrates and proteins through fermentation. Enterotype 2 was enriched in *Prevotella* and *Desulfovibrio*, which can act in synergy to degrade mucin glycoproteins that are present in the mucosal layer of the

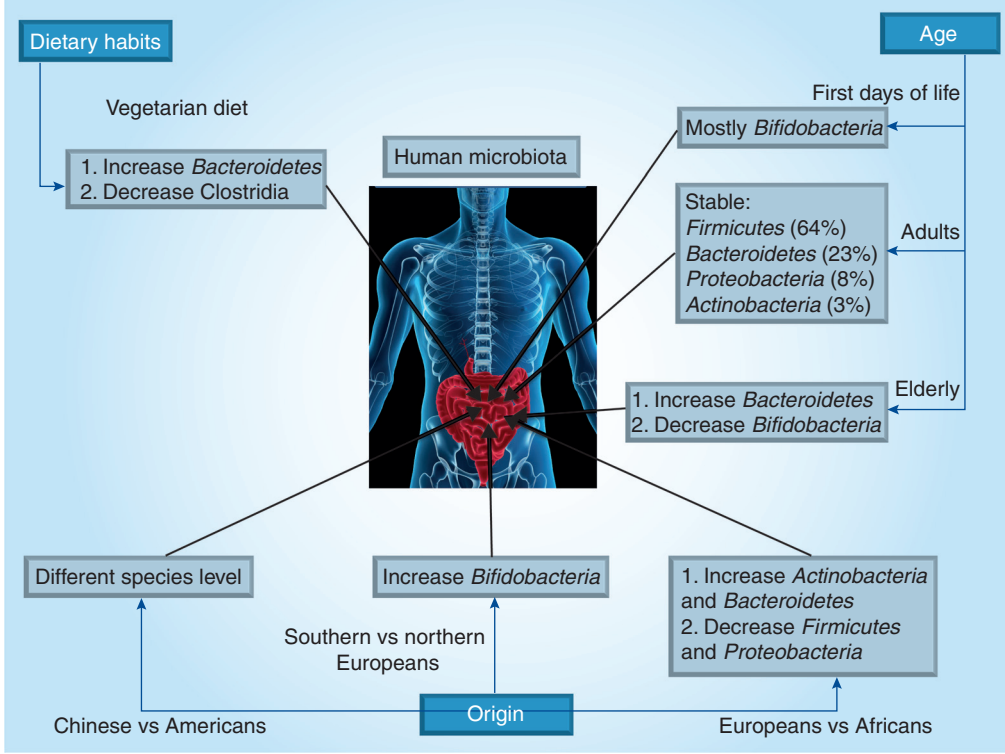

Figure 1. Impact factors for the composition of the human gut microbiota.

gut. Enterotype 3 was the most frequent and was enriched in *Ruminococcus* and *Akkermansia,* which degrade mucins [41]. Moreover, enterotypes 1 and 2 were capable of biosynthesis of different vitamins. The authors proposed that these three enterotypes used different routes to generate energy from fermentable substrates that were available in the colon, reminiscent of a potential specialization in ecological niches or guilds [41].

Effect of the alimentation on human gut flora

Dietary habits are considered to be one of the main factors that contribute to the diversity of the human gut microbiota [42], and the pattern of variation in copy number of the human salivary amylase gene is consistent with a history of diet-related selection pressures, demonstrating the importance of starchy foods in human evolution [43]. *Prevotella*, *Xylanibacter* and *Treponema* were present in the gut flora of children from a rural African village but not from Europe, and the authors of this study hypothesized that the presence of these three genera could be a consequence of high fiber intake, maximizing metabolic energy extraction from ingested plant polysaccharides [39]. These bacteria could ferment both xylan and cellulose through carbohydrate-active enzymes, such as xylanase, carboxymethylcellulase and endoglucanase [39]. Moreover, *Bacteroides* and *Faecalibacterium* species and particularly *Faecalibacterium prausnitzii*, which were found in both children populations, could generally indicate the importance of maintaining a microflora with potential anti-inflammatory capability [39,44]. Liszt *et al.* found that a vegetarian diet affected the intestinal microbiota, especially by decreasing the amount and changing the diversity of *Clostridium* cluster IV [45]. Similar results found by Hayashi *et al.*, who based their studies on RFLP analysis, revealed that the major composition of the vegetarian gut microbiota consisted of *Clostridium* rRNA subcluster XIVa and *Clostridium* rRNA cluster XVIII [46]. Recently, Walker *et al.* tested overweight men with a control diet, diet high in resistant starch or nonstarch polysaccharides and a reduced carbohydrate weight loss diet, over 10 weeks and they found no significant effect of diet upon the proportions of *Bacteroidetes*, *Firmicutes*, *Actinobacteria* or *Proteobacteria* within the fecal microbiota [47]. However, two individual phylotypes, *Eubacterium rectale* and *Ruminococcus bromii*, showed increased proportions on the resistant starch diet while *Collinsella aerofaciens* showed decreased proportions on the weight loss diet [47]. Finally, Wu *et al.* analyzed the fecal samples from 98 individuals and found that fecal communities clustered into enterotypes

Table 1. Weight gain-associated bacterial population shifts in human gut microbiota.

Study	Sample category	Method	Community measured	Major finding	Ref.
Ley *et al.*	Ob	16S clonal sequencing	*Firmicutes*	Significantly reduced level of *Bacteroidetes* in Ob subjects	[5]
	N		*Bacteroidetes*	Correlation between an increase in *Bacteroidetes* and weight loss in Ob subjects	
Zuo *et al.*	Ob	Culture	*Firmicutes*	Significantly reduced levels of *Clostridium perfringens* and *Bacteroides* in the Ob population	[54]
	N		Bacteroidetes		
Schwiertz *et al.*	Ob	qPCR	Firmicutes	Significant increase in *Bacteroidetes* in Ob subjects	[51]
	Ov		*Bacteroidetes*†	Decrease in *Firmicutes*	
	N		*Bifidobacteria*†	Significant decrease in *Bifidobacteria* and *Methanobrevibacter* sp. in Ob subjects	
Kalliomäki *et al.*	Ob/Ov children	FISH	*Bifidobacteria*†	Lower number of bifidobacteria and greater number of *Staphylococcus aureus* predict Ob/Ov phenotype	[58]
			Lactobacilli		
	N children		*Clostridia*		
			Staphylococcus aureus†		
Mai *et al.*	Ob	FISH	*Bacteroidetes*	No significant difference in *Bacteroidetes* levels between Ob and N subjects	[57]
	N	qPCR			
Duncan *et al.*	Ob	FISH	*Firmicutes*	No difference in *Bacteroidetes* levels, independent of diet, in Ob versus N subjects	[59]
	N		*Bacteroidetes*	Significant diet-dependent reduction in *Eubacterium rectale/Roseburia* levels in Ob subjects	
			Eubacterium rectale/ Clostridium coccoides		
Collado *et al.*	Ob pregnant	FCM-FISH and qPCR	*Bacteroidetes*	Higher numbers of *Bacteroides* and *Staphylococcus aureus* in Ov pregnant women	[52]
	N pregnant		*Bifidobacteria*	Correlation between excessive weight gain and high *Bacteroides* levels	
			Staphylococcus aureus†		
Zhang *et al.*	Ob	16S Pyro	*Firmicutes*	More *Bacteroidetes* in Ob subjects (not significant)	[53]
	N		*Bacteroidetes*	Ob microbiota were significantly enriched in *Prevotellaceae*	
			Proteobacteria	Significant increase in *Methanobacteriales* in Ob subjects	
			Actinobacteria	Ob microbiota somewhat enriched in the *Coriobacteriaceae* family of *Actinobacteria*	
			Fusobacteria		
			Verrucomicrobia		

†*Indicates that the difference is significant.*
FCM: Flow cytometry; N: Normal weight; Ob: Obese; Ov: Overweight; Pyro: Pyrosequencing; qPCR: Quantitative real-time PCR.

Table 1. Weight gain-associated bacterial population shifts in human gut microbiota (cont.).

Study	Sample category	Method	Community measured	Major finding	Ref.
Turnbaugh *et al.*	Ob, N twins and mother	16S Pyro of V2 clonal Sanger sequencing 16S Pyro of V6	*Firmicutes*	Significantly reduced levels of *Bacteroidetes* in Ob versus N subjects	[13]
			Bacteroidetes	Significant increase in *Actinobacteria* levels in Ob versus N subjects	
			Actinobacteria	Ob microbiome enriched in genes that belong to *Actinobacteria* and *Firmicutes*	
			Proteobacteria	Nearly half of the lean-enriched genes were from *Bacteroidetes*	
Balamurugan *et al.*	Ob	qPCR	*Bacteroidetes*	Significant increase of *Faecalibacterium prausntzi* levels (belonging to *Firmicutes*) in Ob subjects	[60]
	N		*Bifidobacterium*	No significant difference in *Bacteroidetes* and *Bifidobacterium* levels between Ob and N subjects	
			Lactobacillus acidophilus		
			Eubacterium rectale		
			Faecalibacterium prausnitzii†		
Armougom *et al.*	Ob	qPCR	Firmicutes	Significantly reduced levels of *Bacteroidetes* in Ob versus N subjects	[49]
	N		*Bacteroidetes*†	Significantly higher levels of *Lactobacillus*	
	Anorexic		*Lactobacillus*†	No difference in dietary intake	
			Methanobrevibacter smithii		
Nadal *et al.*	Ob	FISH	*Bacteroidetes/Prevotella*†	Greater weight loss after a multidisciplinary treatment program associated with:	[55]
			Bifidobacterium	Significant reduction of *Eubacterium rectale, Clostridium coccoides* and *Clostridium histolyticum*	
			Clostridium hystolyticum	Correlation with weight	
			Eubacterium rectale/ Clostridium coccoides	Significant increase in *Bacteroides/Prevotella*	
			Lactobacillus/Enteroccocus		
			Enteric group		
Santacruz *et al.*	Ov adolescents	qPCR	*Bacteroides fragilis*	Present after an Ob group submitted to a weight program lost >4 kg	[56]
			Lactobacillus	Significant reduction in *C. coccoides*	
			Clostridium coccoides	Increase in the *Bacteroides fragilis* and *Lactobacillus* groups	
			Clostridium leptum		
			Bifidobacterium		
			Escherichia coli		
			Total bacteria		

†*Indicates that the difference is significant.*
FCM: Flow cytometry; N: Normal weight; Ob: Obese; Ov: Overweight; Pyro: Pyrosequencing; qPCR: Quantitative real-time PCR.

Table 1. Weight gain-associated bacterial population shifts in human gut microbiota (cont.).

Study	Sample category	Method	Community measured	Major finding	Ref.
Santacruz *et al.*	Ob pregnant	qPCR	*Bifidobacterium*†	Significantly reduced *Bifidobacterium* and *Bacteroides* and increased *Staphylococcus* and *Escherichia coli* levels in Ov pregnant women	[62]
	Ov pregnant		*Lactobacillus* group		
			Clostridium coccoides		
			Clostridium leptum		
			Bacteroides†		
			Escherichia coli†		
			Staphylococcus†		
			Total bacteria		

†*Indicates that the difference is significant.*
FCM: Flow cytometry; N: Normal weight; Ob: Obese; Ov: Overweight; Pyro: Pyrosequencing; qPCR: Quantitative real-time PCR.

distinguished primarily by levels of *Bacteroides* and *Prevotella* [48]. They also found that long-term diet, particularly protein and animal fat versus carbohydrate diet were strongly associated with enterotype partitioning. Moreover, in a controlled-feeding study authors found that the microbiome composition changed detectably within 24 h of initiating a high-fat/low-fiber or low-fat/high-fiber diet, but that enterotype identity remained stable [48].

Bacteria species & obesity

The *Bacteroidetes* phylum

Armougom *et al.* found a significant reduction of *Bacteroidetes* proportions in obese, compared with lean and anorexic, individuals [49] and reported lower *Bacteroidetes* concentrations in obese subjects (**Table 1**) [50]. Moreover, the analysis of 16S rDNA sequences from 154 individuals indicated that the microbiota of obese subjects was associated with a decrease in the diversity level and was composed of significantly fewer *Bacteroidetes* [13]. On the other hand, Schwiertz *et al.* quantified bacterial communities in overweight, obese and lean individuals and found a significant increase in the proportions of *Bacteroidetes* in obese and overweight groups [51]. Likewise, before pregnancy, overweight women have a higher number of *Bacteroidetes* than women of normal weight, and excessive weight gain during pregnancy is associated with an increase in *Bacteroidetes* numbers [52]. Assuming that Type 2 diabetes and reduced glucose tolerance is linked to obesity, Larsen and colleagues also found higher levels of *Bacteroidetes* in diabetic patients than in control patients [10]. Using 16S rDNA pyrosequencing, Zhang *et al.* studied the composition of the gut microbiota in morbidly obese, normal-weight and post-gastric-bypass subjects [53]. Their results indicated that the obese microbiota is significantly enriched in *Prevotellaceae,* a subgroup of *Bacteroidetes* [53]. Zuo *et al.*, using culture methods for organisms found in the feces of obese and normal weight participants, found that obese people had fewer cultivable *Bacteroides* than control individuals [54]. Moreover, they found that obese individuals with a Pro/Ala genotype of the nuclear hormone receptor peroxisome proliferator-activated receptor γ2, which modulates cellular differentiation and lipid accumulation during adipogenesis, had lower levels of *Bacteroides* than obese participants with a Pro/Pro genotype [54]. Interestingly, the monitoring of the proportions of two major bacterial communities in obese participants during a weight loss program resulted in linking an

increase in levels of *Bacteroidetes* to weight loss, independent of energy intake [5]. The impact of an obesity treatment program, including a calorie-restricted diet and increase of physical activity on gut microbiota composition in overweight and obese adolescents was reported [55,56]. The FISH method indicated that a significant increase in the ratio of *Bacteroides* and *Prevotella* correlated to weight loss in the adolescent group that exhibited the highest weight loss [55]. Using the same population, the results obtained by FISH [55] were verified by a quantitative PCR (qPCR) method, which detected a notable increase in *Bacteroides fragilis* after the weight loss program [56]. Lastly, Vael *et al.* found that high intestinal *Bacteroides fragilis* concentrations and low *Staphylococcus* concentrations in infants between the ages of 3 weeks and 1 year were associated with a higher BMI in preschool children [23].

Others studies have not found any correlation between the proportions of *Bacteroidetes* and obesity or type of diet. Both qPCR and FISH methods have been applied to subsets of lean and obese subjects, and both have failed to associate a reduced level of *Bacteroidetes* to obesity [57]. In an attempt to study whether the composition of early gut microbiota can affect weight development throughout early childhood, Kalliomäki *et al.* monitored weight, height and bacterial community abundances in children of 6 months, 12 months and 7 years of age. Children who became overweight or obese at 7 years did not present any significant reduction in the proportion of *Bacteroides-Prevotella*, compared with those maintaining a normal weight [58]. The relationships between weight loss and *Bacteroidetes* abundance were examined in adults, but no difference between obese and nonobese subjects was observed [59].

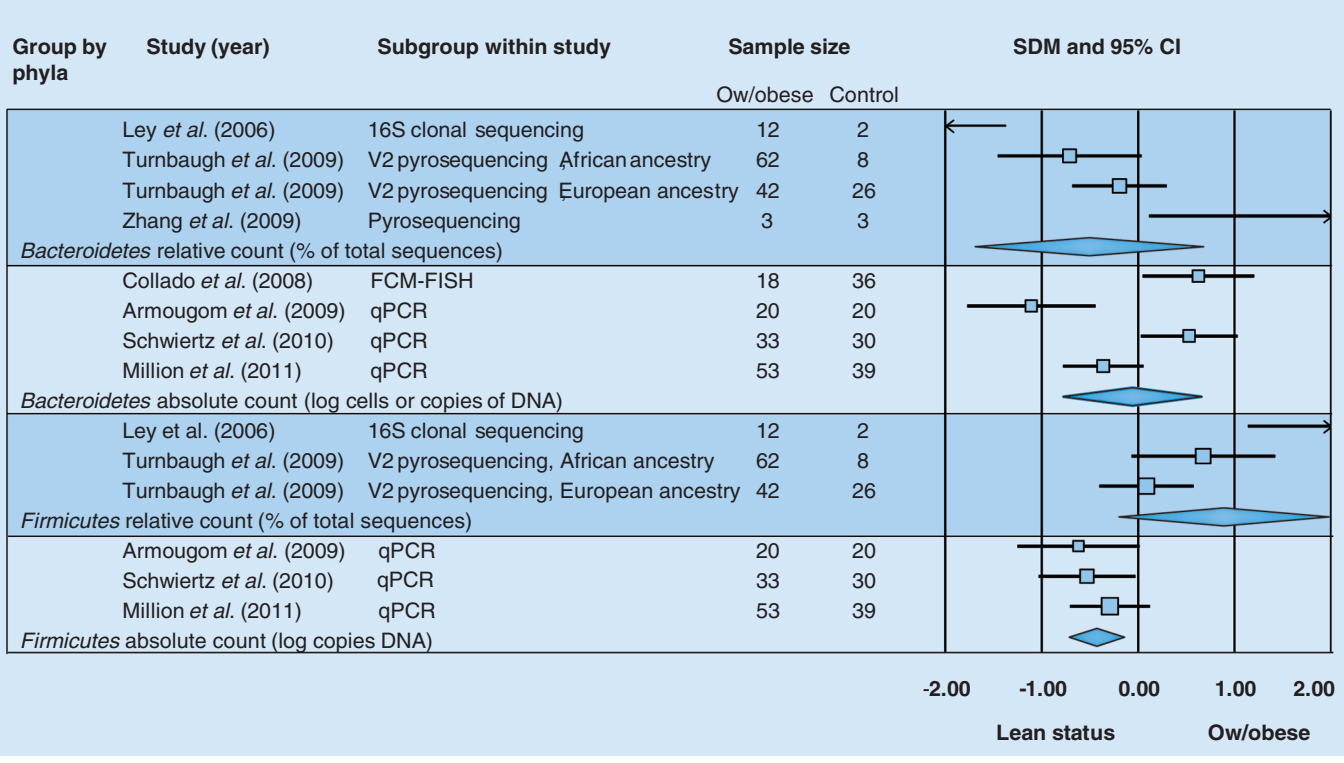

Figure 2. Meta-analysis of the obesity-associated gut microbiota alterations at the phylum level (*Bacteroidetes* and *Firmicutes*) comparing the absolute (abs) or relative (percentage of total sequences) number of sequences (generated by quantitative PCR or cloning/sequencing or pyrosequencing) or cells (flow cytometry-FISH). Meta-analysis was performed with the comprehensive meta-analysis software version 2 [93,94]. Each line represents a comparison between an obese group (right) and a control group (left). The first reported alteration [5] was a decrease in the relative proportion of *Bacteroidetes* (percentage decrease) represented by a deviation of the square (standardized difference in the means) to the left. The size of the square represents the relative weight of each comparison (random model). The length of the horizontal line represents the 95% CI and the diamond represents the summarized effect. The presence of a square to the right and left of the midline means studies with conflicting results corresponding to a substantial heterogeneity (I^2 >50%). Here, the only reproducible and significant alteration at the phylum level is the decrease in the absolute number of sequences of *Firmicutes* in obese subjects. Relative count of *Bacteroidetes* (n = 4; SDM = -0.51; 95% CI = -1.7–0.67; p = 0.40 [I^2 = 81%]); absolute count of *Bacteroidetes* (n = 4; SDM = -0.07; 95% CI = -0.78–0.65; p = 0.86 [I^2 = 85]); relative count of *Firmicutes* (n = 3; SDM = 0.88; 95% CI = -0.21–1.97; p = 0.11 [I^2 = 79%]); absolute count of *Firmicutes* (n = 3; SDM = -0.43; 95% CI = -0.72 to -0.15; p = 0.003 [I^2 = 0%]).
FCM: Flow cytometry; Ow: Overweight; qPCR: Quantitative PCR; SDM: Standardized difference in the means.

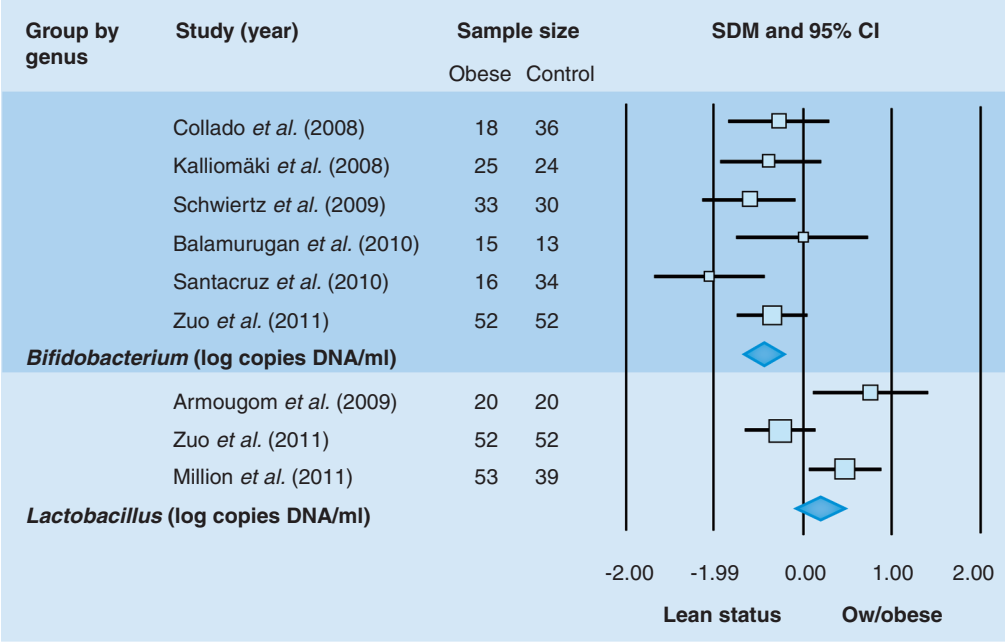

Figure 3. Meta-analysis of the obesity-associated gut microbiota alterations at the genus level for *Bifidobacteria* and *Lactobacilli* comparing the absolute number of sequences generated by genus-specific quantitative PCR. For *Bifidobacteria*, a consistent difference was found by our meta-analysis between 159 obese subjects and 189 controls from six published studies showing that the digestive microbiota of the obese group was significantly depleted in *Bifidobacteria*. Low heterogeneity (I^2 = 17%) shows that this result is very robust. Additional tests have shown that there was no small studies bias (Egger's regression intercept test, p = 0.92; no change after Duval and Tweedie's trim and fill). For *Lactobacilli*, no consistent and significant summary effect was found comparing 127 obese subjects and 110 controls from three studies. *Bifidobacterium* sp. (n = 6; SDM = -0.45; 95% CI = -0.69 to -0.20; p < 0.001 [I^2 = 17%]); *Lactobacillus* spp. (n = 3; SDM = 0.29; 95% CI = -0.31–0.90; p = 0.34 [I^2 = 80%]).
Ow: Overweight; SDM: Standardized difference in the means.

Meta-analysis of the obesity-associated gut microbiota alteration at the phylum level (*Bacteroidetes*) comparing the absolute (abs) or relative (percentage of total sequences) number of sequences (generated by qPCR or cloning/sequencing or pyrosequencing) or cells (flow cytometry [FCM]-FISH) was performed for the seven studies [5,13,49–53]. These studies revealed no difference in the *Bacteroidetes* concentrations between obese people and people of normal weight (Figure 2).

The *Firmicutes* phylum

Ley *et al.* reported that the reduced level of *Bacteroidetes* found in obese humans was counterbalanced by a proportional increase in *Firmicutes* [5]. The greater *Firmicutes* proportion tended to decrease when patients were submitted to a weight-loss program [5]. These results were in agreement with other works, which found that significantly reduced levels of *Clostridium hystoliticum*, *Eubacterium rectale* and *Clostridium coccoides* correlated to weight loss in an obese, adolescent population [55,56]. Moreover, obese, Indian children presented significantly higher levels of *Faecalibacterium prauznitzii* but no difference between the levels of *Bacteroides* and that of *Prevotella*, *Bifidobacterium* species, the *Lactobacillus acidophilus* group or *Eubacterium rectal*, compared with lean children [60]. Duncan *et al.* identified a significant, diet-dependent reduction in levels of *Roseburia-E. rectale*, a group of butyrate-producing *Firmicutes*, for obese patients that were on a weight-loss diet [59]. Zuo *et al.* found a lower amount of *C. perfringens* and a higher proportion of *Enterococci* in obese subjects when compared with normal-weight individuals [54]. Finally, Schwiertz *et al.* found that overweight and obese volunteers exhibited lower cell numbers of the *Ruminococcus flavefaciens* subgroup [51].

Meta-analysis of the obesity associated gut microbiota alteration at the phylum level (*Firmicutes*) comparing the absolute (abs) or relative (percentage of total sequences) number of sequences (generated by qPCR or cloning/sequencing or pyrosequencing) or cells (FCM-FISH) was performed for the five studies

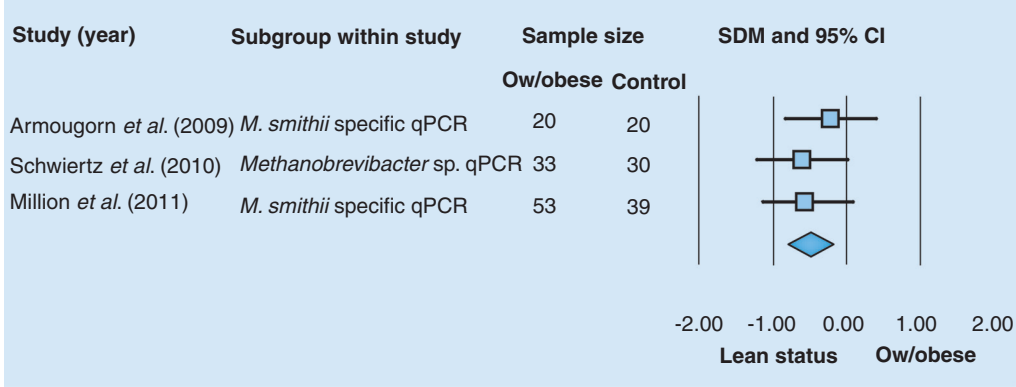

Figure 4. Meta-analysis of the obesity-associated gut microbiota alterations for archaea representatives comparing the absolute number of archaeal sequences generated by quantitative PCR. One study, focused on the *Methanobacteriales* order level, comparing only three obese subjects and three controls, found an increase of this bacterial group in the obese group [53] (square deviated to the right) instead of the three other studies. Our meta-analysis showed, by observing the funnel plot, that this study was an outlier that was subsequently excluded. The comparison of 106 obese subjects and 89 controls including analysis at the *Methanobrevibacter* genus level by Schwiertz *et al.* [51] and at the *Methanobrevibacter smithii* species level [49,50] is justified because it shows a consistent and reproducible effect with a significant reduction of *Methanobrevibacter* sp. in obese subjects (Egger's regression intercept test, p value = 0.39; and Duval's and Tweedie's trim and fill did not change these results). *Methanobrevibacter* sp. (n = 3; SDM = -0.51; 95% CI: -0.79 to -0.22; p = 0.001 [I^2 = 0%]).
Ow: Overweight; SDM: Standardized difference in the means.

[5,13,49–51]. The only reproducible and significant alteration at the phylum level is the decrease in the absolute number of sequences of *Firmicutes* in obese (n = 3; standardized difference in the means [SDM] = -0.43; 95% CI = -0.72 to -0.15; p = 0.003 [I^2 = 0%]) (Figure 2).

Recent studies suggest a role for *Lactobacillus* spp. in weight changes, and the quantification of *Lactobacillus* species in lean, anorexic and obese subjects revealed significantly higher *Lactobacillus* concentrations in nearly half of the obese population [49]. Obese Type 2 diabetic patients displayed significantly higher levels of *Bacilli* and *Lactobacillus* spp. in their gut microbiota [10]. However, an increase in *Lactobacillus* number in an obese, adolescent group after a weight-loss program was also reported [56]. Thuny *et al.* reported significant weight gain in patients with infected endocarditis after treatment with high doses of vancomycin and proposed that *Lactobacillus* spp. that were resistant to vancomycin were responsible for this weight gain [61]. Similarly, Million *et al.* found that *L. reuteri* was associated with obesity [50]. Meta-analysis of the obesity associated gut microbiota alteration at the genus level for *lactobacilli* comparing the absolute number of sequences generated by genus-specific qPCR revealed a nonsignificant summary effect in *Lactobacillus* spp. levels in obese subjects (Figure 3).

The *Actinobacteria* phylum

Recent gut microbiota studies that have been associated with obesity have focused on shifts in *Firmicutes* and *Bacteroidetes* populations. However, the *Actinobacteria* phylum, which is comprised of the *Bifidobacterium* genus as well as other genera, has also been linked to weight gain. Indeed, in an investigation of gut microbial communities of 18 lean or obese twins and their mothers, the obese subjects showed higher levels of *Actinobacteria* [13]. Interestingly, most of the obesity related genes were found to be from *Actinobacteria* (75%), and many of the obesity associated genes that were identified were involved in carbohydrate, lipid and amino acid processing [13]. In addition, the sequencing analysis by Zhang and colleagues revealed that the *Coriobacteriaceae* family of *Actinobacteria* was enriched in the obese microbiota [53].

The fecal concentration of the *Bifidobacterium* genus was reported to be significantly lower in obese subjects when compared with lean subjects [51,52,58,62]. Moreover, Santacruz *et al.* found significantly lower *Bifidobacteria* counts in obese subjects after they had been subjected to a dietary program [56]. Furthermore, Zuo *et al.* found a nonsignificant decrease in the concentration of bifidobacteria between obese and normal weight humans [54]. Meta-analysis of the obesity-associated gut microbiota alteration at the genus level for bifidobacteria comparing the absolute

number of sequences generated by genus specific qPCR revealed that the obese group was consistently and significantly depleted in *Bifidobacteria* (n = 6; SDM = -0.45; 95% CI = -0.69 to -0.20; $p < 0.001$ ($I^2 = 17\%$) (Figure 3). This is extremely important because bifidobacteria depletion seems to be the more reproducible alteration in obese gut microbiota and the best candidate to have an antiobesity effect.

Archaea & obesity

Using the data of Armougom *et al.*, but calculating means of $\log_{10}$ copies DNA/ml of *M. smithii*, we found, contrary to Armougom *et al.*, that there was a decrease in the *M. smithii* load in the obese group, compared with the normal group [49]. Correspondingly, Zhang *et al.* found more *M. smithii* in obese individuals than in lean controls [53], and Schwiertz *et al.* identified lower levels of *M. smithii* in obese subjects compared with lean subjects [51]. However, Million *et al.* recently found higher concentrations of *M. smithii* in nonobese subjects [50]. Overall, methanogenic archaea could indirectly promote caloric intake by the colon and further fat accumulation-related obesity in individuals who were on a high-fiber diet [21]. During the fermentation process, the accumulation of excess H_2 reduces the yield of ATP, which leads to a gradual decrease in the fermentation efficiency [21]. The importance of methanogenic *Archaea* to humans lies in their ability to improve fermentation efficiency by removing H_2 from the gut [21]. It has been speculated that the coexistence of *Prevotellaceae* with methanogenic *Archaea* species in the obese gut allows for greater efficiency of dietary polysaccharide fermentation and therefore increases their conversion into short-chain fatty acids, resulting in their excessive storage [53].

Meta-analysis of the obesity-associated gut microbiota alteration at the genus level for *Methanobrevibacter* spp., main representative of Archaea known in the digestive microbiota, comparing the absolute number of sequences generated by qPCR revealed that obese subjects presented less *Methanobrevibacter* than nonobese subjects (Figure 4). However, the reasons linking methanogens to weight gain still remain unclear. To date, *Methanobrevibacter* is the main representative of archaea in the gut microbiota but archaea could not be extrapolated from *Methanobrevibacter* assessment. This is extremely important since domain-level and genus-level could lead to very different results.

Ability to process polysaccharides

The gut microbiome is also involved in the complex carbohydrate metabolism of food owing to its ability to process indigestible

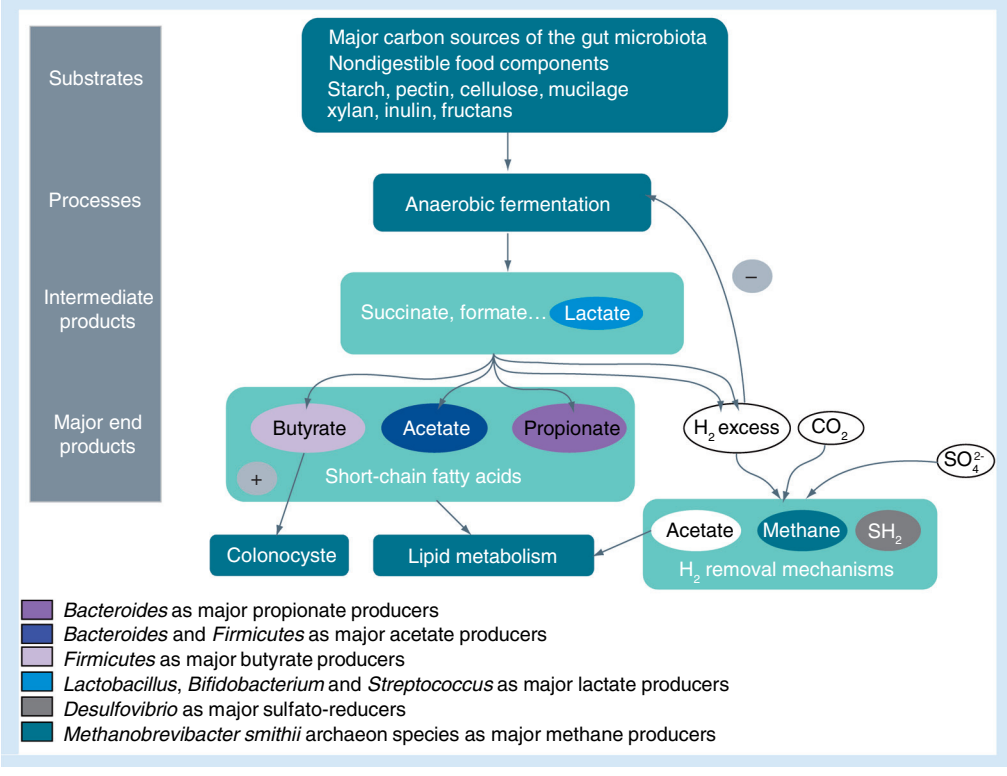

Figure 5. Outline of carbohydrate fermentation by gut microbiota.

components of diets, such as plant polysaccharides [6,13,63]. The human gut microbiome plays an essential role in the catabolism of dietary fibers, the part of plant material in the human diet that is not metabolized by the upper digestive tract, because the human genome does not encode for an adequate carbohydrate active enzyme (CAZymes) (Figure 5). Dietary fibers are the components of vegetables, cereals, leguminous seeds, and fruits that are not digested in the stomach or in the small intestine. Instead, they are fermented in the colon by the gut microbiome and/or excreted in the feces. Additionally, dietary fibers have been identified as strong, positive dietary factors in the prevention of obesity [64]. The human gut bacteria produce a huge panel of CAZymes, with widely different substrate specificities, to degrade these compounds into metabolizable monosaccharides and disaccharides. The array of CAZymes in gut microbes is highly diverse, exemplified by *Bacteroides thetaiotaomicron*, which contains 261 glycoside hydrolases and polysaccharide lyases, as well as 208 homologs of *susC* and *susD* genes, which code for two outer membrane proteins that are involved in starch utilization [65,66]. The CAZymes represent, on average, 2.6% of the sequenced genes in each microbiome [13]. As the human genome encodes, at best, 20–25 digestive enzymes from CAZyme families (i.e., GH1 [lactase], GH13 [α-amylase] and GH31 [maltase, isomaltase and sucrase]), the ability to digest dietary plant carbohydrates resides entirely in gut microbiomes [67]. The CAZymes represented in different human populations that consume different diets may be influenced by their varied cultural traditions. Hehemann *et al.* found that porphyranase and agarase genes are specifically encountered in Japanese gut bacteria and are

Table 2. Major bacteria and archaea in the human gut microbiota and their possible association with obesity.

Representative phyla	Class	Genera	Proven association with obesity	Ref.
Bacteria				
Firmicutes	*Clostridia*	*Clostridium*	Yes	[54,55,56]
		Eubacterium	Yes	[55,59]
		Faecalibacterium	Yes	[60]
		Peptostreptococcus		
		Ruminococcus		
		Roseburia	Yes	[59]
	Bacilli	*Lactobacillus*	Yes	[10,49]
		Enterococcus	Yes	[54]
		Staphylococcus	Yes	[52,58,62]
Bacteroidetes	*Bacteroidia*	*Bacteroides*	Yes	[52,54–56,62]
		Prevotella		
		Xylanibacter		
Proteobacteria	*Deltaproteobacteria*	*Desulfovibrio*		
	Gammaproteobacteria	*Escherichia*	Yes	[62]
	Epsilonproteobacteria	*Helicobacter*		
Actinobacteria	*Actinobacteria*	*Bifidobacterium*	Yes	[51,52,58,62]
Fusobacteria	*Fusobacteria*	*Fusobacterium*		
Synergistetes	*Synergistia*	*Synergistes*		
Spirochaetes	*Spirochaetes*	*Treponema*		
Verrucomicrobia				
Cyanobacteria				
Archaea				
Euryarchaeota	*Methanobacteria*	*Methanobrevibacter*	Yes	[49–51,53]
	Methanobacteria	*Methanosphaera*		

probably absent in the microbiome of western individuals [68]. The authors proposed that consumption of sushi that contains algae from the genus *Porphyramay*, which is associated with the marine bacteria *Zobellia galactanivorans* and *Bacteroides plebeius*, has been the route through which these CAZymes were acquired in human gut bacteria [68,69].

Recently, Benjdia *et al.* hypothesized that sulfatases are critical, evolved fitness factors [70]. To be active, sulfatases must undergo a critical post-translational modification that is catalyzed in anaerobic bacteria by the radical AdoMet enzyme, anaerobic sulfatase-maturating enzyme (anSME). They found that human gut *Bacteroidetes* possessed an anSME gene, and several genes that encoded sulfatases were present within many species, including *B. fragilis*, *Bacteroides dorei* or *Parabacteroides distasonis* [70]. On the other hand, *Firmicutes* did not possess genes encoding predicted sulfatases, and it was proposed that this demonstrated that sulfatases were an important and evolutionary conserved feature among *Bacteroidetes* that inhabited the human digestive tract [70,71].

Gut flora of twins

Turnbaugh *et al.* compared the fecal microbial communities of young, adult female monozygotic and dizygotic twin pairs, who were either lean or obese, along with those of their mothers, to assess the gut microbiota relationship to host weight. Comparisons between all participants showed that obesity was associated with reduced bacterial diversity and a reduced representation of the *Bacteroidetes* [13]. In a more recent study, they found that the majority of species-level phylotypes were shared between deeply sampled monozygotic twins, despite large variations in the abundance of each phylotype [72]. From the gene clusters present in their microbiome bins, only 17% were shared between the two co-twins. Bins exhibited differences in their degree of sequence variation, gene content, including the repertoire of carbohydrate active enzymes present within, and between twins (e.g., predicted cellulases, dockerins) and transcriptional activities [72].

Gnotobiotic mice for the analysis of human gut microbes

Germ-free mice provide a complementary approach for characterizing the properties of the human gut microbiome. Backhed *et al.* found that young, conventionally reared mice have a 40% higher body fat content and 47% higher gonadal fat content than germ-free mice, even though they consumed less food than their germ-free counterparts [73]. When the microbiota of normal mice were transplanted into gnotobiotic mice, there was a 60% increase in body fat within 2 weeks without any increase in food consumption or obvious differences in energy expenditure [73]. Moreover, in a separate study using genetically modified (fasting-induced adipocyte factor [Fiaf]) knockout mice, the same authors showed that gut microbes suppress intestinal Fiaf. Fiaf suppression resulted in increased lipoprotein lipase activity in adipocytes and promoted storage of calories as fat. These findings suggested that the gut microbiota could affect both sides of the energy balance equation, influencing energy harvest from dietary substances (Fiaf) and affecting genes that regulate how energy is expended and stored [74]. Turnbaugh *et al.* were the first to determine that differences in the microbial community could be a factor for obesity [75]. They found that transfer of the gut microbiota from obese (ob/ob) mice to germ-free, wild-type recipients led to an increase in fat mass in the recipients. This led to speculation that the gut microbiota promoted obesity by increasing the capacity of the host to extract energy (calories) from ingested food [75]. Controlled diet manipulation in gnotobiotic mice, which were colonized with a complete human gut (fecal) microbiota,

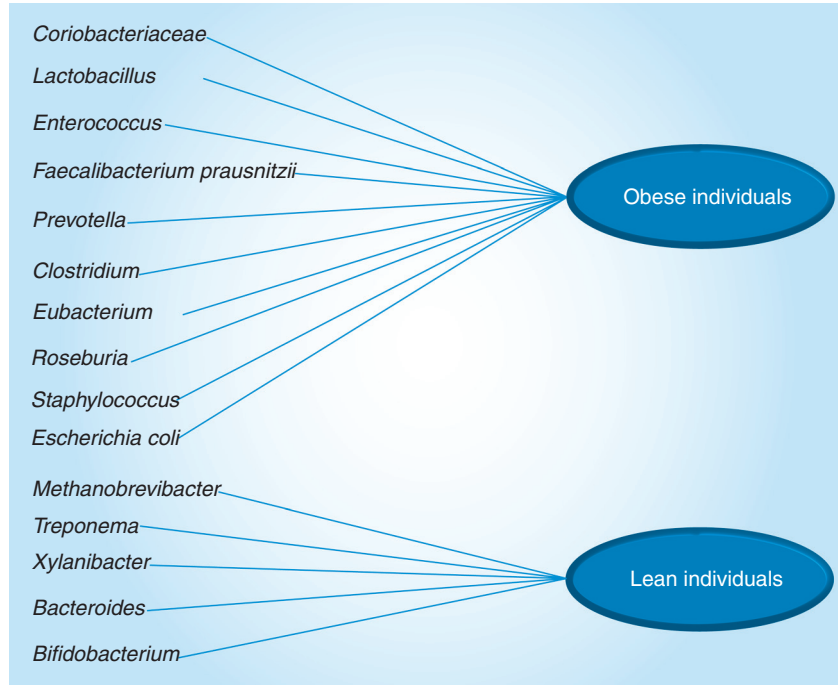

Figure 6. Population of bacteria found to increase in obese and lean individuals.

revealed that the composition of their human gut microbial communities changed dramatically within a single day after the animals were switched from a plant polysaccharide-rich chow to a high-fat, high-sugar "western" diet [14]. Goodman *et al.* developed an approach called insertion-sequencing (INSeq), which is based on a mutagenic transposon that captures adjacent chromosomal DNA to define its genomic location [76]. In this approach, complex populations of tens of thousands of transposon mutants are simultaneously introduced into wild-type or genetically manipulated, germ-free mice in the presence or absence of other microbes. Using this assay, they discovered that *B. thetaiotaomicron* employed the products of five adjacent genes (*BT1957–49*) in response to variations in vitamin B12 levels [76]. Moreover, mice colonized with complete or cultured fecal communities from two human donors displayed significantly greater fat pad to body weight ratios than germ-free controls [77]. Notably, 18 species-level phylotypes were significantly affected when these gnotobiotic mice received a western diet for 2 weeks. Specifically, the relative proportion of representatives of one class of *Firmicutes* (the *Erysipilotrichi*) was increased, and the relative proportion of the *Bacteroidia* class was decreased [77]. Hildebrandt *et al.* found that both wild-type and RELMβ knockout mice were lean on a standard chow diet, but upon switching to a high-fat diet, the wild-type mice became obese, whereas RELMβ knockout mice remained comparatively lean [78]. After the switch to the high-fat diet, the proportions of *Proteobacteria, Firmicutes* and *Actinobacteria* increased, whereas the levels of *Bacteriodetes* decreased [78]. When adult, germ-free, male mice were colonized with *Marvinbryantella formatexigens* and *B. thetaiotaomicron*, it was found that *B. hydrogenotrophica* targeted aliphatic and aromatic amino acids and increased the efficiency of fermentation by consuming reducing equivalents, thereby maintaining a high NAD^+/NADH ratio and boosting acetate production [79]. By contrast, *M. formatexigens* consumed oligosaccharides, did not impact the redox state of the gut and boosted the yield of succinate [79]. Normalized RNA-Seq counts, generated from the cecal contents and fecal samples of the mice revealed that prophages in *M. formatexigens* were completely activated and that two gene pairs were constitutively expressed in all fecal and cecal samples [80]. The authors proposed that a prophage might be liberated from its host cell when that cell is present in a fecal community [80]. Colonization of germ-free mice that consumed a plant polysaccharide-rich or a simple sugar diet with wild-type or anSME-deficient strains revealed that active sulfatase production by *B. thetaiotaomicron* was essential for competitive colonization of the gut, especially when the organism was forced to adaptively forage on host mucosal glycans because complex dietary polysaccharides were not available [70]. The authors proposed that anSME activity and the subsequent activation of sulfatases represented an important pathway that allowed this *Bacteroidetes* species to adapt to life in the gut [70]. Fleissner *et al.* showed that changes in energy expenditure rather than "energy harvest" were responsible for changes in fat deposition and weight gain in mice as they found no difference in body weight gain between germ-free and conventional mice fed a semi-synthetic low-fat diet [81]. By contrast, germ-free mice gained more body weight and body fat than conventional mice on a high-fat diet. Moreover they found that the proportion of *Firmicutes* increased in both mice high-fat and on a western diet. This increase was mainly due to the proliferation of the Erysipelotrichaceae [81]. Murphy *et al.* treated ob/ob mice with a low-fat diet and wild-type mice with either a low-fat diet or a high-fat diet and found that the proportions of *Firmicutes*, *Bacteroidetes* and *Actinobacteria* did not correlate with energy harvesting markers [82]. Higher concentrations of taurine-conjugated bile acids were identified in the livers and intestines of germ-free mice [83] and in those colonized by human baby microbiota [84] compared with conventional animals. Historically, bile acids have been primarily viewed as detergent molecules important for the absorption of dietary fats and lipidsoluble vitamins in the small intestine and the maintenance of cholesterol homeostasis in the liver [83].

Conclusion

Obese and lean subjects presented increased levels of different bacterial populations (**Table 2 & Figure 6**). In addition, a caloric diet restriction impacted the composition of the gut microbiota in obese/overweight individuals and weight loss [5,55,56]. Interestingly, the initial microbiota of overweight adolescents, before any treatment, drove the efficiency of weight loss [56], and differences in the gut composition at infancy could lead to weight gain [23,58]. Studies using gnotobiotic mice have shown that the gut microbiota was critical for normal digestion of

nutrients [74]. It was proposed that the metabolic activities of the gut microbiota facilitated the extraction of calories from ingested dietary substances, helped to store these calories in host adipose tissue for later use and provided energy and nutrients for microbial growth and proliferation [85]. A more recent hypothesis is based on data from vegetarian human populations who presented bacteria that were commonly found in plants, like *B. thetaiotaomicron*, which produced CAZymes and metabolized monosaccharides and disaccharides [6,13,62]. Moreover, it was predicted that other unknown factors in the microbiota and, recently, the manipulation of gut microbial with probiotics, prebiotics, antibiotics or other interventions, were factors for weight gain and obesity [1,86,87], which should be investigated more [88,89]. These results suggest that manipulating the composition of the gut microbiota may prevent weight gain or facilitate weight loss in humans.

Future perspective

During the last few years, an increasing number of studies have related imbalances in the composition of the gut microbiota to obesity. Many studies have reported shifts in the relative abundances of bacterial communities in the gut microbiota of obese relative to normal-weight individuals, and each study has attempted to link obesity with a species- or genus-specific composition profile of the gut microbiota. However, it is possible that the design and/or interpretation of the results has been affected by a conflict of interest of each team. It has recently been shown that published papers in nutrition and obesity research in which the authors were funded by industry were more likely than other papers to contain results or interpretations that favored the industry or company that was producing the product or service that was being studied [90]. Moreover, the heterogeneous methods that were utilized in individual microbiota studies to estimate bacterial proportions prevented rational comparisons of results [12]. Notably, 16S rRNA sequencing-based methods are biased by the heterogeneity of the copy number of the 16S rRNA gene that is present in an individual bacterial genome [91] and can lead to an overestimation of bacterial proportions. However, it is noteworthy that the current 16S rDNA pyrosequencing [53], as well as clonal, Sanger sequencing, studies [5] of gut microbiota within obese populations were not able to detect bacterial concentrations that were below 10^7 organisms per gram of feces [49]. Indeed, the characterization of the 10^{11} bacterial copies per gram of feces that was used in these studies remains superficial. The use of FISH and qPCR methods were dependent on both sensitivity and specificity of the targeted bacterial group. Additionally, the Bac303 probe, which was used in most of the FISH- and qPCR-based studies [55,57–59], underestimated the *Bacteroidetes* proportions because the probe targeted only the *Bacteroides-Prevotella* groups, and it was inadequately sensitive to the *Prevotella* group [92]. Ley *et al.* suggested that it will be interesting to study and compare the effects of these molecular methods using the same sample stool [12]. An integration of mechanistically based investigations and microbial ecology studies using high-throughput sequencing will provide insights into how to best reshape host–microbial interactions to promote weight loss.

Food is a source of bacteria and viruses, and changes in patterns of food consumption results in differences in human gut flora among different groups of people. A question being investigated is whether it is important to identify the source of the gut microorganisms as the most are ingested with food, drinks, and in the course of physical contact and interhuman relationships. Data from agriculture, laboratory animals and humans show that manipulating gut microbiota results in weight modifications and, recently, it was proposed that is necessary to further investigate the effects of routinely adding high amounts of bacteria to food [1,86,87]. In the last few years, the number of published descriptions of the organisms and genes that comprise and manipulate the gut microbiota is increasing dramatically, but these studies have so far been limited to fairly small populations. Moreover, little effort has been made to standardize the microbiota analysis methodology and different sample collection, storage and analysis methods have only been superficially investigated in human studies. This makes it almost impossible to directly compare findings from different groups, limiting our ability to generalize findings. Further well-designed studies should be conducted into how gut microbial communities normally operate, how they shape host physiology, and how they may be altered by probiotic, prebiotic, antibiotic or other interventions. For that reason, massive parallel sequencing technologies and the necessary bioinformatics tools to handle the resulting large datasets should be adapted for human microbiota analysis.

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Human gut microbiota

- The gut microbiota harbors approximately 10^{11-12} microorganisms per gram of content.
- At birth, humans are essentially free of bacteria and over time, in a process of colonization that begins shortly after delivery, the body becomes a host to complex microbial communities.
- 16S rDNA pyrosequencing analysis revealed that geographical origin has an important impact on the intestinal microbiota.
- Dietary habits are considered to be one of the main factors that contribute to the diversity of the human gut microbiota.

Bacteria species & obesity

- Meta-analyses revealed no difference in the *Bacteroidetes* concentrations between obese and humans of normal weight.
- Meta-analyses revealed that obese subjects present less *Firmicutes* than nonobese subjects in their gut flora.
- Meta-analyses revealed that obese subjects presented less *Bifidobacteria* than nonobese subjects.
- Meta-analyses revealed that obese subjects presented less *Methanobrevibacter* spp. than nonobese subjects.

Ability to process polysaccharides

- The gut microbiota plays an essential role in the catabolism of dietary fibers into metabolizable monosaccharides and disaccharides by adequate carbohydrate active enzymes.
- Dietary fibers have been identified as strong, positive dietary factors in the prevention of obesity.
- The human gut bacteria produce a huge panel of carbohydrate active enzymes to degrade dietary fibers into metabolizable monosaccharides and disaccharides.

Gnotobiotic mice for the analysis of human gut microbes

- Germ-free mice provide a complementary approach for characterizing the properties of the human gut microbiota.
- It was first demonstrated in experimental mice models that that differences in the gut microbiota could be a factor for obesity.

Conclusion

- Microbial changes in the human gut are one of the possible causes of obesity.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1. Raoult D. Obesity pandemics and the modification of digestive bacterial flora. *Eur. J. Clin. Microbiol. Infect. Dis.* 27(8), 631–634 (2008).
2. Vasilakopoulou A, le Roux CW. Could a virus contribute to weight gain? *Int. J. Obes. (Lond.).* 31(9), 1350–1356 (2007).
3. Farooqi S, O'Rahilly S. Genetics of obesity in humans. *Endocr. Rev.* 27(7), 710–718 (2006).
4. Speliotes EK, Willer CJ, Berndt SI *et al.* Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat. Genet.* 42(11), 937–948 (2010).
5. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 444(7122), 1022–1023 (2006).

▪▪ **Pioneering study linking obesity and gut microbiota.**

6. Qin J, Li R, Raes J *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464(7285), 59–65 (2010).
7. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. *Trends Microbiol.* 19(7), 349–359 (2011).
8. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol. Rev.* 90(3), 859–904 (2010).
9. Barnich N, Darfeuille-Michaud A. Role of bacteria in the etiopathogenesis of inflammatory bowel disease. *World J. Gastroenterol.* 13(42), 5571–5576 (2007).
10. 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* 5(2), e9085 (2010).
11. Li JV, Ashrafian H, Bueter M *et al.* Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. *Gut* 60(9), 1214–1223 (2011).
12. Ley RE. Obesity and the human microbiome. *Curr. Opin. Gastroenterol.* 26(1), 5–11 (2010).
13. Turnbaugh PJ, Hamady M, Yatsunenko T *et al.* A core gut microbiome in obese and lean twins. *Nature* 457(7228), 480–484 (2009).
14. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* 1(6), 6ra14 (2009).

▪▪ **One of the studies showing that gut transplantation can lead to increased adiposity, establishing the causal link between gut microbiota and obesity.**

15. Ventura M, Sozzi T, Turroni F, Matteuzzi D, van SD. The impact of bacteriophages on probiotic bacteria and gut microbiota diversity. *Genes Nutr.* 6(3), 205–207 (2010).
16. Wang Z, Klipfell E, Bennett BJ *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 472(7341), 57–63 (2011).
17. Moore WE , Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl. Microbiol.* 27(5), 961–979 (1974).

18. Eckburg PB, Bik EM, Bernstein CN *et al.* Diversity of the human intestinal microbial flora. *Science* 308(5728), 1635–1638 (2005).
19. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl Acad. Sci. USA* 104(34), 13780–13785 (2007).
20. Dridi B, Henry M, El Khechine A, Raoult D, Drancourt M. High prevalence of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* detected in the human gut using an improved DNA detection protocol. *PLoS ONE* 4(9), e7063 (2009).
21. Dridi B, Raoult D, Drancourt M. *Archaea* as emerging organisms in complex human microbiomes. *Anaerobe* 17(2), 56-63 (2011).
22. Palmer C, Bik EM, Digiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol.* 5(7), e177 (2007).
23. Vael C, Verhulst SL, Nelen V, Goossens H, Desager KN. Intestinal microflora and body mass index during the first three years of life: an observational study. *Gut Pathog.* 3(1), 8 (2011).
24. Hopkins MJ, Sharp R, Macfarlane GT. Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. *Gut* 48(2), 198–205 (2001).
25. Enck P, Zimmermann K, Rusch K, Schwiertz A, Klosterhalfen S, Frick JS. The effects of maturation on the colonic microflora in infancy and childhood. *Gastroenterol. Res. Pract.* 752401 (2009).
26. Agans R, Rigsbee L, Kenche H, Michail S, Khamis HJ, Paliy O. Distal gut microbiota of adolescent children is different from that of adults. *FEMS Microbiol. Ecol.* 77(2), 404–412 (2011).
27. 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.* 64(10), 3854–3859 (1998).
28. Tap J, Mondot S, Levenez F *et al.* Towards the human intestinal microbiota phylogenetic core. *Environ. Microbiol.* 11(10), 2574–2584 (2009).
29. Turroni F, Foroni E, Pizzetti P *et al.* Exploring the diversity of the bifidobacterial population in the human intestinal tract. *Appl. Environ. Microbiol.* 75(6), 1534–1545 (2009).
30. Woodmansey EJ. Intestinal bacteria and ageing. *J. Appl. Microbiol.* 102(5), 1178–1186 (2007).
31. Woodmansey EJ, McMurdo ME, Macfarlane GT, Macfarlane S. Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. *Appl. Environ. Microbiol.* 70(10), 6113–6122 (2004).
32. Mueller S, Saunier K, Hanisch C *et al.* Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Appl. Environ. Microbiol.* 72(2), 1027–1033 (2006).
33. Zwielehner J, Liszt K, Handschur M, Lassl C, Lapin A, Haslberger AG. Combined PCR-DGGE fingerprinting and quantitative-PCR indicates shifts in fecal population sizes and diversity of *Bacteroides*, *bifidobacteria* and *Clostridium* cluster IV in institutionalized elderly. *Exp. Gerontol.* 44(6–7), 440–446 (2009).
34. Claesson MJ, Cusack S, O'Sullivan O *et al.* Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc. Natl Acad. Sci. USA* 108(Suppl. 1), S4586–S4591 (2011).
35. Rutili A, Canzi E, Brusa T, Ferrari A. Intestinal methanogenic bacteria in children of different ages. *New Microbiol.* 19(3), 227–243 (1996).
36. Dicksved J, Floistrup H, Bergstrom A *et al.* Molecular fingerprinting of the fecal microbiota of children raised according to different lifestyles. *Appl. Environ. Microbiol.* 73(7), 2284–2289 (2007).
37. Lay C, Rigottier-Gois L, Holmstrom K *et al.* Colonic microbiota signatures across five northern European countries. *Appl. Environ. Microbiol.* 71(7), 4153–4155 (2005).
38. Fallani M, Amarri S, Uusijarvi A *et al.* Determinants of the human infant intestinal microbiota after introduction of first complementary foods in five European centres. *Microbiology* 157(Pt 5),1385–1392 (2011).
39. De Filippo C, Cavalieri D, Di PM *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl Acad. Sci.USA* 107(33), 14691–14696 (2010).
40. Li M, Wang B, Zhang M *et al.* Symbiotic gut microbes modulate human metabolic phenotypes. *Proc. Natl Acad. Sci.USA* 105(6), 2117–2122 (2008).
41. Arumugam M, Raes J, Pelletier E *et al.* Enterotypes of the human gut microbiome. *Nature* 473(7346), 174–180 (2011).
42. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 307(5717), 1915–1920 (2005).
43. Perry GH, Dominy NJ, Claw KG *et al.* Diet and the evolution of human amylase gene copy number variation. *Nat. Genet.* 39(10), 1256–1260 (2007).
44. Sokol H, Pigneur B, Watterlot L *et al.* *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc. Natl Acad. Sci. USA* 105(43), 16731–16736 (2008).
45. Liszt K, Zwielehner J, Handschur M, Hippe B, Thaler R, Haslberger AG. Characterization of bacteria, clostridia and *Bacteroides* in faeces of vegetarians using qPCR and PCR-DGGE fingerprinting. *Ann. Nutr. Metab.* 54(4), 253–257 (2009).
46. Hayashi H, Sakamoto M, Benno Y. Fecal microbial diversity in a strict vegetarian as determined by molecular analysis and cultivation. *Microbiol. Immunol.* 46(12), 819–831 (2002).
47. Walker AW, Ince J, Duncan SH *et al.* Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* 5(2), 220–230 (2011).
48. Wu GD, Chen J, Hoffmann C *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334(6052), 105–108 (2011).
49. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. *PLoS ONE* 4(9), e7125 (2009).
50. Million M, Maraninchi M, Henry M, Armougom F, Raoult D. Obesity-associated Gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. *Int. J. Obesity* doi:10.1038/ijo.2011.153 (2011) (Epub ahead of print).
51. Schwiertz A, Taras D, Schafer K *et al.* Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)* 18(1), 190–195 (2010).
52. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am. J. Clin. Nutr.* 88(4), 894–899 (2008).
53. Zhang H, DiBaise JK, Zuccolo A *et al.* Human gut microbiota in obesity and after gastric bypass. *Proc. Natl Acad. Sci. USA* 106(7), 2365–2370 (2009).
54. Zuo HJ, Xie ZM, Zhang WW *et al.* Gut bacteria alteration in obese people and its relationship with gene polymorphism. *World J. Gastroenterol.* 17(8), 1076–1081 (2011).

55. Nadal I, Santacruz A, Marcos A *et al.* Shifts in *clostridia*, *bacteroides* and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. *Int. J. Obes. (Lond.)* 33(7), 758–767 (2009).

56. Santacruz A, Marcos A, Warnberg J *et al.* Interplay between weight loss and gut microbiota composition in overweight adolescents. *Obesity (Silver Spring)* 17(10), 1906–1915 (2009).

57. Mai V, McCrary QM, Sinha R, Glei M. Associations between dietary habits and body mass index with gut microbiota composition and fecal water genotoxicity: an observational study in African American and Caucasian American volunteers. *Nutr. J.* 8, 49 (2009).

58. Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. *Am. J. Clin. Nutr.* 87(3), 534–538 (2008).

▪▪ ***Bifidobacteria* protect children from becoming overweight. This is confirmed by the fact that our meta-analysis found that the obesity-associated gut microbiota are depleted in *Bifidobacteria*. *Bifidobacteria* and *Lactobacillus* strains are currently main candidates for antiobesity probiotics.**

59. Duncan SH, Lobley GE, Holtrop G *et al.* Human colonic microbiota associated with diet, obesity and weight loss. *Int. J. Obes. (Lond.)* 32(11), 1720–1724 (2008).

60. Balamurugan R, George G, Kabeerdoss J, Hepsiba J, Chandragunasekaran AM, Ramakrishna BS. Quantitative differences in intestinal *Faecalibacterium prausnitzii* in obese Indian children. *Br. J. Nutr.* 103(3), 335–338 (2010).

61. Thuny F, Richet H, Casalta JP, Angelakis E, Habib G, Raoult D. Vancomycin treatment of infective endocarditis is linked with recently acquired obesity. *PLoS ONE* 5(2), e9074 (2010).

62. Santacruz A, Collado MC, Garcia-Valdez L *et al.* Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *Br. J. Nutr.* 104, 83–92 (2010).

63. Gloux K, Berteau O, El OH, Beguet F, Leclerc M, Dore J. A metagenomic β-glucuronidase uncovers a core adaptive function of the human intestinal microbiome. *Proc. Natl Acad. Sci. USA* 108(Suppl. 1), S4539–S4546 (2011).

64. Grabitske HA , Slavin JL. Low-digestible carbohydrates in practice. *J. Am. Diet. Assoc.* 108(10), 1677–1681 (2008).

65. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. The carbohydrate-active enzymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* 37(Database issue), D233–D238 (2009).

66. Martens EC, Koropatkin NM, Smith TJ, Gordon JI. Complex glycan catabolism by the human gut microbiota: the *Bacteroidetes* Sus-like paradigm. *J. Biol. Chem.* 284(37), 24673–24677 (2009).

67. Turnbaugh PJ, Henrissat B, Gordon JI. Viewing the human microbiome through three-dimensional glasses: integrating structural and functional studies to better define the properties of myriad carbohydrate-active enzymes. *Acta Crystallogr. Sect. F. Struct. Biol. Cryst. Commun.* 66(Pt 10), 1261–1264 (2010).

68. Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* 464(7290), 908–912 (2010).

69. Kurokawa K, Itoh T, Kuwahara T *et al.* Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res.* 14(4), 169–181 (2007).

70. Benjdia A, Martens EC, Gordon JI, Berteau O. Sulfatases and a radical AdoMet enzyme are key for mucosal glycan foraging and fitness of a prominent human gut. *Bacteroides*. *J. Biol. Chem.* 286(29), 25973–25982 (2011).

71. Berteau O, Guillot A, Benjdia A, Rabot S. A new type of bacterial sulfatase reveals a novel maturation pathway in prokaryotes. *J. Biol. Chem.* 281(32), 22464–22470 (2006).

72. Turnbaugh PJ, Quince C, Faith JJ *et al.* Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. *Proc. Natl Acad. Sci. USA* 107(16), 7503–7508 (2010).

73. Backhed F, Ding H, Wang T *et al.* The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl Acad. Sci. USA* 101(44), 15718–15723 (2004).

74. Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc. Natl Acad. Sci. USA* 104(3), 979–984 (2007).

75. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444(7122), 1027–1031 (2006).

▪ **Pioneering study linking gut microbiota and increased capacity for energy harvest that is one of the putative mechanisms for gut microbiota-associated obesity.**

76. Goodman AL, McNulty NP, Zhao Y *et al.* Identifying genetic determinants needed to establish a human gut symbiont in its habitat. *Cell Host Microbe* 6(3), 279–289 (2009).

77. Goodman AL, Kallstrom G, Faith JJ *et al.* Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc. Natl Acad. Sci. USA* 108(15), 6252–6257 (2011).

78. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA *et al.* High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 137(5), 1716–1724 (2009).

▪ **Proved that diet modifies gut microbiota independently of obesity.**

79. Rey FE, Faith JJ, Bain J *et al.* Dissecting the *in vivo* metabolic potential of two human gut acetogens. *J. Biol. Chem.* 285(29), 22082–22090 (2010).

80. Reyes A, Haynes M, Hanson N *et al.* Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature* 466(7304), 334–338 (2010).

81. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M. Absence of intestinal microbiota does not protect mice from diet-induced obesity. *Br. J. Nutr.* 104(6), 919–929 (2010).

▪ **Showed that modification of gut microbiota is not the only way for diet to induce obesity since germ-free mice are not protected against diet-induced obesity. Diet, gut microbiota and obesity are associated by a triangular causal link.**

82. Murphy EF, Cotter PD, Healy S *et al.* Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut* 59(12), 1635–1642 (2010).

83. Swann JR, Tuohy KM, Lindfors P *et al.* Variation in antibiotic-induced microbial recolonization impacts on the host metabolic phenotypes of rats. *J. Proteome Res.* 10(8), 3590–3603 (2011).

84. Martin FP, Dumas ME, Wang Y *et al.* A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. *Mol. Syst. Biol.* 3, 112 (2007).

85. DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. Gut microbiota and its possible relationship with obesity. *Mayo Clin. Proc.* 83(4), 460–469 (2008).

86. Raoult D. Probiotics and obesity: a link? *Nat. Rev. Microbiol.* 7, 619 (2009).

87. Raoult D. Human microbiome: take-home lesson on growth promoters? *Nature* 454(7205), 690–691 (2008).

88. Gordon JI , Klaenhammer TR. A rendezvous with our microbes. *Proc. Natl Acad. Sci. USA* 108(Suppl. 1), S4513–S4515 (2011).

89. Khoruts A , Sadowsky MJ. Therapeutic transplantation of the distal gut microbiota. *Mucosal. Immunol.* 4(1), 4–7 (2011).

90. Thomas O, Thabane L, Douketis J, Chu R, Westfall AO, Allison DB. Industry funding and the reporting quality of large long-term weight loss trials. *Int. J. Obes. (Lond.)* 32(10), 1531–1536 (2008).

91. Hattori M , Taylor TD. The human intestinal microbiome: a new frontier of human biology. *DNA Res.* 16(1), 1–12 (2009).

92. Hoyles L, McCartney AL. What do we mean when we refer to *Bacteroidetes* populations in the human gastrointestinal microbiota? *FEMS Microbiol. Lett.* 299(2), 175–183 (2009).

93. Borenstein M, Hedges L, Higgins J, Rothstein H. *Comprehensive Meta Analysis Version 2*. Biostat, Englewood, NJ, USA (2005).

94. Borenstein M, Hedges L, Higgins J, Rothstein H. *Introduction to Meta-Analysis*. Wiley, Hoboken, NJ, USA (2009).