Gut Microbiota and Obesity

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Obesity is one of the most prevalent human health problems. Research from the last decades has clarified the role of the imbalance between energy intake and expenditure, unhealthy lifestyle, and genetic variability in the development of obesity. The composition and metabolic functions of gut microbiota have been proposed as being able to affect obesity development.

Keywords: metabolism ; gut microbiota ; dysbiosis ; obesity

1. Introduction

Changes in dietary habits and the increased availability of high-caloric foods have made overweightness and obesity some of the most serious health issues of our era. In 2016, the World Health Organization (WHO) estimated that 39% of individuals older than 18 were overweight, and the worldwide prevalence of obesity almost tripled between 1975 and 2016. It has been reported that nearly 2.8 million deaths annually are a consequence of overweight and obesity-associated conditions: blood hypertension, dyslipidemia, and insulin resistance lead to an increased risk of coronary heart disease, ischemic stroke, type 2 diabetes mellitus, as well as cancer development [1]. Obesity is caused by an imbalance between energy intake and expenditure: increasing the intake of fattening food and other lifestyle changes pushed its prevalence to epidemic proportions. On the other hand, several works have proven a significant genetic role in determining the obesity risk [2][3][4]. On top of the genetic factors clearly contributing to determining body weight and fat mass, the drastic boost in obesity prevalence has also suggested a prominent contribution in the development and maintenance of obesity caused by environmental elements.

In recent years, changes in bacterial strains, hosted in the human intestine, were proposed to have a causative role in obesity [3][5][6]. Intriguingly, the microbiome is a fingerprint of both the environment and human heritable genetic material [7]. In fact, it has been proposed that the genetic pool of the microbiota represents an extension of the nuclear and mitochondrial genomes, leading to the definition of the meta-genome to describe such extension [8].

2. Historical and Current Perspectives

The complex interaction involving the diet, intestinal microbiota, and human host has been investigated for over a century. Acceptance of the germ theory of disease development led to an original classification of a number of human disorders as caused by microbes, including conditions that were eventually going to be reconsidered as non-infectious. The initial proponent of such theories was the immunologist Elie Metchnikoff, considered by many as the father of probiotics. In his 1907 article, ‘‘Essais optimistes’’, Metchnikoff proposed a causative link between microbes and aging mechanisms and suggested a central role in senescence progression for compounds resulting from microbial intestinal putrefaction [9]. Furthermore, he firstly noted the beneficial effect of consuming fermented food on human health. Therefore, he hypothesized that fermented foods could avoid intestinal proteins putrefaction and thus senility.

Over the past century, several studies have demonstrated the influence of gut microbiota on the pathophysiology of many extra-intestinal conditions. More specifically, the exhaustive description of human microbiota’s relationship with health and disease has become the major challenge of research in the twenty-first century [10]. In recent years, the number of annual publications on this topic has almost quadrupled, as compared to 2005, when Eckburg et al. published the first large-scale gut metagenomic study that, starting from genetic fragments, allowed the reconstruction of entire genomic germ profiles [11].

Gut microbiota is the most complex ecosystem in nature since it harbors large bacterial populations in the intestine and colon, with around 1011–1012 microorganisms/gram of the intestinal content and mostly are anaerobes (95% of the total organisms) [12]. The first studies on the composition of intestinal microbiota were based on microscopic observation and culture-based methods, and showed as predominant cultivable species *Bacteroides* spp., *Eubacterium* spp., *Bifidobacterium* spp., *Peptostreptococcus* spp., *Fusobacterium* spp., *Ruminococcus* spp., *Clostridium* spp. and *Lactobacillus* spp. [13]. Subsequently, Gill et al. obtained the first metagenomic sequencing of the distal gut microbiome in two subjects, showing microbial genomic and genetic diversity and identifying some of the distinctive features of this subpopulation of microbiome [14].

To date, genetic tests have led to the generation of large new datasets on gut microbiota, including information on the composition and functional properties of greater numbers of microbial strains. In this frame, US National Institutes of Health (NIH), founded the Human Microbiome Project (HMP) Consortium. HMP follows into the footsteps of the Human Genome Project, being constituted by multiple projects that bring together scientific experts worldwide to explore microbial communities to characterize the composition of the normal microbiome and the relationship with human organism [15]. Characterizing the microbial genes has led to the description of a human microbiome core [16]. It is established by a set of genes shared by microbes colonizing most habitats in humans. Interestingly, core genes present in a limited habitat and in a smaller set of humans can be modified by a combination of factors, such as host genotype, immune system physiology, disease state, lifestyle, diet, and also the presence of other microorganisms. This core microbiome is not present in shared big microbial populations but is involved in several essential metabolic functions for the bugs hosted in our intestine [17].

3. Gut Environment: Microbiota Evolutionary Development

The microbes detected in the human intestinal tract can be divided into three domains based on molecular phylogeny (i.e., 16S ribosomal ribonucleic acid [rRNA] sequence similarities and differences): eukarya, bacteria, and archaea. Eukarya includes organisms whose cells contain complex structures surrounded by membranes, primarily the nucleus. On the other hand, bacteria are the predominant strains of the gut microbiota (**Table 1**). About 90% of the fecal bacteria belong to two of the major phylogenetic lineages: *Firmicutes* and *Bacteroidetes*. However, the gastrointestinal tract of adult humans has been estimated to contain approximately 500–1000 distinct bacterial species [16]. In addition, *Methanobrevibacter smithii* is the dominant methanogenic archaeon species within the microbes in our digestive system [11].

Table 1. Main bacteria and Archaea in the human gut microbiota.

Domain	Phylum	Class	Order	Family	Genus
Bacteria	*Bacteroidetes*	Bacteroidia	Bacteroidales	Bacteroidacee	Bacteroides
				Prevotellacee	Prevotella
					Xylanibacter
				Rikenellacee	Rikenella
	Firmicutes	Clostridia	Clostridiales	Clostridiacee	Clostridium
				Ruminococcae	Faecalibacterium
					Ruminococcus
				Peptostreptococcae	Peptostreptococcus
					Fusibacter
				Eubacteriacee	Eubacterium
				Veillonellacee	Veillonella
				Lachnospiraceae	Roseburia
		Bacilli	Bacillales	Bacillaceae	Bacillus
				Lysteriaceae	Lysteria
				Staphylococcaceae	Staphylococcus
				Pasteuriaceae	Pasteuria

Domain	Phylum	Class	Order	Family	Genus
			Lactobacillales	Lactobacillaceae	Lactobacillus
				Enterococcaceae	Enterococcus
				Streptococcaceae	Streptococcus
	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium
					Gardnerella
			Actinomycetales	Actinomycetaceae	Actynomices
	Proteobacteria	Deltaproteobacteria	Desulfobacteriales	Desulfobulbaceae	Desulfovibrio
		Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia
					Enterobacter
					Klebsiella
					Proteus
		Epsilonproteobacteria	Campylobacteriales	Campylobacteriaceae	Campylobacter
				Helycobacteriaceae	Helycobacter
	Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium
	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Verrucomicrobium
	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes
	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Spirochaeta
					Treponema
	Cyanobacteria	Cyanobacteria			
Archaea	*Euryarchaeota*	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter
					Methanobacterium
					Methanosphaera

More specifically, the subclass distribution of gut microbiota are composed by: Bacteroidetes (23%) that comprise the genus *Bacteroides*, Firmicutes (64%) that includes Bacilli, Clostridia and Mollicutes (the majority of microorganisms in this phylum are closely related to genus *Streptococcus* and *Clostridium*); Proteobacteria (8%), Gram-negative bacteria such as *Escherichia coli* and *Helicobacter pylori*; Fusobacteria, Verrucomicrobia and Actinobacteria (3%) that include species such as *Bifidobacterium* [18][19][20]. Over 20 genera of Bacteroidetes have been described, with *Bacteroidales* being the most studied one, in particular the genus *Bacteroides*. Firmicutes are Gram-positive bacteria, divided into three classes: *Clostridia, Bacilli,* and *Mollicutes* (**Table 1**).

Childhood is characterized by the microbial plasticity that resembles the physiologic process of progressive gut colonization by microbes over time. The colonization of the digestive apparatus begins at birth and is different from individual to individual [21]. This process recognizes three steps: from birth to weaning, from weaning to a normal diet assumption that is characteristic of adulthood, and elderly. More particularly, at birth, the human gut is essentially free from bacteria, but, immediately after delivery, the intestine begins to be populated by a series of microorganisms—this process is influenced by exogenous and endogenous factors (e.g., mother's vaginal and fecal microbiota, environment, skin bacterial flora) [22]. During the first 12–24 h of extra-uterine life, gut colonists are especially facultative anaerobic bacteria such as *Escherichia coli*, *Enterococci* and *Streptococci* [22]. Subsequently, from the second to the third day, these bacteria generate an anaerobic environment promoting the growth of obligate anaerobes (*Lactobacilli* and mainly *Bifidobacteria*), perhaps through reduction of the redox environment potential (low oxygen concentration). Within two weeks, this bacterial population expands from 108 to 1010 per gram of feces and establishes itself as species *Bacteroides* and *Clostridia* [23]. A crucial determinant of gut microbiota development is the infant feeding. Several studies have shown different qualitative compositions of the bacterial flora in the breastfed subjects compared to the artificially fed ones. In breastfed infants, *Bifidobacterium* prevails (60%–90% of the fecal flora) vs. less than 1% of lactic-acid bacteria. In addition, there is a decrease in pH and inhibition of putrefactive flora growth with advantage for fermentative one development. This microbial switch improves intestinal digestive and absorptive functions of nutrients, in particular vitamins, with a consensual stimulation of immune system, namely gastrointestinal associated immune system (GALT), that reduces the risk of contracting allergies [24]. After the first six months of life, the weaning period begins with an enlarged diet composition and the introduction of the solid foods that leads to a further differentiation of microorganisms present in adults [25]. More specifically, these bugs belong to Firmicutes and Bacteroidetes (26). In the first year of life, levels of *Escherichia coli* and *Enterococci* range between 106 and 108 CFU/g of feces—there is a reduction in *Clostridia* and an increase in anaerobic flora, that undergoes a gradual diversification [24][25]. Interestingly, the initial colonization of the intestinal tract by microbes is important for defining the bacterial flora of the adult age. In fact, once the adult microbiota is constituted remains stable, with the exception of possible variations, following several factors such as a change in eating habits or the onset of diseases [26]. In adolescent children, a significantly higher representation of genera *Bifidobacterium* and *Clostridium* has been reported, as compared to adult levels [25][26]. A decline in the microbial abundance and species diversity, has been reported in the elderly, with lower levels of bifidobacteria and higher levels of Enterobacteriaceae [27].

4. Gut Microbiota Distribution and Its Relationship with Obesity

Differences in composition have been noticed in the microbial populations along the gastrointestinal tract [28]. These differences add a horizontal stratification, with the presence of diverse microbial communities in the intestinal lumen, in the layer of mucus of the intestinal crypts and directly adherent to the epithelial cells. In quantitative terms, esophagus and stomach carry the lowest bacterial load and the predominant cultivable bacteria are facultative anaerobes that derive from the oral cavity (e.g., *Streptococci* and *Lactobacilli)*. Bacterial load increases progressively along the intestinal tract as the redox potential drops. Moreover, the genus *Streptococcus* is the most represented among the microbiota of jejunum [28]. However, a significantly higher population of bacteria (108–109/g of feces) characterizes specifically the ileo-cecal area. In fact, the small intestine is enriched by the subgroup Bacillus bacteria (phylum Firmicutes, mainly Streptococcaceae, corresponding to 23% of the genomic sequences identified compared with 5% in the colon). In addition, up to 8% of genomic sequences belong to members of the phylum Actinobacteria and, in particular, to the subgroups Actinomycinaeae and Corynebacteriaceae. In the small intestine, a small percentage Bacteroidetes and Lachnospiraceae has been identified vs. their concentration in the colon [29]. The largest number of bacteria and the vastest microbial diversity (1011–1012/mL of luminal contents) in human gut have been observed in the distal section of the ileum and the colon. The greatest portion is composed by strictly anaerobic, often non-spore-forming, mainly Gram-positive (*Bacteroides* and *Clostridium*). There are also facultative anaerobes such as Lactobacillus, Enterococcus and Enterobacteriaceae [29] [30]. This substantially higher concentration of bacteria is due to a slower motility characterized by anti-peristaltic contractions that allow retention of colonic content for long periods. In addition, the intestinal pH is buffered through the secretion of bicarbonate that makes the environment more favorable to the bacterial colonization [31].

The hypothesis that the intestinal microbiota can constitute to a relevant environmental factor in the pathogenesis of obesity has led to the investigation of gut microbial communities in overweight individuals. The first evidence indicating an association between obesity and intestinal microbes was produced by studies applying DNA sequencing methods on a large scale to allow the screening of the entire gut microbiome. The first link between gut microbial environment and obesity was hypothesized by Ley et al. that analyzed the gut microbiota of leptin-deficient mice at major phyla level [32]. Results from 16S rRNA gene sequencing in mouse models indicated as the two most abundant bacterial phyla were Firmicutes (60%–80%) and Bacteroidetes (20%–40%), and showed how mice homozygous for an aberrant leptin gene *ob/ob*, carried a different proportion of bacteria in the ceca compared to lean wild-type (+/+) or heterozygous (*ob*/+) mice. In particular, the *ob/ob* mice had a 50% decrease in the population of Bacteroidetes and a proportional increase in Firmicutes ($p < 0.05$).

Similarly, Turnbaugh et al. published a study on mouse models using the newer shotgun metagenomic sequencing technique on cecal microbial DNA (*ob/ob*, *ob*/+ and +/+) [33]. This study confirmed the increased ratio of Firmicutes vs. Bacteroidetes in obese mice, as compared to lean ones. Moreover, *ob/ob* mice had a higher proportion of Archaea within

the cecal gut microbial communities. There was also a higher representation of genes involved in energy extraction from food in the obese host microbiota compared to lean host microbes. Works in another mammalian models noticed a lower abundance of Bacteroidetes associated with obesity [34][35]. Other works have associated mouse obesity with specific bacteria, in particular *Halomonas* and *Sphingomonas*, and the reduction in the *Bifidobacteria* number [36]. In order to assess if microbial communities can similarly affect weight gain or loss in humans, several studies have investigated various cohorts of obese and lean individuals, but the results have not always been consistent (**Table 2**).

Table 2. Gut microbial population and obesity: relationship, causality and effects in human studies.

Source	Study Subjects	Comparison	No. of Subjects	Methods	Community Measured	Major Findings
Ley et al. [32]	Human adults	Obese vs. controls	12 obese, 2 normal weight	16S rRNA sequencing	Bacteroidetes Firmicutes	Significantly redu level of Bacteroi obese subjects.
Collado et al. [37]	Pregnant women	Obese vs. lean pregnant	18 overweight, 36 normal weight pregnant women	FCM-FISH qPCR	*Bacteroides Bifidobacteria Staphylococcus aureus*	High numbers of *Bacteroides* grou *S.aureus* in the overweight preg women.
Zhang et al. [38]	Human adults	Obese vs. control vs. after RYGB	3 normal weight, 3 obese, 3 post-gastric bypass	16S Pyrosequencing qPCR	Firmicutes Bacteroidetes Proteobacteria Actinobacteria Fusobacteria Verrucomicrobia	More Bacteroide obese subjects (significant). *Prevotellacee* (p *Bacteroidetes*) a *Coriobacteriacee* (phylum *Actinob* increased in obe Significant increa *Methanobacteria* obese subjects.
Kalliomaki et al. [39]	Human children	Overweight/obese Normal weight	25 overweight: 7 obese, 24 normal weight	FISH	*Bifidobacteria Lactobacilli Clostridia Staphylococcus aureus*	Lower number o bifidobacteria ar greaternumber o *aureus* predict Obese/overweig phenotype.
Duncan et al. [40]	Human male	Obese vs. normal weight	15 obese, 14 lean	FISH	Bacteroides Firmicutes *E.rectale/C. coccoides*	No differences ir *Bacteroides* leve obese and norm weight subjects. Significant diet-dependent reduc *Eubacterium rec coccoides* (phylu *Firmicutes*) level obese subjects.
Turnbaugh et al. [33]	Human twins	Obese and normal twins, mothers	154 subjects: 31 monozygotic twin pairs, 23 dizygotic twin pairs, 46 mothers	16S pyrosequencing V2 and V6 variable region	Bacteroidetes Firmicutes Proteobacteria Actinobacteria	Significantly redu levels of Bacterc in obese and inc level of Actinoba Nearly half of the enriched genes Bacteroidetes.

Source	Study Subjects	Comparison	No. of Subjects	Methods	Community Measured	Major Findings
Armougom et al. [41]	Human adults	Anorexic, normal weight and obese	20 normal weight, 20 obese, 9 anorexic	qPCR	*Lactobacillus M. smithii* Bacteroidetes Firmicutes	Significantly redu levels of Bacterc in obese subject healthy subjects 0.01). Firmicutes are similar in the categories. Sign higher levels of *Lactobacillus.* In of *M. smithii* in a subjects ($p < 0.0$
Mai et al. [42]	Human adults	African American and Caucasian American	98 subjects	FISH qPCR	Bacteroidetes *Clostridia cluster XIV* (Firmicutes)	No significant dif in *Bacteroidetes* numbers betwee and normal-weig subjects.
Nadal et al. [43]	Human adolescents	Before and after 10 weeks of calorie-restricted diet	39 overweight adolescents	FISH	Bacteroidetes/*Prevotella Bifidobacterium C. histolyticum E. rectale/C. coccoides Lactobacillus/En-terococcus*	Greater weight l a multidisciplinar treatment progra associated with: significant reduc *Eubacterium rec Clostridium cocc* and *C. histolytic* significant increa *Bacteroides/Pre*
Santacruz et al. [44]	Human adolescents	Before and after diet and exercise for 10 weeks	36 obese adolescents	qPCR	*Bacteroides fragilis Lactobacillus C. coccoides C. leptum Bifidobacterium Escherichia coli*	After an obese g submitted to a w program lost >4 significant reduc *C.coccoides*; inc the *Bacteroides* and *Lactobacillu*
Schwiertz et al. [45]	Human adults	Obese vs. overweight vs.normal weight	98 subjects: 30 lean, 35 overweight, 33 obese subjects	qPCR	Firmicutes Bacteroidetes *Bifidobacteria*	Significantly incr level of Bacteroi obese subjects a decreased level Firmicutes. Sign decrease in *Bifidobacteria* ar *Methanobreviba* spp. in obese su
Balamurugan et al. [46]	Human children	Obese vs. non obese	15 obese, 13 normal weight	qPCR	Bacteroidetes *Bifidobacterium Lactobacillus acidophilus E. rectale F. prausntzi*	No significant dif in *Bacteroides/Pre* and *Bifidobacter* spp. Significant i of *Fecalibacteriu prausntzi* levels (*Firmicutes* spec obese subjects.

Source	Study Subjects	Comparison	No. of Subjects	Methods	Community Measured	Major Findings
Santacruz et al. [47]	Pregnant women	Overweight/obese pregnant women vs. normal weight women	16 overweight pregnant, 34 normal weight pregnant women	qPCR	*Bifidobacterium* *Lactobacilli* *Bacteroidetes* *Escherichia coli* *Staphilococcus*	Significant reduc *Bifidobacterium* *Bacteroides* nun obese pregnant Increased levels *Staphilococcus* a *coli* in overweigh women.
Abdallah Ismail et al. [48]	Human children and adults	Obese vs. normal weight	79 subjects: 51 obese, 28 normal weight	qPCR	Bacteroidetes Firmicutes	Significantly incr distribution of Bacteroidetes ar Firmicutes in the group.
Furet et al. [49]	Obese after RYGB	Obese subjects enrolled in a bariatric-surgery program	30 obese after RYGB, 13 lean	qPCR	*Bacteroides/Prevotella* *E. Coli F. Prausnitzii* *Bifidobacterium* *Lactobacilli*	*Bacteroides*/*Pre* group was lower obese subjects t control subjects increased after 3 months. *Escheri* species after 3 n and inversely co with fat mass an levels. *F. prausn* species was low subjects with dia and associated negatively with inflammatory ma
Zuo et al. [50]	Human adults	Obese vs. normal weight	52 obese, 52 normal weight	Culture	*Bacteroides Clostridium perfringens*	Significantly redu levels of *Clostric perfringens* and *Bacteroides* in o population.
Payne et al. [51]	Human children	Obese vs. normal weight children	30 subjects: 15 obese, 15 normal weight	qPCR TGGE	Bacteroides Firmicutes *Roseburia/E.rectale* *Lactobacillus* *Bifidobacterium* *Enterobacteriacee F. prausnitzii*	No significant differences for a population teste between obese a normal weight cl
Vael et al. [52]	Human children	Children at 3, 26 and 52 weeks of age	138 subjects	Culture	*Bacteroides fragilis* *Bifidobacterium* *Lactobacillus* *Enterobacteriacea* *Staphylococcus* *Clostridium*	High intestinal *Bacteroides frag* low *Staphylococ* concentrations i between the age weeks and 1 yea associated with risk of obesity la life.

Source	Study Subjects	Comparison	No. of Subjects	Methods	Community Measured	Major Findings
Patil et al. [53]	Human adults	Lean, normal, obese and surgically-treated obese subjects	20 subjects: 5 lean, 5 normal, 5 obese, 5 surgically treated	qPCR	*Bacteroidetes* *Firmicutes*	*Bacteroides* are prominent amon obese subjects.
Zupancic et al. [54]	Human adults	Stratified by BMI	310 adult subjects	16S rRNA pyrosequencing V1-V3	*Bacteroidetes* spp. *Firmicutes* spp.	Bacteroidetes/ Firmicutes ratio associated with metabolic syndr traits.
Xu et al. [55]	Human children	Normal, overweight and obese subjects	175 children: 91 normal, 62 overweight, 22 obese	qPCR	*Bacteroidetes* *Firmicutes*	Reduction of Bacteroidetes le obese group (p = 0.002).No differe Firmicutes level lean and obese (p = 0.628).
Munukka et al. [56]	Premenopausal women	Overweight/obese women with and without metabolic disorders	85 premenopausal women	FISH	*Bacteroidetes* *Bifidobacterium* spp. *Enterobacteriacee E. rectale/C. coccoides F. prausnitzii*	Proportion of *E. rectale/C. cocco* higher in MDG w compared to NM NWG women. C members of *E. rectale/C. cocco* associated with related metaboli disease, not obe se.
Million et al. [57]	Human adults	Obese vs. normal weight	115 subjects: 68 obese, 47 controls	Culture (*Lactobacillus* spp.) qPCR	*Lactobacillus* spp. *Bacteroidetes* *Firmicutes M. smithii*	*L. paracasei* is significantly ass with lean status. *reuteri, L. gasse* significantly ass with obesity. *M.* less abundant in obesity. Bactero are lower in obe significant, p = 0
Simões et al. [58]	Human twins	Obese, overweight, normal weight	20 twin pairs	qPCR DGGE	*Eubacterium rectale group Clostridium leptum group Lactobacillus group Bacteroides* spp.	The abundance diversity of the b groups not differ between normal overweight and individuals. Diet an important rol modulation of th microbiota, in pa *Bacteroides* spp *Bifidobacteria*

Source	Study Subjects	Comparison	No. of Subjects	Methods	Community Measured	Major Findings
Ferrer et al. [59]	Human adolescents	Lean and obese subjects	1 obese, 1 lean individual	qPCR	Bacteroidetes Firmicutes Actinobacteria Proteobacteria	Lower Bacteroid abundance and frequencies of C (*Firmicutes* spp. obese subjects.
Million et al. [45]	Humans adults	Obese, overweight, lean and anorexic subjects	263 individuals: 134 obese, 38 overweight, 76 lean, 15 anorexic	qPCR	Bacteroidetes Firmicutes, *M. smithii* *Lactobacillus* spp. *E.coli*	*L. reuterii* was p correlated with E *smithii* was nega associated with Bacteroidetes w correlated with E
Bervoets et al. [60]	Human children	Obese, overweight and morbidly obese (O/O group) and normal-weight, thinness (C group)	26 overweight/obese, 27 lean	qPCR Mass spectrometry	*Bacteroides* *Bifidobacterium* *Clostridium* *Staphylococcus* *Lactobacillus*	Higher concentr *Lactobacillus* sp obese microbiot Increased conce of Firmicutes an decreased conc of Bacteroidetes obese children.
Tims et al. [61]	Human twins	Concordant and discordant BMI twin pairs	40 subjects: 20 discordant BMI 20 concordant BMI twin pairs	HITChip phylogenetic microarrays	Bacteroidetes Firmicutes Actinobacteria at phylotype level	MZ twins have n similar GI microb compared with u subject. Inverse correlation betw *Clostridium clus* diversity and BM positive correllat between *Eubact ventriosum/Rose intestinalis* and E consistent Bacteroidetes/Fi ratio were obser pair-wise compa lower- and highe siblings.

5. Future Perspectives

The debate on the significance of the correlation between gut microbiota imbalance and obesity is one of the hottest topics in medicine. Although several molecular pathways have widened the view on the causative association between gut microbiota alterations and obesity development, this linkage remains very complex. On the other hand, the obesity pandemic asks for a solid response able to restore the significant gut microbial imbalance present in these patients. Thus, these findings imply the possibility and need for therapeutic manipulation of intestinal microbiota to prevent or treat obesity and its metabolic manifestations. The correlation between Firmicutes/Bacteroidetes ratio and obesity constitutes strong evidence arising from three decades of research in this filed. However, several recent studies have highlighted the complexity of the altered composition of intestinal microbiota in obese patients compared with lean subjects. Therefore, each study has linked obesity to species- or genus-specific composition profiles. The extreme variability of the results can be attributed to the different experimental designs, microbiota fingerprinting, and genome analyses. We must also mention the different populations or sub-populations studied.

Particularly, the heterogeneity of methods used to quantify the levels of gut microbiota does not allow a proper comparison of the results generated by different studies, as every technique is biased by accuracy, sensitivity or specificity issues. Thus, there is the need for a standardization of techniques to be used to detect and classify gut microbiota composition in obese subjects.

In more recent years, the attention of researchers has focused on the understanding of the specific metabolic patterns linked to the obesity physiopathology. Intestinal bacteria are an important part of these integrated functional networks. It has derived an increasing interest of investigators for the impact of gut microbiota modulation by the diet in these metabolic processes.

In conclusion, further investigations using standardized next-generation sequencing technologies should be conducted on the real association of gut microbiota composition and specific obesity-related phenotypes. Moreover, the complex interaction of intestinal bacteria with the host has to be unraveled, as well as the possible effect of variables such as diet, age, gender or physical activity. Future evidences can help, using the modulation of these variables in order to re-shape gut microbiota in a healthier profile. Indeed, it remains possible to directly modulate gut microbiota with probiotics, prebiotics, antibiotics, or other therapeutic interventions. Although several randomized clinical trials on probiotics in obesity setting have been carried out and their results are not yet convincing. Thus, more randomized placebo-controlled are lacking in this topic.

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