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Review Article

Gut Microbiome and Obesity: A Review of Literature -

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ABSTRACT

Obesity and associated adverse metabolic disorders have become the prevalent health issues of our times. This awareness led to assess if there could be other factors involved in the development of obesity, in addition to a prolonged imbalance of energy intake and energy expenditure, related to lifestyle, or a contribution of genetic variability. The relatively recent discovery that the composition and the metabolic function of the gut microbiota affect obesity development has led to a great interest in what is now a challenging research field. Here we will report the current knowledge in terms of the development, distribution and tasks of the intestinal microbiota. Then we will review the literature about the association between intestinal microbiota and obesity, focusing on different population subgroups. Finally, we would point out some items for which gut microbes determine the development of obesity and other associated chronic conditions.

INTRODUCTION

Obesity has become one of the most prevalent health issues of our time. In 2008 the World Health Organization (WHO) estimated that 35% of adults aged 20+ were overweight (BMI ≥ 25 kg/m2) (34% of men and 35% of women). The worldwide prevalence of obesity has more than doubled in 30 years: in 2008, 10% of men and 14% of women in the world were obese (BMI ≥30 kg/m2). Worldwide an estimated 205 million men and 297 million women over the age of 20 were obese – a total of more than half a billion adults. At least 2.8 million people die each year as a result of being overweight or obese: overweight and obesity lead to adverse metabolic effects on blood pressure, cholesterol, triglycerides and insulin resistance, but also on risk of coronary heart disease, ischemic stroke, type 2 diabetes mellitus, as well of cancer [1].

Obesity results from an imbalance between energy intake and expenditure and, obviously, lifestyle changes have driven its prevalence to epidemic proportions. At the same time heritability studies provide evidence for a considerable genetic contribution to obesity risk [2,3]. Throughout, 32 loci are associated with BMI at genome-wide significance [4], but the combined effect of their associated variants on BMI is modest, accounting for only 6%–11% of the genetic variation in BMI [4]. While genetic factors clearly contribute to determine body weight, and hence to the development and maintenance of obesity, the dramatic rise in obesity prevalence over the past decades has, appropriately, turned attention towards other aspects of environment.

Recently, microbial changes in the human gut was proposed to be another possible cause of obesity [5,99]: the twinned observations that obesity may be associated with gut microbiome configuration in humans [6] and that obesity phenotypes can be transmitted via the gut microbiota in rodent models of obesity [7] have focused attention on the role of the gut microbiome in the development of obesity. Intriguingly, the microbiome shares properties with both the environment (it is an close part of the human environment) and genes (it is heritable and contains genetic material) [8]. Indeed, some haveproposed that this microbial genetic material effectively represents an extension of our genome – a "meta-genome"[9]. Hence the gut microbiome represents a forceful candidate as important contributor to the current increase in obesity rates, and accumulating evidences support this role.

Here we will report the current knowledge in terms of the development, distribution and tasks of the intestinal microbiota. Then, we will review the literature about the association between intestinal microbiota and obesity, focusing on different population subgroups. Finally, we would point out some items for which gut microbes determine the development of obesity and other associated chronic conditions.

Historical and current perspectives

The interplay between diet, gut flora (currently termed "microbiota") and human health has been appreciated for over a century. Acceptance of the germ theory of disease led to early attribution of a number of human disorder to microbial sources, including conditions that succeeding generations of physicians have considered to be non-infectious. An initial proponent of such theories, now claimed as the father of probiotics, was the immunologist Elie Metchnikoff. In his 1907 article, "Essaisoptimistes" (published in translation as "The Prolongation of Life: Optimistic Studies") [10], Metchnikoff proposed microbial origins for senility and hypothesized that products of intestinal putrefaction by microbes were responsible. He observed a positive health effects of consuming fermented food, which could provide to avoid such putrefaction and senility.

Study about interactions between the gut flora and organism biology over the past century has demonstrated the influence of the former on numerous extra-intestinal phenotypes. The exhaustive description of human microbiota and their relationship with health and disease are major challenges in the twenty-first century [11]. In recent years it has found a growing interest, by scientists, for this topic, so that the number of annual publications related to human gut microbiota is almost 4 times greater than in 2005, when Eckburg et al. [12] published the seminal large scale gut metagenomic study [13].

The gut microbiota is the most complex ecosystem in nature: it harbors large bacterial populations in the intestine and colon, approximately 1011–12 microorganisms per gram of content, and this is composed of mainly anaerobes (95% of the total organisms). First studies of the gut microbiota com-position was based on microscopic observation and culture methods, and the predominant cultivable species that were identified included Bacteroidesspp., Eubacteriumspp., Bifidobacterium spp., Peptostreptoccocusspp., Fusobacterium spp., Ruminococcusspp., Clostridium spp. and Lactobacillus spp. [14]. The first 16S rRNA study of the human gut microbiome was performed in 1996, with only 1 subject studied (only 31% of identified rRNAs was mapped to known species) [15]. In 2006, Gill et al. performed the first metagenomic sequencing of the human distal gut microbiome, revealing microbial genomic and genetic diversity and indentifying some of the distinctive functional and metabolic attributes encoded in distal gut microbiome (in their report, only 2 individuals underwent sequencing) [16]. The first, large-scale, 16S rDNA sequencing analysis of the gut microbiota by Eckburg et al. [12] revealed a high inter-individual variability at the species taxo-nomic level.

Nowadays DNA-based analyses have expanded the horizon of our knowledge, by generating enormous new data sets that can be extracted for information on the composition and functional properties of vastly greater numbers of microbial communities. In these assumption, US National Institutes of Health (NIH) founded Human Microbiome Project (HMP) Consortium. HMP is a conceptual and experimental extension of Human Genome Project, and it is not a single project [11,17], but an interdisciplinary effort consisting of multiple projects, which collects scientific experts worldwide to explore microbial communities, to characterize the composition of the normal microbiome and the relationship with its human host. HMP, but also other large-scale endeavors (as European Project, Metahit [19]) provide a preliminary understanding of the biology and medical implication of the human microbiota and its collective genes (metagenome). During assessments of microbial genes, the presence of a human "core" microbiome was showed, which is represented by a set of genes present in a given habitat of all, or at least a large part, of the humans. On this core all those genes that are present in a limited habitat and in a smaller set of humans are inserted, and they can be modified by a combination of factors, such as the host genotype, the physiological state (including the properties of the immune system), the disease state, the lifestyle (including diet), the presence of transient microorganism population. This "core" microbiome is not a set of abundant microbial organisms that we all share, but it exist at the level of shared components involved in various metabolic functions [6]. All these findings justify the high inter-individual variability of the human microbiome [19,20].

The gut environment: development and assessment

The gut microbiota can be classified into three domains based on molecular phylogeny (i.e., 16S ribosomal Ribo Nucleic Acid [rRNA] sequence similarities and differences): Eukarya, Bacteria, and Archaea. Eukarya consists of organisms with cells that contain complex structures enclosed within membranes, most notably the nucleus. In contrast, Bacteria are the predominant members of the gut microbiota (Table 1). Most of the bacteria of fecal origin belong to two of the major phylogenetic lineages: Firmicutes and Bacteroidetes(accounting about 90%), even if the gastrointestinal tract in an individual adult human has been estimated to contain approximately 500 to 1000 distinct bacterial species [5,12,16]. Rather Methano brevibactersmithii is the dominant, methanogenic archaeon species in the human gut microbiota [12].

Most of the intestinal microbial communities belongs to the kingdom of Bacteria and Archaea. The first one, most numerous in the intestinal tract, includes many subclasses, otherwise distributed: Bacteroidetes (23%) that comprise the genus Bacteroides, Firmicutes (64%) that includes Bacilli, Clostridia and Mollicutes (the majority of cells in this phylum were closely related to genus Streptococcus and Clostridium); Proteobacteria (8%), Gram-negative bacteria such example Escherichia coli and Helicobacter pylori; Fusobacteria, Verrucomicrobiaand Actinobacteria (3%), which includes species such as Bifidobacterium [21,22]. Bacteroidetesare constituted by approximately 20 genera, Bacteroidalesis the most studied class, in particular the genus Bacteroides.

Firmicutesare Gram-positive bacteria, including 250 kinds divided into three classes: Clostridia, Bacilli and Mollicutes [Table 1].

Infancy is characterized by microbial plasticity, whereby the colonization by microbes can change among the time. The colonization of the gastrointestinal tract begins at birth and it changes, in a variable way from individual to individual [27], substantially at three stage of life: from birth to weaning; from weaning to attaining a normal diet; during old age. At birth, human are essentially free of bacteria, but immediately after birth the intestine begins to be populated by a series of microorganisms that vary, both for the effect of exogenous and endogenous factors (mother's vaginal and fecal microbiota, environment, skin). It was shown that the birth via Caesarean section alters the intestinal microbiome and can be associated with obesity in childhood and adulthood [100]. During the first 12-24 hours of extra-uterine life, gut colonists are especially facultative anaerobic bacteria such as Escherichia coli, Enterococci and Streptococci [23]. Subsequently, the second to third day, these bacteria, maybe by reduction of the redox potential (low oxygen concentration), create anaerobic condition that promote the growth of obligate anaerobes (Lactobacilli and mainly Bifidobacteria). Within 2 weeks the bacterial population expands from 108 to 1010/ g of feces and establish themselves as a species Bacteroidesand Clostridia. In a observational study to investigate the relationship between intestinal microflora and body mass index, Vael et al. [25] found that in the first 3 years of life the Bacteroidesfragilisconcentration continued to increase from the age of 3 weeks until the age of 1 year, instead the populations of Staphylococcus, Lactobacillus, Bifidobacterium, Clostridium decrease from week 3 to week 26 and remained stable until week 52.

A determinant element in developing the gut flora is definitely the infant feeding: some studies have shown the different qualitative composition of the bacterial flora in breastfed subject compared to a artificially fed one. In breast fed infants, for example, Bifido bacterium prevail (60-90% of the fecal flora) compared to a less 1% value of lactic acid bacteria. At the same time occurs a decrease in pH and inhibition of putrefactive flora to the advantage of fermenting. This improves digestive functions and absorption is stimulated immune system and, together with the production of vitamins, reducesthe risk of contracting allergies [24,26]. After the first six months of life, with the beginning of the weaning period, the diversification of the diet and the introduction of solid foods, there is a further differentiation of micro organisms, which are enriched in species even present in adults [27], in particular these belonging to Firmicutes and Bacteroidetes[26]. In the first year of life the levels of Escherichia coli and Enterococci fluctuate between 106 and 108 CFU / g of feces, while there is a decrease in Clostridia and an increase in anaerobic flora which gradually to diversify. The initial colonization is certainly important for defining the bacterial flora in the final adult age, in fact, once constituted, it remains stable with the exception of possible variations following of several factors of different nature such as a change in eating habits or the onset of diseases. While Hopkins et al. [28] found the occurrence, in children between 1 and 7 years of age, of higher numbers of Enterobacteriathan adults, a large-scale German study [29] noticed that total CFU and individual microbial species were highest during the first year of life, decreased within the first 2 years, and then stabilized for the remaining childhood (until 18 years of age). About adolescent children, comparison of intestinal microbiota composition between adolescents and adults revealed a statistically significantly higher abundance of genera Bifidobacterium and Clostridium among first group [30].

A decline in microbiota numbers and species diversity has been reported in old age [31], with reduced number of bifidobacteria, and an increase in Enterobacteriaceae[32]. Bacteroidetesbecome more abundant and Firmicutesless abundant in elderly adults [33,34]. In institutionalized elderly subjects, Zwielehner et al. [34] found a significantly higher numbers of Bacteroidescells than young control,

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
			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

with a reduction and loss of diversity of Bifidobacteria and Clostridium cluster IV associated with age. Instead Claesson et al. [35] observed a clear shift to a more Clostridium cluster IV-dominated community in the elderly.

Microbial composition and distribution

Another interesting point is that the human intestinal microbiota differs in quality along the entire gastrointestinal tract [22]. These differences add a horizontal stratification, with the presence ofdiverse microbial communities in the lumen intestinal, in the layer of mucus in the crypts and directly adherent to the epithelial cells.

number increases progressively along the intestinal tract as the redox potential drops. The microbiota of jejunum appears dominated from species belonging to the genus Streptococcus [22], but only at the ileo- cecal level, there is a significant population of bacteria (108-109/g of feces). At the level of the small intestine is enriched of subgroup Bacillus bacteria (the phylum Firmicutes) (mainly Streptococcaceae, corresponding to 23% of the sequences identified compared with 5% in the colon). It was also found up to 8% corresponding to members of the phylum Actino bacteria and, in particular, of subgroups Actinomycinaeae and Corynebacteriaceae. In the small intestine a small percentage Bacteroidetes and Lachnospiraceae was identified, contrary to what was observed at the colon level [21]. Distal ileum and large intestine are the gastrointestinal tracts with the highest number of bacteria and the increased microbial diversity (1011-1012/mL of luminal contents). The most part is composed from strictly anaerobic, often non-spore-forming, mainly Gram-positive (Bacteroidesand Clostridium), in addition to facultative anaerobes such as Lactobacillus, Enterococcus and Enterobacteriaceae [22,36]. Motility stagnant with retropulsive contractions, which allows to retain the content for long periods, and the pH buffered by secretions of bicarbonate, make the environment more favorable to the colonization of the large intestine by bacteria.

The composition of the gut microbiota in relation to obesity

The finding that the gut microbiota could be considered as environmental factor that modulates obesity has spurred studies to test if gut microbial communities are altered in obese status. The

possible existence of a link between obesity and the gut microbiota became apparent upon the application of DNA sequencing methods on a large scale to facilitate the analysis of the whole gutfrom 16s rRNA gene sequencing revealed that the two most abundant bacterial division in mice were the phylum Firmicutes (60-80%) and the phylum Bacteroidetes (20-40%), and that the ob/ob mice had a difference proportion of bacteria in the ceca compared to lean wild-type (+/+) or heterozygous (ob/+) mice. Particularly, they had a 50% reduction in the abundance of Bacteroidetes and proportional increase in Firmicutes ($p<0.05$). These changes were division-wide (there were no specific subgroup that were preferentially lost or amplified) [37].

Turnbaugh et al. [7] continued this line of studies and published a study using an alternative technique, i.e. shotgun metagenomic sequencing of cecal microbial DNA of mice (ob/ob, ob/+ and +/+). This paper again highlighted an increased ratio of Firmicutes to Bacteroidetesin obese mice compared to lean ones. It was also noted that ob/obmice had an higher proportion of Archaea in the cecal gut microbial communities. They also showed ad enrichment in genes involved in energy extraction from food in the obese host microbiome compared to lean host microbiome, with a greater energy extraction efficiency, less energy left over in feces and greater levels of Short-Chain Fatty Acids (SCFAs), as shown in a section below.

In the following years, the mice, but also pigs, have been extensively used for research on the role of gut microbiota in obesity, even if, in contrast to study performed in humans, studies conducted in animals tend to have less variable outcome. Studies in rats and pigs have reported lower abundance of Bacteroidetesassociated with obesity [38-44]. Some studies have associated the mice obese phenotype with specific bacteria (Halomonasand Sphingomonas) and the reduction in the Bifidobacterianumber [45], other ones have studied canine microbiota experiencing an increase in Clostridialesin ad libitum feeding dogs [46].

The question of whether or not a microbial community can predispose a host to weight gain or loss has been transposed in humans. Several studies comparing different cohorts of obese and leanindividuals have been performed, but the results of these studies have not achieved the same conclusions (Table 2).

a- Bacteria species and obesity: Firstly, many studies tried to identify how the levels of the major phyla (Bacteroidetes and Firmicutes) change in relation to obesity or weight loss. Ley et al. [5], in a pioneering study that linked gut microbiota and obesity in humans, compared the gut flora of lean and obese individuals, through 16s rRNA sequencing of DNA extracted from fecal samples. They found that Bacteroidetes and Firmicutesdivision dominated the microbiota (92.6% of all 16s rRNA sequences), but obese individuals possessed a lower proportion of Bacteroidetes ($p<0.001$) and higher levels of Firmicutes ($p=0.002$) than lean controls, confirming the results established in previous murine studies [37]. After diet therapy the relative abundance of Bacteroidetes increased and the abundance of Firmicutesdecreased, irrespective of diet type.

In 2009 Armougom et al. [47] assessed the gut microbiota of obese, lean and patient suffering from anorexia nervosa, using real-time PCR assay: the study found a significantly reduced levels of Bacteroidetesin obese versus lean ($p<0.01$) or anorexic ($p<0.05$) subjects, whereas Firmicutesdata are similar in the three categories. Intriguingly, the obese group had also a bacterial profile rich in Lactobacillus (not significant differences).

To confirm reported gut alterations and investigate also whether specific bacterial species, like Lactobacillus or Bifidobacterium species, are associated with obesity, in 2012 Million et al. [48], in a large case-control study that used part of patients of the above cited paper, analyzed the intestinal microbiota, not only at the phylum level, but also of Lactobacillus and Bifidobacterium genera at species level. Bacteroidetes was found in not significant lower concentration in obese ($p=0.25$). The result newly reported that there are species-specific variations of Lactobacillus in obesity: L. paracaseiwas significantly associated with lean status, whereas L. reuteriand L. Gasseri were significantly associated with obesity, since confirmed in a subsequent study [49].

Zuo et al. [50] analyzed the composition of cultivable bacteria in obese individuals and their normal-weight counterparts: quantitative bacterial studies demonstrated that the amount of Bacteroides (the major Bacteroidetesgenus) and of Clostridium perfringens was significantly lower in the obese group than in normal-weight one ($p=0.012$ and $p=0.001$ respectively).

Other studies supported the findings of a opposite Firmicutes/Bacteroidetesratio. In 2008 Zhang et al. [51], using 16S rDNA pyrosequencing technology, compared microbial community structures of 9 individuals, 3 of each categories of normal weight, morbidly obese and post-gastric-bypass surgery. The results indicated that H2-producing Prevotellaceae, within the class of Bacteroidetes, were significantly enriched in obese individuals compared to lean controls ($p=0.040$), even if the difference in the relative abundance of Bacteroidetes was not significant between the two groups ($p=0.061$). This study used a very small population, but it has allowed to assert a link with energy homeostasis in humans, justifying the greater capacity to energy harvest from obese subjects.

The debate regarding the possible difference in Bacteroidetes and Firmicutes proportion in obese subjects continued with the study of Schwiertz et al. [52], who found that the median proportion of Bacteroidetes of the total sum of studied species was higher in overweight and obese than in lean volunteers ($p=0.001$ and $p=0.006$ respectively). In addition, both overweight and obese subjects exhibits lower cell numbers of Ruminococcus flave faciens sub group, belonging to bacterial division of Firmicutes. On the whole, a lower ratio Firmicutes/Bacteroidetes was detected. Actually, the methodology was objectionable because the Bacteroidetes proportion was obtained by summing Bacteroides and Prevotellagenera. The study revealed also lower levels of Clostridium leptum group ($p=0.07$) and of the Bifidobacterium genus ($p=0.02$).

Finally, some studies have found no association between intestinal microbiota and changes in body weight. Duncan et al. [53] could not found any relationship between BMI or absolute weight loss, and the relative populations of the major groups of human colonic bacteria, including Bacteroidetes, between obese and non-obese subjects: they hypothesized that the proportion of Bacteroidetes and Firmicutes, at least at the phylum level, have not a key function in determining human obesity. Nevertheless they found a significant diet-dependent reduction in Eubacteriumrectale/C.coccoideslevels, a group of butyrate-producing Firmicutes, in obese subjects on weight loss diet.

The composition of the gut microbiota of both African Americans and Caucasian Americans was investigated by Mai et al. [54]: both

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

Source	Study subjects	Comparison	No. of subjects	Method	Community measured	Major findings
Ley et al., 2006 [5]	Human adults	Obese vs controls	12 obese 2 normal-weight	16s rRNA sequencing	*Bacteroidetes Firmicutes*	Significantly reduced level of Bacteroidetes in obese subjects.
***Collado et al, 2008* [81]**	Pregnant women	Obese vs lean pregnants	18 overweight 36 normal weight pregnant women	FCM-FISH qPCR	*Bacteroides Bifidobacteria Staphylococcus aureus*	High numbers of *Bacteroides* group and *S.aureus* in the overweight pregnant women
***Zhang et al, 2008* [51]**	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 *Bacteroidetes* in obese subjects (not significant). *Prevotellacee* (phylum *Bacteroidetes*) and *Coriobacteriacee* (phylum *Actinobacteria*) increased in obese. Significant increase in *Methanobacteriales* in obese subjects.
***Kalliomaki et al, 2008* [69]**	Children	Overweight/ Obese children Normal children	25 overweight (7 obese) 24 normal weight at 7 y	FISH	*Bifidobacteria Lactobacilli Clostridia Staphylococcus aureus*	Lower number of bifidobacteria and greaternumber of *S. aureus* predict Obese/Overweight phenotype
***Duncan et al, 2008* [53]**	Human male	Obese vs normal weight	15 obese 14 lean	FISH	*Bacteroides Firmicutes E.rectale/ C.coccoides*	No differences in *Bacteroides* level in obese and normal weight subjects. Significant diet-dependent reduction in *Eubacterium rectale/C.coccoides* (phylum *Firmicutes*) levels in obese subjects
***Turnbaugh et al., 2009* [6]**	Human twins	Obese and normal twins, mothers	154 subjectes (31 monozygotic twin pairs, 23 dizygotic twin pairs, 46 mothers)	16s pyrosequencing V2 and V6 variable region	*Bacteroidetes Firmicutes Proteobacteria Actinobacteria*	Significantly reduced levels of *Bacteroidetes* in obeses and increased level of *Actinobacteria*. Nearly half of the lean-enriched genes are from *Bacteroidetes.*
***Armougom et al, 2009* [47]**	Human adults	Anorexic, normal weight and obese	20 normal weight 20 obese 9 anorexic	qPCR	*Lactobacillus M. smithii Bacteroidetes Firmicutes*	Significantly reduced levels of *Bacteroidetes* in obese subjects versus healthy subjects ($p<0.01$). *Firmicutes* data are similar in the three categories. Significantly higher levels of *Lactobacillus.* Increase of *M. smithii* in anorexic subjects ($p<0.05$).
***Mai et al, 2009* [54]**	Human adults	(African American and Caucasian American)	98 subjects: 51 AA and 46 CA (14 lean and 14 obese randomly selected for microbiota analysis)	FISH qPCR	*Bacteroidetes Clostridia cluster XIV (Firmicutes)*	No significant difference in *Bacteroidetes* numbers between obese and normal-weight subjects
***Nadal et al, 2009* [79]**	Obese adolescents	Before and after 10-wk calorie-restricted diet	39 overweight adolescents	FISH	*Bacteroidetes/ Prevotella Bifidobacterium C.histolyticum E. rectale/ C. coccoides Lactobacillus/ En-terococcus*	Greater weight loss after a multidisciplinary treatment program associated with: significant reduction of *Eubacterium rectale, Clostridium coccoides* and *C.histolyticum*; significant increase in *Bacteroides/ Prevotella*
***Santacruz et al, 2009* [80]**	Adolescents	Before and after diet and exercise for 10 wk	36 obese adolescents	qPCR	*Bacteroides fragilis Lactobacillus C.coccoides C.leptum Bifidobacterium Escherichia coli*	After an obese group submitted to a weight program lost >4 Kg: significant reduction in *C.coccoides*; increase in the *Bacteroides fragilis* and *Lactobacillus* group.
***Schwiertz et al., 2010* [52]**	Human adults	Obese vs overweight vs normal weight	98 subjects (30 lean, 35 overweight, 33 obese subjects)	qPCR	*Firmicutes Bacteroidetes Bifidobacteria*	Significantly increased level of *Bacteroidetes* in obese subjects and decreased level of *Firmicutes.* Significant decrease in *Bifidobacteria* and *Methanobrevibacter sp.* in obese subjects.
***Balamurugan et al, 2010* [72]**	Children	Obese vs non obese	15 obese 13 normal weight	qPCR	*Bacteroidetes Bifidobacterium Lactobacillus acidophilus E. rectale F. prausntzi*	No significant difference in *Bacteroides/ Prevotella* and *Bifidobacterium* spp. Significant increase of *Fecalibacterium prausntzi* levels (*Firmicutes* species) in obese subjects.
***Santacruz et al, 2010* [82]**	Pregnant women	Overweight/obese pregnant women vs normal weight women	16 overweight pregnants 34 normal weight pregnant women	qPCR	*Bifidobacterium Lactobacilli Bacteroidetes Escherichia coli Staphilococcus*	Significant reduction of *Bifidobacterium* and *Bacteroides* numbers in obese pregnant women. Increased levels of *Staphilococcus* and *E. coli* in overweight women.

***Ismail et al, 2010* [73]**	Egyptian children and adults	Obese vs normal weight	79 subjects: 51 obese (23 children and 28 adults) and 28 normal weight (17 children and 11 adults)	qPCR	*Bacteroidetes Firmicutes*	Significantly increased distribution of *Bacteroidetes* and *Firmicutes* in the obese group.
***Furet et al. 2010* [62]**	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/ Prevotella* group was lower in obese subjects than in control subjects and increased after 3 months. *Escherichia coli* species after 3 months and inversely correlated with fat mass and leptin levels. *F. prausnitzii* species was lower in subjects with diabetes and associated negatively with inflammatory markers.
***Zuo et al., 2011* [50]**	Human adults	Obese vs normal weight	52 obese 52 normal weight	Culture	*Bacteroides Clostridium perfringens*	Significantly reduced levels of *Clostridium perfringens* and *Bacteroides* in obese population
***Payne et al., 2011* [74]**	Swiss children	Obese vs normal weight children	30 subjects (15 obese and 15 normal weight children)	qPCR TGGE	*Bacteroides Firmicutes Roseburia/ E.rectale Lactobacillus Bifidobacterium Enterobacteriacee F. prausnitzii*	No significant differences for any population tested between obese and normal weight children
***Vael et al., 2011* [25]**	Children	Children at 3, 26 and 52 weeks of age	138 subjects	Culture	*Bacteroides fragilis Bifidobacterium Lactobacillus Enterobacteriacea Staphylococcus Clostridium*	High intestinal *Bacteroides fragilis* and low *Staphylococcus* concentrations in infants between the age of 3 weeks and 1 year are associated with a higher risk of obesity later in life.
***Patil et al., 2012* [55]**	Indian adults	Lean, normal, obese and surgically-treated obese individuals	20 subjecte (5 lean, 5 normal, 5 obese, 5 surgically treated)	qPCR	*Bacteroidetes Firmicutes*	*Bacteroides* are prominent among the obese individuals
***Zupancic et al., 2012* [57]**	Old Older Amish subjects	Stratified by BMI	310 adult subjects	16s rRNA pyrosequencing V1-V3	*Bacteroidetes sp. Firmicutes sp.*	B/F ratio are not associated with BMI or metabolic syndrome traits
***Xu et al, 2012* [76]**	Children	Normal, overweight and obese individual	175 children (91 normal, 62 overweight, 22 obese groups)	qPCR	*Bacteroidetes Firmicutes*	Reduction of *Bacteroidetes* level in obese group (p=0.002). No differences in *Firmicutes* level between lean and obese children (p=0.628).
***Munukka et al, 2012* [58]**	Premenopausal women	Overweight/obese women with and without metabolic disorder	85 premenopausal women	FISH	*Bacteroidetes Bifidobacterium sp. Enterobacteriacee E.rectale/ C.coccoides F. prausnitzii*	Proportion of *Eubacterium rectale/ Clostridium coccoides* is higher in MDG women compared to NMDG and NWG women. Certain members of *E. rectale/ C. coccoides* are associated with obesity related metabolic desease, not obesity per se.
***Million et al, 2012* [48]**	Human adults	Obese vs normal weight	115 subjects (68 obese and 47 controls) *(13 obese and 9 control subjects were part of previous study)*	Culture (Lactobacillus spp) qPCR	*Lactobacillus spp Bacteroidetes Firmicutes M. smithii*	*L. paracasei* is significantly associated with lean status. *L. reuteri, L. gasseri* are significantly associated with obesity. *M. smithii* is less abundant in human obesity *Bacteroidetes* are lower in obeses (not significant, p=0-25)
***Simões et al., 2013* [67]**	Human twins	Obese, overweight, normal weight	20 MZ twin pairs	qPCR DGGE	*Eubacterium rectale group Clostridium leptum group Lactobacillus group Bacteroides spp.*	The abundance and diversity of the bacterial groups not differ between normal weight, overweight and obese individuals. Diet plays an important role in the modulation of the stool microbiota, in particular *Bacteroides spp*. and *Bifidobacteria*
***Ferrer et al, 2013* [79]**	Adolescents	Lean and obese subjects	1 obese and 1 lean individual	qPCR	*Bacteroidetes Firmicutes Actinobacteria Proteobacteria*	Lower Bacteroidetes aboundance and greater frequencies of Clostridia (Firmicutes sp) in obese subject.
***Million et al, 2013* [49]**	Humans	Obese, overweight, lean and anorexic subject	263 individuals (134 obese, 38 overweight, 76 lean and 15 anorexic)	qPCR	*Bacteroidetes Firmicutes M.smithii Lactobacillus spp E.coli*	*L. reuterii* was positively correlated with BMI. *M. smithii* was negatively associated with BMI. *Bacteroidetes* was not correlated with BMI.

Bervoets et al, 2013 **[77]**	Children	Obese, overweight and morbidly obese (O/O group) and normal-weight, thinness (C group) children	26overweight/obese (9 overweight, 7 obese, 10 morbidly obese), 27 lean (21 normal-weight, 5 thinness grade I, 1 thinness grade II children)	qPCR Mass spectrometry	*Bacteroides Bifidobacterium Clostridium Staphylococcus Lactobacillus*	Higher concentration of *Lactobacillus* spp. in obese microbiota. Increased concentration of *Firmicutes* and decreased concentration of Bacteroidetes in obese children.
Tims et al, 2013 **[68]**	Human twins	Concordant and discordant BMI twin pairs	40 subjects (20 discordant BMI and 20 concordant BMI twin pairs)	HITChip phylogenetic microarrays	*Bacteroidetes Firmicutes Actinobacteria at phylotipe level*	MZ twins have more similar GI microbiota compared with unrelated subject. Inverse correlation between *Clostridium cluster IV* diversity and BMI; positive correaletion between *Eubacterium ventriosum/ Roseburia intestinalis* and BMI. No consistent *Bacteroidetes/ Firmicutes* ratio were observed in pair-wise comparison of lower- and higher-BMI siblings.

qPCR and FISH techniques have been applied to analyzed the proportions of Bacteroidetes and Clostridia cluster XIV (Firmicutes), but the results didn't show any association with BMI. However, they observed that the type of diet could influences the gut microbiota: individual that consumed high fat diet had fewer Clostridia, while a fiber-reach diet increased lactic acid bacteria levels.

More recently, other studies have been conducted on this topic: these were performed in unique populations, which differed for particular habits of life, or at genetic and socio-economic status. Patil et al. [55] reported a comparative analysis and quantification of dominant gut microbiota of lean, normal, obese and surgically-treated obese individuals of Indian origin. They detected, by 16s rRNA sequencing, no evident trend in distribution of the predominant bacterial phyla, Bacteroidetesand Firmicutes. At the genus level, dominance of Bacteroides species among the obese individuals was further confirmed by means of RT-PCR, which demonstrated a positive correlation between Bacteroides and BMI (p=0.002).

An interesting progress in the knowledge of gut microbial composition was the development of distinct cluster of enterotypes in the human microbiome. By combining sequencing data from 33 gut microbiomes from different nationalities, three distinct clusters, designated as enterotype, could be identified on the basis of variation in the relative levels of Bacteroides, Prevotella and Ruminococcus [56]. Enterotype 1 was enriched in Bacteroides and co-occurring Parabacteroides, which derive energy from carbohydrates and proteins by fermentation. Enterotype 2 was enriched in Prevotellaand Desulfovibrio, which degrade mucin glycoprotein. Enterotypes 3 is the most frequent and is enriched in Ruminococcusand co-occurig Akkermantsia, able to degrades mucins. Enterotypes did not seem to differ in functional richness, and virtually none of several measured host properties, namely nationality, gender or age, significantly correlates with enterotypes. No correlation was found between BMI and Firmicutes/Bacteroidetesratio [56]. To explore the possible dysbiosis of gut microbiota in obesity, Zupancic et al. [57] studied Old Older Amish subjects, a genetically closed homogeneous population, with an uniform socio-cultural status. By 16s pyrosequencing analysis, they identified three network of interacting bacteria in the human gut, which correlated with the three enterotype of Arumugam [56]: in the first group Prevotellagenus dominated; the second group was Bacteroides-dominated; a Firmicutes dominated group (III) was characterized by diverse Firmicutes genera. Even now, neither BMI nor any metabolic syndrome trait was associated with a particular gut community (p=0.79).

Unlike all previous studies that reported the difference of the intestinal microbiota between obese and lean subjects, Munukka et al. [58] took into account that 25% of obese people are metabolically "healthy" so-called defined, i.e. with a substantially normal lipid and glucose metabolism. Therefore, they conducted a study to assess whether there were differences in the intestinal microbiota of overweight/obese women with and without metabolic disorders, and to investigate whether the fecal microbiota composition was associated with body composition and different biochemical parameters. The participants were divided into three groups: MDG (Metabolic Disease Group), NMDG (Non MD Group) and NWG (Normal Weight Group, as controls). The results showed a greater proportional amount of Eubacteriumrectale-Clostridium coccoides group (belonging to Firmicutesphylum) in MDG women, after adjusting for body weight, compared NMDG and NWG. MDG had also higher proportion of Gram-negative enteric bacteria than NW (p=0.043). Instead, no differences were found in the relative amounts of genus Bifidobacterium, Atropiumcluster, Bacteroides group and F. Prausnitzii among three groups. Above all, larger number of E. rectale-C. Coccoidespositively correlated with body weight, BMI, FM, visceral fat area (all $p<0.01$) and serum trigly cerides ($p<0.05$), while Bacteroideswas inversely correlated with the same parameters ($p<0.05$), but positively with HDL concentration ($p<0.01$). The ratio of E. rectale- C.coccoides group to Bacteroides group, calculated by dividing the proportion of E. rectale- C. coccoidesby the proportion of Bacteroides, was higher in the MDG. These results indicated that members of E.rectale- C.coccoides group are associated with obesity-related metabolic diseases, and not obesity per se.

A recent meta-analysis [59] of the obesity associated gut microbiota alteration at the phylum level (Bacteroidetes and Firmicutes) was performed for several studies which also included other population subgroups evaluated in the review below [5,6,47,48,51,52,81]. The only reproducible and significant alteration at the phylum level was the decrease in the absolute number of sequences of Firmicutesin obese subjects, while no significant differences were found about Bacteroidetes counts.

b- Archaea and obesity: Methanobrevibacteris the main representative of Archaea in the gut microbiota. Firstly Zhang et al. [51] found more M. smithiiin obese individuals than in lean controls. Correspondingly Armougom et al. [47] incorporated into their analysis the quantification of methanogen M. smithii, whose levels were higher in obese group than lean group (1.72 fold increase), but also very increased in anorexic patients. However calculating this

data as means of log10 copies/ml, there was a decrease in M. Smithii load in the obese group [59].

Schwiertz et al. [52] found significantly lower levels of M. Smithii in obese subjects compared to lean ones. More recently Million et al, in two papers, found that M. Smithii was less frequent and significantly less abundant in obese subjects [48,49].

Overall, methanogenic archea could indirectly promote caloric intake by the colon, and the mechanism connecting obesity to methanogens may be, atleast in part, the transfer of Hydrogen gas (H2) from H2-producting bacterium to H2-oxidizing methanogen, which could improve polysaccharides fermentation efficiency removing fermentation intermediates [60]. It was hypothesized that the methanogenic Archaea have a possible H2-producing bacterial partner, like members of Prevotellaceae (phylum Bacteroidetes): this partnership in obese subjects might allow for greater efficiency for dietary polysaccharide fermentation, increasing their conversion into short-fatty acids, resulting in their excessive storage [51].

Actually, a meta-analysis of the obesity associated gut microbiota alteration at the genus level for Methanobrevibacter spp. revealed that obese subjects presented less Methano brevibacter than non-obese subjects [59], highlighting that the reason linking methanogens to weight gain still remain unclear.

c- Bariatric surgery: Bariatric surgery is increasingly employed as an anti-obesity treatment, and for a morbidly obese patient it is the only option available that can deliver substantial and persistent weight loss [61]. The surgery can be performed in different ways in that, in some cases, it involves a reduction in the size of the stomach using a gastric band (as Adjustable Gastric Banding, AGB), in others a part of the stomach is removed (Sleeve Gastrectomy, SG), while another choice involves the creation a small stomach pouch and resecting/re-routing it to the small intestine (Roux-en-Y Gastric Bypass, RYGB), which is the most durable treatment for obesity.

Given the significant changes to gut anatomy and physiology especially in RYGB, several groups have tried to characterize the changes that occur in the distal gut microbiota. In the previously mentioned study, Zhang et al. [51] first investigated these effects comparing 3 obese, 3 lean and 3 post gastric bypass individuals, and they found that RYGB alters the intestinal microbial community in a unique way. Unfortunately the 3 individuals studied was all composed of separate subjects without longitudinal pre- and post-operative assessment, and that makes a difficult interpretation. However, the results showed that surgery leads to an increase in proportion of Gammaproteobacteria (most are Enterobacteriacee) and Fusobacteriaceae, and a proportional decrease in Firmicutes (particularly in Clostridium bacteria) and in methanogens. The authors hypothesized that these results might reflect a double impact of the gut microbial alteration caused by surgical procedure (bypass of the upper small intestine relocates some of typical small intestine microbiota, such Enterobacteriaceae, to the large intestine, and it alters the intestinal micro environment favoring the fast-growing facultative anaerobes over such obligate anaerobes) and the consequent changes in food ingestion and digestion.

Another paper also focused on this topic, but incorporated a large number of patient, i.e. 30 obese subjects who had enrolled in a bariatric surgery program, and 13 lean controls, using a qPCR based analysis [62]. Their results showed the standard increase Firmicutes/Bacteroidetesratio in the obese patient before RYGB, and a subsequent decrease in this ratio at 3 and 6 months postoperatively, in keeping with patient weight loss. Throughout, the authors observed a significant relationships between the amount of F. prausnitzii, E. coli, and Bacteroides/Prevotella and metabolic and inflammatory parameters. The strongest associations were found for the amount of F. prausnitzii, which was negatively correlated with serum concentrations of inflammatory circulating markers (hs-CRP, IL-6,). Predominantly, leptin levels fell in inverse relation to rising levels of E. coli in gut microbiota after RYGB.

Patil et al. [55], in their study of the Indian population, took into consideration also a group of surgically treated patients, in particular, who underwent to SG or AGB, two restrictive bariatric surgery. Treated–obese individuals exhibited comparatively reduced Bacteroidesspp and Archaeal counts, along with reduced fecal SCFA, studied by chromatographic analysis of fecal samples.

Graessler et al. [63] characterized intra-individual changes of gut microbial composition before and 3 months after RYGB by metagenomic sequencing in morbidly obese patients (BMI>40 kg/m2) with T2D. The overall metagenomic RYGB-induced shift was characterized by a reduction of Firmicutes and Bacteroidetes and an increase of Proteobacteria. Twenty-two microbial species and 11 genera were significantly altered by RYGB: particularly they found increased numbers of Proteo bacterium Enterobacter cancerogenus and decreased Firmicutes Faecali bacterium prausnitzii and Coprococcus comes. All these was associated to a significant improvement of weight and of metabolic and inflammatory parameters. However the author assessed that these shifts could have long-term effects on host health with a potential risk of bowel inflammation and colorectal carcinomas, believing need for further prospective studies on this topic.

All these results are very interesting and suggest that this area needs further attention. Nevertheless, other studies have been made on experimental animals. Li et al. [64] examined rats gut microbiome after sham surgery or RYGB. Although different from the previous studies by virtue of its dependence on an animal model, they found the standard decrease in Firmicutes to Bacteroidetes ratio, even if the rats are non obese, so weight loss was from a normal baseline, but moreover a significant increase in phylum Proteo bacteria after RYGB. These results match the findings of Zhang et al. [51], but the proportional increase is greater in rats study.

Very recently Liou et al. [65] increasingly turned to a murine model of RYGB to demonstrate that changes in the gut microbiota after gastric bypass surgery are conserved among humans, rats, and mice, and demonstrated that the underlying cause of much of the microbial response to surgery is due to the reconfiguration of the gastro intestinal tract. Marked changes in gut microbial ecology were observed within 1 week after RYGB, with a pronounced increase in the abundance of the Verrucomicrobia (genus: Akkermansia) and Gamma proteo bacteria (order Enterobacteriales, Escherichia spp). These changes were similar to those observed in the fecal microbiota of human patients that had undergone RYGB. These adjustments were independent of weight change and caloric restriction, were detectable throughout the length of the gastro intestinal tract and were most evident in the distal gut. The most innovative aspect of this study is that it was transferred the microbiota of RYGB- operated mice to non-operated, germ-free mice. The transfer resulted in weight loss and decreased fat mass in the recipient animals, compared with recipients of microbiota induced by sham surgery. These findings

provide the first empirical support for the assert that changes in the gut microbiota contribute to reduced host weight and adiposity after RYGB surgery.

d- Gut microbiota of twins: In 2009 Turnbaugh et al. [6] characterized the gut microbial communities of 154 individuals, consisting of monozygotic or dizygotic twins, concordant for leanness or obesity, and their mother: the aim of the study was to assess the gut microbiota relationship to host genotype and weight. The study revealed that the composition of gut microbiota is more similar between family members than unrelated individuals, however each person's gut microbial communities varies in the specific bacteria lineages, with a similar degree of co-variation existed between monozygotic and dizygotic twin pairs. Obesity was associated with phylum-level changes in microbiota: 16s pyro sequencing analysis of fecal samples revealed a lower proportion of Bacteroidetes and an higher proportion of Actino bacteriain obese compared with lean individuals. Instead, no significant difference in proportion of Firmicutes was observed between obese and lean subjects [6]. In a more recent study the same authors indicated that a majority of species-level phylotypes are shared between deeply sampled MZ cotwins, despite large variations in the abundance of each phylotype. The genetic and transcriptional diversity of the human gut microbiome was remarkable [66].

In more recent years, studies of twins have increased: the interest was directed at the possibility of correlating the gut microbiota to BMI, identifying a possible dependence on the host genotype. Lee et al. [67] compared the composition of the fecal microbiotas of Koreans and US adult twins, to identify the influence that some environmental or genetic factors could have in gut microbial composition. The results did not show a statistically significant overall difference between the two population cohorts. However, diversity was significantly lower in US obese twins that in lean twins, and a similar trend, that did not reach the level of statistical significance, was noted in the smaller Korean sample. This study mostly revealed that interpersonal differences in fecal bacterial community structures were less within a family than between families, and these are comparable for adult MZ and DZ twin.

The previous studies, however, only included twins with concordant phenotypes in terms of BMI. More recently, Simões et al. [68] used qPCR and DGGE to characterize the stool microbiota of 20 Finnish monozygotic twin pairs: 9 twin pairs were concordant and 11 discordant for BMI, and the participants were considered both individually and as twin pairs. The aim of the study was to analyze whether there is a correlation between diet and number or diversity of the predominant bacterial groups in stools (especially Eubacterium rectal group, Clostridium leptumgroup and Bacteroides spp, but also Lactobacilli and Bifido bacteria). The research concluded that the number of bacteria within the different bacterial groups did not differ between BMI groups. Moreover, the study made a thorough investigation about the association of the nutritional intake with the number of different fecal bacteria: individuals with high energy intake had significantly lower numbers of Bacteroides spp ($p=0.007$) and slightly high number of Bifidobacteria ($p=0.02$) than did individuals with lower energy intake. The greater MUFA consumption was associated with lower bifido bacterial numbers ($p=0.0005$); soluble fiber intake had a positive association with the Bacteroides spp numbers ($p=0.009$).

To identify specifically microbiota signature for differences in BMI, Tims et al. [69] compared the microbiota composition in monozygotic twin pairs, that are concordant and discordant in BMI: the design of this study was aimed to define microbiota signatures that correlate directly with BMI differences independent of the host genotype. Fecal microbial diversity and composition was studied in detail using the Human Intestinal Tract chip (HITChip), a phylogenetic microarray that has been benchmarked against several classical 16s rRNA gene-based methodologies. Both monozygotic co-twins concordant and discordant for BMI showed a significantly higher similarity of their microbiota profile compared with random paired subjects ($p=0.001$). Furthermore they found an inverse correlation between Clostridium cluster IV diversity and BMI, and a positive correlation between Eubacterium ventriosum/Roseburia intestinalis and BMI. No consistent Bacteroidetes/Firmicutesratio were observed in pair-wise comparison of lower- and higher-BMI siblings. As other very recent studies, Tims et al. focused mainly on the analysis of the metabolic profile, identifying two distinct ecological networks, which reflected significant differences at the genus-level: lower BMI sibling group was associated with a more abundant network of primary fibers degraders, rich in Oscillosporaguillermondii, while a network of butyrate producers, including Eubacterium ventrosum and Roseburia intestinalis, was more prominent in subjects with higher BMI. The difference in microbial networks suggested a shift in fermentation patterns at the end of the colon, which could affect human energy homeostasis [69].

e- Gut microbiota in children: Childhood obesity is one of the most serious public health challenges of the 21st century. The prevalence has increased at an alarming rate: WHO estimated that in 2010 the number of overweight children under the age of five was over 42 million [1]. This obesity pandemic in children has directed our interest towards the study of the microbiota in lean and obese children.

In a prospective seven-year study to investigate the composition of the fecal microbiota of lean and obese children, Kalliomaki et al. [70] analyzed, by fluorescent in situ hybridization (FISH), the fecal microbiota composition of 25 obese/overweight and 24 normal-weight children, comparing the same composition at 12 years of age in relation to any changes in BMI. The Bifido bacterium numbers during infancy was higher in children remaining at normal weight than were children becoming overweight ($p=0.02$). The observation that bifido bacteria would be protective against children obesity opened the space in studies on use of bifido bacteria as anti-obesity probiotics. In contrast, lower levels of S. aureus was associated with normal weight development. Therefore they showed for the first time that difference in intestinal human microbiota might precede overweight development.

Similarly Luoto et al. [71] confirmed lower bifidobacterial numbers in the gut microbiota ($P = 0.087$) of children who had become obese at age 10, compared to when they were 3 months old. Also early diet seemed to have a role in obesity development. It was distinguished that mothers of children who were normal weight at age 10y had statistically significantly higher mean concentrations of adiponectin in maternal colostrums than mothers of overweight children. Adiponectin is a protein hormone secreted from adipose tissue and the placenta into the blood stream. It has an important role in glucose and fatty acid metabolism and it has a protective effect against metabolic syndrome [72]. In fact obese individuals have low levels of adiponectin.

Balamurugan et al. [73] noticed that consumption of energy, carbohydrates, fat and proteins was not significantly different between obese and non-obese Indian children. Therefore they examined the differences of dominant fecal microbiota in obese children compared with their normal peers, to identify if other component were at play. Quantitative PCR studies showed no significant differences in the levels of Bacteroides-Prevotellagroup, Eubacteriumrectale, Bifidobacterium group and Lactobacillus acidophilus group between obese and non obese Indian children. However obese subjects had significantly higher levels of Fecali bacterium prausnitzii, a representative of Firmicutes which can ferment unabsorbed carbohydrate: it was postulated that the presence of this bacterium in greater numbers of children could lead to increased energy salvage from unabsorbed carbohydrates that would not otherwise contribute to dietary energy intake.

More recently, research on the possible specific role of children gut microbiota at phylum level in development of obesity continued with an Egyptian study [74] which included both children and adults. This study compared the Bacteroidetes and Firmicutes frequencies in the stool of obese and normal weight individuals. The authors found that obesity in Egyptian children and adults was associated with increase in both phyla Firmicute and Bacteroidetes (p=0.03, p=0.05).

A comparison of the microbiota of lean and obese children was carried out by Payne et al. [75], together with the evaluation of the fecal metabolite concentration. Numerical variations in population numbers measure between obese and normal-weight children were not statistically significant for any population tested. Above all, in contrast to some adult studies, they not identified any correlation between Firmicutes/Bacteroides (major genus of Bacteroidetesphylum) ratio and childhood obesity. However the analysis of fecal metabolite concentration revealed significantly lower concentration of intermediate metabolites in obese children, suggesting an exhaustive substrate utilization by obese gut microbiota. They hypothesized that a dysbiosis could be involved in the etiology of childhood obesity, and that the increased Firmicutes/Bacteroidetes ratio observed in obese adults could be a results of this dysbiosis. The same authors assessed the impact of dietary energy on gut microbial communities and metabolism using a three-stage in vitro continuous fermentation model [76]. Two fecal sample, from an obese and a lean children, were inoculate with immobilized fecal microbiota; three different fermentation media were designed to examine the effects of prevalent Western diet on gut microbiota. Media composition reflected obese (high energy), normal weight (normal energy) and anorectic (low energy) child dietary intakes and were alternately supplied to each microbiota during separated fermentation periods. Gut microbial communities demonstrated differential metabolic and compositional adaptation to varied substrate availability. In fact, high energy medium was strongly butyrogenic, resulting in significant stimulation of butyrate-producing members of Clostridia cluster IV. Normal and low energy nutrient loads induced significantly less metabolic activity, with a significantly reduction in fermentable energy. These results suggest a significant metabolic adaptation in response to nutrient load [76], which would lead to microbiota alterations in adults.

Just at the level of the major phyla, Xu et al. [77] conducted a case-control study to see if changes in the intestinal microbiota could be the cause of obesity in Kazakh children. In this case, a negative correlation between Bacteroidetes and Bacteoridetes/Firmicutes ratio with BMI were observed.

Bervoets et al. [78] carry out a genera-level research by quantitative culturing, to identify the concentration of Bacteroidesfragilis, Bifidobacterium, Clostridium, Staphylococcus and Lactobacillus. According to a very recent Spanish study [79] which conducted a thorough comparative metagenomic investigation of gut microbial communities of an obese and an lean adolescent, they detected an elevated Firmicutes/Bacteroidetes ratio in the gut microbiota of obese children and adolescent. Bacteroides fragili group and Clostridium sp were borderline, but not significantly different between obese/overweight group and lean group (p=0.05 and p=0.074 respectively); instead fecal concentration of Lacto bacillus spp were found to be significantly higher in the first group compared to lean children and adolescents (p=0.035). The relevance of this study mainly concerns the association between energy intake and changes in the microbiota. Regardless of BMI status, children and adolescents with higher energy intake possessed high fecal concentrations of Staphylococcus spp, as previous reported by Kalliomaki [70], who showed that a greater fecal concentration of Staphylococcus spp during infancy predicted the development of overweight during childwood. On the contrary, Vael et al. [25] in a prospective study demonstrated that high intestinal Bacteroides fragilis and low Staphylococcus concentrations in infants between the age of 3 weeks and 1 year were associated with an higher risk of obesity later in life.

The age of adolescence has been investigated by Nadal et al. [80] that evaluated the effects of obesity treatment programs on the fecal microbiota composition. In agreement with other previous works [5,81], they found a significantly reduced levels of Clostridium hystoliticum, Eubacterium rectale and Clostridium coccoides (which are part of Firmicutesphylum) correlated to weight loss in obese adolescents. Moreover Santacruz et al. [81] found significantly lower Clostridium and Bifido bacterial levels in obese adolescents after an obesity treatment program.

f- Gut microbiota in pregnant women: Another class of people that has been evaluated on the possible differences in the gut microbiota is that pregnant women. First, Collado et al [82] characterized the gut microbiota in pregnant women according to their BMI, and shows significant differences according to weight, and to normal-weight or overweight before pregnancy. Whereas an overall increased number of bacteria was observed between the first and the third trimester in both group (these increase throughout pregnancy, normal weight and overweight, were significant for each weight group), significant differentiation were observed in microbial community composition of two women groups. Mainly higher numbers of Bacteroides group and Staphylococcus aureus were assessed, by FCM-FISH and qPCR, in overweight state compared to normal-weight women. Furthermore, the microbiota composition also varied in relation to the amount of weight gain during pregnancy. Bacteroides showed a positive correlation with weight and BMI before pregnancy (p=0.005, p=0.023) and with weight gain over pregnancy (p=0.014). More specifically, a one kilogram gain in weight correlated with a corresponding increase in Bacteroides number by 0.006 log units. Instead, Bifido bacterium number seemed to be higher in women who exhibited normal weight gain in pregnancy (p=0.03). Concentration of Clostridium group during the first trimester of pregnancy showed a correlation with BMI (p=0.067).

More recently, Santacruz et al. [83] investigated the fecal microbiota of 50 pregnant women who were assigned into one of two groups, overweight or normal weight, based on their BMI. As with the Collado study, higher numbers of Staphylococcus and lower numbers

of Bifido bacterium were distinguished in overweight women. However, in contrast with the previous study, Bacteroidesnumbers were found to be lower in overweight women. Increased numbers of Enterobacteriaceae (E. coli in particular), were also associated with overweight women. The gut microbiota of women who gained excessive weight during pregnancy underwent similar increases and decreases in microbial numbers as were associated with overweight women. Santacruz et al. also investigated the relationship between gut microbiota composition and biochemical parameters. They found that increased Staphylococcus numbers corresponded with increased serum levels of cholesterol, a rise in numbers of Enterobacteriaceae and E. coli was linked with increased levels of serum ferritin and decreased levels of transferrin, while greater numbers of Bifido bacterium correlated with reduced levels of ferritin, saturation transferring index and increased levels of transferrin and folic acid. Finally, increased Bacteroidesnumbers were associated with increased levels of High Density Lipoprotein (HDL)-cholesterol, folic acid and lower levels of triacylglycerol.

Mechanism linking the microbiota to obesity

All these study have not reached an univocal conclusion, and surely more information about gut microbial species will continue to be defined as technology improves. However, another arisen issue is how these microbial communities are impacting on weight gain, and via which mechanisms they act. A number of mechanisms have been proposed, which are not mutually exclusive.

Gut commensal bacteria avail to several evolutionary advantages: helping to convert ingested complex nutrients to Short Chain Fatty Acid (SCFAs); transforming mucins and dietary fibers into sample sugars for assorption; epithelial proliferation; nutrient metabolism; formation of an crucialdefense barrier in development of systemic and mucosal immune system, producing essential vitamins (such as Vitamin K); activating bio-inactive compounds [16].

Nevertheless the microbiota plays an important role in human adipose tissue, first of all causing an alteration in the energy balance, thanks to the ability to share otherwise indigestible components of the mammalian diet. Germ-free mice provide a complementary approach for characterizing the properties of human gut microbiota, thanks in vivo microbiota transplantation studies. First Backhed et al. [84] in 2004 hypothesized that the microbiota regulates energy storage through host signaling pathways, analyzing Germ-Free (GF) and conventionalized mice. They found that conventionalization of adult Germ-Free (GF) mice with a normal microbiota harvested from the distal intestine (cecum) of conventionally raised animals produces a 57% increase in total body fat content and 61% increase of epididymal fat pads weight, despite reduced food intake (27% less than GF mice). The presence of microbiota increased serum levels of glucose and SCFAs, which induced triglyceride production in the liver, increased adiposity and reduced glucose tolerance. Finally the authors revealed that the presence of the microbiota promoted an increased monosaccharide uptake from the gut and an increased ability to degrade the polysaccharides [84]. They also correlated the increased body fat to a proportional increase in leptin levels. Leptin is an adipocyte- derived hormone, which reduces food intake and increases energy expenditure in mice, thus the microbiota might play an important role in leptin levels (85,86). In a very recent study Lemas et al. have investigated the relations between HM hormones (leptin and insulin) and both the taxonomic and functional potentials of the infant microbiome [101]. Another significant aspect seemed to be the increase in the levels of Lipo-Protein Lipase (LPL) in epididymal fat pads of conventionalized mice. LPL is a key regulator of fatty acid release from triglyceride-rich lipoproteins in muscle, heart, and fat [87]. Increased adipocyte LPL activity leads to increased cellular uptake of fatty acids and adipocyte trigly ceride accumulation. Turnbaugh et al. [7] introduced the fecal contents of the ceca from obese and lean mice into the small intestine of the lean germ free mice, providing an equivalent food intakes. It was observed a greater increase in body fat in the mice that received microbes from ob/obobese donors than in those that received the intestinal contents of lean donors. The authors confirmed that the gut microbiome of ob/ob mice had an increased capacity to ferment polysaccharides compared with the lean associated equivalent: they concluded that the obese microbiome had an increased capacity to harvest energy from ingested food.

In a subsequent study, Backhed et al. [88] showed that the gut microbiota influenced an important gut-derived regulator of host lipid metabolism, known as Fiaf (Fasting-Induced Adipose Factor), a circulating lipoprotein lipase inhibitor whose expression is normally selectively suppressed in the gut epithelium by the microbiota. Germ-free mice had higher levels of Fiaf expression in intestine, but when a normal mice microbiota is administered to germ-free mice, Fiaf production is suppressed in the intestine and a greater proportion of triglycerides are deposited in adipose tissue. The relevance of Fiaf was demonstrated when it was established that germ-free mice lacking Fiaf gained significantly more weight and had significantly greater epididymal fat pads than their wild-type counterpart [87], with higher levels of LPL activity (67%). Germ-free mice were also found to have an increased skeletal muscle and liver AMP Activated Protein Kinase (AMPK), a key enzyme in energy cell status, and the downstream targets involved in fatty acid oxidation (acetyl CoA carboxylase; carnitine palmitoyl transferase). Therefore, germ-free animals are protected from diet-induced obesity by two complementary but independent mechanisms that result in increased fatty acid metabolism, i.e. elevated levels of Fiaf and increased AMPK activity.

SCFA and energy harvest: In the large intestine, gut microbes ferment starch (including resistant starch), unabsorbed sugars, cellulosic and non-cellulosic polysaccharides, and mucins into Short-Chain Fatty Acids (SCFAs) and gases such as CO2, CH4, and H2. The type and quantity of SCFA and gases produced in the gut depend on multiple factors, including diet, especially the availability of non-digested carbohydrates, the gut microbial community composition, gut transit time, and the segment of the colon. The major SCFAs produced as a result of carbohydrate and protein fermentation are acetate, propionate, and buty rate. These SCFA represent an additional source of energy, which was investigated in the previous referred study by Schwiertz et al. [52]. It was notedthat fecal samples of obese subjects had 20% higher mean total SCFA concentration than those from lean volunteers ($p=0.024$). The degree of SCFA increase is considerable, amounting to 41% for propionate ($p=0.024$), 28% for butyrate ($p=0.095$), and a moderate increase of valerate and acetate of 21% and 18% respectively. In 2007 Duncan et al. [89] measured the changes in fecal SCFA in response to changes in dietary intake of carbohydrates, and they found that total SCFA concentrations were lower during consumption of the high protein-low carbohydrate and high protein –moderate carbohydrate diets than during the maintenance period ($p<0.001$). While the concentrations of the predominant SCFAs, i.e., acetate, propionate and valerate, decreased due to the shift from the maintenance to the low carbohydrate diets (50%), butyrate levels decreased even more

dramatically (75%): the relationship between carbohydrate intake and butyrate concentration was linear (r = 0.76, P <0.001). More recently Patil et al. [55] confirmed in their study of Indian population, a significant over production of SCFAs in obese gut (about two fold higher production than lean and normal individuals), demonstrating increased saccharolytic fermentation. This evidence seemed to be associated to elevated archaeal density. On the contrary, the treated-obese subjects exhibited reduced archaeal counts along with reduced fecal SCFAs. In contrast, although Murphy et al. [43] did find that the fecal energy content of ob/ob mice was decreased and cecal SCFA concentrations increased at 7 weeks of age relative to lean controls, these patterns did not continue with time, and mostly fecal acetate levels decreased progressively over time. The Murphy et al. study also indicated that SCFAs concentrations were unrelated to changes in proportions of Firmicutes, Bacteroidetes or Actinobacteria. They concluded that the relationship between the microbial composition and energy harvesting capacity was more complex than previously considered.

Gut microbes and inflammation: Low grade metabolic inflammation is recognized as an important component of obesity and metabolic syndrome and several studies provides evidences that metabolic system are integrated with an increase in pro-inflammatory cytokines, such as TNF-α, typical of obesity-related inflammation and insulin-resistance. Lipo Poly Saccharide (LPS) endotoxin, an essential component of the cell walls of Gram-negative bacteria such as Bacteroidetes, gives acontribute to the increased development of adipose tissues, impacting inflammation grade and insulin resistance. Cani et al. [90] identified bacterial Lipo Poly Saccharide (LPS) as a triggering factor of high-fat diet induced metabolic diseases. More specifically, they showed that high fat feeding caused plasma LPS concentrations to remain high throughout the whole day compared with controls which showed diurnal variations in plasma LPS concentrations (metabolic endotoxemia). To causally link high fat diet increased LPS concentrations to metabolic disease, the authors reproduced LPS concentrations of high fat feeding by continuously infusing LPS or saline in mice for a month. It was then revealed that fasted glycemia, blood glucose, fasted insulinemia, liver triglyceride content and body weight levels were greater in mice infused with LPS than those infused with saline. Moreover, the amount of weight gain and visceral and subcutaneous adipose depots in LPS infused mice was similar to that observed in mice fed a high fat diet. Furthermore metabolic endotoxemia triggers the expression of inflammatory factors similarly to high-fat diet, such as TNF-α, IL-1, IL-6 and PAI-1. Afterwards, the same authors [91] confirmed that metabolic endotoxemia was due to changes in intestinal microbiota, because the antibiotic treatment, which dramatically reduced the local intestinal microbiota, restored normal plasma LPS values in high-fat diet–fed mice. This study also revealed that the high-fat diet significantly increased intestinal permeability by reducing expression of ZO-1 and occludin, i.e., tight junction proteins. Antibiotic treatment reversed this effect, suggesting that gut bacteria affected by antibiotic administration are involved in the control of intestinal permeability and thus the occurrence of metabolic endotoxemia. In accordance with these results, TLR4 (Toll-Like Receptors, which recognizes LPS) deficiency protects from obesity, from visceral and subcutaneous adipose tissue expansion, and glucose intolerance induced by an high fat diet as well as from endoplasmic reticulum stress in the main organs for glucose and lipid metabolism (skeletal muscle, liver and adipose tissue) [92].

Another type of inflammatory molecule that appears to be induced by LPS are Serum Amyloid A (SAA) proteins, suspected mediators of inflammation and atherosclerosis, and which exhibit increased levels in the serum of obese persons [93]. In an interesting study, Saita et al. havedemonstrated the correlation between an enhanced apoE-mediated immune regulation and reduced atherosclerosis in adaptive immunity against gut microbiota [102]. The mouse isoform SAA3 is the most abundant in adipose tissue [94]. Reigstad et al. [95] assessed that SAA3 levels in adipose tissue was significantly higher (9.9 fold) in conventionalized mice (i.e. in presence of gut microbes) than in germ-free mice. They identified epithelial cells and macrophages as cellular sources of SAA3 in the colon and found that colonic epithelial expression of SAA3 may be part of an NF-kB-dependent response to LPS from gut bacteria. The study confirmed that LPS, and potentially other products of the indigenous gut microbiota, might elevate cytokine expression in tissues and thus exacerbate chronic low-grade inflammation observed in obesity.

Gut microbes and enteroendocrine cells: Another way in which energy intake and expenditure are regulated is through endocrine signaling from intestine to brain. Enteroendocrine cells respond to nutrient intake by secreting incretin hormones such as Glucagon-Like Peptide 1 and 2 (GLP-1 and GLP-2). GLP-1 stimulate insulin release from pancreas, slows gastric empty, promotes satiety and weight loss, whereas GLP-2 enhances intestinal glucose transport and reduces intestinal permeability [96,97]. Gut microbiota can regulate entero-endocrine cells and influence the release of gut hormone [96]. As mentioned before, the obesity-associated intestinal microbiome produces more SCFAs from carbohydrate fermentation than lean controls [7]. Entero-endocrine cells express a receptor for SCFAs, GPR41 (a G-protein coupled 41), that can be recovered also in small intestinal, colonic and adipocyte epithelium, and it is necessary for the metabolic effect of these microbial metabolites. Mice lacking Grp41 had reduced levels of the gut hormone PYY, greater gut transit time, lower intestinal absorption of SCFAs from diet, and lower fat accumulation in fat pads: all that was related to a decreased energy harvest from the diet [98].

CONCLUSION AND FUTURE PERSPECTIVE

The debate regarding the implication of the Firmicutesto Bacteroidetes ratio with respect to obesity is still ongoing. This topic is very timely and the goal is to try to respond to what is a real pandemic of obesity. Moreover, from this knowledge, the field is expanded on the possibility of therapeutic manipulation of the intestinal microbiota to prevent or treat obesity and its effects.

Especially in recent years, several studies have been conducted about the composition of the intestinal microbiota in obese compared with lean subjects, and each has linked obesity with species- or genus-specific composition profile. The extreme variability of the results can be attributed to the different designs of the studies or the interpretation of results. We must also consider the specific circumstances of each populations or sub-populations. Mainly, the heterogeneity of methods used to quantify the abundance of the intestinal microbiota makes it difficult to compare the results, as everyone are biased by accuracy, sensitivity or specificity of methods. Furthermore, little effort has been made to standardize the microbiota analysis methodology, making it difficult to compare results between different groups and extend general knowledge. Finally, in recent years the attention was turned to understand not as much the variations at the major phyla-level, but if the obesity correlates with particular metabolic patterns, of which the integrated networks of bacteria are responsible, and especially if differences in the composition of microbiota are mainly related to

diet composition. Further investigations, combined with the ever increasing capacity of next generation sequencing technologies, and the standardization of methods, should be conducted concerning the physiological distribution of intestinal microbes, the interaction with the host, the possible effects on variables such as diet, age, gender or activity, the mechanisms that justify these changes, and the possibility to make alterations using probiotics, prebiotics, antibiotics or other therapeutic interventions. The topic is just beginning.

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