Papel de la microbiota intestinal en la obesidad

Autor: Dña. Elena Blanco Martín

Director/es: Dña. Asunción Seoane Seoane
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ABSTRACT

Obesity is considered the epidemic of today, which limits the quality of life and predisposes to suffer numerous health problems. There is an increasingly evidence of the close relationship between the microbiota and the risk of developing obesity, although the exact mechanism remains unclear as many studies give contradictory results probably due to the multiple conditions that in turn influence the development of obesity such as genetic, sociodemographic and environmental factors.

In this review, reference is made to the importance of the molecular cross-talk of the microbiota with the host, linking it with the development of obesity and metabolic inflammation, as well as the evidence of microbiota plasticity according to diets. Special mention is also made of the central role that the commensal bacterium *Akkermansia muciniphila* has in improving the metabolic profile of the host. Finally, it is pointed out the growing importance of gut microbiota’s manipulation with therapeutic purpose against obesity and related pathologies through interventions like fecal matter transplants, prebiotics or probiotics.

Keywords: gut microbiota, obesity, metabolic syndrome, SCFAs, microbiota-host crosstalk.

La obesidad es considerada una de las epidemias más importantes de hoy en día, que limita la calidad de vida y predispone a padecer numerosos problemas de salud. Cada vez hay más pruebas de la estrecha relación entre la microbiota y el riesgo de desarrollar obesidad, aunque el mecanismo exacto no está claro ya que son muchos los estudios que ofrecen resultados contradictorios probablemente debido a las múltiples condiciones que a su vez influyen en el desarrollo de la obesidad como la genética o los factores sociodemográficos y ambientales.

En esta revisión, se hace referencia a la importancia de la comunicación molecular de la microbiota con el huésped, relacionándola con el desarrollo de la obesidad y la inflamación metabólica así como la evidencia de la plasticidad de la microbiota de acuerdo a las dietas. También se hace mención especial del papel central que desempeña la bacteria comensal *Akkermansia muciniphila* en la mejora del perfil metabólico del huésped. Finalmente, se señala la importancia creciente que ha cobrado la manipulación de la microbiota intestinal con fines terapéuticos contra la obesidad y las patologías relacionadas, a través de intervenciones como trasplantes de materia fecal, prebióticos o probióticos.

Palabras clave: microbiota intestinal, obesidad, síndrome metabólico, AGCC, comunicación microbiota-huésped.
1. INTRODUCTION

Nowadays, obesity is an epidemic that deeply worries around the world and although its etiology is multifactorial (environmental, dietary, lifestyle, genetic and pathological factors), in the recent years it has been giving great importance to the role of the intestinal microbiota in the development of overweight. Short chain fatty acid (SCFA) production, hormones’ stimulation, chronic low-grade inflammation or bile acid metabolism are some of the mechanisms suggested to link the intestinal microbiota with obesity; however, further studies should be done in mice and humans to clarify the cause relationship between microbiota and obesity because there are still many controversies. Moreover, metagenomic studies are essential in order to elucidate concrete functions or mechanisms through which microbiota relates to host metabolic status.

Differences between lean and obese individuals microbiota have led to propose numerous mechanisms that could contribute to host adiposity; as well as experimental activities to check if phenotype is transmissible by fecal matter transplantation (FMT). Anyway, we should not forget that researches have been done mainly in animals with what this means when extending it to humans.

Changes in diet involve changes in microbiota composition and in this way it is thought to be crucial in prevention of diseases related with obesity. One of the links between nutrition, gut microbiota and pathology are thought to be microbial derived metabolites like SCFA, which may protect body against poor metabolic control and inflammatory status associated with Western lifestyles (Morrison and Preston 2016). Researchers are exploring possible therapeutic gut microbiota manipulations not only for obese people but for other pathological diseases like diabetes or metabolic syndrome, with the final aim of finding an effective one that achieves significant changes in the development of the status. Thus, not only microbiota manipulation through diet, but also supplementation with prebiotics or probiotics have been proposed as a treatment for obesity, as well as novel therapeutic approaches like FMT, membrane protein from Akkermansia muciniphila or exogenous peptide tyrosine tyrosine (PYY).

2. DISCOVERING THE HUMAN MICROBIOME

In the 17th century, A. van Leeuwenhoek created powerful lenses with which he observed bacteria from the plaque between his teeth for first time. He called it “animalcules”. Later, in the 20th, Elie Metchnikoff explained that humans are born sterile and later are populated by diverse microbes which increase in quantity along the gastrointestinal (GI) tract (Tropini et al. 2017). For long time, it has been difficult to culture microbiota, since most of bacteria are obligate anaerobes; but technological advances and reduction of sequencing costs have given us a greater knowledge of microbiota composition. In fact, recent development of genetic analysis carried out by culture-independent methods based on 16S rRNA sequencing and metagenomics analysis have begun to catalogue microorganisms in a particular ecosystem (microbiota) and evaluate its genomes (microbiome)(Blum 2017). Through the 16S rRNA gene analysis, evolutionary relationships between organisms can be determined by comparison of their rRNA gene sequences (Namsolleck et al. 2004). However, it is still a challenge to understand the role of each individual member of an ecosystem.

With the aim of revealing microbe’s interactions, the human microbiome project (HMP) and the “Metagenomics of the Human Intestinal tract” (Meta-Hit) Consortium coordinating efforts to carry
out deep sequencing of all microbes (eukaryotes, archaea, bacteria and viruses) that inhabit specific body sites (such as the mouth, throat, airways, stomach, intestine, urogenital system and skin) (Fig.1A). This characterization could have diagnostic, therapeutic and preventive implications, once the composition of the microbiome is categorized according to different diseases, such as in inflammatory bowel disease (IBD), type 2 diabetes (T2D) or necrotizing enterocolitis (Fig. 1B) (Rosenbaum et al. 2015). However, only an average of 10-50% of cultures is successful due to the complexity of culturing the microbiota from the anaerobic environment (Patterson et al. 2016).

As stated above, culture-independence sequencing technologies of microbiota have generated lots of data, but without success in providing information about the function of each population. In consequence, the use of germfree animals has been a key point to improve our understanding about gut microbiota and its interaction with host metabolism and immunity. For example, it is evidenced that in germfree mice there are defects in immune system development with defects in gut lymphoid tissue and cell-turnover rates along with smaller Peyer’s patches and mesenteric lymph nodes (Patterson et al. 2016).

Nevertheless, metagenomics, proteomics and metabolomics approaches (or systematic study of chemical fingerprints that specific cellular processes leave behind) attempt to elaborate a crosstalk map among gut microbes, and between them and the host. These techniques are applied on stool, which allow repeating samples without invasive procedures but with the inability to capture variation in localization and function along the gastrointestinal (GI) tract (Tropini et al. 2017). Nowadays, metabolomics are used to identify biomarkers that could indicate presence of a disease or response to drug intervention, to determine biochemical stresses and to characterize microbial metabolism and human health of disease. Indeed, it has been applied to studies of gut microbiota.

Fig 1. (A) Different microbiomes in humans. (B) Intestinal microbiome in healthy individuals and patients (Blum 2017).
focused on the exploration of disease-related metabolites in order to obtain detailed information on gut metabolic pathways. It was concluded that the microbiota is involved in several biochemical functions associated to physiological or pathological conditions (Table 1) (Vernocchi et al. 2016).

Table 1. Role of gut microbiota metabolites on health and disease.

<table>
<thead>
<tr>
<th>BENEFICIAL MICROBIAL ACTIVITIES</th>
<th>BENEFITS</th>
<th>HARMFUL MICROBIAL ACTIVITIES</th>
<th>DRAWBACKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCFAs and vitamin production, recovery of energy</td>
<td>Nutrients and energy providing</td>
<td>Lipopolysaccharide supply, inflammation</td>
<td>Obesity and metabolic syndrome</td>
</tr>
<tr>
<td>Butyrate production, fermentation of non-digestible fibers</td>
<td>Cancer prevention</td>
<td>Toxins production, inflammation</td>
<td>Cancer promotion</td>
</tr>
<tr>
<td>Antimicrobials production (e.g. bacteriocins, H2O2, acids, etc), intestinal pH regulation, competition of ecological niche</td>
<td>Inhibition of pathogens</td>
<td>Tissue invasion, inflammation, disruption of the gut barrier/homeostasis</td>
<td>Infectious diseases, leaky gut</td>
</tr>
<tr>
<td>Anti-inflammatory vs. pro-inflammatory signals development</td>
<td>Normal GI immune function</td>
<td>Pro-inflammatory vs. anti-inflammatory signals development</td>
<td>IBD, immune disorders</td>
</tr>
<tr>
<td>Non-digestible carbohydrates metabolism</td>
<td>Normal gut motility</td>
<td>Metabolism imbalance</td>
<td>Irritable bowel syndrome, metabolic disease aggravation</td>
</tr>
<tr>
<td>Propionate production</td>
<td>Gluconeogenesis, cholesterol synthesis, inhibition</td>
<td>Acetate production</td>
<td>Cholesterol synthesis, cardiovascular diseases</td>
</tr>
</tbody>
</table>

Mainly there are two ways of designing experimental studies to find strategies that will improve human health by gut microbiota modulation:

- The first one, is based on the collection and comparison of data obtained by multi-omic analysis between healthy and non-healthy people (with metabolic disorders), indicating genes, pathways or molecules as possible targets for therapeutic intervention. These multi-omic approaches are used to increase the understanding of how the microbiota may affect human metabolism. In a second step, in vitro or animal models are used to clarify the underlying mechanisms that lead to each status as well as possible therapies for the modulation of gut microbiota. This is how bases for human intervention trials are established (Sonnenburg and Bäckhed, 2016).
For instance, to generate a testable hypothesis about how specific microbes can affect in a disease state, multi-omic approaches and specific evaluation of microbiota should be made. Then, the created hypothesis first need to be validated in animals, followed by double blind placebo-controlled interventions in humans (Fig.2) (Duranti et al. 2017).

The second way is to begin with human studies as a starting point to identify strategies that modulate the intestinal microbiota from components of the diet, since this intervention is considered “safe”. Subsequently, data processing algorithms (like machine learning) can be used to identify aspects of the clinical profile of individuals, including data about microbiota. After validating these predictive elements in independent cohorts, they can be used to improve human health or to guide mechanistic studies in experimental models (Fig. 3) (Sonnenburg and Bäckhed, 2016).

However, the microscope is still an important tool to determine which bacteria are where, or even how global spatial organization changes in health and disease. Early efforts to visualize the gut microbiota within the colon were based on electron microscopy. Optical microscopy can be used to visualize the spatial organization of the gut microbiota and confocal microscopy is necessary to distinguish individual cells. Interestingly, microscope shows how local environment varies in health and disease, not only by staining intestinal tissues with hematoxylin and eosin but also with specific antibodies or fluorescent probes which permit analysis of specific species or structures such as biofilms (Tropini et al. 2017). Besides, flow cytometry and fluorescence in situ hybridization (FISH) can be used for enumeration of fluorescently labelled bacteria cells. In fact, FISH is a very useful method to enumerate bacteria in complex habitat like human gut and it does not require cultivation of target organisms (Namsolleck et al. 2004).
On the other hand, the Insertion Sequencing (INSeq) method allows to detect the genetic factors that led members of the gut microbiota to flourish within this niche. This approach seeks to find out insertion site and relative abundance of large numbers of transposon mutants in a mixed population of isogenic mutants of a sequenced microbial specie. INSeq can provide gene-level characterization of species which can establish gut robustness or perturbation through diet, disease and clinical treatment. For example, using this approach, a strain-specific and diet-specific determinant (arabinoxylan utilization locus) was discovered in Bacteroides cellulosilyticus WH2 that is decisive for the organism’s fitness during high fat (HF)/simple–sugar feeding (Patterson et al. 2016).

It is important to highlight the role of molecular and cell biology in clinical practice and not only in biomedical research. On the one hand we could make reference to the International “Haplotype Map” Project (HapMap), which produced a genome-wide database of human genetic variation for use in genetic association studies of common diseases. This project was launched in 2002 as a catalog of common genetic variants or single nucleotide polymorphisms (SNPs) that are often inherited together in segments of DNA called haplotypes; and on the other hand the Genome-wide association study (GWAS) can be used as an analysis of genetic variation along the human genome with the aim of linking it with an observable feature. In this way it has been possible to associate SNPs with phenotypic characteristics (like hair or eye color, body mass index (BMI), etc), specific human diseases or different individuals’ response to drug treatment (e.g. to lithium). As we can imagine, this can mean an advance in the diagnosis, treatment and prevention of human diseases, even in the assessment of individual risk to develop a disease. However, it needs a careful evaluation before entering in clinical practice (Blum 2017).

3. THE HEALTHY MICROBIOME

To identify the configuration of microbiome in disease, first is necessary to recognize healthy one. For this we rely on two hypotheses: The first one is based on a “core” of microbes present in healthy individuals; in consequence, its absence would point for dysbiosis. The second one is focused in a “functional core”, which sustain that functions are performed by microbiome within a particular habitat but not necessarily by the same organisms across people (Lloyd-Price et al. 2016). In contrast to what it can be thought due to the high taxonomic variability, functional variability is not so elevated, which suggests that despite a different taxonomic composition between individuals, the metabolic pathways and the functional result are quite constant (Rosenbaum et al. 2015). In this way, the “functional core” includes:

- House-keeping functions required for individual microbial life, like transcription, translation or energy production.
- Specific functions to microbes’ niches such as adhesion to host cell surfaces or creation of compounds concerned with host-microbe interaction.
- Own specialized functions: For example in the gut, the production of short-chain fatty acids (SCFAs), vitamins or essential amino acids, etc.

Furthermore, an important characteristic of a healthy microbiome is the degree of resilience to internal or external changes, which refers to the stress resistance and its ability to recover to a healthy functional profile. So, this consists on a dynamic equilibrium. (Fig.4) (Lloyd-Price et al. 2016).
Healthy gut microbiome has also been associated with high diversity of microbes which leads to temporal stability thanks to functional redundancy, even if the functional potential could be achieved with fewer taxa. For instance, a lack of diversity is observed in obesity, inflammatory bowel disease (IBD), type 1 or type 2 diabetes (T1D or T2D) (Lloyd-Price et al. 2016). We can also find a frequent drastic reduction after antibiotic treatment, with a highly variable recovery dynamics. In healthy individuals after recovery period, relative microbe’s abundance mostly resemble the pretreatment state. This would point about anatomical sites where reservoirs of bacterial cells are located and can proliferate again in the lumen, like for example crypts in colon and appendix (Donaldson et al. 2015).

4. THE INTESTINAL MICROBIAL COMMUNITY

First of all, microbiota means the microbial community of commensal, symbiotic and pathogenic microorganisms (Kim et al. 2016). It is estimated that the human gut microbiota, which has a symbiotic relationship with human host, consists of up to 100 trillion microbes \(10^{14}\) representing a total bacterial mass of 1-2kg and bacteria outnumber human by a ratio of 10:1. However, recent estimations suggest that the number of bacteria in the reference man is \(3.9 \times 10^{13}\) microbes and this slightly exceeds the number of human cells which was estimated around \(3.7 \times 10^{13}\) cells (Postler and Ghosh, 2017). Therefore, these numbers need to be revisited. Interestingly, metagenomics sequencing of fecal samples has identified more genes than human genome, in fact gut microbiome is containing >150 times more genes than human genome (Patterson et al. 2016). Moreover, each person has at least 160 different species, which contrast with the restricted diversity at the phylum level, mainly dominated by two phyla, the Firmicutes (65%, no true outer membrane, mostly Gram-positive) and the Bacteroidetes (25%, outer membrane, Gram-negative). Proteobacteria (5%) and Actinobacteria (3%) are less important (Postler and Ghosh, 2017).

The amount of bacteria differs in every location of the GI tract with a rostro-caudal increasing gradient, as the consequence of chemical and physical factors alongside with the transit time (Fig.5) (Fetissov, 2016).
In the gut, the growth of microbial community is influenced not only by bacteria-intrinsic regulation but diet too, and limited by chemical (like digestive juices that cause bacterial lysis and prevent mucosal adhesions) and physical factors (like intestinal peristalsis that cause elimination of bacteria by feces) of the host. These negative and positive influences determine the relative stability of microbiota at each part of GI tract (Fig. 6) (Fetissov 2016).

5. BIOGEOGRAPHY OF GUT MICROBIOTA

Microbial variations along the length and cross-section of GI tract depend on chemical gradients, oxygen levels, nutrient availability and immune effectors (Fig.7) (Tropini et al. 2017). For example, the small intestine is more acidic and with high levels of oxygen and antimicrobials than the colon, for that reason, fast-growing facultative anaerobes that tolerate this environment will compete with host bacteria or other bacteria for the simple carbohydrates in this region. Another case is the bactericidal properties of bile acids to certain species, which can shape the composition of small intestine mainly. Indeed, it was evidenced a growth of *Firmicutes* (while *Bacteroidetes* decreased) in
mice fed with excess of bile acids (Donaldson et al. 2015). Besides, the shorter transit time in the small intestine compared with the colon makes bacterial adherence an important factor for persistent colonization of the small intestine (Donaldson et al. 2015).

Additionally, some host factors lead to cross-sectional microbial variation of the gut. The colon wall folds over itself and creates inter-fold regions that are different from the central lumen (Fig.7). In mouse studies, laser capture microdissection was used to indicate specific microbial communities. Specifically, *Firmicutes* families *Lachnospiraceae* and *Ruminococcaceae* were enriched in the inter-fold regions, whereas *Bacteroidetes* families, *Prevotellaceae*, *Bacteroidaceae* and *Rikenellaceae* were prevalent in the luminal compartment. Both sites contain lots of mucus as a nutrient source of some bacteria (Donaldson et al. 2015).

As it was stated in the previous section, the adult intestinal microbiota is mainly enriched in bacteria dominated by two phyla: *Bacteroidetes* and *Firmicutes*. But other microbial domains have been identified as well, like archaea genera (*Methanobrevibacter smithii*, which optimize digestion of dietary polysaccharides), viruses (each person has a unique virome consisting primarily of

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**Fig.7.** Microbial habitats in the human lower gastrointestinal tract. The dominant bacterial phyla in the gut are *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia*. The dominant bacterial families of the small intestine and colon reflect physiological differences along the length of the gut. For instance, a gradient of oxygen, antimicrobial peptides (like bile acids) and pH limits the bacterial density in the small intestinal community, whereas the colon carries high bacterial loads. In the small intestine, the families *Lactobacillaceae* and *Enterobacteriaceae* dominate, whereas the colon is characterized by the presence of species from the families *Bacteroidaceae*, *Prevotellaceae*, *Rikenellaceae*, *Lachnospiraceae* and *Ruminococcaceae* (colors correspond with the relevant phyla). A cross-section of the colon shows the digesta, which is dominated by *Bacteroidaceae*, *Prevotellaceae* and *Rikenellaceae*, and the inter-fold regions of the lumen, which are dominated by *Lachnospiraceae* and *Ruminococcaceae*. Cfu (colony-forming units) (Donaldson et al. 2015)
bacteriophages, which serve as means of horizontal gene transfer among distant related bacteria) or fungi (that typically are pathogens, but they can be present also in healthy populations like *Candida, Malassezia* or *Saccharomyces*) (Lloyd-Price et al. 2016).

Actually, direct mutualistic relationships between humans and fungi have been found and these trans-kingdom interactions are responsible for an immune and ecological balance of the healthy microbiome for example *Lactobacillus* control of fungi in the gut. Also, some examples exist about mutualistic relationships between humans and fungi like the probiotic yeast *Saccharomyces boulardii* (Lloyd-Price et al. 2016). This heterogeneous ecosystem indicates that co-evolution of the host with its gut microbial symbionts (either commensals or mutualists) has generated powerful selective mechanisms (Donaldson et al. 2015).

6. GLOBAL VARIATION OF HUMAN GUT METAGENOMES

Gut metagenome is all the genes in the community of gut microorganisms (Boulangé et al. 2016). A study to compare metagenomics of human gut was carried out by M. Arumugam et al., in which a total of 39 samples were analyzed. The majority of these samples belonged to bacteria and they identified 30 most abundant genera and the respective phylum level (Fig. 7). They conclude that *Firmicutes* and *Bacteroidetes* were the dominant phyla and *Bacteroides* were the most abundant, but also the most variable genus (Arumugam et al. 2011).

As it is showed in the box plot (Fig. 8), there is a “long tail effect” corresponding to species in low abundance while there are just a little of predominant species. This ecosystem distribution is the result of the homeostasis by selective pressure from host and microbial competitors. This outcome points to “survival strategies” by which the low abundance microbes share abundant functions (Arumugam et al. 2011).

![Fig. 8. Genus abundance variation box plot for the 30 most abundant genera as determined by read abundance. Genera are colored by the respective phylum (see inset for color key). Inset shows phylum abundance box plot. Genus and phylum level abundances were measured using reference-genome-based mapping with 85% and 65% sequence similarity cutoffs. Unclassified genera under a higher rank are marked by asterisks (Arumugam et al. 2011).](image-url)
During the study, they were able to link the most abundant molecular functions with the most dominant species; but also low abundance genera like *Escherichia* contribute in over 90% of pilus assembly and assists in plasmids’ transfer for protective functions (like antibiotic resistance) (Arumugam et al. 2011). In conclusion, abundance genera cannot reveal the whole functional complexity of gut microbiota.

They also pointed the main contributors for each of the 3 enterotypes: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3), and the co-occurrence networks between these three enterotypes from the Sanger metagenomes (Fig.9). Definitely, there is evidence that enterotypes are not as sharply delimited as blood groups, but they are likely to characterize individuals (Arumugam et al. 2011).

It is significant to note that Arumugam et al. study showed no significant correlation between host properties (like nationality, sex, age or BMI) and enterotypes (exc. Enteroptype 1 which is enriched in Japanese individuals), and what’s more how enterotypes do not seem to differ in functional richness. They only found a negative correlation between age and abundance of *Clostridium* genus, but did not find any correlation between BMI and *Firmicutes:Bacteroidetes* ratio (in contrast with the typical discussion of the relationship between obesity and this ratio) (Arumugam et al. 2011). However, although popular press has focused on the idea of discrete enterotypes, individual’s enterotype can be highly variable with an extraordinary within -and between- diversity. In fact, there are plenty of evidences that support continuous gradients of prevalent taxa rather than discrete enterotypes (Knights et al. 2014).

So, the assumption that individual’s enterotype is relatively fixed over time is not true at all; actually it was found that an individual’s enterotype crosses from one putative presumed enterotype to another (Fig.10A) as it was demonstrated by time series of 1 year’s daily gut microbiota samples from a single individual (Knights et al. 2014). Indeed, it was observed that microbiome of the individual time series occupies nearly every area in the ternary plot (Fig.10B)
which demonstrates that enterotypes are fluid and continuous (Knights et al. 2014). That is why, summarizing microbiota variation into separate clusters would mean they are relatively stable over years, and for the moment, it is not true for healthy subjects (Duranti et al. 2017).

Thus, while members of the gut microbiota can be stable for long time, the community structure and the relative quantity of each member is highly dynamic (Patterson et al. 2016) suggesting the existence of microbial community gradients rather than distinct enterotypes. Hence, the possibility of a microbiome-based classification of human individuals is still subject to debate.

7. ESTABLISHMENT OF GUT MICROBIOTA IN DIFFERENT STAGES OF LIFE

Traditionally, environment in utero has been considered sterile, but DNA based analyses have identified low levels of bacterial species in maternal placenta, amniotic fluid, umbilical cord blood and meconium (Blum 2017, Nogacka et al. 2017). So it has been proposed that an initial colonization process occurs during gestation (Duranti et al. 2017).

After birth there is a fast expansion and successively changing composition of microbiota that becomes relatively stable in adulthood (Blum 2017). The neonatal intestinal tract is rapidly and densely colonized by bacteria from the mother and surrounding environment following the birth (Patterson et al. 2016). Although neonate’s intestinal microbiota has a relative prevalence of Proteobacteria and Actinobacteria (Duranti et al. 2017), its composition depends on several factors (Patterson et al. 2016, Nogacka et al. 2017) such as the delivery method (Natural, which has a larger population of Bacteroides and Bifidobacterium species, versus caesarean section, which are initially colonized with skin bacteria from the mother), the way the baby is fed (breast-fed versus formula-fed babies), the gestational age and an early life antibiotic exposure. Indeed, maternal exposure to antibiotics during pregnancy is associated with decreased bacterial diversity on the first stool of the neonate and reduced abundance of lactobacilli and bifidobacteria in the infant gut; it also affects...
the vaginal microbiota of the mother which could block the later transfer of microbes to the baby during delivery (Nogacka et al. 2017, Rosenbaum et al. 2015).

Moreover, it is suggested that maternal microbiota during gestation also configure the future immune system of the children; and thus, prenatal antibiotics exposition (just with penicillin or chloramphenicol) may be associated with asthma in childhood. In fact, some data advise that the gut resistome (collection of all genes from the gut microbiome that potentially encode for resistance to antibiotics) begins to develop in the utero with the transmission of antibiotic resistance genes from mother to infant. It has also been reported in several studies that early antibiotics exposure could link with allergic disease in later life (“hygiene hypothesis”) and with an increase in body fat and weight gain. Furthermore, it is a pending task to demonstrate the impact of early life antibiotic administration in antimicrobial resistance by gut microbiota. It is known from in vitro studies that exposition of microbial communities to constant antibiotics, they acquire multidrug resistance (Nogacka et al. 2017).

Afterward, gut microbiota continues to develop throughout childhood and adolescence and becomes more stable. It is generally assumed that a 3 years old child’s microbiota closely resembles that of an adult (Patterson et al. 2016). Thus, it stays relatively stable throughout adult life until old age when its composition changes again. Nevertheless, it is crucial to warn that gut microbiota can be altered by changes such as infection, antibiotic treatment, pregnancy or long-term change of lifestyle (Kim et al. 2016).

With regard to obesity in offspring, maternal obesity could be a predictor for child overweight. As it has been demonstrated, child from obese mother had different levels of Faecalibacterium spp., Oscillabacter spp., Blautia spp. and Eubacterium spp. compared to child born from a lean mother. Also, high level of Lactobacillus spp. and low level of Bacteroides spp. during the first 3 months of life may cause child obesity. Concerning human breast milk, it is crucial from a nutritional point of view but also for vertical transmission of bacteria. Breast milk of obese mothers has a reduced microbiota diversity compared to lean mothers who showed higher levels of Bifidobacterium spp. and lower Staphylococcus. In fact, the increased presence of Bifidobacterium spp. in early stages of life may provide protection against overweight (Duranti et al. 2017).

8. SOME ROLES OF GUT MICROBIOTA

Gut microbiota has some important functions like a protective role from harmful bacteria or a direct competition for limited nutrients which gives benefits for the host. It also helps the host immune system to recognize foreign elements and convert otherwise indigestible food into absorbable nutrients and energy for the host (Fig.11) (Kim et al. 2016).

Traditionally, microbiome has been described to has a commensal relationship with the host (nor damaging or helpful) but truly it is often highly mutualistic. For instance, bacteria helps the host in digesting complex food (by converting complex carbohydrates into absorbable substrates) and synthesize some essential vitamins (like B and K) while the host provides the bacteria food and protection. It is interesting to note that, more or less, 10% of metabolites found in mammalian blood are derived from gut microbiota. In a very real sense, humans and their microbiome thus form a composite organism, a so-called holobiont. In the same way, microbiota is able to modulate host energy balance and stores, as will be described later, what could lead to recognize it as an enteroendocrine organ (Postler and Ghosh, 2017).
The important responsibility of microbiota in the protection of host against pathogens can be verified after antibiotic treatment which leads to overgrowth of *Clostridium difficile* and also in germ-free mice which have more risk of infection than conventionally raised mice suggesting that microbiota might play an important role in the protection of the host against pathogens. Here are some ways to clarify the host protection by indigenous microbiota (Kim et al. 2016):

- Mutualistic bacteria can inhibit colonization of pathogens by competing for the same nutrients: *E. coli* commensal strain is in competition with enterohaemorrhagic *E. coli (EHEC)*.

- Some bacterial products can regulate the virulence gene of pathogenic microorganisms: *Bacteroides thetaiotaomicron* encodes fucosidases, which generates fucose from host mucin and subsequently, contributes to the expression of EHEC virulence genes, inducing its colonization.

- Commensal bacteria prevent the attachment of pathogens to the surface of intestinal epithelium avoiding infection at initial stage; in relation to this, it was proved how germ free mice have thinner mucus layer leading to less production of antimicrobial molecules than conventionally raised mice.

- Epithelial barrier function can be improved thanks to some metabolites produced by the microbiota: bifidobacteria produce acetate which inhibits translocation of EHEC Shiga toxin from gut lumen to the blood.

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*Fig. 11. Multiple roles of the microbiota in the gut. Symbiotic microbiota inhibit the colonization of pathogens by competing for the same nutrients, regulating virulent gene through by-product and inducing of production of mucus and antimicrobial peptides from epithelial cells. SCFAs produced by gut microbiota are used as an energy source in the epithelial cells and participate in cholesterol homeostasis in the peripheral tissues and in glycogenesis in the liver. In addition, gut microbiota liberate nutrients from otherwise indigestible food (Kim et al. 2016).*
Gut microbiota is able to produce or modify metabolites that work as signaling molecules for an “intelligent communication system” in the body. “Pharmabiotics” (bioactive metabolites derived from bacteria), contribute to the correct function of epithelial barrier and immune cells. However, many identified metabolites have not been functionally characterized yet (Postler and Ghosh, 2017). These microbiota-associated metabolites can be classified in those produced by the host but modified by gut bacteria, those that are produced by bacteria from dietary components and metabolites that are synthesized de novo by gut microbes.

Some of the metabolites secreted by the host but modified by gut bacteria are secondary bile acids and taurine (Table 2). Intestinal microbes are able to convert the primary bile acids secreted by the host’s liver such as cholic acid and chenodeoxycholic acid to secondary ones such as deoxycholic and lithocholic acid mostly in the colon, by a multi-step process that involves deconjugation and dehydroxylation. Free taurine is released in to the intestinal lumen during the deconjugation process, promoting the integrity of the mucosal epithelium by increasing autocrine production of IL-18. On the other hand, primary and secondary bile acids are signaling molecules for immune system that inhibit the secretion of pro-inflammatory cytokines by macrophages, Kupffer and dendritic cells (Postler and Ghosh, 2017).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Molecular mechanism(s) of action</th>
<th>Origin</th>
<th>Effects on immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Secondary bile acids</strong></td>
<td>Extracellular: activation of GPBAR1 Intracellular: activation of BAR</td>
<td>Derived from host-produced primary bile acids by intestinal microbiota</td>
<td>Inhibit NF-κβ dependent transcription of pro-inflammatory genes in monocytes, macrophages, dendritic cells. Inhibit production of pro-inflammatory cytokine osteopontin by NKT cells</td>
</tr>
<tr>
<td><strong>Taurine</strong></td>
<td>Enhancement of NLRP6 inflammasome activation</td>
<td>Derived from host-produced primary bile salts by intestinal microbiota</td>
<td>Enhances epithelial barrier function and maintenance by promoting epithelial production of IL-18</td>
</tr>
</tbody>
</table>

(Postler and Ghosh, 2017).
The metabolites that are produced by bacteria directly from dietary components are the indole derivatives, polyamines and Short-chain fatty acids (SCFAs) (Table 3). The SCFAs are described in more detail below in next section (see point 10). Indole derivatives are obtained from diet with tryptophan and promote the secretion of IL-22 strengthening the integrity of the intestinal mucosa and inducing antimicrobial peptides secretion from epithelial cells, production of mucins and proliferation of goblet cells. In the same way, polyamines also develop intestinal mucosa, being such its importance that if endogenous production is blocked, maintenance and repair of intestinal epithelium will be affected. On the contrary to previous statement, exogenous polyamines can reduce IL-18 release, a cytokine that promotes epithelial repair and barrier function (Postler and Ghosh, 2017). They also have direct influence on innate immune cells; in fact, the polyamine named spermine inhibits the lipopolysaccharide (LPS) induced expression of pro-inflammatory cytokines, leading to anti-inflammatory effect (Postler and Ghosh, 2017).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Molecular mechanism(s) of action</th>
<th>Origin</th>
<th>Effects on immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole derivatives</td>
<td>Activation of AhR and NR1I2</td>
<td>Derived from dietary tryptophan by different intestinal bacteria</td>
<td>AhR activation promotes maintenance of ILC3 cells, which strengthen integrity of gut mucosa by secreting IL-22. NR1I2 activation also enhances epithelial barrier function</td>
</tr>
<tr>
<td>Polyamines (primarily putrescine, spermidine, spermine)</td>
<td>Unclear. Inhibit expression of pro-inflammatory cytokines in conjunction with AHSG. Also inhibit activation of NLRP6 inflammasome</td>
<td>Derived from arginine by host and bacteria</td>
<td>Enhance development and maintenance of gut mucosa and resident immune cells. Inhibit expression of pro-inflammatory cytokines by LPS-stimulated monocytes and macrophages</td>
</tr>
</tbody>
</table>

(Postler and Ghosh, 2017).
Finally, the main metabolites that are synthesized \textit{de novo} by gut microbes are ATP and Polysaccharide A (PSA) (Table 4). Extracellular ATP secreted by gut bacteria has pro- and anti-inflammatory effects. ATP is consider an endogenous danger-associated molecular pattern (DAMP) that promotes secretion of pro-inflammatory cytokines, chemotaxis and differentiation of naive T cells into T\textsubscript{H}17 cells, but it also inhibits release of IgA into the gut lumen by reducing the number of B-cell activating T follicular helper cells and thus protecting intestinal microbiota from excessive antibody production. On the other hand, PSA, which is produced by the gut bacterium \textit{Bacteroides fragilis}, promotes the secretion of anti-inflammatory cytokine IL-10 by T cells, protecting mice from colitis. As a curiosity, it has also being related to a protective role in IBD and autoimmune encephalomyelitis by increasing ENTPD1 expression on peripheral T-reg (Postler and Ghosh, 2017).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Molecular mechanism(s) of action</th>
<th>Origin</th>
<th>Effects on immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Activation of P2X and P2Y receptors</td>
<td>Actively secreted by subset of intestinal bacteria</td>
<td>Limits numbers of Tfh cells in Peyer’s patches, thus reducing secretion of bacteria-specific IgA by B cells across gut epithelium. Promotes differentiation of T\textsubscript{H}17 cells in gut mucosa. May promote epithelial barrier function by activating NLRP3 inflammasome and subsequent IL-18 secretion by macrophages</td>
</tr>
<tr>
<td>Polysaccharide A (PSA)</td>
<td>Activation of TLR2 on DCs and Tregs. Presentation of PSA fragments with MHC-II to CD4\textsuperscript*</td>
<td>\textit{Bacteroides fragilis} (requested for colonization)</td>
<td>Potent anti-inflammatory effects: induces secretion of IL-10 from CD4\textsuperscript* T cells, directly and indirectly. Skews T\textsubscript{H}1:T\textsubscript{H}2 ratio toward T\textsubscript{H}1 cells</td>
</tr>
</tbody>
</table>

(Postler and Ghosh, 2017).

10. SHORT-CHAIN FATTY ACIDS (SCFAs)

Plant-derived polysaccharides obtained from diet (like resistant starches and dietary fiber) cannot be digested by host enzymes, so anaerobic intestinal microbiota is crucial for its fermentation, resulting in end products called SCFAs (Postler and Ghosh, 2017). The most abundant (95%) SCFAs are: \textit{acetate} (C2), \textit{propionate} (C3) and \textit{butyrate} (C4). The other 5% correspond to branched-chain SCFA like isobutyrate, isovalerate and 2-methyl butyrate, obtained from protein breakdown (Ríos-Covián et al. 2016). Other products of non-digestible carbohydrates fermentation are: lactate, which can be metabolized to acetate, propionate and butyrate by cross-feeding organisms; and formate, which has been linked to methanogenesis and inflammatory processes (Morrison and Preston 2016).
Traditionally, it has been summarized that *Bacteroidetes* phylum supplies most of the acetate and propionate, whereas *Firmicutes* mainly produce butyrate; but actually, it is more complicated than that, as shown in Table 5 (Postler and Ghosh 2017, Ohira et al. 2017).

<table>
<thead>
<tr>
<th>SCFAs</th>
<th>Pathways/Reactions</th>
<th>Producers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>From pyruvate via acetyl-CoA</td>
<td>Most of the enteric bacteria. (Representative of species bacteria) <em>Akkermansia muciniphila, Bacteroides</em> spp., <em>Bifidobacterium</em> spp., <em>Prevotella</em> spp., <em>Ruminococcus</em> spp.</td>
</tr>
<tr>
<td>Wood-Ljungdahl pathway</td>
<td><em>Blautia hydrogenotrophica, Clostridium</em> spp., <em>Streptococcus</em> spp.</td>
<td></td>
</tr>
<tr>
<td>Acrylate pathway</td>
<td><em>Megasphaera elsdenii, Coprococcus catus</em></td>
<td></td>
</tr>
<tr>
<td>Propanediol pathway</td>
<td><em>Salmonella</em> spp., <em>Roseburia inulinivorans, Ruminococcus obeum</em></td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td>Phosphotransbutyrylase/ butyrate kinase route</td>
<td><em>Coprococcus comes, Coprococcus eutactus</em></td>
</tr>
</tbody>
</table>

(A): acetate is the substrate for producing butyrate; (L): lactate is the substrate for producing butyrate.

(Ohira et al. 2017).

### 10.1. Microbial cross-feeding dynamics in SCFAs production

Each SCFA is produced following different routes: Acetate is produced by most enteric bacteria as a result of carbohydrates (CHO) fermentation (from pyruvate, via acetyl-CoA); propionate formation follows three different pathways: succinate, acrylate and propanediol pathway; and finally, butyrate is formed by the butyrate kinase route and the butyryl-CoA: acetate CoA-transferase pathway (Ríos-Covián et al. 2016).

Despite these well-defined routes, there is also an essential microbial cross-feeding dynamics which includes both metabolic and substrate cross-feeding:

- **Metabolic cross-feeding**: The employment by one microorganism of end-products from the metabolism of another one. For example, *Bacteroides thetaiotaomicron* uses polysaccharides and produces acetate, while one of the main producers of butyrate, *Faecalibacterium prausnitzii*, can utilize acetate as a substrate (Ríos-Covián et al. 2016).
Substrate cross-feeding: The use by one bacterium of complex-CHO breakdown products formed by another one. Thus, microorganisms that are incapable of utilizing complex CHO may take advantage. For example, some *Bifidobacterium* that are not able to use inulin-type fructans can grow thanks to oligosaccharides released by primary inulin degraders (Ríos-Covían et al. 2016).

Also, there is evidence of mainly bacterial cross-feeding from acetate to butyrate, at lower extent between butyrate and propionate, and no metabolic flux between propionate and acetate (Fig.12) (Ríos-Covían et al. 2016).

It is important to highlight that each SCFAs has a biological gradient from gut lumen (where they are produced) to host periphery. This will lead to different tissue’s SCFA exposure (Morrison and Preston 2016). While acetate and propionate travel intact across the gut epithelium to the liver, butyrate is metabolized just within the intestinal epithelium where it serves as the main energy source for colonic enterocytes. In the same way, propionate is in part degraded in intestinal mucosa but it also reaches the liver. However, acetate is the only SCFA that is mainly released into systemic circulation (Fig.13) (Postler and Ghosh, 2017).
10.2. Gut integrity and biological effects of SCFAs

In general, SCFAs reduce the luminal pH by inhibiting some pathogenic microorganisms and increasing several nutrients absorption (Ríos-Covián et al. 2016). They also regulate the integrity of epithelial barrier through tight junction proteins (Morrison and Preston 2016).

For example, acetate takes part in the ability of bifidobacteria to inhibit enteropathogens, improves glucose tolerance and plays a role in cholesterol homeostasis in the peripheral tissue (Ríos-Covián et al. 2016, Kim et al. 2016). Moreover, it has little effect on locomotor activity or food intake, but it favors energy harvest and basal thermogenesis (Rosenbaum et al. 2015). On the other hand, propionate is a substrate for liver gluconeogenesis, reduces food intake and promotes locomotor activity (Baothman et al. 2016, Rosenbaum et al. 2015).

The third SCFA, butyrate, serves as energy source for intestinal epithelial cells and boosts healthy colonocytes proliferation. This role is demonstrated in germ-free mice that have an energy-deprived state of the gut epithelium. If butyrate is introduced, the deficit of mitochondrial respiration will be re-established (Kim et al. 2016). On the other hand, butyrate also induces apoptosis in transformed cells by accumulating within cancerous colonocytes and inhibiting tumor cell progression (this is named as “butyrate paradox”). So it is thought to have an important role against the development of colorectal cancer (Ríos-Covián et al. 2016); however, in a mouse model of colorectal cancer, butyrate appears to enhance tumor formation by stimulating hyper-proliferation in those MSH2 deficient colon epithelial cells (Morrison and Preston 2016). It also stimulates mucin production, colon motility and tight-junctions, thanks to an increased expression of claudin-1 and Zonula Occludens-1 (ZO-1) and occluding redistribution, as well as a decreased expression of aberrant ZO-1 (Ríos-Covián et al. 2016, Morrison and Preston 2016).

Finally, the protective effect against diet-induced obesity has been showed with butyrate and propionate; having direct effects on feeding behavior and physical activity too. In addition, low levels of their bacterial producers are observed in ulcerative colitis or children with asthma. It is interesting to highlight that elevated cecal and fecal SCFAs levels have been found in obese mice and humans, what could suggest the existence of decreased colonic absorption in obesity (Baothman et al. 2016).

10.3. SCFAs and the Immune System

SCFAs have anti-inflammatory properties, increase mucin production by globet cells and improve tight junctions. In mice with ulcerative colitis was evidenced that SCFAs mitigate the disease by inducing the secretion of IL-18 which promotes integrity of the intestinal epithelium. They are also agonists for some G-protein-coupled receptors (GPCRs) like FFAR2, a free fatty acid receptor essential for neutrophil chemotaxis at gut mucosa. Exposure to SCFAs lowers pro-inflammatory cytokines production from neutrophils. Thus, FFAR2/- mice are more susceptible to colitis (Postler and Ghosh, 2017).

Moreover, SCFAs promote expansion of both preexisting and de novo regulatory T cells (Treg), responsible for suppressing effector T cells activity and likewise inhibit the histone deacetylases, impeding antibody formation from B lymphocytes (Fig. 14) (Postler and Ghosh, 2017). They also has an essential role on inducing tolerance to commensal bacteria thanks to the ability of reducing intestinal macrophages responsiveness (Morrison and Preston 2016).
Intestinal bacteria convert dietary nutrients to immunomodulatory metabolites. Polysaccharides that cannot be digested by host enzymes, such as cellulose, are metabolized by the intestinal microbiota to SCFAs, green). These exert a plethora of anti-inflammatory effects, such as inducing the expansion and de novo differentiation of regulatory T cells, enhancing the barrier function of the intestinal mucosa through epithelial cells and goblet cells, facilitating production of antibodies by B cells, inhibiting the maturation of dendritic cells and promoting an anti-inflammatory phenotype, and reducing production of pro-inflammatory cytokines by innate immune cells. Dietary tryptophan is degraded to indole derivatives (blue). Indoles promote epithelial barrier function, e.g., by supporting the maintenance of type 3 innate lymphoid cells, which are the primary producers of IL-22. Dietary arginine is metabolized to polyamines (red), which promote the integrity of the intestinal epithelium and reduce the production of pro-inflammatory cytokines by macrophages. DC (dendritic cell), EC (epithelial cell), GC (goblet cell), ILC3 (type 3 innate lymphoid cell), Mφ (macrophage), Nφ (neutrophil), Treg (regulatory T cell). (Postler y Ghosh, 2017).
An important function of butyrate is to reduce inflammation by decreasing LPS translocation and inhibiting macrophage activation or pro-inflammatory cytokine production. We should have in mind that obesity and insulin resistance are associated with an increased permeability of gut barrier that leads to cell wall bacteria components translocation which trigger an inflammatory cascade. In this way, it makes sense to think that butyrate could reduce weight or insulin resistance (Morrison and Preston 2016). Indeed, an experiment with mice was made by giving them butyrate supplementation, and it was evidenced a reduction in diet-induced insulin resistance maybe by increasing mitochondria function and energy spending (Baothman et al. 2016). Butyrate also inhibits nuclear factor kappa β (NF-κβ) activation in macrophages, and it is thought to be the responsible of suppressing TNF-α, IL-6 and myeloperoxidase activity in Kupffer cells as well, suggesting a role in regulating liver inflammation. The anti-inflammatory role of butyrate is also observed in IBD and acute radiation proctitis, with improvement of both diseases. In addition, both butyrate and propionate inhibit the maturation and function of DCs (Morrison and Preston 2016).

10.4. Role of SCFAs on lipid metabolism

The effects of the SCFAs in energy and lipid metabolism suggest an important role of SCFA in the control of metabolic syndrome (Ríos-Covián et al. 2016). In fact, they have a regulatory task in metabolism since they are natural ligands for FFAR2/3 found on enteroendocrine and immune cells (Morrison and Preston 2016). This role of SCFA in regulating gut hormones via FFAR2/3 in the end protects against diet-induced obesity and insulin resistance (Parekh et al. 2014), as it was observed in experimental mice fed with high-fat diet after dietary supplementation with butyrate (Ríos-Covián et al. 2016).

In the liver, acetate is used for de novo lipogenesis and cholesterogenesis, both inhibited by propionate that also is able to reduce visceral and liver fat (Morrison and Preston 2016). Increased circulating SCFA entails a reduced adipogenesis and adipocytes lipolysis which leads to decrease free-fatty acid (FFA) from adipose tissue to liver. They also inhibit lipid accumulation in adipocytes stimulated by insulin. Thus, increasing peripheral SCFA availability in obese would be a novel strategy to regulate FFA flux (Morrison and Preston 2016). However, there is a controversy regarding the role of SCFA in obesity, the so called “Energy harvesting” hypothesis, whereby SCFA add additional calories through fermentation (Morrison and Preston 2016).

10.5. SCFAs and appetite regulation

As it is noted above, the fermentation of dietary fiber to SCFA provides additional energy to the host what could promote obesity; nevertheless, it is well known in epidemiological studies that high-fiber diets prevent rather than promote obesity and it has been linked with an improved appetite regulation (Duranti et al. 2017).

This could be the result of FFAR activation by SCFA which leads to an increased secretion of enteroendocrine hormones such as glucagon-like-peptide 1 (GLP-1) or peptide YY (PYY), both of them responsible for causing satiety (Duranti et al. 2017). For instance, propionate and butyrate appears to induce short-term appetite regulation through PYY and GLP-1 mediated mechanisms, while acetate is thought to reduce appetite by its interaction with the CNS and by stimulating leptin secretion in adipocytes. In any case, further investigations should be done to completely clarify each SCFA’s role (Morrison and Preston 2016, Ríos-Covián et al. 2016).
GLP and PYY are responsible for satiety leading to a diminution of food ingestion. It has been evidenced that subjects with little circulating PYY have lower satiety and in consequence higher fat mass. In fact, some experiments with animals showed that lacking-PYY mice were hyperphagic and become obese, while chronic administration of PYY3-36 to obese mice decreased adiposity (Ríos-Covián et al. 2016, Parekh et al. 2014). That is why it has been proposed as a therapeutic strategy to target the PYY system by altering the microbiome (Parekh et al. 2014).

10.6. Regarding the methodology employed in SCFAs experiments

SCFAs administration was carried out by oral uptake, enema or intraperitoneal injection, but it is still unknown which is the method that most resembles the physiological state. What is clear is that SCFAs concentrations reached by different methods affect experimental outcomes (Postler and Ghosh, 2017).

Another item is the one that refers to the measurement of SCFA production directly in humans, which has been limited to the measurement of stool SCFA output (although it is unclear if it is representative for luminal SCFA production). However, stable isotope techniques hold promise (Morrison and Preston 2016).

11. CONTRIBUTION OF MICROBIOTA TO THE DEVELOPMENT OF DISEASES

Dysbiosis can affect different host systems and it is related with the development of several pathologies. In fact, harmless or even beneficial bacteria in a healthy microbiome can promote chronic diseases like atherosclerosis and obesity when their ecosystem is altered (Postler and Ghosh, 2017). Some of those pathologies are:

11.1 Autoimmune diseases

Previously, it has been exposed how important is microbiota in development of innate and adaptive immune system, but it is also crucial for triggering tolerant responses from the host. Thus, gut dysbiosis is can be related with the pathogenesis of autoimmune diseases like:

11.1.1. Rheumatoid arthritis (RA)

It is well known that bacterial DNA and cell wall components are present in joints of RA patients, which may cause inflammation in genetically susceptible people (Kim et al. 2016). Furthermore, RA-susceptible mice showed alteration of gut microbiota and an increased permeability. So, in order to check if gut microbiota plays a role in RA development, animal models of RA (IL-1 rn -/- mice) raised in germ-free conditions do not develop arthritis and later introduction of Lactobacillus or segmented filamentous bacteria (SFB) indeed induced arthritis supporting the initial hypothesis. However, another experiment evidenced suppression of induced arthritis in germ-free mice which were given E.coli. Thus, additional studies will be required to establish which species have a protective role in RA pathogenesis. It is also interesting to note that RA patients have low levels of Bifidobacterium and Bacteroides.
11.1.2. Systemic lupus erythematosus (SLE)

Intestinal environment can contribute to microbiota composition and development of SLE as it was verified in the following experiment: Mice receiving acidic pH water showed a slower SLE progression and less anti-nuclear antibody (ANA) production than mice receiving neutral water (control mice). Besides, gut microbiome analysis displayed different bacteria abundance between both kinds of mice. However, SLE patients do not have a significant difference in bacterial diversity, although they show dysbiosis with a reduced Firmicutes/Bacteroidetes ratio (Kim et al. 2016).

11.1.3. Ankylosing spondylitis (AS)

AS is closely associated with IBD (>70% patients develop subclinical gut inflammation and 5-10% clinically IBD) and AS patients also have high levels of flagellin antibodies and sulfate-reducing bacterium. The HLA-B27 gene is strongly related with AS pathogenesis, and in HLA-B27 transgenic mice, spondylitis and colitis were abolished in a germ-free state. In contrast, Bacteroidetes introduction induced inflammation indicating that the presence of specific taxa may be enough for AS initiation or progression (Kim et al. 2016).

11.1.4. Inflammatory bowel disease (IBD)

IBD include ulcerative colitis (UC) and Crohn disease (CD), and are characterized by inflammation in mucosal layer of the colon in UC and transmural involvement in CD (Blum 2017). Although it is known that the central event in IBD pathogenesis is the loss of tolerance against the indigenous gut, the precise mechanism is not entirely understood. It is thought that bacterial imbalance of gut lumen leads to inappropriate inflammation, resulting in host cell damage and autoimmunity (Kim et al. 2016).

IBD specific microbiota’s alteration may serve as an indicator for disease predisposition, activity or therapy response. For instance, some of these alterations could be a decreased bacterial diversity and butyrate producing microorganisms such as F.prausnitzii (Morrison and Preston 2016). As well, a decrease of SCFA-producing bacteria in human gut has been associated with IBD and dietary changes that encourage SCFAs production by the microbiome can improve symptoms. In mice, SCFAs have confirmed anti-inflammatory properties in the intestinal mucosa which lead to protective role in IBD prone mice (such knockout IL-10-/- or IL-2-/- mice) (Postler and Ghosh, 2017, Kim et al. 2016). Additionally, it has been showed how E.coli or Enterococcus faecalis trigger chronic intestinal inflammation in IBD prone animals and can generate colitis. On the other hand, Faecalibacterium prausnitzii has anti-inflammatory activity and it is reduced in IBD patients (Kim et al. 2016).

11.2. Neurodevelopmental, psychiatric and neurodegenerative diseases

It has been demonstrated the role of gut microbiota in modulating immune cells in CNS, as well as the activation of peripheral immune cells involved in neuroinflammation, brain injury and neurogenesis. In fact, germ-free mice have more behavior disturbances or neuropathologies like autism spectrum disorders, depression and Alzheimer’s or Parkinson’s disease (PD) (Blum 2017).
Patients with PD have plaques of alpha-synuclein (ASn) both in brain and intestine, and likewise, mice over-expressing ASn develop similar PD neurologic deficits. Also, PD germ-free mice developed less plaques and an improvement of neurological deficits, just like mice treated with antibiotics. As a remarkable point, fecal matter transplantation (FMT) form patients with PD to germ-free mice resulted in neurological deficits resembling PD (Blum 2017).

11.3 Atherosclerosis and thrombosis risk

Atherosclerosis is promoted by systemic pro-inflammatory events that catalogue it as a chronic disease associated with adverse cardiac events (Postler and Ghosh, 2017). It is accelerated by eating food rich in choline, phosphatidylcholine and carnitine (such as meat, egg yolk and high-fat products), being all of them precursors of trimethylamine (TMA) and TMA N-oxide (TMAO). Intestinal TMAO formation is a two-step process: First, generation of TMA by intestinal microbes after food intake (dietary phosphatidylcholine or L-carnitine), and second, the hepatic oxidation of TMA to TMAO by host flavin monoxygenases (FMOs). These molecules not only enhance atherosclerosis but platelet hyperreactivity and thrombotic events (Fig. 15) (Blum 2017).

![Fig. 15. The intestinal microbial metabolite trimethylamine (TMA) and the atherosclerosis/thrombosis risk (Blum 2017)](image)

There are several evidences that support the relationship of these molecules with the risk of developing atherosclerosis. For example, mice fed with choline or TMAO supplemented food promoted atherosclerosis. On the contrary, if gut microbiota was removed with antibiotics treatment, there would be no choline-exacerbation of atherosclerosis, which confirms the main role of microbiota in this disease. However, many fish species contain high amounts of TMAO, and
it is well known how fish-based diet reduces cardiovascular risk; that is why the role of TMAO is not completely clear. Another contradictory result is how L-carnitine supplementation has been linked with a decrease in cardiovascular risk while elevated plasma L-carnitine has been reported with an increased risk (Postler and Ghosh, 2017).

One likely future therapeutic drug to treat or prevent atherosclerosis could be the 3,3-dimethyl-1-butanol (DMB), a choline analog that blocks the intestinal TMA formation through the inhibition of microbial TMA lyase (Fig. 16) (Blum 2017).

![Fig. 16. Inhibition of intestinal microbial trimethylamine (TMA) synthesis by 3,3-dimethyl-1-butanol (DMB) and attenuation of atherosclerosis (Blum 2017).](image)

11.4. Type 1 diabetes (T1D)

It is well-known that T1D is mainly caused by genetic predisposition (HLA genotypes) to pancreatic β-cell autoimmunity. However, family studies have shown that just a fraction of those genetically predisposed persons will develop T1D. For that reason, environmental factors are thought to be involved in triggering this autoimmune response, like diet, viruses or even caesarean section (Patterson et al. 2016).

It is quite remarkable how altering microbiota could help to prevent T1D. For this statement is necessary to explain that MyD88 signaling route is required for autoimmune diabetes development. Thus, the knockout of MyD88 in non-obese diabetic (NOD) mice protect against T1D development. Furthermore, heterozygous MyD88-/- NOD mice, which usually develop diabetes, are protected from this disease when exposed from birth to gut microbiota of a MyD88 knockout donor (Patterson et al. 2016).
11.5. Type 2 diabetes (T2D)

It is believed that gut microbiota of T2D individuals is different from healthy ones, due to a lower production of SCFAs; indeed, they generally have a moderate degree of dysbiosis with a characteristic decrease in butyrate-producing organisms and an increase in opportunistnic pathogens. This inadequate SCFAs levels produce an increase of mucosa permeability allowing gut bacteria (and their cell-wall components like LPS) to cross and enter bloodstream, causing certain grade of endotoxemia. In consequence T2D patients exhibit increased plasma levels of LPS (Postler and Ghosh, 2017). Additionally, T2D was linked to improvement in membrane transport of sugars, oxidative stress responses and branched-chain amino acid transport (Patterson et al. 2016).

Recently, metformin, one of the most widely prescribed T2D therapeutic agents, has been shown to improve the gut microbial profiles in T2D. High-fat fed mice treated with metformin showed high levels of *Akkermansia muciniphila* (which are correlated with an increased mucin production) and negative correlation with glycaemia. In humans, it has been demonstrated how metformin treatment improve butyrate and propionate production; likewise, *Akkermansia* was also found at similar quantity in non-diabetic control than in metformin-treated diabetic patients. Accordingly, it was suggested that *A. muciniphila* may contribute to the antidiabetic effect of metformin indicating that manipulation of the gut microbiota towards *Akkermansia* may be potential treatment for T2D (Patterson et al. 2016).

In addition, one small study of fecal matter transplants (FMTs) from lean healthy donors to obese recipients with T2D, established no reduction weight but an increase in insulin sensitivity (Postler and Ghosh, 2017). Interestingly, butyrate-producing bacteria like *Roseburia intestinalis, Faecalibacterium spp.*, and *Eubacterium hallii* has been projected as potential probiotics in T2D, since it has been demonstrated they decrease metabolic-endotoxemia in T2D through proliferation of colonocytes and tight junction function (Patterson et al. 2016).
12. OBESITY

Obesity is a chronic disease influenced by genetic, sociodemographic and environmental factors (like sedentary lifestyles and dietary habits), and it is considered the 21st century epidemic that has a high impact on morbi-mortality, quality of life and healthcare costs (Serra-Majem and Bautista-Castaño 2013). Recent studies have demonstrated a causal relationship between obesity and gut microbiota in rodents, but numerous factors underlying human obesity (including genetics and lifestyle) make difficult to demonstrate an independent role for gut dysbiosis. However, lots of scientific advances have been done in the management of obesity (Fig.17) (Dao and Clément 2017).

12.1. Diet and obesity

Nowadays, most food contains higher calories and lower nutritional value due to a change in food processing methods and preservation technologies. This westernization and mechanization processes have resulted in prevalence of a sedentary life-style and high fat content diets that directly drive to obesity. Do not forget that nutrients in a balanced diet are distributed in the following way: carbohydrates (55-60%), proteins (15%) and fat (<30%). However, in a typical western USA high fat diet the distribution is: carbohydrate (51,8%), protein (15,4%) and fat (32,8%); high levels of salt, refined sugars and refined vegetable oils (these last two are poor in potassium) are also typical and consequently sodium-potassium ratio can be altered (Duranti et al. 2017).
Dietary fibers like inulin, resistant starch and beta-glucans, can reduce total and LDL-cholesterol by delaying gastric emptying, which may cause satiating effect and thus, help to control caloric ingestion. In fact, inulin regulates gastrointestinal (GI) motility and appetite, reduce fat mass and affect adipose tissue metabolism (Duranti et al. 2017). It is important to point that diets rich in saturated fat are linked to an increased white adipose tissue inflammation and metabolic disease, while diets rich in polyunsaturated fatty acids (fish oil) can neutralize inflammation to promote a lean healthy phenotype (Patterson et al. 2016).

The following table presents additional information taken from the FESNAD (Spanish Federation of Nutrition, Food and Dietetic Associations) and SEEDO (Spanish Association for the Study of Obesity) Consensus Document which may be useful regarding specific dietary factors (DF) that influence weight gain (Table 6) (Serra-Majem and Bautista-Castaño 2013):

<table>
<thead>
<tr>
<th>DF linked to lower BMI</th>
<th>DF associated with higher BMI</th>
<th>DF not linked to BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet with around 50% of the total energy intake composed of complex carbohydrates</td>
<td>High energy diets</td>
<td>Fat intake after adjusting for total energy intake</td>
</tr>
<tr>
<td>High fiber intake within a diet rich in vegetables and fruit</td>
<td>High ethanol intake</td>
<td>Olive oil intake</td>
</tr>
<tr>
<td>Vegetarian or “Mediterranean” diet</td>
<td>Frequent intake of sugared beverages</td>
<td>Addition of nuts to the usual diet</td>
</tr>
<tr>
<td>High intake of whole grains</td>
<td>High intake of meat and processed meat products</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Offering larger portions leads to an increase of individual’s caloric intake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absence of supermarkets with fresh fruit and vegetables, or their far away location (in particular from neighborhoods with low socioeconomic level)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Habitual intake of “fast food” (≥1/week)</td>
<td></td>
</tr>
</tbody>
</table>

(Serra-Majem and Bautista-Castaño 2013)

12.1.1 Role of diet in gut microbiota composition

The availability of the nutrients obtained from diet modulates the composition of gut microbiota, which in turn is involved in some metabolic syndrome and obesity (Duranti et al. 2017). However, gut microbiota composition is not only affected by diet, in fact is also affected by disease state, medications and host genetics; that is why it is in constant change (Fig. 18) (Baothman et al. 2016).

Fig. 18. A diagram showing main factors affecting the gut microbiota composition highlighting the great impact of diet on this composition (Baothman et al. 2016).
It is well known that microbiota extracts energy from dietary components that are not digested by the host and converts that energy to nutrients that the host can absorb, thus varying the energy balance of the host. Actually, microbes that most efficiently employ the components of a given diet gain a growth advantage and displace other species, which can explain the changes in microbiota’s composition upon dietary modification (Postler and Ghosh, 2017).

Dietary patterns can modify human gut microbiota as it is showed in Table 7, which summarizes recent studies that have observed changes in gut microbiota after consuming various types of diets (Baothman et al. 2016).

<table>
<thead>
<tr>
<th>Diet type</th>
<th>Effect on bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>High fat diet</td>
<td>Decrease of genera within the class Clostridia in the ileum. Increase Bacteroidales in large intestine. Increase <em>Lactobacillus</em> spp., <em>Bifidobacterium</em> spp., <em>Bacteroides</em> spp. and <em>Enterococcus</em> spp. Decrease <em>Clostridium leptum</em> and <em>Enterobacter</em> spp. Increase Firmicutes to Bacteroidetes ratio. And increased <em>Enterobacteriaceae</em>. Increase Bacteroidales, Clostridiales and Enterobacteriales.</td>
</tr>
<tr>
<td>Vegetarian diet</td>
<td>Decrease <em>Acteroides</em> spp., <em>Bifidobacterium</em> spp., <em>Escherichia coli</em> and <em>Enterobacteriaceae</em> spp. Decrease <em>Enterobacteriaceae</em> and increase <em>Bacteroides</em>. Increase <em>Bacteroidetes</em> and decrease Firmicutes and Enterobacteriaceae.</td>
</tr>
<tr>
<td>Calorie restricted</td>
<td>Decrease Firmicutes to Bacteroidetes ratio</td>
</tr>
</tbody>
</table>

(Faothman et al. 2016).

Further studies have linked some specific diets with changes in enterotypes, like for example: the consumption of high protein and animal fat diets were associated with _Bacteroides_ enterotype, while _Prevotella_ was with carbohydrates; resistant starch diets provoked an increase of _Ruminococcus bromii, Oscillabacter_ and _Eubacteria rectale_, while an inulin-based diet raised _Bifidobacterium_ genus and _Atopobium_ group but decreased _Bacteroides/Prevotella_ relative numbers (Duranti et al. 2017).

Western-style diet, low in fiber and high in caloric content from sucrose and unsaturated fat, selects for a less diverse microbiota that efficiently extracts energy from these nutrients; while plant-based diet promotes microbial species that ferment fiber (Postler and Ghosh, 2017). High fat (HF) diet has profound effects on gut microbiota composition and associates with injurious metabolic effects. As it was demonstrated, subjects that consumed the least healthy diet had a
greater inflammatory profile; while subjects consuming the healthiest diet had a higher microbial diversity (Patterson et al. 2016). Moreover, abundance of Firmicutes (specifically the Mollicutes class) was linked to diet-induced obesity; while other human researches have connected losing weight under a fat or carbohydrate-restricted diet with a relative abundance of Bacteroidetes (Boulangé et al. 2016).

In a recent diet-switch study, African Americans were fed in a high-fiber low-fat African-style diet and rural Africans in a high-fat low-fiber diet. As consequence, an improved butyrogenesis was observed on low-fat high-fiber feeding alongside an increase of some butyrate producing bacteria as Roseburia intestinalis, Eubacterium rectale and Clostridium symbiosum. However, an increment of CD3+ intra-epithelial lymphocytes and CD68+ lamina propria macrophages on high-fat low-fiber diet, suggested an increased inflammation state (Morrison and Preston 2016). A different research with mice verified that eating low-fat high-fiber diet leads obese-microbiota to fail in colonization of lean mice. This points that diet affect the metabolic phenotype in the host (Patterson et al. 2016).

Interestingly, it has been demonstrated that microbial changes are not only diet-dependent, but sex-dependent too. Diet affects gut microbiota differently in males than females. Anyway, further researches should be done on this subject (Patterson et al. 2016).

### 12.2 Host-gut microbiota cross-talk contribution in metabolic syndrome and obesity

The metabolic syndrome refers to several cardiovascular risk factors that come up from insulin resistance accompanying abnormal adipose deposition and function. It greatly increases the risk of many chronic diseases like cardiovascular disease or T2D and it is widely accepted that metabolic syndrome represent a diet-induced disease; nevertheless, its etiology is multifactorial and also host genetics and environmental factors are involved (Duranti et al. 2017).

These risk factors include hyperglycemia, hypertriglyceridemia, dyslipemia and hypertension, being all of them also associated with activation of the immune system. There are complex mechanisms that link lipid metabolism, inflammation and insulin resistance with obesity (Fig.19), and in fact most people with metabolic syndrome are obese, which suggests that the overload of fat tissue could have a causative role; however, some studies have identified people with normal BMI who exhibit high levels of triglycerides or fat in liver (Boulangé et al. 2016).

![Fig.19](image)

**Fig.19.** Crosstalk between the gut microbiota and the mammalian host in inflammation and metabolism. The gut microbiota can contribute to host insulin resistance, low grade inflammation and fat deposition through a range of molecular interactions with the host and therefore can indirectly participate in the onset of obesity and metabolic diseases (Boulangé et al. 2016).
The excess of calorie intake, fat accumulation and lipotoxicity activate the production of cytokines and cells of the immune system which promote a chronic low-grade inflammatory status. Inflammation impairs insulin action, which contributes to the development of metabolic abnormalities. In fact, activation of pro-inflammatory cytokines contributes to desensitizing insulin signaling pathways. For instance, TNF-α or IL-1β in visceral adipose tissues affects insulin sensitivity by altering the expression of genes encoding insulin receptor substrate proteins (IRS-1), glucose transporter (GLUT4) and PPAR-α (Boulangé et al. 2016).

Patients with metabolic syndrome, obesity or T2D exhibit a significant endotoxemia due to a high intestinal permeability which allows bacteria to enter bloodstream. Pathogen-associated molecular patterns (PAMPs) of invading bacteria such as cell-wall LPS, bind to pattern recognition receptors (PRRs) expressed by immune and adipose cells and trigger pro-inflammatory cytokine production, resulting in a generalized state of chronic low-grade inflammation. Interestingly, mice chronically exposed to low levels of LPS gained weight and became resistant to insulin (Postler and Ghosh, 2017).

An important role of gut microbiota is to regulate metabolic health through modulating LPS infiltration, calorie intake, fat accumulation and insulin action. In consequence, a healthy or dysbiotic gut microbiota will be relevant at the time of reaching metabolic health or disease (Fig.20) (Boulangé et al. 2016).

**Fig.20.** Effects of a healthy gut microbiota and dysbiosis on the gut and metabolic health of the host. A healthy microbiota comprises a balanced representation of symbionts (bacteria with health-promoting functions) and pathobionts (bacteria that potentially induce pathology). A shift toward dysbiosis results from a decrease in symbionts and/or an increase in pathobionts and is likely to be triggered by environmental factors (such as diet, stress, antibiotics and infections). Low bacterial gene counts have also been associated with altered gut microbial functions and dysbiosis and have been linked to increased fat accumulation, lipopolysaccharide-induced inflammation, insulin resistance, obesity and metabolic syndrome. Individuals with these characteristics are more likely to develop metabolic diseases (such as diabetes, cardiovascular diseases and inflammatory bowel diseases). LBP (LPS-binding protein), SCFA (short-chain fatty acid) (Boulangé et al. 2016).
Regarding the association between gut microbiota composition and obesity, it has traditionally been associated with a relative increase in *Firmicutes* at the expense of *Bacteroidetes*, an increase in *Actinobacteria* and a decrease of *Verrucomicrobia* phylum (Duranti et al. 2017). However, there are not universal findings; thus, the worth of *Firmicutes*: *Bacteroidetes* ratio as a biomarker for obesity remains uncertain. (Fig.21) (Patterson et al. 2016).

One hypothesis about the role of microbiota in weight gain is that the increased *Firmicutes*: *Bacteroidetes* ratio in obese people may lead to more efficient hydrolysis of non-digestible polysaccharides in the intestinal lumen which may favor a greater extraction of calories and fat from food (Boulangé et al. 2016). Actually, the link between gut microbiota and weight gain is thought to be caused by an increase in the energy harvesting capabilities of the “obese-microbiota” and in a higher presence of enzymes involved in complex carbohydrate degradation (like glycoside hydrolase enzymes). This microbially-derived energy is in form of SCFA which stimulate the hepatic *de novo* liponeogenesis, modulate the triglyceride storage and promote energy storage (Patterson et al. 2016, Duranti et al. 2017). However, it is important to highlight that although SCFAs are a source of calories for the host, their intestinal production has been associated with reduced inflammation and increased satiety leading to positive metabolic effects (Boulangé et al. 2016).
One research that supports the role of gut microbiota in regulating energy harvest was made in germ-free mice that were inoculated “conventional” microbes, which resulted in similar levels of fat mass than the donor mice (if they received microbiota from an obese one, the facility to use high-caloric nutrients was also transferred resulting in weight gain) (Rosenbaum at al. 2015, Postler and Ghosh, 2017). Therefore, the total of weight gained was different depending on which microbes were inoculated: On the one hand, if microbiota from leptin-deficient mice (Lepob) with high amount of Firmicutes was administrated to germ-free mice, they gained more weight, had lower energy expenditure and higher energy harvest than if “wild-type” microbiota was given; on the other hand, if microbiota from lean mice with high quantity of Bacteroidetes were transferred to their obese litter-mates, it would result in prevention of obesity development (Rosenbaum et al. 2015).

Furthermore, germ-free mice develop less body fat (40%) than conventionally raised mice, despite increasing their food intake (in about 29% more calories) and decreasing their metabolic rate. Germ-free mice also were protected against glucose intolerance and insulin resistance (Patterson et al. 2016, Boulangé et al. 2016).

Intestinal ecosystem of obese individuals is also characterized by having a reduced taxonomic diversity and a decrease in butyrate-producing bacteria. As a consequence, they also have an increase in mucus degradation and high oxidative stress management, which predisposes them to further inflammation. Just as a reminder, butyrate facilitates tight junction assembly enhancing the intestinal barrier and also has anti-inflammatory properties. Both of them prevent from bacterial transport across stressed epithelial cells, preventing for a “leaky gut” (Patterson et al. 2016). The statement that low bacterial richness in obesity might also have a role in the inflammatory status of the host has been supported by some studies that demonstrate how obese people with high bacterial gene count contain more species associated with an anti-inflammatory status (for example, Faecalibacterium prausnitzii) and less of those with a pro-inflammatory status (for example, Bacteroides spp.) than those obese persons with low bacterial gene count, that also have an elevated oxidative stress genes (Boulangé et al. 2016).

Another interesting issue is how Roux-en-Y gastric bypass (RYGB) surgery led to enrichment of Bacteroidetes, Verrucomicrobia and Proteobacteria and relatively greater propionate and lower acetate production (Morrison and Preston 2016). Thus, a new hypothesis was proposed suggesting that weight improvement after RYGB surgery cannot be only explained by the caloric restriction but the change in gut microbiota. In order to demonstrate this, FMT from RYGB-treated mice was introduced into germ-free mice which lead to weight loss and reduction of fat mass (Baothman et al. 2016).

12.2.1. Mechanisms that link gut bacteria and energy metabolism

Studies in germ-free and conventionally raised mice have revealed several ways of linking gut microbiota and energy metabolism (Fig.22) (Boulangé et al. 2016):

- Gut microbiota increases capillaries of the intestinal villi and enhances gut motility, promoting caloric extraction from diet (Boulangé et al. 2016).
- It transformed polysaccharides into digestible compounds such as SCFAs used by colonocytes and the host (Boulangé et al. 2016).
- Microbiota also decreases the intestinal expression of fasting-induced adipose factor (FIAF) which would lead to a greater activity of lipoprotein lipase (LPL). Taking into account that the LPL has a role in hydrolyzing triglycerides and storage the released fatty acids as fat within adipocytes (Boulangé et al. 2016, Parekh et al. 2014).

- In addition, it suppresses the release of adenosine monophosphate-activated protein kinase (AMPK) which leads to downregulation of mitochondrial fatty acid oxidation, glucose uptake and insulin secretion, and up-regulation of lipogenesis, cholesterol and triglyceride synthesis (Boulangé et al. 2016).

- GPCRs (like GPR41) are receptors for SCFAs which stimulate PYY. This promotes gut motility and facilitates nutrient absorption. It was evidenced that GPR41 deficient mice had less body fat than their wild-type littermates; however another study showed an increased BMI in GPR41 knockout mice. That is why more studies should be done (Boulangé et al. 2016).

- Microbiota contributes to obesity phenotype induced by high-fat diet through the regulation of farnesoid X receptor (FXR), responsible for the regulation of bile acid formation and hepatic triglyceride storage (Boulangé et al. 2016).

- It also turns choline to trimethylamine, regulating the bioavailability of choline by the host and leading to a decrease in triglycerides export by VLDL to the organs but indirectly promoting the storage of triglycerides in the hepatocytes (Boulangé et al. 2016).

**Fig.22.** Metabolic and immune interactions between gut microbes and the host in obesity and the metabolic syndrome. The gut microbiota is involved in a molecular crosstalk with the host that modulates host physiology, metabolism and inflammatory status. In particular, the gut microbiota participates in the physiology and motility of the digestive tract and in the digestion of polysaccharides, which directly influences host energy availability. The gut microbiota inhibits fasting-induced adipose factor (FIAAF) in the intestine and monophosphate activated protein kinase (AMPK) in several organs such as the brain and muscle, which results in increasing fat deposition. The short-chain fatty acids (SCFAs) produced by bacteria from polysaccharides interact with G protein-coupled receptors (GPCRs: GPR41, GPR43 and GPR109A), which stimulates gut motility and host immunity. The gut microbiota also contributes to fat deposition through the regulation of the farnesoid X receptor (FXR), the bile acid receptor responsible for the regulation of bile acid synthesis and hepatic triglyceride accumulation. The gut microbiota converts choline to trimethylamine, thus influencing the bioavailability of choline for host use and indirectly affecting phosphatidylcholine production and hepatic triglyceride transport by very-low-density lipoproteins (VLDLs) (Boulangé et al. 2016).
12.3. Gut-brain axis in the regulation of energy balance

Energy homeostasis, a biological process involved in balance between energy intake and expenditure, is mainly coordinated by the brain. The CNS receives two kinds of signals from various peripheral organs: “tonic signals” like leptin or insulin that are released continuously to reflect the amount of body fat, or “episodic signals” that fluctuate depending on the ingestive status of the individual. The GI tract is the responsible for most of the episodic signals that regards about the size and composition of an incoming meal (Bauer et al. 2016).

Enteroendocrine cells (EECs) within the gut epithelium express chemosensory mechanisms on their apical face letting them to react to preabsorptive nutrients. Classically, there have been identified gut peptides’ secretion by specific EECs subtypes of different GI sites (Bauer et al. 2016): Stomach contains X/A-like cells that produce ghrelin and chief cells that produce gastric leptin, the proximal small intestine contains I and K cells that produce cholecystokinin (CCK) and glucose-dependent insulinotropic hormone respectively, and finally, the distal small intestine contains L cells which produce GLP-1/2, oxyntomodulin (OXM) and PYY.

However, recent findings indicate co-expression of several gut peptides trough the intestine suggesting that EECs are really a single cell type producing a different spectra of gut peptides depending on the environment (Bauer et al. 2016). The main mechanisms of action of these gut hormones are exposed in Table 8 (Adamska et al. 2014).

| Table 8. The main mechanisms of action of gut hormones and “adiposity signals” |
|---------------------------------|---------------------------------|
| **Gastrointestinal (GI) hormones- “satiety signals” regulating the beginning, end and intervals between meals** |
| GLP-1 | Incretin effect, satiety regulation, delayed gastric emptying |
| GLP-2 | Affects GI motility and trophic effect in the intestinal tract |
| Ghrelin | Hunger stimulation |
| PYY | Satiety regulation, delayed gastric emptying |
| PP | Affects gastric motility, satiety regulation |
| OXM | Satiety regulation, affects HCl secretion, incretin properties |
| CCK | Affects GI motility, exocrine pancreatic enzyme secretion, secretory function of the gallbladder |
| GIP | Incretin effect |
| Amylin | Affects glucose homeostasis, gastric motility |

| “Adiposity signal” hormones- role in regulating the formation of energy reserves |
|---------------------------------|---------------------------------|
| Insulin | Affects glucose homeostasis, glycogen synthesis |
| Leptin | Regulates energy metabolism |

(Adamska et al. 2014).
Ingested nutrients induce secretion of these gut peptides like CCK, GLP-1 and PYY, which can act locally or far away through endocrine signals. These “satiety signals” are able to maintain energy homeostasis during both feeding and fasting by regulating energy intake or expenditure (Fig. 23). Interestingly, gut peptide receptor antagonists like devazepide, a CCK-1 receptor antagonist, block both inhibition of food intake and neural activation associated with nutrient ingestion (Bauer et al. 2016).

The stomach and proximal small intestine are greatly innervated by vagal and splanchnic nerves with a main role of gut-to-brain signaling (due to the prevalence of afferent pathways over efferent ones). Indeed, vagal afferent fibers situated near EECs surface, express receptors for gut peptides such as ghrelin, leptin, CCK, GLP-1 and PYY which lead to direct neuronal activation; but gut peptides can also activate vagal and spinal afferents indirectly via activation of enteric nervous system (ENS). There are numerous evidences that support the role gut-brain axis has in food intake control. For example, when fasted mice were re-fed, food intake decreases throughout the meal, pointing to a negative feedback signals sent to the brain. This is a way to protect against an excess of incoming nutrients (Bauer et al. 2016).

One of the first signals is the mechanoreception of a food bolus entering the stomach that produces a vagal afferent signal which activates neurons in the hindbrain and leads to a decrease in food intake. Furthermore, the rate of gastric emptying starts with the slow of nutrients arrival into the small intestine, increasing the distention of the stomach after food ingestion. Release of CCK, GLP-1 and vagal activation achieve this slowing of gastric emptying. Nutrients in the intestine can also per se suppress food intake, independently of gastric emptying effects (Bauer et al. 2016).
As it was evidenced, nutrient sensing is weakened in obese people since they express a reduction of both postprandial levels of gut peptides and reduce sensitivity to them. For instance, a low vagal sensitivity to nutrients, CCK and GLP-1 were found in obese rats, promoting overeating and weight gain (Bauer et al. 2016).

12.3.1. Control of energy homeostasis by the endocannabinoid system (ECS).

Endocannabinoids are endogenous lipid-based retrograde neurotransmitters that resemble the action of cannabinoid D9-tetrahydrocannabinol (D9-THC), the principal active component of Cannabis sativa. The endocannabinoid system (ECS) encompasses endocannabinoids, the proteins that regulate their production and degradation, and the receptors through which they signal. The main endocannabinoids are the N-arachidonoyl ethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), which target the cannabinoid receptors CB1 and CB2 (Silvestri and Di Marzo, 2013).

The endocannabinoids release occurs immediately within the CNS, with no intermediate storage in vesicles, being perfect mediators for responding in real-time to any change in the feeding state of a person. They regulate appetite and food intake by modulating, via activation of CB1 receptors, the activity of hypothalamic neurons and then, the release of feeding-regulated neuropeptides. In fact, endocannabinoid levels increase within the hypothalamus and accumbens in response of fasting, returning to normal after refeeding. This regulation is mediated in part by the action of anorexigenic hormones like leptin (which decrease endocannabinoid levels) and orexigenic ones, ghrelin or glucocorticoids (which increase endocannabinoid levels) which they are deregulated during obesity resulting in high hypothalamic endocannabinoid tone. Interestingly, high levels of 2-AG in hypothalamus (which goes along with an impair leptin signaling) may also be involved in peripheral metabolic dysfunctions, such as adipose tissue accumulation and hepatic glucose production causing insulin resistance (Silvestri and Di Marzo, 2013).

Activation of the ECS can also affect the reward and reinforcement circuits of the mesolimbic system and brainstem neurons, interacting with dopaminergic and opioidergic pathways, therefore participating in homeostatic and hedonic aspects of food intake (leading to a preference for highly palatable food). For example, D9-THC increases sucrose-induced hedonic activity and dopamine release into the nucleus accumbens, whereas CB1 antagonism decreases dopamine in this nucleus. To demonstrate that the main result from CB1 activation is a feeding stimulus, a study with mice was developed. In that study, rodents increased feeding after an injection of AEA or 2-AG, even if they were previously satiated. Furthermore, it was showed how CB1 and μ-opioid blockade resulted in hypophagia and reduced body weight in rodents. Equally important is how the orexigenic effect of ghrelin is also lost in rodents in which CB1 receptors are pharmacologically or genetically inactivated (Silvestri and Di Marzo, 2013).

Finally, recent studies suggest that also CB2 receptors, which were initially considered to be devoid of a metabolic function, could have a role in energy homeostasis control, mainly in the inflammatory aspects of obesity and T2D (supported by the high expression of these receptors in immune cells) and perhaps in the physiological control of hepatic lipogenesis and glucose tolerance at both peripheral and central levels. However, much work is still required to fully understand the exact role of CB2 (Silvestri and Di Marzo, 2013).
Having in mind a possible pharmacological modulation of brain endocannabinoid activity that may let a better control in overeating in obese, a clinical trial with CB1 blockers was carried out. Unfortunately this trial had to be stopped due to psychiatric side effects. It was initially designed to diminish food intake and body weight rather than a potential therapy for the metabolic syndrome, but emerging knowledge about the role ECS have over lipid and glucose metabolism in several peripheral organs (like liver or adipose tissue), have opened a window to future therapies to reduce metabolic risk factors linked with obesity (Silvestri and Di Marzo, 2013).

13. MODULATION OF GUT MICROBIOTA WITH THERAPEUTIC PURPOSES

Food components of diets, in particular, polysaccharides operate as primary modulators of the composition and function of the microbiota. Since diet is the main contributing factor to gut microbial composition, it can be considered to prevent or alleviate diseases by altering microbial gut composition (Fig. 24) (Duranti et al. 2017). Such an empirical approach can be used to improve human health and can be also compatible with emerging focus on precision health. Thus, the hypothesis that obesity can be controlled by modulating the gut microbiota may guide the way to future therapeutic interventions such as the selection of specific gut bacterial strains to control energy intake and reduce overweight or metabolic syndrome (Boulangé et al. 2016, Duranti et al. 2017). Nevertheless, in order to treat obesity, many dietary strategies have failed to preserve long-term results in lowering body weight and in some way facilitate weight regain. It has been suggested that a persisting core of microbiota may predispose the individual to weight-gain cycles on the post-diet period (Duranti et al. 2017). By contrast, the change in gut microbiota towards that of a lean individual is linked with weight loss (Rosenbaum et al. 2015).

Fig. 24. Schematic representation of the diet-microbiota-obesity correlations. Interactions between diet and gut microbiota in lean and obese subjects: nutrition, energy intake and microbiota modulation are reported. For lean individuals, possible microbiota enterotypes are shown. For obese individuals, obesity-correlated diseases and possible dietary manipulations are illustrated (Duranti et al. 2017).
Another novel therapeutic approach to overcome obesity is the fecal matter transplants (FMTs) which has a role in reshaping the gut ecosystem after antibiotic treatment or in *C. difficile* infection too (Postler and Ghosh, 2017, Boulangé et al. 2016). FMT was also used in people with metabolic syndrome and it showed an increased insulin sensitivity and butyrate-producing intestinal microbiota after 6 weeks (Boulangé et al. 2016, Parekh et al. 2014).

13.1. Prebiotics and probiotics used in prevention and treatment of obesity

**Prebiotics**

They are oligosaccharides or short-chain polysaccharides found in common products such as vegetables and whole grain cereals, and can be added in yoghurt. They stimulate the growth and/or activity of beneficial bacteria present in GI tract. They reach colon still intact and then they are used as fermentable substrates for intestinal bacteria, promoting a change in microbiota that will prevent for fat accumulation (Duranti et al. 2017). For example, inulin stimulates the growth of bifidobacteria and may reduce caloric intake and fat mass in animals. Additionally it was evidenced in Ob/Ob mice fed with high-carbohydrate diet supplemented with oligofructose how they increased bifidobacteria and lactobacilli, improving epithelial tight junctions in gut and lowering gut permeability, systemic endotoxemia and inflammation (Boulangé et al. 2016). Prebiotics were also linked with lower hepatic lipid levels as it could be shown after galactosyl-oligosaccharides (GOS) supplementation in healthy mice. They also seemed to modulate immune function in elderly people, to reduce markers of the metabolic syndrome in overweight adults and to decrease hunger with a greater satiation after a meal, as well as an increasing of plasma GLP-1 and PYY (Boulangé et al. 2016, Rosenbaum et al. 2015).

However, it was not yet demonstrated the anti-obesity effects of prebiotics. Actually, intervention with dietary inulin-type fructans (ITF) in obese women selectively modified the gut microbiota composition but it did not elicit any significant change in either host metabolism or body weight (Boulangé et al. 2016, Duranti et al. 2017).

**Probiotics**

They are live microorganisms that, when used as food supplements in adequate amounts, confer a health benefit on the host by improving intestinal microbial balance (Boulangé et al. 2016). The most commonly used probiotics are *Bifidobacterium* and *Lactobacillus*, which are typical bacteria of human gut (Duranti et al. 2017).

A study carried out in mice fed with high fat diet (HFD), evaluated the result of supplementation with both lactobacilli and bifidobacteria for 12 weeks. They significantly diminished HFD-induced weight gain, improved glucose-insulin homeostasis and reduced hepatic steatosis. There was also a reduction of pro-inflammatory macrophage infiltration into adipose tissue (Duranti et al. 2017).

In humans, probiotics have shown a positive effect on glucose metabolism. In fact, in a placebo-controlled study of 60 overweight healthy Indian individuals, the VSL#3 probiotic mix (*Bifidobacterium, Lactobacillus* and *Streptococcus*) decreased systemic glucose and insulin levels (Boulangé et al. 2016). Also, in a human clinical trial randomized and double-blind, it was
demonstrated that overweight women that received a probiotic mix reduced abdominal fat and increased antioxidant enzyme activity (Duranti et al. 2017).

It is worth mentioning that numerous probiotics exhibit bile salt hydrolase (BSH) activity, which have caused a reduction in host weight gain, cholesterol levels and liver triglycerides in mice. So it has been suggested that microbial metabolism of bile acids may also play a role in regulation of host weight gain (Duranti et al. 2017).

13.2. Role of Akkermansia muciniphila and Faecalibacterium prausnitzii

Akkermansia muciniphila is a Gram-negative anaerobe that belongs to the Planctomycetes-Verrucomicrobia-Chlamydiae superphylum. A.muciniphila has been found to inhabit the gastrointestinal tracts of more than 90% of adult subjects analyzed, and it constitutes 1 to 4% of the fecal microbiota. It is capable of using intestinal mucins, the highly glycosylated proteins of the epithelial mucus layer, as its sole source of carbon and nitrogen (Reunanen et al.2015). In fact, mucus degradation by A. muciniphila is known to result in the liberation of oligosaccharides and subsequent production of acetate, which becomes available to microorganisms in the vicinity of the intestinal mucosa.

Obesity has been linked to changes in energy metabolism and deterioration of the intestinal mucosal and this is consistent with the fact that microbiota is considered an important regulator of mucosal barrier function. An example of a regulator could be Akkermansia muciniphila, as it is decreased in obese and diabetic mice, while its reintroduction improves epithelial barrier function and reduces diet-induced obesity (Postler and Ghosh, 2017). Thus, it is suggested to have a protective role against obesity by controlling fat storage, adipose tissue inflammation and glucose metabolism(Patterson et al. 2016); indeed, A.muciniphila has an inverse correlation with obesity, low-grade inflammation, T2D, IBD, cardiometabolic and liver diseases (Fig.25) (Cani and de Vos, 2017). Moreover, fewer A.muciniphila cells have been detected in ulcerative colitis and Crohn’s disease patients than in healthy individuals (Reunanen et al. 2015). It is significant to note that antidiabetic treatment such as metformin was associated with an increase of this bacteria (Cani and de Vos, 2017).

Accordingly to the preventing function on obesity that A.muciniphila has, it was shown that daily oral supplementation of live A.muciniphila protected mice against diet-induced obesity by lowering up to 50% their body weight without altering the diet. They did not show insulin resistance or infiltration of inflammatory cells (CD11c). In a similar research, Patrice D.Cani and Willem M.de Vos also found re-established the endogenous production of antimicrobial peptides along with an increased production of endocannabinoid lipids with anti-inflammatory function(Cani and de Vos, 2017). A.muciniphila was also able to restore normal mucus layer thickness thus improving endotoxemia at similar levels to those observed after prebiotic supplementation with oligofructose or inulin although in this case food intake was also reduced (Cani and de Vos 2017, Patterson et al. 2016).

It is remarkable that a novel therapeutic application could be carried out with a purified membrane protein from A.muciniphila (Amuc_1100) which replicates the previous effects (Postler and Ghosh, 2017). In relation with diet, mice fed with fish oil had higher levels of members of the genera Lactobacillus and Akkermansia, while mice fed with lard had increased levels of Bilophila (which has been shown to exacerbate colitis in susceptible models) (Patterson et al. 2016).
Another bacterium that was found reduced in patients with obesity, metabolic dysfunctions and IBD was *Faecalibacterium prausnitzii*. In fact, obese patients who followed a probiotic formula or a dietary supplementation with starch had high levels of *F. prausnitzii*. It was suggested to have protective effects in acute and chronic inflammatory diseases, and it was associated with decreased gut permeability by promoting barrier function. *F. prausnitzii* was proposed to be used as a novel probiotic for gut dysfunction or inflammation (Patterson et al. 2016).

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**Fig. 25.** Effects of *A. muciniphila* and derived products on host metabolism. *A. muciniphila* has been found to be lower in several conditions such as during obesity, diabetes, intestinal inflammation, liver diseases or chronic alcohol consumption. This is associated with an altered gut barrier function leading to an increased plasma LPS levels and eventually triggering low grade inflammation and metabolic disorders. *A. muciniphila* alive or pasteurized as well as Amuc_1100 (a protein implicated in the formation of pili) has been shown to restore gut barrier function likely by acting on TLR2 and restoring appropriate tight junction expression. All these results are associated with an increased mucus later thickness and an improvement of metabolic disorders. It is worth noting that an exploratory human investigation has shown that *A. muciniphila* is apparently safe (Cani and de Vos 2017).
14. CONCLUSIONS

Obesity is a major problem worldwide and it is considered one of the most serious public health challenges of the 21st century. Diet, lifestyle and microbiota play an important role in the development and evolution of obesity and many details of the complex relationship between microbiota, diet and host should be taken into account. A better understanding of the impact of specific microbes on host physiology will be crucial in order to develop future therapeutic strategies to prevent obesity.

However, obesity is not only microbiota-driven; in fact there is no obvious cause-effect relationship. This might be attributable to different studies’ methodologies (fecal samples, mice vs. humans, etc), diets, lifestyle or genetics that interfere with significant results. For instance, it is important to highlight how studies done in humans have failed to discern if microbiota modification is a cause or a consequence of changes in fat mass (Rosenbaum et al. 2015). Actually gut microbes are changing continuously and every new research describes any conflicting results to the last; that is why larger cohorts and more studies are necessary to support previous content in this work. Anyway, some clues are shown about microbiota composition and their function in different host status thanks to metagenomic analysis and rRNA sequencing, showing variations in gut microbiome diversity in metabolic disease. Indeed, microbial gut composition has been linked to other multiple diseases like T1D, T2D, inflammatory diseases or atherosclerosis risk.

Recent studies have shown the association between dietary intake and microbiota composition suggesting that gut microbiota can be modulated by dietary supplementation with prebiotics or probiotics. Simultaneously, healthy eating with high-fiber low-fat diet and physical activity can improve health to manage obesity.
BIBLIOGRAPHY REFERENCES


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AhR</td>
<td>aryl hydrocarbon receptor.</td>
</tr>
<tr>
<td>Akk</td>
<td>Akkermansia muciniphila.</td>
</tr>
<tr>
<td>AMPK</td>
<td>activated protein kinase.</td>
</tr>
<tr>
<td>AS</td>
<td>ankylosing spondylitis.</td>
</tr>
<tr>
<td>ASn</td>
<td>alpha-synuclein.</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate.</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index.</td>
</tr>
<tr>
<td>CCK</td>
<td>cholecystokinin.</td>
</tr>
<tr>
<td>CD</td>
<td>Crohn disease.</td>
</tr>
<tr>
<td>CHO</td>
<td>carbohydrates.</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system.</td>
</tr>
<tr>
<td>DAMP</td>
<td>danger-associated molecular pattern.</td>
</tr>
<tr>
<td>DCs</td>
<td>dendritic cells.</td>
</tr>
<tr>
<td>DF</td>
<td>dietary factors.</td>
</tr>
<tr>
<td>ECS</td>
<td>endocannabinoid system.</td>
</tr>
<tr>
<td>EECs</td>
<td>enteroendocrine cells.</td>
</tr>
<tr>
<td>EHEC</td>
<td>enterohaemorrhagic E.coli.</td>
</tr>
<tr>
<td>FFA</td>
<td>free-fatty acid.</td>
</tr>
<tr>
<td>FIAF</td>
<td>fasting-induced adipose factor (FIAF).</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization.</td>
</tr>
<tr>
<td>FMT</td>
<td>fecal matter transplantation.</td>
</tr>
<tr>
<td>GI</td>
<td>gastrointestinal.</td>
</tr>
<tr>
<td>GLP-1</td>
<td>glucagon-like peptide 1.</td>
</tr>
<tr>
<td>GPCRs</td>
<td>G-protein-coupled receptors.</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome-wide association study.</td>
</tr>
<tr>
<td>HapMap</td>
<td>haplotype map.</td>
</tr>
<tr>
<td>HF</td>
<td>high fat.</td>
</tr>
<tr>
<td>HFD</td>
<td>high fat diet.</td>
</tr>
<tr>
<td>IBD</td>
<td>inflammatory bowel disease.</td>
</tr>
<tr>
<td>INSeq</td>
<td>Insertion sequencing.</td>
</tr>
<tr>
<td>LPL</td>
<td>lipoprotein lipase.</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide.</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor Kappa Beta.</td>
</tr>
<tr>
<td>NOD</td>
<td>non obese diabetic.</td>
</tr>
<tr>
<td>OXM</td>
<td>oxyntomodulin.</td>
</tr>
<tr>
<td>PAMP</td>
<td>pathogen-associated molecular pattern.</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson disease.</td>
</tr>
<tr>
<td>PRRs</td>
<td>pattern recognition receptors.</td>
</tr>
<tr>
<td>PSA</td>
<td>polysaccharide A.</td>
</tr>
<tr>
<td>PYY</td>
<td>peptide YY.</td>
</tr>
<tr>
<td>RA</td>
<td>rheumatoid arthritis.</td>
</tr>
<tr>
<td>RYGB</td>
<td>Roux-en Y gastric bypass.</td>
</tr>
<tr>
<td>SCFAs</td>
<td>short-chain fatty acids.</td>
</tr>
<tr>
<td>SFB</td>
<td>segmented filamentous bacteria.</td>
</tr>
<tr>
<td>SLE</td>
<td>systemic lupus erythematosus.</td>
</tr>
<tr>
<td>SNPs</td>
<td>single nucleotide polymorphisms.</td>
</tr>
<tr>
<td>T1D/T2D</td>
<td>type 1 or type 2 diabetes.</td>
</tr>
<tr>
<td>TMA</td>
<td>trimethylamine.</td>
</tr>
<tr>
<td>TMAO</td>
<td>TMA N-oxide.</td>
</tr>
<tr>
<td>Treg</td>
<td>regulatory T cells.</td>
</tr>
<tr>
<td>UC</td>
<td>ulcerative colitis.</td>
</tr>
<tr>
<td>ZO-1</td>
<td>zonula occludens-1.</td>
</tr>
</tbody>
</table>
BIOLOGICAL CLASSIFICATION SYSTEM OF ORGANISMS:

Example: Taxonomic classification of *A. muciniphila*

**Domain:** Bacteria

**Kingdom:** Eubacteria

**Phylum:** Verrucomicrobia

**Class:** Verrucomicrobiae

**Order:** Verrucomicrobiales

**Family:** Verrucomicrobiaceae

**Genus:** *Akkermansia*

**Species:** *A. muciniphila*
Table 9. Overview of the composition of human microbiome

<table>
<thead>
<tr>
<th>Phylum</th>
<th>% Microbiome</th>
<th>Genus or species</th>
<th>Relevant function</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major phyla  (&gt;1% of most individuals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firmicutes</td>
<td>≈60-65%</td>
<td><em>Clostridium</em>, <em>Eubacterium</em>, <em>Faecalibacterium</em>, <em>Lactobacillus</em>, <em>Roseburia</em>, <em>Ruminococcus</em></td>
<td>Some species ferment fiber into butyrate; other functions range from symbionts to pathogens. Butyrate production</td>
<td></td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>20-25%</td>
<td><em>Alistipes</em>, <em>Bacteroides</em>, <em>Parabacteroides</em>, <em>Porphyromonas</em>, <em>Prevotella</em></td>
<td>Polysaccharide degradation</td>
<td>Increased in protein-rich, high-meat diets</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>5-10%</td>
<td><em>E.coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>3%</td>
<td><em>Bifidobacterium</em>, <em>Collinsella</em></td>
<td>Vitamin biosynthesis</td>
<td>Common probiotic</td>
</tr>
<tr>
<td>Minor phyla  (&lt;1% of most individuals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaea</td>
<td>&lt;1%</td>
<td><em>Methanobrevibacter</em>, <em>Methanosphaera</em></td>
<td>Both convert hydrogen gas to methane (methanogens)</td>
<td></td>
</tr>
<tr>
<td>Deferribacteres</td>
<td>&lt;1%</td>
<td></td>
<td>Degrade iron</td>
<td>Increased in gastrointestinal bleeding</td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>&lt;1%</td>
<td><em>Fusobacterium nucleatum</em></td>
<td>Proinflammatory colonic tumorigenic factor</td>
<td>Increased in high-meat diets</td>
</tr>
<tr>
<td>Melainabacteria</td>
<td>&lt;1%</td>
<td></td>
<td>Synthesize vitamins B and K, ferment carbohydrate into ethanol, lactate and formate</td>
<td>Increased in high-plan diets, present in groundwater</td>
</tr>
<tr>
<td>Spirochaetes</td>
<td>&lt;1%</td>
<td><em>Treponema</em></td>
<td></td>
<td>More common in rural communities and in high-fiber diets</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>&lt;1%</td>
<td><em>Akkermansia muciniphila</em></td>
<td>Degrade mucin, diminish inflammation and increase gut butyrate and mucus layer thickness</td>
<td></td>
</tr>
</tbody>
</table>

(Rosenbaum et al. 2015)