Human gut microbiota and immune system

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Healthy human adults possess about 100 trillion bacteria, archaea, fungi and viruses in their gut. The identification and characterization of these microorganisms are important to elucidate the role of microbiota composition in human disease. Most of them, called core microbiota, belong mainly to the phyla *Bacteroidetes* and *Firmicutes,* which have pathways for carbohydrate and amino-acid central metabolism. Benefits from gut microbiota to the host in steady state include extraction of nutrients from diet and protection against enteropathogens. In another way, perturbation of this balance is caused by many factors and can lead to immune dysregulation and susceptibility to diseases.The chronic inflammatory condition present in obese people is a typical link existing between adipose tissue and immune response, however the use of viable microorganisms is a potential manner of treating gastrointestinal disorders. This review summarize the efforts performed by many authors to understand the complex interplay between the host and microorganisms, as also present the important new findings in respect to fortification of immune system against pathogens through the gut microbiota.

Keywords: adipose tissue; inflammation; immunonutrition; microbial resistance.

1. Introduction

Human beings can be considered "superorganisms", since in addition to all their own cells, they host a tenfold number of microbial cells (bacteria, archaea, fungi and viruses). Microorganisms, however, are very small in size, occupying only 1-3% of the mass of the human body [1]. Most of them are hundreds of individual bacterial species of different strains with high genetic diversity residing in the gut, and results in peaceful coexistence with mutual benefits for both, microbiota and host healthy [2]. The consortium of those single-celled organisms provides the host with a range of metabolic capabilities that would not be accessible otherwise, while the host provides the nutrients and environment in which the bacteria can thrive. Therefore, the gastrointestinal tract (GIT) receives a strong influence by such microbiota, specially depending of its profile – this virtually impacts all aspects of human physiology and biology [3-6] –, and this is highly variable among individuals as a result of a multifactorial process that involves succession over time, the host genetics and lifestyle choices such as diet (4, 7, 8]. Different structural patterns, such as the ratio between *Firmicutes* and *Bacteroidetes* Phyla present in the gut, for instance, have been associated with metabolic diseases.

Therefore, the use of animal models is crucial to understand the relation of microbiota and the human host. The yeast *Saccharomyces boulardii*, for example, is able to limit inflammation and infection in the GIT in healthy intestine of mice [6]. Other way to study how microbiota can act is through gnotobiotic mice, *i.e.* animals that have been colonized with a defined microbiota [9] that can modulates many aspects of the host, like bone-mass density, fat storage, intestinal angiogenesis and the development of an immune response [1]. Finally, given that several gut functions and immune genes are conserved between zebrafish and mammals, this small fish is an interesting model organism to investigate fundamental processes underlying intestinal inflammation and injury, being attractive to immunologists and oncologists [10].

The microbial communities hosted by the human gut comprise a very promising and fascinating area. Disruption of the establishment of a stable normal gut microbiota may be associated with, or even contribute to, the pathogenesis of disease [11, 12]. It is crucial knowing the microbiota to understanding the development of gut functions, gut-brain axis and some health disorders and diseases, as well as their treatment and prevention.

2. Usual terms and animal models

Although the terms "microbiota" and "microbiome" are often used interchangeably [13], in the present paper, **microbiota** is referred to all organisms that comprise the microbial community from a specific habitat – the gastrointestinal tract, in the case. Bacterial cells are the most cited integrant of microbiota, but bacteriophages, fungi, protozoa, and viruses that live inside and on the human body are also part of it. The microbiota possesses its own functions, *i.e.*, modulating expression of genes involved in mucosal barrier fortification, angiogenesis, and postnatal intestinal maturation. While the microbiota represents the complex collections of microbial communities that colonize a host, **microbiome** is the collection of genes encoded by members of the microbiota.

The relationship between the host and its microbiota is the result of millions of years of co-evolution and, therefore, is generally mutually beneficial (symbiotic) and fundamental for human health. When unfavorable changes happen and the composition of gut microbiota is altered, we call it **dysbiosis**. Such condition may be associated with several clinical

disorders such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), obesity, autoimmune diseases and allergies [3,4,7,12]. According to DeGruttola (2016) [14], dysbiosis can be categorized into three different types: 1) Loss of beneficial organisms, 2) Excessive growth of potentially harmful organisms, and 3) Loss of overall microbial diversity.

In another way, diet plays a central role in shaping the microbiota and the majority of these data relate to probiotics found on yogurts. **Probiotics** are live nonpathogenic microorganisms – mainly bacteria, but utilization of eukaryotes has been investigated – with putative beneficial effects on the host, once they help with digestion and offer protection from harmful bacteria (in contrast to antibiotics that counteract microbial activity) [6]. Those characteristics has been stimulated numerous studies to investigate the use of probiotic bacteria for also the delivery of gastrointestinal therapeutics. In this way, intestinal specimens might contribute to the identification of microbial biomarkers for health and disease. In contrast, **prebiotics** are nondigestible carbohydrates that act as food for probiotics [15]. When probiotics and prebiotics are combined (in fermented dairy products, for example) they then form a **synbiotic**.

Much of the focus on studying microbioma and immuno-pathogenesis of chronic gut inflammation has been performed through sampling of faecal microbiota, although many metabolic functions also occur in the small intestine.

Under certain conditions, the intestinal barrier function can be impaired or overwhelmed, allowing bacteria and endotoxins within the GIT to reach systemic organs and tissues, a process termed **bacterial translocation**. Disturbances of this system have been implicated in a wide range of disorders, including obesity and autism [3]. For this purpose, the animal models are frequently used. Mice represent an usual system to study the effects of gut microbes on host gastrointestinal physiology. Three types of mice can be defined: conventional, germ-free and gnotobiotic. Animals with microorganisms naturally present on them are referred to as **conventional** (or **wild type**), while **germ-free** mice are born and raised in sterile conditions. To avoid any contamination, at birth they are removed from the mother by Caesarean section and live in the isolators with germ-free foster mothers. So, the animals never come into accidental contact with microorganisms. However when those animals are deliberately in contact with a defined microbiota, we denominate them **gnotobiotic** animals, which provide an excellent system for controlling host genotype, microbial community composition, diet, and housing conditions [1,9,10].

To investigate the metabolic functions of each microbiota, the transplantation is usually performed between conventional/gnotobiotic animals to germ-free, but Ellekilde *et al*. (2014) [16] recently proposed to transfer different microbial compositions to conventional antibiotic-treated mice (rather than borned germ-free mice). The authors showed that microbiota was efficiently introduced, established and changed in the alternative donors. When experiments are performed using adult human fecal microbiota into germ-free mice, it is denominated **humanized mouse gut microbiota**. Alternatively, characteristics such as small size, high fecundity and immune genes conservation between zebrafish and mammals – mainly involved in epithelial proliferation, promotion of nutrient metabolism, and innate immune responses – have allowed this animal to be an effective model. The transparent body of zebrafish early in life (< 2 weeks) also facilitates the *in vivo* tracking of immune responses in a complete organism [17]. A growing amount of studies continues to show the power of the zebrafish model when investigating conserved pathways in gut epithelial homeostasis and inflammation [10,18]. Both strategies (mice and zebrafish) are complementary and necessary to understand the human microbiome.

3. Composition of the gut microbiota

The important role of the resident microbiota in human health has gained increased recognition over the past few decades. Historically, the human fetus has been considered microbiologically sterile, although some new research challenges that assumption and has been demonstrated that efflux of commensal bacteria through the placental barrier may occur [1, 7]. In this way, early colonization of the infant gastrointestinal tract, *i.e.* during pregnancy, delivery and lactation, is undoubtedly an important factor for infant health and may have additional benefits in later life – infants delivered by cesarean section have a reduced number of bacteria compared with vaginally delivered infants [4,7]. Following birth, human milk supports a protective microbiota in the infant GIT, characterized by low-species diversity (mainly *Bifidobacterium* spp. and *Lactobacillus* spp.). This contributes to the development and regulation of the gut function as well as to the maintenance of intestinal barrier, the protection against infection and challenges the immune system, promoting also tolerance to certain foods [19, 20]. Around the age of 1-2 years old, the infant gut microbiota undergoes a shift and the stable adult microbiome begins to emerge, supporting further the significant role of the diet in influencing this microbial community [21].

Therefore, the association between gut microbiota and health indicators is not attributable to a single microorganism, but rather to an ecosystem that influences the complicated interaction between host biology and environment [2, 11, 13, 14]. So, it is not possible to define a "normal microbiome", as healthy individuals can harbor different microbial consortia, as the United States of America (USA)- National Institutes of Health (NIH) has studied. The Human Microbiome Project (HMP), established in 2008 as an interdisciplinary effort of United States, European Community and Asia and other areas worldwide, is an attemptative to perform a comprehensive analysis of the human microbiome in health and/or disease (HMP-aims in the **Box 1)**. However, the HMP is not a single project and the

Metagenomics of the Human Intestinal Tract (MetaHIT) emerged to characterize the microbial communities in this specific habitat of human body [22].

Box 1. Stated aims of the United States of America (USA) -National Institutes of Health (NIH) and Common Fund of Human Microbiome Project (HMP).

- ❖ Development of a reference set of 3,000 isolate microbial genome sequences;
- ❖ Initial 16S & metagenomic whole genome shotgun studies to generate an estimate of the complexity of the microbial community at each body site, providing initial answers to the questions of whether there is a "core" microbiome at each site;
- ❖ Demonstration projects to determine the relationship between disease and changes in the human microbiome;
- ❖ Development of new tools and technologies for computational analysis, establishment of a data analysis and coordinating center (DACC), and resource repositories;
- ❖ Examination of the ethical, legal and social implications (ELSI) to be considered in the study and application of the metagenomic analysis of the human microbiota.

Each person has a distinct (and highly) variable microbiota, but a conserved set of gut colonizers [23, 24]. 25Zambom de Souza *et al.* (2015) [25] determine whether oral supplementation with L-glutamine (GLN) modifies the gut microbiota composition in overweight and obese adults, and after 14 days of supplementation, adults in the GLN group exhibited statistically significant differences in the *Firmicutes* and *Actinobacteria* phyla compared with those in the L-alanine (ALA) control group. The ratio of *Firmicutes* to *Bacteroidetes*, a good biomarker for obesity, decreased in the GLN group from 0.85 to 0.57, whereas it increased from 0.91 to 1.12 in the ALA group. At the genus level, *Dialister*, *Dorea*, *Pseudobutyrivibrio*, and *Veillonella*, belonging to the *Firmicutes* phylum, had statistically significant reduction.

Bacterial species whose proportional representation defined a healthy gut microbiota, were identified by Subramanian *et al.* (2014) [8], during the first two years of children with healthy growth, from Dhaka (Bangladesh), using monthly fecal samples and a machine-learning-based approach to 16S rRNA generated datasets. As reviewed by Donaldson *et al.* (2016) [26], even the microbiota in the mucus layer differs from that of the intestinal lumen. In fact, microbiota is biogeographically stratified within the gut on different spatial scales and axes, but in general (healthy intestine), the obligate anaerobes predominate because oxigen is naturally low in this organ [22]. However, in dysbiosis there is a decrease of anaerobic population and an increase in facultative anaerobes, meaning that oxygen as well as reactive oxygen species may be one of the causal factors of disturbance on microbial population.

The gut microbiota encompasses predominantly the bacterial phylotypes, *Bacteroidetes* and *Firmicutes* [1, 8, 22, 23].

Bacteroidetes are Gram-negative, anaerobic, non-sporulating bacteria that secrete many enzymes that degrade carbohydrates, present at approximately 10^9 cells per gram in the lower intestine, are represented by the genera *Bacteroides*, *Prevotella* and *Xylanibacter* [27]. *Firmicutes* are Gram-positive, anaerobic, spore-forming bacteria that ferment simple sugars to produce a variety of short chain fatty acids (SCFAs). The *Firmicutes* phylum, present at only moderate densities (10^6-10^8 cells per gram), is represented principally by lactic acid bacteria such as *Lactobacillus* (from which several strains are probiotics) and *Enterococcus* as well as *Clostridium* species [19, 27].

Other phyla also contribute (*Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia*), but they are relatively less present. Since the control of microbial populations indicates the health of the host, in stress/disease status, composition of microbiota is severely altered (**Figure 1**).

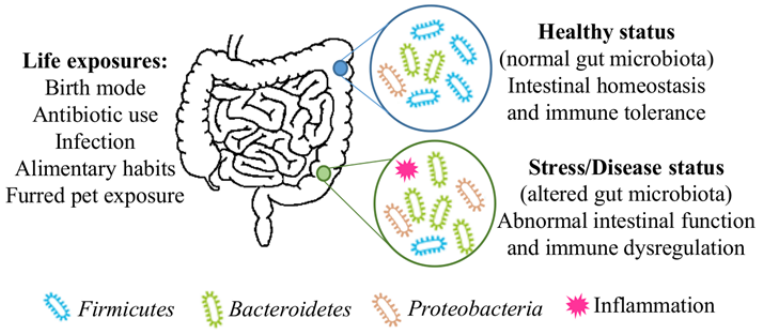

Figure 1. Conditions that influence the composition of gut microbiota and characteristics of the organ in healthy or stress/disease status.

4. Microbial importance to immunity

The host and the GIT microbiota are in constant communication, and due to their both complexity, especially in respect to the immune system, it is difficult to define the specific mechanism underlying the functional effects of pro- and prebiotics [7, 15]. It is established that germ-free mice exhibits a severely underdeveloped intestinal immune system as compared to conventionally raised mice, so their importance can not be subestimated. Select members of the microbiota have been shown to have dramatic and specific effects on the host immune system through their intimate interactions with the host. For example, *Akkermansia muciniphila* is a beneficial strain, once has been shown to have a protective effect against obesity and type 2 diabetes in both humans and mice, lowering body fat mass, improving glucose homoeostasis, decreasing adipose tissue inflammation and increasing gut integrity [3, 28]. *A. muciniphila* produces a variety of fermentation products, including SCFAs, through mucin degradation, and these are energetic substrates to other bacteria and to the host, and may contribute to the expansion of other beneficial species. Palm *et al.* (2015) [12] propose that these immunologically important commensals can be split into two broad categories, depending on their functional effects on the immune system: a) Inflammatory commensals: microorganisms that stimulate inflammatory/effector immune responses; and b) Immunoregulatory commensals: which primarily stimulate immunoregulatory response rather than inflammatory.

The SCFAs acetate (C_2), propionate (C_3) and butyrate (C_4) are the main metabolic fermentation products of anaerobic bacteria in the human intestine, being found at different concentrations, according to the part of the organ: ~ 13 mM in the terminal ileum, ~130 mM in the caecum and ~80 mM in the descending colon [24, 26]. Essentially, they act as fuel for intestinal epithelial cells (5 to 10 % of human basal energy requirements are provided by SCFAs) and other tissues, including liver and muscle – their transport occurs predominantly via the portal vein after intestinal absorption –, but also modulate different processes in the gastrointestinal tract such as electrolyte and water absorption [2].

These SCFAs (**Table 1**) have been recognized as protective against gut inflammation and acts on leukocytes and endothelial cells, through at least two mechanisms: 1) activation of endogenous free fatty acid receptor (FFAR), like FFAR2 and FFAR3 [which are otherwise designated as GPR41 and GPR43 respectively, because they belong to G-protein coupled receptor family of receptors, which leads to an increase of the expression of the satiety hormone, the polypeptide YY (PYY), and to an increase of the intestinal motility]; and 2) inhibition of histone deacetylase (HDAC). SCFAs also regulate several leukocyte functions, including production of cytokines (TNF-α, IL-2, IL-6 and IL-10), eicosanoids and chemokines (e.g., MCP-1 and CINC-2), as well as they modify the recruitment of circulating leukocytes to the inflammatory site [13, 29].

Table 1. Main functions of short-chain fatty acids (SCFAs) produced by microbiota in human gut.

SCFAs	Functions
Propionic	It is used in gluconeogenesis, reduces the intake of food and cholesterol synthesis, with a favorable effect on leptin gene expression
Acetic	It acts as a substrate for synthesis of cholesterol and also takes part in the *de novo* synthesis of lipids in liver. Plays a role in prevention of weight gain through an anorectic effect, inflammation, metabolic dysregulation, and it is the most predominant gut-produced SCFA in peripheral blood
Butyric	It is source of energy for colonocytes, increases insulin sensitivity (in mice) and possesses obesity-related antiinflammatory action (in humans); it can give protection against diet-induced obesity without causing hypophagia, may protect against colon carcinoma, and increase the leptin gene expression

Because the GIT is one of the main portals of pathogen entry, there is an important defense function associated to the gut, in addition to other physiological functions [16, 26, 29]. Selected manipulation of the immune system has the potential to alter gut microbiota composition to make it inherently less pro-inflammatory (*i.e.* more diverse and with a reduced level of innate immune activators), reducing susceptibility to and/or severity of intestinal inflammation development [30]. The gut and the liver are closely connected also [7]. A well functioning link between the gut and the liver is dependent on both an intact intestine and a liver in balance with respect to immunologic response and metabolism of endogenous and exogenous compounds. The importance of the intestinal microbiota in immune system development reveal that intestinal structure and function are impaired mainly through decreased IgA secretion, decreased number and function of intraepithelial lymphocytes and reduced lymphatic tissue [9].

Dollé *et al*. (2016) [30] also propose that IgA may be used as a target to shape the intestinal bacterial community in order to maintain a beneficial relationship between the host and the microbiota. In a previous work, Palm *et al.* (2015) [12] found that known inflammatory commensals like *Helicobacter* sp. and *Prevotellaceae* were uniquely highly coated with IgA in dysbiotic mice. So, the functional categorization of the microbiota based on IgA-coating can be useful to identify novel immunologically important members of the microbiota in both mice and humans.

Beyond microbiota itself, the mucus layer overlying the intestinal epithelium forms a protective blanket that keeps gut microorganisms from touching intestinal walls, helps to control the levels of blood sugar and fats, and prevents the invasion and systemic spread of bacteria and endotoxins (by prevention of those harmful byproducts from stimulating an immune response) [18, 23, 26], which are mostly lipopolysaccharide (LPS) from the cell walls of Gram-negative bacteria and found at low concentrations in the blood of healthy persons [15, 26, 31]. Since the intestinal mucosal barrier is the first line of defense against luminal microbiota, this degradation may be an intermediate step towards dysbiosis and some diseases [30].

5. Dysbiosis in inflammatory conditions

Inflammation is a natural defence reaction of the body against injury that can be triggered off by both internal and external factors and can be involved in conditions not previously linked with it. In respect to inflammatory bowel diseases (Crohn disease and ulcerative colitis), it may be caused by dysbosis associated with more complex interactions between the host and the entire intestinal microbiota rather than the "one-microbe-one disease" concept [12, 17, 29]. A characteristic pattern of such type of inflammation is a decrease in commensal bacteria diversity. In obesity, for example, there is an increase in the *Firmicutes*/*Bacteroidetes* ratio, which are the two most abundant groups in the normal flora [3].

The intestinal microbiota has also been considered an important component of type 1 diabetes (T1D) development [31]. However, whether it plays a protective or pathogenic role in the disease remains controversial. It has been shown that, in rodent models, diabetes is aggravated upon administration of antibiotics [32], whilst other studies have shown the exact opposite [33]. In humans, subclinical immune activation [34] and signs of impaired T reg subsets [35] in the intestine of T1D patients were found. Additionally, it has been shown that T1D diabetic patients have alterations in the epithelial barrier of the gut, the so-called "leaky gut" [36], which also indicates a possible correlation between the gut microbiota and T1D development. It has been shown that microbe-associated molecular patterns derived from the microbiota activate the host innate immune system via pattern-recognition receptors, such as TLRs and nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs) present in intestinal epithelial and myeloid cells [37]. The nucleotide-binding oligomerization domain containing 2 (NOD2) is an intracellular receptor that belongs to the NLR family and recognizes the bacterial component muramyl dipeptide (MDP), playing a role in maintaining gut homeostasis [38]. It also induces a proinflammatory immune response by myeloid cells, thus promoting immunity against a variety of viral and bacterial pathogens [39].

Costa *et al.* (2016) [40] evaluate the role of the NOD2 receptor in the pathogenesis of T1D using the multiple low doses (MLD)-streptozotocin (STZ) mouse model, and demonstrated that STZ-injected WT mice display an altered gut microbiota, *i.e.*, there is an increase of the genera *Bacteroides* (phylum *Bacteroidetes*), *Oscillospira* (phylum *Firmicutes*), *Sutterella* (phylum *Proteobacteria*) and *Bifidobacterium* (phylum *Actinobacteria*). This was accompanied by the translocation of commensal bacteria from the intestinal tract to the pancreatic LNs (PLNs) in STZ-induced T1D.

This translocation is responsible for the activation of the NOD2 receptor in myeloid cells inside the PLNs, thereby driving the differentiation of pathogenic T helper cells 1 (Th1) and Th17 cells, which in turn contributes to the pathogenesis of the disease. The WT mice treated with broad-spectrum antibiotics (Abx) were fully protected from STZ-induced T1D, which correlated with the abrogation of bacterial translocation to the PLNs, and when Abx-treated STZ-injected WT mice received the NOD2 ligand MDP, both hyperglycemia and the proinflammatory immune response were restored. Therefore, the authors demonstrate that the recognition of bacterial products by NOD2 inside the PLNs contributes to T1D development, establishing a new putative target for intervention during the early stages of the disease.

The obesity is a serious medical condition that can cause complications such as heart disease, metabolic syndrome, cancers, high blood cholesterol and pressure and even type 2 diabetes (T2D) or *vice-versa* [41, 42]. Such condition represent a major public health threat to both developed and developing countries and it is associated with low grade chronic inflammation (the link seems to be adipose tissue itself, more specifically the aggregation of macrophages) [12, 14, 41]. Ingestion of high-fat diet increases gut permeability, metabolic endotoxemia, systemic inflammation and recruitment of microglia and macrophages, and exacerbates choroidal neovascularization [17]. Evidences of inflammation in obese tissues in mice revealed increased levels of the cytokine TNF-α in adipose tissue compared with lean controls, as also an increased infiltration of immune cells into the metabolic tissues [43]. In contrast, in the lean state, immune cells in adipose tissues – primarily resident M2-like macrophages together with T regulatory (Treg) cells and eosinophils – can synthesize IL-10, IL-4, and IL-13 and help to maintain an anti-inflammatory environment that contributes to the insulin-sensitive state [30].

The control of obesity and T2D, *i.e.*, insulin resistance at the molecular level, begin in the gastrointestinal tract [11, 41]. In experiments on Swiss mice, Carvalho *et al.* (2012) [42] studied animals fed with a hyperlipid diet (with very high levels of fat), and it was seen that the mice developed insulin resistance after three days. The animals also had an increase in lipopolysaccharide (LPS) circulation and absorption by Gram-negative bacteria from the gut microbiota.

Then, the insulin (hormone produced by the pancreas that stimulates glucose uptake by cells and acts in the metabolism of lipids and proteins) could not adequately transmit its signal to cells, before the animals becoming obese –

their muscles captured less glucose after the onset of the hyperlipid diet. One of the possible keys to the development of insulin resistance in these mice prior to their becoming obese is the LPS present in the outer membranes of Gram-negative bacteria could activate the serine protein kinases responsible for serine phosphorylation of insulin receptor substrate IRS1 and IRS2, which leads to changes in the structures of these proteins such that they can no longer interact with insulin receptors (insulin resistance) and therefore tyrosine phosphorylation can not take place.

The authors confirm these findings after performing another experiment in which mice were treated with antibiotics to reduce gut microbiota, and a control group with normal gut flora, both fed with a hyperlipid diet. The Verrucomicrobia phylum, which has two Gram-negative sister phyla, *Chlamydiae* and *Lentisphaerae*, was present in low percentages in the control mice, but showed considerable prevalence in animals subjected to a high-fat dietary (0.03 % *vs*. 13.65 %). Almost all *Bacteroidetes*, *Firmicutes* and *Verrucomicrobia* were eradicated from the intestinal lumen of antibiotic-treated mice, but these changes were accompanied by a striking increase in *Proteobacteria*, which was almost the sole phylum present (97 %). Even with the high prevalence of Gram-negative bacteria (*Proteobacteria*), the reduction in circulating levels of LPS is related to the striking reduction in the overall content of bacteria in the intestinal lumen, consequently reducing its uptake, in addition to improving the intestinal barrier function. Therefore, the high fat diet modulates the gut microbiota, and this modulation caused an increase in LPS intake and consequently induced insulin resistance before the animals developed obesity.

It has been evidenced that the gut microbiota serves as a pivotal contributing factor in the development of diet-related obesity (or simple obesity, SO), *i.e.,* transplantation of gut microbiota from genetically obese animals into germ free wild type mice, confers parts of the obesity phenotype to the recipients [44]. While transplantation experiments of gut microbiota from obese mice or humans can induce significantly higher fat accumulation in recipient mice than transplantation of gut microbiota from lean donors, the removal of gut microbiota (by using cocktails of broad-spectrum antibiotics) prevented fat accumulation even in genetically obese mice [45]. Such kind of transplant in their current form is not a practical cure for obesity; there are too many risks, including the transfer of bacterial infections from donor to recipient, but these transplants do confirm the impact of bacterial composition on blood sugar regulation in humans. Despite its role in genetically predisposed obesity (GPO) in humans has not been characterized so far, the dysbiosis is similar in both SO and GPO (**Table 2**). Identifying and understanding all relevant strains in the gut microbiota can lead to the discovery of new biomarkers of predictive and diagnostic value, as well as new targets for effective interventions in humans. The beneficial microbe *Faecalibacterium prausnitzii*, in particular, is less abundant in obese patients or with Chron's disease [46]. This specie is a major butyrate-producing bacteria in the intestines [14].

Table 2. Prevalence of microorganisms in health (normal gut) and dysbiosis (obesity, autism and Chron's disease).

Condition	Microbiota
Normal gut	Presence of the Phyla *Firmicutes*, *Bacteriodetes*, *Actinobacteria*, *Verrucomicrobia* and the specie *Faecalibacterium prausnitzii*
Gut in obesity	Phyla *Bacteroidetes*, *Verrucomicrobia* and the species *Faecalibacterium prausnitzii* at reduced abundance; Phyla *Firmicutes* and *Actinobacteria* in higher proportions
Gut in autism spectrum disorder	Significant reduction of *Bacteroidetes* and increase of the bacterial genera: *Collinsella*, *Corynebacterium*, *Dorea*, *Clostridium* and *Lactobacillus*; increase of the yeast *Candida spp.*
Gut in Chron's disease	Increase of *Ruminococcus gnavus*, and decrease of *Faecalibacterium prausnitzii*, *Bifidobacterium adolescentis*, *Dialister invisus*, and an unknown of *Clostridium cluster* XIVa

The Prader-Willi syndrome is the most common known of GPO in children [28, 41]. Once it causes a wide range of symptoms including constant hunger (hyperphagia), the treatment is based in an energy-restricted diet with low level of carbohydrates, which can lead to production of toxic metabolites by the gut bacteria. To investigate how the dietary habits can influence this condition, Zhang *et al.* (2015) [11] tested changes in the diet with dramatically increased levels of non-digestible carbohydrates (whole grains, traditional Chinese medicinal foods and prebiotics) in two groups of obesity children (SO and GPO). The research proved that almost all relevant bioclinical parameters evaluated (serum glucose, total cholesterol and free fatty acids, for example) indicate a significant alleviation of the metabolic deteriorations in all children after 30 days of the dietary intervention.

Products of microbial metabolism directly affect intestinal function but may also affect the adipose and muscle tissue, as well as liver and brain [5, 46]. So, an area that still lacks much information is the origin (or mechanism of initiation) of inflammatory signaling in Autism spectrum disorder (ASD). ADS, commonly referred to as Autism describes a range of conditions characterized, in varying degrees, by communication difficulties, social and behavioural challenges, and repetitive behaviours. People with ADS often suffer from gastrointestinal problems such as inflammatory bowel disease and "leaky gut." In a recent paper, Strati *et al.* (2017) [47] characterized the gut microbiota

associated with autism, disclosing an altered microbiota community (**Table 2**). The authors observed a significant reduction of *Bacteroidetes* and abundance of the yeasts *Candida* spp. in autistic people, when compared to neurotypical subjects, being both groups submitted to a Mediterranean-based diet, and none antibiotic, probiotic or prebiotic during the three months prior to the samples collection. This altered gut microbiota can cause the synthesis of neurotoxins, which may interfere with neurodevelopment. The gut microbiota was able to modulate the immunological responses to *Candida* in the GI tract by providing tryptophan-derived aryl hydrocarbon receptor ligands that stimulate the immune system, principally ILC3 cells, to produce IL-22. Together with IL-17, IL- 22 avoids the excessive proliferation of fungal commensals in the gut (including *Candida*). It is therefore possible that alterations of the gut microbiota in ASDs could lead to an expansion of the *Candida* population preventing from full restoration of the bacterial community structure. Another group of microorganisms strongly linked to ADS is the *Clostridiales*, exactly because of the production of propionic acid, which can cross the physiological barrier between the gut and the blood and permeate into the blood-brain barrier [5].

In this way, food is a major factor that shapes the proportional representation of organisms present in the gut microbial community and its gene content [8]. So, the dietary habits are considered one of the main factors contributing to the diversity of human gut microbiota. Whole grains, for example, are concentrated sources of dietary fiber, resistant starch, and oligosaccharides, as well as carbohydrates that escape digestion in the small intestine and are fermented in the gut by a bacterial community able to use xylane, xylose, and carboxymethylcellulose resulting in the formation of short-chain fatty acids along with gases like CO_2 and H_2. In a study published in 2010, De Filippo *et al.* [44] investigated the traditional rural African diet by the characterization of the fecal microbiota of 14 healthy children from a Boulpon village (BF) in Burkina Faso. They compared it with that of 15 healthy European children (EU) living in Florence, Italy. While the diet of BF children is predominantly vegetarian (*i.e.* low in fat and animal protein and rich in starch, fiber, and plant polysaccharides) and permits them to have a rich microbiota in their guts (like strains of the genera *Xylanibacter*, *Prevotella*, *Butyrivibrio* and *Treponema*), the Italian children – with high consumption of sugar, animal fat, and calorie-dense foods – had less microbial biodiversity. In a recent paper, Lisko *et al.* (2017) [48] reported a monitoring of the human gut microbiota following dietary yogurt consumption (250 g of yogurt per day) during 42 consecutive days. Every week, the authors examined the temporal changes of GI microbial communities of five healthy human subjects – a person strongly lactose intolerant was the control – using a combination of molecular techniques, such as T-RFLP and qPCR analysis. In this study, they proved that the probiotic *Lactobacilli* survived the GI passage and the relative abundance of *Proteobacteria* decreased to 68 % (initially was 99 %) of the detected bacteria taxa, with increased abundance of *Firmicutes* and *Actinobacter*.

While specific *lactobacilli* and *bifidobacteria*, broadly used as probiotic supplements in foods, are beneficial because they involve inhibition of pathogen adherence to the mucosa (others advantages are the improvement of barrier function of the intestinal mucosa, production of bacteriocins, increase of mucosal IgA production and reduction of mucosal proinflammatory cytokine secretion), the pathogenic bacteria such as *Helicobacter pylori*, *Entamoeba histolytica* and *Pseudomonas aeruginosa* have mechanisms that allow them to invade or utilize mucus associated nutrients by reduction of mucin disulfide bonds or utilizing proteases [48]. In people with diabetes, the mucus layer is thinner than in healthy ones and inflammatory process is frequent on those patients. A decrease in the population of *bifidobacteria* species in the gut causes the tight junctions between the cells of the gut lining to loosen [49]. The loose junctions increase the gut's permeability and allow LPS from these microbes to leak through the gut wall [16]. The resulting metabolic endotoxaemia causes a low-grade inflammation and can induce a number of metabolic disorders (**Figure 2**).

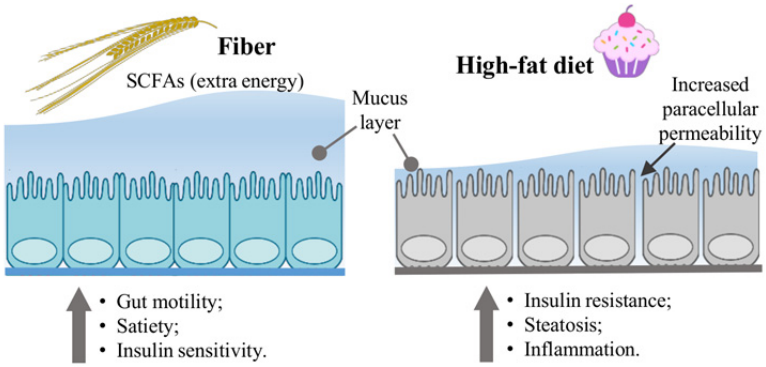

Figure 2. Characteristics of gut as consequence of dietary habits.

From the above discussion, it seems that by the same way the dietary habits can negatively alter a microbiota from gut, it is possible to stimulate the growth of healthy microorganisms, even at cases of extreme situations. It is well established that malnutrition is not caused solely by lack of food, but also by impaired utilization of the food that is ingested. As demonstrated by Subramanian *et al.* (2014) [8], the severe acute malnutrition (SAM) is associated with

significant relative microbiota immaturity in children and the condition can be partially ameliorated following two widely used nutritional interventions: Khichuri-Halwa or Ready to use Therapeutic Food (RUTF). The first one consists of the standard cereal-based foods (rice, lentils, green leafy vegetables, and soybean oil) and RUTF is a ready-to-use paste of peanut that does not need to be mixed with water. Next generation probiotics using gut-derived taxa may also be required in addition to food-based interventions [46, 48]. This is an excellent proof that diet has a dominating role in shaping gut microbiota and changing key populations may transform healthy gut microbiota into a disease-inducing entity [1, 13, 27].

6. Conclusions

The microorganisms residing in the gastrointestinal tract comprise a dynamic community and provide the host with a sort of immunologic stimuli, nutrients and vitamins. Changes in dietary habits are very important determinants for a healthy microbiota characterized by the Phyla *Firmicutes*, *Bacteriodetes*, *Actinobacteria*, *Verrucomicrobia* and the species *Faecalibacterium prausnitzii*. Currently, the functional study of gut microbial ecology uses animals such mice and zebrafish with meta-omics approach. In the future, the progress towards a functional understanding of the microbiota will permit engrafting microorganisms from healthy donors into a patient recipient to restitute microbiota within the gut ecosystem.

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