UNIVERSITY OF COIMBRA

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The Role of Gut Microbiota on Obesity Development

Dissertação realizada no âmbito do Mestrado em Biotecnologia Farmacêutica orientada pela Professora Doutora Cláudia Cavadas e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

Dissertation for the Master Degree in Pharmaceutical Biotechnology, directed by Professor Cláudia Cavadas and presented to the Faculty of Pharmacy, University of Coimbra.

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September, 2013

Acknowledgments

It is with immense gratitude that I acknowledge the help, advices, and availability of my Professor, Cláudia Cavadas. I am grateful that she has suggested so interesting and promising theme.

I would also like to thank my family and friends for their unconditional support.

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Abstract

Obesity is a disease characterized by the excess of body fat with increasing incidence worldwide. Its aetiology has a multifactorial character comprising a wide range of mechanism both at a central level and at a peripheral one, in which different organs are involved.

Recently, studies suggest that gut microbiota contributes to the development of obesity. On the other hand, there is increasing evidence suggesting that gut microorganisms and their metabolic products can influence the food metabolism, particularly the energy harvest and fat uptake, intestinal barrier function, gut hormones, as well as immunologic responses and inflammation. Thus, any modifications in gut microbiota composition can influence energy expenditure, satiety, and food intake. In addition to diverse approaches destined to improve the obesity status by controlling of the weight, the gut microbiota seems to be an important target to take into account on the treatment of obesity and related diseases, so its modulation can influence the weight gain and weight loss. A better understanding of the effect of different members of the gut microbiota upon host physiology is needed to establish these relationships.

In present dissertation it will be made a brief review of the main mechanisms that have the preponderant role in aetiology of obesity at level of different organs. It is intended to emphasize the agent that seems to have a great contribution to the progression of obesity – the gut microbiota, as well as highlight the microorganisms that apparently have the greatest impact on obesity. It will be reviewed the scientific publications that address microbiota's functions and mechanisms underlying the impact of gut microbiota on obesity development, in order to better understand the points where the possible therapeutic agents might act. Also it will be briefly presented some measures to combat obesity, including those that involve the gut microbiota.

Resumo

A obesidade é uma doença caracterizada pelo excesso de gordura corporal cuja incidência tem aumentado a nível mundial. A sua etiologia tem um carácter multifactorial, que compreende uma grande variedade de mecanismos, tanto a nível central como a nível periférico, onde os diferentes órgãos estão envolvidos.

Recentemente, vários estudos apontam para o facto de a microbiota intestinal contribuir para o desenvolvimento de obesidade. Assim, há cada vez mais provas que sugerem que os microrganismos do intestino, bem como os seus produtos metabólicos podem influenciar o metabolismo. Especialmente são influenciadas a extração de energia e a absorção de gordura, a função da barreira intestinal, as hormonas intestinais, bem como as respostas imunológicas e inflamação. Deste modo, qualquer alteração na composição da microbiota intestinal pode influenciar o gasto energético, a saciedade e a ingestão de alimentos. Estando a microbiota intestinal relacionada com a redução da massa corporal e consequentemente com a diminuição do estado de obesidade, é um possível alvo importante a considerar para o tratamento da obesidade e das doenças relacionadas. Para tal é fundamental compreender os efeitos específicos de diferentes microrganismos intestinais na fisiologia do hospedeiro.

Com a presente dissertação pretende-se fazer uma revisão dos mecanismos principais que têm o papel preponderante na etiologia da obesidade a nível de diferentes órgãos. Pretende-se dar ênfase à microbiota intestinal, bem como realçar os microrganismos que têm o maior impacto sobre a obesidade. Serão analisadas as publicações científicas que abordam as funções da microbiota e os mecanismos subjacentes ao impacto da microbiota intestinal no desenvolvimento da obesidade, a fim de identificar os pontos onde os possíveis agentes terapêuticos poderiam atuar. Também são sucintamente apresentadas algumas medidas de combate à obesidade, incluindo as que envolvem a microbiota intestinal.

Abbreviations

α-MSH	Alpha-Melanocyte-Stimulating Hormone
AgRP	*Agouti*-Related Protein
AMPK	AMP-Activated Protein Kinase
APJ	Apelin G Protein-Coupled Receptor
CART	Cocaine- and Amphetamine- Regulated Transcript
CB	Cannabinoid receptor
CCK	Cholecystokinin
FIAF	Fasting Induced Adipose Factor
GCGR	Glucagon Receptor
GIP	Gastric Inhibitory Polypeptide
GLP-	Glucagon-Like Peptide-
GRP	G Protein-Coupled Receptor
IL-	Interleukin–
LPL	Lipoprotein Lipase
LPS	Lipopolysaccharides
mRNA	Ribonucleic Acid Messenger
NEFA	Non-Esterified Fatty Acids
NF-Kb	Nuclear Factor Kappa B
NOD	Nucleotide-Binding Oligomerization Domain
NPY	Neuropeptide Y
NTS	Nucleus Tractus Solitary
PP	Pancreatic Polypeptide
PYY	Peptide YY
SCFA	Short-Chain Fatty Acid
TLR	Toll-Like Receptor
TNF-α	Tumour Necrosis Factor Aipha
ZO-1	Zonula Occludens-1

1. Introduction

Obesity is a metabolic disease, which prevalence and incidence are worldwide increasing in children, adolescents and adults [1, 2]. This disease doubled since 1980, and has reached epidemic levels [3] with particularly high rates in developed countries [1, 4]. In the last decade, the amount of affected individuals increased rather than 30% worldwide [5], reaching on average 30–35% of the general population the United States of America [6], 17% in United Kingdom [1] (or by another researchers about 25% [6]) and in the years 2003-2005 - 54% in Portugal [4]. It is predicted that by the year 2030 half of the American adult population will be obese [7]. It is particularly worrying that this obesity epidemic achieves also children and adolescent populations. The current prevalence of this disease is round about 7–10% in these age groups and it is predicted that this rate will be at least double by 2025 [6].

In 1998 obesity was declared by the World Health Organization [4] as a chronic disease of serious complications [1, 4], associated to high rates of morbidity and mortality [1, 4, 8]. For these reasons, this disease requires the attention of public health officials [4], as well as preventive measures and development of effective therapies. With the increasing prevalence of obesity and co-morbidities (diabetes mellitus type 2, cardiovascular disease [2, 9-18], certain types of cancer, and other diseases [9, 10, 13, 16-18] there are enormous economic and personal costs for its treatment [6] as well as decreased life expectancy [2, 19]. In Portugal 3.5% of health expenditures are related to obesity [4].

Obesity is defined as the excessive accumulation of body fat (adipose tissue) [12, 13] that depending on it distribution [14] may induce disease [20] due to chronic low-grade inflammation, insulin resistance [12, 18], and endothelial dysfunction [12].

A person is considered obese if she has an excess of rather than 20% of “ideal" weight, where this "ideal" weight contemplates the height, age, sex and physical constitution of each individual [11]. The body mass index is a useful measure of overweight and obesity that relates body weight to height, and reflects (in adults) total body fat mass [11, 13]. National Institutes of Health defines obesity as a body mass index ≥ 30 [1, 9, 11]. As body weight comprises the mass of muscle, bone, fat and water (in excess or not), which is reflected in body mass index [13]. Very muscular people may have a high body mass index but without risk to their health, not being considered as obese [11].

Obesity is not an alone disease. Obesity comprises diverse conditions and has multiple causes, i.e., it is multifactorial, and is influenced by genetic (including family history) and environmental factors [9, 13].

The dramatic increase in the prevalence of obese people over the past two decades is largely attributed to the characteristics of modern life, such as excessive intake of "tasty" caloric food, high in sugar and fat, as well as a insufficient physical exercise [9, 10, 17, 18, 20] and lead to disturbances in the mechanisms of energy homeostasis [8] due to imbalance between caloric intake and caloric spent [13, 20], being the excess of calories stored in the form of glycerides in adipose tissue [21]. The balance is only verified if the energy that enters into the body in the form of food is equal to the energy expended in exercise and basal metabolism, including breathing and digestion [1, 20]. The positive energy balance is the primary physiological cause of obesity, where the excess energy is reserved in the form of fat into adipocytes [1].

Central obesity [13, 22 – 25], in which the fat is accumulated preferentially in the upper part of the trunk, when compared with the peripheral obesity in which the fat distribution is mainly in hip region, is more clearly linked to metabolic disorders of lipids and carbohydrate [9], leading to the metabolic syndrome [24] that is a risk factor for developing of diabetes mellitus type II and cardiovascular diseases [19, 22, 23, 26, 27]. Thus, central obesity is linked to cardiovascular complications [14, 22, 24], such as cerebrovascular accident (stroke) [3, 23], myocardial infarction and congestive heart disease and other diseases such as gout, atherosclerosis, osteoarthritis [9] sleep apnoea [3, 9, 14], chronic kidney disease [9, 19], diseases of the gallbladder and liver [3, 9, 23], asthma, gynaecological complications [9, 23], infertility [9, 28], some types of cancer [3, 9, 19, 23] and psychological disorders [29, 30].

It is important to find new and effective ways to treat this disease, or at least to reduce the associated risks.

Although there is a daily variation in food intake and energy expenditure, the body weight remains stable for relatively long periods of time [8].

The polymorphism of the genes involved in the control of signalling pathways of hunger / satiety and metabolism, as well as mutations in several receptors of signalling molecules can predispose to obesity [9]. Thus, the genetic background determines inter-individual differences regarding energy expenditure and capacity of its storage [3, 31]. The "thrifty genotype" hypothesis suggests that people predisposed to obesity possess genes that promote the efficient storage of ingested food as body fat for use in periods of eventual

undernutrition [31]. From twin studies it is estimated that up to 80% of the variance in body mass index might be genetically determined. These genetic contributions seem to be especially important in individuals with severe or early-onset forms of obesity [32], however, the evidence suggests that also external factors contribute for the host response, as well as the intestinal microbiota has an important role (Fig. 1) [3].

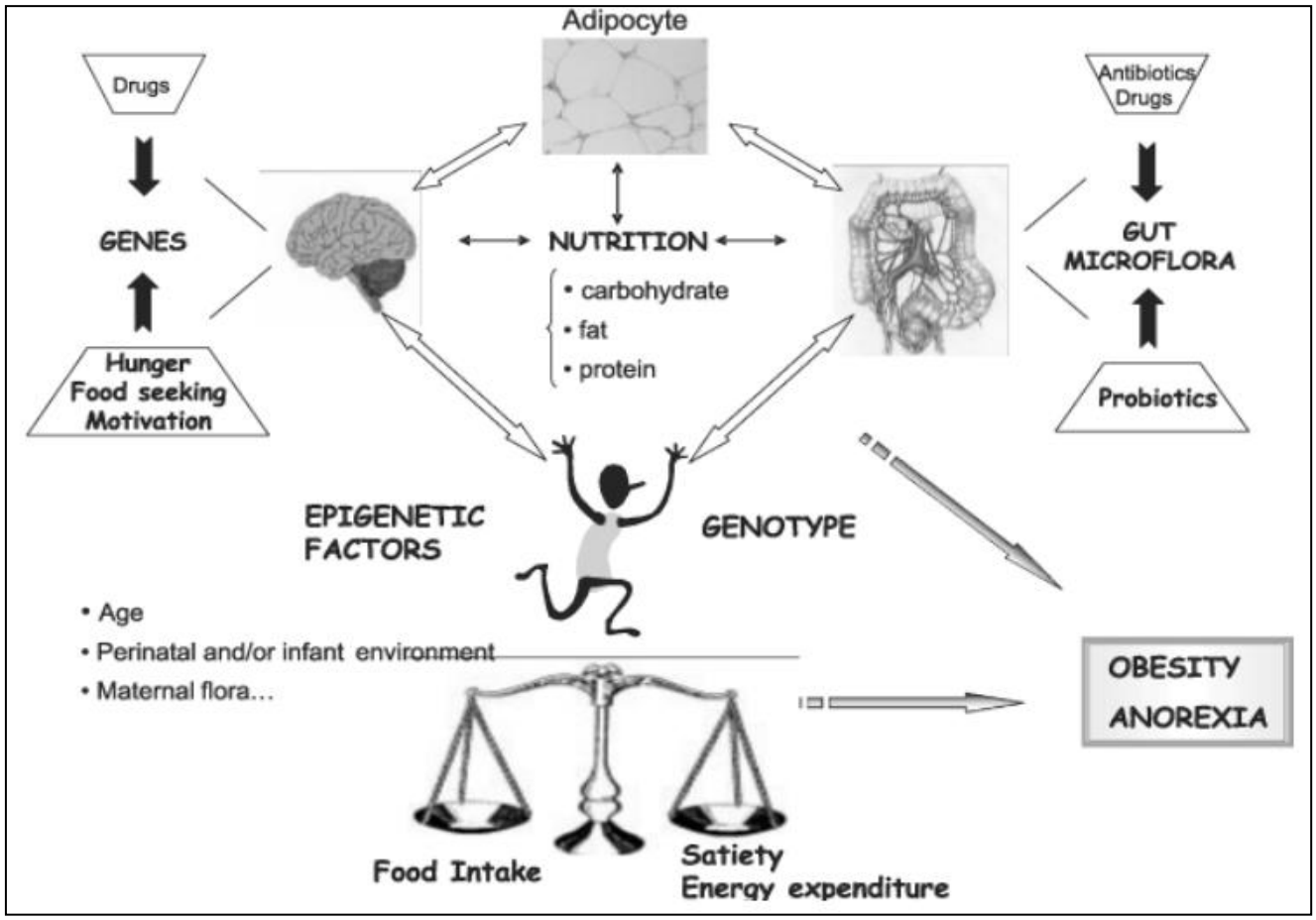

Figure 1 – Factors involved in the regulation of the control of nutrition state. Genes, gut microbiota as well as its modulating factors, type of diet, and other external factors contribute to energy homeostasis. Genes, including its epigenetic variations, in addition to drugs and psychological effects have a powerful control on energy balance. The formation and functionality of adipose cells is strongly modulated by our diet. Also, our diet can modify perfectly balanced ecosystem of intestinal commensal microbiota, which is also influenced by drugs and prebiotic agents. The set of signals from different organs is integrated and together with environment factors determine the energetic balance, which perturbation leads to metabolic disorders such as obesity [21].

A set of mechanisms for the regulation of satiety occurs at various organs such as the stomach, intestine, pancreas, adipose tissue and brain that together contribute to metabolic homeostasis.

2. Mechanisms of satiety regulation at nervous system

The regulation of food intake is very complex because it comprises the interaction of several hormones, signalling molecules from peripheral tissues and neural signals related to the intake and absorption of food that should be subsequently processed in the brain (Fig. 2).

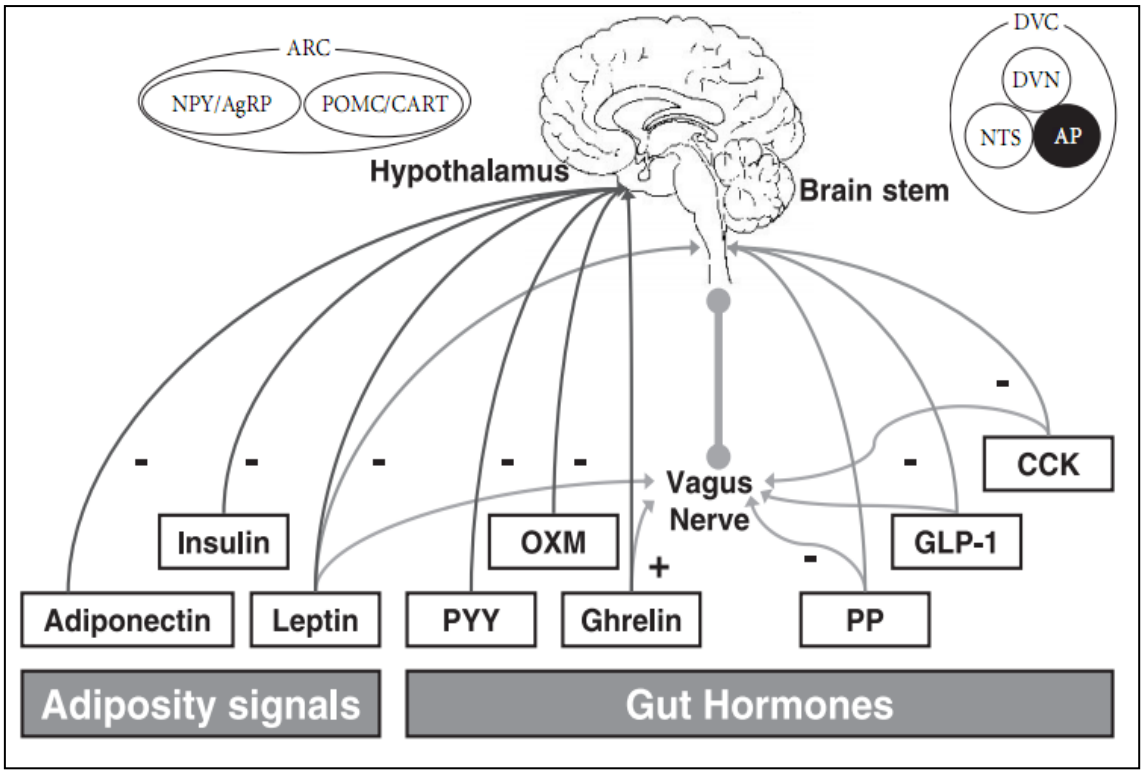

Figure 2 – Peripheral signals from adipose tissue, pancreas, and the gastrointestinal tract implicated in the physiological regulation of food intake. It is schematically represented the major signalling pathways that influence central circuits in the hypothalamus and brain stem to produce a negative (-) or positive (+) effect on energy balance, thus regulating food intake. ARC – Arcuate nucleus; NPY/AgRP - neuropeptide Y and agouti-related peptide; POMC/CART – pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript; DVC - dorsal vagal complex; DVN - the dorsal motor nucleus of vagus; NTS - the nucleus tractus solitary; AP - area postrema; GLP-1 - glucagon-like peptide-1; CCK - cholecystokinin; PP - pancreatic polypeptide; PYY - peptide YY; OXM, - oxyntomodulin [8].

2.1. Nervous system structures involved in the regulation of satiety

The brain together with the related to the gastrointestinal tract structures is essential for the regulation of appetite, energy homeostasis and body weight [8, 18, 33], and several regions in the brain involved in this regulation are closely interrelated.

2.1.1. Arcuate nucleus

The arcuate nucleus has the extreme importance in the integration of peripheral signals in appetite regulation [17]. These peripheral signals converge on the central nervous system via the activation of afferent fibres of the vagus nerve that projects to the brainstem or mainly by direct action on brain receptors mostly located in the arcuate nucleus of the hypothalamus [34]. Due to not have a complete blood-brain barrier, arcuate nucleus is thought to be the first place of the hypothalamus where the peripheral hormones act [1, 8, 34], modulating its activity.

The neurons of this nucleus extend to other hypothalamic areas, where the release of orexigenic or anorexigenic peptides adjusts the intake and expenditure of energy in order to maintain stable body weight [8]. Thus, the arcuate nucleus has two distinct populations of neurons [1, 8, 17, 18, 35], which act together to regulate the feeding behaviour [17]. Neurons activated by orexigenic molecules (such as ghrelin) are projected to the centres regulating the hunger [1], particularly to the paraventricular nucleus of hypothalamus and lateral hypothalamic area (Fig. 3) [17, 18]. In these centres, the neurons from the arcuate nucleus express neuropeptide Y (NPY) and Agouti-related protein (AgRP) [1, 8, 17, 18, 35] that exert their orexigenic effect and can also inhibit the anorexigenic neurons [1, 8]. The neurons activated by anorexigenic hormones such as leptin and insulin [1] express the cocaine-and amphetamine-regulated transcript (CART) and alpha-melanocyte-stimulating hormone (α-MSH) - the product of pro-opiomelanocortin [1, 18] and exert its anorexigenic effect by projecting to the satiety centres, such as paraventricular nucleus of hypothalamus, dorsomedial hypothalamic nucleus and ventromedial nucleus (Fig. 3) [8].

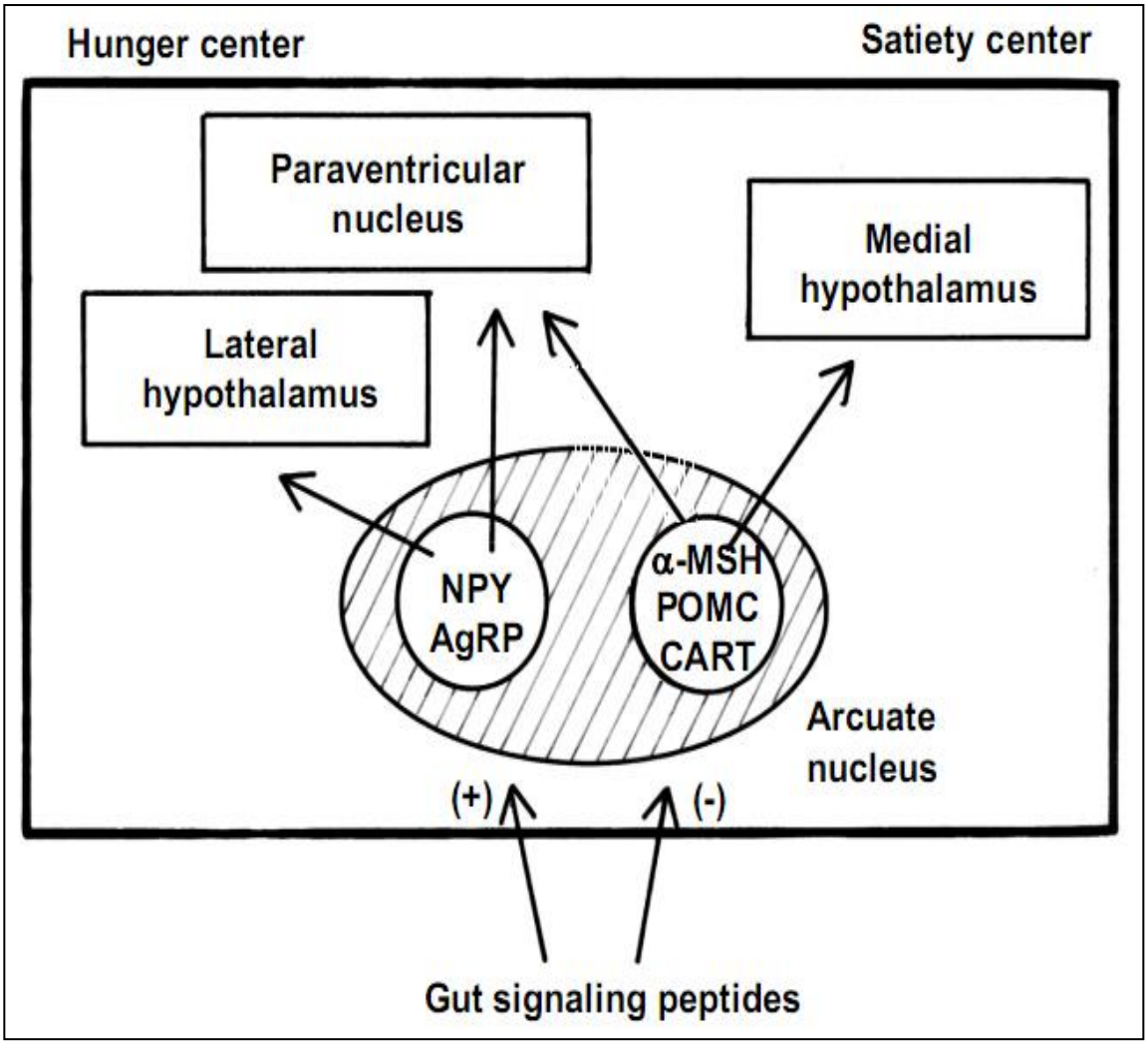

Figure 3 – Signalling of orexigenic and anorexigenic molecules in Hypothalamic Arcuate Nucleus. Orexigenic molecules signal through NPY/AgRP neurons stimulate food intake in Lateral hypothalamus or Paraventricular Nucleus and anorexigenic molecules signal through POMC-α-MSH neurons induce satiety in Paraventricular Nucleus or regions of Medial hypothalamus [1].

Thus, the arcuate nucleus of the hypothalamus integrates different signals coming from adipose tissue and gut, by altering the activity of neurons expressing NPY/AgRP and neurons expressing melanocortin/cocaine-and amphetamine-regulated transcript (CART). These neuropeptides circuits, as previously referred, project to downstream nuclei, and modulate energy intake and its expenditure to maintain a stable body weight (Fig. 4) [2].

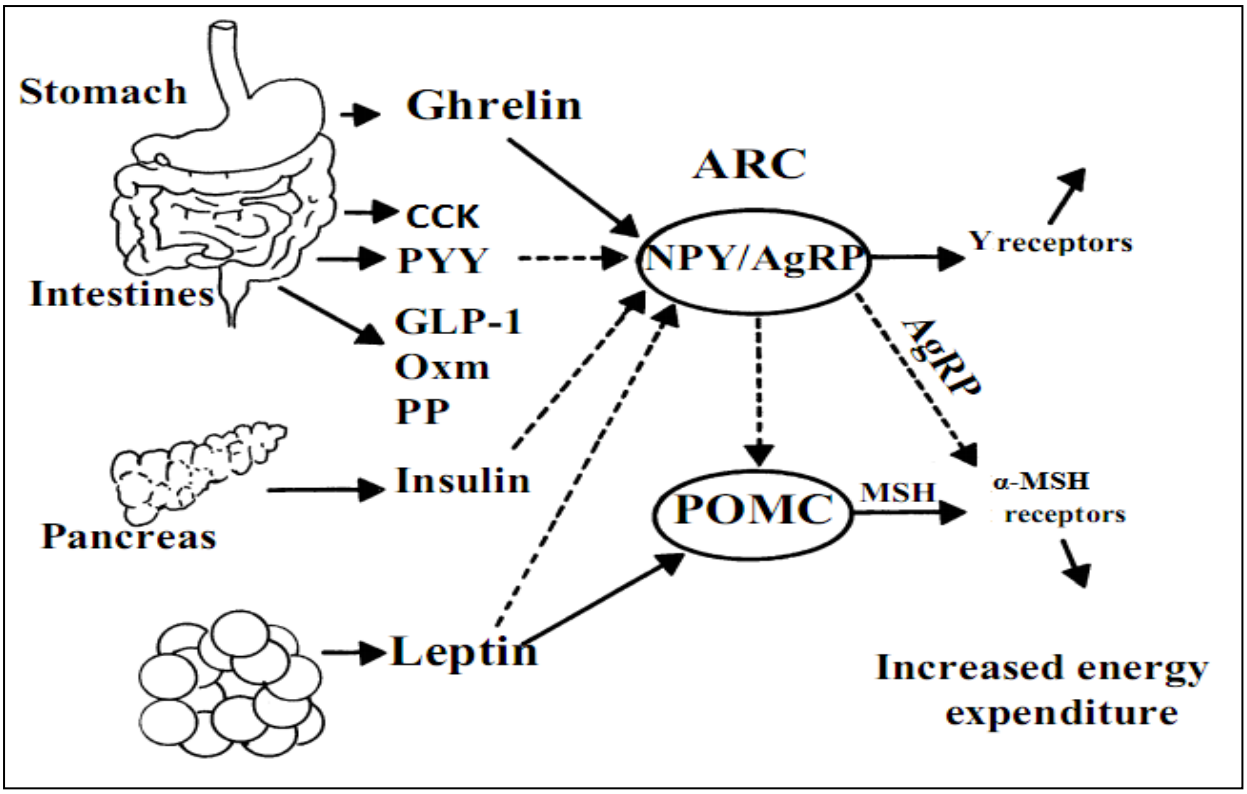

Figure 4 – Schematic presentation of role of signalling molecules on energy homeostasis. Peripheral molecules such as leptin, ghrelin, peptide YY (PYY), oxyntomodulin(OXM), glucagon-like peptide-1 (GLP-1), pancreatic polypeptide (PP), and cholecystokinin (CCK) after crossing the blood-brain barrier exercise its effect on brain regions, namely on neurons of arcuate nucleus. Thus, peripheral signals acts on activity of neurons expressing neuropeptide Y (NPY)/ agouti-related protein (AgRP) and neurons expressing melanocortin (α-MSH) / cocaine- and amphetamine-regulated transcript (CART), thus modulate the energy homeostasis [1].

2.1.2. Paraventricular nucleus of hypothalamus

The paraventricular nucleus of hypothalamus is another brain area, important for the regulation of appetite, body weight and energy balance [18], being the centre of satiety or hunger, depending on the neurotransmitters that will act on it. Melanocortin receptor agonists such as α-MSH injected into the paraventricular nucleus of hypothalamus produce anorexigenic effect leading to reduced appetite, whereas orexigenic peptides, such as NPY [1, 8, 36], and AgRP lead to food intake by the inhibition of anorexigenic pathway mediated by α-MSH [1, 8] and CART on this nucleus [1].

2.1.3. Ventromedial nucleus of hypothalamus

The ventromedial nucleus of hypothalamus is also involved on regulation of appetite, body weight [37] and energy homeostasis. Lesions of this nucleus cause hyperphagia, central hypogonadism and obesity [8]. Animals that have a poor development of this cerebral structure have deregulation of energy balance [33]. The ventromedial nucleus receives projections from arcuate nucleus neurons expressing NPY / AgRP and α-MSH [8, 18], and in turn projects its own neurons to other brain regions such as the dorsomedial hypothalamic nucleus and the nucleus tractus solitary [8]. The ventromedial nucleus is a satiety centre [1, 15], because the injection of anaesthetics in it leads the satiated animals to ingest food [11]. The changes in the transport channels of glucose in the ventromedial nucleus may be related to disturbances in the energy balance [33], given this nucleus enhances its signalling pathways after oral administration of glucose [38]. In animal models of obesity induced by cerebral lesion of ventromedial hypothalamus, an alteration of intestinal microbiota was also observed, suggesting a major impact of this compartment in the control of intestinal microbiota [39].

2.1.4. Dorsomedial nucleus of hypothalamus

The dorsomedial hypothalamic nucleus is also involved in the modulation of food intake and energy balance [8], given the lesion of its neurons results on hyperphagia and obesity [8, 18], although less dramatically than in damage to the ventromedial nucleus. The injection of orexigenic peptides, such as NPY in this nucleus enhances food intake [8].

2.1.5. Nucleus tractus solitary

The vagus nerve transmits satiety signals through afferent fibres to the brainstem, namely to the nucleus tractus solitary (NTS). NTS modulates the satiety pathway, because a neuronal activity in this nucleus was detected after a protein or sucrose postage, although different subpopulation of neurons was activated [34].

NTS controls meal size by integrating the gastrointestinal satiety signals with the signalling pathways of leptin [10, 18]. The neurons of this nucleus process information from the ascendant gastrointestinal tract, from neurons of medial hypothalamus activated by leptin and direct leptin signalling on them. NTS neurons that express leptin receptors are located in the medial region of NTS - a key zone of afferent vagal projection of gastrointestinal

system. A significant proportion of these neurons is activated by signals of satiety and intestinal distension, triggering the neurophysiologic response to produce satiety [10].

Leptin delivered to the central nervous system, when present in NTS produces a strong physiological response concerning the reduction of appetite and body weight, even with a low pronounced gastric distension. It was also shown that administration of exogenous leptin into the neurons of medial nucleus tractus solitary reduces the appetite [10].

2.1.6. Lateral hypothalamic area

The lateral hypothalamic area is called the hunger centre [1] or centre of feeding [15] because it stimulates the food intake. Damage on this area leads to decreased appetite, weight loss [15], and may eventually lead to starvation and death [8], whereas its stimulation results on hyperphagia. This hunger centre seems to remain always active, and its activity is permanently inhibited by produced satiety after food intake [1].

2.2. Signalling molecules of nervous system involved in the regulation of satiety

The way how cerebral structures interact with each other is not yet clarified [15]. However, it is believed that the nuclei involved on the energy control are regulated by neuronal circuits [8], wherein the signalling peptides [15] such as NPY, ghrelin, leptin and α-MSH that influence the feeding behaviour and energy expenditure, play an important role in this interconnection [40].

2.2.1. Neuropeptide Y

Neuropeptide Y is one of the most abundant neurotransmitter in the central nervous system [8], expressed in diverse brain centres, especially in the arcuate nucleus [18]. NPY is the most potent known orexigenic neuropeptide, and its hypothalamic levels reflect the nutritional status, being higher during the fasting state and lower after food intake [8]. Furthermore, the production of this neuropeptide in the arcuate nucleus is regulated by circadian cycle. Its concentrations are highest during the day and decrease to baseline overnight [17]. Into the hypothalamus, NPY acts upon the neurons of the paraventricular

nucleus of the hypothalamus [17, 18] and lateral hypothalamic area thus producing the response to the feeding [17].

Injections of NPY in the third ventricle or paraventricular nucleus of the hypothalamus cause accentuated hyperphagia and severe obesity. The central administration of this peptide also suppresses sympathetic nervous activity, reducing energy expenditure. The effects of NPY on feeding are mediated by the combination of several receptors [8, 18].

Under physiological conditions, neurons of the arcuate nucleus that release NPY are controlled by hormonal signals such as leptin [17, 35], insulin [17] and ghrelin [1]. The administration of insulin or leptin suppresses NPY expression and reduces the food intake [17], whereas ghrelin has the opposite effect [41].

2.2.2. Melanocortin system

The melanocortin system consists on the bioactive products resulted from the cleavage of the pre-hormone pro-opiomelanocortin [8] including alpha-melanocyte-stimulating hormone, and respective receptors [42, 43] expressed in the arcuate nucleus, ventromedial nucleus, and paraventricular nucleus of hypothalamus [8] and also consist on the leptin sensitive neurons that express NPY and AgRP [43] - antagonist of α-MSH [8, 18, 41-43].

The hypothalamic pro-opiomelanocortin expression is regulated by nutritional status, with low levels in the fasting state that are restored by the exogenous administration of leptin or approximately 6 hours after a meal. α-MSH increases sympathetic nerve activity, oxygen consumption and energy expenditure [8], being the potent inhibitor of appetite, whereas the AgRP stimulates appetite [1, 41, 42] by antagonizing the effects of α-MSH, but its expression is inhibited by leptin [41, 42].

The suppression of the leptin receptor in the AgRP and POMC neurons in mice leads to obesity, because the leptin acts on these neurons in order to maintain the body weight, although other nuclei / neurons are necessary to mediate its actions [42]. In humans, the genetic mutation or abnormal processing of POMC results in obesity due to lack of α-MSH [8].

2.2.3. Cocaine- and amphetamine-regulated transcript

The cocaine- and amphetamine-regulated transcript peptide is an abundant neurotransmitter in the hypothalamus and is involved in stress, appetite, reward,

cardiovascular function and bone remodelling. This peptide was found in brain regions that are relevant to appetite control, such as ventromedial nucleus, lateral hypothalamic area, arcuate nucleus, paraventricular nucleus of hypothalamus and nucleus tractus solitary [18, 44]. Neurons that express CART are involved in energy homeostasis, accordingly the acute food privation is compensated by the reduction of its expression in the arcuate nucleus and consequently reduces the caloric expenditure [8, 44]. The anorexigenic effect of this peptide is strongly associated with peripheral leptin that stimulates its expression [44].

It is believed that mutations in CART genes are linked to eating disorders, such as obesity and anorexia. CART injected to mice into the third brain ventricle inhibits both, normal and NPY-induced feeding [44], whereas the antibodies against this peptide increase the nocturnal feeding [8]. The intraventricular cerebral injection of the CART also reduced the food intake [41], gastric motility, secretion and emptying, decreased plasma levels of insulin and leptin, and increased lipid oxidation that limits the fat storage. However, injection of high doses of CART into some hypothalamic nuclei such as the arcuate nucleus and ventromedial nucleus increases food intake [8, 41, 44]. The different responses to the different local of administration of discussed signalling molecule are probably due to the activation of distinct neuronal circuits [44] that involve different populations of neurons [8] - orexigenic or anorexigenic.

2.2.4. Endocannabinoid system

The endocannabinoid system is the signalling system was firstly "identified" in the 1990s, and then the innate ligands have also been isolated [45], being the most widely studied endocannabinoids anandamide and 2-arachidonoylglycerol [45, 46], but several other similar endogenous substances have also been identified [46]. Endocannabinoids are endogenous lipid mediators with wide range of biological effects and potentially can be generated in all cell types - in the brain as well as in various peripheral tissues. Endocannabinoid mediators and its cellular receptors, as well as several proteins involved in its synthesis, release, transport, and degradation constitute this signalling endocannabinoid system [46]. Endocannabinoids exert their biological effects via two main cannabinoid receptors, the CB_1 and CB_2. Previously, it was thought that CB_2 receptors are mainly expressed in immune and hematopoietic cells and immunomodulatory effects was to it attributed, whereas CB_1 receptors are mainly present in the central nervous system being responsible for cannabis effect. However, it was demonstrated the presence of CB_1 receptors in peripheral tissues such as myocardium, coronary artery endothelial and smooth

muscle cells, adipose tissue, and the liver [46]. Human peripheral blood immune cells, such as lymphocytes B and T, monocytes and natural killer cells, express CB_1 and CB_2 receptors [46]. Importantly, the CB receptors expression in immune cells can be modulated by various inflammatory agents, as bacterial lipopolysaccharide, resulting in activation of these cells, with probable triggering of endocannabinoids production [46].

It has been known since antiquity that use of cannabis in its various forms increases appetite, particularly for palatable foods, and can also result in significant weight gain [47]. The discovery of endocannabinoids has raised the question of their potential involvement in the physiological control of appetite and energy metabolism.

The relation of endocannabinoids to appetite control was demonstrated by the ability of low doses of anandamide to increase food intake, when administered either systemically or into the ventromedial hypothalamus, and this effect could be attributed to stimulation of CB_1 receptor [47].

Interestingly, endocannabinoid activation of hypothalamic nuclei, such as the paraventricular nucleus, may also occur indirectly via CB_1 receptors on peripheral afferent nerve terminals, most likely located in the gastrointestinal tract [47]. Definitive evidence for the involvement of endocannabinoids in the control of food intake has been provided through the use of CB_1 receptor-deficient mice, which were found to eat less than their wild-type littermates [47].

It was shown that the absence of leptin leads to augmented endocannabinoid activity [47]. Actually, the endocannabinoids amount in the hypothalamus is higher in leptin-deficient mice and rats that lowering after a leptin administration. This fact suggests that endocannabinoids are part of the leptin-regulated neural circuitry related to regulation of appetite. Consequently, administrated leptin may similarly suppress the endocannabinoid levels, so the inverse correlation between plasmatic concentrations of leptin and anandamide in the brain regions involved in appetite control was observed [47].

3. Mechanisms of satiety regulation at pancreatic level: signalling molecules

Besides the structures belonging to central nervous system, visceral organs also play important roles on obesity aetiology and the pancreas is one of these organs.

The pancreas is the endocrine organ that in addition to secrete the pancreatic juice with digestive enzymes contributes to digestion and absorption of the nutrients. It also synthesizes hormones that play an important role for the control of energy homeostasis, including that take a part of the body's mechanism of processing and regulating the bloodstream glucose levels.

3.1. Insulin

The hormone insulin, produced by β-cells of the islets of Langerhans of the pancreas, was the first signalling molecule related to obesity described [8]. This hormone is positively correlated with the energy balance [8, 48]. Similarly to leptin hormone, insulin concentrations are depend on the body's sensitivity to it and are related to the mass and distribution of adipose tissue, being the visceral adiposity the determining factor [8]. However, in contrast to the leptin levels that are relatively insensitive to food intake, insulin secretion increases after meals [8].

Insulin plays the crucial role on biology of adipocyte. Insulin promotes adipocyte triglyceride stores, and lipogenesis, being the lipolysis inhibitor molecule [27]. On the other hand, towards the clinical manifestations of obesity, the intracellular lipids contribute to insulin resistance together with tumour necrosis factor - alpha (TNF-α) and Interleukine-6 (IL-6) produced in macrophages and adipocytes, respectively, that reduce insulin action [49]. In turn, given the fat mobilization is rapidly suppressed by insulin [27], the hypeinsulinemia as sequence of insulin resistance leads to downregulated fatty acid release [50].

Adipocytes also release the non-esterified fatty acids (NEFAs) into blood, so obese people have elevated concentrations of this non-endocrine product [49, 50]. NEFAs are the vehicle by which triacylglycerol stored in adipose tissue is transported to its sites of utilization [50]. Circulating NEFAs reduce adipocyte and muscle glucose uptake and its high levels may also contribute to insulin resistance in the muscle and liver. NEFAs also raise hepatic glucose output [49] and NEFAs concentrations are associated with insulin resistance, seen in obesity [50]. Given adipocytes lipolysis is inhibited by insulin, insulin resistance can

lead to NEFA elevation, which, in turn, induces additional insulin resistance as part of a vicious cycle [49].

On the reverse of the medal, insulin decreases the appetite and the body weight, being an anorexigenic signal on the central nervous system. Insulin circulate in proportion to body fat levels and access the hypothalamus via specialized uptake system [51], being transported across the blood-brain barrier by its receptors (saturable mechanism) in proportion to its blood concentration [8]. The insulin receptors are widely distributed in the brain, particularly in regions involved in food intake control [8, 48, 52], such as the arcuate nucleus, dorsomedial nucleus, paraventricular nucleus [8, 48] and vetromedial hypothalamic nucleus [48, 52], which neurons have insulin receptors with the insulin-responsive glucose transporter [52]. The stimulation of these regions by insulin intracerebral delivery can modulate the expression of neuropeptides that regulate food intake [8, 52]. Thus, insulin, together with hormones leptin and ghrelin (produced and released by adipose adipocytes and stomach, respectively) controls food intake and energy balance by regulating orexigenic and anorexigenic pathways by neurons in the arcuate nucleus of the hypothalamus [8, 51].

Insulin inhibits expression of arcuate nucleus neurons that express orexigenic NPY and AgRP [8, 51], and promotes derived peptide production [51]. Inhibiting of orexigenic neurons reduces the appetite, once the insulin infusion into the lateral ventricle inhibits the food intake and results in weight loss over a period of weeks [1, 8, 52]. In the other hand, the brain intraventricular administration of anti-insulin antibodies showed increases the appetite and body weight [1], being mice with the lacking neuronal insulin receptors obese and resistant to insulin actions [52]. It has been reported that intracerebral delivery of insulin can also suppress hepatic glucose production. In addition, models in which neuronal insulin receptors were specifically deleted support the role of central insulin signalling in the development of glucose intolerance and insulin resistance [52].

It will be important to identify the signalling pathways and transcription factors that could allow for such discordant actions of insulin.

Although insulin resistance is characteristic of obesity, it is not established that all of insulin's actions are impaired in individuals in these conditions. It is possible that hepatic lipogenesis and lipid storage are performed in excess in adipose tissue, whereas other insulin effects are impaired [27].

For example, the antilipolytic effect of insulin requires much lower insulin concentrations than stimulation of insulin-responsive glucose transporter. Hence, even in insulin-resistant states in which glucose transport is impaired, sensitivity to insulin's

antilipolytic effect is relatively preserved, resulting in maintenance or expansion of adipose stores [27].

3.2. Pancreatic polypeptide

The pancreatic polypeptide (PP) is a peptide mainly produced by "PP" peripheral cells of islets of Langerhans [1, 8, 19]. This peptide is also produced by the exocrine pancreas and distal gastrointestinal tract. Plasma concentrations of pancreatic polypeptide show variation throughout the day, with lower levels in the early hours of the morning and high levels at night [8]. PP release is also depended on the nutritional status – it is low in fasting state that increases with the food intake [1, 16] reaching the maximum levels after 1 up to 2 hours [16], which remains during about 6 hours [1]. The main stimulus for PP release is the intake of proteins and lipids [1], being this stimulus proportional to the caloric intake [8, 16, 35]. This polypeptide is also released during the abundant physical exercise and may be responsible for the reducing of appetite afterwards. The gastric distension and intestinal hormones such as ghrelin, motilin and secretin increase the circulating levels of PP, whereas somatostatin inhibits the release of this signal molecule [1, 8]. Plasma concentrations of PP are inversely related to obesity, being this molecule elevated in anorexic and reduced in obese subjects [8, 16]. This peptide hormone exerts its effects through specific receptors and inhibits pancreatic exocrine secretion and gastrointestinal motility [1], however, its effects on appetite depend on the route of administration. The PP has anorexigenic effect when administered peripherally and orexigenic one toward central administration [16]. Its peripheral administration showed to reduce food intake about 25% over 24 hours in human volunteers of normal weight, to diminish the rate of gastric emptying without causing resistance, and showed also to decrease body weight in animals [1, 8, 16]. At the bloodstream this peptide is not able to cross the blood-brain barrier, but exerts its anorexigenic effect through the area *postrema* which has the incomplete barrier [8, 18]. PP influences the appetite via vagal brainstem, once its anorexigenic effect is reduced after vagotomy. Thus, pancreatic polypeptide also exerts anorexigenic effects by NPY and orexin regulation [8, 18]. The peripheral infusion of PP had no influence on plasma concentrations of ghrelin, peptide YY (PYY), glucagon-like peptide-1 (GLP-1), leptin and insulin, thus being anorexigenic effect of pancreatic polypeptide independent of changes in the levels of these molecules [1]. In contrast, the PP injected centrally (in the third ventricle) increases the

appetite [1, 8, 16, 35] and rate of gastric emptying [35]. This difference may be due to activation of different receptors [16]. However, the receptors that mediate these actions and the mechanisms involved are unclear [8, 16], and it is not excluded that the effects of pancreatic polypeptide on food intake are secondary to its effect on the rate of gastric emptying [1, 35].

4. Mechanisms of satiety regulation at gastrointestinal tract level.

In addition to the central nervous system, the enteric nervous system is located in the tissue sheaths of lining of the oesophagus, stomach, small intestine and colon, and has approximately more 100 million nerve cells than the spinal cord. Smaller and less complex than the brain, the "brain" of the intestine contains from 70 up to 85 percent of the cells of the immune system of the body, being an independent central data processing that involves complex circuit of neurons, neurotransmitters and neuromodulators [21].

As mentioned above, the enteric nervous system is constantly interacting with both, adipose tissue and the central nervous system, and the regulation of food intake at central level involves enteric receptors and hormones [21].

4.1. Mechanisms of satiety regulation at the stomach

For many years it was thought that the stomach was the principal organ regulating appetite. It is now known that it is one of many organs that contribute to the physiological control of feeding. Gastric distension is a signal that controls satiety immediately and stops the food intake [15]. Its effect depends on the amount of volumetric strain, regardless of the type of the ingested food during a meal course, once this effect can also be observed as a result of artificial "feed" by using the air [15, 53]. The gastric distension stimulates the receptors that generate signal during gastric filling [15, 53, 54], reaching the tractus solitary via the vagus nerve [15, 54].

The mechanisms that control the physiological time interval between meals are unclear, but the rate of gastric emptying and signal peptides delivered by the gastrointestinal tract as a result of food intake play an important role [15].

The peptides cholecystokinin (CCK) and peptide YY inhibit gastric emptying and prolong the feeling of satiety associated with gastric distension [15, 53]. In addition to these peptides, gastric epithelial cells, although in little amount, produce another signalling molecule - leptin [15], which plays an important role in the control of satiety.

4.2. Mechanisms of satiety regulation at intestinal level

In addition to stomach, the intestine plays a crucial role in the regulation of food intake and energy balance. Furthermore, the intestine also contains a vast number of microorganisms, collectively defined as microbiota [21] that plays an important role on the mechanisms involved on obesity development.

4.2.1. Mechanic regulation

Intestinal tract can send signals that are integrated and processed in specific regions of the hypothalamus and brain stem via vagal afferent neurons that are naturally insert in the gastrointestinal tract. These signals are the response to intestinal mechanical deformation, pH, tonicity and macronutrients [55].

One of the mechanisms regulating the satiety at the small intestine is mediated by duodenal osmoreceptors that towards a high osmotic irrigation produce satiety. After the meal, these receptors promote the release of the hormone CCK where the nervous system produces its satiating action [15].

During the intestinal distension caused by meal, the receptors of the jejunum spontaneously begin its physiological activity in response to food that moves through the mucosa. These receptors respond to the mucosa stimulation, and send the signals to the connection of satiety centres in the brain [17].

Indeed, the vagus nerve has a direct effect on the small intestine, influencing the ingestion of the food, given its removal (vagotomy) suppresses the effects of satiation of feeding in rabbits and rats by itself [17], and also suppresses satiating activity of cholecystokinin and of intestinal mechanoreceptors [8].

4.2.2. Intestinal signalling molecules

The gastrointestinal tract is an endocrine organ that produces a great number of hormones that play important roles in several control pathways over alimentation.

4.2.2.1. Cholecystokinin

The cholecystokinin is a peptide widely expressed throughout the gastrointestinal tract, being present in greater amounts in the duodenal and jejunal mucosa [1] and also in the peripheral nerves and brain neurons [8]. CCK is a neurotransmitter that regulates the

behaviour of the recompense, anxiety, memory, and satiety with its receptors distributed in vagal afferent nerves. CCK was the first peptide shown to inhibit food intake in animals and humans [1, 15, 56]. This molecule is locally quickly released after the ingestion of food entering into the bloodstream. Although CCK plasma levels remain elevated for 5 hours after the releases [8], this molecule has a short-term effect that does not surpasses some minutes [8, 53], which confirms the fact that CCK is released in high amounts. This peptide increases intestinal motility [8], stimulates the release of pancreatic enzymes and bile [8, 54] and inhibits rate of gastric emptying [8, 15, 53]. On the other hand, CCK reduces meal duration and the ingested amount of the food in humans and animals [8, 18, 54] thus potentiating the effect of gastric distension [8, 15]. This peptide when administered intracerebrally inhibits food intake in animals [15]. The effect of CCK on food intake and body weight may also result from its interaction with other molecules involved in obesity control, such as leptin that synergistically increases the satiating effect of this signalling molecule [8, 56].

4.2.2.2. Peptide YY

In addition to cholecystokinin, peptide YY is another signalling molecule that controls food intake at gastrointestinal tract level for the short period of time [1, 8]. PYY is released from intestinal L-cells after food ingesting and is present in increasing concentrations in distal portions of intestine - mucosa of the ileum, colon [1, 8, 53, 41] and rectum [1, 53, 54]. Its release depends on the intake of calories, reaching the concentration more elevated up to a maximum of 1 to 2 hours after the meal, which remain elevated for some hours [8, 41]. The food composition influences the circulating levels of PYY - intake of food reach in fat causes a greater increase of plasma concentrations of this peptide compared to proteins or carbohydrates [1, 8, 41]. Circulating levels of this polypeptide are also influenced by other signals. The gastric acid, the CCK, bile salts, and bombesin increase its concentration and glucagon-like peptide-1 decrease [1, 8], whereas gastric distension has no any effect on the circulating levels of PYY [8]. Peripheral administration of PYY inhibits gastric motility [54] and slows the rate of gastric emptying, as well as gastric and pancreatic secretions [1, 8] and increases intestinal absorption of fluids and electrolytes. PYY administered peripherally is effective in reducing of food intake and weight control in animals and humans [1, 8, 54]. Intravenous administration of PYY reduced about 30% the subjective hunger and food intake in individuals of normal weight. PYY passes through the blood-brain barrier and inhibits the activity of more than 90% of the neurons of the arcuate nucleus [8] in particular activity of

neurons that express agouti-related protein [1] and NPY, and this anorexigenic effect is mainly mediated by inhibition of neuronal Y2 receptor [1, 18, 41]. PYY activates anorexigenic neurons of the arcuate nucleus that express POMC and reduces neuronal orexigenic activity of NPY, thus reducing appetite [8, 41]. The peripheral administration of PYY reduces plasma levels of orexigenic pre-prandial ghrelin [1, 8]. However, the anorexigenic effect of PYY depends on stress. Both stress and PYY alter the food intake, but when the appetite is inhibited by stress, no additional appetite inhibition occurs with the administration of this polypeptide [8, 35]. Contrary to peripheral administration, the central administration of this intestinal molecule increases the appetite. Its injection into the third, fourth and lateral cerebral ventricles, hypothalamic paraventricular nucleus or into the hippocampus stimulates the food intake in rodents [1, 8], by increasing expression of NPY / AgRP neurons in the arcuate nucleus. The discrepancy in the effects between these two routes of administration of PYY is not yet explained [1].

4.2.2.3. Ghrelin

Ghrelin is produced and released predominantly by gastric cells [7, 51, 54] and also by duodenum [7, 8], ileum, caecum, and colon [8]. At the peripheral level, ghrelin stimulates the release of stomach acid and gastrin [1, 54]. This hormone has also gastro-protective activity against various irritants [1]. This hormone has strong orexigenic properties [1, 8, 18, 48, 54] and increased spontaneous food intake [8, 48, 51].

Ghrelin signalling pathway is mediated by arcuate nucleus neurons [51], particularly by enhancing activity of ones that express the orexigenic neuropeptides NPY / AgRP [51, 54] and by reducing the activity of others anorexigenic neurons that express pro-opiomelanocortin [51].

The ghrelin effect of promoting appetite by its peripheral and central administration [48, 51] has been attributed to stimulation of AMP-activated protein kinase (AMPK) enzyme that is activated during fuel deficiency to promote catabolic and inhibit anabolic pathways. Actually, hypothalamic expression of the active form of AMPK or its pharmacological activation in hypothalamus increases food intake and expression of AgRP and NPY mRNA. However, the downstream signalling in hypothalamic neurons, capable of integrating anorexigenic and orexigenic signals through AMPK pathways to mediate control of orexigenic neuropeptides is unclear [51].

Furthermore, ghrelin increases the sensation of hunger via the central nervous system by stimulating the release of human growth hormone that has a potent action on the

increasing of appetite and increasing of body mass [54]. Plasma levels of ghrelin are regulated by food intake, and increased just before each meal [8, 48]. People with fixed mealtimes have higher ghrelin levels in fasting state that rapidly decrease after a meal [1, 8] and that turn to increase after the gastric emptying before the next meal [1]. A rise before each meal and a rapid decrease after eating, supporting a role of ghrelin in meal initiation [48] The postprandial reduction of ghrelin is regulated by caloric intake and by circulating nutritional signals such as glucose, not having, however, the gastric distension any effect on ghrelin levels, given the water intake does not inhibit the release of this hormone [8]. However, in obese individuals the levels of ghrelin do not decrease in the postprandial period, which leads to the continuous food intake and aggravates the situation. Anorexia and diet-induced weight loss in obese individuals increased plasma ghrelin levels [8, 48], which turn to decrease after weight gain [8]. In rodent, ghrelin also increases food intake and adiposity when administered exogenously [7].

4.2.2.4. Glucagon-like peptide-1

The glucagon-like peptide-1 is a hormone of very short half-life [8, 54] that potentiates insulin biosynthesis and release [8], and, in the other hand, reduces the postprandial glucagon secretion [41, 54] to keep postprandial blood sugar levels stable [1]. GLP-1 is produced and released by L-cells of the mucosa of the ileum and colon in response to food ingestion [1, 8, 41].

The pattern of GLP-1 secretion is biphasic with an initial rapid rise which occurs 15 up to 30 minutes after the meal ingestion, and another peak of secretion occurs a little later as a result of direct interactions between nutrients and L-cells. The composition of the meal results in a different release profile of this peptide. Meals rich in sugar and fat exerted more potent stimulation of the secretion of GLP-1 while protein-rich meals did not stimulate the release of this peptide so markedly [57].

The levels of circulating GLP-1 are inversely proportional to body mass [8], and obese people have lower concentrations of this peptide [1]. GLP-1 inhibits food intake [1, 8, 41] and decreases the rate of gastric emptying [1, 41]. Obese people have a rapid rate of gastric emptying and low levels of GLP-1 [1, 54], the satiety in these individuals is less pronounced and the beginning of the next meal is early [1]. This fact contributes to the progression of obesity disease. The replacement of levels of GLP-1 can restore the satiety, because despite of the obese people have the lower levels of GLP-1 they remain sensitive to its anorexigenic action [8].

The activation of the intestinal cells that produce GLP-1 leads to improved blood glucose levels and insulin response, reduces fat mass and participates in satietogenic effect of the prebiotics [3].

4.2.2.5. Glucagon-like peptide-2

Glucagon-like peptide-2 (GLP-2) is peptide produced by the mucosal L-cells of the intestine and specific neurons located in the brainstem, which actions are mediated by GLP-2 receptors. These receptors are expressed in the gastrointestinal tract cells, in the neurons of the enteric nervous system and in the central nervous system at hypothalamus and nucleus tractus solitary. The main stimulus for the GLP-2 release is the nutrients presence in the intestinal lumen, particularly fats and carbohydrates [58].

GLP-2 affects the gastrointestinal motility in humans and rodents - it inhibits gastric emptying. However, the importance of GLP-2 upon the food intake control remains unclear. Although GLP-2 is able to decrease food intake in rodents towards the central administration, it seems to not affect the food ingestion after peripheral administration in rodents or avian species, probably due to the activity of GLP-2 degrading enzymes [58].

Glucagon-like peptide-2 is a growth intestinal factor with anti-inflammatory activities that stabilizes the intestinal barrier function [59]. On the other hand, an excessive secretion of GLP-2 that is co-secreted with the GLP-1 [3, 60] is implicated in systemic low-grade inflammation [3]. An increased production of endogenous Glucagon-like peptide-2 has been associated with enhanced mucosal barrier function by restoring the tight junction protein expression and its distribution [60].

4.2.2.6. Gastric inhibitory polypeptide

In 1886 it was showed that olive oil added to food inhibited rate of gastric emptying and acid secretion. In 1930 this mixture was proposed to induce the release of a compound that was named as "enterogastrone", from the small intestine and gastric acid secretion and gastric emptying could be inhibited by intravenous infusion of extracts of intestinal mucosa. Later, this factor was isolated from the duodenum and jejunum [31], and was found to be produced in specific endocrine cells named K-cells [5, 31]. Based on its effects the name "gastric inhibitory polypeptide" was proposed in 1971. The gastric inhibitory polypeptide (GIP) is one of the incretin hormones, composed by 42 amino acids [5, 31], and is released from the duodenum and jejunum in response to ingestion of a meal containing glucose or fat

and potentiates glucose induced insulin secretion. GIP is also referred as glucose-dependent insulinotropic polypeptide [5, 31], therefore, the principal action of GIP is the stimulation of glucose-dependent insulin secretion [5].

Probably, GIP may be involved in type 2 diabetes mellitus and obesity. Plasmatic concentrations of GIP are elevated in obese and diabetic humans and also in leptin deficient obese rodents [32]. In fact researchers showed that inhibition of GIP prevented insulin resistance and obesity induced by high fat diet [31]. The authors propose a model for overnutrition in which excessive fat intake leads to hypersecretion of this insulinotropic peptide, increased nutrient uptake into fat cells resulting in obesity [31].

4.2.2.7. Oxyntomodulin

The oxyntomodulin is a peptide released postprandially in the distal intestine [1, 35] by the same cells that release GLP-1 (from the L-cells of the gut) [16, 18] in proportion to caloric intake [8, 16] and is also released the brain [1]. The levels of oxyntomodulin have diurnal variation with low levels in the morning and high at night [8]. Oxyntomodulin decreased the gastric secretion and emptying rate, the pancreatic secretion and intestine uptake of glucose [1]. Furthermore, oxyntomodulin suppresses appetite and food intake in rats and human healthy volunteers [1, 8]. These actions of oxyntomodulin similarly to GLP-1 may be mediated by GLP-1 receptors expressed in the hypothalamus, lung, heart, pancreas, gastrointestinal tract and kidney [1, 8, 16, 18, 35]. Although oxyntomodulin inhibits food intake in rodents and in human, the exact signalling pathways mediating its anorectic action remain uncertain. Oxyntomodulin has a weak affinity for both the GLP-1 receptor and the glucagon receptor (GCGR) [1, 8, 61]. It has been proposed that oxyntomodulin modulates energy homeostasis just trough the GLP-1 receptor agonism, given the acute anorectic effects of this peptide was annulled in rodents towards the antagonist co-administration for this receptor [61]. The additional body weight loss observed during the study in which GCGR was blocked the during the oxyntomodulin infusion was, at least in part, due to activation of the GCGR. Although oxyntomodulin acts as GLP-1 receptor agonist, the weak affinity of oxyntomodulin for GLP-1 receptor compared to its cognate ligand cannot explain the anorexic effect of oxyntomodulin. Other effects of oxyntomudulin including its improvement of β-cell function and stimulation of heart rate and energy expenditure appear to be independent of GLP-1 signalling pathway [61]. Besides, it have been suggested that this peptide can also suppress plasma levels of ghrelin [1, 8]. In fact, the peripheral administration of oxyntomodulin reduces plasma concentrations of ghrelin in 44% of human and 20 % of

rodents [8], thus suggesting that this peptide may play a physiological role in the regulation of energy balance [8, 16].

4.2.2.8. Bombesin

Bombesin is a peptide widely distributed in the mammalian intestine [8, 15], which plasma levels markedly increase after the feeding [8]. This peptide mediates responses, such as inhibition of gastric emptying and inhibition of food intake [62]. This peptide may act as a neurotransmitter and stimulates the release of cholecystokinin [15]. However, its satiating effect is not only due to the release of CCK, given peripheral injections of bombesin [63] or central ones [8] reduce appetite via the vagus nerve and also via the activation of visceral afferent fibres [62, 63] in an independent way of the effects of cholecystokinin [8]. Actually, the vagus and the visceral afferent fibres that communicate with the feeding brain areas are necessary to induce the reduction of meal size and to prolong the intermeal interval by bombesin. In addition, one-tenth of dose of bombesin is necessary to reduce meal size and prolong the intermeal interval when administered into the left gastric artery that supplies the stomach, or into the cranial mesenteric artery that supplies the intestine when comparing with intraperitoneal administration. Thus, additional researches would be necessary to elucidate a potential neurocrine or an endocrine f action of this peptide [62].

5. Mechanisms of satiety regulation at adipose tissue - signalling molecules

For many years it was thought the adipose tissue had the unique function of energy storage. Today it is known that adipose tissue is an active endocrine organ [8, 21, 64, 65] that produces several hormones (adipokins) such as leptin, adiponectin and resistin, integrates multiple signalling pathways [64, 65].

Adipose tissue consists of mature fat cells adipocytes, small blood vessels, connective tissue, nervous tissue and pre-adipocytes in different stages of development. Adipose tissue consists on about 50-70% of adipocytes, 20-40% of the stromal vascular cells (which include pre-adipocytes, fibroblasts, and mesenchymal stem cells) and 1-30% of infiltrated macrophages [66].

All of these components work as an integrated unit, which allows the accumulation of fatty acid into adipocytes after a meal and aliment distribution for the whole body between meals [21]. The excess of adipose tissue mass is the result of an increase of the size of existing fat cells due to lipid accumulation, and also increased number of adipocytes [21, 49] due to the proliferation of its precursors - pre-adipocytes. Thus, due to environmental factors such as the nutritional state, the new adipocytes are generated lifelong. Thus, although the number of adipocytes present in an organism is largely determined by the process of adipogenesis - formation of mature adipocytes from precursor cells, the base number of adipocytes is established during the adolescence [21].

The increase of adipose tissue mass in obesity, in addition to the single increase in number and size of adipocytes [21], which become to be producers of inflammatory cytokines [66], is accompanied by a progressive infiltration of macrophages [21, 64, 66].

Under normal conditions, the adipocytes store lipids and regulate metabolic homeostasis, and may release the lipids that modulate inflammation and participate in the neutralization of pathogens. The macrophages in its function of immune cells shall care about the elimination of pathogens and secrete inflammatory cytokines and chemokines thus participating in the inflammatory response [66].

Under certain conditions (for example, overeating and obesity), in addition to macrophages, the adipocytes, which co-locate the adipose tissue, also participate in innate immune response [1]. Pre-adipocytes act as cells of immune system exhibit phagocytic and antimicrobial properties, and differentiate to macrophages [66]. These accumulated macrophages are the mainly responsible cells for the regulation of genes related to inflammation, such as TNF-α or interleukin-6 that provides pro-inflammatory condition to

adipose tissue [21]. Thus, adipocytes, pre-adipocytes and macrophages express the same cytokines (i.e. tumour necrosis factor - alpha, interleukin-6) [66].

As it referred before, the obesity is characterized by a low-grade inflammation of unclear origin [57]. Inflammation can result from infiltration of macrophages in diverse organs such as adipose tissue, liver and muscle, which promotes the secretion of pro-inflammatory factors. However, the exact role of macrophages and the source and type of triggering factors of the immune response in this specific context will continue to be an issue to discuss [60]. Nonetheless, it is known that the metabolic pathways are functionally integrated with immune system and its responses, and the relevance of role played by the innate immune system in the pathogenesis of metabolic diseases is increasingly recognized. It could be observed by the example of mice fed with a high-fat diet, in which an activation of macrophages resident in the liver promotes hepatic resistance to insulin and glucose intolerance. The selective depletion of these immune cells without affecting macrophages of adipose tissue restores insulin sensitivity and improves (decreased) liver fat accumulation [67].

5.1. Leptin

One of the most important peptide hormones of adipose tissue is a leptin. Leptin has several neuroendocrine and immune functions and is responsible for controlling appetite, body weight over the long term, adiposity and energy homeostasis [8, 40]. The most part of leptin is secreted by adipose tissue, and the rest of this peptide is produced by the gastric epithelium [1, 8] where it protects the gastric mucosa against diverse topic irritant and ulcer agents acting at least in part, by increasing the flow of gastric mucosal blood [1]. Circulating levels of leptin are correlated with the degree of obesity and food restriction leads to decrease in its concentrations [8, 68] that can be reversed by food intake or insulin administration [8]. The increase in postprandial circulating leptin has origin in the stomach [40]. The leptin crosses the blood-brain barrier via receptor that modulates its bioavailability and activity, and then acts in the medial hypothalamus [8, 40]. The leptin binds to leptin receptor in POMC neurons and in NPY/AgRP neurons in the arcuate nucleus [68, 69, 70]. Thus, leptin stimulates α-MSH and coordinately inhibits AgRP in order to regulate food intake [40]. The absence of leptin has significant effects on food intake, body weight and endocrine function leading to hyperphagia, obesity, neuroendocrine and immune disorders in mice which may be normalized by its administration [8]. Similarly, leptin deficiency in

children and adults leads to severe obesity and hypogonadism [8] and defects on its receptor change body weight and endocrine function, also leading to early-onset hypogonadism and morbid obesity [8, 68]. Nonetheless, obesity and hypogonadism provoked by defects on leptin receptors are less severe than one in subjects with very low plasma levels of this peptide [8]. Despite of mentioned above, obesity is characterized by resistance to leptin actions, given only a small proportion of obese animals and human are leptin-deficient. Most of these individuals have high leptin plasma levels but without suppressing appetite [1, 8, 49, 69], contributing reduced sensitivity for the aetiology of this disease. The resistance to this signalling compound results from both, its poor transport across the blood-brain barrier, and from the defects on signalling in leptin-responsible neurons [1, 8]. The exogenous leptin reduces plasma levels of ghrelin and *vice-versa* [1].

Moreover, some data suggest that leptin regulates intestinal microbiota. The molecular pathways that correlate this molecule to microbiota are unknown, but evidence suggest that leptin action would be performed via it central effects or its via the induction of obesity [39].

5.2. Adiponectin

Adiponectin is another protein secreted by the adipose tissue [4, 71] with 1000 fold higher plasma concentrations than insulin and leptin [8]. Adiponectin plasma concentration is inversely proportional to the stage of obesity [4, 71, 72] and is significantly increased after weight loss induced by caloric restriction [8, 72] or gastric surgery [8]. The function of adiponectin is not fully understood but is thought to be involved in regulating of energy homeostasis, mediated by the hypothalamus. Thus, adiponectin seems to increase energy expenditure and weight loss [4] and its reduced levels may contribute to the pathogenesis of obesity [8, 71].

5.3. Resistin

Resistin is a cytokine produced by macrophages [65, 73, 74] that infiltrate the adipose tissue [25, 34] and stimulate the secretion of adipokins [73], but according to some authors, this cytokine can also be produced by the adipocytes [8, 75]. Resistin contributes to insulin resistance obesity and diabetes [8, 74]. Although its role in the pathogenesis of obesity

disease remains to be defined, in humans resistin circulating levels decrease after weight loss [8].

5.4. Apelin

Apelin is also synthesized and secreted by adipocytes. Apelin and mRNA of its receptors are widely expressed in different tissues [76]. This peptide has been proposed as the new key involved in the regulation of diverse physiological functions [64], both, in the central nervous system and in the periphery [64, 76]. This peptide plays a key role in the cardiovascular system acting on the cardiac contractility, blood pressure, vessel formation and cells proliferation [64]. Different studies have also indicated an emerging role of apelin in energy metabolism. A central administration of this bioactive molecule was shown to reduce food intake in rodents, but other reports have demonstrate the contrasting effects, however, apelin showed to be up-regulated in obese humans and mice [76] Serum levels of molecule in question are related to nutritional status and plasmatic levels of insulin in rodents and humans. Furthermore, apelin plasmatic concentrations are elevated in obese and hyperinsulinemic people and mice when compared to lean individuals [64, 76]. Interestingly, apelin has been shown to controlling the glucose homeostasis [64].

It has been suggested that the inflammation may participate in the apelin production and modulation of expression of its receptors, although the mechanisms of regulation of apelin are not fully understood [64].

6. Brain-gut axis

As previously described, there are many structures, mechanisms/pathways and molecules involved on feeding regulation. The schematic summary of the principal components discussed is present on figure 5.

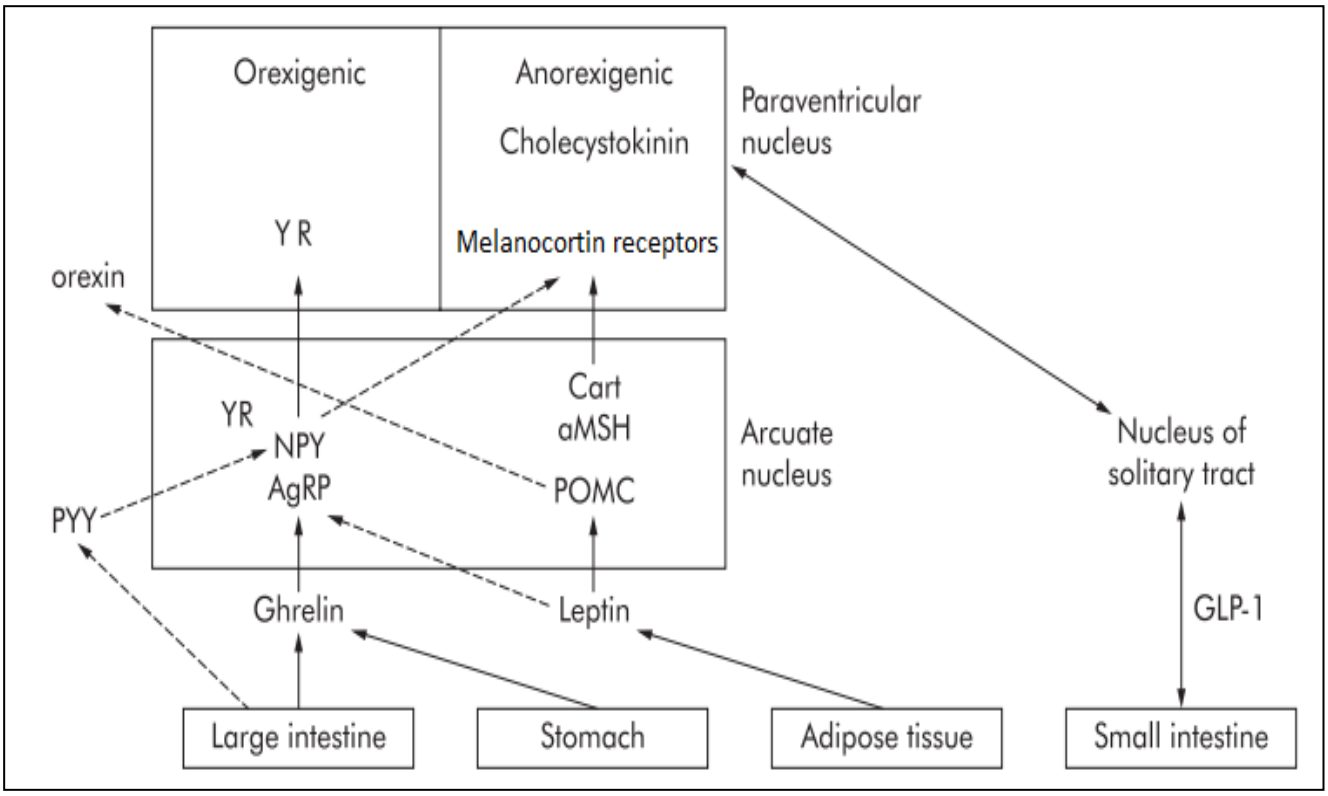

Figure 5 – A schematic representation of the complex interrelation between peripheral and central (hypothalamic) signalling molecules for regulation of feeding. Orexigenic pathways stimulation results in food intake and stimulation of anorexigenic pathways leads to satiety. Continuous lines represent stimulating pathways, dot lines - suppressive actions. NPY - neuropeptide Y; AgRP - agouti related peptide; POMC - pro-opiomelanocortin; PYY - peptide YY; YR - postsynaptic NPY receptors; Cart – cocaine- and amphetamine- regulated transcript; GLP-1 glucagon-like peptide-1 [55].

These pathways are stimulated or inhibited in function of diverse factors such as genetic, type of alimentation, among others, as it was yet mentioned and it is now known there are a number of physiological and environmental predispositions subjacent to the "traditional" unexplored risk factors for obesity and associated metabolic disorders, such as gut microbiota. The development of obesity is the continuous interference between the intestine and brain [77]. Commensally microbiota plays an important role in the

communication made between the gastrointestinal tract and brain structures through different mechanisms that involves the participation of diverse signalling molecules, and contribution of this microbiota to the brain and gut communication has been increasingly valued [78].

The brain-gut axis is a bidirectional communication between the complex central nervous system and the gastrointestinal tract, and that is vital for the maintenance of homeostasis conditions (energy balance) [77, 79]. The “human microbiota" modifies this bidirectional communication because microbiota is very early introduced in the life of its host [79]. The interaction between gut microbiota, gut and central nervous system is named as "brain-gut-enteric microbiota axis" [78, 79]. The brain-gut-enteric microbiota axis includes the central nervous system, neuroendocrine and neuroimmune systems, the sympathetic and parasympathetic divisions of the autonomic nervous system, the enteric nervous system and gut microbiota [77]. Therefore, the human microbiota complicates the system of control of energy balance, which is already one of the more highly integrated and complex systems of our body [79].

The importance of the role played by the commensally microbiota, in our overall well-being and in the brain-gut axis, is increasing [77, 80] (Fig. 6).

It has been demonstrated a link between gut and brain development, once the axenic mice exhibited an increased motor activity and reduced anxiety, when compared with mice with a normal intestinal microbiota but free of specific pathogens [39, 80]. In this context, the intestinal microbiota has recently been proposed as an environmental factor responsible for weight gain and altered energy metabolism that accompanies the obese state. Vagus nerve is also involved in the direct communication between brain and gut microbes [77, 79], since this nerve can be stimulated by bacterial products such as endotoxin, or by inflammatory cytokines - interleukin-1β and TNF-α [81].

Gaining a better insight about the link between the gut microbiota and host metabolism, and the elucidation about specific bacterial phyla, genera or species related to the development of fat mass can open the door to new therapeutic strategies and find a new target for the treatment of obesity and associated morbidities [1, 3].

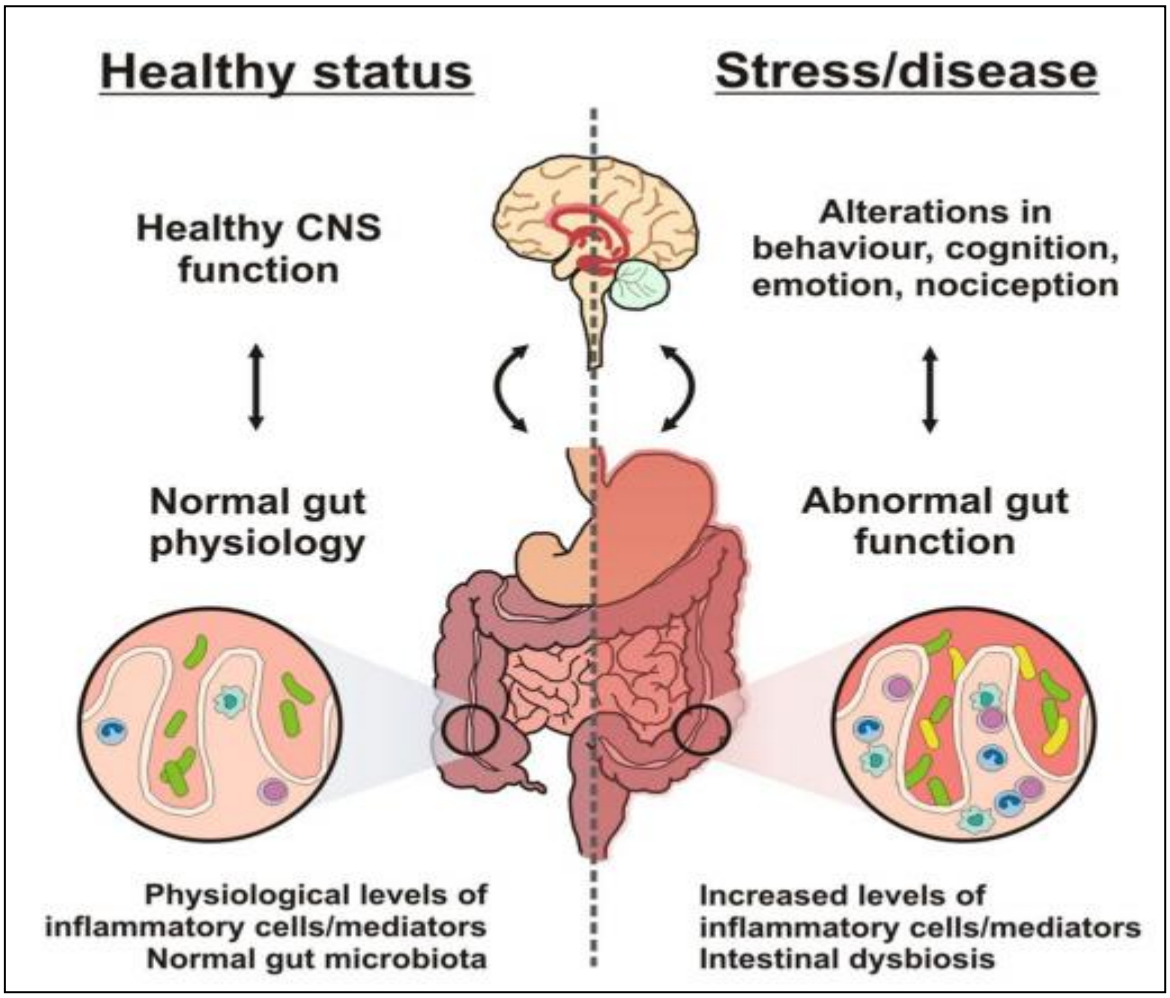

Figure 6 – Brain-gut-enteric microbiota axis communication in health and disease. A healthy gut microbiota is essential for normal intestinal physiology and appropriate signalling along the brain–gut axis. However, intestinal dysbiosis can adversely influence gut physiology leading to inappropriate brain–gut axis signalling and associated consequences for central nervous system functions and disease states. In turn, tress at the level of the central nervous system can also impact on gut function and lead to perturbations of the microbiota [12].

7. Human gut microbiota: global vision

The composition of the intestinal microbiota and the exact role of different microorganisms in the intestine are still poorly defined [64]. However, gut microbiota is being increasingly recognized as an important factor that links genes, environment and immune system, once about of 50% to 55% of the human genome has evolved in a close relationship with the microbial community, generating a strong genetic dependence [39, 67].

The microbial community can be seen as a "metabolic organ" that consists on different cell lineages, which are communicating by signalling ways of cell-to-cell, cell-to-host and self-repair and which are extraordinarily adapted to our physiology [66, 81].

The gut microbiota is nourished by nutrients with changes on its ecological structure that was ingested absorbed by its host. Every microorganism that lives inside its host interacts with other present microorganisms in the same habitat. Therefore, the excess or lack of nutrients can alter the metabolic activity of a given bacterium, which will produce excessively or not produce at all the essential metabolites or deleterious ones to the neighbouring bacteria [39].

7.1. Gut microbiota studies

The knowledge about the species and function of the human gut microbiota is increasing, but it is still based on few studies and little is known about its variation across the world [82]. Gut microbiota was extensively studied by culture-based methods [83]. However, the conventional culture techniques can detect only 30% of total intestinal bacteria [67], because most of phylotypes have no representative that could be cultivable [21]. In addition, a lot of the intestinal dominant microorganisms cannot be put into culture medium [21, 84] due to different reasons, including reduced selectivity of growth medium, unknown needs for bacteria growth, stress imposed by culture processes, the need of completely anoxic conditions and the difficulty in simulating the interactions of bacteria with each other and with the host cells [67].

Recently, methods of characterization of microbial diversity that are not dependent on growth cultures have considerable technological advances [64, 83]. These approaches are based in more facilitated genetic identification and classification of bacteria [67] and helps assess the functional contribution of microbes for the metabolism of its host [64]. In

particular, the technological development of metagenomic methods based on direct sequencing of small ribosomal subunits of DNA sequences [39, 81, 83] and sequenced material amplifier [39, 83] leading to important advances in data collection of great weight, and defining the gut microbial population [39, 81]. This metagenomic approach represents a method that produces results of "gold standard" [83], and characteristics of this method permit identification of phyla, classes, orders, families, genera and species of bacteria, when comparing the material obtained from a database [39].

Technology evolves rapidly, and new generations of sequencing with different bioinformatics analyses allow a performing of analysis of the microbiome even faster. These techniques use the platforms to sequence a diverse set of samples with an average of several millions of readings per sample, and its capacity is continuously increasing. This technique confirmed the existence of a "core" microbiota (pattern) more or less similar among individuals [39].

The human microbiome is highly complex and diverse [21]. The gastrointestinal tract is the most heavily colonized body organ that hosting a massive microbial ecosystem and have a greater number of microorganisms in humans [85].

Although the exact composition of the gut microbiota is unknown and the proportion of non-recognized species increase from birth to old age [21], advances in metagenomic technologies described above have recently begun to unravel our microbial partners [60].

Apart from described methods of microorganisms identification, and in the scope of microorganisms role in obesity, the strategy recently adopted to offer a great potential for studying of environmental influences on the microbiota is the mice "humanization" (colonization of the mice gastrointestinal tract with human microorganisms) [59] or studies with axenic and convectional/convencionalizated and mice [77] (transference of entire bacterial microbiota isolated from conventionally created animals [80]). Among different strategies used to study the role of the gut microbiota, the use of axenic animals seems to be the one, that more conclusions can provide [77]. From a simple comparison of the germ-free mice physiology with that of conventional ones, useful information about how bacteria can modulate the host metabolism is obtained [80]. Use of this strategy is based on sterile environment inside the womb during prenatal development and surgical delivery to replace the normal birth process, in order to eliminate the postnatal colonization of the gastrointestinal tract. Subsequent comparison of these animals with their conventionally colonized counterparts allows drawing some conclusions about the morphological and physiological parameters that can be influenced by microorganisms [77], since the absence of

intestinal microbiota leads to alterations in intestinal morphology and physiology [60]. Studies impact of the microbiota with axenic and conventionally colonized rodents are the rationale ones, because rodents follow a similar colonization pattern to humans [77]. However, in axenic model, results can be inconsistent and controversial because it can be influenced by the species of animal used (mouse *versus* rat), their strain, diet and sample sizes in different studies [85].

7.2. Gut microbiota composition

Humans and other mammals are colonized by a complex and dynamic bacterial community. In humans, the number of microbes that colonize the mucosal surfaces exceeds 10 times the total number of human cells [77, 81, 21, 83, 60]. It is believed that the entire microbial genome contains rather than 100 [21, 66, 83, 86] or 150 times more genes than the human genome [59, 77], with 3.3 millions of genes [87]. These data support the surprising concept that the genes in the humans are not 100% human, but 90% microbial and only 10% human [60]. Therefore, it is recognized that this "small inner world" of the intestine performs the important biological functions that cannot be performed by humans without these microorganisms [60, 81]. Thus, this complex symbiosis, as well as its development is probably dependent on genetic host-microbe interactions and on environment. This "microbial organ" contributes to our homeostasis through multiple metabolic functions and different mechanisms control [60].

The bacterial density gradually increases along the gut [66, 67]. The stomach and duodenum have a low number of microorganisms [66, 81], typically less than 10^2 colony-forming units per ml of the sample in the stomach and less than 10^3 colony-forming units in duodenum [80, 81], increasing to 10^4 in the jejunum, up to 10^7 in the ileum [67, 80], and reaching 10^{11} - 10^{12} units in the more distal digestive tract as in colon [67, 80, 81]. The figure 7 schematically represents bacterial abundance on different parts of intestinal tract, as well as the most common bacteria.

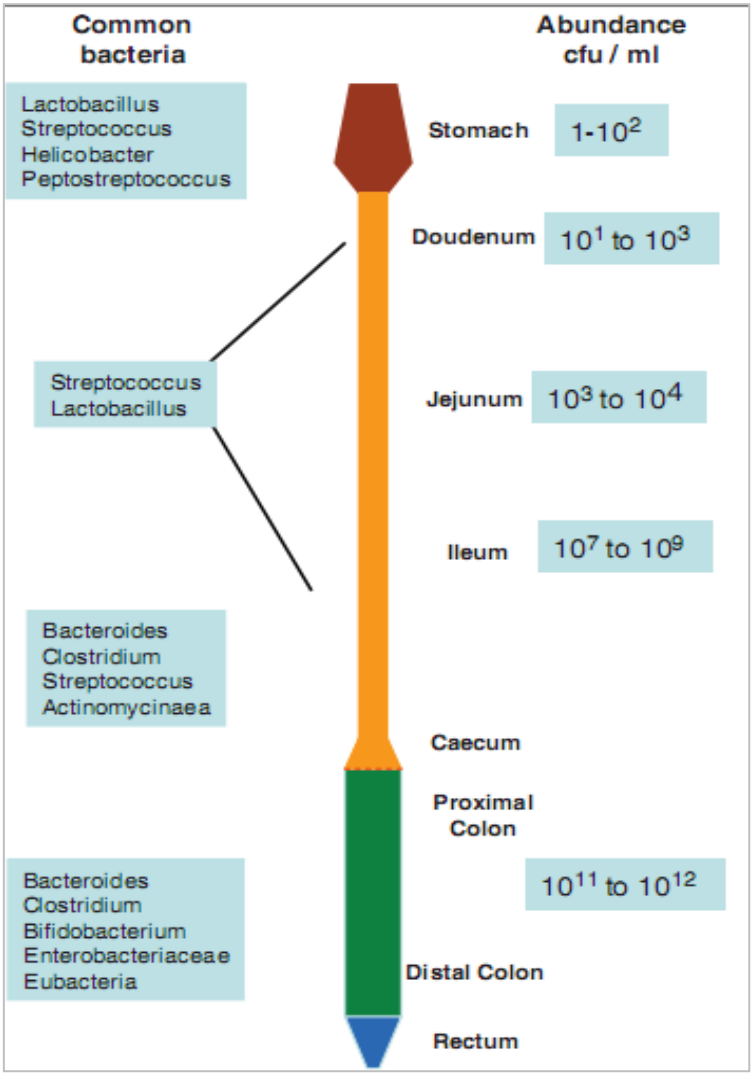

Figure 7 – Representation of bacterial type and abundance on different gut compartments. It is schematically present the common bacteria for each gut compartment as well as its abundance. The bacterial abundance is low in the stomach, increasing along the gastrointestinal tract and is more elevated in the distal portion of the intestine [80].

The human microbiota has about 160 different individual species [77, 60]. Gut microbiota also includes viruses, *Archaea*, unicellular eukaryotes (such as yeast) [12, 17] and protozoa [77], so the microbiota of mammals is highly variable at lower taxonomic levels [87].

The ribosomal sequencing technique was used to demonstrate that 90% of the bacteria belong to two phyla: the *Firmicutes* (mostly represented by *Clostridia* genus [80]) and *Bacteroidetes* [60, 66, 80, 81, 83, 87]. From metagenomic studies of mucosa and faecal samples [83], in which in a greater detail has been analyzed the gut microbiota of mammals, it was demonstrated that human microbiota is mainly composed by four phyla of bacteria:

Gram-negative - *Proteobacteria* (8% [83, 84]) and *Bacteroidetes* (20-40%) [59, 86, 88] that include about 20 genera [57, 67] and Gram-positive [59, 77] - *Actinobacteria* (3% [86] or 5% [83]) and *Firmicutes* (60-80% [86, 88]). The gut microbiota composition also includes *Fusobacterium* (1% [83]), *Verrucomicrobia* [77, 83] and disqualified bacteria near to cyanobacteria [66], and at least 1800 genera and 16000 phylotypes at the species level have so far been identified [83].

Into the domain of Bacteria, the *Firmicutes* phylum is the largest one and contains more than 200 genera, including *Bacillus, Lactobacillus, Mycoplasma* [57, 67], *Eubacterium, Peptostreptoccocus, Ruminococcus* [86] and *Clostridium* [57, 67, 86, 88]. This last genus constitutes approximately 75% of all *Firmicutes* [88]. Other great phylum is *Bacteroidetes*, and its most common species are *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron, Bacteroides caccae, Bacteroides fragilis, Bacteroides Putredinis* (of *Bacteroidaceae* family), and other species of *Prevotellaceae* family [66]. Another predominant species on human intestine are *Bifidobacteria* spp. (*Actinobacteria* phylum) and *Fusobacteria* spp. (*Fusobacteria* phylum) [86]. In the domain *Archaea*, *Methanobrevibacter smithii*, was the most prevalent specie [66, 80].

The gastrointestinal tract is composed of specialized compartments, such as the mouth, oesophagus, stomach, small intestine, large intestine (colon), rectum and anus. Each of these compartments has unique physiological functions and anatomical structures [85]. The chemical environment inherent to these locals results in influence of the prevalence and diversity of microorganisms in different areas of the gastrointestinal tract [67, 85]. Jejunum has a predominance of gram-negative aerobic and some obligate anaerobic bacteria while colon has predominance of anaerobes [67]. It is estimated that the colon alone contains more than 70% of all microbes of the human body [81].

The jejunal samples show a distinct composition of communities, including an abundance of bacteria that belong to the *Streptococcus* genus [67], while the vast majority of the microorganisms prevalent in the colon belong not only to *Firmicutes*, but also to the *Bacteroidetes*, [67, 83] *Actinobacteria* and *Proteobacteria* phyla, with relatively low numbers belonging to *Fusobacteria*, *TM7* and *Verrucomicrobia*. *Archaea* and fungi may also be present on colon colonization communities [85]. In the distal ileum and rectum *Firmicutes* and *Bacteroidetes* microbial groups were also predominant [83]. Thus, the microbial composition differs in each compartment [67, 81, 85] as shown in table 1 [85].

Table I – Composition of gut microbiota with its proportion in different gut compartments [85].

Bacterial Phylum	Stomach	Distal Gut	Sublingual Patch	Distal Oesophagus
Fusobacteria	≈ 3.3%		≈ 14.0%	≈ 2.5%
Actinobacteria	≈ 9.0%		≈ 10.9%	≈ 4.5%
Firmicutes	≈ 25.3%	≈ 50.8%	≈ 26.1%	≈ 69.6%
Proteobacteria	≈ 51.9%	<1%	≈ 13.4%	≈ 2.5%
Bacteroidetes	≈ 10.5%	≈ 47.7%	≈ 9.3%	≈ 20.2%
Verrucomicrobia		<1%		
TM7*			≈ 1.5%	≈ 1%
Spirochaetes			≈ 21.3%	
Deffeibacteres			≈ 3.5%	

**TM7* is candidate phylum, because there is insufficient information to call it a new species.

Each individual is colonized by unique strains, which generally remain constant over the time. Genetics, diet, immune status, infections, gastrointestinal diseases, as well as administration of antibiotics and other medications influence or even determine the microbiota uniqueness of each individual [83].

Dietary factors, namely dietary habits are the determining factor that contributes to the composition and diversity of the human gut microbiota [59, 84, 86]. An example that can prove this fact is the discovery of *Prevotella*, *Treponema* and *Xylanibacter* genera in the intestinal microbiota of children of African rural village, genera which do not exist in the European population. Presence of these microorganisms is thought to be a consequence of fibre intake, once these bacteria maximize energy extraction from ingested plant polysaccharides and protect against inflammation and infections [59]. Results of some studies have also shown that a vegetarian diet affects the composition of the human gut microbiota. The changes on diversity and decreased amount of *Clostridium* group IV and presence predominantly of groups *Clostridium* XIV and XVIII was observed on individuals with vegetarian diet [86].

The mechanisms and effects that different environments, including the variety of diets throughout the world, can exert, and how gut microbial composition and its function are affected are poorly understood [59].

Profile of intestinal microbes undergoes changes with the alteration of composition of diet from rich in carbohydrates to a "Western" diet , that is loosely defined as one high in

saturated fats, red meats, "empty" carbohydrates (junk food) and low in fresh fruits and vegetables, whole grains, seafood and poultry. As demonstrated by diverse studies this diet makes also the mice to become obese [3].

The genetic background of our microbiota may determine how certain dietary components are processed and in turn these genetic factors in addition to diet and other environmental factors can mould the gut microbiota [59].

The "construction" of the intestinal microbiota begins at birth and its composition undergoes significant changes throughout life [21], and the initial colonization and ecology of the gut does not seems to be random, but rather, pre-programmed genetically. However, there is growing evidence that the microbial ecology may be influenced by several factors including epigenetic, type of birth delivery, exposure to antibiotics, neonatal nutrition, nutrition in adulthood, stress, age, specific events, including bacterial infection. Therefore, while the gene can predict to some extent the microbial composition of human gut, diverse extrinsic factors contribute to the development of unique microbial fingerprint of each individual, and his consequent possible susceptibility to different diseases (dependent on microbial composition) [87].

The gastrointestinal tract is rich in molecules that can be used as nutrients by microorganisms, and therefore have the potential to be heavily colonized by different bacteria, both harmful and beneficial, so that the mucosa of the gastrointestinal tract is continuously exposed to an environment rich in foreign substances such as food particles and antigens of microbial origin [81].

With an increase count of microbial population and subsequent nutrient depletion, the gut habitat becomes occupied with a certain types of bacterial species. The ability of other species to occupy this habitat will depend on its ability to utilize nutrients more efficiently, or ability to modify these nutrients to better adapt it to their own metabolic capabilities [21].

7.2.1. Child gut microbiota

During the early life, the composition of microbes changes with age [80] and diet type of infant nutrition [80, 86]. The hospitalization, prematurity and administration of antibiotics can determine the microbial composition of the gut during childhood [86].

At birth, humans have practically no bacteria, but the colonization process begins immediately after birth and continues still adulthood, with hosting of new complex microbial communities over time [86].

7.2.1.1. Common child microbiota

The infant microbiota is characterized by heterogeneity and instability until it becomes more stable and resembles to the adult microbiota [87].

The foetus is sterile in the uterus and is colonized by microbes during its passage through the birth canal [66, 67, 80, 87], acquiring the intestinal and vaginal mother´s microbiota, including *Bacteroides* spp*., Bifidobacteria* spp*., Lactobacilli* spp., and *Escherichia coli* [87]. On the other hand the infants delivered by caesarean section are colonized by environmental microorganisms associated with skin [87], coming from their mother, from the air, and transferred by the healthcare professionals team [67, 79]. As a result, babies born by caesarean section have fewer *Bifidobacteria* and *Bacteroides* spp. - two species that was shown to protect against obesity, and are most often colonized by *Clostridium difficile* [66, 67] and *Staphylococci* spp. that persist in childhood [87], when compared with infants of normal delivery birth. The initial intestinal microbiota of infants born by caesarean section may be disturbed for up to 6 months after birth, while children born by vaginal delivery take up to a month to get their intestinal microbiota established [66]. After birth (normal delivery or caesarean section), the bacteria from the mother's mouth, skin and milk [57, 66, 67, 79, 81, 87] as well bacteria from surrounding environment enter to the intestine of newborn [57, 66, 81, 87]. After this, microbiota colonization suffers dynamic changes [57, 67] due to continuous exposure to different environmental bacteria [67] and probably due to the influence of the diet. In addition to the type of infant nutrition, other factor that can influence the composition of the intestinal microbiota in newborns is the measures of hygiene [66].

Bacteria that first colonize an individual can induce or modulate gene expression in epithelial cells to create a favourable habitat for themselves, thus preventing the growth of other type of bacteria that could be introduced later into the gut ecosystem. The initial colonization - immediately after the birth, can therefore be very relevant for the final composition of gut permanent microbiota in adults [66] and make an impact on the occurrence of metabolic diseases [39].

The gastrointestinal tract is firstly colonized by facultative aerobic and then by anaerobic microorganisms. When first colonizer populations of bacteria are growing, they consume oxygen and create an anaerobic environment. During the first week of life, it is created a favourable environment for the strict anaerobic species, mainly for once which belong to the *Bifidobacterium, Bacteroides, Clostridium*, and *Ruminococcus* genera. Since the

number of these anaerobic bacteria expands, facultative bacteria cannot support the environmental changes caused by competition and therefore decrease in number [66].

After birth the number and diversity of strict anaerobes increases as a result of diet and environment [77]. However, it is considered that the hospital environment affects the colonization rate by *Clostridium difficile*. In fact, it is believed that neonates are generally colonized by these anaerobic spore-forming microorganisms, mainly due to the hospital setting because the prematurity and hospitalization are associated with an increased prevalence and counting of *Clostridium difficile*. An antibiotic treatment of children has been associated with a decrease in the number of *Bifidobacteria* and *Bacteroides* genera, but in all cases, all children are initially colonized by a large number of *Escherichia coli* and B group *Streptococcus* [66]. The acquiring of methanogenic *Archaea*, not only can occur due to available favourable conditions in the intestine for their permanent colonization, but also due to environmental contamination [86].

In babies that are breastfed, the intestinal microbiota is dominated by bifidobacteria [57, 81]. The growth of bifidobacteria in infants is probably favoured by the presence of growth factors in breast milk [66, 83], like neutral oligosaccharides with prebiotic effect. On the other hand, infants that are fed with milk substitutes have different gut microbiotas [21], and are most often colonized by species from *Enterobacteriaceae* family, *Bacteroides* spp., *Streptococci* spp., *Clostridium difficile* [67] and *Escherichea coli* [66], when compared with breastfed infants. The last ones are predominantly colonized by *Staphylococci* spp., *Streptococci* spp., *Lactobacilli* spp., and *Bifidobacteria* spp. [67], although according to some authors, *Lactobacilli* counts are significantly lower than in formula-fed infants [66]. Similarly, the addition of prebiotics to the infant's diet stimulates the growth of endogenous beneficial bacteria [81]. In formula-fed babies, relatively high amounts of propionate and butyrate are produced, and in contrast, breastfed infants' microbiota produces high amounts of lactate and acetate that restricts the growth of potential pathogens such as *Escherichia coli* and *Clostridium perfringens* [81].

In babies from three months old to one year the population of *Bacteroides fragilis* increases, while the populations of *Staphylococci* spp., *Lactobacilli* spp., *Bifidobacteria* spp., *Clostridium* spp. and total anaerobes decrease, being an amount of these bacteria stable in about of one year old infants [86].

Children between 1 and 7 years old have a higher number of bacteria belonging to *Enterobacteriaceae* family, when compared to adults. It is considered that about of one year old until the second year of life, the intestinal microbiota of the child begins to resemble the

adult microbiota [66, 86]. This age is coinciding with the introduction of the adult diet. After the introduction of solid food and weaning, the microbiota of infants becomes similar to that one of children fed with formula [66]. Thus, with this transition, when baby is weaned from milk [67, 81] - a diet rich in fat [67] to solid food diet [81, 67] rich in carbohydrates [67], the composition and complexity of the gut microbiota changes and becomes mature [67]. In a large scale study it also was concluded that during the first two years of life the changes of the predominant genera of microbiota were significant, whereas between 2 and 18 years of life the changes were not so significant, with stable levels of *Bifidobacterium* and *Lactobacillus* genera. In another study it was found that the *Bifidobacterium* and *Clostridium* genera were more abundant in adolescents when compared to adults [86].

7.2.1.2. Microbiota and child obesity.

Researches about origins of obesity development and associated metabolic disturbances showed that disruptions on perinatal development that are however still unknown, increase the risk of predisposition to obesity [66].

Higher numbers of *Bifidobacteria* spp. was found in children who had a normal weight at 7 years old compared to overweight children. In addition, it was found that the levels of *Staphylococcus aureus* were lower in children who have maintained normal weight than in children who become overweighting few years later [3, 39, 67, 83]. Given this fact, it was proposed that *Staphylococcus aureus* can trigger the low-grade inflammation and can contribute to obesity development [3, 83].

The impact of widespread use of antibiotics in children is associated with a decrease of the number of antiobesogenic *Bifidobacterium* and *Bacteroides* genera. After an antibiotic treatment the new growth of *Bifidobacteria* spp. was slow, whereas *Bacteroides* spp. were generally not re-established [67]. The undesirable variations in gut microbiota in early life may confer an increased risk of developing of obesity in adulthood [57].

Once it is known that the altered composition of microbiota, called a gut dysbiosis, can lead to altered immune function and increased risk of diseases, intestinal colonization between childhood until the next years of life can be a critical control point during which development of the immune tolerance and susceptibility to disease is occurring, as a result of responses to enteric bacteria [87]. As the babies' gut first colonization is usually derived from their mothers during childbirth, and given the microorganisms be known today to be related to obesity, it can be speculated that the transmission of microbes responsible for the obese phenotype occurring from mother to child, in addition to feeding, that can also

modulate the composition of the intestinal microbiota of an infant [5]. So it can be concluded that the factors that modulate the microbiota composition in early life can have preventive or therapeutic implications for adult obesity [67].

7.2.2. Adult gut microbiota

After its transformation to the adult type, the composition of the gut microbiota is relatively stable over time [39, 66, 86] and is quite similar among subjects [39, 86] to allow affirming the existence of said microbiota "core" [39]. At any certain point the gut microbiota encompasses species that permanently or temporarily colonize the tract, and permanent microorganisms seem to present a restricted set of highly adapted bacteria [66].

It has been found that among *Bifidobacteria* spp., the *Bifidobacterium bifidum* and *Bifidobacterium pseudolongum*, are prevalent in adults, while colonization *by Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium adolescentis* and *Bifidobacterium pseudocatenulatum* is not depended on age of subjects [86]. Moreover, differences in the composition relative to the proportion of bifidobacteria can be explained by the host's specificities or related to differences on diet [21].

The final composition of microbiota is influenced by prior colonization, physiology of the host and also by lots of environmental factors, so it becomes evident the importance of the initial colonizing microbial community in the eventual microbial composition of the gut of adult subjects [83]. Several studies have also shown that the individual's genotype influences the "basic" composition of microbiota. Microbial populations are an inheritance from progenitors, once mothers, sisters and descendants have similar microbial communities and the genome of gut microbiota is "shared" by family members with a high degree of similarity [5]. For example, the microbiota of monozygotic twins that live separately [57, 83] or even of members of the same family [80] is much more similar than the microbiota of unrelated individuals [57, 80, 83]. Likewise, the environment does not appear to significantly influence the composition of the microbiota, because the spouses did not have a significantly greater similarity on bacterial communities when compared to unrelated individuals, even though they live in the same environment and have similar dietary habits [57, 83].

In studies in individuals from different countries and a continent it have been identified [3] that the microbiota of an individual, regardless of the host characteristics such as age, nationality, gender and body mass index, belongs to one of three main "enterotypes", [3, 87] which differ in their phylogenetic composition and functional characteristics [87]. This

enterotypes were identified by the variation at the level of one of the three following genera - *Bacteroides*, *Prevotella* and *Ruminococcus* [3, 82, 86]. The enterotypes are the well-balanced and defined microbial community compositions but are not so clearly delimited as, for example, human blood groups. Nevertheless, the enterotype characterizes its host, accordingly to studies that report the intestinal microbiota is stable in individuals and can be restored after its perturbation [82]. The different genera of bacteria with its unique characteristics present in different types of enterotypes suggest that enterotypes use different routes and pathways to obtain energy from the food, namely from the fermentable substrates available in the colon. Thus, bacteria of each enterotype have its potential specialization in ecological niches [82, 86]. From results obtained in some studies, it can be said that Enterotype 1 has rather bacteria from *Bacteroides* genus [82, 86], which co-occurrence of other types of bacteria (Fig. 8a) [82].

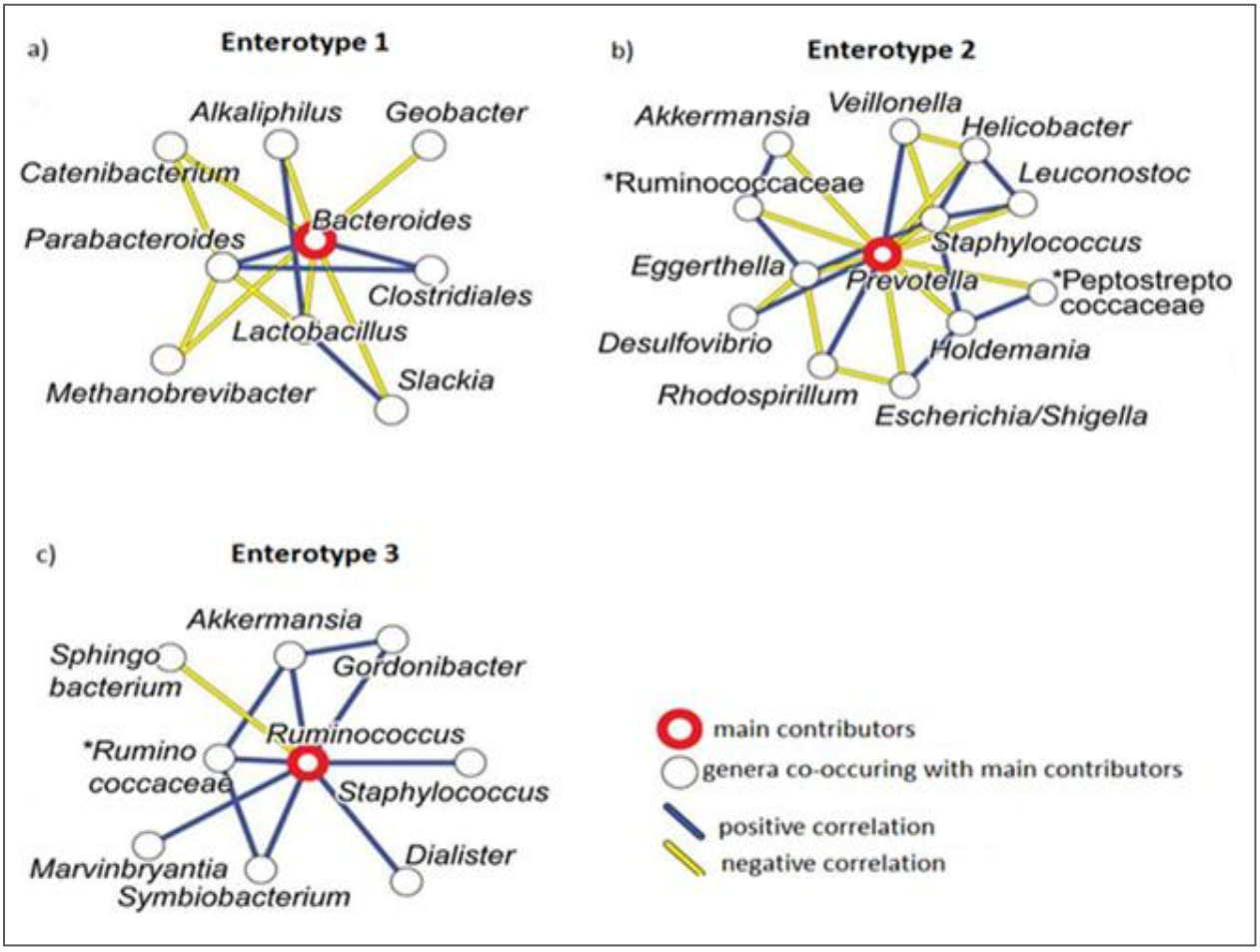

Figure 8 – Networks of three human gut enterotypes with co-occurrence. Unclassified genera are marked by asterisk [82].

The bacterial constituents of this enterotype seem to derive energy primarily from carbohydrates and proteins through fermentation process [82, 86]. This finding is supported by the fact that genera related to enterotype 1 have a very extensive saccharolytic capacity and also have genes encoding enzymes, such as galactosidases, hexosaminidases and proteases involved in the degradation of these substrates in addition to glycolysis and pentose phosphate pathways that are enriched in this enterotype [82].

Enterotype 2 showed to have rather bacteria from *Prevotella* with the main co-occurring of bacteria from *Desulfovibrio* genera (Fig. 8b), which can act synergistically to degrade mucin glycoproteins present in the mucosal layer of the gut [82, 86]. *Prevotella* spp. are known as mucin-degrader and *Desulfovibrio* spp. could enhance the rate of limiting step - mucin desulfation by removing the sulphate [82].

Enterotype 3 is the most frequent one and is enriched in *Ruminococcus* as well as co-occurring predominantly by *Akkermansia* genera bacteria (Fig. 8c). Both genera comprise species able to degrade mucins [82, 86]. The membrane transporters of most of sugars were shown to be also augmented, suggesting the efficient binding of mucin and its subsequent hydrolysis as well as uptake of the resulting simple sugars by bacteria belonging to these genera [82].

In addition to the conversion of complex carbohydrates into absorbable substrates, the gut microbiota is also beneficial to the human host by producing vitamins. Although all enterotypes presented to have the vitamin metabolism pathways [82], enterotypes 1 and 2 were capable of biosynthesis of different vitamins [82, 86]. Biotin, riboflavin, pantothenate and ascorbate were shown to be produced by bacteria from enterotype 1, and thiamine and folate by bacteria from enterotype 2. These phylogenetic and functional differences among enterotypes thus reflect the possible different impact on its inter-relations with the hosts [82] and probable host responses to environment factors.

Microbial sets form in humans functional groups that can differently respond to diet or medication, and may represent the future direction of functional characterization of microorganisms [87]. The current definition of enterotypes will undergo upgrading over near future. This classification is useful to provide a better picture of microbial properties with impact on health, xenobiotic and nutritional metabolism and may be an indicator of disease susceptibilities. Although the current knowledge about enterotypes is far to be complete, the potential uses of this new classification of microbiota may define a standard for disease-associated bacterial microbiota [80].

7.2.3. Elders microbiota

After the transformation to the adult form, the gut microbiota remains relatively constant up to about 60 years old [57]. However, microbiota "background" of elderly individual undergoes changes and becomes different, when compared to microbiota of young adults [39, 77]. In elderly people, the diversity of microbiota compound species is reduced [86] and there is a higher proportion of *Bacteroides* spp. and abundance of different groups of *Clostridium* genera [39]. In one study, higher proportions of microorganism of *Enterobacteriaceae* family were found in all elderly volunteers regardless of their geographic location [21].

8. Function of gut microbiota

The microbiota has undergone an evolution of adaptation in human beings [8].

The interaction of epithelial cells with microbes and released components by them, including its metabolites, is a key mediator of the interaction between epithelial and other cell types. The exchange between bacteria and epithelium may differ in small and large intestine because of anatomical differences, and because of difference in secreted mucus layer that covers this epithelium. Intestinal microbes utilize the nutrients and produce metabolites that influence a wide range of human metabolic phenotypes, including susceptibility to conditions such as obesity [39].

Microbiota and its host are living in close symbiotic relationship: the microbiota performs nutritional, metabolic and protection functions that make it essential for its host, while the host provides conditions, for example nutrients, for microbiota growth [91]. A schematic summary of probable relation between the intestinal microbiota and metabolism are shown on figure 9 [57].

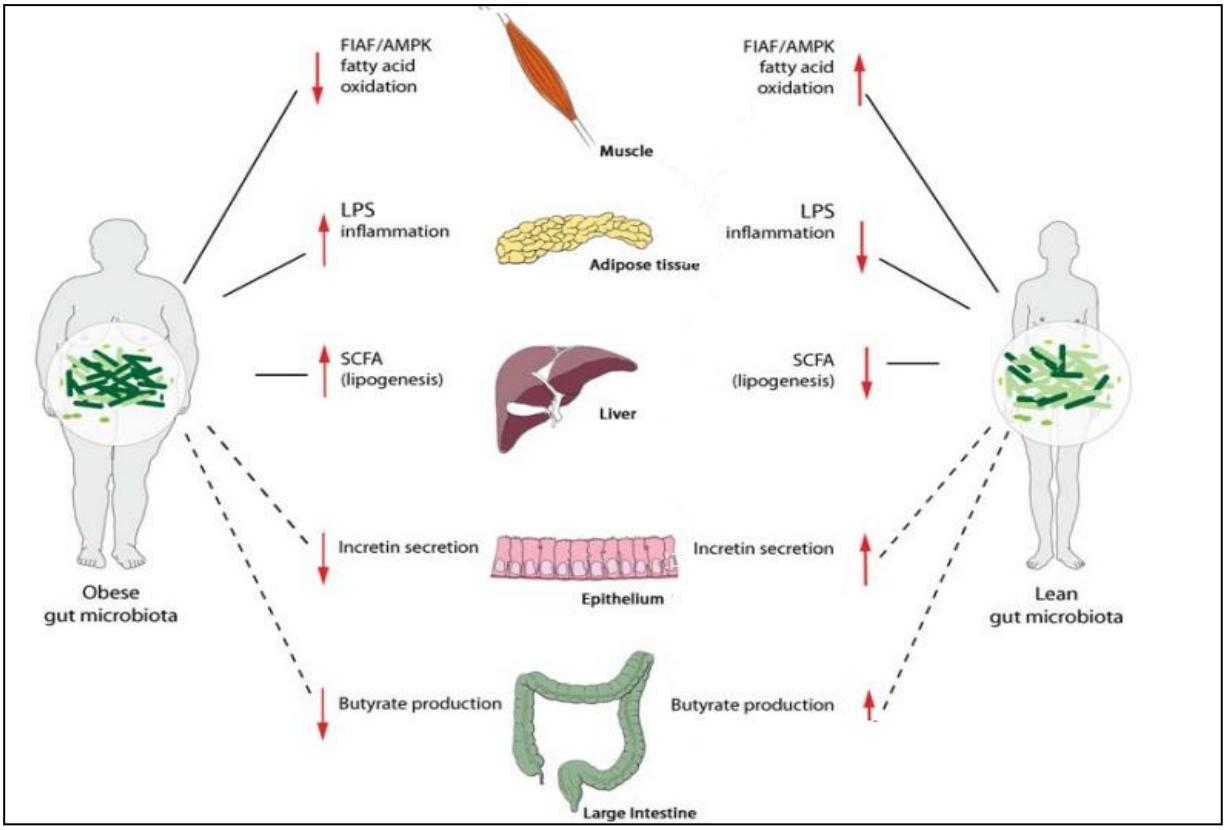

Figure 9 – Relationship between intestinal microbiota and diverse metabolic functions. Intestinal microbiota may influence the fatty acids oxidation, inflammation triggered by bacterial lipopolysaccharides, short-chain fatty acids production and its storage into adipose tissue and incretin secretion [57].

The microbial community exploits the medium of the host, but also presents different advantages [66], and the interactions between host and bacteria are implicated in a variety of host functions, including intestinal development and function [83].

To better understand the role or function of the bacterial communities, it was necessary to study bacterial gene expression. It was confirmed the great variability between individuals. Only about 300 000 commonly shared microbial genes were found in more than half of the examined subjects. Each individual carried around 500 000 bacterially encoded genes, being most of it rare genes, shared by less than half of the sampled group. Near to 2.5% of the encoded microbial genes that were more similar within individuals of the same family may be attributed to encode the functional group of enzymes involved in carbohydrate metabolism. It was detected the minimal gut microbiome present in most bacteria that are responsible for functions necessary for the bacterial surviving in the host intestine. However, a great part of genes were observed to have the unknown functions. On the other hand, the principal objective was identify the minimal gut metagenome coding for functions involved in the homeostasis of the host ecosystem. Most of these common bacterial genes that interestingly were coded by species present in lower number and not by the most abundant ones, showed be involved in the digestion of complex sugars and its posterior fermentation. This fact suggest that relatively small microorganisms group, for example *Archaea*, might be crucial for the establishment of a well gut function. It should be noted that there are many genes that will belong to both groups - necessary for surviving of bacteria and for host intestinal function, so the microbiome core should exhibit bacterial functions necessary to ensure the survival and successful symbiosis in the gut. In other study microbial mRNA expression showed stable representation of genes involved in nutrient processing, energy harvest, and biosynthesis of cellular components. Lipid and amino-acid metabolism were poorly reflected in the expression pattern [80].

It is known that microbiota is contributing to the maturity of the intestinal epithelial layer, the mucosal innate immune system, enteric nervous system and the intestine vascular system [39]. So, the gut microbiota affects the metabolism by increased energy extraction, modulation of immune system and by altered lipid metabolism, and the physical presence of both bacteria and bacteria's metabolites are responsible for these effects [85]. These functions constitute the protection offered by the intestinal microbiota – maintenance of a physical barrier against colonization or invasion by luminal pathogen microorganisms [66, 21], such as *Escherichia coli,* and bacteria from *Clostridium, Salmonella* and *Shigella* genera [83], and facilitates digestion and assimilation of nutrients [21], including the energy extracting

from non-digestible constituents such as cellulose. Other function is the synthesis of short-chain fatty acids (SCFAs) [66] that are involved in the development of enterocytes and colonocytes [83] and stimulate the development of the immune system [66] providing signals to immune surveillance [21, 83] at the interface of the mucosa in the intestinal lumen [21]. Intestinal microbiota has also other important metabolic function, as the metabolism of xenobiotics once it makes the decomposition of dietary toxins, drugs and carcinogens, and further affects gastrointestinal motility [83], given the presence of bacteria being essential to normal gastrointestinal gut motility [77].

Despite of significant interpersonal variation in gut microbiota, there seems to be a balance that confers health benefits, and a change in beneficial bacteria may negatively influence the well-being of the individual. Several factors can alter the gut microbiota, such as infection, disease, diet and antibiotics. However, the intestinal microbiota tends to revert to its composition that was established in childhood, when the factor that led to changes disappears [77].

8.1. Metabolism

Despite of variations in the composition of the microbiota between and within individuals, there is a functional stability that covers all basic biochemical reactions such as the degradation of carbohydrates, fermentation and synthesis of micronutrients [21, 66, 83]. The intestinal bacteria can produce a large number of vitamins such as of the B and K groups, synthesize amino acids and perform the biotransformation of bile thanks to microbial enzymes that is important for the metabolism of glucose and cholesterol; [81]. Also, other molecules, such as glycoesphingolipids [21], as well as mucins of the host can be degraded by bacterial enzymes [21, 81].

Intestinal microbiota can affect energy homeostasis of the host, modulating its ability to extract energy from nutrients [66] as non-digestible food components (takes part in the extraction of the maximum nutritional value from the diet [21, 77]) and store it as a fat [66] by *de novo* hepatic lipogenisis and storage of fatty acids in adipocytes. The intestinal microbiota influences the expression of genes that are expressed in the gut of the host, which control the absorption of fatty acids, oxidation and fat storage [60, 83]. The summary of potentially dangerous and beneficial functions of microbiota in the host are shown in Table 2 [66].

Table 2 –Functions of intestinal microbiota and associated performed functions [66].

Type of Function	Functions
Metabolic function	Extraction of energy from oligosaccharides, sugar alcohols and non-digestible carbohydrates (fermentation of resistant starch, cellulose, hemicellulose, non-starch polysaccharides, pectins and gums).
	The fermentation of carbohydrates in SCFA (acetate, propionate, butyrate), lactate, ethanol, succinate, etc.
	Conversion of endogenous and dietary nitrogen compounds into ammonia and microbial protein.
	The water and salt absorption
	Proteolysis of amino acids in order to form branched chain fatty acids (isobutyrate, 2-methylbutyrate, isovalerate e), NH_3, phenols, indoles and amines.
	Synthesis of vitamins B and K
	Membership of complex lipids and cholesterol
	Metabolism of xenobiotics.
Barrier function	Protection against penetration of pathogens.
Trophic function	Modulation of cell proliferation, differentiation and apoptosis - stimulation of intestinal angiogenesis.
Immunologic function	Migration and maturation of lymphoid precursor cells.
	Development and maturation of IgA plasma cells)
	Modulation of the local and systemic immune response (oral tolerance).
Regulation of fat storage	Modulation of lipogenesis and fatty acid oxidation.

The intestinal microbiota provides the biochemical pathways for the fermentation of substrates such as non-digestible fibres [81]. In other words it provides to host a large amount of enzymes - glycoside hydrolases [59, 67, 86, 88], which are not encoded in the human genome, but that [59, 86, 88] participate in food digestion [59, 86, 88]. It was demonstrated that axenic animals were absent of bacterial enzymes necessary to polysaccharides digestion [80]. Thus, the gut microbiota is essential for the metabolism of complex carbohydrates such as plant polysaccharides [67, 86], so nutrients that were not digested in the upper intestine are then fermented by intestinal microbes [3, 89]. These complex carbohydrates, called as dietary fibres, are part of vegetables, cereals, seeds of legumes and fruits and other vegetables that enter in the human diet and are not digested in the superior digestive tract (stomach or the small intestine), because as it was said, the human genome does not encode suitable enzymes for processing of this kind of carbohydrates. Dietary fibres are fermented in the colon by the intestinal microbiota and / or excreted in the faeces [80, 86]. A high variety of active enzymes over carbohydrate is

produced by the human gut bacteria, and is called CAZymes [86, 90]. These CAZymes then degrade carbohydrate complex compounds in monosaccharides and disaccharides [86]. The matrix of CAZymes of intestinal microbes is highly diverse and has specificities for many substrates [86, 90]. While the human genome, that encodes a maximum of 20-25 digestive enzymes of CAZyme families, such as lactase, amylase, maltase, sucrase and isomaltase [86], *Bacteroides thetaiotaomicron*, for example, can express different hydrolases and digest different food polysaccharides [91]. It is believed that gut microbiota contains 261-glycosyl hydrolases, and lyases for polysaccharides, as well as homologues of 208 genes that encode two membrane proteins involved in the utilization of starch [86, 90]. The intestinal microbiota's CAZymes of individuals from different geographic populations are influenced by different traditional diets. It was discovered that the porphyranase and agarase genes are found in the intestinal microbiota of the Japanese and are probably absent in the microbiome of Western individuals [86, 90]. The authors proposed that consumption of sushi that contains algae of the genus *Porphyramay*, which is associated with the marine bacterium *Bacteroides plebeius* and *Zobellia galactanivorans* led to the acquisition of these CAZymes in the human intestine [86, 90]. Fermentation or metabolism of these indigestible substrates of plants leads to the growth of microbes that perform this fermentation [81].

The prevalent end products that result from this intestinal bacterial fermentation of complex carbohydrates from the diet are monosaccharides and short-chain fatty acids - acetate, propionate and butyrate [21, 57, 67, 88, 91] with a ratio of approximately 60%, 20%, 20%, depending on the nature of the fibre [91].

The caecum and ascending colon are the priority places for fermentation processes where bacterial populations produce higher concentrations of volatile fatty acids [81] with its subsequent absorption by stimulating the absorption of minerals [83] and water [81, 83]. The final products of fermentation of these polysaccharides in addition to the above-mentioned SCFAs are also water, gases - CO_2, H_2, CH_4 [91], isobutyrate, 2-methyl-butyrate, lactate, ethanol, succinate, etc. The colonic bacteria utilize butyrate as the source of energy, and most of it is completely metabolized. The acetate (main SCFA produced in the colon) serves as a substrate for the biosynthesis of cholesterol [81].

Regarding the non-bacterial fraction of gut microbiota, it has been argued that *Archaea* may contribute to the health status of the human gastrointestinal tract, thanks to the unique nature of its metabolic feat [80].

8.2. Barrier function of gut microbiota

Intestinal mucus layer results from a balance of mucin secretion and degradation. The mucin layer creates a barrier to pro-inflammatory compounds and avoids the absorption of diverse antigens. Furthermore, the intestinal structure and function are also assured by the gut microbiota (Fig. 10). Evidence indicate that butyrate (SCFA secreted by these microbes) induces secretion of mucin, antimicrobial peptides, and other factors that enhance the defence barrier in the colon, besides, all fatty acids of short-chain have a protective effect on the intestinal epithelium [81].

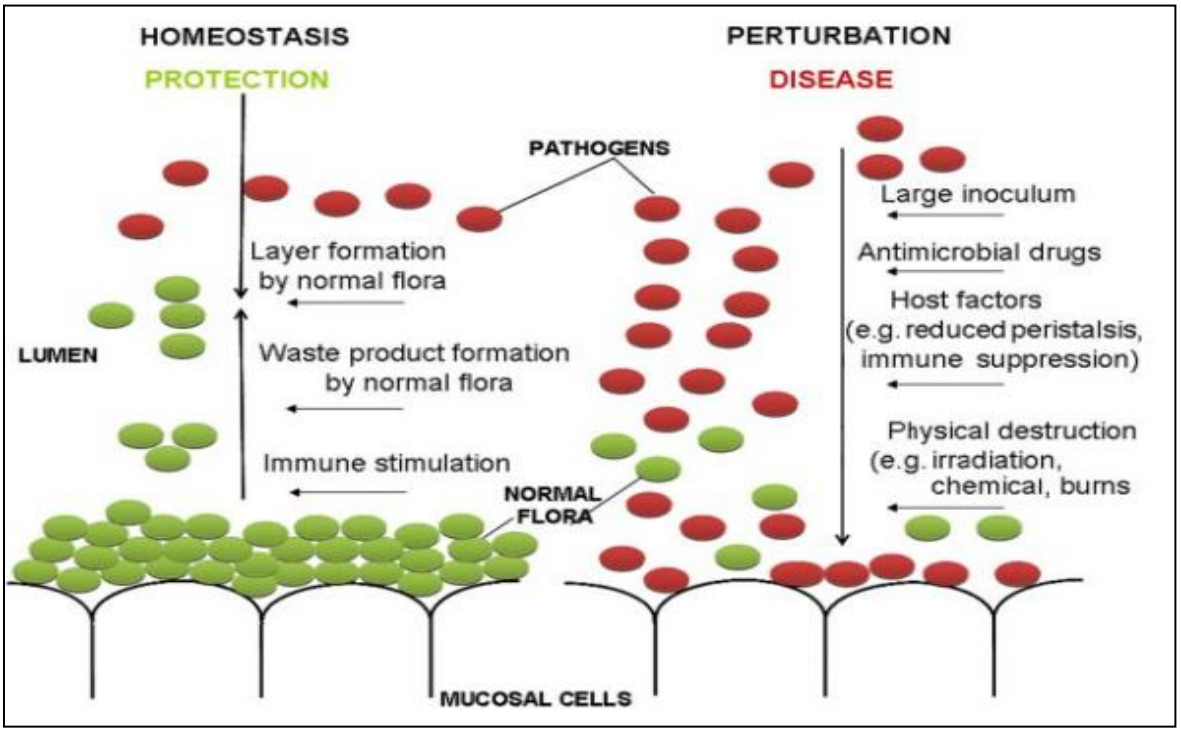

Figure 10 – Microbiota gut barrier homeostasis and perturbation. Gut protection is performed by commensally microbiota, conferring a protective physical layer to enterocytes (mucosal cells). The healthy microbiota layer prevents from invasion by external pathogens, also providing helpful metabolic products and almost none immune stimulation. Towards the external stimulus, host and environmental factors, the alteration and perturbation of the gut microbiota balance leads to disease [66].

It is possible to directly protect the host against pathogenic bacteria, because many of commensally microorganisms produce antimicrobial compounds [67 ,81, 87] and are competing for nutrients and binding sites on the surface of the intestine [81, 87], preventing

attachment of pathogens (colonization resistance) and maintaining proper intestinal pH [87]. When this balance between bacterial populations is suitable, microbiota plays a role of protective barrier, preventing against colonization and multiplication of pathogen microorganisms and precluding development of gastrointestinal pathologies [81, 91].

Recent data suggest that changes in the composition of the intestinal microbiota and gut barrier function play a critical role in the development of inflammation associated with obesity [64].

Moreover, the development of microvasculature of intestinal villous is dependent on the microbes that colonize the intestine. This fact was demonstrated by studies, which used germ-free mice with its subsequent colonization by *Bacteroides thetaiotaomicron*, and the importance of gut microbes on the development of intestinal structure, and morphology was demonstrated [81]. *Bacteroides thetaiotaomicron* induce the expression of genes that regulate the barrier function, such as ones that are implicated on vascularisation of the intestinal epithelium and nutrient digestion and absorption [91].

The recognition of the commensally microbiota by toll-like receptors (TLRs), which will be discussed in more detail later, is necessary to induce epithelial cell proliferation, i.e. the acceleration of reparation of epithelial surface after injury [77].

8.3. Immune surveillance

The intestinal microbiota plays a crucial role in the development of systemic and local immunity [87].

In addition to stimulation and production of antimicrobial substances (bacteriocins), competition for the same biological niche and preventing the replication of other bacterial community that may be pathogenic, the modulation of epithelial pro-inflammatory transcription factor – nuclear factor kappa B (NF-kB) and stimulation of immunity mediated by B and T cells [67, 87], especially CD4+ T cells in Peyer's patches and mucosal mesenteric lymph nodes are the activities of gut microbiota [87]. Thus, gut microbiota can induce the production of antibody of mucosal immunity - immunoglobulin A, pro-inflammatory cytokines such as Interleukin 12, or anti–inflammatory cytokines (e.g. Interleukin -10) [67].

Regulation of compounds involved on immunity response is the mechanisms by which the intestinal microbiota participates in maintenance of the integrity and combating of external threats. Besides, butyrate resulted from microbial fermentation regulates cell

growth and differentiation, inhibits the cell transformation during the cell growth process and help to reversion of cell with neoplasic phenotype to non-neoplasic phenotype [81].

The importance of the intestinal microbiota in development of the immune system is highlighted by studies with axenic mice. These studies show that the intestinal structure and functions are impaired by a decrease of immunoglobulin A secretion, decrease of number and function of intraepithelial lymphocytes and reduced lymphatic tissue [87]. Thus, microbiota is essential for the development of the gut-associated lymphoid tissue and plays an important role in the formation of immune assembly of gastrointestinal tract [77].

In the postnatal period and adulthood, the presence of microbiota is necessary to induce regulatory mechanisms designed to maintain balance in both mucosal and systemic immunity, so that we can be tolerant to harmless bacteria but produce appropriate responses to pathogenic bacteria [87]. Some bacterial species as segmented filamentous bacteria, which strongly adhere to the intestinal epithelium can largely induce the maturation of T cells and increase its number in both small intestine and colon [39, 67, 87].

The innate immune system, as a first line of defence, tries to keep the layer of the intestinal mucosa free of microorganisms through phagocytosis of invading pathogenic bacteria. Secondarily, B lymphocytes secrete immunoglobulin A in mucosa, which is going to be specifically targeted against invasive pathogens [39]. The immunoglobulin A secretion is a part of this immunologic assembly, being the controlled inflammation seen as a consequence of bacterial colonization [77]. In comparison with the conventional animals, axenic animals have reduced plasmatic cells and immunoglobulin A levels, and also reduction of expression of gut markers for macrophage activation, of the major histocompatibility complex in epithelial cells, nitric oxide, and the histamine levels in the small intestine was observed [77]. Moreover, germ-free animals have fewer dendritic cells, and there is evidence that shows that bacteria play a role in development of B cells [81].

Paneth cells are secreting cells found in small clusters on the basis of Lieberkühn crypt of epithelium in the small intestine. These cells have the function of maintenance of intestinal homeostasis trough detection of enteric bacteria by toll-like receptors [21, 77]. When bacteria are detected by TLRs, multiple antimicrobial factors are expressed. Thus control of transposition of the intestinal barrier by the commensally and pathogenic bacteria is performed [77], and imbalance of this signalling pathway can lead to the initiation of inflammatory bowel diseases [21]. Paneth secretory cells are known to express and release large variety of antimicrobial peptides into the lumen of the intestine [39, 77]. These peptides belongs to the defensin family members or to family of RNAse [39], including α-

defensins and lysozyme C, but complete microbiome is essential to produce an integral set of these peptides [77]. It follows that an important defence mechanism is the immune system that can regulate the secretion of antimicrobial molecules as defensin and so can shape the microbial community. The expression of these molecules is induced, for example, by *Bacteroides thetaiotaomicron* - a predominant member of the intestinal microbiota. Mice with absent corresponding genes have different gut microorganisms, showing a mechanism whereby commensally bacteria influence intestinal microbial ecology of the gut and form the innate immunity [39].

Follicles of Peyer's patches, involved in immune surveillance in the intestinal lumen and that facilitate the generation of mucosal immune responses against pathogens, are reduced in number and in size in axenic animals [77]. Moreover, in these animals, the mesenteric lymph nodes are also smaller and present cell rarefaction and have no germinal centres. However, the reconstitution of intestinal microbiota is sufficient to restore the mucosal immune system [77]. This fact confirms that there is a dynamic relationship between microorganisms and the immune system [81].

The intestinal mucosa destroys external threats due to the signalling capacity of innate immune system through the toll-like receptors, in which the bacterial ligands, such as specific microbial macromolecules like lipopolysaccharides (LPS) [77, 81], polysaccharide, lipoteichoic acid [77], flagellate and peptidoglycan [81] are recognized by these toll-like receptors and affect normal function and development of the mucosal immune system [77].

Many species of bacteria have specific effects on their host. The development of T regulatory and T helper 1 and 2 cells is also dependent on the signals emitted by intestinal bacteria, because the short-chain fatty acids, such as butyrate, demonstrated the ability to inhibit NF-kB [81]. In particularly, it is known that the polysaccharide A associated with *Bacteroides fragilis* is able to activate CD4+ T cells, and promote the balance between T helper1 and T helper 2 cells. Mono-colonization with *Bacteroides fragilis* promotes T regulatory cells proliferation and induces anti-inflammatory cytokine - Interleukine-10 (IL-10), that results in protection against chemically induced colitis. *Bifidobacteria* spp. enhances the maturation of the mucosal immunoglobulins A, whereas colonization with *Bacteroides fragilis* regulates the response to lipopolysaccharides during childhood [87]. *Bifidobacterium* and *Lactobacillus* genera are gram-positive bacteria that were able to combat listerial infections *in vitro* [77]. In one study, a strain of *Lactobacillus salivarius,* which produces a bacteriocin *in vivo*, significantly protects mice from infection with the pathogen *Listeria monocytogenes* [77]. *Lactobacillus* spp. inhibited infection through the combination of acid

production and secretion of a unidentified protein, while the infection inhibition by *Bifidobacteria* spp. was attributed to segregation of extracellular protein compound [77].

Another important group of bacteria are *Clostridium coccoides* and *Clostridium leptum*. Colonization with *Clostridia* species protects against inflammatory bowel disease in axenic mice and strongly promotes the production of regulatory T cells that produce IL-10 [87]. *Clostridium coccoides* is the main producer of short-chain fatty acids, particularly butyrate that in addition to be a source of energy has also a protect function against harmful inflammatory responses [87].

However, towards the absence of intestinal microbiota, the main members of receptors of the toll-like family have profiles of low or absent expression in the gastrointestinal tract, that compromising the adequate immune response to pathogens [77]. The basic mechanism of the mucosal immune system is innate immunity and its ability to distinguish the feature of potentially pathogenic microbes from harmless antigens. This distinction is achieved through pattern recognition receptors – the above mentioned toll-like receptors. Recognition of microbiota by TLRs is essential for induction of inflammation and immune response [77], but it remains to be understood how the host distinguishes danger from homeostatic signals [80]. In addition to toll-like receptors, nod-like receptors [39] and nucleotide-binding oligomerization domain (NOD) also recognize microbe-associated molecular patterns [80].

Nod-like receptors are important group of defence molecules that form cytoplasmatic complexes known as inflammasomes that are able to sense hazard signals [80], and that activate the inflammatory processes. Therefore, nod-like receptors are components of innate immune system, being the intracellular receptors for bacterial DNA, which also recognize other fragments, such as peptidoglycans, and can be considered as targets for controlling of inflammation [39].

Members of the NOD family – NOD1 and NOD2 can recognize the microbial molecules, but the exact role of these proteins in the pathogenesis of diseases remains unknown [80].

Toll-like receptors are highly conserved family of receptors that are present on the surface or cytoplasm of epithelial cells of the innate immune system and each of them recognize characteristic molecules [39, 77, 80, 85]. In humans, some of toll-like receptors (TLR1, 2, 4, 5, 6 and 10) are expressed on the cells surface [85]. So, these receptors play a crucial role in the innate immune system of the host defence against pathogens and are also required for intestinal homeostasis [39]. Recognition of the pathogens by specific toll-like

receptors and its consequent activation triggers a cascade of events, starting among the other pathways with the activation of the signalling system of NF-kB, that results on production and release of protection peptides - cytokines [77, 81] and phagocytes [81] as well as on increased T cell activation [77]. The result can be a protective response to commensally bacteria, an inflammatory reaction against pathogens or triggering of apoptosis [81].

The toll-like receptors 5, highly expressed in epithelial cells of the intestinal mucosa [85, 87], bind to bacterial flagella through the recognition of bacterial flagellin and are involved in mediating of immune response. [85, 87]. Moreover, there is evidence that they also protect against metabolic syndrome [77].

Interestingly, the toll-like receptor 2 can also recognize a wide variety of molecules, including structural lipids, lipoproteins [85] and lipid-containing bacterial lipopeptides [5, 60] found on the surface of bacteria, and in fact, several independent studies have investigated the role of toll-like receptor 2, giving a causal link to development of diet-induced obesity and metabolic disturbs to this pathogen-associated receptor [60]. Moreover, the expression and induction of toll-like receptor 2 was found to be under direct control of bacterial lipopolysaccharides, and can also be induced by tumour necrosis factor α [60]. These data support the idea that different toll-like receptors can respond to the presence of bacterial structures [85].

Regarding toll-like receptor 4, it is located on the surface of immune cells such us monocytes, macrophages, Kupffer cells and preadipocytes, as well as on non-immune cells such us adipocytes, hepatocytes and endothelial cells [66].

Taking into account these data a new set of problems can arise from this close connection of metabolic and immunologic systems [66].

9. Gut microbiota and health disturbances

The ability of intestinal microbiota to perform their functions can be affected by specific factors inherent to host, such as reduced peristalsis, immune suppression, or by environmental factors [66]. Regular consumption of anti-inflammatory drugs, laxatives, chemicals, alcohol or the application of radio- or chemotherapy [91] as well as burns will cause changes in gut microbiota [66]. The administration of antibiotics even for a short period of time significantly alters the balance of intestinal bacterial species for long-term [21, 57], returning to the original composition microbiota around after 4 weeks [57]. Antibiotics can drastically reduce the dominant populations with occurrence of antibiotic-associated diarrhoea in individuals more susceptible [21] and the development of opportunistic infections such as due to *Clostridium difficile* is also favoured [91]. These factors can modulate immunity, homeostasis of the gut and cause a disturbance in the bacterial composition and cause disease [66], being another not less important environmental factor - western diet responsible by making changes in microbial ecology with an impact on health [21].

The complex and still poorly characterized interaction between the intestinal microbiota and innate immune system may be involved in metabolic dysfunction [59] given the metabolic diseases be accompanied by a change in the composition of the gut microbiota in animals and humans [39].

Signalling molecules released into the intestinal lumen from cells in the lamina *propria*, which are under control of the central nervous system may result in changes in gastrointestinal motility, secretion and intestinal permeability, and can affect the gastrointestinal environment in which bacteria reside. The stress also induces intestinal permeability and allows bacteria and bacterial antigens to cross the epithelial barrier. The epithelial barrier permeation can activate the mucosal immune response, which in turn alters the composition of the microbiota. It was shown that acute stress causes an increase of paracellular permeability of the colon, involving mast cells, overexpression of interferon gamma and decreased expression of tight junction proteins [77].

In genetically and diet induced metabolic syndrome mice, in addition to producing profound global changes in glucose tolerance, the bacterial species that confer barrier protection – *Bifidobacteria* spp., were reduced and the species that produce endotoxins such as ones belonging to *Desulfovibrionaceae* family (order of *Proteobateria* phylum) were increased. Thus, the diet seems to be a strong member for the structural changes compared with the microbial genetic alterations. It is indeed surprising that the common "subject-

matter" is associated with diseases or conditions as diverse as obesity and inflammatory bowel disease, wherein the microbial complexity is substantially reduced in comparison with the intestinal microbial communities in healthy individuals [59].

Many diseases, such as allergies [86, 91] some cancers [86] as for example colorectal cancer, arthritis [91], diabetes [86] and obesity [39, 57] can be associated with changes in the intestinal microbiota ecology.

9.1. Gut microbiota and intestinal diseases

The bacterial genome is considered as important pathogenic factor involved in diverse diseases [57] and the quantitative and qualitative changes in the intestinal microbiota can result in diseases of the gastrointestinal tract, such as chronic inflammatory bowel disease, constipation or diarrhoea [57, 81, 91].

Intestinal microbiota was altered in patients with inflammatory bowel disease and was also significantly altered in biopsies taken from inflamed mucosa when compared with healthy sites. Nonetheless, it is not yet clear whether these changes are responsible for the appearance of diseases or are the result of inflammatory response and considerable changes in the tissues of the gut [77].

Patients with chronic inflammatory bowel disease have their intestinal microbiota with very low diversity of *Firmicutes*, when compared with healthy people. Thus, the indigenous microbiota is considered the main factor that triggers inflammatory bowel disease. Indeed, it was reported that a lower proportion of *Firmicutes* can lead to an increased number of gram-negative bacteria with pro-inflammatory activities and that some *Firmicutes* due to producing of large amounts of butyrate may interfere with NF-kB activation cascade, and thereby inhibiting inflammation [21].

9.2. Gut microbiota and obesity

There are physiological and environmental factors underlying the risk causes for obesity and for associated metabolic disorders [85].

Although the cause of obesity is often the ingesting of more calories than expended, the differences in the gut microbial composition in humans may be an important factor that affects energy homeostasis [88]. Therefore it is proposed that high-fat diet itself and not obesity may contribute to changes in the composition of the microbiota [3], which in turn possible has a role in the development of metabolic disorder and leads to obesity (Fig. 11).

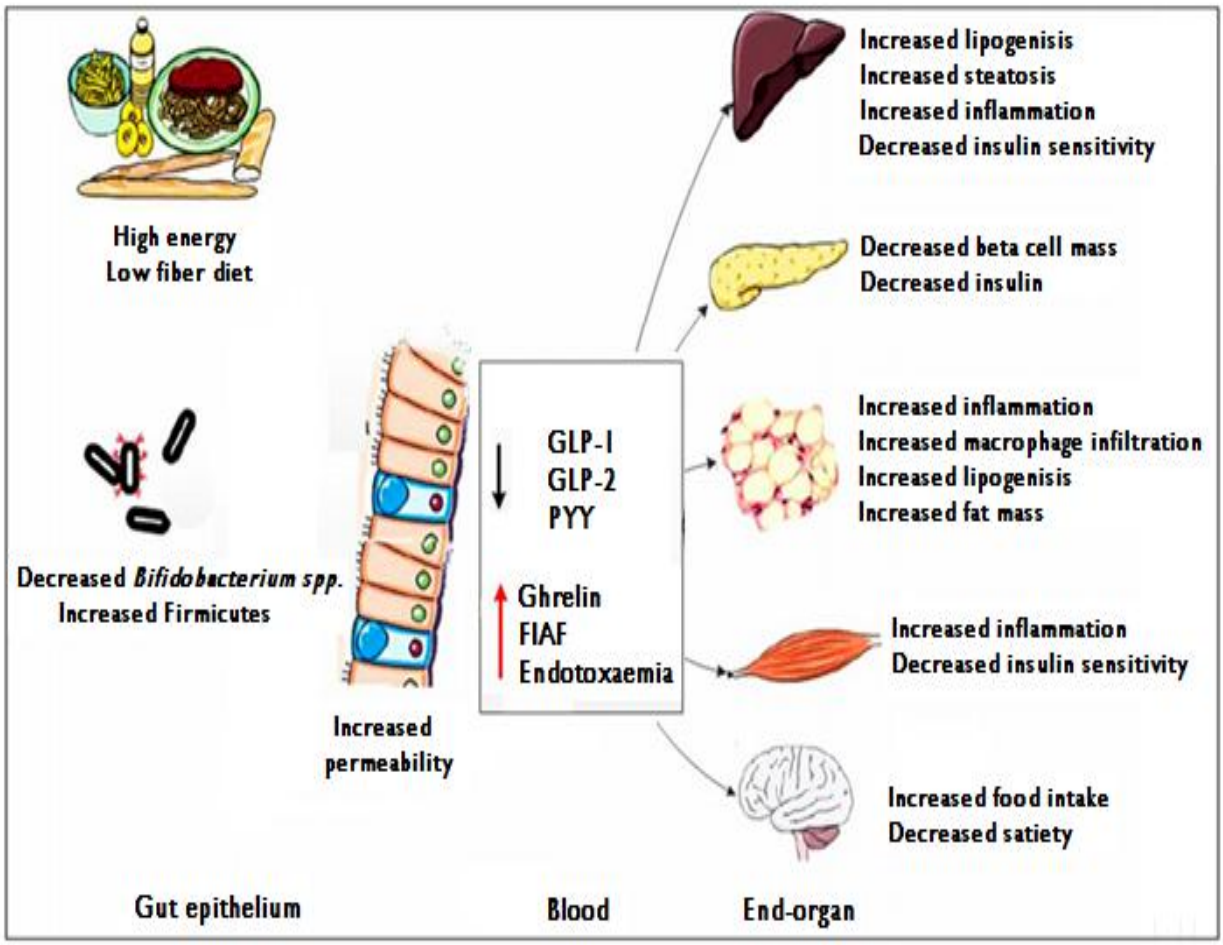

Figure 11 – Gut microbiota alterations and metabolic disorders. It is schematically represented mechanism whereby alteration of the gut microbiota with a high-fat low-fibre diet leads to metabolic endotoxaemia with increased lipid storage and decreased insulin sensitivity. High-fat diet feeding changes gut microbiota, promotes increased intestinal permeability and consequent metabolic endotoxaemia with decreased anorexigenic and decreased orexigenic molecules promotes metabolic disorders (insulin resistance, steatosis, adipose tissue macrophages infiltration, etc.). GLP-1 – glucagon-like peptide-1; GLP-2 - glucagon-like peptide-2; PYY – polypeptide YY [83, 92].

Given the importance of microorganisms in the digestion process and metabolism of its host [59], gut microbiota has recently been proposed as an environmental factor

responsible for weight gain [85], altered energy [85, 91] and lipid metabolism [91] that are associated with obesity and related diseases [59]. In other words individuals predisposed to obesity have microbial communities that promote more efficient extraction and storage of energy from a given diet, when compared to those communities of lean individuals [39, 88]. However, this mechanism has been challenged by other studies that suggest that the relation between the bacterial composition and capacity of energy extraction is more complex than previously considered [39].

9.2.1. Gut microorganisms related to obesity

In obesity the changes of microbiota are strongly associated with increased ability to energy extraction from the diet, with the influence of expression of host genes particularly those that regulate the metabolism of lipids and glucose on periphery, and promotion of low-grade systemic inflammation and insulin resistance [79]. At any approach, the weight loss is accompanied by changes in the intestinal microbiota [5]. In fact, in obese and lean individuals, a high calorie intake of about 2400-3400 kcal/day is accompanied with rapid changes in intestinal microbiota [3].

The colonization of axenic mice has substantially changed the transcription of several mediators in the gut, especially on epithelial cells, thereby regulating key gut functions, such as the absorption of nutrients, mucosal barrier function, angiogenesis and metabolic functions [59]. The conventionalization (colonization with "normal" microbiota) of germ-free mice promotes weight gain and development of fat mass [67]. Thus, apparently the presence of microbes itself increases the energy extraction from the diet [57], because axenic mice after 8 weeks of feeding with high fat and carbohydrates diet increased weight and fat mass significantly less than conventionalized mice [67]. In fact, it was found that young conventionally created mice have about 40% more fat than axenic mice, although they had lower energy intake [57, 67, 91]. Also, studies with colonization of germ-free mice with the microbiota of conventional mice demonstrate that this "conventionalization" results in an increase of about 60% of body fat mass [5, 57, 59, 67, 77, 91], increased insulin resistance [57, 59, 67, 77], altered fasting glucose and insulin concentrations, induction of hypertrophy of adipocytes [91] and hepatic triglyceride count [59, 67 91], although without changes on energy consumption or expenditure [67]. Axenic mice beside the weight gain were also resistant to high fat diet induced obesity and comorbidities [3, 7, 66, 77, 80], metabolic syndrome [39, 59] and glucose intolerance induced by Western diet [67] when compared with conventional mice. This resistance was probably due to the fact that colonization of

axenic animals alters the metabolism of fatty acids [77]. However, on other study using another strain of axenic mice, animals have gained more weight and body fat than the "normal" mice towards the similar diet regarding the caloric intake and fat, but with a different composition of ingredients [7]. Another interesting fact results from a comparison of two types of colonization. The colonization of mice with microbiota of obese animals results in higher gain of weight than in mice colonized with microbiota of non-obese animals [39, 60, 66, 67, 89]. The total body fat mass gain was, respectively, 60% and 40% [66]. However, regardless the type of transferred microbiota (from obese or non-obese animals), the fat mass gain was accompanied by increased blood glucose, insulin and leptin levels [91]. Furthermore, axenic animals that received the obese caecal microbiota extracted more calories from food when compared with transplantation of lean microbiota [66, 67, 87]. These results are probably caused by differences in the microbiota between lean and obese mice [87]. It can be concluded that in obese individuals there is an increase of efficiency of energy extraction by microbiota from the diet [57, 67], and the composition of intestinal microbiota varies, depending on the state of obesity [57, 59, 85].

Gut microbiota can be involved in the development of low-grade inflammation classically associated with metabolic diseases such as obesity [3]. However, little is known about the effects of nutrition on the ability to induce specific microbial populations that can confer a protection and prevention from specific diseases or, conversely, that is harmful and cause diseases [87]. Even less is known about other constituents of the microbial microbiota of the gut such as viruses and fungi, which can together with bacteria affect the metabolism physiology and immunity in general of their host [59].

There has been proposed a set of microorganisms of intestinal microbiota that can be linked to obesity (Table 3). And different types of bacteria are present in obese and in lean individuals (Fig. 12) [86].

Table 3 – Principal Bacteria and Archaea in human gut microbiota and its probable relation to obesity [86].

Representative phyla	Class	Genera	Associated with obesity
Bacteria			
Firmicutes	*Clostridia*	*Clostridium*	Yes
		Eubacterium	Yes
		Faecalibacterium	Yes
		Peptostreptococcus	
		Ruminococcus	
		Roseburia	Yes
	Bacilli	*Lactobacillus*	Yes
		Enterococcus	Yes
		Staphylococcus	Yes
Bacteroidetes	*Bacteroidia*	*Bacteroides*	Yes
		Prevotella	
		Xylanibacter	
Proteobacteria	*Deltaproteobacteria*	*Desulfovibrio*	Yes
	Gammaproteobacteria	*Escherichia*	
	Epsilonproteobacteria	*Helicobacter*	
Actinobacteria	*Actinobacteria*	*Bifidobacterium*	Yes
Fusobacteria	*Fusobacteria*	*Fusobacterium*	
Synergistetes	*Synergistia*	*Synergistes*	
Spirochaetes	*Spirochaetes*	*Treponema*	
Verrucomicrobia			
Cyanobacteria			
Archaea			
Euryarchaeota	*Methanobacteria*	*Methanobrevibacter*	Yes
		Methanosphaera	

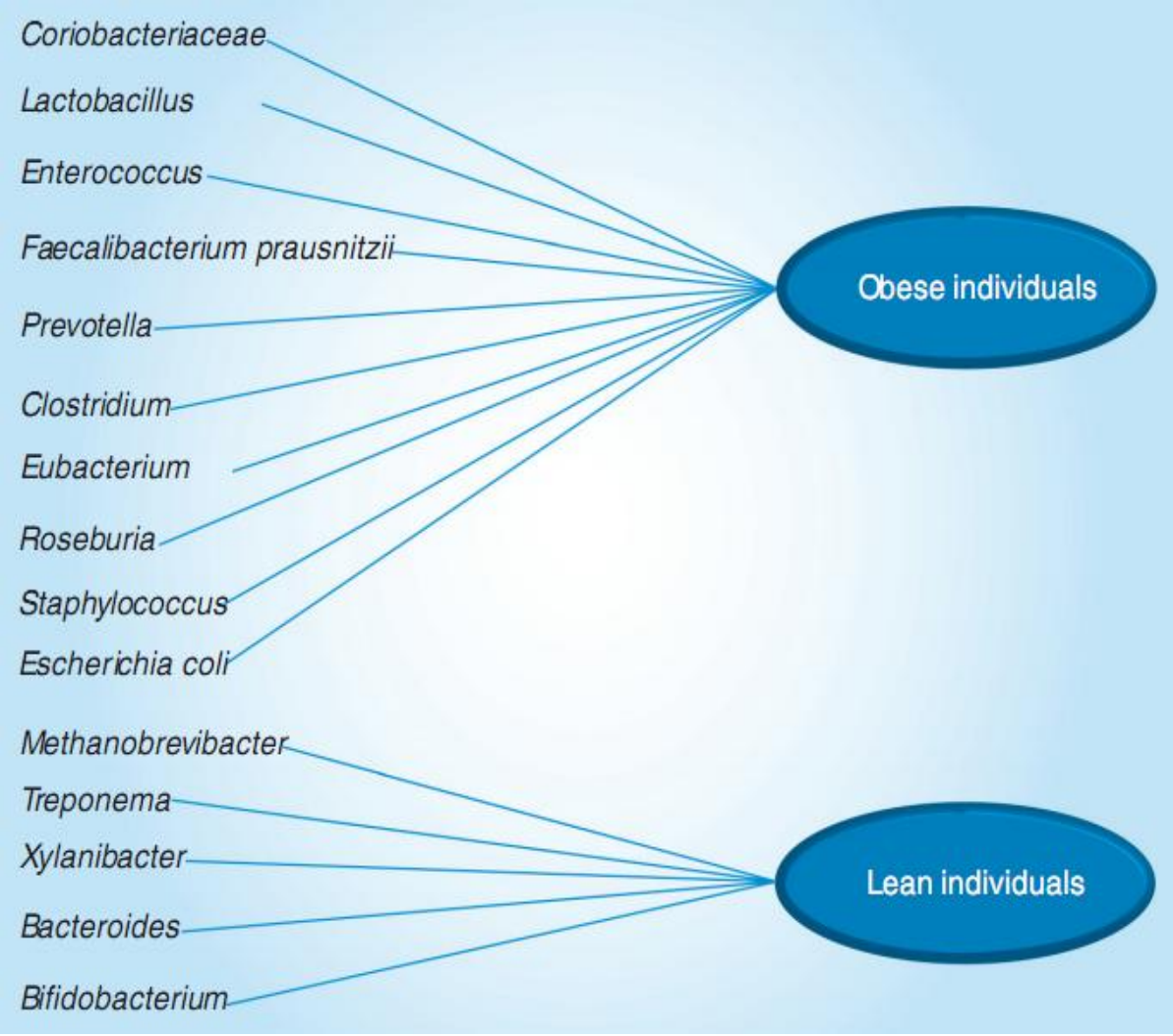

Figure 12 – Different type of bacteria present on obese and lean individuals [86].

9.2.1.1. Obesity and *Bacteroidetes*

Studies with volunteers suggested that 42% of lean genes [66] that are involved in processing of lipids, carbohydrates and amino acids [66, 86] were derived from *Bacteroidetes* [66].

In obese human subjects submitted to weight loss over a period of 12 months, the percentage of *Bacteroidetes* was associated with an increase of the total faecal bacteria from about 2% up to 20% [89]. Particularly, a considerable increase of *Bacteroides fragilis* correlated with a reduction in body weight It was detected [5, 86, 85]. Indeed, in a study of overweight adolescents a caloric restriction and exercise for 10 weeks resulted in a body

weight loss (rather than 4 kg) followed by an increase in the number of *Bacteroides fragilis* [5, 85].

Although the count of bacteria from *Bacteroidetes* phylum was reduced in obese individuals, it was observed that a specific subgroup of *Bacteroidetes* - *Prevotellaceae* family was significantly increased in obese individuals [3, 39, 59, 86]. Furthermore, *Bacteroides thetaiotaomicron* that is a highly obligate anaerobe present on gut microbiota of adult individuals seems to have a diverse foraging activity [5], such as saccharolytic fermentation contributing to adiposity development [80].

9.2.1.2. Obesity and *Firmicutes* – class of *Mollicutes*

Mollicutes is a class of bacteria that influences the energy balance of its host and development of obesity [5]. In obesity-induced mice an increased *Firmicutes* seems to due mainly to the class of *Mollicutes* [57, 67, 80, 85], belonging to *Erysipelotrichaceae* family [80], given *Mollicutes* were predominant in mice with induced by western diet rich in carbohydrates obesity [5]. Increase of this type of microorganisms towards the western diet was accompanied by induced expression of genes involved in the transport and processing of sugars in the intestine [5, 85], as phosphotransferase system with mechanisms for fructose and mannose metabolism [5] leading to rather energy extraction from the diet [67]. *Mollicutes* population have also significantly reduced on mice upon submission to carbohydrate and fat restricted diet [5].

9.2.1.3. Obesity and *Firmicutes* – *Lactobacillus* spp.

Even regarding the *Firmicutes*, the number of bacteria belonging to *Lactobacillus* genus in obese patients is higher in comparison with its number in lean individuals [3].

Nearly half of the obese population have concentrations of *Lactobacilli* species greatly increased and patients with Diabetes Mellitus Type II have also higher levels of *Lactobacillus* spp. together with *Bacilli* spp. It was reported a significant weight gain in patients with endocarditis after treatment with high doses of vancomycin, which was proposed to be responsible to *Lactobacilli* spp. that resisted to the effect of this antibiotic [86].

Paradoxically, weight loss induced by caloric restriction and exercise in overweight adolescents also promoted the *Lactobacilli* spp. number increase. High levels of *Lactobacilli* spp. that accompanies the obesity state may come from the fact that they are part of the phylum *Firmicutes* that are increased on obese subjects. However, although studies are still

scarce, the administration of *Lactobacillus gasseri* decreases visceral and subcutaneous fat mass and body mass index of obese individuals [3].

9.2.1.4. Obesity and other *Firmicutes*

The mice fed with a low-fat but rich in plant polysaccharides diet when fed with a "Western" diet, the composition of its microbiota has changed with notable overgrowth of *Firmicutes*, including *Clostridium innocuum*, *Eubacterium dolichum*, *Catenibacterium mitsuokai* and *Enterococcus* spp. and a significant reduction in several *Bacteroides* spp. [87].

In the study with pigs, it was observed an increase in relative abundance of bacteria from *Firmicutes* phylum with diet intervention, being found a positive correlation with *Firmicutes* and weight-gain [93].

Bacteria related to *Roseburia* spp. and *Eubacterium rectal*, together with *Clostridium coccoides* are an important group of *Firmicutes* that contribute to polysaccharide breakdown in the colon and are major producers of butyrate [86, 94]. Body weight gain, fat mass development, fasting glycaemia, glucose tolerance, cholesterolemia were negatively correlated with these bacteria [95]. It is known that diet-induced obesity in mice led to markedly reduced *Eubacterium rectale* and *Clostridium coccoides* [3, 60]. It is thought that *Roseburia* and *Eubacterium rectale* group bacteria play a key role in the formation of butyrate, and probably has a positive impact on the health of the colon, showed a marked reduction in their share with the reduction of the total carbohydrates and non-starch polysaccharides intake [89]. Indeed, *Roseburia* spp. and *Eubacterium rectale* were decreased through high-fat feeding and were restored by fiber supplementation [95].

9.2.1.5. Obesity and *Bacteroidetes* / *Firmicutes* rate

It has been proposed that there are two major phyla of bacteria found in humans – *Firmicutes* and *Bacteroidetes* [59, 89], responsible for more efficient energy extraction from the diet resistant (non-digestible) components [59, 89]. Several studies showed that high-fat diet results in a decrease numbers of *Bacteroidetes* while *Firmicutes* and *Proteobacteria* increase. The ratio *Firmicutes* / *Bacteroidetes* helps determine state of obesity [3, 5, 59, 84, 93], given obese mice have rather than 50% of *Firmicutes* than *Bacteroidetes* when compared to non-obese mice [5, 57, 77, 83, 85, 88, 91] or their lean brothers. It should be noted that in the last case the increasing of rate *Firmicutes* / *Bacteroidetes* is not related to differences in the food consumption [83]. The change in the abundance of these two dominant divisions

was also associated with mice deficiency of leptin [5, 59, 67, 85] or related to genetic ablation of the leptin receptor gene [39].

Human obese individuals also have more *Firmicutes* and fewer *Bacteroidetes* [5, 57, 59]. In study with volunteers the change of the ratio of *Firmicutes* and *Bacteroidetes* was accompanied with a parallel increase in the concentration of propionate in obese and overweight subjects [5]. Other studies show that fat and carbohydrates restrictions can cause a decrease on the *Firmicutes* [57] and increase of *Bacteroidetes* number [57, 87], or only decrease of the number of butyrate producing *Firmicutes* [5].

However, there are other studies that did not show any relationship between the proportions of *Bacteroidetes* and type of diet or obesity [86] as well as the ratio of *Firmicutes* versus *Bacteroidetes* and body mass index [80]. Although there are studies that can contradict the relation of change on the ratio of colonizing bacteria to obesity [85], many studies with animals support this fact.

The changing back of *Firmicutes* and *Bacteroidetes* ratio can be correlated with caloric restriction [77, 91], with weight loss [66, 83, 86] or also with the return to low fat and high polysaccharide content diet [67, 83].

The amount of energy in the faeces in the proportion of consumed calories was also positively correlated with abundance of *Bacteroidetes* phylum and negatively correlated with the abundance of phylum of *Firmicutes* [3, 85].

The high-fat diet decreases bacteria belonging to *Bacteroidetes* and increases bacteria belonging to *Firmicutes* and *Proteobacteria* phyla regardless the obese or non-obese phenotype of animals, suggesting that the fat content of the diet, and not so much obese phenotype of the individual, is the major factor of changes observed in the microbiota of obese people [91].

The loss of body weight in obese adolescents, results in reductions in *Clostridium histolyticum* and *Clostridium coccoides*, as well as in *Eubacterium rectale* [5, 86] and in increased counts of the group of *Bacteroides* and *Prevotella* species [86]. A positive correlation was also observed towards the ratio of *Bacteriodes* and *Prevotella* genera to *Clostidium coccoides* and *Eumacterium rectale* species and glucose levels [87].

9.2.1.6. Obesity and *Proteobacteria*

Resistant and predisposed to obesity mice had an overall decrease of bacteria with high-fat diet, however, the number of *Enterobacteriales* (*Proteobacteria* phylum) increased in

mice prone to develop obesity with a diet rich in fat [85]. Furthermore, the mice with leptin receptor deficiency or ablation have also increased *Proteobacteria* counts [5, 39, 59].

Some authors showed that consumption of safflower oil have stimulated the growth of δ-*Proteobacteria* with increasing of bacterial genes responsible for chemotaxis and flagella development, giving to these bacteria a competitive advantage over other groups of bacteria that colonize the gastrointestinal tract. However, another study showed that a diet rich in saturated fat as milk, and not in safflower oil, caused the growth of δ-*Proteobacteria*, in particular *Bilophila wadsworthia* at caecum [87]. These controversial observations may result from different area of the gastrointestinal tract investigated the caecum and colon are different in terms of functions as well as in microbial composition [87].

9.2.1.7. Obesity and *Actinobacteria –Bifidobacteria* spp.

Obesity is also related to higher proportion of *Actinobacteria* in comparison with the normal or anorexic individuals [67, 80, 86]. It is considered that the *Actinobacteria* phylum that among different genera includes the *Bifidobacterium* genus has been associated with weight gain [86]. This affirmation is proposed because most of genes (75%) related to obesity were found to be from *Actinobacteria* [66].

Bacteria from *Bifidobacterium* genus were present in higher quantity in normal-weight than in overweight individual [3]. Some reports have shown that the weight loss can be associated with reduced amounts of *Bifidobacterium bifidum* and *Bifidobacterium breve* and increased counts of *Bifidobacterium catenulatum* [3]. In study of caloric restriction and exercise that leads to weight loss, regarding the *Bifidobacterium* spp, it was just observed a significant reduction of *Bifidobacterium longum*, but the ratio of *Bifidobacteria* / *Clostirdium coccoides* has increased [5]. The level of *Bifidobacterium* genus was also decreased after weight loss following the surgery performed in obese individuals [3]. Although it was found *Bifidobacteria* decreases its quantity in obese people submitted to restrict diet, the faecal quantity of the *Bifidobacterium* genus was reported to be significantly lower in obese subjects when compared with lean ones [86]. It was also demonstrated by some studies that diet-induced obesity (high-fat and low on carbohydrates diet) in rodents in addition to *Bacteroides* -related bacteria has also markedly reduced the number of *Bifidobacterium* cells [3, 60, 83, 95].

Obese mice with a mutation in the leptin receptor have also showed a lower amount of total bacteria and particularly of *Bifidobacterium* genus when compared to healthy mice. In

these mice it was detected strains of *Halomonas* and *Sphingomonas* genera, which have been related to the obesogenisis with the production of acetate [5].

It is important to take into account that, *Bifidobacteria* spp. represent an important and complex group of bacteria whose presence is often associated with beneficial effects for health [3], and its depletion in obese subjects seems to be a change more reproducible in intestinal microbiota, being so that the best candidate to produce the anti-obesity effect [86].

9.2.1.8. Obesity and *Archaea*

In an analysis of the changes of gut microbiota associated with obesity concerning *Methanobrevibacter* spp., - a principal known representative of *Archaea* on microbiota, it was found that obese individuals have *Methanobrevibacter* spp. in greater quantity than the non-obese ones [39, 86]. Although it is known that *Archaea* can increase the efficiency of bacterial fermentation [57] and is related to the enhancing of energy absorption in obese people [39], the exact mechanism of weight gain caused by methanogens remains still unclear [86].

An association of *Bacteroides thetaiotaomicron* with saccharolytic fermenting microorganism [80] with - *Methanobrevibacter smithii*, was found in mice fed with diet rich in fructans. *Methanobrevibacter smithii* influences the specificity of the fermentation and storage of the fat. Mice co-colonized with these two types of microorganisms have also showed an increase on levels of ethyl, leading to up-regulation of fatty acid synthase, and contributing also to adiposity when compared to axenic controls [5, 80].

9.2.2. Changes on gut microorganisms - genetics and nutrition

The question that remained for some time without answer is "the obese phenotype is secondary to changes in the microbiota or, otherwise the microbiota alters as a sequence of obesity?" It was shown that the change in composition of intestinal microorganisms is a cause and not a consequence of obesity or altered eating habits [67]. However, it should be realized that it cannot be said with absolute certainty that the microbiota changes is the cause and not a consequence of obesity. Therefore, diversity of microorganism core can have an origin and be dependent on the genetic background, but deviations from this core can in turn be associated with different physiological states, such as obesity or leanness [39].

An example proved that the microbiome structure rapidly changes in response to a change of a diet from one of low fat and high herbal content to a high on fat and sugar content diet, modifying both, available metabolic pathways and gene expression [39].

The diet and age are important not only to define the composition of the intestinal microbiota, but also its ability to modulate energy extraction [59].

Dietary changes are associated with 57% of the bacterial variation in intestine, while the genetic background is responsible only for 12% of this variation in animals, suggesting that the diet is determinant on the composition of the intestinal microbiota, and influences its diversity and profiles [85, 87]. Changes in principal microbial populations can lead to transformation of a healthy intestinal microbiota into entity that induces disease. For example, the "Western" diet that is rich in fat and sugar causes dysbiosis affects both the metabolism of the host gastrointestinal tract and immune homeostasis [87]. However, the precise mechanism by which high-fat and high-sugar diets alter the functionality of intestinal microbiota requires further detailed studies. Within the context of obesity, it seems that the genes can determine the initial composition of the gut microbiota, being on the other hand the diet its potent modulator [85].

A comparative study of different eating habits, such as herbivore, omnivore and carnivore demonstrated that the acquisition of a new diet is enough to radically change the intestinal microbiota, with the triggering the evolution of new species [39]. In fact, both, diet and phylogeny shape modulate a variety of bacteria, which increases from carnivore to herbivore and to omnivore. In addition, a modern lifestyle alters the intestinal microbiota of humans to the similar one to primate omnivores [39]. The intake of specific nutrients can really changes the composition of the intestinal microbiota [3]. For example, vegetarianism alters the gut microbiota in humans because large amounts of fibre can lead to increased production of short-chain fatty acids by microbes with consequent decrease of intestinal pH. [87].This decrease prevents the growth of potentially pathogenic bacteria such as *Escherichia coli* and other members of the family *Enterobacteriaceae*. Interestingly, it also was found that European children have their microbiota depleted in *Bacteroidetes* and enriched on *Enterobacteriaceae* when compared with rural African children. Authors have proposed that this difference is due to low dietary fibre intake by Europeans [87].

It was evaluated the effects produced by high-carbohydrate meal, fat saturated meal, or orange juice meal in healthy subjects in inflammatory markers. The expression of NF-kB, TNF-α and interleukin-1beta significantly increased after glucose and cream (saturated fat) intake. Plasma concentrations of toll-like receptor 4 and LPS were enhanced only after ingestion of cream, but orange juice did not change these markers. Moreover, when orange juice was added to a high fat and carbohydrate content meal, it prevented the postprandial rise of endotoxin LPS and related inflammatory markers in plasma [67]. An excessive intake

of fructose also increased the levels of portal endotoxin, plasma inflammatory cytokines, hepatic steatosis, and resistance to insulin, when compared with control groups who drank only water [67]. These data suggest that different nutrients induce different inflammatory response and endotoxaemia, and fat, and possibly fructose have a higher potential to induce these events [67]. Furthermore, the ingestion of complex carbohydrates reduces the risk of colonization by pathogenic species such as *Mycobacterium avium* and bacteria of *Enterobacteriaceae* family when compared with high-fat or protein diet. Complex carbohydrates also benefit an increased of levels of *Bifidobacteria* spp. such as *Bifidobacterium longum*, *Bifidobacterium breve* and increased counts of *Bifidobacterium catenulatum*. On the other hand, refined sugars mediate the overgrowth of opportunistic bacteria such as *Clostridium perfringens* and *Clostridium difficile*, and increasing of bile flow [87].

The change in eating behaviour was associated with an increased intestinal permeability to lipopolysaccharide. Changes in intestinal microbiota were also associated with a different susceptibility to antimicrobial treatment. The mice fed with a high content of fat diet were more sensitive to antibiotic treatment, suggesting that the new microbial ecology after changes in dietary content is much more fragile [39].

The short-chain fatty acids resulted from microbial fermentation as stated above, contribute to the total energy obtained from the diet. However, on the other hand, the energy derived from a "non-digestible" carbohydrate which undergoes microbial conversion to short-chain fatty acids is lower than that resulting from an equivalent amount of sugar absorbed directly into the small intestine. In addition, the non-digestible carbohydrates can also increase the satiety [89].

The consumption of formula supplemented with the fish oil has the ability to change the microbial composition on the children. However, it is unknown whether these microbial changes might be durable or transient [87].

In following table (table 4) it is resumed the way how different kinds of feeding can change the components of the microbial microbiota [87].

Table 4 – Bacteria affected by different types of diet [87].

Diet	Affected bacteria	Effect on bacteria
High-fat	*Bifidobacteria* spp.	Decreased (absent)
High-fat and high-sugar	*Clostridium innocuum*, *Catenibacterium mitsuokai* and *Enterococcus* spp.	Increased
	Bacteroides spp.	Decreased
Carbohydrate-reduced	*Bacteroidetes*	Increased
Calorie-restricted	*Clostridium coccoides*, *Lactobacillus* spp. and *Bifidobacteria* spp.	Decreased (growth prevented)
Complex carbohydrates	*Mycobacterium avium* subspecies *paratuberculosis* and *Enterobacteriaceae*	Decreased
	Bifidobacterium longum subspecies *longum*, *Bifidobacterium breve* and *Bifidobacterium thetaiotaomicron*	Increased
Refined sugars	*Clostidium difficile* and *Clostidium perfringens*	Increased
Vegetarian	*Escherichia coli*	Decreased
High n-6 PUFA from safflower oil	*Bacteroidetes*	Decreased
	Firmicutes, *Actinobacteria* and *Proteobacteria*	Increased
	δ-Proteobacteria	Increased
Animal milk fat	*δ-Proteobacteria*	Increased

9.2.3. Gut microbiota and mechanisms related to obesity

Gut microbiota affects the metabolism by increased energy extraction [85, 67], modulation of the immune system and altered lipid metabolism. The physical presence of both bacteria and its metabolites are responsible for these effects [85].

In addition, other mechanisms relate the intestinal microbiota with obesity, such as chronic endotoxaemia of low grade [67] that leads to increased levels of TNF-α and IL-6 in adipose tissue [60], regulation of the composition of tissue biologically active fatty acids and modulation of secretion intestinal peptides [67].

Through the genetic sequencing of microbiome, it was verified that its wholesale contains important information related to the metabolism of carbohydrates and amino acids, secretion and transportation systems [5].

Different systems or proteins are involved in the control of metabolism of fatty acids, inflammation, the intestinal barrier function, intestinal motility, oxidation and storage of

nutrients. Fasting induced adipose factor (FIAF), the endocannabinoid system and tight junction proteins - zonula occludens-1 (ZO-1), and Occludin are affected by microbe colonization of the intestine and are changed after modulating of the gut microbiota due to dietary composition [83].

In summary, changes in microbiota can affect the balance of the host immune system and lead to increased translocation of bacterial antigens into metabolically active tissues. This would result in a chronic inflammation and disability of metabolic functions leading to insulin resistance, fat deposition on the liver and excessive development of adipose tissue [87]. On the figure 13 is shown the cascade of events that are proposed to evidence that the gut microbiota is involved in the onset of metabolic disorders associated with obesity. Nutritional ("western" diet) and genetic obesity are associated with gut microbiota dysbiosis, leading to the enhanced gut permeability. This gut barrier alteration favours the metabolic endotoxaemia, triggering the low-grade inflammation that characterize the state of obesity [60].

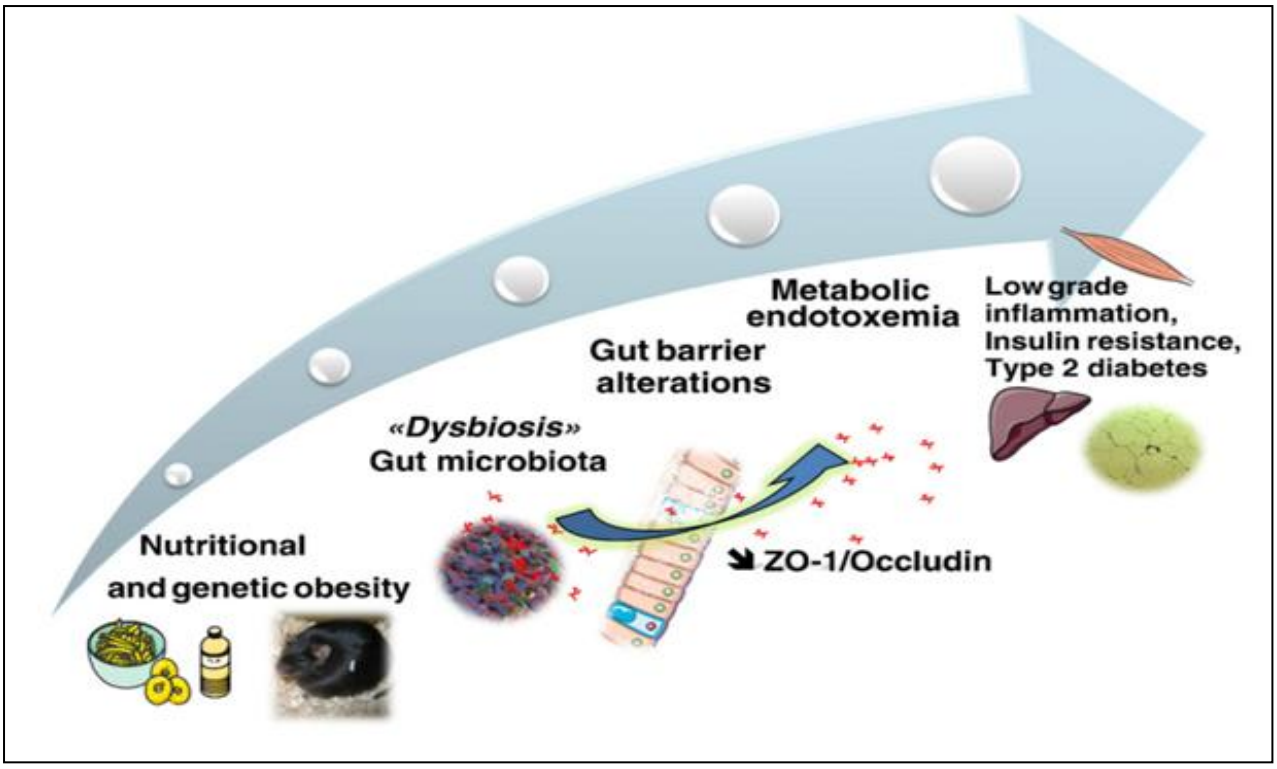

Figure 13 – Relation of intestinal microbiota and low-grade inflammation. The intestinal microbiota is involved in the cascade of events leading to metabolic disorders associated with obesity. Nutritional and genetic obesity is associated with intestinal dysbiosis, leading to the augmented gut permeability due to alterations on tight junction proteins (zonula occludens-1, ZO-1, and Occludin) distribution, promoting metabolic endotoxaemia with low-grade inflammation [60].

9.2.3.1. Gut microbiota promotes efficient food energy uptake

Gut microbiota interacts with host epithelial cells, influencing the energy storage and expenditure [59]. In particular, the distal intestine has been recently recognized as an ecosystem in which each component of the microbial content is involved in the redistribution of energy, by its ability to facilitate the extraction of energy in the form of short chain-fatty acids or chemical transformations. Many bacterial proteins are responsible for the active transport, uptake and metabolism of a wide range of polysaccharides in the human intestine supplied through the diet [5].

Several genes are involved in metabolism of carbohydrates, such as those encoding outer membrane proteins and ones that participate in binding of starch to the bacterial surface, allowing its digestion by α-amylase [21].

Energy storage is affected by bacterial fermentation [21, 57], and the mechanism by which the gut microbiota affects the weight, is the increased uptake of energy from dietary fibre. The microbiota of obese mice has produced more short-chain fatty acids by increased fermentation of polysaccharides coming from the food [85].

The greater ability to extract energy from nutrients observed in obese mice seems to be related to the presence of genes encoding enzymes that break down food non-digestible polysaccharides with consequent increased production of fermentation end products - mainly short-chain fatty acids [66]. Furthermore, in addition to the lower energy content of faeces of obese mice than of lean animals, the presence of the higher concentrations of fermentation products on caecal, namely acetate and butyrate suggest that there is additional absorption of short-chain fatty acids by the intestine of obese mice [5, 85]. Thereby, an increase of energy extraction from dietary fibre may partly contribute to excessive weight gain in obese mice [57]. However, despite of axenic and conventionalized mice had different energy content in their faeces (reduced on lean animals), in one study was found that this difference was not statistically different even though a similar energy intake was observed. This observation suggests that there is another addition way, distinct from a different capacity in energy harvesting, could be responsible for the weight gain induced by the intestinal microbiota [66].

Still regarding the capacity of energy harvesting, the process of extraction and absorption of energy from alimentation is really rather efficient in the obese animals probably due to the fact that the number of genes dedicated to the hydrolysis of polysaccharides is much higher in bacterial community in obese animals than in lean controls [91].

It is now widely accepted that there are great differences of microbiota and their profiles of fermentation between lean and obese phenotypes [5]. It is noted that there are genetic differences between obese and lean mice, because a higher concentration of acetate and butyrate in the caecum of obese mice was observed, while the propionate had no significant difference between the two groups [85]. The short-chain fatty acids resulted from this intestinal bacterial fermentation of complex carbohydrates from the diet are an important source of energy because its absorption stimulates *de novo* synthesis of triglycerides in the liver [3, 67, 88]. Although both acetate and propionate are absorbed from the colon [96], the role of lipogenesis is attributed primarily to acetate [91]. Acetate is captured by peripheral tissues, but can also be used as a substrate for lipogenesis in adipocytes [21, 91] and hepatocytes, and when it is captured by the liver, it is the preferred substrate for gluconeogenesis and for synthesis of cholesterol and triglyceride. However, data from both human and animal studies suggest that propionate inhibits this process, having an opposing effect on lipid metabolism [91, 96]. Propionate inhibits gene expression of hepatic enzymes involved in this *de novo* lipogenesis [91], and this fatty acid has also been demonstrated to stimulate leptin expression and increase its release from adipose tissue in mice, and suppress the pro-inflammatory factor resistin in human adipose tissue depots [5].

The short-chain fatty acids resulted from intestinal bacterial fermentation act as ligands for at least two receptors - G protein-coupled receptor 41 (GRP-41) and G protein-coupled receptor 43 (GRP-43) [67, 77, 79, 83]. The mRNA of GRP-43 was detected in several tissues, but with highest expression in immune cells such as neutrophils, monocytes, peripheral blood mononuclear cells, B cells, polymorphonuclear cells and, although with the less mRNA expression, was also detected on skeletal muscle, adipose tissue, terminal ileum, and colon. On the other hand GRP-41 is more widely distributed than GRP-43, and a high level of its expression can be found in adipose tissue, pancreas, spleen and lymph nodes [85]. These receptors have recently been identified in human colon enteroendocrine L cells that express peptide YY [5, 67, 77, 79, 85]. While GPR-41 binds preferentially to the short-chain fatty acids with bigger chain length [85] and present an increasing specificity to propionate> butyrate> acetate, GPR-43 is equally potent for these three fatty acids [5].

Mice that do not express receptors for short-chain fatty acids, namely GRP-41 when colonized with glycolytic bacteria such as *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii* have a lower weight gain [3], increased circulating levels of PYY, lower stomach emptying time and less energy extraction [59] when compared to mice that express these

receptors. This fact suggests that short-chain fatty acids can promote weight gain through binding to its receptors [3, 59].

Obese mice that are rather colonized by methanogenic *Archaeas* have the ability to improve the efficiency of fermentation by removing of hydrogen from the intestine [59, 86]. This happen because the excessive accumulation of hydrogen reduces the energy yield, leading to a gradual decrease in the fermentation efficiency [86]. In addition, it was suggested that the coexistence of methanogenic species such as bacteria from *Prevotellaceae* family and *Archaea* (*Methanobacteriales* order) in obese subjects allows the more efficient energy capture due to transference of hydrogen between bacteria. The hydrogen transfer that prevents its excessive accumulation allows capture of energy [5] by fermenting of polysaccharides from diet more efficiently, with consequent increasing of their conversion to short-chain fatty acids that result in their excessive storage [86].

Studies with *Methanobrevibacter smithii* and *Bacteroides thetaiotaomicron* showed that their co-colonization not only increased the efficiency of energy extraction, but also changed the specific bacterial fermentation of polysaccharides, thereby increasing the adiposity when compared to mice colonized with a one of this types of microorganisms alone [59]. In addition, the *Archaea Methanobrevibacter smithii* (methane-producer) consumes the molecular hydrogen produced in the colon by fermentation (reduces the acidity of the lower intestine [80]). The elimination of this gas reduces the intraluminal gas pressure and facilitates the growth of other bacterial populations, thereby making the process of fat storage rather more efficient [91].

9.2.3.2. Gut microbiota promotes the increased energy storage

The intestinal microbiota can modulate the hepatic and systemic lipid metabolism of its host by modifying the conjugative ability of bile acids, with direct impact on emulsive and absorption properties of bile acids, with indirect influence on the fat storage on the liver and on properties of lipid peroxidation by bile acid signalling [67]. The transference of microbiota of obese mice has also resulted in the transmission of characteristic of adiposity [5].

Intestinal microbiota is involved in increase of energy storage through various mechanisms (Fig. 14) [60]. It increases uptake and storage of triglycerides in circulation into adipocytes, so adipocytes size in axenic animals is lower than in conventional animals [91]. This increased uptake occurs by suppressing the expression of intestinal inhibitor of circulating lipoprotein lipase - fasting induced adipose factor [5, 57, 67, 85] also known as angiopoietin-like protein 4, which is produced by adipose brown and white tissue, liver and

intestine [21, 59]. Lipoprotein lipase is an enzyme involved in cellular uptake of fatty acids from lipoproteins and accumulation of triglycerides into adipocytes [91]. The angiopoietin-like protein 4 inhibits the absorption of fatty acids from lipoproteins rich in triglycerides that circulate in white adipose and muscle tissues [83], stimulates the lipolysis that is resulting in elevated levels of triglycerides and lipoproteins in plasma, with a consequent reduction of quantity of stored fat [7]. FIAF inhibits lipoprotein lipase activity, thus reducing the release of fatty acids from circulating triglycerides [60].

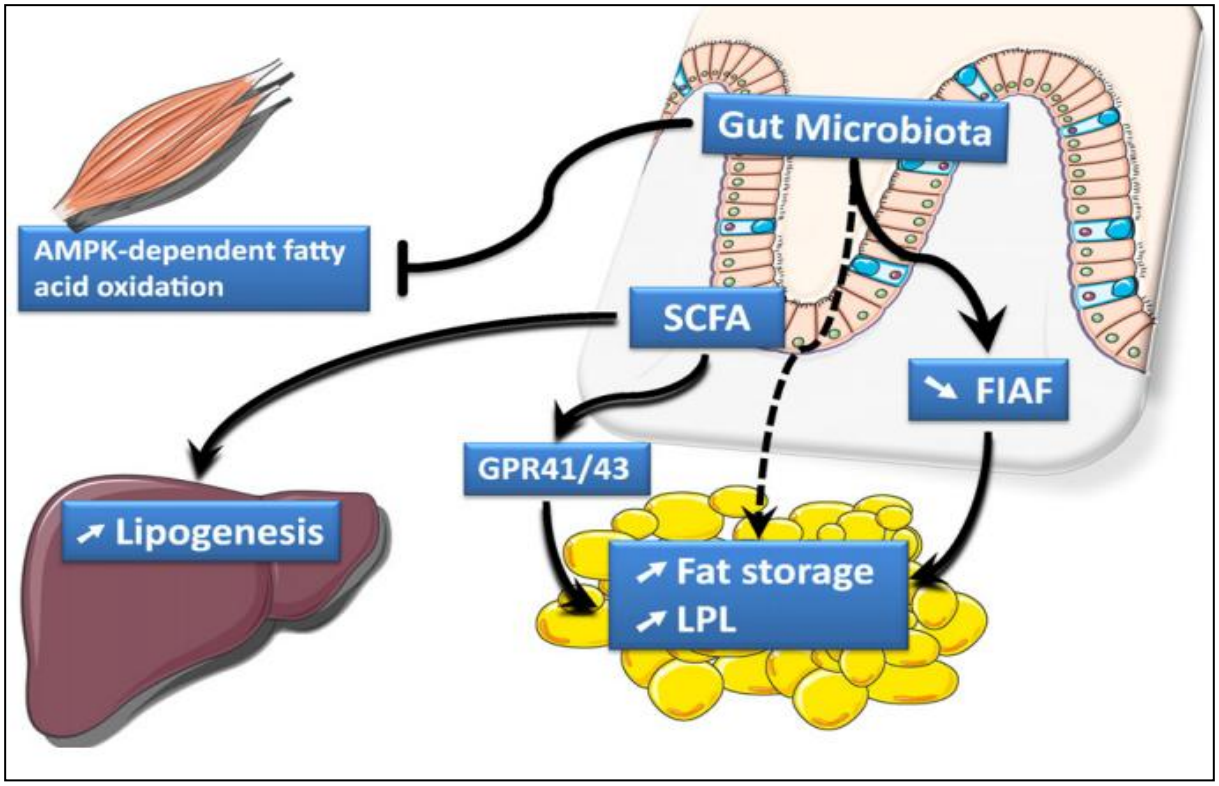

Figure 14 – Gut microbiota promotes the increased energy storage. Due to higher short-chain fatty acid production and absorption, the intestinal microbiota provides to the host lipogenic substrates, thus promoting hepatic lipogenesis and fat storage. For example, by suppressing intestinal Fasting Induced Adipose Factor (FIAF), this microbiota influences the enzyme lipoprotein lipase (LPL). Furthermore, SCFA take part in the fat storage by acting through receptors GPR41 and GPR43, and finally, in response to a high-fat diet, the gut microbiota inhibits AMPK-dependent fatty acid oxidation. Moreover, it should be noted that other direct or indirect mechanisms exist (dotted line) [60].

Fasting induced adipose factor is a key modulator of fat storage induced by microbiota [57, 59, 91], that it inhibits lipoprotein lipase activity [3, 80] – an enzyme that catalyze the release of fatty acids and triglycerides from circulating lipoproteins in muscle and adipose

tissue. After intestinal colonization by microorganisms of the mice, an expression of FIAF is suppressed in the gut, particularly in the intestinal epithelium, and results in consequent overall increase in the activity of LPL [3, 60, 66, 83]. This phenomenon causes a larger storage capacity of hepatic triacylglycerol in the liver [88, 91], as well as increased storage of triglycerides in the adipocytes [66]. Inhibition of LPL by FIAF results predominantly on a decrease of the energy extraction from the diet [7] and on the lower capacity of the triglycerides stored in adipocytes [91]. Fasting induced adipose factor appears to have an important role in the regulation of central metabolism of energy [59], because it was significantly up-regulated (produced in excess) in axenic mice [7]. This overproduction leads to the blockage of cleavage of fatty acids for their capture and also leads to overregulation of fatty acids oxidation, resulting in reduction of its stored quantity [85]. Thus, the suppressed activity of FIAF and the not inhibition of LPL activity in germ-free mice may be one of mechanisms that explains the resistance of these animals to obesity [39, 60].

Conventional transgenic mice that express fasting induced adipose factor are also resistant to obesity [91], but axenic mice without fasting induced adipose factor were not shown to be resistant to high-fat diet and increased their weight [85]. Germ-free knockout to this factor animals has also gained more weight, with a parallel reduction in the genes encoding the enzymes for mitochondrial fatty acid oxidation [5, 66].

A study suggested that the mechanism related to fasting induced adipose factor is not universally associated with the development of fat mass related to gut microbiota [60]. For example, it was showed that axenic mice exposed to high fat diet have higher levels of mRNA expression of intestinal FIAF, when compared with conventionalized mice [60]. Thus, the determination of the exact physiological contribution of intestinal fasting induced adipose factor, as well as its regulation by specific populations of bacteria and its metabolites, needs to further investigation [85].

Fasting induced adipose factor is also a potent inhibitor of angiogenesis, and thus, the intestinal microbiota has a profound ability to influence intestinal angiogenesis. Therefore, intestinal FIAF may serve as a local member for the control of angiogenesis, instead of circulation metabolism [7].

The pathway AMP-activated protein kinase can also explain the resistance of lean phenotype or germ-free mice fed with a high fat diet [39, 59, 60, 66]. AMPK, is a key enzyme (conserved from yeast to humans) that controls the state of cellular energy [59]. The lean phenotype of axenic mice even fed with a "western" diet (high-fat and sugar) is associated with increased levels of phosphorylated AMP-activated protein kinase in skeletal muscle and

liver [39, 59, 66] and increased number of its targets involved in the oxidation of fatty acid, such as acetyl-CoA carboxylase [39, 59].

Indeed, AMPK positively regulates fatty acid oxidation and glucose uptake by muscle tissue, inhibits fatty acid synthesis, gluconeogenesis on the liver [66], glycogen storage and increases hepatic insulin sensitivity [59].

Therefore, the gut microbiota can affect fatty acid oxidation in skeletal muscle via metabolic pathways that involve AMPK. The exact mechanism by which the microbiota makes the signalling to the liver or skeletal muscle through AMPK is not clear, but it seems to be independent on fasting induced adipose factor [59]. This mechanism seems to work by suppressing the oxidation of the fatty acid driven from AMPK in liver and skeletal muscle [60]. Thus, the axenic mice have the protection from diet-induced obesity by two independent but complementary mechanisms (FIAF and AMPK pathways), that result in an increase in fatty acid metabolism [39].

In addition to FIAF and AMPK pathways, short-chain fatty acids' receptors seem also contribute to fat depot. One study showed that GPR-43 knockout mice are resistant to diet-induced obesity [60] and other data revealed that acetate and propionate can stimulate the development of white adipose tissue through activation of its receptor GRP-41 [3].

There are other mechanistic explanations for the resistance of axenic mice to deposition of fat and diet-induced obesity that comprises a number of pathways, including decreased hepatic *de novo* lipogenesis [7]. Free of intestinal microorganisms mice had an increased activation of hepatic and muscle oxidation pathways of fatty acids [67]. Likewise, the transplantation of intestinal microorganisms have increased capillary density in the villi of the small intestine [60, 66, 67, 91] thereby improving the absorption of monosaccharides [60] from the gut into the portal blood [21, 59, 66, 67] and its posterior hepatic uptake [91]. It is results on the induction of lipogenesis [21, 59] and accumulation of triglycerides in liver and adipose tissue [59]. In these mice there was a notable change in the expression of genes that regulate lipogenesis and the "fuel sensor", being the *de novo* lipogenesis also up-regulated due to the increased availability of substrates - short-chain fatty acids and monosaccharides resulted from digestion of polysaccharides [66].

Furthermore, it was found that the intestinal microbiota induces significant increase of the expression of two key enzymes of fatty acid biosynthesis - acetyl-CoA carboxylase and fatty acid synthetase [21, 60], as well as the expression of genes of transcription of factors involved in responses to insulin and glucose of lipogenic hepatocytes [21].

9.2.3.3. Poor integrity/high permeability of barrier, inflammation and endotoxaemia

Among the causes potentially involved in the development of metabolic endotoxaemia, numerous studies support the idea that mutuality bacteria-host takes control of the intestinal barrier function [60].

The weight gain and development of obesity were positively associated with intestinal inflammation, reinforcing the idea that the intestinal inflammation may be an early "marker" for the development of obesity and associated disorders due to a high fat diets [3].

Rats Sprague-Dawley with the tendency for weight increasing show the ileal inflammation, decreased intestinal alkaline phosphatase activity and increase of the activation of the innate immune system in the luminal wall, when compared with the rats resistant to obesity. These data suggest that it is likely that the inflammatory environment is an integral part of the development of obesity [85].

In human beings, high fat diets as western-style with duration of over a month may induce a very significant increase in the levels of endotoxin in plasma, suggesting that endotoxaemia can be developed in patients with dysfunction of gastrointestinal barrier related to dysbiosis [87]. High fat diets can surely affect epithelial integrity consequently leading to harmful intestinal permeability and systemic inflammation [59] due to decreased expression of genes encoding tight junction proteins [85]. In fact, the diet rich in fat and gut microbiota are required to induce the intestinal inflammation [3].

In a series of experiments on mice fed with high fat diet, it has been shown that a diet that increases endotoxaemia, favours an increased colonizing by Gram-positive bacteria instead of Gram-negative ones [83]. In addition to high-fat diet, the protein-rich diets increase the activity of bacterial enzymes such as β-glucuronidase, azoreductase and nitroreductase, which produce toxic metabolites that cause inflammatory responses [87].

Microbiota affects the host inflammatory condition in animals and humans [67]. Certain components of the gut microbiota are able to promote systemic low-grade inflammation, insulin resistance, and increased risk of cardiovascular events through a mechanism that involves increased exposure to bacterial products coming from the gut, a condition that is called "metabolic endotoxaemia" [66], being this chronic endotoxaemia a probable inductor of obesity [83].

Intestinal epithelial cells act as sensors for microbial products [59] and are the main pathways by which bacteria interact with host when these bacteria or its components and metabolites enter the blood circulation of the host [85].

Commensalism is the tolerance of the "innocent" bacteria which are found on the epithelial surface or inside of the mucus. In the other hand, bacteria that penetrate the epithelial barrier should be rapidly eliminated. However, in order to respond appropriately, taken into account a large number of microorganisms in very close proximity with the host tissue, the intestinal mucosa and immune system are highly adapted. Mucosal immune responses are induced by a small number of commensally organisms which penetrate the specialized sites, such as Peyer's patch or isolated lymphoid follicles where these microorganisms are taken up by dendritic cells or are phagocytised and destroyed by macrophages by triggering the innate immunity [21].

Dendritic cells induce mucosal response with Immunoglobulin A on the surface of B cells, in particular in the Peyer's patches. As the dendritic cells loaded with commensally bacteria cannot penetrate rather than the mesenteric lymph nodes, the inducing of the immune response towards the commensally bacteria is confined to the mucosa, while the systemic immune system remains relatively ignorant towards these organisms [66].

The intestinal epithelium can also directly detect pathogenic and commensally bacteria [66] by recognizing of different types of bacterial components [85] (conserved structures), viruses and fungi, and in general, activate host pro-inflammatory pathways of alert to the infection [66] by via of pattern recognition receptors.

Pattern recognition receptors recognize bacterial structures, such as LPS from the outer membrane of Gram-negative bacteria [66, 85], lipoteichoic acid from cell wall of Gram-positive bacteria [66], lipoproteins and peptidoglycans, in order to trigger the cascade of signalling of the immune system towards a pathogen [85]. Thus, these molecules generally trigger the innate immune response through the following steps: 1) the detection of a microbial organism; 2) transduction of recognition event; and 3) induction of appropriate effectors responses [66].

A question that remains without answer is how the human gut distinguishes between pathogens and commensally bacteria. Recognition of bacteria mediated by TLR is relatively crude, in which TLRs are unable to identify the bacteria, either at the level of species or genera, or distinguish pathogens hazardous from harmless components of commensally microbiota.

It is known that the contact between the bacteria and the epithelium seems to be necessary to activate the anti-inflammatory response of the host, triggered by commensally bacteria against the pathogens [66]. Likewise, certain intestinal bacteria are also known to suppress unnecessary inflammatory responses thereby contributing to the maintenance of

immune homeostasis [97]. An example is the case of *Bacteroides thetaiotaomicron*, which has shown to markedly attenuate the inflammatory response of the enterocytes induced by *Salmonella enteritidis,* by selectively antagonization of the nuclear transcription factor kB. The intimate contact between commensally microorganisms and epithelial cells seems to play a central role in regulating of the response against pathogenic agents [66].

Mice fed with a high-fat diet for a short period of time (2 weeks) showed metabolic endotoxaemia because it was observed an increase of 2 up to 3 fold in blood lipopolysaccharides. However, the levels of blood lipopolysaccharides were much lower than those observed during acute infection [39].

The proinflammatory effects of a diet rich in fat has mainly been attributed to inflammatory properties of dietary fatty acids [60], such as palmitic acid [60, 66], and lauric acid [66]. Different studies have suggested that saturated fatty acids promote insulin resistance and trigger an inflammatory response that leads to low-grade inflammation through signalling mechanism of LPS receptors – toll-like receptor-4 [60] and toll-like receptor-2 [66] found in adipocytes and macrophages, that can contribute to inflammation of the adipose tissue verified in obesity states [60]. Surprisingly, these molecular pathways play a crucial role in integrating of metabolic and immune responses, triggered upon infection by compounds derived from gram-negative bacteria, namely lipopolysaccharides [60]. Thus, the fatty acids are in fact involved in the stimulation of the innate immune system, but probably together with the initial stimulation of toll-like receptor-4 complex and posterior stimulation of toll-like receptor-2 by lipopolysaccharides [60]. An interaction with the toll-like receptor 4 with bacterial lipopolysaccharides triggers the exacerbated proinflammatory response of innate immune system, resulting in damage to target organs [101], such as liver and blood vessels [39, 66].

Toll-like receptors-5 are highly expressed in epithelial cells of the intestinal mucosa and are involved in mediating the immune response through the recognition of bacterial flagellin [79, 87], whereas both, toll-like receptors -2 and -4 are expressed in preadipocytes and mature adipocytes [66].

Evidences show that the toll-like receptor 5 protects not only against infections, but also against metabolic syndrome [39]. This hypothesis is sustained by the fact that knockout or genetically deficient in toll-like receptors 5 mice have higher weight [80], showed hyperphagia and developed the changed profile of the intestinal microbiota. This altered profile has been associated with metabolic syndrome [39, 85, 87] characterized by greater deposition of visceral fat, dyslipidemia, hypertension and insulin resistance [85] and also

associated with dysbiosis and low-grade inflammation which have even worsened after a high-fat diet [87]. In fact the cause of the metabolic syndrome in toll-like receptors 5 knockout mice has been demonstrated be caused by intestinal microbiota, because the transplantation of the altered microbiota to the axenic mice caused metabolic syndrome [85, 80] and these marking had disappeared when mice were treated with antibiotics [80]. So, it was here shown that toll-like receptors 5 control hyperphagia, hyperlipidemia, hypertension, insulin resistance and increased adiposity, through mechanisms which however require inflammation [39] and mice without these type of receptors showed develop metabolic syndrome. Besides, several researching groups have shown that the lack of toll-like receptor 2 prevents the development of obesity induced by high fat diet, hepatic steatosis and insulin resistance in mice [5].

Endotoxaemia may play a key role in the pathogenesis of obesity and related inflammatory conditions, and food intake affects the levels of endotoxin in plasma [67].

In *in vitro* and animal models an increase of pro-inflammatory cytokines such as TNF-α led to insulin resistance [3]. It has been shown that bacterial lipopolysaccharides present in the membrane of intestinal bacteria, being the major components of Gram-negative bacteria [3, 66, 67, 85] as endotoxin and induces strong immune responses [66]. These bacterial components cause the secretion of pro-inflammatory cytokines [57], leading to the development of inflammation [3, 67, 39, 85] in response to high-fat diet [118] and are implicated in the development of obesity [3, 67]. Bacterial lipopolysaccharides are continuously released into the intestine when the lysis of the bacteria is occurring [57]. In fact, slow and continuous subcutaneous infusion of this bacterial endotoxin caused the majority of the first signs described for metabolic syndrome [39] as the excessive weight gain and insulin resistance in mice [57, 60, 85, 87, 91] due to development of fat mass - visceral and subcutaneous at about 30% and 40%, respectively [86]. This phenomenon occurred due to the accumulation of macrophages in white adipose tissue [87] and disturbances in the metabolism of energy [85]. So, here it was described the same conditions with the same abnormalities to ones of metabolic endotoxaemia induced by diet high in fat [66, 67, 85]. In addition, an inflammatory response in muscle, adipose tissue and liver are similar to ones produced by effect of increased expression of TNF-α, IL-1, IL-6, and an inhibitor plasminogen activator [66].

In contrast, the mice treated with antibiotics [91] that have bacterial lipopolysaccharide receptor TLR-4 or part of its machinery removed [39, 60], or mice knockout to this receptor tend to be resistant to this chronic inflammatory state [57, 67, 85, 91], obesity and

resistance to insulin [85, 91]. In turn, the serum levels of bacterial lipopolysaccharides are increased to twice on obese individuals or who ingest considerable quantities of fat [3, 60, 67, 91]. Elevated levels of these bacteria compounds are achieved via the processes that involve an increase formation of chylomicrons - lipoproteins that transport triglycerides and cholesterol from the intestine to other tissues. This mechanisms presuppose the decreased intestinal barrier integrity [3, 67], as in the case of compromised intestinal tight junctions which increase the possibility of absorption and translocation of bacterial products [85], or via the decreasing of activity of alkaline phosphatase - the enzyme responsible for cleavage of LPS in the intestine (Figure 15) [3].

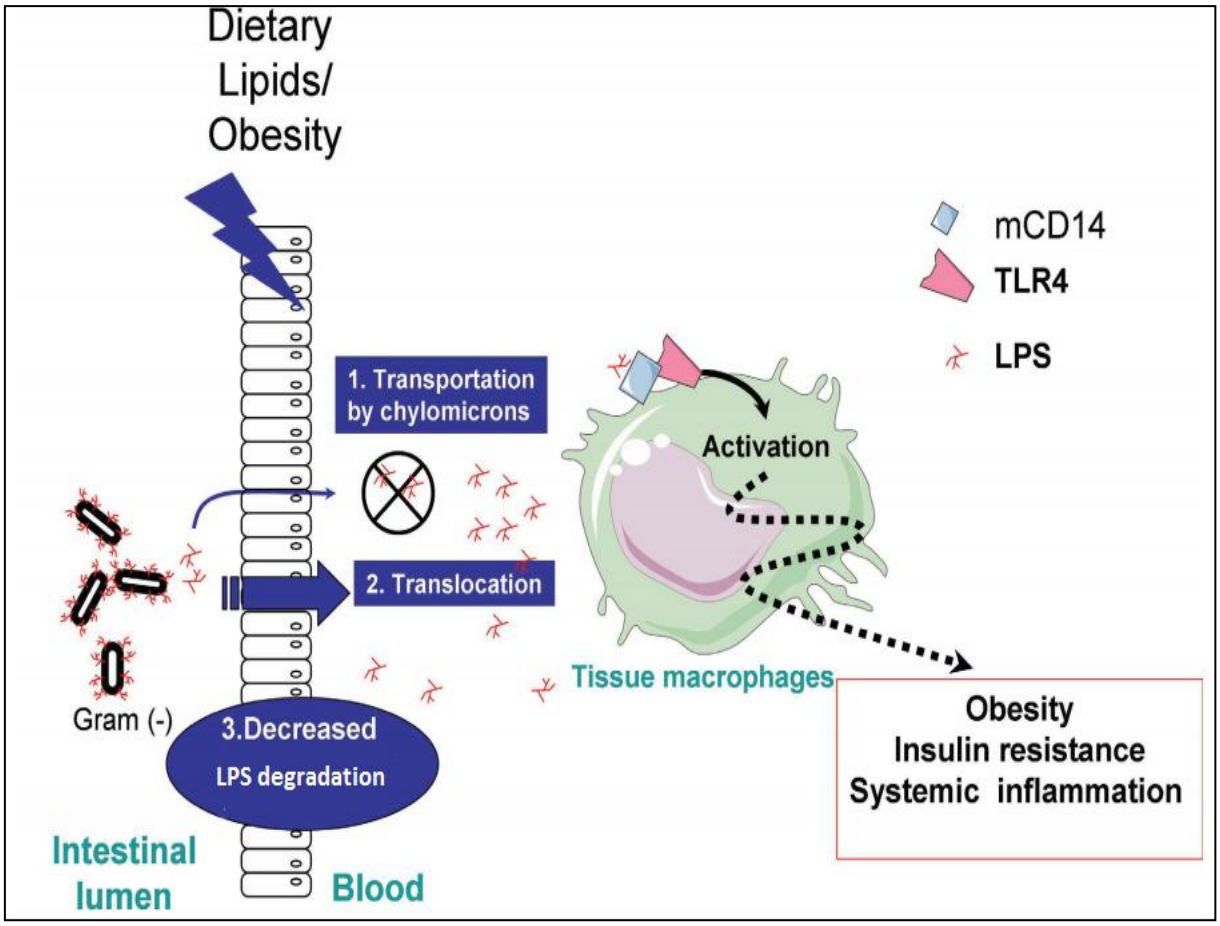

Figure 15 – Occurrence of endotoxaemia towards the high-fat diet and obesity. Increased lipopolysaccharides levels are common in obese individuals. This is mostly due to processes involving the transport of lipopolysaccharides from the gut to the blood. This process are an increase in chylomicron-driven transport of bacterial lipopolysaccharides, a rupture of the gut barrier integrity leading to abnormal gut permeability, and a decrease in processes involved in intestinal lipopolysaccharides degradation. The increased levels of lipopolysaccharides (endotoxaemia) thereby activate the macrophages in the different tissues leading to a low grade inflammation involved in the metabolic alterations occurring in obesity [3].

When the chylomicron formation occurs during the fat absorption, bacterial lipopolysaccharides are embedded in it, and passes through the barriers of the intestine into the lymphatic system and then into the bloodstream [66, 85]. It has been demonstrated in humans that the increase of chylomicron formation due to a meal with a high fat content leads to higher postprandial transportation of bacterial lipopolysaccharides, when compared with a meal of low-fat or no meal at all [85]. To prove this fact, it have been made studies on animal and cell models, in which was found that the formation of chylomicrons stimulated by feeding with a high-fat content meal have indeed significantly increased the bacterial lipopolysaccharides transport from the intestinal lumen into the circulation or enterocytes [85]. Thus, bacterial lipoglycansis conveyed by lipoproteins, reaches target organs and causes the inflammation [39], being its target tissues the adipose tissue [66], the liver and the endothelium of blood vessels [39, 66].

In adipose tissue these bacterial lipopolysaccharides trigger inflammation in macrophages and preadipocytes – sites that release pro-inflammatory molecules and chemokines. In the liver, hepatocytes undergo bacterial lipopolysaccharides biliary clearance or trigger inflammation in Kupffer cells [66]. It can be concluded that the inhibition of the secretion of chylomicrons can effectively reduce metabolic endotoxaemia and could be benefit for metabolic disorders associated with obesity [67].

The plasma concentration of bacterial lipopolysaccharides can also be regarded as a risk factor, as this component of bacterial membrane was present in excess in the blood of apparently healthy individuals, but ones who consumed more alimentary fat than protein or carbohydrates. These bacterial endotoxins trigger the inflammation (figure 16), that is proven by the fact that adipocytes exposed to these bacterial endotoxins showed inflammation [39]. This bacterial lipoglycans induce the release of proinflammatory molecules and inhibits the expression of adiponectin in preadipocytes and macrophages [66].

An increase of the plasma concentration of this bacterial endotoxins can be acute, induced by a single lipid absorption in humans and in mice and appears to depend on increased intestinal permeability trough a mechanism related to glucagon-like peptide-2. Bacterial lipopolysaccharides, besides being absorbed from the gut during the synthesis of chylomicrons can be exchanged with other lipoproteins and can be chronically transported to its target tissues with its consequent inflammation. However, in an acute situation, an administration of "flash" of lipoproteins can dampen cell-associated bacterial toxin and reduce its impact on the acute phase of inflammation [39].

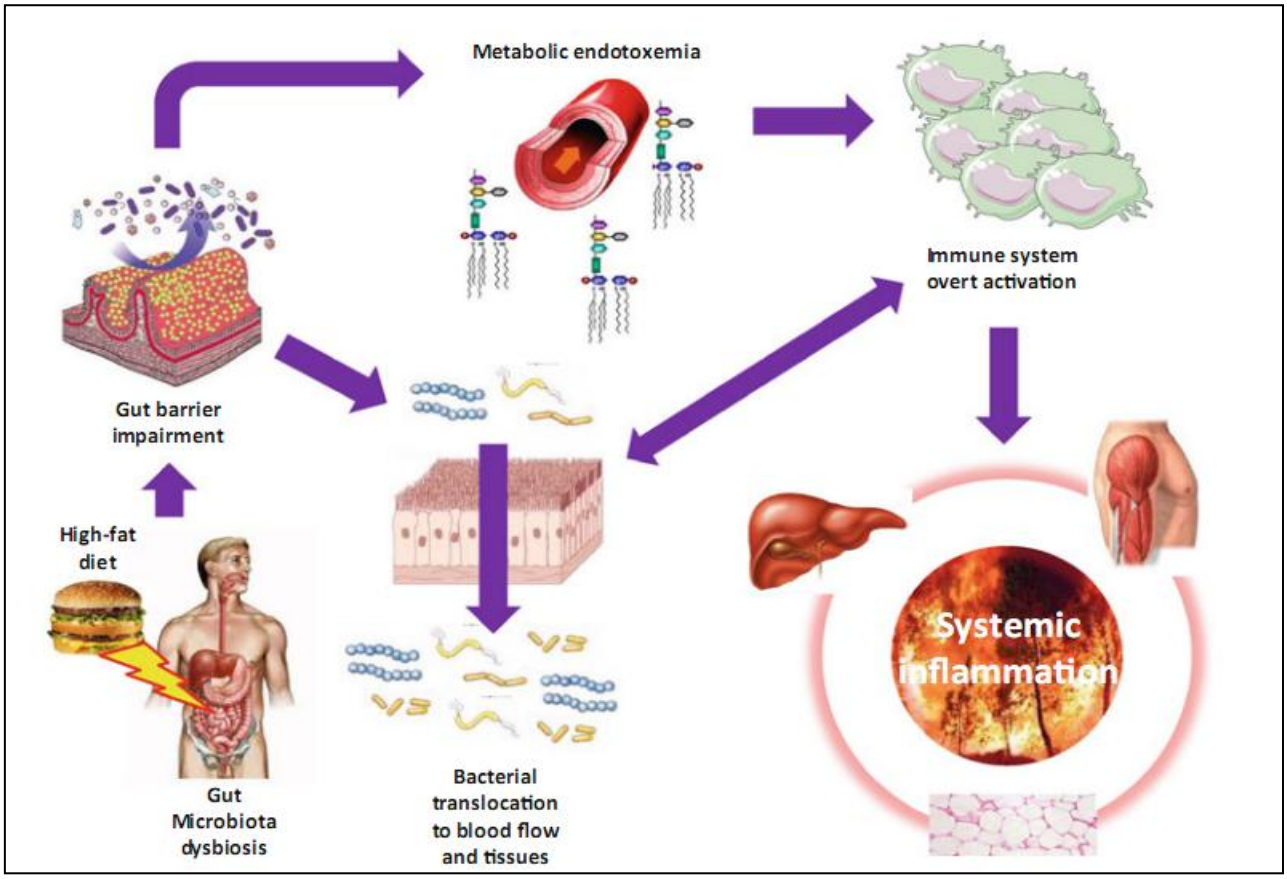

Figure 16 – The inflammatory triggering trough gut microbiota dysbiosis and consequent metabolic impairments. Inspite of the origin of metabolic diseases be multifactorial, deleterious feeding habits have the major influence. This directly modifies intestinal ecology - increases intestinal permeability, leading to an increased circulating concentration of bacterial lipopolysaccharides, called metabolic endotoxaemia. The inflammatory bacterial fragments can translocate toward target tissues such as the blood, the liver, the adipose depots or the arterial wall and interact with cells from the immune system to generate the chronic low-grade inflammation, implicated on the development of metabolic diseases [98].

Lipopolysaccharides were associated with a significant reduction in adipocyte size but with the increased number of macrophages incorporated on the adipose tissue, when compared with a diet of high fat content that causes an increase of the size of adipocytes in wild type mice. So, it can be concluded that bacterial lipoglycans are associated with hyperplasia of adipose tissue and enhanced differentiation of preadipocytes into adipocytes, whereas the feeding with high fat can mainly cause a chronic tissue hypertrophy [66].

The long-term exposure to bacterial outer membrane endotoxaemia compounds lead to tolerance to anorectic effect of this endotoxin. The causes and mechanisms of a different response, and tolerance to anorectic effect of LPS are unknown but may be related to

differences in hormone levels (leptin and ghrelin), genotype of the host, or the chemical composition of lipopolysaccharides [66].

The lipid A of bacterial lipoglycans is disaccharide that is fixed to LPS in the bacterial membrane. It is responsible for most of the toxicity of gram-negative bacteria and is an important marker for the recognition of invasion by gram-negative bacteria in the host. The antigen O is repetitive polymer of glycan, and its composition varies from strain to strain. Thus, the ability of bacterial lipopolysaccharides to promote the low-grade inflammation and metabolic disorders could be different, depending mainly on lipid A and also on the composition of the O-antigen chains. However, no data are available to confirm this hypothesis [66].

Microorganisms can be responsible for weight gain caused by the intake of fat, low-grade inflammation and development of insulin resistance, in which LPS can reasonably trigger these conditions due to presence of properties described above. Some researchers wanted to revoke this hypothesis, and as a first step, wanted to rule out any relationship between dietary fat and these bacterial endotoxins. In this study, wild mice were fed with a high-fat diet and changes were observed in the levels of circulating LPS. Its daytime quantities suffered alteration in relation to food intake, and the peak of endotoxins were reached at the end of the hours of darkness, corresponding to the postprandial period, and the concentrations were lower during the daylight hours [66].

Levels of immunoprotein CD14 and interleukine-6 – markers of acute inflammation, were elevated after a mixed meal containing fat, in combination with higher levels of bacterial lipopolysaccharides [85]. Thus, after the high fat meal it is occurred a development of an inflammatory state and postprandial activation of endothelial cells [91].

In one study it was demonstrated that after 4 weeks of high fat feeding, the mice exhibited an obese phenotype, accompanied with a change in the composition of the gut microbiota (reduction of *Eubacteria* spp. and *Bifidobacteria* spp.) and increased levels of circulating bacterial endotoxaemic cell wall compound about two to three times [67]. All together these data suggested that bacterial endotoxins are related to meal composition.

In fact, diverse food has an impact on microbial production and intestinal absorption of bacterial lipoglycans. The high-fat diet showed to alter the composition of the gut microbiota - increases gram-negative and reduces the gram-positive bacteria [91] and reduces the expression of tight junction proteins in epithelial cells - occludin and zonula occludens -1 [67, 91], leading to increased intestinal permeability and thus high concentrations of LPS resulting on metabolic endotoxaemia [66, 67, 91]. Indeed, changes in the distribution and localization

of ZO-1and occludin on intestinal tissue are associated with an increased permeability of the intestine that frequently occurs in obese rodents [60]. This suggests that the presence of fat in the meal and its absorption may have a predominant role in the entry of bacterial lipopolysaccharides in portal blood that does not happen towards an isocaloric diet rich in carbohydrates [67].

The animals with high-fat diet, increased body mass and endotoxaemia levels have pro-inflammatory cytokines, such as TNF-α, IL-1betta and IL-6 increased not only on the plasma but also on liver, adipose tissue and muscle [91]. The postprandial increase of endotoxaemic part of the outer bacterial membrane in plasma induced by a meal rich in carbohydrates and fat have the same role and the same degree of onset of systemic inflammation as caused by increased TNF-α and IL-6 on adipose tissue. This increase was not observed after a meal rich in fibre and fruits [67].

What about studies in human beings, circulating concentrations of bacterial lipopolysaccharides complex was positively correlated with fat intake but not with other nutrients. In healthy individuals, with a meal high in fat, the endotoxaemia has acutely increased to the concentrations that are sufficient to activate the release of TNF-α of monocytes in the cultures of human aortic endothelial cells [67].

High-fat diet has demonstrated be able to decrease the number of *Bifidobacteria* spp. and increase bacterial lipoglycans in plasma. Treatment with antibiotics or dietary intervention with oligofructose reduce glucose intolerance, decrease weight gain and inhibit inflammation in mice. These results suggest that changes in the gut microbiota may be responsible for increased endotoxaemia in response to a high fat diet, which in turn cause the development of diabetes mellitus and obesity [57]. Gut *Bifidobacteria* spp. and *Lactobacilli* spp. in mammals can synthesize from conjugated linoleic acid the free from of its bioactive isomers, which have anti-diabetic, anti-atherosclerotic, immunomodulator, and anti-obesity properties. The supplementation with *Bifidobacterium breve* and linoleic acid resulted in reduced expression of pro-inflammatory cytokines, tumour necrosis factor α, interleukine-6, and interferon-γ [67]. The specific prebiotic compounds, such as oligofructose can affect the structural composition of the intestinal microbiota towards high-fat diet, and can improve the metabolic parameters of inflammation [59].

An administration of microorganisms from *Bifidobacterium* genus reduced the levels of bacterial lipopolysaccharides in mice and improves barrier function of the mucosa. *Bifidobacteria* spp. do not degrade glycoproteins of intestinal mucosa as another pathogenic

bacteria do, and promote a healthier microvilli environment by preventing permeability and bacterial translocation [66].

Furthermore, the stimulation of short-chain fatty acids receptors limits the inflammation in experimental models of colitis, arthritis and asthma. Axenic mice that do not produce short-chain fatty acids due to lack of bacteria which ferment dietary fibre, show exaggerated inflammation, similar to mice that do not express receptors for these fatty acids. Thus, the receptors can provide a molecular relation between diet, gastrointestinal bacterial metabolism, and immune and inflammatory responses [59].

Another possible relation of the intestinal microbiota to the chronic inflammation can be due to decreased levels of butyrate in the plasma in obese individuals, and its consequent low bioavailability, since butyrate has anti-inflammatory properties. The main groups of butyrate-producing bacteria are species from *Roseburia* genus and *Eubacterium rectale* and *Faecalibacterium prausnitzii* species belonging to *Firmicutes* phylum. Many studies show that intake of fermentable carbohydrates can influence the production of butyrate. Particularly, diets containing high levels of non-digestible carbohydrates stimulate the growth of certain butyrate producing bacteria, and therefore, cause higher plasma levels of the butyrate. The older publications report that increased plasma levels of butyrate enhances insulin sensitivity and increases energy expenditure in animal models of diet-induced obesity [57].

9.2.3.4. Modulation of signalling molecules

Intestinal microbiota influences the release of some main transmitters that are operating on brain-gut axis and modulate food intake and energy balance. These transmitters are short-chain fatty acids, peptide YY, serotonin, ligands of endocannabinoid system and ghrelin [79]. It was shown that axenic mice had a decreased intestinal expression of satiety peptides such as cholecystokinin, glucagon-like peptide-1 and peptide YY and had the decreased plasmatic expression of gastrointestinal hormone levels such as leptin and PYY when compared to normal controls. These decreased hormonal levels were accompanied by alterations in plasma biochemical markers that mimic a fasting state, with increased fat metabolism and decreased circulating glucose. These results suggest that in germ-free mice occurs as a compensatory mechanism for their decreased energy stores. However, lower levels of ghrelin on blood circulation for both fasted and re-fed condition was also observed in axenic animals compared to normal ones, and interestingly, re-feeding decreased plasma ghrelin in control group and not in germ-free mice [7].

Axenic mice show an increased expression of receptors for sweet food in the proximal intestine, which was associated with the increase of sucrose intake. The mode of participation of receptors for nutrients in the increasing of caloric intake in axenic animals is unknown. However, the activation of receptors that respond to nutrients leads to the release of intestinal peptides of satiety such as cholecystokinin, glucagon-like peptide-1 and peptide YY [83].

The conventionalization of axenic mice results on significant changes in hormone levels, particularly on the increase of circulating levels of glucose, insulin and leptin [66]. As mentioned above, secretion of glucagon-like peptide-1 is reduced and delayed in obese individuals in comparison to the lean control. Thus, the difference in composition of the intestinal microbiota in obese humans may possibly contribute to reduced secretion of this peptide [57].

The pattern of secretion of glucagon-like peptide-1 is biphasic, with a quick initial rise that occurs 15 up to 30 minutes after eating a meal. This quick increase occurs before the nutrients reaching the ileum, where glucagon-like peptides-producing cells are located. This fact turns unlikely the proposal that glucagon-like peptide-1is released only by the direct action of nutrients on L cells [57], and here the intestinal microbiota may play an important role. The fermentation of prebiotics by the gut microorganisms increases the production and concentrations of the GLP-1 in the caecum due to promoting of L-cells differentiation [57, 67]. In addition, there are data that suggest that the intestinal microbiota can modulate intestinal barrier integrity and endotoxaemia through glucagon-like peptide-2, which is co-secreted with GLP-1 by enteroendocrine L cells [67].

The role of combating of obesity played by fermentable fibre is mediated through sort-chain fatty acids and regulation of expression of gut hormones [67, 77, 79]. Emerging evidence indicates that SCFA, in addition to its other physiological functions, are signalling molecules that can help to explain that gut microbiota has a role in the control of obesity [85], because they may perform signalling functions in the human body [3]. The binding of SCFAs to these G protein-coupled receptors stimulates the secretion of peptide YY [7, 67, 77, 79], which inhibits the motility of the bowel and reduces the intestinal transit, thereby increasing the nutrient uptake [67, 77], including short-chain fatty acids itself [77]. Thus, it is not surprising that the decrease in supply of SCFA in the distal intestine due to lack of microbes, resulting in decreased circulating PYY levels [7]. Since the L-cells are also responsible for the production and secretion of GLP-1, it is likely that short-chain fatty acids can affect the insulin secretion through multiple pathways and mechanisms. In support to this

hypothesis, the GRP-41-deficient mice showed reduced insulin secretion in a test of tolerance to oral glucose [85], and the effect of fermentative bacteria on enhanced host fat storage was diminished in mice GRP-41 lacking [80].

It was also demonstrated in mice, that propionate can stimulate leptin expression and increase its release from adipose tissue, as well as can suppress resistin expression [5], propionate to induce expression of leptin gene [85].

GRP-41 knockout mice colonized with a microbial community specially destined to fermentation resisted to gain fat mass when compared to their littermates of wild type [60]. Deficient mice for the same receptor when co-colonized with *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii* had shown the lower leptin and peptide YY levels. Although the amount of food intake was not affected, the extraction of calories from the diet was reduced in these "deficient" mice. Once PYY is the regulator of the gut motility, its lower levels resulted on increased intestinal transit with reduced absorption of short-chain fatty acids, which eventually led to reduced hepatic lipogenesis in mice knockout for short-chain fatty acids receptors. Thus, the results confirm that GRP-41 is the factor regulated by gut microbiota, which plays an important role in energy balance [5].

One additional mechanism potentially involved in the impact of the gut microbiota in the development of obesity and related disorders is endocannabinoid system [60]. Intestinal microbiota contributes to regulating of the endocannabinoid system tone in adipose tissue, in both, physiological and pathological situations. Furthermore, obesity is characterized by increased responsiveness of the endocannabinoid system in humans and rodents [60, 64]. There is an altered expression of cannabinoid receptor 1 and increased levels in plasma and on adipose tissue of signalling endocannabinoids molecules. It is known that bacterial lipopolysaccharides can stimulate the synthesis of endocannabinoids *in vivo* and *in vitro* and the CB_1 blockade (pharmacologically or genetically) protects against obesity, fatty liver (steatosis) and low-grade inflammation through mechanisms poorly known [60].

Specific changes in gut microbiota of axenic mice in comparison with conventional ones lead to selectively decrease of the endocannabinoid system activity in the colon and adipose tissue. In both cases, diet-induced obesity and genetic obesity in mice, the endocannabinoid system is over-activated in the intestine and adipose tissue. It was noted that this system, and more specifically CB receptor, controls the intestinal barrier function, since the block of this receptor in obese mice showed reducing of gut permeability through better distribution and location of the tight-junction proteins (occludin and ZO-1) [60]. Anandamide is an endogenous ligand of CB_1 and CB_2 receptors. It was found a significant

increase in anandamide levels (about 50%) in adipose tissue and the reduced levels of the enzyme that degrades anandamide - fatty acid amide hydrolase [64].

It was shown that the activation of the endocannabinoid system by a cannabinoid receptor agonists significantly decreased the expression of apelin and its G protein-coupled receptor – APJ. In lean wild-type mice, it was observed that the *in vivo* inhibition of anandamide degradation by administration of a potent inhibitor of fatty acid amide hydrolase, have significantly reduced the apelin and APJ expressions. These data support the idea that the endocannabinoid system regulates apelinergic system under physiological conditions [64].

Recent evidence suggest that under pathophysiological conditions, such as obesity, the peripheral apelinergic system seems to be deregulated [64, 76] in humans and mice [76]. To date, the mechanisms of regulating of the expression of apelin and its receptor in adipose tissue of obese mice are not fully understood, but it has been shown that the inflammation participates in the regulation of expression of apelin and its receptor. The stimulation of adipose tissue explants with cannabinoid agonist reduces the expression of apelin and APJ. The apelin expression is regulated by bacterial lipopolysaccharide in pathological conditions. Taking into account the above said, endocannabinoid system and inflammation contribute to the regulation of apelin and its receptor [64]. Although there are two different mechanisms of regulation of apelinergic system - endocannabinoid system tone and metabolic endotoxaemia, both are clearly related to the composition of the intestinal microbiota. The endocannabinoid system has also demonstrated the potential to mediate the influence of microbiota on intestinal permeability [59].

9.2.3.5. Other mechanism potentially relates gut microbiota and obesity.

Recent observations are also indicate that the consumption of oxygen was 25 to 40% lower in conventional mice than in the axenic - the conclusion which was independently confirmed in another study. The comparison of energy expenditure in gnotobiotic mice colonized with different gut microbiota would be necessary to demonstrate the role of gut microbiota in energy expenditure [85].

Some molecules are related to obesity state, even if it is only marker, without direct apparent relationship [39].

It was demonstrated that the urinary metabolic phenotype differs between obese patients and healthy controls [99].

The metabolites derived from the intestinal microbiota as hippuric acid, trigonelline, 2-hydroxyisobutyrate [39, 99], and xanthine contribute to the classification and were

responsible for discrimination between obese and lean individuals [39] because its amount were altered in obese patients. In these patients, 2-hydroxyisobutyrate levels were higher, whereas trigonelline and hippuric acid levels were lower than in controls [99]. The causal role of these molecules could not be demonstrated, but it can be at least as biomarkers of obesity [39].

In fact, some data suggested that the metabolite hippurate originated by gut microbes, can be the great promise as a diagnostic marker for individuals with risk of obesity. The production of hippurate requires both, mammalian and microbial metabolism [85].

Urinary hippurate is recognized as a marker of the activity of intestinal microbiota. Interestingly, urinary hippurate, acetate and propionate were found at lower levels in obese and insulin resistant Zucker rats than wild-type rats. In humans, the amount of urinary hippurate discriminates patients with morbid obesity and insulin resistant from control subjects matched by age. In particular, the urinary profile of metabolites was characterized with a lower level of hippurate in obese subjects when compared to lean control groups [85].

However, the intake of polyphenols found in fruits and vegetables can affect the amount of urinary hippurate. Therefore, both dietary and metabolic activities of intestinal microorganisms should be taken into account when association between urinary hippurate and metabolic diseases are examined [85].

The trigonelline have some effects on enzymes related to diabetes and obesity. The administration of trigonelline to diabetic rats exerted *in vivo* inhibitory effects on the key enzymes of lipid metabolism (digestion and absorption) in the small intestine, such as lipase. This fact shows the antiobesogenic action of this molecule, also contributing for effects against hyperlipidemia and heart diseases, too [98].

The high serum uric acid (hyperuricemia) is closely associated with visceral fat accumulation and various metabolic disorders including glucose intolerance and other conditions that all together constitute the named metabolic syndrome. In mice, increased uric acid production in obese adipose tissue could contribute to the observed hyperuricemia. The experiments involving organ culture indicated that there is significantly higher production of uric acid by entire adipose tissue of obese mice than of ones from the control group. Actually, the plasma levels of xanthine which is the precursor of uric acid are increased in those mice [100].

The gut microbiome of obese individuals has depletion of genes involved in microbes motility, such as genes encoding the chemotaxins, motility proteins, and flagellar assembly,

and has increased expression of that ones, which encode glycosidic hydrolases, capable to break down the non-digestible polysaccharides from meals [67].

10. Interventions to combat obesity

The traditional pharmacological therapies for obesity combat, although having the initial success in achieving the weight loss, are usually subject to counter-regulation. This fact is due to the multiplicity and complexity of mechanisms involved in appetite regulation and energy homeostasis. The mechanisms that regulate energy balance have considerable overlap with many other physiological functions, and are influenced by social, hedonic and psychological factors that can limit the effectiveness of pharmacological interventions. It is therefore unsurprising that anti-obesity drug discovery programmes have been replete with false starts, failures in clinical development, and revocations due to adverse effects that were not fully appreciated at the its release [56]. So, other methods with the objective of reduce weight gain or promote the weight losses should be taken into consideration.

10.1. Gastric bypass

To date, there is only one available effective treatment for morbid obesity - gastric bypass surgery [39, 56, 85], that consistently achieves and sustains substantial weight loss associated with a drastic change in the gut microbiota [39], and also with comorbidity reduction and enhanced survival [56]. The metabolic phenotype disappeared after weight loss induced by bariatric surgery [39]. The obesity was associated with a higher number of bacteria of the family *Prevotellaceae* before this surgery. After surgery these bacteria have reduced its levels to equal ones of lean subjects, whereas other bacteria, such as of family *Enterobacteriaceae* and genus *Akkermansia* were increased. In another clinical study, the number of bacteria of faecal of *Escherichia coli* specie was increased after gastric bypass surgery. The gastric surgery also influences the profile of intestinal microbiota in animals, leading to increased proportion of *Enterobacter hormaechei.* Given that both species, *Escherichia coli* and *Enterobacter hormaechei* belong to the family of *Enterobacteriaceae*, a proportional increase of *Enterobacteriaceae* result of gastric bypass surgery. However, it is not clear whether the changes in populations of *Enterobacteriaceae* are related to rapid weight loss and improvement of insulin sensitivity as the result of this operation [85].

In mice after bypass gastric surgery substantial changes in the principal intestinal microbial phyla were observed. It was reported a higher concentrations of *Proteobacteria* (52

times), as well as lower concentrations of *Firmicutes* (4.5 times) and *Bacteroidetes* (double), when compared to "sham" control-operated animals [83].

It was found that gastric bypass surgery strongly changes the gut microbiota - led to reductions in the levels of specially dominant in normal-weight and obese individual *Firmicutes* [3, 5, 39, 57, 59] and proportional increase of levels of *Gammaproteobacteria* (members of the *Enterobacteriaceae*) [3, 5, 39, 57].

However, owing to concerns about perioperative mortality, surgical complications and the frequent need for reoperation, these procedures tend to be reserved for the morbidly obese people [56]. Alternative strategies to surgery should develop interventions that can reduce body weight by decreasing the consumption or absorption of food, and/or by increasing energy expenditure.

10.2. Physical activity

It is known that the most variable component of energy expenditure is physical activity, and is representing from 20 up to 50% of total energy expenditure, and an evaluation of an individual's activity behaviour is critical to any therapeutic assessment. When physical activity alone is used as the treatment of obesity, the weight losses are modest and ranging about of 2 up to 3 kg [55].

The physical activity seems to play a critical role in maintaining the lost weight with a positive association between the level of activity and the degree of maintained weight loss. This may be explained by additional expending of energy, improving of maintenance of lean tissue, or by the positive psychological influence on continued behaviour changes [55].

The recommendations suggest the practice of moderate physical activity about of 30 minutes on at least five days of the week. However, recent evidence has highlighted a longer duration of daily activity required to maintain lowered weight and prevent weight regain, suggesting that 45 up to 60 minutes per day may be necessary [55].

10.3. Dietary treatment

Dietary treatment that leads to the weight loss improves most of medical complications associated with obesity. It is known that weight loss arises from changes in the adipose cells by delipidation process, with the shrinkage and adoption of elongated or star

shape. The non adipose cells of adipose tissue also undergo changes, but the exact molecular mechanism of weight loss by calorie restriction remains still unknown [21].

There are different types of diet, including the low-carbohydrate, low- and very-low-fat diets, *inter alia* [101], but the key ones are the low-fat, particularly saturated fat diets, increased wholegrain fibre carbohydrates diet, increased fruit and vegetables diet and eventually one that consider portion sizes. However, it should be clear that no dietary treatment will be the totally appropriate for all individuals and there is a need to match treatments to a patient's necessities and preferences [55].

10.3.1. Low-carbohydrate diets

Despite controversy surrounding the low-carbohydrate high-fat, diets, individuals submitted to it may lose weight because the intake of fat and protein is self-limiting and overall caloric intake is decreased. It is suggested that high-carbohydrate meals resulting in a decreased sense of satisfaction than meals that contain adequate fat, resulting in increased hunger and increased food intake [101]. The weight loss seen with low-carbohydrate diet was possibly due to the increased thermic effect of the high protein content. Postprandial thermogenesis is doubled on high-protein diets and contributes to an additional loss about of 90 kcal /day. Nevertheless, these types of diets are not recommended by the American Heart Association [102].

Considering the role of nutrients and environmental factors that control adipogenesis, it was demonstrated that fatty acids provided from diet can promote or prevent adipogenesis, depending on its chain length, degree of saturation, and also according to the location of adipose tissues. Polyunsaturated fatty acids of the ω-6 series are more adipogenic (both, *in vitro* and *in vivo*) in comparison with the ω-3 series. Likewise, diet rich in ω-3 fatty acids has demonstrated to protect against obesity by favouring oxidation in detriment of the storage of fat [21].

Nowadays the low-carbohydrate high-protein diets have popular demand for the managing weight. Typically, this kind of diet contains a high proportion of proteins, unrestricted fats, particularly saturated ones, and a severe restriction of carbohydrates (refined and complex). It is not proved yet that the low-carbohydrate high-protein diets lead to effective long term weight loss, because the insufficient evidence is currently available [55]. The American Heart Association and the British Dietetic Association have published some warning statements against the adoption of these types of diets. More investigations

about the safety and efficacy of high-protein low-carbohydrate diets are needed, preferentially with large randomised controlled trials over an extended period of time [55].

10.3.2. Low-and very low- fat diets

Low-fat high-carbohydrate diets which contain about 10%–15% of fat [102] with only 30% of energy is obtained from it, play a central role in the dietary management of overweight and obese subjects [55] and originally designed to prevent or reverse heart disease [102]. These diets contain a high proportion of complex carbohydrates, fruits, and vegetables [101]. The reduction in dietary fat about 10% leads to 3–4 kg of weight loss in normal or overweight subjects and to 5–6 kg of weight loss in the obese ones. However, the relative effectiveness of low-fat diets compared with low-fat and energy restricted approaches remains still unclear [55] because these diets are naturally high in fibre and caloric density is low, so individuals submitted to these types of diets consume fewer calories and lose weight [101]. Actually, the availability of maintenance of moderate weight loss (3–4 kg) over time using a low-fat high-carbohydrate diet as part of treatment has been demonstrated [55]. Very-low-fat diets have a very high carbohydrate and fibre content. The American Heart Association alerts about the use of this type of diets because its high carbohydrate content can increase triglyceride levels. In addition, the diets can contain over twice (40–70 g/day) of the recommended amount of fibre, that can compromise the absorption of zinc, calcium and iron [102].

10.3.3. Low-calorie diets

Fasting or food restrictions with diets with low-calorie content are efficient therapeutic method to promote fatty mass loss in obese patients [21].

Diets that reduce overall caloric intake result in loss of body weight and body fat [101]. In general, low-calorie diets are high in carbohydrates (55%–60% of total daily energy intake), low in fat (< 30% of energy intake) [102]. The approach "fixed energy-deficit diets" has become increasingly common and is widely used by dieticians. This approach is based on estimated individual energy requirements by calculating of basal metabolism, adjusting for physical activity and subtracting an energy deficit that is round of 600 kcal per day [55] (500–1000kcal/day). So, daily caloric intake should be about of 1000–1200 kcal/day for women and 1200–1400 kcal/day for men [102], or according to other authors, the low calorie diets should comprise from 800 up to 1500 kcal per day [55]. The inclusion of diverse methods

for restricting food intake has been recommended for weight loss and maintenance [55]. It was reported that low-fat diets without intentional restriction of energy intake resulted in greater weight loss [102]. Fixed energy-deficit diets showed to induce 0.5 kg per week of weight loss [55]. The use of low calorie diets during the period about six months has been associated with a mean weight loss of 8% [55].

The more severe is obesity - the higher requirements of energy is observed and the larger energy deficit is inflicted. So, there is evidence that suggest that modest energy reductions about 600 kcal per day may improve adherence and is recommended as a dietary option for weight losses and maintenance [55].

10.3.4. Very low-calorie diets

Very low-calorie diets are formula foods providing about of 450 up to 800 kcal per day, used as the single source of nutrition [55, 102] replacing all meals and snacks [55] or specific foods, including meat, fish and poultry [102]. These diets need to contain proteins of high biologic value (high in essential amino acids) to preserve lean body mass, essential fatty acids, and vitamin and mineral supplements. Very-low-calorie diets result in an average weight loss of 1.5–2.5 kg per week, compared with 0.4–0.5 kg with low-calorie diets. The average weight loss at 12–16 weeks on very-low-calorie diets is about 20 kg, compared with 8 kg on low-calorie diets [102]. Diets providing such low-energy intakes are often associated with a feeling of fatigue, constipation, nausea, and occasionally diarrhoea. A more serious side effect is the development of gallstones associated with the rapid weight loss as 1–2 kg per week. Due to the potential adverse effects of this type of diets, it are usually reserved for patients with severe obesity – with body mass index equal or rather than 35 kg/m^2 with associated comorbidities and which need the rapid weight loss, that justify the use of this type of diet [55, 102] or for whom other approaches have failed [102]. In addition, there is evidence that suggests very low-calorie diets are not more effective in long term management than more moderate dietary strategies [55, 102].

10.3.5. Meal replacement diets

Meal replacement programmes comprise the control of products portion (shakes, bars, soups, and pastas) that replace two meals (and snacks) per day, allowing an inclusion of only one regular meal composed by “healthy” foods. This type of diet generally provides from 1200 up to 1600 kcal per day. A recent study confirmed the efficacy of this approach

when compared with conventional energy restricted diets. In the short period of time (months), the weight loss was more accentuated in the treated group with meal replacements when compared with the control group, and this difference and was still evident passed one year [55].

10.3.6. Low glycaemic food diet

Low glycaemic index foods (foods with low effect of on postprandial glycaemia) are another approach that can be used to treat or at least control the overweight and obesity. This kind of food is more slowly absorbed and seems to be able to reduce hunger, increase satiation, and reduce energy intake. However, it is premature to specifically recommend low glycaemic index diets for weight losses and maintenance, but the promotion of certain constituents of a low glycaemic index diet, such as increased ingestion of wholegrain cereals, fruit and legume as part of a conventional energy restricted diet for the long term controlling of obesity seems to be appropriate [55].

Along the written, it was mainly mentioned the role of fat and sugars on the composition of intestinal microbiota and consequent effects over obesity. But it should be remember is possible that not only macronutrients, but also micronutrients and phytochemicals present in small concentrations in the diet, can interact with gut microbiota and modulate host physiology [3].

The lifestyle changes in the form of dieting and/or exercise *per se* do not generally produce marked or sustainable weight loss [56], so it should be resort to other methods.

10.4. Probiotic / prebiotic approach

Antibiotics, prebiotics and probiotics can offer the handling of the intestinal microbiota.

The term "prebiotic" was introduced by Gibson and Roberfroid in 1995, in which it was defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and / or activity of one or limited number of bacteria in the colon" [83]. A prebiotic is a selectively fermented ingredient that results in specific changes in the composition and / or activity of the gastrointestinal microbiota [81]. In other words, prebiotics act as "fertilizers" for the colonic microbiota and increasing the growth of beneficial commensally microorganisms [83]. The prebiotics are oligosaccharides composed

of 4 up to 10 monomers of hexose [81] that are non-digestible, for example, inulin and oligofructose. These monomers are fermented by the colonic microbiota [67] and enhance the growth of *Bifidobacteria* and *Lactobacilli* species [67, 91]. Furthermore, these prebiotic compounds promote the growth of specific strains capable of digesting polysaccharides and provide additional energy to the host, since the total mass of bacteria in the colon are increasing [60].

The modulation of the gut microbiota by using of the prebiotics in obese mice, favourably acts on the intestinal barrier, decreases the LPS-induced endotoxaemia towards the high-fat diet and systemic inflammation of the liver [83].

The probiotics, according to the currently adopted definition are "a preparation or product containing live microorganisms that upon its administration in adequate amounts can alter the microbiota and confer health benefits to the host" [21, 24, 67, 79, 81, 83]. Probiotic bacteria - microorganisms generally recognized as safe, are mainly belonging to bacteria of the family of lactic acid, and are known to confer beneficial effects to human or animal health. Probiotics have also a stimulating effect on colonic absorption of water and sodium, together with the calcium, magnesium, iron and zinc, by the way of absorption of short-chain fatty acids such as butyrate. The protective effects of probiotics could also be explained by their role in the modulation of mucosal immune response. Indeed, probiotics have different specific immunomodulatory capabilities *in vitro* that can be correlated with its potential anti-inflammatory action *in vivo* [21].

Symbiotic are the combination of probiotic and prebiotic products administered together [81, 83].

10.4.1. Probiotics: lactic acid bacteria - *Lactobacillus* and *Bifidobacterium* genera

Decrease of obesity and related diseases can be mediated trough the modulation of endocrine function of the gut. The proximal small intestine mucosa is highly sensitive to changes in microbial composition caused by probiotics [3].

The known beneficial bacteria, which have a long association with the health, are lactic acid producers, belonging to the genera *Lactobacillus* and *Bifidobacterium*. These bacteria can positively affect the health, and therefore, the restoring of the balance by these bacteria in order to treat and prevent the diseases should be advantageous [81]. The most consistent effect of health benefit conferred by family of lactic acid bacteria is concerned to improving of the lactose digestion [21]. As regards the use of probiotics in general, there are important differences of strain to strain that can determine its effects [66].

Probiotics belong to a number of genera including *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus* and *Enterococcus* [83].

It is known that the number of *Bifidobacteria* spp. is inversely correlated with the development of fatty mass, glucose intolerance, and the level of bacterial lipopolysaccharides [83]. The population of *Bifidobacteria* spp. is lower in obese individuals than in lean ones. These findings suggest that the *Bifidobacteria* can play the role in the development of obesity and its associated co-morbidities [81].

Concerning the *Lactobacillus* genus, it comprises more than 90 species, and the most commonly used to this purpose include *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus reuteri* and *Lactobacillus plantarum* [83].The observed anti-inflammatory effects of *Lactobacilli* spp. after its systemic or oral administration suggest that mechanisms of protection of these bacteria can involve regulation of cell populations, being the example showed on recent studies that probiotics improve the murine colitis by inducing regulatory T cells [21]. *Lactobacillus rhamnosus* can modulate the immune system by preventing the induction of IL-8 by TNF-α in epithelial cell lines of human colon, and by modulating the inflammation trough generation of regulatory T cells, having also these bacteria the effect on the intestinal motility [78]. In addition to have anti-inflammatory properties *Lactobacillus rhamnosus* has been suggested as a candidate of interference with the central nervous system signalling through the vagus nerve, allowing the communication between the viscera and the brain [80].

The administration of probiotic demonstrates a decrease in adiposity in different animal models of obesity. For example, the size of adipose cells (and body fat) was reduced in obese mice administrated with the specific strains of *Lactobacillus plantarum* or *Lactobacillus paracasei* [3]. Also, the administration of probiotics such as *Lactobacillus rhamnosus* and *Bifidobacterium lactis* to little children have reduced their body mass index and prevented the excessive weight gain during the first year of life [3]. On other study, when mice were fed with fruit juice containing *Lactobacillus plantarum* and *Bifidobacterium lactis*, the results showed that circulating levels of leptin were decreased due to this probiotic administration [81].

The fasting induced adipose factor that inhibits the absorption of circulating fatty acids of triglyceride-rich lipoproteins in white adipose tissue and muscle tissue [81] showed an increase in its circulating levels in mice fed with a high-fat diet but supplemented with *Lactobacillus paracasei*, that was accompanied by a significant reduction on body fat [59, 81]. *Lactobacillus paracasei* regulates the expression of this factor in cell lines of colonic epithelial cells and its oral administration to germ-free mice also resulted on increased circulating

levels of fasting induced adipose factor [59]. The obese individuals who were administered the *Lactobacillus acidophilus* and *Lactobacillus gasseri* showed a decrease in fatty mass. In the group that consumed *Lactobacillus gasseri*, the abdominal and visceral fat, as well as the fatty mass of subcutaneous areas as well as the body weight have significantly decreased [81].

One probiotic strain of *Lactobacillus gasseri* - SBT2055 showed to have an impact on health of volunteers with high body mass index and visceral fat. An administration of milk containing *Lactobacillus gasseri* strain SBT2055 reduced the weight and abdominal adiposity. This suggests that this strain has a beneficial effect on human health. The mechanism(s) by which this benefit is obtained are unknown, but in another study in rodents, milk fermented by this strain of *Lactobacillus gasseri* (LG2055) showed to restrict dietary fat absorption in rat intestine [80].

In one study an increase in the serum levels of fast induced adipocyte factor caused in mice by administration of *Lactobacillus paracasei* subsp. paracasei F19 were associated with diminished weight gain regardless of high-fat fed. Although some studies had reported lowered levels of mRNA expression of fast induced adipocyte factor in the intestinal tissue towards bacterial exposure, these contradictory results may be explained by fact that the exposure of mice to the whole bacterial community results in a decrease in intestinal fast induced adipocyte factor levels, while introduction of a single defined bacterial species has the contrary effect. It happened because just certain types of bacteria (as paracasei F19) seem to be able to overregulate the FIAF expression, and could have a therapeutically role in weight management [80].

Whereas probiotics are considered as safe, just a few prebiotics have received approval from the Food and Drug Administration as "Generally Recognized as Safe" [83].

All probiotics should be used with specially caution in critically ill patients and immune-compromised ones. The effectiveness of probiotics can be specific to a particular strain and the obtained results can be due to using of a particular probiotic (or combination) and its effect cannot be extrapolated to the other one. Furthermore, results of clinical trial may be depended on the potency (concentration) and measures used to ensure "bioavailability" of the bacteria [83]. Therefore, the bacteria of *Lactobacillus* genus, (belonging to the phylum of *Firmicutes*), is subject to controversial results [60]. This discussion is related to the potential association of *Lactobacilli* and obesity. To date, this debate remains unresolved, but it is probable that this association can exists, in which some specific species can play the protective role against obesity development, while other ones are actually associated with weight gain. A simple analogy can be proposed to the commensally *Escherichia coli* strains

which can be viewed as potential pathogens, while another specific strain, such as *Escherichia coli* strain of Nissle 1917 is known to have positive impact on intestinal inflammation [60].

Although *Helicobacter pylori* is not prebiotic bacteria, infected mice have different feeding behaviour when compared to healthy controls: the infected mice showed an early satiety [81].

The patients with liver disease that were administrated probiotics and symbiotic, have reduced intestinal permeability and endotoxaemia and can serve as a model for the reduction of metabolic endotoxaemia [83].

10.4.2. Prebiotic fibre

The reason for the use of prebiotics such as oligosaccharides such as galacto-oligosaccharides, inulin derivatives, including fructo-oligosaccharides and soluble fibre is based on its ability to stimulate the growth of beneficial bacteria (*Bifidobacteria* and *Lactobacilli*) in the intestine in order to generate the fermentation products like short-chain fatty acids with its anti-inflammatory effects. This effect is intended from short-chain fatty acid binding to its receptors on leukocytes, with the view to reduce the appetite, and to simulate the binding sites of pathogenic agents that are coating the surface of epithelial gastrointestinal cells, and thus prevent the adhesion of enteric pathogens and consequent infection [66].

As it was mentioned above, the diet enriched with a specific prebiotic non-digestible carbohydrates (oligofructose) strongly promotes intestinal short-chain fatty acids production in mice and thereby has reduces body weight gain, fatty mass development and severity of diabetes [60].

Prebiotics act as substrates for *Bifidobacterium* genera and will favour their growth and performance of its anti-inflammatory functions [39].

When prebiotics such as inulin-type fructans were administered to mice, it was used by the intestinal bacteria as energy substrate [81] trough fermentation by a number of bacteria in the colon [83]. The number of *Bifidobacteria* spp. have significantly increased [81, 83], and there was an inverse correlation with the levels of bacterial lipopolysaccharides, glucose tolerance and the development of fatty mass. In addition, the administration of prebiotics has prevented over-expression of several genes related to the host adiposity and inflammation [81]. This increase of genes expression occurs within a few days, but rapidly (after one week) disappears upon removal of prebiotic compounds. In other study the single dose of prebiotic (for example inulin) has significantly increased the plasma levels of

postprandial glucagon-like peptide-1 and decreased plasma levels of ghrelin. This finding contradicts the previous idea that persistent and prolonged modulation of the intestinal microbiota it is necessary to establish an effect on the endocrine function of the intestine [60].

The extent of increase of the number of *Bifidobacteria* is also dependent on its initial number in the intestine, where the mother´s milk containing oligosaccharides with prebiotic properties contributes to the increase in the number of *Bifidobacteria* spp. [83].

The prebiotic approach seems to be interesting, given the inulin-type fructans have been shown to increase in healthy volunteers *Faecalibacterium prausnitzii* - bacteria that can modulate inflammation and diabetes in obese individuals [60]. Clinical trials that used prebiotics as inulin and arabinoxylan, have showed positive results in diabetic, overweight and obese populations [81].

In the other hand, in mice whose diet is rich in fat with fructan supplement that favours the fermentation process, led to reduction of genes expression that respond to short-chain fatty acids in adipose tissue (gene that encode its receptors), and thus, prevent the development of fatty mass [3]. One clinical study in which inulin-type fructans of short-chain were administered as supplements for 3 months, have showed a reduction on food intake, weight gain and development of fatty mass in obese subjects, which highlights the fact that prebiotics promote weight maintenance [3].

Interestingly, administration of a prebiotic fibre was associated with behavioural changes in dietary intake [60]. The addition of the inulin-type fibre (10% of the diet) have decreased the food intake [3] due to the mechanisms related to altered expression or content of various intestinal hormones related to the regulation of energy balance, namely the increase of production and concentration of anorexigenic peptides - PYY and GLP-1 in the caecum [7, 57, 60, 67, 91]. The minor release of ghrelin that improves the condition of diabetes induced by high-fat diet is also observed towards the inulin-type fibre administration [3, 7, 57, 60, 79, 91]. Modulation of intestinal peptides, such as increased levels of GLP-1, GIP and PYY due to prebiotic fructans was also observed in healthy individuals [3].

As it was referred before, the GLP-2 is co-released with the GLP-1. In addition to GLP-1, prebiotics also promote the production and release of GLP-2 and the concentrations of its active forms in the portal vein were detected. GLP-1 participates in decreases on appetite, fat mass, and on hepatic insulin resistance related to prebiotic, while the GLP-2 contributes to reducing of the permeability of the intestinal wall and endotoxaemia that are associated with obesity [83]. However, the pharmacological or genetic deletion of GLP-1

blocked the beneficial effects of prebiotics on weight gain, glucose metabolism, and activation of the inflammatory pathway [67].

Several studies have shown that administration of short-chain oligosaccharide prebiotics such as oligofructose, as it was referred, decreases food intake and fatty mass development by accumulation of triacylglycerol in serum caused by a high-fat diet and thus protects against the body weight gain [57]. Furthermore, oligofructose (as well as pre- and probiotics in general [67]) showed to promote satiety in healthy humans [57], reduce caloric intake, improve the greater responses to GLP-1 and PYY and reduce the postprandial inflammatory responses [67]. In two separate studies, fermentation of prebiotics by gut microbiota has been linked to reduced hunger and increase satiety, thus reducing the total energy consumption about 10% [60].

The improvement of metabolic inflammation in obese mice, in addition to involve changes in the microbiota [59], also exerts protective effects on the intestinal barrier function [91]. In comparison with the carbohydrate diet [91], the prebiotic carbohydrate diet (as well as systemic antibiotics [59]) increased the proportion of intestinal *Bifidobacteria* and *Lactobacilli*, and protects obese subjects against the alteration of intestinal permeability [59, 91] – have maintained tight junctions integrity and the intestinal barrier function [67], as well as diminished endotoxaemia, liver and systemic inflammatory cytokines [59, 67, 91] and oxidative stress [67, 91]. In fact, the animals that suffer modulation of the gut microbiota by treatment with prebiotics showed an increased production of glucagon-like peptide-2 in the colon [85, 83], that is associated with increased expression of ZO -1 [83], and mechanisms mediated by endocannabinoid receptors [85], thereby improving the barrier function of the mucosa by decreasing of intestinal permeability [83, 85], leading to a decrease levels of LPS in plasma [83].

In genetically obese mice this alteration with prebiotics on intestinal microbiota was really associated with a significant improvement in gut permeability measured *in vivo*, and this phenomenon was in fact associated with increased expression of the mRNA and a better distribution of tight junction proteins. Although these data strongly suggest that modulation of intestinal microbiota with prebiotics in obese mice can positively act on the intestinal barrier, the mechanism by which probiotics improve the permeability of the intestine, especially in the context of obesity remained to be elucidated [60]. Nonetheless, the effect on intestinal integrity is thought to be probably indirect, mediated by the generation of volatile fatty acids during the fermentation [91] and subsequent stimulation of the release of intestinal glucagon-like peptide-2 [59, 67 93]. Furthermore, it was investigated the role of

specific intestinal peptide, glucagon-like peptide-2, involved in controlling of proliferation of intestinal epithelial cells and in the integrity of the barrier function [60], and the treatment of animals with glucagon-like peptide-2 agonist showed similar beneficial effects to these peptide [59]. In fact, the role of GLP-2 on the protective effect of prebiotics was investigated by blocking it receptors, with the concomitant presence of changes in the intestinal microbiota associated with prebiotics. Indeed, the GLP-2 antagonist have completely blocked the main characteristics of prebiotic treatment, that leads to conclusion that without a functional receptor for glucagon-like peptide-2, treatment with prebiotics was not able to reduce metabolic endotoxaemia, hepatic inflammation and oxidative stress markers [60].

Collectively, these data support the concept that specific alterations of intestinal microbiota on improving intestinal permeability and inflammatory tone are performed through a glucagon-like peptide-2-dependent mechanism [60].

More importantly, it was found that the intestinal microbiota changes by prebiotics can promote the normalization of response of the endocannabinoid system in both, the intestine and adipose tissue. [60]. In fact, the responsiveness of the endocannabinoid system of intestine was normalized, thus reducing its permeability, endotoxaemia, and metabolic development of fatty mass [3, 60]. In addition, there was detected a reduction in the absorption of LPS due to improved expression and activity of proteins involved in intestinal barrier function [3].

However, it should be noted that, since there is a strong correlation between the composition of the intestinal microbiota and crucial elements of the barrier function of the gut (for example, glucagon-like peptide-2, and the endocannabinoid system), the direct involvement of specific intestinal microbes and microbial metabolites remains unclear [60].

The intake of prebiotic fructans of inulin type during the one-year period has demonstrated a significant benefit in maintaining of the body mass index. It was demonstrated that daily intake of *yacon* syrup for more than 120 days increases satiety and reduces body weight, waist circumference and body mass index in obese premenopausal women [3].

Other non-digestible carbohydrates, such as resistant starch, are capable to increasing the *Actinobacteria* and specific types of *Bifidobacterium* genus as *Bifidobacterium adolescentis* in humans. This resistant starch have also been proposed as nutrients capable of controlling blood glucose and food intake by changing the profile of gut hormones such as glucagon-like peptide-1 [3].

Prebiotics showed also increase the villus height, crypt depth and increase the thickness of the mucosa layer of jejunum and colon [60].

Different components of the diet, including wheat fibre, inulin, starch or oat, affect glucose uptake, increase concentrations of incretin and GLP-1 [59].

10.5. Antibiotics

Changes on intestinal microbiota associated with the intake of antibiotics have resulted in reductions in induced inflammation by metabolic endotoxaemia and obesity [5].

An administration of antibiotics cancelled negative effects in mice fed with a high-fat diet that had increased intestinal permeability and decreased expression of genes encoding tight junction proteins [85].

The use of antibiotics in order to alter the composition of the intestinal microbiota in genetically obese mice have reduced the body weight, fasting glucose levels and have improved glucose tolerance, suggesting that the intestinal microbiota can be a new target for the treatment of metabolic diseases such as obesity. The concomitant diminution of LPS improves the effects of glucose reduction by antibiotics [57]. It was demonstrated that the changes on the intestinal microbiota with antibiotic treatment protects against the development of fatty mass induced by diet, glucose intolerance, insulin resistance and inflammation [60], although there is study, in which changes in the intestinal microbiota induced by antibiotic treatment did not provoke changes in body weight or body fatty mass, despite of improvement of insulin sensitivity correlated with reduced production of lipopolysaccharides, TNF-α and elevated secretion of adiponectin [5].

Obese or fed with a high-fat diet mice treated with antibiotics (norfloxacin and ampicillin) show changes in its gut microbiota, with high suppression of aerobic and anaerobic bacteria in the caecum [66]. It was observed the improvement in insulin resistance, fasting glucose [59] and glucose tolerance [5, 59] and it was reduced the hepatic steatosis [5] together with a reduction in systemic metabolic endotoxaemia [59, 67, 85] and inflammatory parameters in treated obese mice, when compared with obese mice from control group [59]. Mice treated with ampicillin and neomycin had reduced caecal content of lipopolysaccharides [67], and all improved above mentioned parameters are closely correlated with an improvement of the obese phenotype, however, it is important to reveal the potential long-term consequences of antibiotic therapy in various stages of life [59].

11. Proposals and future therapeutic perspectives

Taking into account all discussed facts, the new findings certainly will lead to the identification of new therapeutic strategies not only for the treatment but also for prevention of metabolic diseases. The identification of biomarkers capable of predicting the development of diabetes and overweight, in the absence of any risk factor would also be developed [39].

The intestinal microbiota and toll-like receptor-4 emerge as potential therapeutic targets for obesity and associated comorbidities. Thus, new therapeutic strategies directed to modulate the intestinal microbiota, the microbiota-host interactions, and LPS-TLR-4 machinery may be presented in the near future and its effectiveness would be evaluated in different studies [66], and the lower plasma lipopolysaccharides levels may be also a useful strategy to control the inflammation associated with metabolic diseases [91].

The interaction into intestinal microbial world with its host and its mutual regulation is becoming to be an important topic of biomedical research and provide new perspectives on the relationship between microbiota, metabolism, metabolic syndrome and obesity [59].

12. Conclusion

Overweight and obesity are the fifth leading risk for global deaths. It is urgent to develop new strategies to treat and prevent obesity.

Gut microbiota represent a promising therapeutic strategy to revert or prevent an obese state [80]. Indeed, there are several mechanisms that relate gut microbiota to obesity, including the metabolic process of nutrients, maintenance of gut barrier function and immunologic surveillance. Moreover, the beneficial actions on obesity can be due to energy capture modulation, release and processing of neurotransmitters, parasympathetic activity and changes in expression profiles of different signalling pathways [79]. Also, it should be taken into consideration that due to the complex nature of the gut environment, it is very unlikely that just one a phylogenetic change in the gut microbiota could be responsible for the obesity development [80]. The interpretation of diverse data emphasize that in obese mice a shift of *Bacteroidetes versus Firmicutes* rate occurs; in humans this shift seems to be more complicated, and it is very difficult to reach a consensus about the influence of a specific group of microbiota on human body weight changes [80].

Some researchers were able to demonstrate a relationship between type of diet and microbiota, concluding that composition of gut microorganisms is influenced by changing of dietary factors [57, 80] and that intestinal colonization seems to be dependent on presence of a single component in the food [80]. However, the correlation between changes in the gut microbiota and weight gain in humans has confounding factors (sex, age, diet, environment, antibiotic treatments, disease history, and finally genetic heterogeneity). So, the variability observed in different studies about the microbial composition may be due to this variety of factors, including inherent ones to studies. These factors are volunteer cohort, duration of study, methods of sample preparation, and techniques of analysis (detection) [80].

A better understanding and evaluation of the link between human intestinal microbiota, metabolic products and food-related behaviours in healthy and obese humans will help to develop new guidelines for humans feeding at various stages of their life, in order to globally improve human health, and establish forms to prevent or treat different diseases related to alimentation [79, 59]. In this approach, the investigation of the impact of the intestinal microbiota in programming or "re-programming" obesity development and adult intestinal microbiota modulation by microbial strains represents research priorities. These studies will reveal the full therapeutic potential of introduction of gut bacteria probably soon after birth

in order to prevent obesity and associated pathologies. Also, the role of probiotics, prebiotics or antibiotics administration should be better investigated to improve the epidemic of overweight and obesity [79].

To implement the modulating of so promising agent of obesity improvement – gut microbiota, it is necessary more studies to answer the following questions: is the gut microbiota a decisive responsible factor of obesity and not its consequence, and is it possible to treat obesity modifying only gut microbiota?

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