

Microbiota and LPS-induced obesity inflammation: therapeutic implications

Raffaela Pero¹, Giovanna Fico², Olga Scudiero¹ and Sonia Laneri³

1 Department of Molecular Medicine and Medical Biotechnology, University of Naples “Federico II”, 80131 Naples, Italy. 2 ASL Napoli 3 Sud, Naples, Italy; 3 Department of Pharmacy, University of Naples “Federico II”.

Corresponding author:

Raffaela Pero: Department of Molecular Medicine and Medical Biotechnology, University of Naples “Federico II”, Via S. Pansini, 5, 80131 Naples, Italy. E-mail addresses: pero@unina.it

Running title: Microbiota and LPS-induced obesity

Abstract

Obesity and chronic low-grade inflammation are becoming global epidemics. The dysbiosis has a specific role in the metabolism and energy stocks of the host. The discovery that a low-grade of inflammation could be directly connected to the intestinal microbiota metabolic endotoxemia (elevated levels of plasma lipopolysaccharides) has allowed the identification of novel mechanisms involved in the control of the intestinal barrier. In this review, it will analyze the latest news to explain how human symbiotic microorganisms participate in the growth of the fat reserves and promote insulin resistance as a low-grade inflammation. Besides, it will discuss new treatments with probiotics and prebiotics as a promising therapeutic approach to reverse the host's metabolic changes linked to dysbiosis observed in obesity.

Keywords: LPS, microbiota, inflammation, intestinal permeability, obesity

Introduction

Obesity is a chronic degenerative disease with multifactorial pathogenesis and is characterized by fat mass excess and complications in various parts of the body. Obesity is the leading cause of preventable death and is one of the most severe public health problems of our century.

Before the twentieth century, obesity was a rare condition; in 1997, the World Health Organization (WHO) has officially recognized obesity as a global epidemic disease. In the XXI century, the condition is increased around the world, both in developed and in developing countries. The only region of the world where obesity is not prevalent in the area of sub-Saharan Africa.

According to data provided by the World Health Organization (WHO), in 2014: 2 billion people (>20 years) worldwide was overweight. Besides, since 1980 the number of obese people in the world is doubled and, to date, 200 million men and 300 million women are obese according to data from WHO, 2016.

Childhood obesity has reached alarming: data covering 144 countries, the worldwide prevalence of overweight and obesity in preschool children aged 0 to 5 years old increased from 4.2% in 1990 to 6.7% in 2010 and is projected to reach 9.1% (about 60 million) by 2020. The severity of these data lies in the fact that obese children are likely to become obese adults (Cauchi et al. 2016). Recent studies demonstrated that the economic costs of obesity and overweight represent 2-7% of total healthcare costs. The obesity prevention programs have reduced the cost of treatment of diseases related to it. However, people are living longer and therefore, the cost of medical expenses is increased. Obesity can lead to social stigmatization and disadvantages in employment. When compared with their counterparts of healthy weight, obese workers have on average higher rates of

absenteeism from work and thus increasing costs for employers by reducing productivity (Robertson et al. 2014). At present, the intestinal microbiota has been recently proposed as an environmental factor involved in energy homeostasis and body weight (Backhed et al. 2007; Ley et al. 2006). Here, it will review how the microbiota is associated with obesity and identify some of the remaining challenges in understanding the mechanisms underlying this association.

Obesity complications

Obesity increases the risk of many physical and mental diseases. Complications are either directly caused by obesity or indirectly related to it, through mechanisms sharing a common cause such as a poor diet or sedentary lifestyle. The strength of the link between obesity and specific conditions is various. One is with type 2 diabetes. Excess of fat body underlies 64% of cases of diabetes in men and 77% of cases in women. The complications fall into two broad categories: i) attributable to the effects of increased fat mass (osteoarthritis, obstructive sleep apnea, social stigma); ii) due to the increased number of fat cells (diabetes, cancer, cardiovascular disease, nonalcoholic fatty liver disease).

The increase in body fat alters the body's response to insulin, carrying resistance to it. Increased fat also creates a proinflammatory and prothrombotic state. These complications, therefore, affect almost all organs and apparatus of the body: i) urinary tract: incontinence, hypogonadism, glomerulosclerosis; ii) cardiovascular: arterial hypertension, ischemic diseases, heart failure, chronic pulmonary heart, IVLC, venous varices; iii) neurological: stroke cerebri, idiopathic intracranial hypertension, Meralgia paresthetica; iv) respiratory: chronic respiratory failure restrictive, obstructive sleep apnea, Pickwick syndrome, asthma; v) endocrine: metabolic syndrome, type 2 diabetes mellitus, dyslipidemia, hyperuricemia, polycystic ovarian syndrome (PCOS), hyperandrogenism, amenorrhea/infertility, gynecomastia. vi) psychiatric: changes in mood, binge eating, sexual dysfunction; vii) tumoral: breast, uterus, ovaries, colon, prostate; viii) musculoskeletal: osteoarthritis, Ernie discs, valgus knees, flat-footedness; ix) cutaneous: stretch marks, dermatitis, abdominal hernia x) gastro-intestinal: gastro-oesophageal reflux disease (GERD), hepatic steatosis, steatohepatitis (NAFLD), cholelithiasis (Bray et al. 2004).

Classification of obesity

The fat body is stored mostly in the subcutaneous adipose tissue. In the male, the distribution of fat in the subcutaneous occurs preferentially in the neck, face, shoulders, torso, abdomen while in the female it occurs in the neck, breast area, abdomen, pelvic girdle, the trochanteric region, the

peritrocantheric region and the medial aspect of the knee. Obesity can be classified according to: i) Morphology; ii) Pathogenesis; iii) BMI (Sweeting 2007).

Obesity Morphology

The classification based on the morphology can be classified into four types:

- a) Obesity Type I: iper gynoid (female), gynoid (male): the bi-humeral diameter is less than bi-trochanteric diameter. It is also called also iper-gynoid in the female and gynoid in the male. This because the fat distribution is similar to that of women (in the lower half of the body). There is also talk of ipotenic obesity or pear-shaped obesity.
- b) Obesity Type II: android (female), iper-android (male); the bi-humeral diameter is higher than that of bi-trochanteric. It is also called android in the female and the male iper-android because the fat distribution is similar to that of man (in the upper half of the body). This obesity is defined as apple obesity.
- c) Obesity Type III: ortho-gynoid (female), ortho-android (male). It presents intermediate characteristics between type I and type II. The bi-humeral and bi-trochanteric diameters are superimposed bi-trochanteric, and the increase of diameters bi-humeral, thoracic and abdominal perimeter is proportional. The fat distribution assumes a lateral aspect, that occurs both in the upper half, both in the lower half of the body and this obesity has the overall appearance of a parallelepiped, of a cylinder.
- d) Obesity Type IV: monstrous. It presents large enough to distort the shape of the body (Bjontorp 1984).

Obesity BMI

The Body Mass Index (BMI) is calculated by dividing your weight in kilograms by the square of height in meters: [BMI = weight (kg)/(height) 2 meters]. It represents a morbidity index against cardiovascular disease, bone and joint, dyslipidemic, respiratory, cancer (adenocarcinoma of the colon, breast cancer, ovarian cancer, prostate adenocarcinoma) and metabolic disorders (diabetes mellitus). The farther away a weight/height balanced more increases the risk of mortality.

The BMI is a useful biometric data to determine whether the weight/height relationship is balanced. The BMI can be unbalanced by: i) weight gain; ii) decrease in body weight. Normal BMI range is between 18.7 and 23.8 for females and between 20.1 and 25 for males. Higher values indicate: i) an overweight [male: (BMI = 25.1-30); Female: (BMI = 23.9- 28.6)]; ii) obesity grade 1 [male (BMI = 30.1-35); Female: (BMI = 23.9- 28.6)]; obesity grade 2 [male (BMI = 35.1-39.9); Female: (BMI = 23.9- 28.6)]; obesity grade 3 [male (BMI = 40 and over). Female: (BMI = 23.9- 28.6)]. Lower values

indicate: i) Malnutrition [male (BMI = 15.1-20); Female: (BMI = 23.9- 28.6)]; ii) severe malnutrition [male (BMI = 10.01-15). Female: (BMI = 23.9- 28.6)] (Gray et al. 1991).

Obesity pathogenesis

The classification based on the morphology can be: i) primary: are not known precisely the mechanisms that led to its decision. ii) secondary: caused by a known cause and includes the following forms: i) iatrogenic causes: corticosteroids, tricyclic antidepressants; ii) neuroendocrine causes: hypothalamic syndrome, hypothyroidism, Cushing's syndrome, PCO insulinoma and hyperinsulinism, GH deficiency; genetic causes: Prader-Willi syndrome, Bardet-Biedl syndrome, Ahlstrom's syndrome, Carpenter syndrome, Cohen syndrome, pseudohypoparathyroidism (Inukai 2013).

Microbiota and obesity

The intestinal microbiota is considered an important environmental factor that can affect the predisposition of the accumulation of adipose tissue. In this context, scientific literature has developed the new concept of "MicrObesity" (microbes/obesity) to understanding the specific relationship between dysbiosis and metabolic impact in obese patients. The new molecular biology technologies such as metagenomics and metaproteomics are doing light on the large diversity of intestinal bacteria. In 2010, Qin et al., have published the genome sequencing of human gut microbiota to clarify how these microbes affect our health and to develop new therapies and diagnostic tests for various diseases (Qin et al. 2010). This study has identified over 1,000 bacterial species. Among these bacteria, 90% of phylotypes is a member of two phyla (Bacteroides and Firmicutes), followed by Actinobacteria and Proteobacteria (Eckburg et al. 2005; Quin et al. 2010). Everyone has at least 160 species of microbes in their intestines with two components: a "hardcore" strongly linked to a genetic predisposition, containing microbial species that remain stable over time, and a "changing group" that can change during the life concerning environmental conditions, food or use of antibiotics. This variable group could explain why some people are affected by intestinal diseases or are prone to obesity.

Recent studies demonstrated that gut microbiota has a definite role in the management of homeostasis energy and extraction of calories ingested, and helps to store these calories in the fat tissue of the subject to later use. This studies firstly comes from Backhed et al. who first described the effects of the gut microbiota transplant from obese mice into mice germ-free (Backhed et al. 2004) and Ley et al. who demonstrated the different composition of the gut microbiota in genetically obese (ob / ob) and lean mice (Ley et al. 2006). Subsequent studies have shown that

colonization of germ-free mice with gut microbiota of ob/ob mouse causes a higher weight gain and energy extraction than the colonization with gut microbiota of lean mouse (Murphy et al. 2010). Moreover, Ridaura et al. (Ridaura et al. 2013) have shown that mice treated with the obesity gut microbiota present significantly increased adiposity, further providing direct evidence for the presence of a transmissible obesity microbiota.

Factors modifying the microbiota

Various factors modify the microbiome. However, the more significant factor in the changing of the intrapersonal and interpersonal plasticity of the intestinal microbiome is the diet (Voreades et al. 2014).

In humans, different studies indicate an increase in the Firmicutes and a decrease in the Bacteroidetes phyla to be associated with obesity. Armougom et al. have shown that obese people have a higher ratio of Firmicutes to Bacteroidetes, suggesting a correlation of this ratio to body weight (Armougom et al. 2009). Chronic exposure to a high-fat diet (HFD) could be changed the composition of the colon microflora in mice, leading to a reduction in the levels of *Bifidobacterium* and *Lactobacillus* and an increase in the levels of Firmicutes and Proteobacteria that include pathogenic species (Luoto et al. 2011; Backhed et al. 2004). Moreover, it was also observed a decrease of *Bacteroides* (phylum Bacteroidetes) and an increase of *Bacillaceae*, *Clostridiaceae* and other representatives of phylum Firmicutes. Also, in the gut of obese people, it has been reported the presence of Actinobacteria and H₂-oxidizing methanogenic Archaea (Hildebrandt et al. 2009; Turnbaugh et al. 2008; Zhang et al. 2009).

It has suggested that a signaling cascade triggered by LPS/TLR4/CD14-dependent mechanism, in turn, activates the expression of TLR-2, to activate an inflammatory response of the innate immune system. In particular, the presence of LPS at low concentrations in the blood has connected to metabolic disorders. This chronic low endotoxemia has been called "metabolic endotoxemia." The increase in the LPS could occur through an increase in the formation of chylomicrons (high-fat diet), a reduction in the integrity of the intestinal barrier and a decrease in the activity of alkaline phosphatase enzyme responsible for cutting intestinal LPS (Delzenne et al. 2013).

Obesity and inflammation

Obesity is associated with a state of chronic low-grade inflammation in which exist increased circulating levels of proinflammatory cytokines (Pereira et al. 2014) and macrophages infiltration of adipose tissue, generating a local inflammation that rapidly reaches the systemic circulation disrupting the insulin pathway, in the host tissues, resulting in insulin resistance. Therefore, insulin

is unable to increase the absorption of glucose by target tissues, thereby reducing the inhibition of lipolysis in adipose tissue and thus increasing the release of free fatty acids (FFA) in the blood. (Yuntao et al. 2015). Cani et al. hypothesized that LPS, derived from the intestinal microbiota through lysis of Gram-negative bacteria, could be an early factor in the development of low-grade inflammation by binding to the CD14/TLR-4 complex at the surface of innate immune cells (Cani et al. 2007a). More precisely, high-fat diet not only increases systemic exposure to potentially pro-inflammatory fatty acids but also facilitates the development of metabolic endotoxemia (Cani and Delzenne, 2007; Cani et al. 2007b) (Fig. 1).

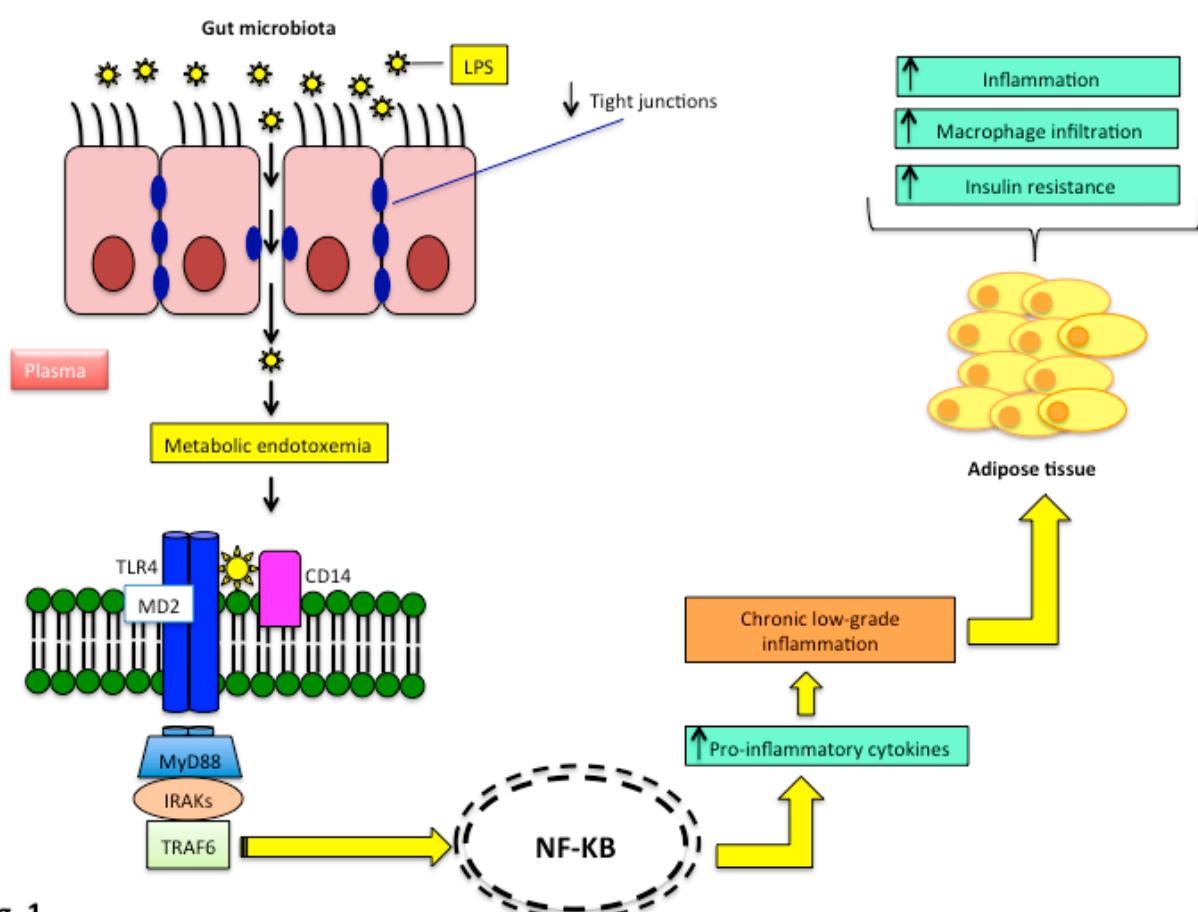


Fig. 1

Fig. 1 Schematic overview of the possible interaction between gut microbiota, host innate immunity and LPS-induced low-grade inflammation in adipose tissue.

Subsequently, various experiments have demonstrated that gut microbiota can start the inflammatory processes associated with obesity and insulin-resistance through modulation of plasma levels of LPS. This is the first study that has postulated a connection between the gut microbiota and a high-fat diet discovering a microbial dysbiosis as an inducer of intestinal inflammation and weight gain in mice. A high-fat diet increased the plasma levels of LPS (endotoxemia, metabolic) of two or three times. In mice lacking the CD14 co-receptor TLR-4: (CD14-/-), after four weeks of high-fat feeding, these mice exhibited an increased body weight, a low-grade inflammatory status (liver, muscles and adipose tissue) as well as a change in gut microbiota composition (reduction of Bifidobacteria and Eubacteria spp.). This study has also shown that chronic metabolic endotoxemia produced by chronic subcutaneous infusions of LPS (that mimic the metabolic endotoxemia) significantly reduces inflammation and insulin resistance. Moreover, in the absence of a receptor for LPS (CD14 -/-), the mice were resistant to obesity-induced diet and related disorders even when they are fed a normal diet, suggesting that CD14 may modulate insulin sensitivity under physiological conditions. In particular, LPS bind the plasma LPS-binding protein (LBP), which activates CD14 that is located in the plasma membrane of macrophages (Cani et al. 2008). Recently it has been proposed that fatty acids can stimulate an inflammatory response through the activation of the LPS receptor (toll-like receptor-4 [TLR-4]) that sends signals to adipocytes and macrophages, that may contribute to inflammation of the adipose tissue obesity (Shi et al. 2006; Suganami et al., 2007a, b), involving activation of NF- κ B and JNK by TLR signaling and mediates insulin resistance by phosphorylation of IRS-1 (Verges et al. 2008; Cani et al. 2008). LPS also regulate the nucleotide oligomerization domain (NOD)-like receptors present in macrophages and dendritic cells, which cooperate with TLRs to induce NF- κ B. In addition, LPS is able to recruit other effector molecules, such as nucleotide-binding domain leucine-rich repeat containing (NLR) protein, adaptor protein ASC, and caspase-1, which are involved in the activation of the innate immune system (Tanti et al. 2012). Although many data support the thesis of a mechanism activated by the complex LPS-TLR-4/ CD14, some emerging evidence supports the concept that other TLRs might be involved in the development of insulin resistance and a low-grade inflammation in obesity.

TLR-2 recognizes a large number of lipidic molecules of the Gram-positive bacterial cell wall, including the bacterial lipopeptide (Lien et al., 1999). In addition the expression and the induction of TLR-2 are directly controlled by LPS, but can also be induced by TNF- α and CD14 (Lin et al., 2000). Up-regulation of TLR-2 in the presence of low levels of microbial products is an important mechanism by which the immune system increases its response to recent infections (es. LPS) (Nilsen et al., 2004). So it has been proposed that metabolic endotoxemia lead to the activation of

TLR-2, and thus the amplification of signals of LPS/TLR-4/CD14 complex to stimulate the inflammatory response. Several studies have suggested that saturated fatty acids promote the low-grade inflammation (Shi et al. 2006; Suganami et al. 2007). It may be suggested that fatty acids are deeply involved in the stimulation of the innate immune system, but probably in conjunction with initial stimulation of LPS of the complex TLR-4/CD14 and a consequent TLR-2 stimulation. Toll-like receptor 5 (TLR5) recognizes bacterial flagellin and is highly expressed on the intestinal mucosa. TLR5 deficient mice (TLR5KO1) had alterations in gut microbiota composition that develop metabolic syndrome including hyperlipidemia, hypertension, insulin resistance, and increased adiposity. Also, transfer of microbiota from TLR5KO1 to wild-type mice conferred similar metabolic changes (Zhang et al. 2016).

TLR-9 recognizes bacteria-derived cytosine phosphate guanine (CpG)-containing DNA and can be involved in induction of obesity and adipose tissue inflammation. Mice lacking TLR9, compared to wild-type (WT) mice, exhibit excessive weight gain with development of obesity-associated glucose intolerance and insulin resistance under a high-fat diet (HFD) condition. This study shows that M1 macrophages and TH1 cells accumulate significantly more in the VAT than of TLR9-deficient mice, resulting in the increased levels of proinflammatory cytokines and chemokines. In vitro experiments suggested that cfDNA released from degenerated adipocytes increased M1 macrophages expression via TLR9 in wild-type macrophages (Nishimoto, 2016).

MyD88 (Myeloid differentiation primary-response gene 88) is a central signalling adaptor for the majority of TLRs. MyD88 play a role in the interaction between gut microbes and the host in obesity. Deleting MyD88 in intestinal epithelial cells protects against diet-induced obesity, glucose intolerance and HFD-induced metabolic endotoxemia, thereby supporting the hypothesis that the deletion improves metabolic inflammation. In addition, gut microbiota transplantation into germ-free recipient mice may be transfer this protection, suggesting that targeting intestinal epithelial MyD88 constitutes a putative therapeutic approach for obesity and associated disorders (Duparc et al. 2016). These findings shown that modulation of the immune system is integrated with pathogen-sensing systems (e.g. TLRs) and support the emerging view that the gut microbiota contributes to the inflammation and metabolic disease.

LPS and intestinal permeability

Several studies support the idea that a host-bacterial mutualism represents an essential key in the control of the intestinal barrier functions (Brun et al. 2007; Cani et al. 2008; Cani et al. 2009b; De La Serre et al. 2010; Muccioli et al. 2010).

Under physiological conditions, the intestinal epithelium acts as a continuous and effective barrier that prevents bacterial translocation of LPS. Microbiota represents the primary source of LPS, and the primary site of LPS entry is through the gut. The small intestine contains epithelial secretory cells (Paneth cells) confined to the bottom of the crypts. These cells release a wide variety of AMPs (mainly alpha and beta-defensins) and enzymes in response to Gram-positive or Gram-negative bacteria but also LPS itself detected in the intestinal lumen (Ayabe et al. 2000). AMPs are capable to target LPS directly or to induce bacterial death, and therefore LPS release. After the bind to AMPs, LPS is not able to prime TLR-mediated inflammation. AMPs also target bacteria through binding to their cell membrane and this binding results in its permeabilization and consequently bacteria death (Bevins et al. 2011). Moreover, LPS itself can increase intestinal permeability through a TLR-4-dependent upregulation of mCD14 expression inducing a perpetuation of inflammation once it has crossed the intestinal barrier (Guo et al. 2013). However, different endogenous and exogenous factors are associated with an alteration of the protective function of the intestinal barrier. Some lines of experimental have recently proposed that changes in the distribution and localization of Zonula Occludens-1 (ZO-1) and Occludin (two proteins of tight junctions) in intestinal tissue are associated with increased intestinal permeability and low-grade systemic inflammation, which are found in obese mice (Brun et al. 2007; Cani et al. 2008; Cani et al. 2009a; De La Serre et al. 2010; Muccioli et al. 2010).

Also, it has been investigated the role of a specific intestinal peptide involved in controlling the proliferation of epithelial cells and in the integrity of the intestinal barrier, called 2-glucagon-like peptide (GLP-2) (Jeppesen et al. 2001; Chiba et al. 2007; Dubè and Brubaker, 2007). Increased endogenous production of GLP-2 was associated with the improved functionality of the mucosal barrier through the restoration of the expression and distribution of proteins of the tight junctions (Cani et al. 2009b). The massive presence of the mucus-degrading bacterium *A. muciniphila* represents a direct relationship with the thickness of the mucus layer, and administration of this bacterium to obese mice reversed HF diet-induced metabolic disorders (Everard et al. 2013).

It is also identify the endocannabinoid (eCB) system as a determinant of gut barrier function. The eCB system mediates communication between adipose tissue and the gut microbiota. In fact, obesity is also characterized by an altered expression of cannabinoid receptor 1 (CB1 mRNA) and increased levels of eCB in plasma and adipose tissue. (Engeli et al., 2005; Blüher et al. 2006; Muccioli et al. 2010). Some evidence suggest that LPS stimulates the synthesis of eCB (in vivo and in vitro) (Di Marzo et al., 1999; Liu et al. 2003; Hoareau et al., 2009) and that the genetic or pharmacological block of the CB1 receptor protects from obesity, from steatosis and low-grade inflammation through mechanisms not yet resolved (Osei- Hyiaman et al. 2005; Gary-Bobo et al.

2007; DeLeve et al. 2008). eCB system, inflammation, and obesity are interrelated, and the gut microbiota and barrier functions converge in a molecular mechanism in which specific changes of the intestinal microbiota selectively diminish the activity of the eCB system in the colon, regulating gut permeability and plasma LPS levels (Muccioli et al. 2010). More specifically the CB1 receptor controls the function of the intestinal barrier. For example, blocking the CB1 receptor in obese mice reduces intestinal permeability through the improvement of distribution and localization of tight junction proteins (ZO-1 and Occludin).

Moreover, there is a reciprocal exchange between eCB and intestinal microbiota involved in the regulation of lipogenesis (Muccioli et al., 2010). Additionally, changes in the intestinal microbiota, through the use of prebiotics in ob/ob mice, promote the normalization of the responsiveness of the system eCB both gut and adipose tissue. These effects are strongly associated with decreased intestinal permeability and metabolic endotoxemia and increased adipocyte differentiation and lipogenesis.

Obesity therapy

Label drug therapy

Obesity drug therapy can be done through the official pharmacopeia drugs, which can be: i) label: Rimonabant, Sibutramine, Orlistat or ii) off-label: Branched Chain Amino Acids, metformin, acarbose, cholestyramine, Detastrano, Chitosan, fluoxetine, bupropion.

I) Rimonabant blocks the CB1 receptors for endocannabinoids. It is a drug with anorexiant effect. (Fong et al. 2009). The CB1 receptors are present in the brain, especially in the basal ganglia, globus pallidus, and substantia nigra and, in smaller quantities, in the cerebellum, hippocampus, caudate nucleus, putamen, hypothalamus, and amygdala. They are also identified, but with lower density, in the lungs, liver, kidney and male and female reproductive cells. Stimulation of CB1 receptors determines the euphoric effects of cannabinoids but also antiemetic, antioxidant, hypotensive, immunosuppressive, anti-inflammatory, analgesic, antispasmodic actions and appetite stimulation. Endocannabinoids are produced by multiple biosynthetic neuronal cells. The process of biosynthesis is activated by a stimulus that causes the depolarization of the cell membrane. The anandamide is formed following the enzymatic hydrolysis, catalyzed by a phospholipase type D, by a phospholipid precursor, the N-arachidonoyl- phosphatidylethanolamine (NAPE). The biosynthetic pathways leading to the formation of 2-arachidonoylglycerol (2-AG) provide for the formation of a diacylglycerol that is then hydrolyzed to 2-AG by a phospholipase type C. Unlike other neuromediators, they are not stored in vesicles but are synthesized on demand from membrane phospholipid precursors. Once synthesized, the endocannabinoids are immediately released from

the cell and bind to cannabinoid receptors on neighboring cells or the same cell that produced them, acting as autocrine or paracrine mediators. In particular, it has been suggested that endocannabinoids behave as retrograde messengers: synthesized in the postsynaptic cell, would activate the CB1 receptors of the axon of the presynaptic cell. Degradation mechanisms inactivate endocannabinoids or recycled controlled enzymatically. Endocannabinoids are a class of bioactive lipids essentially consisting of anandamide (AEA), 2-arachidonoylglycerol (2-AG), 2-arachidonyl glyceryl ether (noladin, 2-AGE), virodhamine, N-arachidonoid dopamine (NADA). Endocannabinoids constitute a neuromodulation system capable of regulating neuronal excitability, by inhibition of communication through tight junctions or interactions with the GABA-ergic transmissions, serotonergic, glutamatergic and dopaminergic.

Endocannabinoids are generally produced in the postsynaptic level and after that are released go to act in a retrograde on the presynaptic neuron, reducing the increase of the intracellular calcium and thereby inhibiting the release of neuropeptides generally of inhibitory type.

In food control, GABA, in the postsynaptic level acts to stimulate satiety. The activation of the endocannabinoid in the postsynaptic neuron level, which can be induced by the feeling of pleasure caused by the vision or the brief taste of a tasty sweet, attenuates the feeling of satiety caused by GABA stimulates and consequently the trend of the power recovery. The endocannabinoid system then modulates the reward properties of food by acting on mesolimbic specific areas in the brain. Hypothalamus, the CB1 receptors, and endocannabinoids are integrated components of the network which controls appetite and food intake. Rimonabant drug being a CB1 antagonist produces a series of positive effects on energy homeostasis including a reduction in the sense of hunger, reduction in fat mass and improved plasma profile (Pertwee 2006). The European Medicines Agency (EMEA) has shown an absolute contraindication to the use of Rimonabant major depression or taking antidepressants, because of the risk of a psychiatric side.

II) sibutramine reduces the sense of hunger by acting centrally as an inhibitor of the reuptake of norepinephrine and serotonin (anorectic action). In March 2002 the Health Minister decreed the withdrawal, due to deaths related to the assumption of this drug. In August of the same year, it was readmitted. In January 2010 it was again banished from the market because the risks outweigh the benefits. Today is back on the market (Heal et al. 1998).

III) Xenical is a drug to lipid-lowering action. It is a derivative of Lipstatin, a potent natural inhibitor of pancreatic lipases isolated from the bacterium *Streptomyces toxytricini*. The active principle, taken orally and not absorbed by the intestinal mucosa, reaches the gastrointestinal environment, where, given its chemical structure, can selectively interact with the pancreatic lipase, inibendole through a covalent bond. The inhibition of these enzymes prevents dietary lipids

introduced via the diet, to be adequately digested into fatty acids and glycerol, and then absorbed through the intestinal mucosa. The undigested fat, and the active ingredient and its metabolites, are subsequently excreted through feces, significantly changing the texture and the oiliness of the same (Chanoine et al. 2005).

Off-label drug therapy

I) Branched amino acids (BCAA: Branched-Chain Amino Acid) are a group of three of the nine essential amino acids (EAA) consisting of leucine, isoleucine, valine. They are called branched-chain because their structure forms branches, and account for about 35% of the essential amino acids in muscle proteins and 40% of the amino acids required by mammals. As one of the nine essential amino acids, the body is unable to synthesize them, in that they must be hired through protein foods such as meat, or specific supplements. The combination of these three essential amino acids representing approximately one-third of skeletal muscle in the human body in the form of proteins, although BCAAs are present in skeletal muscle in free form (not protein) in limited amounts. Unlike many other amino acids, BCAAs are metabolized only in skeletal muscle, because the BCAA aminotransferase enzyme is not present in the liver where instead many other amino acids are converted (Lu et al. 2013). During a period of caloric restriction, for body fat loss it is necessary to be able to achieve this result while preserving the skeletal muscle. However, caloric restriction leads to an energy deficit, and this quickly leads to a degradation of muscle proteins or muscle protein catabolism (Muscle Protein Breakdown, MPB), because the catabolic enzymes break down muscle proteins for BCAA. This self-destructive effect is thus to degrade some whole protein filaments to obtain only a small fraction represented by the three branched-chain amino acids. During the low-calorie diets, muscle mass can be saved by increasing protein intake. Providing a dose of BCAAs to provide for the energy demand of BCAAs in a low-calorie diet, it is possible to get an inhibition of dependent catabolic processes of skeletal muscle. Recent studies show that individuals who take large amounts of BCAA in their diet have lower levels of obesity, lower body weight, and improved body composition. It seems that leucine is capable of increasing the energy expenditure and improve glucose tolerance. A recent review has shown that the BCAA, leucine in particular, "appear to have particular reducing obesity effects" because they reduce food intake and body weight, activating the gene mTOR signaling, through the streets of AKT or PKB (Torres-Leal et al. 2001). mTOR (mammalian target of rapamycin) is a protein kinase that phosphorylates serine and threonine, which regulates the growth, proliferation, motility and cell survival, protein synthesis and transcription. mTOR is involved in all metabolic processes, anabolic and catabolic and has an essential role in the regulation of energy balance and body weight. It is

activated by amino acids, glucose and insulin and other hormones that regulate metabolism. The hypothalamic mTOR acts as a sensor for leucine in particular, but also for other amino acids. BCAA, through the activation of mTOR, prove to optimize the levels of certain hormones that lead to muscle growth, minimizing those that cause the opposite effect, that is the catabolism or muscle degradation. In fact, BCAA can: i) minimize the levels of cortisol caused by stress induced by exercise. The low levels of cortisol can have a favorable effect, because cortisol is the hormone responsible for the degradation of muscle protein, inhibits the absorption of amino acids, and can induce the accumulation of fat. The BCCA are therefore able to stimulate the biosynthesis of muscle proteins; ii) to have a stimulatory action on the hormone growth hormone (GH), another anabolic hormone; iii) reduce insulin resistance; activate lipolysis; iv) reduce the feeling of hunger. This product, taken by mouth, is rapidly absorbed from the gastrointestinal tract. Already after 10 minutes the levels in the blood of the three amino acids increased significantly (Cota et al. 2006).

II) Metformin is an oral hypoglycemic agent. Metformin reduces blood glucose: reducing the production of glucose by the liver (decrease of gluconeogenesis); favoring the increase in glucose consumption by peripheral tissues (increased glycolysis); reducing the intestinal absorption of glucose (He et al. 2009).

III) Acarbose is a pseudo tetrasaccharide of microbial origin with hypoglycemic action and is an inhibitor of intestinal enzymes (alpha-glucosidase) designated for hydrolysis of carbohydrates. In this way, acarbose reduces the increases postprandial blood glucose. It is a drug to lipid-lowering action and a resin capable of sequestering bile acids (Domecq et al. 2015).

IV) Chitosan is a drug to lipid-lowering action made from purified chitin obtained from shellfish. It is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). One study performed on obese subjects showed that change in BMI was not significant (Mhurchu et al. 2004).

V) Fluoxetine is a drug with anorectic action. Avoid associations with anticoagulant drugs. It is a selective serotonin reuptake inhibitor (SSRI) in central neurons (Uguz et al. 2015).

VI) Bupropion is a selective inhibitor of neuronal reuptake of catecholamines (norepinephrine and dopamine) with minimal effect on the re-uptake of indolamines (serotonin) and does not inhibit monoamine oxidase. The mechanism by which bupropion promotes the ability of patients to abstain from smoking is unknown. It is presumed that this action is mediated by noradrenergic and dopaminergic mechanisms (Ali et al. 2016).

Targeting the microbiome

Prebiotics

Prebiotics are non-digestible compounds, fibers or carbohydrates not digested by human enzymes, which through decomposition by microorganisms present in the intestine regulate the composition and the activity of the microbiota. In obese patients, the administration of fructans or arabinosilani stimulates the production of hormones in the digestive tract improving the sense of satiety or blood glucose. This integration of prebiotics also allows reducing fat mass and inflammation, as well as the transit of LPS through the intestinal barrier. Besides, prebiotics contribute to the increase of so-called "good bacteria" in the microbiota (particularly *Bifidobacteria*), which strengthen the protection of intestinal function. A recent study, by transferring the microbiota, has noted that administration of *Prevotella copri*, the most abundant *Prevotella* species in their study, improved glucose metabolism in a fibre-specific manner suggesting that individualized treatment programmes based on the microbiota may provide novel treatment strategies for obesity and correlate metabolic diseases (Kovatcheva-Datchary et al. 2015).

Probiotics

Probiotics are microorganisms that produce a beneficial effect on health only if they remain alive when reaching the intestine. Studies in overweight adults, the administration of probiotics for a time of 3-12 weeks, have led to a weight loss. In other studies, weight loss has been achieved without caloric restriction. In one study, patients who were taking probiotics following a low-calorie diet lost more weight than those who were content to follow the diet. In some studies, the authors point out that the weight loss is accompanied by a reduction in fat mass and waist (Sáez-Lara et al. 2016). Moreover, there are also studies in pregnant women, in different periods of pregnancy and some of these women until the end of breastfeeding. In one of these studies, it was noted a reduction in the incidence of gestational diabetes through the intake of probiotics. In another study, however, women who had been administered probiotics, six months after birth, showed a lesser amount of adipose tissue in the abdomen. Finally, in some pregnant women that have taken probiotics in the last four weeks of gestation, it was also shown a benefit for their children until the age of 6 months (Sáez- Lara et al. 2016). Obesity has a negative impact on the health and welfare of the people who are affected. Therefore, any road to prevention should not be overlooked. Some prebiotics and probiotics are doing their part. Administration of *Lactobacillus reuteri* increased insulin secretion by promoting incretin release in obese glucose-tolerant subjects (Simon et al. 2015) and effectively reduces dietary fat absorption (Chung et al. 2016). Administration of *Christensenella minuta* altered the microbial ecology and protected mice from obesity (Goodrich et al. 2014). Despite promising

results in mouse experiments, it is not clear how these microbes may affect metabolism in humans, and the mechanism of action is yet to be determined.

Conclusions

Recently the presence of LPS in the plasma has been linked to obesity-associated metabolic disorders, increasing the scientific interest for this molecule. LPS originates primarily from the gut microbiota. In the host, to avoid the presence of LPS at the visceral level, multiple mechanisms limit the close contact of LPS or LPS-bearing bacteria with IECs or reduce LPS toxicity by modifying its structural components, limiting further interactions with TLR4. Understanding these multiple mechanisms is crucial to the design of efficient and targeted solutions for the prevention and cure of LPS-associated diseases especially obesity and metabolic related disorders. To investigate whether the microbiota is altered before the onset of disease, prospective studies are required, which may further indicate that the microbiota contributes to rather than reflecting metabolic disease. These results may provide the basis for human intervention studies. However, given the genetic and microbial diversity in the human population and the complex microbiota–diet interactions, individualized treatment strategies are likely to be required. In particular, the selection of specific gut bacterial strains and the enhancement of the gut microbial ecology represents a promising therapeutic approach to control energy intake and reduce the prevalence of obesity and the metabolic syndrome.

The content of the review is original, and it has not been published or accepted for publication, either in whole or in part, in any form, is not under consideration for publication elsewhere. I disclose any potential sources of conflict of interest. If the paper is accepted, it will not subsequently be published in the same or similar form in any language.

References

- Ali KF, Shukla AP, Aronne LJ (2016) Bupropion-SR plus naltrexone-SR for the treatment of mild-to-moderate obesity. *Expert Rev Clin Pharmacol* 1:27-34
- Armougom F, Henry M, Vialettes B, Raccah D, Raoult D (2009) Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. *PLoS ONE* 4: e7125
- Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ (2000) Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol* 1: 113-118

Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A (2004) The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 101:15718–15723

Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci U S A* 3:979-984 Bevins CL, Salzman NH (2011) Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat Rev Microbiol* 9: 356–368

Bjontorp P (1984) Morphological classifications of obesity. What they tell us, what they don't. *Int. J of Obesity* 5:525-533

Blüher M, Engeli S, Klöting N, Berndt J, Fasshauer M, Bátka S, Pacher P, Schön MR, Jordan J, Stumvoll M (2006) Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 11:3053-3060

Bray GA (2004) Medical consequences of obesity. *J Clin Endocrinol Metab* 89: 2583–2589

Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, Martines D (2007) Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 2: G518-G525

Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R (2007b) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 7:1761-1772

Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat-diet-induced obesity and diabetes in mice. *Diabete* 6:1470-1581

Cani PD, Delzenne NM (2007) Gut microflora as a target for energy and metabolic homeostasis *Curr Opin Clin Nutr Metab Care* 6:729-734

17

Cani PD, Delzenne NM (2009a) Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. *Curr Opin Pharmacol* 6:737-743

Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM (2007a) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxemia. *Diabetologia* 11:2374- 2383

Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM (2009 b) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 8:1091-1103

Cauchi D, Glonti K, Petticrew M, Knai C (2016) Environmental components of childhood obesity prevention interventions: an overview of systematic reviews. *Obes Rev*

Chanoine JP, Hampl S, Jensen C, Boldrin M, Hauptman J (2005) Effect of orlistat on weight and body composition in obese adolescents: a randomized controlled trial. *JAMA* 23:2873-2883

Chiba M, Sanada Y, Kawano S, Murofushi M, Okada I, Yoshizawa Y, Gomi A, Yatsuzuka M, Toki A, Hirai Y (2007) Glicentin inhibits internalization of enteric bacteria by cultured INT-407 enterocytes. *Pediatr Surg Int* 6:551-554

Chung HJ, Yu JG, Lee IA, Liu MJ, Shen YF, Sharma SP, Jamal MA, Yoo JH, Kim HJ, Hong ST (2016) Intestinal removal of free fatty acids from hosts by Lactobacilli for the treatment of obesity. *FEBS Open Bio* 1:64-76

Cota D, Proulx K, Smith KA, Kozma, SC, Thomas G, Woods SC, Seeley RJ (2006) Hypothalamic mTOR signaling regulates food intake. *Science* 312:927-930

de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE (2010) Propensity to high- fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am J Physiol Gastrointest Liver Physiol* 2:G440-G448

DeLeve LD, Wang X, Kanel GC, Atkinson RD, McCuskey RS (2008) Prevention of hepatic fibrosis in a murine model of metabolic syndrome with nonalcoholic steatohepatitis. *Am J Pathol* 4:993-1001

Delzenne NM, Neyrinck AM, Cani PD (2013) Gut microbiota and metabolic disorders: How prebiotic can work? *Br J Nutr* 109 Suppl 2:S81-S85

Di Marzo V, Bisogno T, De Petrocellis L, Melck D, Orlando P, Wagner JA, Kunos G (1999) Biosynthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in circulating and tumoral macrophages. *Eur J Biochem* 1:258-267

Domecq JP, Prutsky G, Leppin A, Sonbol MB, Altayar O, Undavalli C, Wang Z, Elraiayah T, Brito JP, Mauck KF, Lababidi MH, Prokop LJ, Asi N, Wei J, Fidahussein S, Montori VM, Murad MH (2015) Clinical review: Drugs commonly associated with weight change: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2:363-370

Dubé PE, Brubaker PL (2007) Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. *Am J Physiol Endocrinol Metab* 2:E460-465

Duparc T, Plovier H, Marrachelli VG, Van Hul M, Essaghir A, Ståhlman M, Matamoros S, Geurts L, Pardo-Tendero MM, Druart C, Delzenne NM, Demoulin JB, van der Merwe SW, van Pelt J, Bäckhed F, Monleon D, Everard A, Cani PD (2016) Hepatocyte MyD88 affects bile acids, gut microbiota and metabolome contributing to regulate glucose and lipid metabolism. *Gut* 5:pii

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005). Diversity of the human intestinal microbial flora. *Science* 308:1635-1638

Engeli S, Böhnke J, Feldpausch M, Gorzelnik K, Janke J, Bátka S, Pacher P, Harvey-White J, Luft FC, Sharma AM, Jordan J (2005) Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* 54:2838-2843

Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD (2013) Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* 110: 9066-9071

Fong TM, Heymsfield SB (2009) Cannabinoid-1 receptor inverse agonists: current understanding of mechanism of action and unanswered questions. *Int J Obes* 33:947-955

Gary-Bobo M, Elachouri G, Gallas JF, Janiak P, Marini P, Ravinet-Trillou C, Chabbert M, Cruccioli N, Pfersdorff C, Roque C, Arnone M, Croci T, Soubrié P, Oury-Donat F, Maffrand JP, Scatton B, Lacheretz F, Le Fur G, Herbert JM, Bensaid M (2007) Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese Zucker fa/fa rats. *Hepatology* 46:122-129

Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT, Spector TD, Clark AG, Ley RE (2014) Human genetics shape the gut microbiome. *Cell* 4:789-799

Guo S, Al-Sadi R, Said HM, Ma TY (2013) Lipopolysaccharide causes an increase in intestinal tight junction permeability in vitro and in vivo by inducing enterocyte membrane expression and localization of TLR-4 and CD14. *Am J Pathol* 182: 375–387

Gray DS, Fujioka K (1991) Use of relative weight and Body Mass Index for the determination of adiposity. *J Clin Epidemiol* 44:545-550

He L, Sabet A, DjedjosS, et al (2009) Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell* 137:635-646

Heal DJ, Aspley S, Prow MR, Jackson HC, Martin KF, Cheetham SC (1998) Sibutramine: a Novel Anti-obesity Drug. A Review of the Pharmacological Evidence to Differentiate It from D-

amphetamine and D-fenfluramine. International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity 22:S18-S28

Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, Knight R, Ahima RS, Bushman F, Wu GD (2009) High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 137:1716-1724

Hoareau L, Buyse M, Festy F, Ravanan P, Gonthier MP, Matias I, Petrosino S, Tallet F, d'Hellencourt CL, Cesari M, Di Marzo V, Roche R (2009) Anti-inflammatory effect of palmitoylethanolamide on human adipocytes. *Obesity* 17:431-438

Inukai T (2013) Symptomatic obesity classification, pathogenesis, diagnosis and therapy. *Nihon Rinsho* 2:291-296

Jeppesen PB, Hartmann B, Thulesen J, Graff J, Lohmann J, Hansen BS, Tofteng F, Poulsen SS, Madsen JL, Holst JJ, Mortensen PB (2001) Glucagon-like peptide 2 improves nutrient absorption and nutritional status in short-bowel patients with no colon. *Gastroenterology* 120:806-815

Kovatcheva-Datchary P, Nilsson A, Akrami R, Lee YS, De Vadder F, Arora T, Hallen A, Martens E, Björck I, Bäckhed F (2015) Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella. *Cell Metab* 6:971-982

Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. *Nature* 444:1022-1023

Lien E, Sellati TJ, Yoshimura A, Flo TH, Rawadi G, Finberg RW, Carroll JD, Espevik T, Ingalls RR, Radolf JD, Golenbock DT (1999) Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J Biol Chem* 47:33419-33425

Lin Y, Lee H, Berg AH, Lisanti MP, Shapiro L, Scherer PE (2000) The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. *J Biol Chem* 32:24255-24263

Liu J, Batkai S, Pacher P, Harvey-White J, Wagner JA, Cravatt BF, Gao B, Kunos G (2003) Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-kappaB independently of platelet-activating factor. *J Biol Chem* 45:45034-45039

Lu J, Xi G, Jia W, Jia W (2013) Insulin resistance and the metabolism of branched-chain amino acids. *Front. Med* 7:53-59

Luoto R, Laitinen K, Nermes M, Isolauri E (2011) Impact of maternal probiotic-supplemented dietary counseling during pregnancy on colostrum adiponectin concentration: a prospective, randomized, placebo-controlled study. *Early Hum Dev* 88:339-344

Mhurchu CN, Poppitt SD, McGill AT, Leahy FE, Bennett DA, Lin RB, Ormrod D, Ward L, Strik C, Rodgers A (2004) The effect of the dietary supplement, chitosan, on body weight: a randomised controlled trial in 250 overweight and obese adults. *Int J Obes Relat Metab Disord* 28:1149-1156

Muccioli GG, Naslain D, Bäckhed F, Reigstad CS, Lambert DM, Delzenne NM, Cani PD (2010) The endocannabinoid system links gut microbiota to adipogenesis. *Mol Syst Biol* 6:392

Murphy EF, Cotter PD, Healy S, Marques TM, O'Sullivan O, Fouhy F, Clarke SF, O'Toole PW, Quigley EM, Stanton C, Ross PR, O'Doherty RM, Shanahan (2010) Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut* 59:1635–1642

Nilsen N, Nonstad U, Khan N, Knetter CF, Akira S, Sundan A, Espevik T, Lien E (2004) Lipopolysaccharide and double-stranded RNA up-regulate toll-like receptor 2 independently of myeloid differentiation factor 88. *J Biol Chem* 38:39727-39735

Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátka S, Harvey-White J, Mackie K, Offertáler L, Wang L, Kunos G (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 5:1298- 1305.

Pereira SS, Alvarez-Leite JI. (2014) Low-Grade Inflammation, Obesity, and Diabetes. *Curr Obes Rep* 4:422-431

Pertwee RG (2006) The pharmacology of cannabinoid receptors and their ligands: an overview. *Int J Obes* 30 Suppl 1: S13–18

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J; MetaHIT Consortium, Bork P, Ehrlich SD, Wang J (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 4:59-65

Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 341:1241214

Robertson C, Archibald D, Avenell A, Douglas F, Hoddinott P, van Teijlingen E, Boyers D, Stewart F, Boachie C, Fioratou E, Wilkins D, Street T, Carroll P, Fowler C (2014) Systematic

reviews of and integrated report on the quantitative, qualitative and economic evidence base for the management of obesity in men. *Health Technol Assess* 3:1-424

Nishimoto S, Fukuda D, Higashikuni Y, Tanaka K, Hirata Y, Murata C, Kim-Kaneyama JR, Sato F, Bando M, Yagi S, Soeki T, Hayashi T, Imoto I, Sakaue H, Shimabukuro M, Sata M (2016) Obesity induced DNA released from adipocytes stimulates chronic adipose tissue inflammation and insulin resistance. *Sci Adv* 3:e1501332

Sáez-Lara MJ, Robles-Sánchez C, Ruiz-Ojeda FJ, Plaza-Díaz J, Gil A (2016) Effects of Probiotics and Synbiotics on Obesity, Insulin Resistance Syndrome, Type 2 Diabetes and Non-Alcoholic Fatty Liver Disease: A Review of Human Clinical Trials. *Int J Mol Sci* 13:17

Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 11:3015-3025

Simon MC, Strassburger K, Nowotny B, Kolb H, Nowotny P, Burkart V, Zivehe F, Hwang JH, Stehle P, Pacini G, Hartmann B, Holst JJ, MacKenzie C, Bindels LB, Martinez I, Walter J, Henrich B, Schloot NC, Roden M (2015) Intake of *Lactobacillus reuteri* improves incretin and insulin secretion in glucose-tolerant humans: a proof of concept. *Diabetes Care* 10:1827-1834

Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, Kotani H, Yamaoka S, Miyake K, Aoe S, Kamei Y, Ogawa Y (2007) Role of the Toll-like receptor 4/NF-κB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler Thromb Vasc Biol* 1:84-91

Sweeting HN (2007) Measurement and Definitions of Obesity In Childhood and Adolescence: A field guide for the uninitiated. *Nutr J* 6: 32

Tanti JF, Ceppo F, Jager J, Berthou F (2012) Implication of inflammatory signaling pathways in obesity-induced insulin resistance. *Front Endocrinol* 3:181

Torres-Leal FL, Fonseca-Alaniz MH, Teodoro GF, de Capitani MD, Vianna D, Pantaleão LC, Matos-Neto EM, Rogero MM, Donato J, Tirapegui J (2011) Leucine supplementation improves adiponectin and total cholesterol concentrations despite the lack of changes in adiposity or glucose homeostasis in rats previously exposed to a high-fat diet. *Nutr Metab* 8:62

Turnbaugh PJ, Backhed F, Fulton L, Gordon JI (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 3:213–223

Uguz F, Sahingoz M, Gungor B, Aksoy F, Askin R. (2015) Weight gain and associated factors in patients using newer antidepressant drugs. *Gen Hosp Psychiatry* 1:46-48

Verges B, Duvillard L, Lagrost L, Vachoux C, Garret C, Bouyer K, et al. (2014) Changes in lipoprotein kinetics associated with type 2 diabetes affect the distribution of lipopolysaccharides among lipoproteins. *J Clin Endocrinol Metab* 99: E1245-E1253

Voreades N, Kozil A, Weir TL (2014) Diet and the development of the human intestinal microbiome. *Front Microbiol* 5:494

Yuntao B and Qinghua S (2015) Macrophage recruitment in obese adipose tissue. *Obes Rev* 2: 127-136

Zhang W, Hartmann R, Tun HM, Elson CO, Khafipour E, Garvey WT (2016) Deletion of the Toll-Like Receptor 5 Gene Per Se Does Not Determine the Gut Microbiome Profile That Induces Metabolic Syndrome: Environment Trumps Genotype. *PLoS One* 3:e0150943

Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, Parameswaran P, Crowell MD, Wing R, Rittmann BE, Krajmalnik-Brown R (2009) Human gut microbiota in obesity and after gastric bypass. *PNAS* 106:2365-2370