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Abstract

Obesity-related disorders derive from a combination of genetic susceptibility and environmental factors. Recent evidence supports the role of gut microbiota in the pathogenesis of obesity, type 2 diabetes and insulin resistance by increasing energy harvest from diet and by inducing chronic, low-grade inflammation, as emerged by animal models and human data provided mechanistic insight into the interactions between gut microbiota and their host. Several studies describe characteristic differences between composition and activity of gut microbiota of lean individuals and those with obesity. Despite this evidence, some pathophysiological mechanisms remain to be clarified.

We will discuss mechanisms connecting gut microbiota to obesity and fat storage, and the potential therapeutic role of probiotics and prebiotics.

Key-words: microbiota, endotoxin, obesity, probiotics, prebiotics.

Introduction

Obesity-related disorders are related to energy homeostasis and inflammation; gut microbiota are involved in several host metabolic functions and may play an important role in this context through several mechanisms: increased energy harvest from the diet, regulation of host metabolism and modulation of inflammation.

Human gut flora comprises at least 10^{14} bacteria belonging to 3 bacterial phyla: the gram positive Firmicutes and Actinobacteria and the gram negative Bacteroidetes. Firmicutes is the largest bacterial phylum and comprises over 200 genera, including Lactobacillus, Mycoplasma, Bacillus and Clostridium species[1].

Although each subject has a specific gut microbiota, a core human gut microbiome is shared among family members despite a different environment[2]; nevertheless, the microbiome dynamically changes in response to some factors, including dietary nutrients, illness and antibiotic use.

In this review, we will discuss the interaction of gut microbiota with host metabolism and the impact of manipulating microbiota composition on the pathogenesis and the treatment of obesity.

Association between gut microbiota and obesity: pathophysiological mechanisms.

Several data suggested that gut microflora plays a role in the regulation of host energy homeostasis.

Animal models suggest obesity is associated with alteration of gut microbiota: germ-free mice have less total body fat than conventionally raised mice. The colonization of germ-free mice with a normal microbiota (composed mainly of Bacteroides and Clostridium genera) results in an increase in total body fat, hepatic triglycerides, fasting plasma glucose and IR, despite a reduced food intake[3]. Similarly, conventionalization of germ-free mice with flora from obese donors induced a greater increase in total body fat than colonization with microbiota from lean mice[4].

.Moreover, germ-free mice were protected against the Western diet-induced insulin resistance and gained less body weight and fat mass than conventionalized mice[5].

Genetically obese leptin-deficient ob/ob mice harbour a significantly higher percentage of Firmicutes and a 50% lover percentage of Bacteroidetes, compared with their wild-type littermates fed the same polysaccharide-rich diet[6]. Consistently, in the high-fat/high-sugar Western diet mice, a model of dietary obesity, the development of obesity was associated with an enrichment in Firmicutes at the expense of the Bacteriodetes, as compared with mice receiving a low-fat/high polysaccharide diet[7]. Metagenomic analysis of the obese microbiome showed a depletion of genes involved in motility and an enrichment in genes enabling the capacity of extract energy from the diet, including glycoside hydrolases, phosphotransferases, β-fructosidase and in other transport proteins and fermentation enzymes further processing breakdown products.

Although Bifidobacterium is not a predominating phylum in the gut, it seems to play an important role in host metabolism. In mice, a high-fat diet led to a reduction in Bifidobacterium , associated with increased fat mass, insulin resistance and inflammatory activity[8].

Gut microbiota is connected to metabolic disorders also through the modulation of the innate immune system. Mice genetically deficient in Toll-like receptor 5 (TLR5), a component of innate immune system in the gut, developed hallmark features of metabolic syndrome, including hyperlipidemia, hypertension, insulin resistance and increased adiposity, associated with changes in the composition of the gut microbiota; Transplantation of microbiota from TLR5-deficient mice to wild-type germ-free mice conferred many features of metabolic syndrome to the recipients [9].

Mechanisms connecting gut microbiota to obesity-related disorders are described in Table 1.

Increased energy harvest from the diet

Nutrient absorption and gut motility can be modulated by short chain fatty acids (SCFAs), the major end-products of bacterial fermentation. SCFAs (propionate, acetate, butyrate) represent more than 60% of energy content of carbohydrates from the diet[10] and are ligands for Gpr41 and Gpr43, two G protein-coupled receptors that induce intestinal secretion of peptide YY (PYY) and leptin.

Gpr41 functional deletion was related with a reduction in PYY expression, a faster intestinal transit rate and a reduction of energy uptake from the diet[11]. Consistently, Grp43-deficient mice showed lower total body fat and improved insulin sensitivity; moreover, GPR43 inhibition was associated with higher energy expenditure accompanied by higher core body temperature and increased food intake[12].
Collectively, these findings disclose the pivotal role for Gpr41 and Gpr43 in mediating microbiota regulation of energy harvest from the diet.

Regulation of host energy storage

In conventionalized mice, microbiota promotes absorption of monosaccharides from the gut lumen[5]. Increased carbohydrate availability promotes de novo lipogenesis in the liver and the adipose tissue by stimulating carbohydrate response element binding protein (ChREBP)- and sterol response element binding protein 1 (SREBP-1)-mediated transcription of genes encoding two rate-limiting lipogenetic enzymes: acetyl-CoA carboxylase (Acc)-1and fatty acid synthase (Fas)[13]. This mechanism leads to an accumulation of triglycerides in the liver and in adipose tissue.
Fasting-induced adipose factor (Fiaf), also named angiopoietin-like protein 4, is an inhibitor of adipose lipoprotein lipase (LPL) produced by enterocytes, hepatocytes, skeletal myocytes and adipocytes in response to fasting, peroxisome proliferator-activated receptor (PPAR)-γ activation and inflammatory prostaglandins PGD2 and PGJ2[14]. Fiaf also modulates fatty acid oxidation in skeletal muscle and in adipocytes, increasing the nuclear transcription factor peroxisomal proliferator-activated receptor coactivator (PGC)-1α, a coactivator of genes encoding key enzymes involved in mitochondrial fatty acid oxidation[15].
Gut microbiota affect storage of circulating triglycerides into adipocytes by regulating intestinal secretion of Fiaf: conventionalization of germ-free mice suppressed intestinal expression of Fiaf in differentiated villous epithelial cells in the ileum; consistently, germ-free Fiaf-KO mice fed a high-fat, high-carbohydrate diet were not protected against diet-induced obesity[5]. Specific microbiota has different effects on expression of Fiaf: mice fed a high-fat diet supplemented with Lactobacillus paracasei showed increased levels of Fiaf

and displayed significantly less body fat and reduced triglyceride levels. In co-culture experiments, Lactobacillus also induced Fiaf gene expression[16]. These data suggest that modulation of Fiaf through manipulating gut flora could be an important therapeutic target.

Microbiota may regulate the fatty acid metabolism also by affecting adenosine monophosphate (AMP)-activated protein kinase (AMPK) activation. AMPK stimulates fatty acid oxidative pathways in the liver and the skeletal muscle through activation of mitochondrial enzymes such as acetylCoA-carboxylase and carnitinepalmitoyltransferase I, and reduces hepatic glycogen-synthase activity and glycogen stores, improving hepatic and muscle insulin sensitivity[17].

Gut flora may have an inhibitory effect on AMPK-regulated fatty acid oxidation, as germ-free mice present a persistent activation of hepatic and muscle AMPK, while AMPK activity and related metabolic pathways were suppressed in conventionalized mice[5].

Regulation of chronic low-grade endotoxinemia and host inflammatory response

Chronic activation of the immune system is linked to the development of obesity and type 2 diabetes; toll-like receptor 4 (TLR4)-activated inflammatory pathway has been specifically connected with the low-grade chronic inflammation which characterizes obesity-related disorders.

Gram-negative microbiota may affect host metabolism through lipopolysaccharide (LPS), which binds the complex of CD14 and TLR4 at the surface of innate immune cells, activating inflammatory pathways implicated in the pathogenesis of obesity, insulin-resistance and type 2 diabetes mellitus[18].

Beside LPS, free fatty acid (FFA) and products from dying cell can bind TLR4 and stimulate inflammatory response in cell expressing TLR4 (gut immune cells, adipocytes, endothelial cells, tissue macrophages, hepatocytes, and hepatic Kupffer and stellate cells). The hepatic Kupffer cells may have an independent role in this contest: in mice, high-fat diet promotes the activation of Kupffer cells, resulting in insulin-resistance and glucose intolerance, while selective depletion of these cells restores hepatic insulin sensitivity and improves whole-body and hepatic fat accumulation, without affecting adipose tissue macrophages[19,20].

Metabolic endotoxiemia is also associated with nonalcoholic steatohepatitis (NASH), through hepatic inflammasome activation: a recent study reported, in mouse model of NASH, a saturated fatty acids up-regulation of the inflammasome that lead to sensitization to LPS-induced inflammasome activation[21]. LPS administration modifies the gut microbiota composition (reduction of Bifidobacteria and Eubacteria spp) and determinates metabolic effects, such as systemic insulin-resistance, increased plasma and hepatic triglyceride content and in reduction of HDL levels [22][23]; mice fed a high-fat diet shown the same change in microbiota, associated with a low grade elevation in circulating LPS levels (metabolic endotoxemia)[22]. Consistently, LPS receptor deletion or changes of gut microbiota composition induced by antibiotic administration prevented the metabolic alteration of a high-fat diet[22].

Modification in gut microbiota composition results in change of metabolic endotoxiemia level: prebiotic fermentable oligofructose administration increased the intestinal proportion of Lactobacilli and Bifidobacteria in ob/ob mice, restored normal intestinal permeability through stimulation of epithelial tight-junction proteins, and reduced systemic endotoxiemia, in association with enhanced intestinal GLP-2 levels[24].

Gut microbiota modulates the gut-derived peptide secretion, promoting L-cell differentiation in the proximal colon of rats and increasing glucagon-like peptide 1 (GLP-1) secretion in response to a meal in healthy humans[2]; deletion of GLP-1 abolished the beneficial effects of prebiotics on weight gain, glucose metabolism and inflammatory pathway activation [25]. Furthermore, gut microbiota may modulate gut barrier integrity and endotoxinemia through glucagon-like peptide-2 (GLP-2), a 33-amino acid peptide with known intestinotrophic properties, which is cosecreted with GLP-1 by enteroendocrine L cells.

Ob/ob mice treated with prebiotic + carbohydrates diet presented an increased circulating GLP-1 and GLP-2, which were associated with an altered gut flora composition(increased proportion of Lactobacilli and Bifidobacteria), restored tight junction integrity and intestinal barrier function and lowered endotoxinemia[24]. Administration of a GLP-2 antagonist prevented these effects, which were mimicked by the administration of a GLP-2 agonist, suggesting that GLP-2 could mediate the effects of prebiotics[24].

Microbiota such Bifidobacterium and Lactobacillus may exert an anti-inflammatory effect through the synthesis of bioactive isomers of conjugated linoleic acid (CLA), which show anti-diabetic, anti-atherosclerotic, hypocholesterolemic, hypotriglyceridemic and immunomodulatory activity[26, 27].

In different mammalian models, dietary supplementation of linoleic acid + Bifidobacterium breve altered the profile of polyunsaturated fatty acid composition, resulting in higher intestinal, hepatic and adipose tissue content of c9,t11 CLA; the animals also present a higher adipose tissue concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two omega-3 polynsatured fatty acids with anti-inflammatory and lipid-lowering properties[28] . These changes were associated with a reduced expression of pro-inflammatory cytokines, such as TNF-α, interleukin-6 interleukin-1β and interleukin-8, accompanied with a higher anti-inflammatory interleukin-10 secretion.

Finally, also SCFAs elevation could result in a reduction of the inflammation and an improvement of insulin sensitivity. Butyrate shows anti-inflammatory properties which could improve epithelial permeability[29]. Acetate raised plasma PYY and GLP-1 and suppressed pro-inflammatory cytokines[30].

Collectively, these data suggest that endotoxinemia is involved in the pathogenesis of obesity-related diseases, is affected by dietary nutrient composition, and may be modulated by manipulation of gut microbiota composition.

The role of vitamin D

Vitamin D deficiency has been associated with allergic diseases development and increased body mass index[31] .

Vitamin D plays a role in immunomodulation and a decreased vitamin D uptake has been correlated with a change in fecal microbiota composition in one study[32], though this association needs to be confirmed in larger cohorts.

Mice lacking the vitamin D receptor (VDR) present chronic, low-grade inflammation in the gastrointestinal

tract[33] and the absence of the VDR results in enhanced inflammation in response to normally nonpathogenic bacterial flora[34]. Moreover, intestinal VDR has also been shown to negatively regulate bacterial-induced intestinal NF-κB activation and to attenuate response to infection, suggesting that the vitamin D may affect the impact of intestinal flora on inflammatory disorders[35].

The role of gut microbiota in human obesity

Obese humans show an increase in Firmicutes/Bacteroidetes ratio; dietary- or surgically-induced weight loss results in a reduction in this ratio, with a proportion of bacteroidetes and firmicutes similar to that found in lean humans, irrespective of the type of diet (fat or carbohydrate restricted[36, 37, 38, 39, 40] .

A metagenomic analysis of 154 individuals, including monozygotic and dizygotic twins concordant for leanness or obesity, and their mothers, also showed that obesity was associated with a relative depletion of Bacteroidetes and a higher proportion of Actinobacteria compared with leanness[2]. Consistently, one prospective study found that children with lower proportion of Bifidobacterium and higher levels of Staphylococus aureus in their infancy gained significantly more weight at seven years[41].

The aforementioned changes in gut microbiota composition in human obesity were not uniformly found by different investigators. Some Authors reported no differences or even lower ratios of Firmicutes to Bacteroidetes in obese human adults compared with lean controls, however, significant diet dependent reductions in a group of butyrate-producing Firmicutes were found[38, 42] . Arumugam et al. investigated the phylogenetic composition of 39 faecal samples from individuals representing 6 nationalities. They characterized three clusters of individual microbiotal composition referred to as enterotypes that were not nation- or continent-specific. They identified three marker molecules that correlate strongly with the host's body mass index, two of which are ATPase complexes, supporting the link found between energy harvest and obesity in the host and suggesting the importance of metagenomic-derived functional biomarkers over phylogenetic ones[43].

Changes in energy harvesting from diet is also associated with the uptake of SCFAs, end-products of bacterial fermentation: in obese humans, the amount of SCFAs in faecal samples was greater than in lean subjects[42], although the diets rich in nondigestible fibres decrease body weight and severity of diabetes [44]; these contradictory findings could be explained by the anti-inflammatory effects of butyrate.

Furthermore, another pathway has been better studied in humans: the linkage between microbiota and systemic inflammation. LPS administration induces acute inflammation and systemic insulin resistance, stimulating the systemic and adipose tissue expression of pro-inflammatory and insulin resistance-inducing cytokines [45].

Consistently in healthy human subjects, total energy intake and high-fat/high-carbohydrate meal, but not fruit/fibre meal, can acutely increased plasma LPS levels, coupled with enhanced TLR4 expression[22, 46.]

In summary, the different pathophysiological factors that explain the association of microbiota with metabolic disturbances have not been studied in depth in human in comparison with animal models, although growing evidences link gut microbiota with endotoxemia and energy harvest from diet.

Therapeutic targets

The mechanisms connecting gut microbiota to obesity could have relevant implications for treatment.

Probiotics

Probiotics are food-supplements that contain living bacteria such as Bifidobacteria, Lactobacilli, Streptococci and non-pathogenic strains of E. Coli. When administered, they confer beneficial effects to the host because of changes in the gut microbiota that are transient and diminish gradually with time after cessation [47]. Different studies suggest that probiotics influence the intestinal lumen rather than the gut-epithelium, possibly explaining the transient effect of probiotics [48 49]. This thesis was tested by Goossens et al: they compared the effects of consuming Lactobacillus Plantarum on the microbial colonization of faeces and biopsies from the ascending colon and rectum[48]. Within faecal samples, the amount of Lactobacilli was

significantly increased. However, the biopsies did not confirm a growth of Lactobacilli. Recently van Baarlen et al described changes in the expression of up to thousands of genes in duodenal biopsies after administration of three types of Lactobacilli[50]. Alterations in the gut microbiota as a result of probiotics are commonly observed but evidence showing that probiotica administration directly affects inflammatory state has only recently been demonstrated in humans [5152]. In contrast, studies on the effects of probiotics on characteristics of T2DM are mostly performed in animal models, reporting beneficial effects by various strains of Lactobacilli on characteristics of T2DM[47]. Both anti-diabetic and anti-inflammatory effects of Lactobacillus casei in diet-induced obese mice were recently described[53]. In addition diet-induced obese mice showed a reduction in body weight gain after they were supplemented with Lactobacillus Rhamnosus PL60 plus an adequate diet [54]. In the same way, Kang et al studied the effects of Lactobacillus gasseri BNR17 on diet-induced overweight rats; they found that the percent increase in body weight and fat pad mass was significantly lower in the BNR17 group[55]. Although these animal findings are interesting, the relevance of lactobacilli supplementation for the control of adiposity is a matter of debate. In order to clarify the effect of Lactobacillus-containing probiotics on weight, Million et al performed a meta-analysis of clinical studies and experimental models [56]. They included 17 RCTs in humans, 51 studies on farm animals and 14 experimental models and they concluded that Different Lactobacillus species are associated different effects on weight change that are host-specific. In particular Lactobacillus fermentum and Lactobacillus ingluviei were associated with weight gain in animals; Lactobacillus plantarum was associated with weight loss in animals and Lactobacillus gasseri was associated with weight loss both in obese humans and in animals.

Prebiotics

Prebiotics (mostly oligosaccharides) are non-digestible but fermentable food ingredients that selectively stimulate the growth or activity of one or multiple gut microbes that are beneficial to their human hosts[47]. The beneficial metabolic effects of prebiotics are in part mediated by a reduction in metabolic endotoxiemia. In a physiological situation, Bifidobacteria are capable of lowering LPS levels[57 58].

Interestingly, the number of Bifidobacteria was inversely correlated with the development of fat mass, glucose intolerance and LPS level[57]. High-fat diets promote the growth of LPS-producing gut microbiota and subsequently restrict the amount of Bifidobacteria. Bifidobacterium spp and Lactobacillus spp. are extremely sensitive to the administration of certain prebiotics[59]. Prebiotics containing oligofructose (OFS) specifically stimulate the growth of these intestinal bacteria[60], [61]. OFS administration completely restored Bifidobacteria spp and normalized plasma endotoxin levels, leading to improved glucose tolerance, increased satiety and weight loss in human subjects[8, 62, 63]. Besides modulating endotoxemia, OFS can alter metabolism in various other manners. Cani et al. showed that effects of OFS were mediated via a GLP1-dependent pathway[64]. High-fat-fed diabetic mice on OFS treatment exerted improved glucose tolerance, diminished body weight and a decrease in endogenous glucose production. Either adding the GLP-1 receptor antagonist exendin 9-39 (Ex-9) or using GLP-1 knockout mice resulted in a complete lack of the OFS-mediated beneficial effects, thus showing the causal role of GLP-1 in this pathway in animals. Attempts to translate these findings to human subjects are ambiguous, showing that OFS tends to dose-dependently decrease energy intake and increase PYY plasma concentrations[63, 65], but reported effects on satiety are conflicting[44]. Everald et al found that in ob/ob mice, prebiotic feeding decreased Firmicutes and increased Bacteroidetes phyla, and improved glucose tolerance, increased L-cell number and associated parameters (intestinal proglucagon mRNA expression and plasma glucagon-like peptide-1 levels), and reduced fat-mass development, oxidative stress, and low-grade inflammation. In high fat–fed mice, prebiotic treatment improved leptin sensitivity as well as metabolic parameters[66]. Furthermore, OFS fermentation directly affects SFCA butyrate synthesis from extracellular acetate and lactate, implicating the therapeutic potential of prebiotics[67]. In addition, insulin-type fructance also decreased the activity of the endocannabinoid system (by reducing the expression of cannabinoid receptor 1, restoring the expression of anandamide-degrading enzyme and decreasing anandamide levels in the intestinal and adipose tissues), a phenomenon that contributes to an improvement barrier function of the gut and adipogenesis [68]. Finally insulin-type fructan prebiotics counteract the overespression of GPR43 in the adipose tissue, which is related to a decrease rate of differentiation and a reduce adipocyte size [69].Thus, available evidence supports

the hypothesis that prebiotics can influence metabolic disturbances. The beneficial effect on clinical end-points in metabolic disturbances remains to be demonstrated in large prospective randomized controlled trials.

Conclusion

Increased consumption of foods with high energy is involved obesity development, which is a well known risk factors for T2DM and cardiovascular disease.

Several studies demonstrated gut microbiota can modulate host energy homeostasis and adiposity, through different mechanisms: energy harvest from diet, fat storage and expenditure, incretins secretion and systemic inflammation.

Although experimental data suggested gut microbiota manipulation with probiotics and prebiotics can beneficially affect host adiposity and glucose metabolism, their effects are transient and diminish gradually after cessation . In this review we analyzed the potential gut microbiota-driven pathways that could represent novel target for treatment of obesity.

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Table I. Gut microbiota modulation of host energy homeostasis: mechanisms

MECHANISMS	MEDIATORS	METABOLIC EFFECTS
Reduced intestinal transit rate	Production of SCFA , that increase Gpr41-/Gpr43-mediated PYY secretion	**Increased energy harvest from the diet**
Polysaccharide degradation to monosaccharides	Microbial transport proteins and enzymes	**Increased CHO absobtion and portal flow**
Increased glucose absorption	Increased intestinal Glut1 expression	
Increased monosaccharides portal low	Increased capillaries density in intestinal villi	
Increased de novo lipogenesis	ChREBP and SREBP-1 mediated expression of lipogenic enzyme	**Increased hepatic/adipose Tg contents**
Increased adipociyte uptake of circulating FFA	Increased adipose LPL activity through reduction of intestinal Fiaf secretion	
Reduced FFA oxidation	Reduced Fiaf-induced (PGC)-1α and AMPK-induced expression of mitochondrial FFA oxidative enzymes	**Reduced hepatic/muscle FFA oxidation**
Regulation of GLP-2 secretion	Modulation of intestinal L-cell activity	**Modulation of intestinal barrier function**
LPS production	LPS-TLR4-mediated induction of hepatic/adipose/macrophagical pro-inflammatory cytokines SOCS-1, SOCS-3, IL-6, TNF-α, MCP-1	**Modulation of systemic/ hepatic/adipose inflammation**
Modulation of gut barrier integrity	Stimulation of L-cell differentiation and GLP-2 secretion	
Regulation of hepatic/adipose fatty acid composition	Increased linoleic acid conversion to c9,t11 CLA , increased hepatic and adipose contents of DHA and EPA	**Modulation of tissue composition of fatty acid**