Linking Gut Microbiota and Inflammation to Obesity and Insulin Resistance

Obesity and insulin resistance are the major predisposing factors to comorbidities, such as Type 2 diabetes, nonalcoholic fatty liver disease, cardiovascular and neurodegenerative diseases, and several types of cancer. The prevalence of obesity is still increasing worldwide and now affects a large number of individuals. Here, we review the role of the gut microbiota in the pathophysiology of insulin resistance/obesity. The human intestine is colonized by ~100 trillion bacteria, which constitute the gut microbiota. Studies have shown that lean and overweight rodents and humans may present differences in the composition of their intestinal flora. Over the past 10 years, data from different sources have established a causal link between the intestinal microbiota and obesity/insulin resistance. It is important to emphasize that diet-induced obesity promotes insulin resistance by mechanisms independent and dependent on gut microbiota. In this review, we present several mechanisms that contribute to explaining the link between intestinal flora and insulin resistance/obesity. The LPS from intestinal flora bacteria can induce a chronic subclinical inflammatory process and obesity, leading to insulin resistance through activation of TLR4. The reduction in circulating SCFA may also have an essential role in the installation of reduced insulin sensitivity and obesity. Other mechanisms include effects of bile acids, branched-chain amino acids (BCAA), and some other lesser-known factors. In the near future, this area should open new therapeutic avenues for obesity/insulin resistance and its comorbidities.

Over a trillion microorganisms influence the functioning of the human body. For every human cell, there are an estimated 10 micro-organismal cells colonizing the whole surface of the human body in contact with the external environment (59, 99). The greatest concentration of microorganisms is found in the gastrointestinal tract, and they consist mostly of bacteria. Bacterial distribution in the gastrointestinal tract varies according to the region, and it is influenced by pH, oxygen, and nutrient availability (106).

The gut microbiota plays an important role in normal intestinal function and maintenance of the health of the host. It produces a large number of enzymes involved in the ability to extract energy from the host’s diet and to deposit energy in fat stores (7, 60, 102a, 119). However, this is dependent on a balance between potentially pathogenic bacteria and numerous nonpathogenic microorganisms that promote health (47). The commensal bacteria in the gut can provide the benefits of an effective extra organ, extracting energy from cellulose digestion, improving the development and maturation of the intestinal and systemic immune system (116).

Interactions between the human organism and intestinal microbiota start at birth, and from then on, the composition of the gut microbiota undergoes several changes. Children breastfeeding, for example, acquire a high profile of *Bifidobacterium* in their early days. As a consequence, a high amount of acetate and lactate are produced, which restricts the growth of pathogenic bacteria, such as *Escherichia coli* and *Clostridium perfringens* (34, 121). On the other hand, children fed formula have a predominance of *Clostridium* (8, 45). Studies on gut microbiota transplants in which donor and recipient received various types of diets showed that the gut microbiota is easily modified by dietary change (82).
Therefore, the diet is clearly an important factor in regulating the composition of the gut microbiota. However, some studies suggest that the gut microbiota can also change with age. May this be due to changes in the diet of the elderly or a direct effect of aging? Furthermore, different bacteria can perform the same metabolic function, which can render it difficult to identify an ideal microbial profile. On the other hand, it can explain some of the variability found among the composition of the microbiota in similar hosts (118).

Although there are still many questions about the gut microbiota that need to be answered, it is known that the gut microbiota plays different roles in metabolism. The gut microbiota improves energy extraction from the diet, modulates plasma levels of lipopolysaccharide (LPS), which may begin a chronic low-grade inflammation, leading to obesity and Type 2 diabetes (T2DM), and modulates some host genes and proteins that regulate the stock and energy expenditure (10, 116). Therefore, in this article, we will discuss how the composition of the gut microbiota can induce the development of obesity and insulin resistance.

The Obese Microbiome

Metagenomic analyses of lean mice and human volunteers showed that almost all bacteria present in the distal gut and feces belong to two main bacterial phyla, *Bacteroidetes* and *Firmicutes*, and most studies show a predominance of *Bacteroidetes* over *Firmicutes* (61, 117, 119), although this is not uniformly observed (31, 52, 98, 128). However, most of the studies showed that in genetically obese ob/ob mice, in diet-induced obesity (DIO) mice, and in obese humans, this proportion is changed with a great increase in bacteria from the phylum *Firmicutes* (18, 60, 73, 79, 120). This is not the only pattern observed, and some studies have shown the opposite. Obese and overweight subjects and obese mice have reduced prevalence of *Firmicutes* and enhanced prevalence of *Bacteroidetes* (6, 23, 25, 46, 98). Although this issue has not yet been completely addressed, it is possible that the interactions between genetic and environmental factors also exert great influence on the composition of the microbiota in the obese. To better understand these controversies, it is important to mention that the composition of the gut microbiota is not constant and can show changes within an individual. The host genome has a central role in determining the composition of the intestinal microbiota, but many geographic and environmental factors, such as diet, lifestyle, hygiene, and use of medications, can contribute to changes in this microbiota. With these controversies related to phyla in obesity in mind, we believe that the analyses at other taxonomic levels might have a more prominent role in establishing more specific relationships between the microbiota and obesity.

Analyzing the composition of the microbiota in obese mice and humans downstream from phylum to genus, it recently has been demonstrated that the percentage of *Akkermancia muciniphila* is reduced in obesity and that reconstitution with this bacterium can improve insulin action and glucose tolerance (33).

Although most studies have investigated the composition of the microbiota in obese patients or those with metabolic syndrome, the time course of changes in the microbiota has not yet been determined. However, in a late phase, i.e., in patients with T2DM, the same pattern of alterations in the microbiota is also found. By using large metagenome-wide association studies in different populations, Karlsson et al. (52) and Qin et al. (80) showed a decrease in the percentage of *Roseburia* and *Faecalibacterium prauznitzii*, which are butyrate-producing bacteria, in the gut microbiota of patients with T2DM compared with that of healthy subjects. However, these finds are not definitive and should be reproduced in other cohorts. It is important to mention that these alterations in intestinal microbiota composition may also be secondary in T2DM because, in these patients, it is not rare to find altered gastrointestinal motility or bacterial overgrowth.

Microbiota-Induced Fat Gain and Insulin Resistance

Previous data have shown that germ-free mice on a high-fat diet gain less weight than control mice on the same diet and are protected from insulin resistance. The mechanisms that account for this protection are not completely known. It was also shown that AMPK (5’ adenosine monophosphate-activated protein kinase) activity was enhanced in the muscle and liver of germ-free mice. This result contributes to enhanced fatty acid oxidation and energy expenditure, leading to lower body weight gain. Moreover, germ-free mice infected with the gut microbiota content of conventionally raised mice presented an increase in body weight and fat content, associated with insulin resistance and glucose intolerance (7). In addition, transplantation of intestinal microbiota from ob/ob to germ-free mice enhanced the adiposity associated with insulin resistance (120). These data indicate that there is a cause-effect relationship between the microbiota, fat content, and insulin resistance.

All of this information led to different hypotheses to explain the mechanisms by which changes in microbiota composition can induce obesity. The
first hypothesis suggested that the intestinal bacteria of *ob/ob* mice are capable of removing more energy from the diet (35, 127), probably due to enzymes produced by such bacteria that are very efficient in dietary nutrient degradation. Although interesting, this is not confirmed in other animal models of obesity. Most of the mechanisms proposed over the past 10 years may have an important role in the link between the microbiota and insulin sensitivity, and are related to LPS, SCFA, bile acids, and BCAA (FIGURE 1). At this point, we would like to emphasize that, following a gut microbiota transplant, the lean animal that received gut microbiota from an obese mouse developed more severe insulin resistance than would be expected from weight gain alone, suggesting that the mechanisms of insulin resistance are, at least in part, independent of weight gain (18).

Another critical point is that most of the studies converge to demonstrate that the gut microbiota has a causal role and/or can predispose to diet-induced obesity. Recently, Zhao used Koch’s postulates to discuss the causal relationship between the gut microbiota and obesity and concluded by a chain of causation that a cause-effect relationship was very clear (129).

**LPS and Altered Intestinal Barrier in Insulin Resistance**

It is important to point out that the two most prevalent phyla belong to different groups in clinical classification in accordance with Gram staining, i.e., *Firmicutes* are Gram-positive and *Bacteroidetes* are Gram-negative bacteria. Gram-negative bacteria contain LPS (1), a strong activator of toll-like receptor 4 (TLR4), a receptor of the group of toll-like receptors, which is expressed in most cells and macrophages and recognizes pathogen-associated molecular pattern (PAMP). The binding of LPS to TLR4 activates an extensive cell signaling pathway that induces the inflammatory response and cytokine expression and secretion (69) (FIGURE 2).

Data from different sources have shown that circulating levels of LPS are elevated in obese rodents and humans (17, 26, 76). Although this may seem a paradox, because in the microbiota of obesity there is an increase in the percentage of *Firmicutes*, which are Gram positive, it has been shown that this increase in LPS is directly related to increased intestinal permeability. This altered permeability is probably due to reduced expression of zonula occludens-1 (ZO-1), claudin, and occludin, proteins that compose the tight junction, creating a gut

**FIGURE 1. High-fat-diet-induced insulin resistance**

High-fat diet modulates microbiota and induces alteration in intestinal barrier associated with an increase in absorption and circulating levels of LPS and branched-chain amino acid (BCAA) and a reduction in acetate, propionate, and butyrate (SCFA) and secondary bile acids. LPS induce subclinical inflammation, insulin resistance, and an increase in adipose mass. An increase in circulating BCAA is associated with a fivefold increased risk of developing T2DM. A decrease in SCFA affects tight-junction protein expression, contributing to increased intestinal permeability. Secondary bile acids activate glucagon-like peptide-1 (GLP1) secretion, which can protect against insulin resistance. These alterations contribute to install insulin resistance.
epithelial barrier that impedes the bacterial population and products from the intestinal lumen from reaching the circulation. The breakdown of tight-junction function leads to LPS translocation, which may be an early factor in the development of inflammation and insulin resistance in humans and mice (4, 12, 13, 17, 18) (FIGURE 2). In addition to modulation of the intestinal barrier, it has been shown that LPS is transported along with chylomicrons into the circulation, contributing to the explanation of why a high-fat diet can increase the uptake of this lipid (44). As will be discussed below, a protective effect of SCFA on the intestinal barrier is well established, and a reduction in the population of bacteria that produces butyrate may contribute to altered intestinal permeability (122).

One important point that should be emphasized is whether an increase in circulating LPS levels is able to induce, besides subclinical inflammation and insulin resistance, an increase in adipose mass. Previous data showed that the infusion of LPS for 1 mo in control mice on chow diet is able to induce not only inflammation, insulin resistance, and glucose intolerance but also obesity. In this regard, it is important to point out that, in diet-induced insulin resistance, there is a tissuespecific regulation of insulin signaling, with adipose tissue showing an increase in glucose uptake and lipid synthesis, despite insulin resistance in the hypothalamus, muscle, and liver (84). Moreover, previous data showed that the MIRKO mice (knockout of insulin receptor only in muscle) develop mild obesity and metabolic syndrome (55).

**LPS Signaling**

It is interesting that the interaction of LPS with TLR4 is a very specific and high-affinity interaction, and occurs not only in macrophages but in almost all cells of the body. In DIO and other conditions related to altered intestinal permeability, TLR4 is involved in the inflammatory response that culminates in insulin resistance and metabolic derangement, since those responses are attenuated in mice with genetic alterations in this pro-
tein’s activity (15, 23, 56, 70, 76), such as in TLR4 loss-of-function C3H/HeJ mice (115), in CD14−/− mice (16) and in TLR4−/− mice (100) (FIGURE 1). In addition to LPS, fatty acids can activate TLR4, although not directly (32, 96). Recent lines of evidence indicate that a major carrier of FFAs in the circulation, FetA (hepatic protein fetuin-A) (24) acts as a ligand of TLR4 (78). In this regard, it was demonstrated that, in obese individuals and rodents, circulating levels of FetA are increased and correlate with body weight (51, 86) and, more importantly, FetA−/− mice are protected from obesity and insulin resistance induced by aging (67, 68). Taken together, these data suggest that both Fetuin A and TLR4 are essential for FFA-induced insulin resistance. Whether the modulation of Fetuin A in diet-induced obesity is dependent on microbiota modulation deserves further exploration.

In the signaling pathway activated by TLR4, serine kinases (JNK, IKKbeta, and IKKepsilon) have important roles in the induction of insulin resistance through serine phosphorylation of IRS-1 (38, 124). This posttranslational modification of IRS-1 has been considered as an insulin resistance marker (37, 71, 83, 95, 115). In addition to this classical mechanism of insulin resistance, the increase in circulating LPS, via TLR4, leads to increased expression of inducible nitric oxide synthase (iNOS) (107, 110). The increase in iNOS expression induces protein S-nitrosation/S-nitrosoylation, in which NO (nitric oxide) reacts with protein cysteine residues, altering protein function (103, 104). Thus LPS may also impair insulin signaling by the S-nitrosation/S-nitrosoylation of IR, IRS-1, and Akt in insulin-sensitive tissues (22, 77, 101). Very recently, it was demonstrated that S-nitrosation/S-nitrosoylation is an early and central phenomenon in the induction of ER stress, which is also considered an important molecular mechanism of insulin resistance (40). Genetic disruption of iNOS and its pharmacological inhibition attenuates insulin resistance in models of obesity or sepsis (20, 21, 80, 88). Another important mechanism of subclinical inflammation in obese and obese diabetic humans is an increase in TNF-α, IL-6, and MCP1, which activates NF-κB. Hotamisligil and Spiegelman (48) and Folli and collaborators initiated these studies in mice and have demonstrated that these mechanisms are also critically important in humans with obesity and T2DM (28, 72, 114).

**Inactivation of TLR4 in Different Tissues Protects From Insulin Resistance**

Although macrophages express large amounts of TLR4, the expression of this receptor is not restricted to cells of the immune system. Tissues and cells with relevant metabolic roles in insulin action, such as muscle, adipocytes, and hepatocytes, also express TLR (62, 102). In the past years, data from bone marrow transplantation, tissue-specific pharmacological blockade, and tissue-specific knockout mice investigated the role of each of these cells and tissues in the insulin resistance induced by obesity. Most studies that used bone marrow transplants indicated that the absence of TLR4 in bone marrow-derived cells protected from diet-induced insulin resistance.

When liver-specific TLR4 knockout mice were fed a high-fat diet for 16 wk, they presented improved glucose tolerance and insulin sensitivity, associated with an improvement in insulin-induced insulin signaling in liver. It is interesting that these animals also presented less macrophage infiltration in adipose tissue and reduced inflammatory cytokine expression in this tissue.

Our laboratory previously demonstrated that a direct effect of TLR4 mutation on muscle tissue may also have a role in protection against diet-induced insulin resistance. We used isolated muscle from control mice and showed that palmitate was able to induce activation of the TLR4 receptor and downstream kinases, such as IKKβ and JNK, and also reduced insulin signal transduction and glucose uptake. However, this sequence of events was not observed in isolated muscle from C3H/HeJ and TLR4−/− mice, suggesting that a loss of TLR4 function protects muscle from fatty acid-induced insulin resistance (50).

The relationship between TLR4 activation and modulation of lipid metabolism in adipose tissue is not completely understood. Although it is expected that TLR4 activation might increase lipolysis, this might not happen because, in insulin resistance of diet-induced obesity, adipose tissue is usually protected from insulin resistance (84). On the other hand, recent data has shown that TLR4 activation may also induce triglyceride synthesis (50).

In accordance with the important role of the hypothalamus in integrating insulin signaling and glucose metabolism, blocking TLR4 in obese mice specifically in the hypothalamus improves peripheral insulin action. Taken together, these data suggest that the integration of TLR signaling in different tissues and cells may account for the insulin-antagonizing effect of this receptor (123).

**TLRs and Other PAMPS**

Besides TLR4, other TLRs may also have a role in the molecular mechanisms by which changes in the microbiota can induce insulin resistance. Unexpected results observed in two other TLR-deficient mice, TLR2−/− (18) and TLR5−/− (125), reinforce the importance of the intestinal microbiota in the induction of insulin resistance. These two knockout mice, depending on the environ-
ment in which they grow up, develop obesity and insulin resistance on chow diet, a phenomenon related to modulation of the gut microbiota (18, 125). Since TLR2<sup>−/−</sup> in some situations can be genetically protected from insulin resistance, we can hypothesize that changes in the microbiota can overcome some genetic protection, and can by itself induce insulin resistance and contribute to the development of obesity and T2DM.

Other PAMPs and damage-associated molecular patterns (DAMPs), such as the inflammasome, seem to be related to intestinal epithelial integrity. The inflammasome is a complex of proteins that recognizes a wide range of stress signals, bacterial infections, and damage in general. When activated, the inflammasome induces caspase-1 activation, proinflammatory cytokine secretion, and cell death (105). It is important to mention that inflammasome proteins are activated in macrophages by LPS, which can have an important role in insulin resistance (85, 97).

Viral infections also activate pattern recognition receptors (PRRs) and the double-stranded RNA-activated protein kinase receptor (PKR), which trigger an immune response (43, 94). It has been described that PKR is also activated by LPS (14, 49) and that PKR knockout mice are protected from diet-induced obesity and insulin resistance (19). It is tempting to speculate that, besides LPS, some constituents or products of the gut microbiota or some viral products from the intestine can integrate the PKR signaling pathway with metabolic effects on insulin resistance, but this has not yet been investigated.

**Short-Chain Fatty Acids Derived From the Gut Microbiota and Insulin Sensitivity**

Gut microbiota fermentation degrades nondigestible carbohydrates to produce short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate (65), in the cecum and colon (27). It is important to mention that the production of SCFA demonstrates metabolic cooperation among the bacterial community, since no bacterial genus can hydrolyze all kinds of nutrients, suggesting that the entire bacterial community has a role (63). In the intestine, SCFA are absorbed through passive diffusion via MCT1 (monocarboxylate transporter 1) (64).

Their first function is as an energy source for colonic epithelial cells, accounting for 60–70% of their fuel, mainly attributed to butyrate (27), but this SCFA may also play an important role in cell growth and differentiation (5, 87). On the other hand, acetate may be used as a cholesterol or fatty acid precursor, and propionate is a substrate for gluconeogenesis (2, 29). Other molecules, such as conjugated linoleic acids (CLA) (30, 57), metabolites, or bile acids (109), and gases, such as methane and H<sub>2</sub>S (91), with metabolic regulatory functions can also be released by gut bacteria but might have minor roles in mammal physiology compared with SCFA.

SCFA are also important in epithelial barrier function maintenance (9). Butyrate increases mucus production and also affects tight-junction protein expression, that is, zonulin and occludin, contributing to reduced intestinal permeability (11, 81). Moreover, acetate has more pronounced effects on epithelial protection, and the inhibition of GPR strongly attenuates the effects of acetate in terms of epithelial survival and integrity (108).

Acetate and butyrate can increase fatty acid oxidation and energy expenditure in humans, reducing body weight (108). We and others have shown that the administration of acetate activates 5′-AMP-activated protein kinase (AMPK), increasing fatty acid oxidation and energy expenditure (54, 89). This result was associated with improvement in insulin resistance and glucose tolerance in mice fed a high-fat diet (23, 92). Butyrate administration is able to increase brown adipose tissue mass and UCP1 expression (42). SCFA can also modulate satiety, since acetate infusion induces an increase in the circulating levels of GLP-1 and PYY in overweight women (39). It is possible that the effects of SCFA on appetite regulation are mediated by GPR41 and depend on the intestinal transit rate (93).

It is well known that SCFA have anti-inflammatory effects, in part through inhibition of NF-κB activation in the host immune cells by binding to G-protein-coupled receptors 43 and 41 (GPR43 and GPR41) (3, 41, 90, 111), and GPR43 has an essential role in mediating acetate-induced anti-inflammatory stimuli (66). Related to this anti-inflammatory effect, it was recently demonstrated that butyrate can induce the extrathymic generation of anti-inflammatory Treg cells. These data are relevant because it has been demonstrated that, in white adipose tissue, an increase in Treg cells can reduce macrophage infiltration in adipose tissue, which seems important to improve insulin resistance (36) (FIGURE 3).

**Bile Acids and Branched-Chain Amino Acids**

In the liver, bile acids are conjugated to glycine, reaching the entero-hepatic circulation following reuptake in the ileum. This allows the intestinal bacteria to act on these primary bile acids. These secondary bile acids act through GPR TGR5, which is a different receptor from that of primary bile acids (FXR). The activation of TGR5 by secondary bile acids activates GLP1 secretion from L-cells in
the intestine, and this secretion can protect against insulin resistance (112).

An increase in circulating BCAA is associated with a fivefold increased risk of developing T2DM (74). In addition, there are clear correlations between circulating BCAA and insulin resistance, and the supplementation of BCAA to a high-fat diet also impairs insulin action (58, 113, 126). The mechanisms by which BCAA can induce insulin resistance are complex and controversial (75), but also complex and not completely known are the reasons for this increase in BCAA in situations of obesity and insulin resistance. This increase may be related to altered peripheral amino acid metabolism, but the gut microbiota has been shown to be important for the supply of leucine, isoleucine, and valine (BCAA) to mammalian hosts. In this regard, it is reasonable to assume that altered microbiota composition in obesity and in T2D may contribute to the elevations in BCAA. Bile acids produced in response to high-fat diet possibly promoted the growth of Clostridium; thus a higher production of

FIGURE 3. SCFA and the generation of anti-inflammatory Treg cells
SCFA have anti-inflammatory effects reducing the secretion of proinflammatory cytokines and chemokines, and possibly reducing local macrophage infiltration. Butyrate can induce the extrathymic generation of anti-inflammatory regulatory T cells (Treg) cells. Propionate promotes Treg differentiation and IL-10 production. In white adipose tissue, an increase in Treg cells can reduce macrophage infiltration in adipose tissue, improving insulin resistance. M1, macrophage subtype 1; M2, macrophage subtype 2.
BCAA by proteolysis is related to the increased number of Clostridium. This an indirect effect of lipids acting on the microbiota.

Conclusions

Diet induced-obesity promotes insulin resistance by mechanisms independent and dependent on gut microbiota. In this review, we have discussed several mechanisms that contribute to explaining the link between intestinal flora and insulin resistance/obesity. The LPS from intestinal flora bacteria can induce a chronic subclinical inflammatory process and obesity, leading to insulin resistance through activation of TLR4. The reduction in circulating SCFA may also have an essential role in the installation of reduced insulin sensitivity and obesity. Other mechanisms include effects of bile acids, branched-chain amino acids (BCAA), and some other lesser-known factors. In the near future, this area should open new therapeutic avenues for obesity/insulin resistance and its comorbidities.

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