THE ROLE OF GUT MICROBIOTA IN OBESITY, DIABETES MELLITUS AND EFFECT OF METFORMIN: NEW INSIGHTS INTO OLD DISEASES

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Abstract

There is recent growing evidence that abnormalities in the microbiota composition can have a major role in the development of obesity and diabetes and that some actions of metformin may be mediated by gut bacteria. Several mechanisms have been found. A reduced microbial diversity is associated to inflammation, insulin-resistance and adiposity. In particular, a rise in the *Firmicutes*/*Bacteroidetes* ratio is related to a low-grade inflammation and to an increased capability of harvesting energy from food. Interestingly, high-fat-diet favors the growth of bacteria capable of extracting more energy from food. Changes in some metabolites, such as short-chain fatty acids (SCFAs), produced by gut microbiota, and decreased amounts of the *Akkermansia muciniphila* are associated with the presence of type 2 diabetes. Among the mechanisms by which metformin acts on glucose metabolism and on the cardiovascular risk, some of them are due to positive effects on gut microbiota. A shift towards positive SCFAs produced by bacteria, an increase in some bacterial strains, including *A. muciniphila*, and some actions on bile acids mediated by microbiota have been described. All these recent advances have been reported and discussed.

Introduction

The microbiota is a complex and heterogeneous ecosystem of taxonomically identified and unidentified microorganisms residing in several districts of the human body, being over 70% localized in the gastrointestinal tract and typified by two dominant bacterial *phyla*, namely *Bacteroidetes* and *Firmicutes*. The microbiota lives in a symbiotic relationship with its host and is characterized by a considerable inter-individual variability. The most dramatic changes in its composition take place during early childhood and are influenced by several variables including type of delivery (vaginal or cesarean), diet, environment [1]. Given that the number of microbial genes (a.k.a. microbioma) has been estimated 150-fold higher than the number of genes in the human genome, the microbiota seems to carry out more than 98% of the genetic activity of the organism, as if it were a "second genome" [2]. Moreover, it can be also considered as a metabolic "organ"; indeed, not only facilitates harvesting of nutrients and energy from the ingested food but is able, as well, to produce a wide range of bioactive compounds, such as vitamins and short-chain fatty acids (SCFAs), and it is implicated in the regulation of several metabolic processes. Consequently, it is not surprising that abnormalities in the microbiota composition are often linked to the presence of common metabolic diseases, such as obesity and type 2 diabetes (T2D) [3].

Role of the microbiota in obesity and diabetes

In the last four decades, the global prevalence of obesity has almost reached 650 million of people, a number that is 6 times more than what observed in the 1990s and that cannot be only explained by an increased caloric intake and a reduced physical activity [4]. To this regard, it has been suggested that the gut microbiota, and particularly its changes in the composition and biodiversity, can play a relevant role in the development of metabolic diseases [5]. For instance, a high-fat diet (HFD) promotes in mice the development of systemic endotoxemia and inflammation, favors the growth of bacteria capable of extracting more energy from the ingested food, and triggers insulin resistance and obesity [3]. Notably, HFD leads, for example, to a drop in *Lactobacillus* genus (which, instead, has positive effects on the gastrointestinal barrier function) and to an increase in microbial populations (i.e. *Oscillibacter*) that release pro-inflammatory cytokines (primarily TNF-α and IL-6) and alter negatively the gut barrier [6]. In contrast, germ-free (GF) mice fed with HFD are protected from increased gut inflammation and exhibit decreased adiposity with respect to conventionally raised mice fed with the same diet [7]. Nevertheless, GF mice transplanted with the microbiota of

obese mice become obese within the next 2 weeks [8]. In humans, most of the published studies indicate that a rise in the *Firmicutes*/*Bacteroidetes* ratio is related to an augmented low-grade inflammatory status and to a more elevated capability of harvesting energy from food. Further, these studies are in agreement with the concept that a diminished microbial diversity is associated with a higher insulin resistance, inflammation and adiposity [4, 9].

With respect to diabetes, during the last ten years, several studies have also pointed out changes in gut microbiota composition or function in T2D patients, where the dysbiosis mostly depends on a depletion in butyrate-producing bacteria (i.e. *Faecalibacterium prausnitzii* and *Roseburia intestinalis*) coupled with an enrichment of opportunistic pathogens (i.e. *Escherichia coli*, *Bacteroides caccae*, *Clostridium ramosum*, *Clostridium symbiosum*, *Clostridium hathewayi* and *Eggerthella lenta*) [10, 11]. To this regard, it should be emphasized that changes in the metabolites produced by gut microbiota may be associated with the development of T2D and insulin resistance. In particular, SCFAs, such as butyrate, acetate and propionate, are endowed with important metabolic functions and are critical for gut health [3, 12]. Indeed, SCFAs display beneficial effects on peripheral tissues, such as adipose tissue, liver and skeletal muscles, leading to an improvement of insulin sensitivity [13, 14]. Specifically, butyrate also improves gut barrier integrity by increasing the transcription of mucin and claudin-1, and regulating the expression of epithelial tight-junction proteins [10, 15]. Moreover, together with propionate, butyrate has been shown to activate intestinal gluconeogenesis resulting in metabolic benefits in energy homeostasis, such as decreased adiposity and body weight, and a better glucose control, including a reduction in hepatic glucose production [16].

One of the most abundant single species in the human intestinal microbiota is *Akkermansia muciniphila*, a mucin-degrading bacterial strain, which has gained considerable attention since decreased amounts of the bacterium have been linked to obesity, insulin resistance, diabetes and other cardiometabolic disorders in rodents and in humans [17]. Of interest, *A. muciniphila* has recently been shown to delay the onset of type 1 diabetes in diabetes-prone animals probably as a result of its modulatory action on the immune system [18]. However, although oral administration of *A. muciniphila* to mice fed with HFD significantly improves glucose homeostasis, the antidiabetic properties of this bacterium have not been clearly demonstrated in humans due to its growth requirements and oxygen sensitivity that render the use of living *A. muciniphila* unsuitable for putative therapeutic opportunities [11]. Nonetheless, Plovier and collaborators recently faced this issue finding that the pasteurized *A. muciniphila* versus the live bacterium is endowed with an

enhanced capacity to reduce fat mass development, insulin resistance and dyslipidemia in mice. Although it remains to be clarified how pasteurization improves *A. muciniphila* action, they also showed that the outer membrane protein Amuc_1100 is specifically implicated in the bacterium-to-host interaction via the Toll-like receptor 2 signaling, and that this protein partially recapitulates the effects of *A. muciniphila* towards insulin resistance, obesity and gut barrier alteration [19]. These data pave the way for future human researches exploring *A. muciniphila* as a therapeutic approach in the management of metabolic diseases.

Metformin: a new outlook for an old antidiabetic drug

Metformin is an orally administered drug that has been employed for more than 60 years as a first-line antidiabetic compound, either alone or in combination with other anti-hyperglycemic drugs, due to its safety profile and favorable cardiovascular outcomes. Although the possible development of a rare adverse event, lactic acidosis, sometimes limits its use.

The glucose-lowering effect of metformin has been primarily ascribed to a reduction of hepatic gluconeogenesis. Nevertheless, in spite of elevated levels of metformin accumulating in the human intestinal mucosa, much lower concentrations of the drug are detected in the plasma (up to 300 times lower) [20]. Further, after an oral administration, metformin half-life in blood is around 3-4 hours, which appears inconsistent with the duration of its glucose-lowering effect. In addition, the glucose-lowering effect of the newly developed delayed-release metformin is similar to that of the same dose of the extended-release metformin, in spite of a much lower bioavailability [21]. Therefore, the possibility exists that the metabolic benefits linked to metformin treatment may in part depend upon its action in the gut (Figure 1). Accordingly, metformin-dependent glucose lowering is stronger following intradoudenal *versus* intravenous administration of the drug [22]. Concerning the underlying molecular mechanism, in both humans and rodents, metformin has consistently been shown to augment the blood levels of the incretin hormone glucagon-like peptide 1 (GLP-1) produced by enteroendocrine L cells that, through several pathways (i.e. glucose-dependent insulinotropic and glucagonstatic effects, slowing of gastric emptying), improves blood glucose homeostasis [22]. Of note, a recent study documented that a 24 hours exposure to metformin directly stimulates GLP-1 secretion from intestinal L cells [23].

However, accumulating evidence also underscores the ability of metformin to reshape the gut microbiota promoting, as well, a shift towards SCFAs-producing bacteria in T2D individuals [11, 24]. A double-blind study carry on treatment-naïve T2D patients demonstrated that, after 4 months,

metformin induces significant changes in the relative abundance of more than 80 bacterial strains compared to placebo, where most of the changes were observed in the *Firmicutes* and *Proteobacteria* phyla. Moreover, fecal transfer from metformin-treated donors to HFD-fed GF mice improved glucose tolerance, proving that metformin-altered microbiota can produce glucose-lowering effects. Of interest, the same authors also found an enhanced abundance of *Akkermansia muciniphila* and, in *in vitro* experiments, they demonstrated that metformin directly increases the growth of this bacterium [25]. This is in agreement with other studies showing that metformin dramatically increases the abundance of *A. muciniphila* up to 20% of the total microbiota [14, 26]. Finally, transcriptome analyses on fecal samples from treatment-naïve patients cultured with metformin in a gut simulator documented that metformin directly affects gut microbiota regulating the expression of genes encoding metalloproteins or metal transporters [25].

In a very recent study performed on newly diagnosed T2D patients naively treated with metformin for only 3 days, it was documented that even a short treatment with the drug is able to reshape gut microbiota composition, where the genus *Bacteroide*s is characterized by the largest decrease in abundance and being *B. fragilis* the most changed. Indeed, the authors also showed that metformin inhibits, in a dose-dependent fashion, the growth of *B. fragilis.* In addition, transplanted stool from subjects with T2D treated with metformin to microbiota-depleted mice fed with HFD caused, on one side, a decrease in *B. fragilis* abundance and, on the other, an improvement in glucose intolerance and insulin resistance, indicating that the beneficial effects of metformin can be transferred by stool transplantation [27]. A modulation of the gut microbiota by metformin has been also reported to improve the metabolic profile of aged obese mice, suggesting that the drug may have a therapeutic impact on metabolic disorders also in elderly individuals [28].

Another target of metformin action is represented by bile acids that, thanks to their amphipathic structure, facilitate emulsification and absorption of dietary lipids and fat-soluble vitamins. Notably, bile acids have been involved not only in the hepatic regulation of cholesterol metabolism but also in glucose homeostasis [29]. The inhibition of bile acid resorption by biguanides has been acknowledged since the 1970s [30], and the resulting rise in fecal bile acid secretion, which is coupled to an increase in bile acid synthesis from cholesterol, likely accounts for metformin-induced lowering in serum cholesterol levels [31]. In humans, the primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized in the liver from cholesterol and secreted as glycine and, to a lesser extent, taurine conjugates into the intestine. CDCA is further converted to the secondary bile acid ursodeoxycholic (UDCA) by gut microbial 7α/β-dehydrogenation and

transported back to the liver via the enterohepatic circulation. Here, most of UDCA is conjugated with glycine and taurine to generate, respectively, GUDCA (glycoursodeoxycholic acid) and TUDCA (tauroursodeoxycholic acid), which are transported into the gut and successively reabsorbed into the ileum epithelial cells. Overall, about 95% of secreted bile acids is reabsorbed from the intestine, mainly as conjugated acids. Bile acids deconjugation is carried out by bacteria endowed with bile salt hydrolase (BSH) activity, thus preventing their active reuptake. Metagenomic analyses revealed that, in the human gut, BSH is present in all major bacterial divisions and archaeal species including *Bacteroides*, *Lactobacilli*, *Bifidobacteria* and *Clostridium* [32].

After metformin treatment, Sun and colleagues found that especially the levels of the conjugated bile acids GUDCA and TUDCA were elevated. To this regard, they demonstrated that GUDCA and TUDCA were negatively correlated with *B. fragilis*, and that its BSH activity was strongly reduced following metformin administration. Further, they documented that GUDCA significantly increases the levels of GLP-1. Of interest, Sun and collaborators also proved that GUDCA and TUDCA are potential antagonists of the nuclear receptor FXR (farnesoid X receptor) [27], which controls bile acid homeostasis, glucose and lipid metabolism, and it is implicated in several metabolic diseases [33]. Indeed, oral administration of GUDCA strongly reduces intestinal FXR signaling and alleviates some metabolic endpoints in established obese mice, thus suggesting that the GUDCA-intestinal FXR pathway can represent a potential novel target to counteract metabolic disorders in humans [27].

Collectively, these data strongly indicate that the gut, and especially the microbiota, is a key site of metformin action in the control of glucose homeostasis. Moreover, given that metformin treatment has also been associated with a significant decrease in all-cause mortality and incidence of age-related diseases, the drug has been even proposed as an anti-aging molecule [24, 34]. Therefore, further knowledge on its action will help to better address the therapeutic targets of this widely employed antidiabetic molecule.

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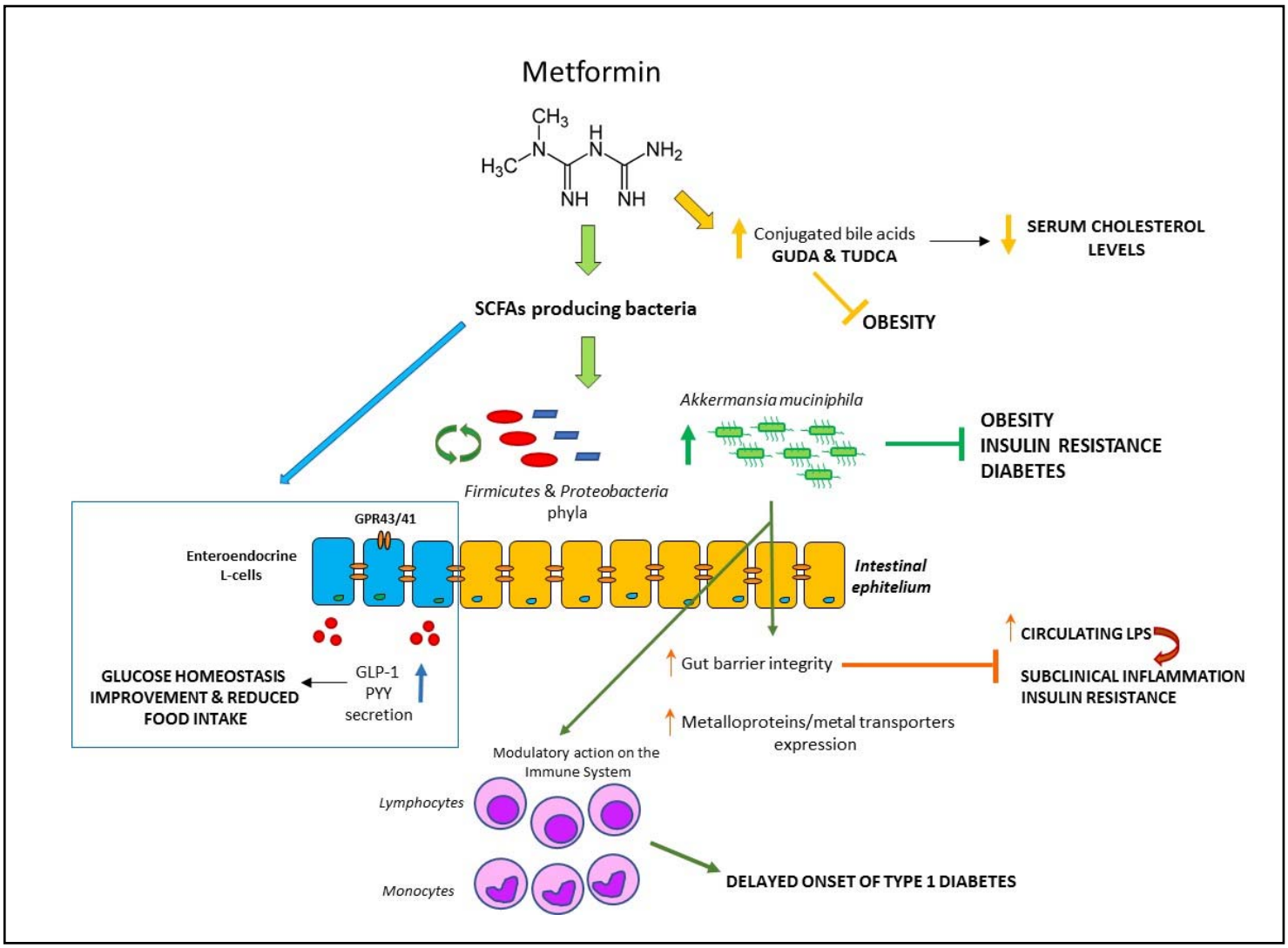

Figure 1. Main effects of metformin in the gut. Metformin is able to reshape the gut microbiota promoting a shift towards short-chain fatty acids (SCFAs)-producing bacteria, where most of the changes have been observed in the *Firmicutes* and *Proteobacteria* phyla. SCFAs, through the G-protein-coupled receptors GPR-41 and GPR-43, lead to the secretion of intestinal peptides implicated in glucose homeostasis or food intake, such as glucagon-like peptide 1 (GLP-1) or peptide YY (PYY). SCFAs also improve gut barrier integrity; indeed, dysfunctions in gut barrier expose the host to high levels of translocated lipopolysaccharide (LPS) that promotes the production of pro-inflammatory cytokines (primarily TNF-α and IL-6), which in turn trigger subclinical inflammation and insulin resistance. Specifically, metformin enhances the abundance of *Akkermansia muciniphila*; accordingly, decreased amounts of the bacterium have been linked to the development of obesity, insulin resistance, and diabetes. Further, *A. muciniphila* seems to have a modulatory action on the immune system which may account for a delayed onset of type 1 diabetes. Moreover, metformin treatment regulates the expression of genes encoding metalloproteins/metal transporters, and increases the levels of the conjugated bile acids GUDCA (glycoursodeoxycholic acid) and TUDCA (tauroursodeoxycholic acid). This latter effect likely accounts for metformin-induced lowering in serum cholesterol levels.