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Perspective

Metformin and the Gut Microbiome in Diabetes

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Diabetes is a worldwide epidemic. According to the most recent WHO fact sheet, the prevalence of diabetes among adults has risen from 4.7% to 8.5% in the last 34 years. Diabetes is a multifactorial disease that results from interaction of genetic, epigenetic, and environmental factors. Metformin has been used extensively as a first-line therapy for Type 2 diabetes (T2DM)[3] and is the most prescribed antidiabetic drug in the US. Metformin is a biguanide derivate and has pleiotropic effects *(1)*. Metformin has been shown to inhibit hepatic glucose production, upregulate peripheral glucose uptake in both the liver and skeletal muscle, and improve insulin sensitivity *(1)*. In addition, T2DM patients on metformin exhibit an improvement in their lipid profiles, and prolonged benefits include a reduction in the micro- and macrovascular complications that are increased in patients with diabetes. Many of these pleiotropic effects of metformin have been ascribed to its activation of the enzyme adenosine monophosphate–activated protein kinase (AMPK) in 2 sites, the skeletal muscle and the liver. One should note, however, that in animal AMPK knockout models, the effects of metformin are still not attenuated *(1)*. This observation has rekindled interest on the effects of metformin on gut pharmacology. About 15%–25% of patients on metformin have metformin-associated gastrointestinal side effects, and some of these patients cannot tolerate metformin at all due to lactic acidosis *(2)*.

Metformin is orally consumed as the hydrochloride salt and absorption of metformin is mainly in the small intestine *(2)*. Metformin also increases glucose uptake and utilization in the intestine, and causes increased lactic acid production in enterocytes *(2)*. In addition, in the liver, metformin inhibits conversion of lactate to pyruvate, and subsequently the buildup of lactate results in increased release of lactate into circulation. Furthermore, metformin increases GLP-1 release from the L cells of the ileum, and in some studies inhibits its degradation by the enzyme DPP-4 *(2)*. Metformin has also been shown to decrease bile acid absorption and could thus indirectly result in improvement of dyslipidemia. However, in some cases the increased luminal bile salt concentration has an osmotic effect, resulting in diarrhea in metformin-treated patients *(2)*. Despite its adverse effects of causing lactic acidosis, diarrhea, nausea, and vomiting, metformin's benefits far outweigh these risks. Recent studies have shown that metformin, in addition to regulating hepatic gluconeogenesis and improving hyperglycemia via modulation of AMPK, regulates pathways independent of AMPK, resulting in improvement in the metabolic derangement seen in diabetes and its complications. These effects of metformin appear to be mediated by alterations in diet and the associated microbiome *(1)*.

The recent literature points to the important role of the gut microbiota in regulating and harvesting energy from the diet and from metabolism and the effects of the microbiota on immune modulation. The human gut microbiota comprises several trillion organisms of more than a thousand heterogeneous species. Several studies have reported alterations in the gut microbiome in insulin-resistant states such as diabetes and metabolic syndrome, and these alterations have been implicated in their pathogenesis. Mice kept in germfree conditions are protected against diet-induced obesity and have increased liver and muscle AMPK activity. In the presence of obesity and a high-fat diet, there is a significant increase in the relative abundance of certain microbiota, especially of the phylum Firmicutes, with associated decreases in the Bacteriodetes phyla *(3)*. The proof of principle studies involving the transplantation of gut microbiota from obese mice to germfree mice resulted in a significant increase in body fat and increased insulin resistance compared to their lean counterparts. Previous studies have shown that metformin therapy is associated with marked changes in microbiota, especially in high fat diet conditions, suggesting a possible interaction between dietary composition, metformin and the composition of the gut microbiome *(2)*. In particular, metformin treatment results in increases in the prevalence of *Akkermansia muciniphila*, a gram-negative anaerobic bacterium that degrades mucin. In addition, *Akkermansia* as well as metformin has been shown to upregulate the expression of several endocannabinoids such as acylglycerols, controlling inflammation in the intestine, which in turn leads to an improvement in gut barrier function that attenuates the metabolic deregulation produced by high fat diets *(2)*. However, this has not replicated in other studies. In a metagenome-wide association study, samples from pa-

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[3] Nonstandard abbreviations: T2DM, Type 2 diabetes; AMPK, adenosine monophosphate–activated protein kinase.

tients with T2DM demonstrated increased abundance of *Akkermansia (4)*. It is worth noting that these different results from different studies may be attributable to the fact that the gut microbiome composition can change rapidly during the course of treatment with antidiabetic drug, such as metformin, which has not been accounted for in many studies.

Forslund et al. *(5)* recently published a very elegant and comprehensive study by combining multicountry (Danish, Swedish, and Chinese) data sets available from 784 human gut metagenomes. This analysis involved nondiabetic controls (n = 554), metformin untreated T2DM (n = 106), metformin treated T2DM (n = 93), and type 1 diabetes patients (n = 31). Importantly, treatment information was obtained on all patients. Diabetic patients who were not taking metformin had different bacteria from patients without diabetes and those with diabetes who were taking metformin. Using a unique supervised learning module for classification, regression, and outline detection, Forslund et al. were able to identify the diabetic patients that were treated with only metformin based on their gut microbiome composition. When data sets were analyzed after controlling for metformin treatment, a common finding was the reduction in butyrate (*Roseburia, Clostridialis* species) and propionate-producing taxa across all country data sets and an increase in *Escherichia coli* seen in Danish and Swedish cohorts, whereas the data from the Chinese cohorts showed increased *Escherichia* in both the diabetic individuals and controls. These authors effectively demonstrated that individuals who were taking the medication had a greater abundance of *E. coli* and lower amounts of *Intestinibacter*. The increase in *Lactobacillus* in diabetic patients was lost when controlling for metformin treatment. Furthermore, in metformin-untreated T2DM, there was enrichment in redox genes such as catalase, as well as ribose. Among the amino acids, metformin untreated T2DM had increased amounts of the amino acids tryptophan, glycine and threonine (which are important in glutathione metabolism), and exhibited decreases in genes regulating arginine and threonine degradation, and pyruvate synthase. To delineate the effects of dysglycemia from effects on the microbiome, Forslund et al. also compared the metagenomic data of 31 patients with T1DM and nondiabetic controls. This enabled them to disentangle the effects of glycemia from those of the T2DM specific microbiome. These authors further demonstrated that changes in gut microbiome in metformin untreated T2DM were associated with the onset of progression of T2DM and could not be solely attributed to alterations in glycemia.

The recent metagenomic analysis by Forslund et al. indicates partial microbial mediation of both the therapeutic and adverse effects of metformin. Thus, metformin appears to have very important effects on the human gut microbiome that contribute to its pleiotropic beneficial antidiabetic effects, but an individual's tolerance to metformin can also be influenced by their inherent gut microbiome. Since the microbiome profile can be altered by various factors, we believe multicountry longitudinal studies on nondiabetic controls, at risk individuals, and diabetic individuals controlled for metformin with extensive diet and sedentary lifestyle information will be helpful to shed light on causality and its dual effects. Future antidiabetic treatment regimens should target modulation of bacterial species that cause changes in amino acid metabolism, redox status and inflammation. Such studies will pave the way for novel precision medicine based approaches in treating diabetes and its complications.

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