Name of Journal: *World Journal of Diabetes*

Manuscript Type: LETTER TO THE EDITOR

To the editor,

We would like to thank the reviewers for their comments, as these comments strengthened our Letter to the Editor. All changes were highlighted in yellow throughout the manuscript.

Reviewer 1 – There are some major points that have to be addressed

1. The aim of the paper is unclear, the message is not significant.

Response: We added more data on the potential targets of gut microbiota-derived metabolites and current pharmacological and non-pharmacological approaches in diabetes mellitus. We anticipate improving the focus of our Letter.

2. The core tip should be included in the main text.

Response: We included the core tip in the main text

3. Valid conclusions should be stated.

Response: Thank you for your suggestion. We completely reformulated the conclusions.

Reviewer 2 - Based on a review paper published in World Journal of Diabetes recently, the authors wrote a letter to the editor. Overall, the letter mainly summarizes the context of the review and brings some studies that show the unbeneficial effect of gut microbiota-derived metabolites.

1. However, the major concern is that the authors did not mention how to target these metabolites and the associated literature studies as the title shows the potential targets of treatment.

Response: Thank you very much for highlighting this question. We added the main pharmacologic and non-pharmacologic strategies that could target the metabolites derived from gut microbiota (reference 11-27).

2. In addition, there are some minors in this short letter. Minors:

2.1 Conflict of interest is needed

Response: The disclosure was added to the manuscript.

2.2 Grammar errors, such as in abstract, Insulin resistance (IR) is a global pandemic metabolic disease that progress (> progresses) to type 2 diabetes

mellitus., bacterial-derived metabolites, and so on; Typo errors such as proprionate > propionate;

Response: We apologize for these grammar errors and typo. They were all corrected.

2.3 Abbreviation: STAT3.

Response: We added the meaning of STAT3, Signal Transducer and Activator of Transcription 3.

2.4 Reference: World J Diabetes. 2021;12(6): 685-915. The page number looks not correct.

Response: Thank you for pointing out that error. We have already corrected it.

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Gut microbiota-derived metabolites are novel targets for improving insulin resistance

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Abstract

Insulin resistance (IR) is a global pandemic metabolic disease that progresses to type 2 diabetes mellitus. Gut microbiota-derived metabolites have broad effects locally and in distinct organs, in particular skeletal muscle, adipose tissue, and liver, that can modulate glucose metabolism. Therefore, the therapeutic potential of current pharmacological and non-pharmacological approaches used to treat diabetes mellitus can be tested to target specific metabolites derived from intestinal bacteria, which may ultimately ameliorate the hyperglycaemic burden.

Key words: Insulin resistance; gut microbiota; metabolites; host metabolism; metabolic organs; novel targets

Conflict of interest statement

The authors declare no conflicts of interest

TO THE EDITOR,

We read the recent publication by Jang HR and Lee HY (1) on the relationship of mechanisms linking the gut microbiota-derived metabolites to insulin resistance published in this journal with great interest.

The gut microbiota plays a key role in metabolic diseases. Gut microbiota-derived metabolites are found in different dietary sources, including carbohydrate (acetate, propionate, butyrate, and succinate), protein (hydrogen sulfide, indole, and phenylacetic acid), and lipids (resveratrol-, ferulic acid-, linoleic acid-, catecin- and berries-derived metabolites). Insulin signalling pathways are directly targeted by these metabolites. Therefore, gut microbiota-derived metabolites, in particular the short chain fatty acids (SCFAs), increase glucose uptake and lipid oxidation in skeletal muscle, whereas in liver SCFAs decrease lipogenesis and gluconeogenesis, increasing the lipid oxidation through activation of PI3K-AKT-PKB (phosphatidylinositol 3-kinase - serine/threonine protein kinase B) and AMPK (adenosine monophosphate-activated protein kinase). In adipose tissue, SCFAs stimulate adipogenesis and thermogenesis, inhibit lipolysis and attenuate inflammation. Therefore, an increase in energy expenditure and fat oxidation occurs in whole-body. Collectively, these findings pave the way for the development of novel drugs or for investigation of the therapeutic potential of drugs currently used to treat insulin resistance, targeting the gut microbiota-derived metabolites.

Notably, based on compelling evidence for the existence of host microbiota crosstalk and metabolic diseases, substantial body of literature, either in preclinical models or in clinical studies (2-4), has linked intestinal microbiota to the pathophysiology of insulin resistance and type 2 *diabetes mellitus* (T2DM).

This present article elegantly highlights the potential role of specific microbiota-derived compounds in insulin-responsive tissues, acting as risk factors or protectors for the development of insulin resistance and the importance of the muscle-liver-adipose tissue axis interaction.

Although the authors documented so well the potential role of some bacterial metabolites as regulators of metabolic functions in the body, such as SCFA derived from carbohydrates (propionate, butyrate, and acetate) and the protein- and lipid-derived metabolites, in modulating pathways of insulin signalling, the impact of these bacterial metabolites on host metabolism warrants further investigation.

Despite mentioned the importance of succinate, a metabolite of the tricarboxylic acid cycle produced by both microbiota and the host (5), in the improvement of glucose homeostasis through activation of intestinal gluconeogenesis (6), Serena et al demonstrated that obese individuals exhibit high levels of this circulating metabolite (5). Furthermore, the imbalance of higher relative abundance of succinate-producing bacteria (*Prevotellaceae* and *Veillonellaceae*) and lower relative abundance of succinate-consuming bacteria *Odoribacteraceae* and *Clostridaceae*) may promote an increase in succinate levels and, ultimately, an impaired glucose metabolism.

These authors also pointed out succinate as having a potential role in obesity and metabolic-associated cardiovascular disorders. Importantly, succinate acts as an immunogenic molecule, named damage associated molecular patterns (DAMPs). This molecule is recognized by immune cells and through its G-protein coupled receptor (succinate receptor 1/SUCNR1 or GPR19) stabilizes hypoxia-inducible factor-1α (HIF-1α), which promotes the pro-inflammatory differentiation of T lymphocytes, and synergizes the effects with Toll-like receptor ligands in dendritic cells for the production of cytokines (7,8). Collectively, these findings may promote an enhancement of insulin resistance and DM burden.

In addition, broader investigations on the physiological implications of hydrogen sulfide (H_2S) and the role of sulfur-reducing bacteria from the intestinal microbiota in better host glycemic control would be relevant (9). H_2S metabolite may protect from oxidative stress by restoring reduced glutathione (GSH) and scavenging of mitochondrial reactive oxygen species, inducing pro-survival/angiogenesis signalling pathway (STAT3, Signal Transducer and Activator of Transcription 3), and promoting immunomodulation (inhibition/activation of NF-κB) and vasodilation (activation of K_{ATP} ion channel) (10). However, the balance between therapeutic and harmful effects of H_2S should be considered when targeting that metabolite, as H_2S either endogenous or exogenous, as well as the one produced by the gut microbiota, promotes or inhibits a variety of characteristics in mucosal microbiota biofilms (11). Depending on H_2S concentration, in particular when gut microbiota produce excessive amount, may cause mucus disruption and inflammation in colon and contribute to cancer. Conversely, low levels of H_2S directly stabilize mucus layers, prevent fragmentation and adherence of the microbiota biofilm to the epithelium, inhibit the release of invasive opportunistic pathogens or pathobionts, and prevent inflammation and tissue injury (11). Moreover, H_2S overproduction is a causative factor in the pathogenesis of ß-cell death in DM due to increased levels of reactive oxygen and nitrogen species, whereas its deficiency, as a result of increased H_2S consumption by hyperglycaemic cells, may lead to endothelial dysfunction, and to kidney and heart diseases (12).

As we learn more about gut microbiota-derived metabolites, we will better understand how to target these metabolites. Thus, acetate, which is involved in host energy, substrate metabolism, and appetite via secretion of the gut hormones (glucagon-like peptide [GLP] and peptide YY), may be increased by oral acetate administration (vinegar intake), colonic acetate infusions, acetogenic fibers and acetogenic probiotic administration (13). These strategies may both decrease whole-body lipolysis and systemic pro-inflammatory cytokine levels, and increase energy expenditure, insulin sensitivity, and fat oxidation, which contributes to the weight control and glucose homeostasis. Probiotics (live microorganisms) act as microbiome modulators and confer a health benefit, as demonstrated by the capacity of selected probiotic strains (lactobacilli and enterococci) to increase SCFA production, in particular propionate and butyrate (14). As reviewed elsewhere, probiotic administration (*Bifidobacterium pseudocatenulatum, Lactobacillus plantarum* or the formula VSL#3) in preclinical models of obesity led to an increase in the intestinal barrier function, a reduction in the endotexemia, an acceleration in the

metabolism, and a suppression in the body weight gain and insulin resistance via modulation of the gut microbiota composition and SCFA production (15). Probiotics may also ameliorate glucose homeostasis and lipid profile in diabetic mice (15).

From clinical point of view, obese children treated with the probiotic *Lactobacillus casei* shirota for six months presented loss of weight, improved lipid metabolism, and exhibited an increase in the number of *Bifidobacterium* spp and acetate concentration in the feces (16). Likewise, type 2 diabetic individuals treated with the probiotic containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp lactis BB-12 for six weeks ameliorated glucose and lipid profile, which was associated with lower levels of systemic inflammation and increased concentration of acetate (17). Additionally, modification of gut microbiota by dietary weight loss intervention decreases circulating succinate levels and improves the metabolic profile in a cohort of individuals with type 2 DM and obesity (6).

Pharmacologic interventions or xenobiotics may also have effects on gut microbiota. Metformin is the most frequently administered medication to treat patients with insulin resistance and type 2 DM. This drug may alter the gut microbiota composition through the increase in the *Bacteroidetes* and *Verrucomicrobia* phyla and in the mucin-degrading *Akkermansia muciniphila*, *Bacteroides* and *Escherichia* genera, as well as in the SCFA butyrate and propionate production, pointing out to important effects in the maintenance of the integrity of the intestinal barrier, regulation of the bile acid metabolism and improving glucose homeostasis (18,19). Importantly, Metformin may have these benefits in newly-diagnosed DM (20).

Sodium glucose cotransporter 2 inhibitors (SGLT2i) represent the most recently approved class of oral medications for the treatment of type 2 DM. Dapagliflozin decreased the *Firmicutes*-to-*Bacteriodetes* ratio in diabetic mice, which was correlated to an improvement in the vascular function (21). In a rodent model of type 1 DM, inhibition of SGLT2 reduced the intermediate metabolite succinate and increased the SCFA butyrate levels, as well as decreased norepinephrine content in the kidney (22). Hence, the impact of SGLT2 inhibitors on the gut microbiota is an area of active research.

Likewise, GLP-1 agonists reduced the abundancy of the species of the *Firmicutes* phylum (*Lachnospiraceae*, *Clostridiales*) and increased the abundance of the species representing the *Proteobacteria* (*Burkholderiales bacterium YL45*) and *Verrucomicrobia* (*Akkermansia muciniphila*), as well as *Firmicutes* (*Clostridiales*, *Oscillospiraceae*) phyla in obese mice (23). In particular, body weight loss was correlated to increased abundance of *Akkermansia muciniphila*, a mucin-degrading SCFA-producing specie, whose abundance is decreased in obesity and has a negative correlation to markers of gut permeability and inflammation. Notably, the GLP-1 agonist liraglutide can prevent weight gain by modulating gut microbiota composition in both obese and diabetic obese animals (24).

In the cardio-metabolic disease setting, lipid-lowering drugs, such as statins, may also play an important role in modulating gut microbiota. In vitro studies documented increased levels of SCFA production, including propionate, butyrate, and acetate

(25). These drugs may increase the abundance of the *Bacteroides*, *Butyricimonas*, and *Mucispirillum* genera, which was associated with a decrease in the inflammatory response, including lower levels of IL-1β and IL-6, and higher levels of TGFβ-1 in the ileum, and improved hyperglycaemia (26). In humans, obesity was associated with a microbiota signature based on the abundance of the *Bacteroides* genus profile, displaying the lowest abundances of *Akkermansia* and of *Faecalibacterium*, as well as a decrease in the butyrate production potential (27). Importantly, statin therapy resulted in a lower prevalence of a pro-inflammatory microbial community type in obese individuals.

In conclusion, gut microbiota imbalances and maladaptive responses have been implicated in the pathology of insulin resistance, DM, and obesity (28). Host-gut microbiota interaction is suggested to play a contributory role in the therapeutic effects of antidiabetics, statins, and weight loss promoting drugs. Therefore, additional studies combining untargeted metabolomics and proteomics are essential to identify further microbial metabolites or proteins and to determine how they interact with the host targets in improving host metabolism.

ACKNOWLEDGEMENTS

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