

Response to Reviewers' Comments:

Reviewer #1: This review article conducted the mechanisms linking gut microbial metabolites to insulin resistance (IR). It is an interesting title. Please take care of the concerns below.

Response: We would like to thank Reviewer #1 for his/her kind and thoughtful comments regarding our manuscript and for stating that these were "It is an interesting title".

Comments to Author:

1. Hepatokines were ignored in this report. Why?

Response: We agree that hepatokines are also an important factor in the pathogenesis of insulin resistance. However, as requested by reviewer #2, we changed the structure of manuscript and drastically reduced the parts for reviewing insulin resistance, and focused on the mechanisms related to microbial metabolites only. In this view, we couldn't find proper references for hepatokines regulated by bacterial metabolites.

2. The intestinal gluconeogenesis (IGN) is participated. What is the functional stimulator in addition to food? Please add into the discussion.

Response: We thank to you for this suggestion. There are several animal studies reported that gastric bypass surgery increased intestinal gluconeogenesis and suppresses hepatic gluconeogenesis in diabetic animal model (Troy S et al., Cell Metabolism, 2008; Yan Y et al., Obesity Surgery, 2016). The detailed mechanism how gastric bypass surgery increases IGN is not clear, but we agree it would be worthy mention in this review. We have now included this and accordingly revised the Intestine section with text marked in red.

In addition to this, we further detailed the mechanisms of a gut-brain neural circuit by food in regulation of IGN, how the peptides digested from protein-rich diet act on μ -opioide receptors and and increases cAMP levels, which induces the expression of IGN-related genes (Durauffourd C et al., Cell, 2012; De Vadder F et al., Neurogastroenterol Motil, 2015) in the Intestine section with text marked in red.

3. The potential gut microbiota involved in IR did not mention in clear. Are they associated with the metabolites only for IR?

Response: Reviewer #1 astutely points out that it is important to mention the potential gut microbiota involved in IR. We have rewritten the text in Conclusion section and add "...in addition to SCFAs, which has been studied a lot in the past, recent studies have found some candidates that protein-derived (e.g., hydrogen sulfide, Indole-3-carboxylic acid, and phenylacetic acid) and lipid-derived microbial metabolites (e.g., KetoA and

CLA) can play a role in the pathogenesis of insulin resistance." to highlight this.

4. Chronic application of antibiotics in relation with IR is important. Please conduct it in detail.

Response: This study is of interest, however, the comments by reviewer 1 is too broad to be covered by our manuscript. As you are well aware, bacterial colonization is highly diverse depending on antibiotics treatment, and further complexed for their metabolites. Substantial further reviews will be required to fully summarize this field, which is well beyond the scope of our manuscript. It would be very interesting if WJD have special topics about this in future. In our manuscript, we only briefly mentioned about TMAO, which is completely suppressed in both antibiotics-treated humans and mice but the plasma of TMAO levels return to normal after the withdrawal of the antibiotics (Tang WH et al., The New England journal of medicine, 2013; Wang Z et al., Nature, 2011). It is added in the Liver section.

5. In this report, dietary microbial metabolites were the major targets. Please add it in the title.

Response: Thanks for this suggestion. We revised the title from “microbial metabolites” to “gut microbial metabolites” and added “diet-derived” in Table to point this out.

6. SCFAs were the major metabolites and mainly produced from what kinds of microbiota in gut? It was not indicated in the conclusion.

Response: As requested by reviewer #1, we now have kinds of SCFAs-producing bacteria in the Brown adipose tissue section.

We appreciate that the comments by the reviewer#1 were insightful but definitively answering each question would be a separate set of studies unto itself. Thus, we hope that the reviewer will now acknowledge that we have made a reasonable attempt to address the questions raised with revisions and now feels that this paper

Reviewer #2: This research focused on mechanisms linking microbial metabolites to insulin resistance, after check the pubmed, there are 10 articles about Gut Microbiota and Type 2 Diabetes, this manuscript focus on insulin resistance, also important. But I think some places need further revise.

Response: We would like to thank Reviewer #2 for his/her comments regarding our manuscript and for stating that these were “this manuscript focus on insulin resistance, also important.”.

Comments to Author:

1. The structure of the article needs to be optimized, such as the principal phenomena of insulin resistance this part need simplify, because this knowledge was acknowledged by us. After the Effects of gut bacterial metabolites on the pathogenesis of insulin resistance this part, I think must conclusion the mechanism most good add a Figure, also give some treatment method now from the references.

Response: As requested by Reviewer #2, we optimized the structure of the article, and drastically reduced the parts for reviewing insulin resistance, and more focused on the mechanisms specifically related to microbial metabolites. We also have added Figure 1 with 4 sections for skeletal muscle, liver, adipose tissue and intestine. Some of clinical studies with SCFA are added in Brown adipose tissue section at page 11 and the Conclusion section is revised and marked in red colored text.

2. Figure 1 cannot represent the meaning, insulin result muscle normal insulin action and resistance? In which condition? Very confused.

Response: We thank to you for this suggestion. As requested by Reviewer#2, we now have the new Figure 1, represents insulin action and microbial metabolites action for each organ.

3. English I think need further polish although looks good.

Response: Yes, we have carefully read manuscript and revise some typos accordingly.

We appreciate that the comments by the reviewer#2 were helpful to improve the quality of this paper. We hope that the reviewer will now acknowledge that we have made a reasonable attempt to address the questions raised with revisions and now feels that this paper

EDITORIAL OFFICE'S COMMENTS

Response: We thank to your kind comment to improve the quality of this paper. We have revised the paper and upload documents according to Editorial office comments.

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor: 1 Scientific quality: The manuscript describes a minireviews of the mechanisms linking microbial metabolites to insulin resistance. The topic is within the scope of the WJD.

(1) Classification: Grade B and Grade C;

(2) Summary of the Peer-Review Report: This review article conducted the mechanisms linking gut microbial metabolites to insulin resistance (IR). It is interesting. However, the questions raised by the reviewers should be answered; and - **Done**

(3) Format: There is 1 table and 1 figure.

(4) References: A total of 105 references are cited, including 20 references published in the last 3 years; - **We replaced some of cited references to more recent reviews, now the revised paper has 39 cited references published in last 3 years.**

(5) Self-cited references: There are 2 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations that are closely related to the topic of the manuscript, and remove other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated; and

- **We added 2 self-cited references, and the revised paper has 4 self-cited references.**

(6) References recommend: The authors have the right to refuse to cite improper references recommended by peer reviewer(s), especially the references published by the peer reviewer(s) themselves. If the authors found the peer reviewer(s) request the authors to cite improper references published by themselves, please send the peer reviewer's ID number to the editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately.

2 Language evaluation: Classification: Grade B and Grade B. A language editing certificate issued by Editage was provided. 3 Academic norms and rules: No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an invited manuscript. The study was supported by 2 grants. The topic has not previously been published in the WJD. 5 Issues raised:

(1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

- Yes, we uploaded the approved grant application forms.

(2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

- Yes, we uploaded the original figure in ppt format.

(3) The column should be review; and - **OK**

<< Revised manuscript for Reviewers >>

Name of Journal: *World Journal of Diabetes*

Manuscript Type: MINI REVIEW

Mechanisms linking gut microbial metabolites to insulin resistance

Jang HR *et al.* Microbial metabolites and insulin resistance

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Abstract

Insulin resistance is the rate-limiting step in the development of metabolic diseases, including type 2 diabetes. The gut microbiota has been implicated in host energy metabolism and metabolic diseases and is recognized as a quantitatively important organelle in host metabolism, as the human gut harbors 10 trillion bacterial cells. Gut microbiota break down various nutrients and produce metabolites that play fundamental roles in host metabolism and aid in the identification of possible therapeutic targets for metabolic diseases. Therefore, understanding the various effects of bacterial metabolites in the development of insulin resistance is critical. Here, **we review the mechanisms linking gut microbial metabolites to insulin resistance in various insulin-responsive tissues.**

Key words: Insulin resistance; Skeletal muscle; Liver; Adipose tissue; Intestine; Gut bacterial metabolites;

Core tip: Since the gut microbiota has been implicated in host energy metabolism and metabolic diseases, understanding mechanisms linked to insulin resistance is a first step in discovery of new drugs and novel targets against metabolic diseases. Here, **we review the mechanisms linking gut microbial metabolites to insulin resistance in major target tissues of insulin.**

Introduction

Insulin resistance is a pathological state in which tissues do not respond normally to insulin in the process of glucose metabolism. Insulin is an endocrine hormone that binds to insulin receptors on the plasma membrane of target cells, which induces an anabolic response to nutrient availability^[1]. Insulin directly regulates glucose homeostasis by acting on skeletal muscle, the liver, and adipose tissue. These tissues have different functions in metabolic homeostasis that are regulated via tissue-specific insulin signaling pathways. In skeletal muscle, insulin stimulates glucose uptake and storage by increasing the expression of glucose transporters and glycogen synthesis^[1, 2]. In the liver, insulin activates glycogen synthesis and *de novo* lipogenesis and suppresses gluconeogenesis^[1, 2]. In adipose tissue, insulin suppresses lipolysis and increases both glucose and fatty acid uptake and lipogenesis^[1, 2]. In the insulin-resistant state, peripheral glucose disposal is impaired and hepatic gluconeogenesis and adipose lipolysis are not suppressed by insulin. Insulin resistance increases circulating glucose level, which results in increased insulin production in β cells as a compensatory response and hyperinsulinemia, leading to a vicious cycle that promotes further insulin resistance^[1, 3]. Non-treated and prolonged insulin resistance causes hyperglycemia and type 2 diabetes, and can lead to its complications including hyperlipidemia, metabolic syndrome, nonalcoholic fatty liver disease, and cardiovascular diseases^[3, 4].

Various factors have been implicated in the pathogenesis of insulin resistance, including genetic predisposition, aging, obesity, and a sedentary lifestyle. More recently, the gut microbiota has been considered to be a key factor leading to the insulin resistance^[5]. The gut microbiota regulates host dietary intake, energy metabolism, and energy expenditure^[6]. Changes in the composition of the intestinal bacteria might alter energy metabolism and exert various effects on the important metabolic organs, such as skeletal muscle, the liver, and adipose tissue^[6]. In addition, the gut microbiota produces thousands of metabolites that accumulate in the

gastrointestinal system and can be transferred to distant organs^[7]. Lots of recent metabolomics studies examined the association of gut microbiota-derived metabolites with metabolic disease and their effects on host metabolism^[8-10]. Therefore, understanding the various effects of bacterial metabolites in the development of insulin resistance becomes critical for discovering novel targets and developing new drugs against metabolic diseases. **In this review, we review studies that provide evidence for a relationship between gut bacterial metabolites and insulin resistance, and summarize current mechanisms linking gut microbial metabolites to the development of insulin resistance in various metabolic organs, including skeletal muscle, liver, adipose tissue, and intestine.**

Effects of gut bacterial metabolites on the pathogenesis of insulin resistance

We split this section into four parts for each metabolic organ, and briefly describe the pathophysiology of insulin resistance first followed by further discussions on current mechanisms linking gut microbial metabolites to the development of insulin resistance. The mechanisms for each organ are graphically presented in Figure 1 and the studies for each metabolite are summarized in Table 1.

Skeletal muscle

Skeletal muscle is the primary organ for glucose disposal, accounting for up to 70% of glucose uptake in our body^[11]. Insulin promotes glucose uptake in skeletal muscle by translocating the glucose transporter (GLUT) 4 to the plasma membrane^[12]. In insulin-sensitive skeletal muscle, the insulin receptor substrate 1-phosphoinositide-3-kinase (PI3K)-AKT arm of the insulin signaling cascade is activated, which increases glucose uptake and glycogen synthesis^[1]. In insulin-resistant skeletal muscle, proximal insulin signaling events are impaired, which blocks the function of insulin

to translocate GLUT4 to plasma membrane and to stimulate glycogen synthesis^[1]. Furthermore, when calorie loads exceed the glucose uptake capacity of skeletal muscle, the circulating glucose mostly returns to the liver, triggering hepatic *de novo* lipogenesis^[3], which causes ectopic fat deposition in the liver and other tissues, further exacerbating insulin resistance^[13]. Therefore, impaired glucose uptake in skeletal muscle has been considered as a major culprit of type 2 diabetes^[14-16] and targeted as a therapeutic strategy against insulin resistance^[17, 18].

A recent study suggest that microbial products derived from phenolic acids may increase glucose uptake in skeletal muscle under insulin-stimulated condition^[19]. Microbiota-produced phenolic metabolites are derived from ferulic acid, resveratrol, and berries. The ferulic acid-derived metabolites, ferulic acid 4-O-sulfate and dihydroferulic acid 4-O-sulfate, and the resveratrol-derived metabolites, *trans*-resveratrol 4'-O-glucuronide and *trans*-resveratrol 3-O-sulfate, increased 2-deoxy-D-[1-¹⁴C(U)]-glucose uptake in LHCN-M2 human skeletal muscle cells^[19]. Isovanillic acid 3-O-sulfate, which is primarily derived from berries, increased glucose uptake in myotubes through GLUT4-PI3K-AKT-dependent mechanisms and stimulated dose-dependent glucose uptake^[19]. On the other hands, a study has been shown that bacterial-derived metabolite-complex can decrease glucose uptake, though the exact composition of the complex is not defined^[20]. Gut bacteria-derived extracellular vesicles (EVs), which are phospholipid spherical bilayer, are ubiquitously produced by gram-negative bacteria^[20]. **Especially, *Pseudomonas panacis*-derived EVs increasing in a high-fat diet (HFD)-fed mice compared to regular chow-fed mice as well as gut microbe-derived EVs from HFD-fed mice stools induced insulin resistance**, including impairment of insulin signaling both *in vitro* and *in vivo*, and impairment of glucose uptake by decreasing insulin-dependent GLUT4 translocation, both in myotubes and adipocytes^[20].

Insulin-independent glucose uptake is also activated by microbial metabolites. Activation of AMP-activated protein kinase (AMPK) in response to exercise regulates

the translocation of GLUT4 storage vesicles and promotes insulin-independent glucose uptake^[21]. In particular, 5-(3,5-dihydroxyphenyl)- γ -valerolactone has been shown to increase GLUT4 translocation via activation of AMPK through an insulin-independent pathway in skeletal muscle both *in vitro* and *in vivo*^[22]. The antidiabetic green tea catechin (-)-epigallocatechin gallate (EGCG) is further degraded by *Flavonifractor plautii*^[23], and several gut bacteria-derived EGCG metabolites, including 5-(3,5-dihydroxyphenyl)- γ -valerolactone, 4-hydroxy-5-(3,4,5-trihydroxyphenyl)valeric acid, 5-(3,4,5-trihydroxyphenyl)- γ -valerolactone, and 5-(3-hydroxyphenyl) valeric acid, have been shown to promote 2-deoxy-glucose uptake in myotubes *in vitro*^[22]. It has been reported that *Flavonifractor plautii* was decreased in fecal microbiota of subjects with mild fasting hyperglycemia^[24].

Liver

The liver is a central organ that coordinates whole-body metabolism, including carbohydrate, lipid, and protein metabolism. The liver is responsible for gluconeogenesis, glycogenolysis, glycogen synthesis, and *de novo* lipogenesis^[1]. In contrast to skeletal muscle, hepatic glucose uptake is not regulated by insulin but blood glucose levels because GLUT2, a transporter with a high K_M for glucose, is abundantly expressed in the liver and not translocated by insulin stimulation^[25]. Rather than regulating the glucose uptake, in the liver, insulin suppresses hepatic glucose production by reducing the transcription of gluconeogenic enzymes^[26, 27] and induces a shift from net glucose production to net glucose storage by simultaneous regulation of glycogenolysis and glycogen synthesis^[2]. However, in an insulin-resistant state, these regulations are not controlled by insulin, and the non-suppressed hepatic glucose production under insulin stimulated condition has been considered as a marker for hepatic insulin resistance ^[1, 28]. It has been reported that propionate, a gut microbial product derived from carbohydrate fermentation, regulate hepatic gluconeogenesis under insulin stimulated condition^[29]. Previously, stable isotope

studies in both humans and animals have showed that propionate is used as a gluconeogenic substrate in the liver rather than being directly oxidized^[30, 31]. Recently, it was reported that propionate effectively suppresses hepatic glucose production in both presence and absence of long chain fatty acid by increasing the expression of gluconeogenesis-related genes, including *G6PC* and *PCK1*, via the G protein-coupled receptor (GPCR) 43-mediated AMPK signaling pathway under insulin-stimulated condition as well as increases AKT phosphorylation in HepG2 hepatocyte^[29]. In addition to insulin-stimulated condition, it has been reported that gut bacterial metabolites regulate hepatic glucose production under non-insulin stimulated basal condition^[32, 33]. Hydrogen sulfide, a product of protein fermentation, is not only generated in the body but also produced by **sulfate-reducing bacteria, including *Desulfovibrio*, *Desulfobacter*, *Desulfomonas*, and *Desulfobulbus***, in the colon^[32, 34] and affects the basal hepatic glucose production. Under basal condition, this metabolite impairs glucose homeostasis by stimulating gluconeogenesis via increased phosphoenolpyruvate carboxykinase activity and decreased glucokinase by reducing glycogen synthesis in HepG2 human hepatoma cells^[32]. **In type 2 diabetes patients, it has been reported that the plasma hydrogen sulfide levels were reduced compared to healthy subjects^[35, 36], suggesting clinical association of microbial metabolites in hyperglycemia.** Trimethylamine N-oxide (TMAO), which is known as a gut bacterial metabolite derived from choline, **is converted by hepatic enzymes from trimethylamine, a choline-derived microbial metabolite, in liver^[37]. The production of TMAO is completely suppressed in both antibiotics-treated humans and mice but the plasma of TMAO levels return to normal after the withdrawal of the antibiotics^[38, 39].** It has been reported that TMAO increases with insulin resistance in both humans and animals^[40-42]. In mice, TMAO treatment promoted glucose intolerance, while a reduction of TMAO prevented glucose intolerance^[33]. Under basal condition, the treatment of TMAO in mice activated PKR-like ER kinase (PERK) and increased gluconeogenic gene expression, **including *G6pc* and *Pck1*, via transcription factor forkhead box protein O1**, which promoted hyperglycemia^[33, 43].

In addition to glucose metabolism, insulin also controls lipid metabolism in the liver. Since insulin normally promotes net hepatic *de novo* lipogenesis, one might expect decreased *de novo* lipogenesis in an insulin-resistant state; however, hepatic insulin resistance is highly associated with hepatic steatosis^[1, 3], and *de novo* lipogenesis is consistently elevated in insulin-resistant liver tissue^[3]. This phenomenon has been termed “selective hepatic insulin resistance,” as glucose metabolism is affected by insulin resistance but lipid metabolism is not affected^[44]. The increased *de novo* lipogenesis could be partly accounted by hyperinsulinemia, but still there are selective insulin resistance between glucose and lipid when considers the action of insulin *per se*^[3, 4]. Nevertheless, microbial metabolites can regulate the hepatic lipid metabolism. Phenylacetic acid is a microbial metabolite derived from aromatic compounds and produced by *Bacteroides* spp., which have aromatic amino acids fermentative activities^[45]. Plasma phenylacetic acid positively correlates with the nonalcoholic fatty liver disease activity score in humans^[9, 46]. Phenylacetic acid induced the accumulation of hepatic triglycerides both in cellular and animal studies ^[46]. The metabolite also reduced insulin-induced AKT phosphorylation in human primary hepatocytes^[46]. In contrast, short chain fatty acids (SCFAs), including acetate, propionate, and butyrate, decreased hepatic lipid accumulation. Administration of all three SCFAs in HFD-fed mice decreased not only total body fat content, without a change in food intake, but also the expression of genes related to hepatic lipogenesis and fatty acid synthase^[47]. In addition, hepatic lipid oxidation capacity in SCFA-fed mice was increased via upregulation of mitochondrial uncoupling protein (UCP) 2 expression and activation of AMPK^[47-49]. The SCFA acetate inhibited fatty acid synthesis in the liver via activation of AMPK. Oral administration of acetate stimulated the phosphorylation of AMPK, which inactivates carbohydrate-responsive element-binding protein^[50], and in turn modulates the transcription of lipogenic genes in the liver^[51]. Acetate also suppressed the increases in whole-body fat mass and hepatic lipid accumulation by increasing the expression of genes encoding peroxisome proliferator-activated receptor (PPAR) α and fatty acid oxidation-related proteins through AMPK α 2 in the

liver^[52]. In mice, acetate treatment improved liver mitochondrial function by increasing the number of cristae, the location of the electron transport chain, per mitochondria, and the expression of complexes III, IV, and V^[53]. Another SCFA, butyrate, increased mitochondrial mass and area and improved fatty acid oxidation in the liver of HFD-fed mice^[49].

Adipose tissue

Adipose tissue is an energy storage organ^[5]. In adipose tissue, insulin-stimulated glucose uptake also occurs via GLUT4 translocation, which is greatly reduced in insulin resistant condition, such as obesity and type 2 diabetes^[54]. A linoleic acid-derived fatty acid generated by gut lactic acid bacteria, 10-oxo-12(Z)-octadecenoic acid (KetoA), induced adipocyte differentiation via activation of PPAR γ , and increased the production of adiponectin and insulin-stimulated glucose uptake in 3T3-L1 murine adipocytes^[55]. However, physiologically, adipose tissue is not quantitatively significant in insulin-stimulated glucose disposal because it accounts for <5% of blood glucose uptake in our body^[16]. Rather than glucose metabolism, insulin may have more critical roles in lipid metabolism of adipose tissues, thus the suppression of lipolysis is an important function of insulin in adipose tissue^[4]. Failure to suppress lipolysis in insulin-resistant adipose tissue increases circulating free fatty acids and glycerol^[15, 56], and affect in hepatic glucose production^[1]. These increased levels of circulating free fatty acids lead to an increase in ectopic fat accumulation in the liver and muscle, further exacerbating insulin resistance^[15]. In addition, the glycerol released from adipose tissue serves as a gluconeogenic substrate and stimulates hepatic gluconeogenesis^[1]. Suppression of lipolysis also reduces the levels of acetyl-CoA, an allosteric activator of pyruvate carboxylase, decreases pyruvate carboxylase activity^[57]. As a result, gluconeogenic flux, involving glycerol and acetyl-CoA, is diminished, resulting in decreased hepatic gluconeogenesis^[57]. Therefore, the regulation of lipolysis in adipose tissue is considered a therapeutic strategy against

insulin resistance^[58]. In the SCFAs, it has been reported both acetate and propionate stimulate adipogenesis and inhibit lipolysis via activation of GPCR43 but not GPCR41^[59, 60]. Acetate might inhibit basal and beta-adrenergic receptor-mediated intracellular lipolysis **via attenuation of hormone-sensitive lipase phosphorylation in human adipose tissue-derived** adipocytes and lead to a reduction in non-esterified fatty acid release^[61]. Injection of acetate into fasted mice led to decreased plasma free fatty acid levels via activation of GPCR43^[60].

Adipose tissue also functions as an endocrine organ and releases adipokines, lipids, and cytokines, which regulate whole-body metabolism^[62]. Adipose tissue can secrete molecules associated with improved insulin sensitivity, including adiponectin and branched fatty acid esters of hydroxyl fatty acids^[63]. Chronic low-grade inflammation occurs in obese individuals with insulin resistance, which is mainly induced by adipose tissue inflammation^[64]. Inflammation of adipose tissue is caused by macrophage infiltration, which impairs the insulin sensitivity of insulin target organs, resulting in insulin resistance^[65]. TMAO, a microbial metabolite derived from choline, promoted adipose tissue inflammation in HFD-fed mice by increasing mRNA and serum levels of monocyte chemoattractant protein-1 (MCP-1), the proinflammatory cytokine, and decreasing mRNA and serum levels of interleukin (IL)-10, the anti-inflammatory cytokine, in adipose tissue^[43]. Conversely, the SCFAs propionate and butyrate improved adipose tissue inflammation. Propionate may have a directly beneficial effect on adipose tissue in overweight subjects, as it reduced the mRNA expression and secretion of inflammatory cytokines and increased the mRNA expression of genes involved in lipogenesis (*e.g., LPL, SREBP1c*) and glucose uptake (*e.g., GLUT4*)^[66]. Butyrate suppressed lipolysis and inflammatory responses, including the upregulation of tumor necrosis factor- α , MCP-1, and IL-6, which are generated by the interaction of adipocytes and macrophages^[67]. It has been reported that gut bacterial metabolites derived from protein fermentation have anti-inflammatory effects in adipose tissue. Indole-3-carboxylic acid **and indole**, a tryptophan-derived microbial metabolites, **are decreased in the cecal contents of HFD-**

fed mice compared to regular chow-fed mice^[10]. These metabolites increased energy expenditure and improved insulin sensitivity by decreasing the expression of the *microRNA miR-181*, which is upregulated in high-fat diet feeding and increases white adipose tissue (WAT) inflammation^[10].

Brown adipose tissue and whole body energy expenditure

Unlike WAT, because brown adipose tissue (BAT) is responsible for energy expenditure by burning fatty acids to produce heat, it is an important organ that effects on whole body energy metabolism^[68]. Similarly increasing beige adipocytes in white adipose tissue, a process termed “browning,” results in increased heat production and energy expenditure^[69]. Therefore, enhanced BAT activity and browning of WAT are important for energy expenditure and are thought to influence insulin sensitivity^[68, 69]. It has been reported that gut bacterial metabolites derived from carbohydrates and fatty acids increase energy expenditure via browning of WAT and/or enhancing the function of BAT. SCFAs, including acetate, propionate, and butyrate, which are products of dietary fiber fermentation by gut bacteria. Acetate is mainly produced by *Bifidobacteria* and *Lactobacillus* and propionate is largely produced by *Bacteroides* and *Veillonella*, such as *Bacteroides eggerthii*, *Bacteroides fragilis*, and *Veillonella parvula*^[70, 71]. Butyrate is mostly produced by anaerobic bacteria, including *Faecalibacterium prausnitzii*, and *Eubacterium rectale*^[72]. Acetate enhanced beige fat differentiation of white adipocytes *in vitro*^[73]. Acetate and butyrate promoted browning in adipocytes^[53, 74]. In obese diabetic mice, acetate also induced browning of adipocytes by increasing thermogenesis-related gene expression, altered adipocyte morphology, and increased the thermogenic capacity of adipose tissue, independent of BAT, in HFD-fed mice^[53, 73]. Butyrate exerted an anti-obesity effect in animal models by strengthening the function of BAT. This anti-obesity effect occurs by increasing in energy expenditure and fat oxidation through upregulation of the expression of thermogenesis-related genes in BAT, such as peroxisome proliferator-activated

receptor- γ coactivator 1- α (PGC1 α) and UCP1^[48, 74]. All SCFAs stimulated lipid oxidation by activating AMPK in the liver and adipose tissue^[47]. Similarly, in skeletal muscle, acetate improved oxygen consumption by increasing the expression of lipid oxidation-related genes and AMPK activity in animal models^[75]. Butyrate increased oxygen consumption and energy expenditure both *in vitro* and *in vivo*. These effects are caused by activation of AMPK and inhibition of histone deacetylases, which activate PGC1 α and subsequently increase the expression of PPAR δ , thus promoting fatty acid oxidation and increasing the proportion of type I muscle fibers, which are characterized by their high oxidative capacity^[48]. In contrast to animal models, treatment of both lean and metabolic syndrome subjects with butyrate had no effect on BAT function^[76], and open to debate over the single treatment. However, infusion of SCFA mixtures of acetate, propionate, and butyrate increased fat oxidation and whole-body energy expenditure in overweight/obese men^[77, 78]. These effects were observed following treatment with each SCFA alone as well as mixtures of the SCFAs. Propionate increased whole-body energy expenditure and fat oxidation in healthy and overweight/obese humans^[79]. These findings suggested that SCFAs affect whole-body energy expenditure through a combination of mechanisms in different tissues, including skeletal muscle, the liver, and adipose tissue.

There are several studies show that fatty acid-derived microbial metabolites effect on energy expenditure. KetoA, a linoleic acid-derived microbial metabolite, has been suggested as the regulator of host energy metabolism. The anti-obesity effect of KetoA is shown via the activation of transient receptor potential vanilloid 1 (TRPV1), a member of the TRPV channel family, which has been reported to be important for the regulation of energy metabolism in adipocytes^[80, 81]. KetoA-induced TRPV1 activation enhanced energy expenditure by increasing the function of both BAT and WAT in diabetic mice^[81]. Conjugated linoleic acid (CLA) is also mainly produced from linoleic acid by lactic acid bacteria, and enhanced energy expenditure via increase in the expression of UCPs genes in adipose tissue^[82, 83]. Mice fed CLA-producing bacteria, *Lactobacillus rhamnosus* PL60, are prevented from diet-induced obesity and hepatic

steatosis^[84].

Intestine

The small intestine takes up glucose from the intestinal lumen mainly through sodium-glucose cotransporter 1 and transports glucose from enterocytes to the blood via GLUT2^[85]. Although it has been reported that insulin inhibits the translocation of GLUT2 from the basolateral surface to the apical epithelial membrane, the role of insulin in intestinal glucose uptake is unclear^[85, 86]. Insulin signaling has been implicated in both increased and decreased glucose uptake from the intestinal lumen to the enterocytes in both humans and *in vitro* studies^[87]. Beside the glucose uptake, insulin's action on lipid metabolism and gluconeogenesis seems to be well established in intestine. Insulin modulates lipoprotein metabolism in the intestine and suppresses lipoprotein secretion^[86]. Production of apolipoproteinB48-containing chylomicrons by the small intestine increases insulin resistance^[86, 88]. Like liver, the intestine shows gluconeogenic capacity. Intestinal glucose production is suppressed by insulin and increased in insulinopenic states, such as a 48-hour fasting and type 1 diabetes^[89, 90]. In a postprandial state, intestinal gluconeogenesis (IGN) accounts for about 5–7% of the total endogenous glucose production^[91–93]. A protein-rich diet increases the expression of genes involved in IGN in the intestine and the regulatory enzymes in gluconeogenesis and glutaminase^[94]. **In intestine, the peptides digested from protein-rich diet act on μ -opioid receptors presenting in the portal vein nerves^[94], which signals to the brain and releases of the neuromediator vasoactive intestinal peptide from brain during the postprandial period^[95]. This neuromediator activates adenylate cyclase and increases cAMP levels, which induces the expression of IGN-related genes^[95]. The IGN-related enzymes are progressively induced during the postprandial period, and the amount of enzyme is maintained during the post-absorptive period^[94, 96]. In addition, because protein-rich diets provide major IGN substrates, including glutamine and glutamate, these substrates can be utilized by IGN induced during the**

post-absorptive period^[97]. It was recently reported that IGN protects against diabetes and obesity by suppressing hepatic gluconeogenesis and positively regulating glucose homeostasis^[91, 92]. Gastric bypass surgery also has been reported to increase IGN and suppress hepatic gluconeogenesis in diabetic animal model^[93, 98].

It has been reported that IGN is induced by the SCFAs and succinate, the gut microbial metabolites derived from carbohydrate fermentation^[91, 92]. The SCFA propionate, as a gluconeogenic substrate, activated G6Pase activity and increased IGN gene expression via vasoactive intestinal peptide released from brain through a gut-brain neural circuit involving the free fatty acid receptor 3-dependent stimulation^[91, 95]. Propionate showed the strongest capacity to induce intestinal glucose production among SCFAs^[91]. Another SCFA, butyrate increased the levels of ATP, a substrate of adenylate cyclase, which promoted the production of cyclic AMP^[91]. Cyclic AMP functions as an intracellular messenger that stimulates the expression of genes involved in gluconeogenesis^[99]. Through this mechanism, butyrate promoted gluconeogenesis in enterocytes^[91]. Microbial-derived succinate not only showed an anti-obesity effect but also improved glucose tolerance and insulin sensitivity. Succinate functioned as a gluconeogenic substrate such as propionate, and it was shown to promote activation of gluconeogenesis in the intestine of high-fat and high-sucrose diet-fed mice^[92]. In addition, succinate-fed wild-type mice showed a decreased capacity for hepatic glucose production, and this suppression of hepatic gluconeogenesis was absent in succinate-fed *G6pc* intestinal-specific knockout mice^[92]. In humans, it has been reported that succinate-producing bacteria, including *Bacteroidaceae* and *Prevotella*, were found to be increased in fecal samples of patients with non-alcoholic steatohepatitis^[92, 100].

Conclusions

Since the association between microbiome and metabolic diseases in the last 20 years has been increasingly revealed, microbial metabolites are considered to be the link between microbiome and metabolic diseases. This review summarized the role of microbial metabolites in the major mechanisms representing insulin resistance in each tissue. Through this, in addition to SCFAs, which has been studied a lot in the past, recent studies have found some candidates that protein-derived (e.g., hydrogen sulfide, Indole-3-carboxylic acid, and phenylacetic acid) and lipid-derived microbial metabolites (e.g., KetoA and CLA) can play a role in the pathogenesis of insulin resistance. However, metabolites by gut bacteria are highly diverse depending on intestinal environments (e.g. dietary substrates, host enzyme, acidity, temperature, and antibiotics), yet only limited number of metabolites have been identified and functionally studied in metabolic diseases. Indeed, according to Human Microbiome Database, over 100 thousand of metabolites are existed in our body, but only hundreds are counted as bacterial-specific (<https://hmdb.ca/statistics>).

What makes this even more challengeable is the complex etiology of insulin resistance. In glucose and lipid metabolism, each organ is highly interrelated. Muscle insulin resistance can divert ingested glucose into the liver, and increases hepatic de novo lipogenesis and gluconeogenesis. Adipose tissue insulin resistance can release lipogenic and gluconeogenic substrates to liver as well intestinal IGH reversely control the hepatic gluconeogenesis. In order to develop bacterial metabolites as a therapeutic agent for insulin resistance in humans, not only 1) clarifying the exact mechanism of action for which stage of insulin resistance, but also 2) understanding metabolic complexities between multiple organs should be conducted in parallel. Nevertheless, insulin resistance is a common prerequisite for various metabolic diseases, the discovery of metabolites that specifically act on insulin resistance is a strategy to overcome metabolic diseases in terms of more fundamental etiology and early prevention, and more research should be conducted.

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Figure 1

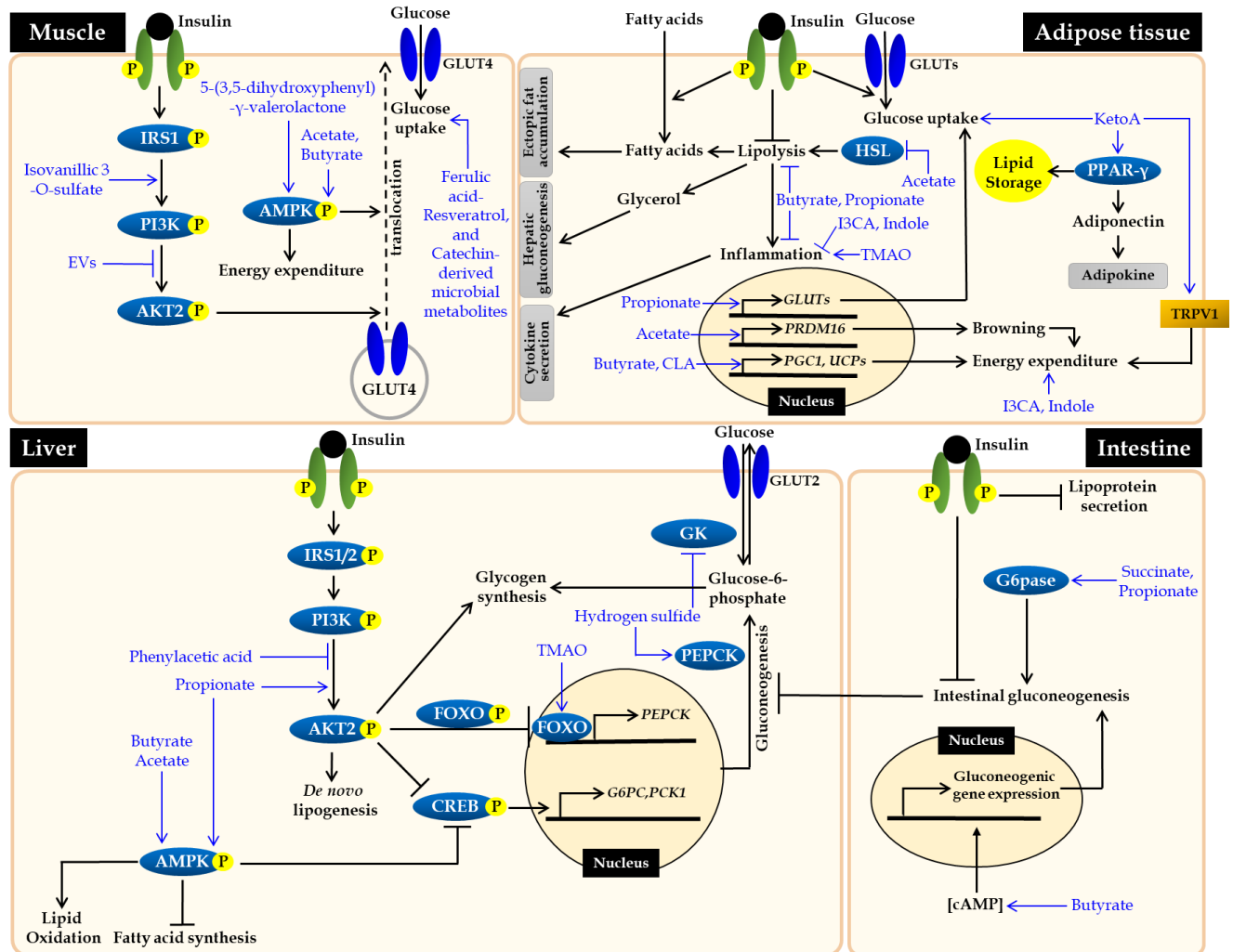


Figure Legends

Figure 1. The mechanisms linking microbial metabolites to insulin resistance. In skeletal muscle, insulin stimulates glucose uptake by translocating glucose transporter (GLUT) 4 via insulin receptor substrate (IRS)-phosphoinositide-3-kinase (PI3K)-AKT signaling. Isovanillic 3-O-sulfate increases glucose uptake by activating PI3K-AKT pathway. Gut bacteria-derived EVs decrease glucose uptake by inhibiting AKT phosphorylation. Glucose uptake is also increased via AMP-activated protein kinase (AMPK) activation, insulin-independent. 5-(3,5-dihydroxyphenyl)- γ -valerolactone activates AMPK phosphorylation, which enhance glucose uptake. Although mechanisms have been unknown, other ferulic acid, resveratrol, and catechin-derived microbial metabolites also enhance glucose uptake (left upper panel).

In liver, insulin activates glycogen synthesis and *de novo* lipogenesis and suppresses gluconeogenesis via IRS-PI3K-AKT signaling. Propionate increases the phosphorylation of both AKT and AMPK, which suppresses gluconeogenesis. Hydrogen sulfide stimulates gluconeogenesis via PEPCK activation and reduces glycogen synthesis via the inhibition of GK activity. TMAO increases gluconeogenesis via PERK-FOXO1 pathway. Phenylacetic acid inhibits AKT phosphorylation. All short chain fatty acids (SCFAs), including acetate, propionate, and butyrate, activate AMPK phosphorylation, which lead to decrease lipid accumulation (left lower panel).

In adipose tissue, insulin stimulates glucose and fatty acid uptake and suppress lipolysis. Failure to suppress lipolysis in insulin-resistant adipose tissue increases circulating free fatty acids and glycerol, which lead to an increase in ectopic fat accumulation in the liver and muscle and stimulates hepatic gluconeogenesis. 10-oxo-12(Z)-octadecenoic acid (KetoA) increases insulin-stimulated glucose uptake and energy expenditure via TRPV2 activation. KetoA also increases the production and secretion of adiponectin via PPAR- γ activation. TMAO increases inflammation in adipocyte. Indole and I3CA have anti-inflammatory effects. CLA enhances energy expenditure by increasing the expression of uncoupling proteins (UCPs) genes. All

SCFAs inhibits lipolysis. Acetate inhibits lipolysis by suppressing HSL and stimulates also browning by increasing the expression of browning-related genes. Butyrate and propionate attenuate inflammation. Propionate increases glucose uptake by increasing GLUT4 expression. Butyrate enhances energy expenditure by upregulating PGC1 and UCPs genes (right upper panel).

The intestine, as discussed in this review, is an organ that actively interacts with gut bacteria and accumulates microbial metabolites. Intestinal lipoprotein secretion and gluconeogenesis are suppressed by insulin. In intestine, propionate and succinate act as gluconeogenic substrate, which activate gluconeogenesis via G6Pase activation. Butyrate increases cAMP levels, which upregulates the expression of gluconeogenic genes and increases gluconeogenesis. Through this mechanisms, increased intestinal gluconeogenesis suppresses hepatic gluconeogenesis (right lower panel).

Black lines represent insulin resistance-related events and blue lines represent action of metabolites. Grey boxes represent the effects of adipose tissue on other tissues. EVs, Extracellular vesicles; PEPCK, Phosphoenolpyruvate carboxykinase; GK, Glucokinase; TMAO, Trimethylamine N-oxide; PERK, PKR-like ER kinase; FOXO1, Forkhead box protein O1; CREB, cAMP-response element binding protein; G6PC, Glucose 6-phosphatase catalytic subunit; PCK1, Phosphoenolpyruvate carboxykinase 1; TRPV1, Transient receptor potential vanilloid 1; PPAR, Peroxisome proliferator-activated receptor; I3CA, Indole-3-carboxylic acid; CLA, Conjugated linoleic acid; HSL, Hormone-sensitive lipase; PRDM16, PR domain containing 16; PGC1, Peroxisome proliferator-activated receptor-gamma coactivator 1; G6Pase, Glucose 6-phosphatase; cAMP, Cyclic adenosine monophosphate.

Table 1. The effects of **diet-derived** gut bacterial metabolites on the pathogenesis of insulin resistance in various organs.

Category	Metabolite	Target organ	Effects	Ref
Carbohydrate				
Fiber-derived	Acetate	Skeletal muscle	Increased lipid oxidation <i>in vivo</i>	[75]
		Liver	Decreased lipogenesis <i>in vivo</i>	[47, 51]
			Increased lipid oxidation <i>in vivo</i>	[47, 51-53]
		Adipose tissue	Stimulated adipogenesis <i>in vitro</i>	[60]
			Inhibited lipolysis <i>in vitro</i> and <i>in vivo</i>	[59-61]
			Increased browning <i>in vitro</i> and <i>in vivo</i>	[53, 73]
	Propionate	Whole body	Increased energy expenditure and fat oxidation <i>in vivo</i> and in humans	[47, 77, 78]
		Liver	Suppressed gluconeogenesis <i>in vitro</i>	[29]
			Decreased lipogenesis <i>in vivo</i>	[47]
			Increased lipid oxidation <i>in vivo</i>	[47]
		Adipose tissue	Increased adipogenesis <i>in vitro</i>	[60]
			inhibit lipolysis <i>in vitro</i> and <i>in vivo</i>	[59, 60]
			Improved inflammation <i>in ex vivo</i>	[66]

		Intestine	Promoted gluconeogenesis <i>in vivo</i>	[91]
		Whole body	Increased energy expenditure and fat oxidation <i>in vivo</i> and in humans	[47, 77, 79]
	Butyrate	Skeletal muscle	Increased lipid oxidation <i>in vitro</i> and <i>in vivo</i>	[48]
		Liver	Decreased lipogenesis <i>in vivo</i>	[47]
			Increased lipid oxidation <i>in vivo</i>	[47-49]
		Adipose tissue	decreased lipolysis <i>in vitro</i>	[67]
			Improved inflammation <i>in vitro</i>	[67]
			Increased thermogenesis <i>in vivo</i>	[48, 74]
		Intestine	Promoted gluconeogenesis <i>in vitro</i> and <i>in vivo</i>	[91]
		Whole body	Increased energy expenditure and fat oxidation <i>in vivo</i> and in humans	[47, 48, 77]
	Succinate	Intestine	Promoted gluconeogenesis <i>in vivo</i>	[92]
Protein				
Protein-derived	Hydrogen sulfide	Liver	Increased gluconeogenesis <i>in vitro</i>	[32]
			Decreased glycogen synthesis <i>in vitro</i>	[32]
	Indole	Adipose tissue	Increased inflammation <i>in vivo</i>	[10]

	Indole-3-carboxylic acid	Adipose tissue	Increased inflammation <i>in vivo</i>	[10]
	Phenylacetic acid	Liver	Increased lipogenesis in <i>ex vivo</i> and <i>in vivo</i>	[46]
Lipid and Others				
Linoleic acid-derived	10-oxo-12(Z)-octadecenoic acid	Adipose tissue	Induced adipogenesis <i>in vitro</i>	[55]
			Increased thermogenesis <i>in vivo</i>	[81]
	Conjugated linoleic acid	Adipose tissue	Increased energy expenditure	[82-84]
Ferulic acid-derived	Ferulic acid 4-O-sulfate and Dihydroferulic acid 4-O-sulfate	Skeletal muscle	Increased glucose uptake <i>in vitro</i>	[19]
Resveratrol-derived	<i>Trans</i> -resveratrol 4'-O-glucuronide and <i>Trans</i> -resveratrol 3-O-sulfate	Skeletal muscle	Increased glucose uptake <i>in vitro</i>	[19]
Berries-derived	Isovanillic acid 3-O-sulfate	Skeletal muscle	Increased glucose uptake <i>in vitro</i>	[19]
Catechin-derived	4-hydroxy-5-(3,4,5-trihydroxyphenyl) valeric acid, 5-(3,4,5-trihydroxyphenyl)- γ -valerolactone, and 5-(3-hydroxyphenyl) valeric acid	Skeletal muscle	Increased glucose uptake <i>in vitro</i>	[22]
Catechin-derived	5-(3,5-dihydroxyphenyl)- γ -valerolactone	Skeletal muscle	Increased glucose uptake <i>in vitro</i> and <i>in vivo</i>	[22]
Bacteria-derived	Extracellular vesicles	Skeletal muscle	Decreased glucose uptake <i>in vivo</i>	[20]
Choline-derived	Trimethylamine N-oxide	Liver	Increased gluconeogenesis in <i>ex vivo</i> and <i>in vivo</i>	[33, 43]

Adipos e tissue	Promoted inflammation <i>in vivo</i>	[43]
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