The Gut Impacts Diabetic Management Tomorrow: The Recent Messages from Intestine and Microbiota

Abstract

The number of diabetic patients tremendously increased worldwide constituting a grave health problem at present. Lifestyle changes including high-fat high-sugar diet and poor physical activity affect diabetes risk. Persistent, low-grade inflammatory responses in obese patients with metabolic syndrome are considered to play a cardinal role in the development and progression of type 2 diabetes. Emerging experimental and clinical evidence indicates that gut dysbiosis, intestinal barrier dysfunction and resultant metabolic endotoxaemia are closely related to the inflammation, insulin resistance and finally cardiovascular events in patients with type 2 diabetes. Gut microbiome transmitted from mother to child at birth is profoundly affected by eating habits in life thereafter. In the faeces of type 2 diabetics, relatively higher abundance in endotoxin producing gram-negative bacteria and lower abundance in butyrate-producing bacteria were reported. As butyrate is an important energy source and protector of intestinal barrier, its defect may enhance intestinal hyperpermeability and endotoxaemia. Inflammation in the adipose tissue provokes detrimental effects on other organs through secreted pro-inflammatory cytokines. Activation of Toll-like receptor 4 in immune cells such as macrophages evokes inflammation and insulin resistance, finally leading to an impairment of insulin signalling and β-cell failure. Inflammatory changes in the arterial vessels and liver lead to two life-threatening states, ischemic heart disease and liver cirrhosis, respectively. Meticulous management strategies to improve gut dysbiosis may pave the way for effective pharmacotherapy and lower the morbidity and mortality of type 2 diabetes. Biguanide derivative metformin is known to have an anti-inflammatory effect in addition to its glucoregulatory function. There is a possibility that two newly developed diabetic drugs, dipeptidyl-peptidase-4 (DPP-4) inhibitors and sodium-glucose co-transporter 2 (SGLT2) inhibitors, may have some undetermined effect on inflammation, which is worth investigating. This review summarizes the bulk of latest information on type 2 diabetes, endotoxaemia and gut dysfunction.

Keywords: Type 2 diabetes, Gut dysbiosis, Intestinal permeability, Endotoxaemia, Inflammation, Prognosis, Dietary therapy, Metformin, Dipeptidyl-peptidase-4 (DPP-4) inhibitors, Sodium-glucose cotransporter (SGLT)-2 inhibitors

Introduction

The estimated number of diabetic patients is rising globally, from 108 million in 1980, to 422 million in 2014 [1]. Besides genetic predisposition [2-4], this tremendous explosion of diabetic patients should be attributable to life style changes, diet and nutritional status and physical activity. It is now clear that the progressive pancreatic β-cell failure that drives the deterioration of metabolic control over time begins early and may be present before the diagnosis of diabetes [5]. Obesity
and pre-diabetes as underlying risk factors for type 2 diabetes and associated complications should be intensively targeted in the diabetes prevention programme. Although lifestyle optimization is fundamentally indicated for all overweight and obese patients with prediabetes, needed pharmacotherapy can be initiated simultaneously for preserving B-cell function in type 2 diabetics [6].

Recently the potential role of the intestinal epithelial barrier dysfunction and increased permeability associated with gut dysbiosis has been described in variable human diseases, from the intestinal diseases such as inflammatory bowel disease [7-12] and irritable bowel syndrome [13,14] to liver [15-24], pancreas [25-27], kidney [28-30], heart [31,32] brain [33-35] and systemic autoimmune and allergic diseases [36-38]. Among them, type 2 diabetes mellitus is one of the most popular entities. The gut barrier derangement related to dysbiosis may induce the intestinal translocation of bacterial fragments and the development of "metabolic endotoxaemia", leading to systemic low-grade inflammation and insulin resistance [39].

Adipose tissue is a massive source of bioactive substances known as adipocytokines (adipokines) [40]. Adipokine dysregulation attributable to endotoxaemia or excessive fatty acids may induce obesity-related metabolic disorders, called metabolic syndrome, a cluster of metabolic abnormalities, including type 2 diabetes, hypertension, hyperlipidemia, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) [40-42]. The imbalance between excessive pro-inflammatory adipokines such as tumor necrosis factor-α (TNF-α) and defective anti-inflammatory adipokines such as adiponectin is a cardinal pathogenetic finding in the adipose tissue of obese subjects with metabolic syndrome [43].

The pathogenesis of type 2 diabetes is closely related to the intestine. The basic lifestyle management begins with dietary therapy, which should be based on the knowledge of gut microbiome, because diet itself directly influences gut microbiota. Needless to say, the effect of diabetic pharmacotherapy is strengthened by ideal diet control. This review introduces latest knowledge of gut dysbiosis, intestinal hyperpermeability, endotoxaemia and their implications in the management of type 2 diabetes.

Gut Dysbiosis and its Effects

Gut microbiota, a complex intestinal "superorganism", affects host metabolic balance modulating energy absorption, gut motility, appetite, glucose and lipid metabolism, as well as hepatic fatty storage [39]. Small intestinal bacterial overgrowth (SIBO) is a condition in which colonic bacteria translocate into the small bowel due to impaired microvilli function, which causes a breakdown in intestinal motility and gut homeostasis [44,45]. Most controlled trials demonstrated higher prevalence of SIBO (50~77.8% vs. 9.1~31.2%) in NAFLD patients compared with healthy subjects [20,46-50]. SIBO was also reported to be associated with enhanced hepatic expression of Toll-like receptor (TLR) 4 and release of interleukin (IL)-8 in NASH patients [48].

In accordance with these findings, SIBO was also reported in 43% of diabetic patients with chronic diarrhea, and 75% had a significant improvement in their symptoms after being treated with antibiotics [51]. Additionally, in a group of 82 diabetic patients, of those who had carbohydrate malabsorption on an oral glucose tolerance test, 75% were diagnosed with SIBO [52,53].

Accumulating data in animal and human studies suggest that obesity and type 2 diabetes are associated with a profound gut dysbiosis [54]. Ley et al. [55] first reported in their preliminary study that obese patients had higher abundance of Firmicutes and lower abundance of Bacteroidetes than did lean controls. Turnbaugh et al. [56] showed that the microbial changes affect the metabolic potential of the mouse gut microbiota in the way that the obese microbiome has an increased capacity to harvest energy from the diet. They found that microbiota transplantation from obese mice fed a high-fat diet to lean germ-free recipients promoted greater fat deposition than recipients from lean donors [57]. They further established the human gut ecosystem in mice by transplanting human faecal microbial communities into germ-free mice and demonstrated that the high-fat, high-sugar diet altered microbiome gene expression [58]. These mice had increased adiposity and their microbiota showed an increased abundance of Erysipelotrichi class of bacteria and the Bacteroidetes (mainly Enterococcus) within the Firmicutes phylum as well as a decreased abundance of members of the Bacteroidetes [58]. In contrast to these clear-cut experimental data, the succeeding clinical investigations on the gut microbiome have given variable and sometimes contradictory results as summarized in my previous review [19]. Several studies [59-61] even reported an increased Bacteroidetes/Firmicutes ratio in obese subjects.

In addition to microbial cells or microbial structural components, microbial metabolites also affect the health and disease of the host [19]. Human colonic microbiota break down substrates such as resistant starch and nonstarch polysaccharides (major components of dietary fiber), which are not completely hydrolyzed by host enzymes in the small intestine [62]. The main fermentation products ensuing from this fiber breakdown are the short chain fatty acids (SCFAs) : acetate, propionate, and butyrate [62].

Changes in intestinal microbiota associated with clinical studies on type 2 diabetes are summarized in Table 1. As pointed out by the previous review [63], the results are again variable, while interesting common microbiota features are noted. Larsen et al. [64] reported that faeces in type 2 diabetes patients were relatively enriched with endotoxin producing gram-negative bacteria, belonging to the phyla Bacteroidetes and Proteobacteria. Chinese patients with type 2 diabetes were characterized by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria (Clostridiales sp. S53/4, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis and Roseburia inulinivorans etc.) and an increase in various opportunistic pathogens, as well as an enrichment of other microbial functions conferring sulphate reduction and oxidative stress resistance [65]. Another large scale study from Sweden also revealed decreased butyrate-producing bacteria such as Roseburia and Eubacterium eligens.
in female patients with type 2 diabetes [66]. Second study from China [67] showed a lower abundance of butyrate-producing *F. prausnitzii* in prediabetic subjects than those with normal glucose tolerance. Among SCFAs, butyrate is an important energy source for intestinal epithelial cells and has potent effects on a variety of colonic mucosal functions, reinforcing the colonic defence barrier and decreasing oxidative stress [68]. Butyrate was also reported to enhance the intestinal barrier by regulating the assembly of tight junctions (TJs) by the activation of AMP-activated protein kinase (AMPK) [69]. Decreased *Roseburia* and *Eubacterium* compared with healthy controls have been noted in patients with symptomatic atherosclerosis, defined as stenotic atherosclerotic plaques in the carotid artery leading to cerebrovascular events [70]. The counts of the *Clostridium cocoides* group, *Atopobium* cluster, and *Prevotella* were significantly lower, while the counts of total *Lactobacillus* were significantly higher in faecal samples of Japanese type 2 diabetic patients than in those of control subjects [71].

Of note, Zhang et al. [72] further reported significantly lower abundance of *Akkermansia muciniphila* in type 2 diabetic patients. Although *A. muciniphila* is a relatively low-abundance bacteria, it has the potential to affect host metabolism profoundly. This mucin-degrading bacterium in the mucus layer was reported to reverse high-fat diet-induced metabolic disorders: fat-mass gain, metabolic endotoxaemia, adipose tissue inflammation, and insulin resistance [73]. *A. muciniphila* also increases the intestinal levels of endocannabinoids that control the inflammation, gut barrier, and gut peptide secretion [73]. Overweight and obese adults with higher gene richness and *A. muciniphila* abundance exhibited the healthiest metabolic status, particularly in fasting

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**Table 1:** Changes in intestinal microbiota on patients with type 2 diabetes. ("↑" means increased abundance of bacteria in patients vs. normal subjects, "↓" means decreased abundance of bacteria in patients vs. normal subjects, Superior numbers are related reference numbers and Butyrate-producing bacteria are marked with asterisk (*))

| Phylum      | Class              | Order              | Family                  | Genus          | Species                  |
|-------------|--------------------|--------------------|-------------------------|----------------|--------------------------|
| Firmicutes  | Bacilli↑, 64       | Lactobacilales     | Lactobacillace↑, 67     | Lactobacillus↑, 66, 72 | *L. gasseri*↑, 66       |
|             |                    |                    |                         |                | *L. plantarum*↑, 72      |
|             |                    |                    |                         |                | *L. reuteri*↑, 72        |
| Aerococcales| Abiotrophia↑, 67   |                    |                         |                |                          |
| Streptococcales| Streptococcus↓, 67 |                    |                         |                |                          |
| Enterococcales| Enterococcus      |                    | Clotridiaceae↑, 64, 66  | Clotridium↓, 64, 66 | *Clotridiales ss3/4↓, 65↑, 67 |
| Clostridia  | Clotridiale↑, 66, 67|                    |                         |                | *C. cocoides*↓, 71       |
|             |                    |                    |                         |                | *C. hathewayi*↑, 65      |
|             |                    |                    |                         |                | *C. ramosum*↑, 65        |
|             |                    |                    |                         |                | *C. symbiosu*↑, 65       |
|             |                    |                    |                         |                | *C. clostridioforme*↑, 66 |
|             | Sporobacter↑, 67   |                    |                         |                |                          |
|             | Subdoligranulum↑, 67|                    |                         |                |                          |
| Peptostreptocooccus| Eubacterium↑, 67 |                    |                         |                | *E. rectale*↑, 65        |
|             |                    |                    |                         |                | *E. eligens*↑, 66        |
| Ruminococcales| Ruminococcus↑, 67 |                    |                         |                |                          |
| Faecalibacterium|                |                    |                         |                | *F. prausnitzii*↓, 65, 67|
| Lachnospiraceae| *Roseburia*        |                    |                         |                | *R. intestinales*↓, 65   |
|             |                    |                    |                         |                | *R. inulinivoran*↓, 65   |
|             | *Roseburia_272*↓, 66|                    |                         |                |                          |
| Dorea       |                    |                    |                         |                |                          |
| Actinobacteria| Coriobacteriaia  | Coriobacteriales    | Coriobacteriaceae↓, 65  | Coriobacterium| *A. muciniphila*↓, 67    |
|             |                    |                    |                         |                |                          |
|             |                    |                    |                         |                | *Collinsella*↑, 67, 75   |
|             | Eggerthellae      | Eggerthellaceae     | Eggerthella            | E. fermentelian↑, 65 |
| Verrucomicrobia| Verrucomicrobiae↓, 67| Verrucomicrobiae    | Verrucomicrobiae       | Akkermansia     | A. muciniphila↓, 67      |
| Bacteroides | Bacteroidia        | Bacteroidales       | Bacteroidaceae       | Bacteroides↓, 67| B. intestinalis↓, 66     |
|             |                    |                    |                         |                | B. caccae↑, 63          |
| Proteobacteria| β-proteobacteria↑, 64|                    |                          |                |                          |
|             | γ-proteobacteria↑, 64| Pasturellales      | Pasteurellaceae         | Haemophilus↓, 67| E. coli↑, 65             |
|             |                    |                    |                         |                |                          |
|             | Enterobacteriales  | Enterobacteriaceae  |                          |                |                          |
|             |                    |                    |                         |                |                          |

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plasma glucose, plasma triglycerides and body fat distribution. Individuals with higher baseline *A. muciniphila* displayed greater improvement in insulin sensitivity markers and other clinical parameters after 6-week calorie restriction [74].

Recently, Lambeth et al. [75] studied the characteristics of the gut microbiome in prediabetes and type 2 diabetes, compared with non-diabetic subjects. *Collinsella* and an unknown genus belonging to family *Enterobacteriaceae* were significantly increased in type 2 diabetes. The increase in *Collinsella* in type 2 diabetes, also reported in the study by Zhang et al. [67], has been associated with symptomatic atherosclerosis in the study by Karlsson et al. [70]. Sato et al. [71] found that the faecal concentrations of total organic acid, acetate and propionate were significantly lower and that the level of faecal total organic acids were closely correlated with carbohydrate intake, and negatively correlated with the intakes of total fat and saturated fatty acids in patients with type 2 diabetes. Organic acids in faeces promote elimination of *Escherichia coli* O-157 [76] and SCFAs stimulate the release of anorectic hormones like glucagon like peptide-1 (GLP-1) and PYY from intestinal L-cells [77,78]. Taken together, the low faecal levels of organic acids might be harmful by causing worsening of glycemic control through the reduction of postprandial incretin secretion and the increased susceptibility to infection in type 2 diabetics [71].

### Intestinal Permeability

The intestinal barrier prevents the entry of pathogenic microorganisms and toxic luminal substances while regulating the absorption of nutrients, electrolytes and water from the lumen into the circulation [33]. These functions are preserved by a complex multilayer system including a mucus layer and a monolayer of epithelial cells interconnected by TJs. An intact intestinal barrier prevents the permeation of antigens, endotoxins, pathogens, and other pro-inflammatory substances into the human body, whereas intestinal dysintegrity allows their entry, which may trigger local or systemic inflammation and disease [79]. Assessment of intestinal barrier function in humans is currently possible by using intestinal permeability assays, and by the assessment of biomarkers of epithelial integrity such as soluble adhesion molecules, or bacterial markers like circulating endotoxin.

NAFLD patients were reported to present increased gut permeability characterized by disruption of the intercellular TJs with decreased TJ protein ZO-1 expression, which is likely to be the underlying mechanism of translocations of bacteria and their products [20]. Intestinal permeability is also increased in children with NAFLD, and correlates with the severity of steatohepatitis [21].

Horton et al. [80] recently reported that intestinal permeability measured with 51Cr-EDTA urinary recovery significantly increased in patients with type 2 diabetes and that it was correlated to increased levels of systemic inflammatory markers such as high-sensitivity C-reactive protein, IL-6 and TNF-α. Zhang et al. [81] noted that serum levels of zonulin, a useful marker of intestinal permeability [82], was significantly increased in newly diagnosed Chinese Type 2 diabetic patients, and the zonulin level was associated with dyslipidemia, inflammation and insulin resistance. Circulating zonulin level was also positively correlated with body mass index (BMI), fasting insulin, triglycerides, and IL-6 levels, and negatively correlated with insulin sensitivity in Caucasian male patients [83]. Genetically obese ob/ob and db/db mice showed enhanced intestinal permeability, profoundly modified distribution of occludin and ZO-1 in the intestinal mucosa together with higher circulating levels of inflammatory cytokines and portal endotoxaemia compared with lean control mice [84]. In mice fed high-fat diet, not only bacterial products but also complete living bacteria can be translocated from the intestinal lumen towards adipose tissues [85].

### Metabolic Endotoxaemia

Lipopolysaccharide (LPS), often referred to as endotoxin, represents the major constituent of the outer cell membrane of gram-negative bacteria and crosses the gut mucosal membrane to enter the circulation and directly stimulates inflammatory pathways. It can cross the deranged paracellular TJ or can be taken up by the enterocytes coupled with damaging lipoproteins, because it has a strong affinity for chylomicrons [86].

Studies in both animal models and humans have shown that a high-fat diet can modulate the gut microbiota and increase circulating levels of endotoxin [63]. Morbidly obese patients with the highest postprandial hypertriglyceridemia showed a significant increase in endotoxin levels in serum and the chylomicron fraction after the fat overload [87]. Although baseline endotoxin level was already significantly higher in patients with type 2 diabetes and impaired glucose tolerance (IGT) than in nonobese control subjects, ingestion of a high-fat meal further led to a significant rise in endotoxin levels in type 2 diabetic, IGT, and obese subjects [88]. This suggests that a continual snacking cumulatively promotes their pro-inflammatory conditions in type 2 diabetic and IGT subjects due to the constant exposure to endotoxin [88].

Besides paracellular leakage of endotoxin across the intestinal epithelium, there exits more physiological route of endotoxin entry. It has been demonstrated that enterocytes can internalize gram-negative bacteria through TLR4 and endotoxin through myeloid differentiation protein-2 (MD-2)-dependent mechanism [89-91]. Endotoxin is transported to Golgi compartment of the enterocyte, where newly assembled chylomicrons are located before their basolateral secretion [92-94]. Chylomicrons rapidly bind endotoxin [95] and promote endotoxin uptake by enterocyte [94,96]. In fact, the morbidly obese patients with the highest postprandial hypertriglyceridemia showed a significant increase in endotoxin levels in serum and the chylomicron fraction after the fat overload [87]. As chylomicrons inhibit endotoxicy and cell activation [95], excess endotoxin attributable to paracellular leakage may strongly enhance inflammatory states in type 2 diabetics. In accordance with these findings, a large cohort study revealed that endotoxaemia was strongly associated with cardiometabolic disorders [97].

The lack of endotoxin tolerance in macrophages of type 2 diabetes can be explained by the fact that low-dose subclinical
endotoxaemia induces low-grade inflammation via IRAK-1 and Tollip and fails to activate the classical nuclear factor-κB (NFκB) pathway causing an anti-inflammatory resolution [98]. Moreover, as described below, obesity reduces the production of adiponectin [43], which is known to promote endotoxin tolerance [99,100].

Figure 1 summarizes relationships of gut dysbiosis, intestinal hyperpermeability and endotoxaemia to the progression of metabolic syndrome and type 2 diabetes.

**Low-Grade Inflammation**

Increased intestinal permeability is thus considered to induce microbial translocation, metabolic endotoxaemia [101] and low-grade inflammation in patients with obesity, NASH and type 2 diabetes. Low-grade chronic inflammation in these patients was probably triggered by activation of TLR2 and TLR4. TLR4 is activated by endotoxin and fatty acids, which results in activation of NFκB and release of pro-inflammatory cytokines such as IL-6, IL-1β, TNF-α, and monocyte chemotactic protein-1 (MCP-1) [42].

Human adipose tissue is an active site of innate immune response, through activation of TLRs and downstream NF-kB signaling [102]. It also contains a large number of macrophages and thus may work as a first line of defence against superficial wounds. To cope with marked positive energy balance, the adipose tissue in obese subjects is destined to develop chronic low-grade inflammation, which induces secondary effects on other organs like muscle and liver through the inflammatory adipocytokine [102]. The adipose tissue in obese and NASH patients can be expanded with both hyperplasia and hypertrophy, coupled with increased macrophage infiltration [103], where adipocytes and recruited macrophages trigger the inflammatory responses via overexpression of TLR2, TLR4 and MyD88 [102,104]. In contrast, anti-inflammatory, anti-diabetic and anti-atherogenic adiponectin expression is suppressed in the adipose tissue of obese subjects [105]. Its production is reduced in subjects with visceral fat accumulation and its plasma levels are negatively correlated with visceral adiposity [43].

Leptin is an adipokine whose major effects are to reduce food intake and to increase energy expenditure [106]. Although total leptin levels are elevated in obese patients, its action is not amplified due to the condition called leptin resistance [107]. Rajala et al. [108] demonstrated that pair-fed leptin receptor-deficient (db/db) mice displayed significant changes in expression of various antimicrobial peptides and a shift in faecal microbial composition toward a decrease in the Bacteroidetes to Firmicutes ratio. These suggest that the leptin receptor signaling has a role in modulating microbiota composition, although it is not still clear whether leptin signaling regulate antimicrobial peptides, which in turn regulates the microbiota, or does leptin signaling directly regulate the microbiota [109]. It is plausible that defective leptin signaling may enhance SIBO or gut dysbiosis together with dietary habit in obese diabetic patients. Clinical data are lacking to support the issue at present, but this is worth investigating.

Low-grade inflammation in metabolic syndrome forms NAFLD in the liver. It includes a spectrum of pathological changes ranging from the simple fatty liver (NAFL) through NASH to fibrosis, cirrhosis, and hepatocellular carcinoma [20]. Diabetic patients have a twofold to threefold higher risk of dying of chronic liver diseases, mainly associated with a non-virus and non-alcohol-related etiology, which is largely attributable to NAFLD [110]. Patients with NASH revealed endotoxaemia and overexpression of TLR4 protein in the liver [111,112] associated with pro-inflammatory cytokine release and systemic inflammation. Plasma endotoxin levels and hepatic TLR4 mRNA expression were proved to be higher in NASH patients compared with NAFL patients [113]. SIBO relevant in NASH patients is also associated with enhanced hepatic expression of TLR4 and release of IL-8 [48].

Type 2 diabetic subjects also revealed significantly increased TLR2, TLR4 mRNA and proteins in the peripheral blood mononuclear cells (PBMCs) compared with control subjects [114]. The increased TLR expressions were correlated with body mass index (BMI), homeostasis model assessment-insulin resistance (HOMA-IR), glucose, HbA1C, and free fatty acid in the blood [114]. Another study [104] showed that increased TLR2 and TLR4 expressions in PBMCs were also correlated with TNF-α and IL-6 expressions in PBMCs and fasting blood glucose and HbA1c levels in the blood. The elevated circulatory ZO-1 and endotoxin levels were correlated to inflammatory markers and poor glycemic/lipid control [115].
Diabetic complications are also associated, in part, with the release of endogenous TLR ligands that lead to activation of TLR signaling [116]. TLR1, 2, 4, and 6 mRNA expressions were increased significantly in wounds of diabetic patients compared with non-diabetic wounds [117]. Although several experimental studies have suggested that other pattern recognition receptors NOD-1 and TLR9 are also related to low-grade inflammation and insulin resistance, no study has demonstrated so far the increases in their ligands, peptidoglycan moieties and bacteria-derived cytosine phosphate guanine (CpG)-containing DNA, respectively, in obese or diabetic patients [94]. In addition to endotoxin, glucose solution itself stimulated TLR4 expression and induced TNF-α and IL-6 secretion in the abdominal subcutaneous adipose tissue and isolated abdominal subcutaneous adipocytes [118]. It induced similar TLRs expression and cytokine secretion of PBMC [119].

**Insulin Resistance and β-cell Dysfunction**

Insulin as a main regulator of glucose homeostasis initiates its biological effects through activation of the insulin receptor. After tyrosine autophosphorylation, insulin receptor substrate (IRS)-1 and IRS-2 bind and activate phosphatidylinositol 3-kinase (PI3-K), which increases serine phosphorylation of Akt, leading to glucose transport in the muscle and adipose tissue, glycogen synthesis in the muscle and liver and lipogenesis in the adipose tissue [94]. Glucose, lipids and endotoxin are major three factors evoking low-grade inflammation and insulin resistance [102]. Lipotoxicity with raised circulating free fatty acids are associated with increased insulin resistance [102]. TLRs, especially TLR2 and TLR4, induce insulin resistance, which is pivotal in the pathogenesis of obesity and metabolic syndrome [42]. Enhancement of inflammatory pathways due to TLR activation leads to an impairment of the insulin signaling, such as decreased phosphorylation of the insulin receptor, IRS and Akt, as well as increased inhibitory serine phosphorylation of IRS-1 [94]. Activation of TLR4 by endotoxin in preadipocytes increases the expression of TNF-α and IL-6, which impair the insulin signaling in adipocytes [120]. Endotoxin can promote the expression of iNOS and thus interfere with the insulin signaling [121]. Excessive production of nitric oxide worsens insulin resistance by hampering lipoprotein lipase (LPL) activity and increasing lipolysis and circulating fatty acids [94,122].

Low-grade inflammation and innate immune system activation further lead to β-cell failure [116]. TLR4 expression is elevated in fat, muscle and pancreatic islet cells, including β-cells, and resident macrophages in insulin-resistant mice [123]. Expression of TLR4 in db/db mouse islets increased in parallel with hyperglycemia, which was associated with increased expression and secretion of TNF-α, IL-1 and IL-6 [123]. Endotoxin impairs insulin gene expression (PDX-1 and MafA mRNA levels) of human and rat islets via TLR4 and NF-kB signaling [124]. Importantly, the effects of endotoxin on the insulin gene in human islets are observed at concentrations similar to the circulating levels during endotoxaemia, which suggests that direct repression of the insulin gene might contribute to the metabolic disturbances associated with alterations of the gut microbiota [124].

On the other side, TLR4 or TLR2 deletion improved diet-induced insulin resistance and inflammation of adipose tissue in mice [116]. Diabetic islets have 40% fewer TLR4 positive β-cells, but twice the number of TLR4 positive macrophages as compared to healthy islets [125]. The TLR4 responsiveness is elevated in the diabetic mouse islets, which is mainly mediated by newly recruited macrophages [125]. The TLR4 positive macrophages induce apoptosis of β-cells and induce β-cell dysfunction measured as reduced glucose stimulated insulin secretion in mouse islets [125].

**Possible Relation of Pharmacotherapy to Gut Microbiota.**

**Metformin**

A biguanide derivate metformin has been used widely in the treatment of type 2 diabetes for over 50 years. It offers the major clinical advantage of not inducing hypoglycemia or weight gain and ameliorates hyperglycemia with remarkable cardiovascular safety [126]. The main effect of this drug is to decrease hepatic glucose production, mostly through a transient inhibition of the mitochondrial respiratory-chain complex 1 [127]. The resulting decrease in hepatic energy status (reduction of ATP and accumulation of AMP) activates the AMPK, a cellular metabolic sensor, providing a generally accepted mechanism for metformin effect on hepatic gluconeogenesis [127]. However, metformin has pleiotropic effects beyond glucose reduction, including improvement of lipid profiles and lowering microvascular and macrovascular complications associated with type 2 diabetes [128]. These effects have not been totally ascribed to AMPK activation, because intravenous metformin is less effective than oral medication, which suggests important gut pharmacology of the drug [128]. Napolitano et al. [128] settled an on-off study of metformin administration in type 2 diabetic patients and found that metformin withdrawal was associated with a reduction of GLP-1 and elevation of serum bile acids, especially cholic acid and its conjugates. Microbiota abundance of the phylum **Firmicutes** was positively correlated with changes in cholic acid and its conjugates, while **Bacteroidetes** abundance was negatively correlated with them [128]. This means that metformin is considered to enhance GLP-1 secretion and suppress serum bile acids levels and faecal **Firmicutes/Bacteroidetes** ratio.

Interestingly, metformin is known to stimulate mucin formation just like **Akkermansia muciniphila**. Metformin treatment induces intestinal mucin 2 and mucin 5 expression, and increases **Akkermansia** in an in vitro culture system [129]. Metformin and **Akkermansia** administration were also associated with the downregulation of elevated IL-1β and IL-6 mRNA expression in visceral adipose tissue of mice fed a high-fat diet, suggesting that metformin like **Akkermansia** improves the metabolic profile of diet-induced obesity by ameliorating low-grade tissue inflammation [130].
the plasma and tissue levels of TNF-α and IL-6 and increased activating transcription factor-3 (ATF-3) expression in spleen and lungs, which further supports that metformin exhibits anti-inflammatory action in macrophages [131]. Moreover, high-dose metformin provides anti-inflammatory effects, protects against oxidative stress and extends the lifespan of middle-aged mice by approximately 6% [132].

Atherosclerosis-associated cardiovascular diseases are the major complications of diabetes, where inflammation plays a pivotal role [133]. Metformin has been admitted as the first choice drug for most patients with type 2 diabetes, because its reduces cardiovascular morbidity and mortality [134]. Further human study may be needed concerning its effects on gut dysbiosis and inflammatory parameters in relation to cardiovascular advantage.

**DPP-4 inhibitors**

The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 are secreted from enteroendocrine cells in the gut and regulate physiological and homeostatic functions related to glucose control, metabolism and food intake [135]. Native GLP-1 has a half-life of less than 2 min as a result of degradation by dipeptidyl-peptidase-4 (DPP-4) mainly secreted by endothelial cells and rapid renal clearance of both the intact and degraded GLP-1 molecules [136,137]. DPP-4 inhibitors are newly developed anti-diabetic drugs that improve glucose metabolism by raising the concentration and duration of active GLP-1 [138,139]. In this way, DPP-4 inhibitors stimulate glucose-dependent insulin secretion and suppress glucagon secretion [6].

DPP-4 on the other hand acts as a multifunctional regulatory protease for cytokines, chemokines, and neuropeptides involved in inflammation, immunity, and vascular function [140]. Anti-inflammatory effects together with anti-diabetes property has opened up a new possibility for the use of DPP-4 inhibitors. Makdissi et al. [141] reported that DPP-4 inhibitor sitagliptin exerts a potent anti-inflammatory effect on type 2 diabetic patients. After 12 week of sitagliptin administration, the mRNA expressions of TNF-α, TLR4, TLR2, JNK-1, IKKβ, and NLRP3 inflammasome, TLR4 and IL-1β expression were significantly attenuated in obese mice [141]. In diabetic apolipoprotein E-deficient mice fed high-fat diet, alogliptin attenuated diabetes-augmented IL-6 and IL-1β expression [142]. In diabetic apolipoprotein E-deficient mice fed high-fat diet, alogliptin attenuated diabetes-augmented IL-6 and IL-1β expression in atherosclerotic plaques and inhibited TLR4-mediated upregulation of IL-6, IL-1β, and other pro-inflammatory cytokines by mononuclear cells [133]. Linagliptin induced a down-regulation of the pro-inflammatory markers cyclooxygenase-2 and macrophage inflammatory protein-2 (MIP-2) expression in healing wounds from ob/ob mice [143]. Concerning the effect against NASH, 5-week administration of sitagliptin effectively attenuated methionine/choline-deficient diet-induced steatohepatitis, where it suppressed the expression of cytochrome P450 2E1 (CYP2E1), 4-hydroxy-2-nonenal (4NHE), fibronectin and α-smooth muscle actin (α-SMA) in the liver and attenuated the inflammatory changes of adipose tissue [144].

We have reported that sitagliptin markedly inhibited liver fibrosis in rats via suppression of activated hepatic stellate cells (HSCs) [145]. These suppressive effects were associated with dephosphorylation of ERK1/2, p38 and Smad2/3 in the HSCs. Although direct anti-inflammatory actions of DPP-4 inhibitors have been reported in *in vitro* studies using aorta tissue [139], mononuclear cells [133], macrophages [142], endothelial cells [146] and adipocytes [147], their relation to gut dysbiosis, intestinal permeability or metabolic endotoxaemia have not been studied. Mashitani et al. [148] reported that alogliptin for 12 months significantly suppressed serum ferritin levels in type 2 diabetics with relatively low HbA1c tertile. Although the results is meaningful in the point that DPP4-inhibitors may be helpful to prevent the disease progression in patients with NAFLD and type 2 diabetes, the mechanism of decrease in ferritin was not discussed. The effect of DPP4-inhibitors on metabolic endotoxaemia may deserve further evaluation, because both iron and endotoxin are taken up by Kupffer cells [149] and close correlation has been found between plasma endotoxin and serum ferritin levels in patients with advanced liver disease [150, 151].

**SGLT2 inhibitors**

There are two main sodium-glucose cotransporters (SGLTs), SGLT1 and SGLT2. SGLT1 enables the small intestine to absorb glucose and contributes to the reabsorption of glucose filtered by the kidney. SGLT2 is responsible for reabsorption of most of the glucose filtered by the kidney [152]. Various SGLT2 inhibitors have been accepted as a new class of treatment for Type 2 diabetes [153]. By decreasing renal glucose absorption, these agents target hyperglycemia independent of insulin secretion or insulin sensitivity [154]. On the bases of this unique mechanism of action different from existing antidiabetic agents currently on the market [154], they are also expected as a safe and effective combination drug with other agents, including insulin, and incretin-based therapies [154].

On the other side, there are wide variety in their selectivity for SGLT2 relative to SGLT1: canagliflozin 160 fold, ipragliflozin 570 fold, dapagliflozin 1,200 fold, luseogliflozin 1,770 fold, and the highly selective empagliflozin 2,700 fold [155]. In spite of this highly renal selectivity, empagliflozin was surprisingly associated with lower rates of all-cause and cardiovascular death and lower risk of hospitalization for heart failure [156]. Heart failure–related endpoints appeared to account for most of the observed benefits in this study [6]. The cardioprotective effect of this 3-year empagliflozin administration cannot be explained by slight decrease of HbA1c level observed in the study.

In experimental studies, administration of empagliflozin not only improved hyperglycemia but also normalized endothelial function of aortic rings and reduced oxidative stress in aortic vessels of diabetic rats induced by streptozotocin [157]. Another study confirmed that empagliflozin significantly improved markers of oxidative stress 8-hydroxydeoxyguanosine (8-OHdG) in the kidney of streptozotocin-induced diabetic rats [158]. It suppressed inflammatory and fibrotic gene expression such as...
MCP-1, intercellular adhesion molecule-1 (ICAM-1), plasminogen activator inhibitor-1 (PAI-1), and transforming growth factor-β (TGF-β) in the diabetic kidney [158]. Interesting enough, renal SGLT1 gene expression was completely suppressed in the study [158]. Although the authors did not analyze SGLT1 in the intestine, there was a possibility that empagliflozin inhibit it, thereby decreasing the rate of intestinal glucose absorption.

SGLT1 is predominantly expressed in the small intestine and transports glucose and galactose across the apical membrane in a process driven by a sodium gradient created by Na⁺/K⁺-ATPase [153]. Elevated mRNA and protein levels for SGLT1 have been reported in the intestine of obese subjects and type 2 diabetics [159,160]. Intestinal SGLT1 inhibition lowers and delays the glucose excursion following carbohydrate ingestion and augments GLP-1 and peptide YY secretion [153]. The latter is likely due to increased glucose exposure of the colonic microbiota and formation of metabolites, such as L cell secretagogues [153]. An increase in colonic microbial production of SCFAs enhances barrier function of the colonic epithelium and may suppress metabolic endotoxaemia. Taken together, the above cardioprotective effect of empagliflozin might be explained by its anti-inflammatory actions not only in the kidney but also in the intestine.

Although the results of succeeding trials of dual SGLT1/2 inhibitors should be carefully evaluated, the probable effects of SGLT2 inhibitors on intestine, gut dysbiosis and metabolic endotoxaemia may deserve further investigation.

Conclusion

The implications of gut microbiota can be also exemplified in other treatment modalities for type 2 diabetes. Beneficial metabolic effects of a probiotic VSL#3 on rats fed high-fat diet has been reported, on the bases that it can increase SCFA butyrate, which stimulates the release of GLP-1 [78]. Enrichment of gut microbiota with Lactobacillus reuteri was proved to increase insulin secretion in glucose-tolerant volunteers [161]. The striking effect of bariatric surgery on body weight and metabolic state were related to profound gut microbial changes: decreased abundance of Firmicutes and increased abundance of Bacteroides-Prevotella group, γ-Proteobacteria and F. prausnitzii [39,162,163], some of which were directly linked to the reduction in low-grade inflammation [163]. As stated above, gut dysbiosis is likely to play a key modulatory role on the disease progression of type 2 diabetes. Marked technological progress in the studies of gut microbiota has opened a novel area of research field in diabetology. However, we should be aware of its limitations and always try to refine the method. Studies using the direct measurement of microbiota function such as metagenomic, transcriptomic, and metabolomic assays (i.e. the metabiome) are needed to determine whether changes in bacterial function rather than composition are related to health and disease. Nevertheless, we can further tune the better microbial composition of patients by a skillful diet therapy to get a maximum effect of each pharmacotherapy. Interestingly enough, some probiotics are reported to increase adiponectin levels [164,165] and to enhance its receptor AdipoR2 gene expression [166] in experimental animals.

The progress in gut microbiology with emerging analytical technologies has a power to bring about a paradigm shift in the diabetes treatment of tomorrow. There is a great possibility that meticulous management of gut microbiota and intestinal functions may suppress metabolic endotoxaemia and inflammation and finally improve the prognosis of diabetic patients. By all means, lifestyle optimization is essential for all patients with diabetes [6]. Dietary treatment on the basis of latest knowledge on the food-microbiome interaction may improve the effects of diabetic drugs and finally suppress the morbidity of diabetes and related cardiovascular and hepatic events in risky diabetic patients.

Conflict of Interest

The author has no conflict of interest to declare relevant to the subject of this review article and every statement in it.
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