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Sodium butyrate supplementation ameliorates diabetic inflammation in db/db mice

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Abstract

Endotoxemia has been recognized to be closely accompanied with type 2 diabetes mellitus (T2DM) and is responsible for many diabetic complications. Recent study suggests the potential role of butyrate, a short-chain fatty acid (SCFA) from microbiota metabolite, on T2DM. Gut-leak is a key event in diabetic-endotoxemia. To investigate if butyrate could ameliorate diabetic-endotoxemia, both in vivo and in vitro experiments were carried out in the present study. The effect of butyrate supplementation on blood HbA1c and inflammatory cytokines were determined in db/db mice; gut barrier integrity and expression of tight junction proteins were investigated both in vivo and in vitro. Oral butyrate administration significantly decreased blood HbA1c, inflammatory cytokines and LPS in db/db mice; gut barrier integrity and intercellular adhesion molecules were increased as detected by HE staining, immunohistochemistry and Western blot. By gut microbiota assay, ratio of Firmicutes:Bacteroidetes for gut microbiota was reduced by butyrate. In Caco-2 cells, butyrate significantly promoted cell proliferation, decreased inflammatory cytokines’ secretion, enhanced cell anti-oxidative stress ability and preserved the epithelial monocellular integrity, which was damaged by LPS. The present findings demonstrated that butyrate supplementation could ameliorate diabetic-endotoxemia in db/db mice via restoring composition of gut microbiota and preserving gut epithelial barrier integrity.

Introduction

Type 2 diabetes mellitus (T2DM) has been recognized as an epidemic disease with low-grade chronic inflammation as its pivotal characteristic. Epidemiological studies show that inflammatory cytokines and LPS dramatically elevated in T2DM population (Gomes et al. 2017). Under this setting, the notion ‘diabetic-endotoxemia’ is suggested, and it has been recognized as a risk factor that is closely accompanied with both the onset and the progress of T2DM (Gomes et al. 2017). Although the source of this endotoxemia was not fully elucidated, increasing studies established that gut-leak played pivotal role in it (Denis et al. 2015) and preserving gut integrity might have significant effect on reversing this syndrome. At present, it is believed that gut dysbacteriosis might be the ringleader that contributes to gut-leak (Qin et al. 2012). The dysbiosis of gut microbiota has the potential to affect host metabolism and energy.
storage (Turnbaugh et al. 2006), increase gut permeability and, as a consequence, give rise to metabolic endotoxia and higher plasma lipopolysaccharide (LPS). However, there is still a long way to unveil this riddle concerning the underlying mechanism between dysbacteriosis and diabetic-endotoxia.

Metabolites from gut microbiota, mainly short-chain fatty acids (SCFAs) which are the metabolic products of intestinal bacterial fermentation of undigested carbohydrates, have been found to be significantly decreased in T2DM population (Qin et al. 2012). SCFAs can serve as the energy source for gut epithelial cells, couple with their receptors to function and promote the secretion of glucagon sample peptide 1 and 2 in intestinal L cells and so forth (Yadav et al. 2013). There are mainly three forms of SCFAs namely acetate (60%), propionate (25%) and butyrate (15%) (Huang et al. 2017a). Our previous study found that SCFAs could inhibit both high glucose and LPS-induced oxidative stress and inflammatory damage in glomerular mesangial cells (Huang et al. 2017b), indicating their potential function in modulating diabetic-endotoxia.

Although butyrate is the least component of SCFAs, it has been recently reported to have immunomodulatory effects on intestinal macrophages and thereby conferring them hypo-responsive to commensal microbiota residing in the colon (Chang et al. 2014). By identifying approximately 60,000 type-2-diabetes-associated markers, Qin et al. (2012) showed that the abundance of some universal butyrate-producing bacteria decreased, strongly suggesting the key role of butyrate in the progression of T2DM. Researchers found that butyrate-addition diets enhanced gut integrity and protected the animal from diabetes (Mariño et al. 2017). However, to our knowledge, effects of oral administration with butyrate on preserving gut integrity and ameliorating diabetic-endotoxia have not yet been investigated in T2DM. Researchers found that butyrate-addition diets enhanced gut integrity and protected the animal from diabetes (Mariño et al. 2017).

Materials and methods

Materials

Metformin and sodium butyrate (But) were purchased from GBC BIOtechnology (Guangzhou, China) and Meilun Biological Technology (Dalian, China) respectively. Lipopolysaccharide (LPS), which was derived from Escherichia coli 055:B5, was purchased from Sigma (product number: L2880). DMEM, fetal bovine serum (FBS), trypsin, MEM non-essential amino acids solution and t-glutamine were obtained from Gibco. 3-(4,5)-dimethylthiazol(-z-y1)-3,5-diphenyttetrazoli- umromide (MTT), ELISA kits for IL-1β IL-8, MCP-1 and TNF-α were purchased from Cheng Lin biotechnology company (Beijing, China). Primary antibodies against zona occludens protein-1 (ZO-1), intercellular adhesion molecule-1 (ICAM-1) and occludin were purchased from Santa Cruz. FITC-conjugated secondary antibody (Rabbit Anti-Goat IgG) was supplied by Boster Biotechnology company (Wuhan, China). Twenty-four-well transwell cell culture plates (hanging insert well diameter 6.5mm, membrane area 0.3cm²) were obtained from Corning (Corning, NY, USA). The electrical resistance detection system (Millicell ESR-2) was bought from Millipore. Insulin detection kit was derived from Applygen (Beijing, China). The other materials and agents were from commercial sources.

Animals

All animal care and investigation were approved by Macau University of Science and Technology. The experiments were performed in 7-week-old male db/db mice weighing 20–30g and male C57BL/6 mice weighing 15–20g supplied by Cavens Lab Animal Co. Ltd. (Changzhou, China), and high-fat diet (2g/10 g) or normal chow (2g/10g) diet was respectively administrated to db/db mice or C57BL/6 mice. All animals were housed in an animal house with 12-h daylight cycle and 25°C. Before drug intervention, all animals were kept in animal house for 3 days to make them adapt for the environment.

Diabetic animals were randomly divided into three groups as follows: (1) model group (n=6); (2) intervention group in which animals were orally treated with sodium butyrate (0.5 g/kg/day, n=6); (3) positive control group in which animals were orally administrated with metformin (0.15 g/kg/day, n=6). Six C57BL/6 mice were set as normal control. Each day, all animals were administrated with drugs as mentioned above or 0.45 mL normal saline (for normal and model animals) by gavage for 5 continuous weeks.

During the period of drug administration, body weight was assayed by weighing-machine every 7 days. At the end of the experiment, blood samples were collected for biochemical examination, fecal samples were collected for microbiome analysis; the animals were killed, and pancreas and colon samples were collected for histological evaluation.
Hematoxylin-eosin staining and immunohistological evaluation of colon and pancreas

Colon or pancreas was fixed in 4% paraformaldehyde for 24 h and then paraffinized. For observation, the sections (0.4 μm) were stained with HE solution for 5 min, and the histopathological images of colon were observed under a light microscope (Olympus).

For immunohistological evaluation of the colon, sections were firstly incubated with primary antibodies including ZO-1 (1:200) and ICAM-1 (1:200), and then biotin-conjugated secondary antibody and streptavidin-biotin-enzyme complex were administrated to the sections to develop brown deposit (positive staining).

Western blot

Total protein was extracted from colon of db/db mouse using RIPA Lysis Buffer. Protein concentrations were assessed using BCA Protein Assay Kit (Bioworld Technology, USA). Western blot was performed as previously described (Gao et al. 2013).

Gut microbiota composition analysis

Fecal was suspended in Tris-EDTA buffer supplemented with 10% sodium dodecyl sulfate (SDS) and DNA was collected by standard method. The V3-V4 regions of the bacterial 16S rRNA gene and Phusion High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs) were used for DNA amplification. PCR products were extracted after 2% agarose gel electrophoresis. Sequencing libraries were generated by a TruSeq DNA PCR-Free Sample Preparation Kit (Illumina). Finally, the sequence was analyzed on HiSeq2500 PE250 platform.

Cell culture

Human epithelial colorectal adenocarcinoma (Caco-2) cells were purchased from the American Type Tissue Collection. The cells were cultured according to the method described in our previous report (Guo et al. 2016).

Cell viability evaluation by MTT assay

Caco-2 cells at exponential phase were seeded into 96-well cell culture plates and treated with vehicle (as control), different concentration of LPS, But, LPS+But, and Met for 24 h. Then, cells were incubated with MTT (5 mg/mL) for 4 h and formazan was dissolved by DMSO. A spectrophotometer (TECAN, Seestrasse, Switzerland) was used to test the absorbance at wavelength of 490 nm.

ELISA assay

For blood sample as collected in the animal study, blood HbA1c, ALT, AST, Cr, BUN, LPS, insulin, inflammatory cytokines including MCP-1, TNF-α and IL-1β were determined by kits according to the protocol provided by the manufacturers.

For in vitro experiment, Caco-2 cells were incubated with vehicle (as control), LPS (100 μg/mL), But (5 mM/L), LPS (100 μg/mL) + But (5 mM/L), or LPS (100 μg/mL) + Met (0.5 mM/L) for 24 h and the cell culture supernatant was collected; levels of inflammatory cytokines including IL-1β, IL-8 and TNF-α were assayed by ELISA method according to the manufacturer's protocols.

Cell anti-oxidant activity evaluation

Caco-2 cells were seeded into 24-well cell culture plates and administrated with vehicle (as control), LPS (100 μg/mL), But (5 mM/L) or LPS (100 μg/mL) + But (5 mM/L) for 24 h. ROS and MDA were detected by the detection kits according to the manufacturer's protocols. In general, the culture supernatant was collected and MDA was analyzed by the spectrophotometer, and the level of ROS in the cells was observed by the fluorescence microscope (OLYMPUS).

Immunofluorescence analysis

Caco-2 cells at exponential growth state were seeded on the slide and were administrated with vehicle (as control), LPS (100 μg/mL), LPS (100 μg/mL) + But (5 mM/L) and LPS (100 μg/mL) + Met (0.5 mM/L) for 24 h. After being gently washed with PBS solution, the cells were fixed with 4% paraformaldehyde. After being blocked with rabbit serum for 30 min at room temperature, the cells were incubated with primary antibodies including ZO-1 (1:100), Occludin (1:100) or ICAM-1 (1:100) for 24 h at 4°C followed by PBS washing for three times (3 min/time). Secondary antibody conjugated with fluorescein isothiocyanate (FITC) was applied to observe the proteins’ expression under a fluorescence microscope.

In vitro gut epithelial barrier model construction and trans-epithelial electrical resistance evaluation

An in vitro gut epithelial barrier model was constructed using cell culture inserts according to our previous report (Guo et al. 2016). In general, Caco-2 cells at exponential growth state were seeded on the upper insert of Transwell cell co-culture system for 21 days; then vehicle...
Butyrate supplementation ameliorates T2DM Y-H Xu et al.

(as control), LPS (100μg/mL), LPS (100μg/mL)+But (5mM/L) and LPS (100μg/mL)+Met (0.5mM/L) were added into the upper well of the system for 24h. Trans-epithelial electrical resistance (TEER) across Caco-2 cell monolayer was assayed with a Millicell-ERS electric resistance system (Millipore) to determine monolayer integrity of the cells.

Statistical analysis

All experimental data were obtained from at least three independent experiments, and the data were expressed as mean ± standard deviation (s.d.). For data analysis, SPSS 19.0 software was applied by one-way ANOVA method. P<0.05 indicated statistical significance.

Results

Butyrate administration significantly decreased blood glucose and lowered animal body weight

Butyrate supplementation did not significantly affect the general condition of the diabetic animals throughout the experiment, but the body weight was lowered compared with model group (Fig. 1A). Moreover, we also observed significant effect of butyrate on lowering glucose (vs DM group, P<0.05, Fig. 1B) and satisfactory effect on decreasing HbA1c to the level that was comparable to the normal mice (Fig. 1C). It was interesting that butyrate administration also increased blood insulin level compared with DM mice (P<0.05, Fig. 1D).

![Figure 1](https://doi.org/10.1530/JOE-18-0137)

Influence of drug intervention on the animal metabolism. (A) Body weight, (B) blood glucose, (C) HbA1c, (D) insulin, (E) ALT, (F) AST, (G) Cr and (H) BUN were determined. Diabetic animals were administrated with sodium butyrate (But) or metformin (Met) for 5 weeks, the concerned parameters were evaluated. ‘Normal’ indicates normal animal without diabetes, ‘DM’ indicates diabetic animal without drug intervention. Each group had 6 mice.

*P<0.05, **P<0.01. A full color version of this figure is available at https://doi.org/10.1530/JOE-18-0137.
Renal and liver function

To initially evaluate whether butyrate supplementation may deliver possible side effects on renal and liver function, parameters of these two important organs were assayed. As shown in Fig. 1E, F, G and H, the level of blood aspartate aminotransferase (ALT and AST, for liver function), blood creatinine (Cr) and urea nitrogen (BUN) (for renal function) in db/db mice were strikingly elevated (P<0.01, vs normal mice); while butyrate administration significantly decreased blood Cr and BUN, indicating butyrate has beneficial effect on preserving kidney from diabetic damage. Unexpectedly, we also observed that butyrate supplementation slightly increased blood AST while increased ALT in db/db mice (Fig. 2E and F), the underlying mechanism deserves further investigation.

Blood evaluation for diabetic inflammation

Diabetes has been recognized as a low-grade inflammatory disease and inflammatory cell infiltration is one of the characteristic. In our study, we found that the amount of WBCs (Fig. 2A) and inflammatory cells including neutrophils (NE, Fig. 2B), abnormal lymphocytes (ALY, Fig. 2C) and monocytes (MONO) (Fig. 2D) were dramatically increased compared with normal mice, and butyrate administration slightly decreased the level of WBC and ALY.

Gut microbiota analysis

The most important source for blood LPS is gut microbiota. As one of a metabolic product for microbiota, we would like to know if butyrate supplementation could have some influence on the composition of gut microbiota. As shown in Fig. 4A, the microbiota composition was varied among groups in that the diversity of microbiota was reduced in diabetic animals. By analyzing specific composition of microbiota, we observed Firmicutes, Bacteroidetes, Tenericutes and Cyanobacteria are the four most important bacteria in healthy mice at Phylum level (Fig. 4B), Bacteroidetes and Tenericutes were reduced while Verrucomicrobia was increased in diabetic mice, and butyrate administration significantly increased amounts of Bacteroidetes, and reduced ratio of Firmicutes:Bacteroidetes (Fig. 4C).
Histological examination

As graphed in Fig. 1D, butyrate supplementation increased blood concentration of insulin, we wonder if it has some effects on pancreatic islet β cells. For this aim, we observed morphological changes of pancreas by HE staining. We found although insulin was not significantly decreased in diabetic animal, the islet was obviously shown with atrophy; while both MET and butyrate supplementation restored function of islet β cells (Fig. 5A); the exact effect and mechanism of butyrate on β cells is still under investigation.

As LPS cannot be generated by the organism; by HE staining, we observed distinct gut epithelial barrier

Figure 4
Alteration of gut microbiota composition. (A) Venn diagram for microbiota composition among groups. (B) Heatmap of microbiota abundance. (C) Top 10 microbiota among groups. A full color version of this figure is available at https://doi.org/10.1530/JOE-18-0137.
damage in diabetic gut in that the gut villus continuity was interrupted and infiltrated with inflammatory cells, and the physical barrier of gut was severely damaged (Fig. 5B); expectedly, butyrate administration obviously enhanced the gut integrity to the state even better than the normal, in that inflammatory cell infiltration was reversed and the histological structure was improved.

To further demonstrate our finding, immunohistochemistry and Western blot were applied to determine the effects of butyrate on expression of cellular adhesion molecules including ZO-1 and ICAM-1 on gut. Butyrate administration significantly increased the expression of ZO-1 (Fig. 5C, D, E and F), while the inflammatory adhesion molecule ICAM-1 was decreased (Fig. 5G, H, I and J).

**Figure 5**
Histological examination and Western blot after drug intervention. Diabetic animals were administrated with sodium butyrate (DM+But) or metformin (DM+Met) for 5 weeks; at the end of the experiment, the animal pancreas and gut sample were collected. Hematoxylin-Eosin (HE) staining for (A) pancreas and (B) gut. Immunohistochemistry and Western blot were carried out to evaluate gut integrity and inter-cellular-adhesion molecules including (C and E) ZO-1 and (G and I) ICAM-1 expression. (D), (F), (H) and (J) are the statistical charts of ZO-1 and ICAM-1. ‘Normal or NC’ indicates normal animal without diabetes, ‘DM’ indicates diabetic animal without drug intervention. (The experiments were repeated for three times and characteristic images were shown; Magnification: 200× for HE images, and 200× or 400× for immunohistochemistry images as indicated). *P<0.05, **P<0.01. A full color version of this figure is available at [https://doi.org/10.1530/JOE-18-0137](https://doi.org/10.1530/JOE-18-0137).
Butyrate promoted proliferation of gut epithelial cell line Caco-2 cells

To validate our above findings and further elucidate mechanism of butyrate on enhancing gut barrier integrity, a series of in vitro experiments were further carried out on human gut epithelial cell line, Caco-2 cells. As the inflammation nature for diabetic-endotoxemia, we firstly assayed role of LPS on the viability of Caco-2 cells by MTT method, and found LPS concentration-dependently inhibited cell viability, and it possessed significant effect at 100 μg/mL (Fig. 6A). Then, we evaluated effects of butyrate and metformin on cell viability and found butyrate can promote cell viability at 5 mM/L (Fig. 6B) while metformin possessed dose-related effects on cell viability (Fig. 6C). Moreover, we observed that butyrate could concentration dependently reverse effects of LPS on cell viability (Fig. 6D).

Butyrate increased gut epithelial monocellular integrity

Intercellular adhesion molecules play key role in binding the cells together. As shown in Fig. 7A, B, C and D, molecules including ZO-1, Occludin and ICAM-1 were mainly expressed and located on the cell membrane; LPS stimulation dramatically decreased their expression, and both butyrate and metformin could reverse this reduction. More importantly, effects of butyrate on these molecules are more significant than that of metformin.

To comprehensively evaluate capacity of butyrate on preserving LPS-induced gut epithelial barrier integrity, we constructed an in vitro Caco-2 cell monolayer barrier model according to our previous report (Guo et al. 2016) and analyzed trans-epithelial electrical resistance (TEER) variance upon butyrate incubation. As shown in Fig. 7E, LPS significantly increased the epithelial-leak and decreased TEER by about 70%, while both butyrate and metformin incubation could enhance the barrier integrity and elevate level of TEER.

Inflammatory cytokines and oxidative stress were reduced by butyrate

To verify role of butyrate on LPS-mediated inflammatory cytokines, the cells were treated with LPS followed by butyrate. As graphed in Fig. 8, secretion of inflammatory cytokines including IL-1β and TNF-α (Fig. 8A and B) was significantly inhibited while anti-inflammatory cytokine IL-8 (Fig. 8C) was strikingly promoted. This finding indicates that butyrate may re-balance the pro- and anti-inflammatory factors.

Oxidative stress damage accompanies with the progression of diabetes and diabetic-endotoxemia, and superoxide anion can directly induce cell death thus promote gut-leak. We noticed in the present study that butyrate administration to the cells reduced level of both reactive oxygen species (ROS, Fig. 9A and B) and malondialdehyde (MDA, Fig. 9C) and showed satisfactory effects against oxidative damage.

Discussion

Metabolic-endotoxemia has now been recognized as a risk factor that is closely accompanied with both the onset and the progress of T2DM (Gomes et al. 2017). Although there is still conflict concerning the source of endotoxemia, scholars from both basic research and clinical physician have achieved a consensus that inhibiting endotoxemia is helpful to attenuate the development of diabetes (Cani et al. 2007a). According to the reports concerning the characteristics of db/db mice, 6- to 8-week-old animals are widely applied to observe early-stage signs and symptoms...
Butyrate supplementation ameliorates T2DM

Y-H Xu et al.

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As early intervention is the key to effectively ameliorate development of T2DM when the body is not severely damaged and db/db mice at 7-week-old are applied.

We found in the present study that butyrate administration decreased blood glucose and HbA1c levels and reduced the animal body weight. Although its exact function on obesity and fatty acid metabolism is still controversial (Rumberger et al. 2014, Goffredo et al. 2016, Lu et al. 2016), we believe butyrate supplementation helps to modulate energy balancing. On the one hand, butyrate preserves pancreatic islet β cell functions, and thus may help to delay disease development. On the other hand, butyrate might reduce energy intake via activation of FFAR-3 (Lin et al. 2012) and vagal afferent neurons (Goswami et al. 2018), and positively modulate mitochondrial function (Rose et al. 2018) and energy expenditure (Si et al. 2018).

Recent studies suggest that gut barrier dysfunction and gut-leak play pivotal role in mediating of T2DM (Allen et al. 2004, Bates et al. 2005, Li & Zhang 2011). As early intervention is the key to effectively ameliorate development of T2DM, the present study is designed to investigate potential influence of butyrate supplementation on early-stage T2DM when the body is not severely damaged and db/db mice at 7-week-old are applied.
diabetic-endotoxemia ([Denis et al. 2015]). Defects in the gut barrier will result in a paracellular influx of luminal antigens and toxins ([Groschwitz & Hogan 2009]) and lead to diabetic-endotoxemia. Furthermore, the gut barrier may be an important origin for inflammatory elements that would activate inflammatory cells and enlarge the production of cytokines. Since [Hotamisligil et al. (1993)] firstly proposed strategy of neutralizing-inflammatory cytokines on ameliorating development of DM, 'anti-inflammation' has been labeled as one of the index that evaluating therapeutic efficacy in clinic. Although many efforts have been paid to ease inflammatory status in diabetic population, the overall effect is limited.

As gut microbiota gains intense notice due to their co-mechanism with the host, much concern at present for preventing inflammation are focused on re-balancing the composition between good and bad bacteria within gut. Based on this theory, strategy such as fecal microbiota transplantation ([Udayappan et al. 2014]) has been successfully applied; however, there exist problems including microbiota resistance and donor–recipient compatibilities ([Li et al. 2016]), furthermore, potential risk including behavior change in receipts should be noticed ([De Palma et al. 2017]). In fact, one of the important factors that participating in the health-preserving function of microbiota is their metabolites and the most important ones are short-chain fatty acids (SCFAs). SCFAs possess multiple beneficial functions ([Huang et al. 2017a]) and some scholars even believe that these metabolites may be developed as potential target for drug design and therapy ([Obrenovich et al. 2016]).

Recent evidence suggests that the amount of butyrate-producing bacteria is reduced in T2DM population ([Qin et al. 2012]) and consequent to a series of diabetic-related events, including endotoxemia; so how about additional supplement with butyrate could help to prevent these unwanted events. To demonstrate our hypothesis, we carried out the present study. We observed that butyrate treatment significantly ameliorated diabetic-endotoxemia, the gut barrier integrity was enhanced and inflammatory oxidative stress was inhibited. The underlying mechanism should be attributed to the following aspects.

**Inhibiting inflammatory cell infiltration**

Dysbacteriosis under T2DM setting will dramatically increase the production of LPS within the gut lumen ([Cani et al. 2007b]) and thereafter induce a series of damages to the gut–epithelial barrier. Moreover, a unique defect in this barrier is enough to trigger the development of chronic gut inflammation ([Blumberg et al. 1999]). As the impairment of the epithelial barrier will facilitate the entrance of pathogens or toxins from the intestinal lumen to the mucosa, it is currently recognized as one of the early events in intestinal inflammation ([Vivinus-Nébot et al. 2014]). In T2DM population, gut dysbacteriosis accompanied with inflammatory cells and gut epithelial cells activation will exacerbate inflammatory cytokines production and thereafter induce oxidative stress and damage to the gut barrier integrity ([Sanchez-Munoz et al. 2008]). From this aspect of view, blocking the vicious cycle of cell death–inflammation–oxidative stress will help to ameliorate diabetic gut leak and endotoxemia.

Existing evidence from both *in vitro* and *ex vivo* studies has indicated the role of SCFAs on inflammation ([van der Beek et al. 2017]); however, most of studies are conducted under settings of inflammatory bowel disease. Studies found that in both mice and human, SCFAs application is helpful to reduce weight gain ([Lin et al. 2012, Chambers et al. 2015]). In this sense, butyrate application may be helpful to modulate the organism’s metabolism. This phenomenon was also observed in the present study in that body weight in db/db mice was controlled after butyrate supplementation; moreover, level of blood glucose and plasma HbAlc was also reduced compared with model group. As glucose metabolism dysfunction has been well recognized to be important promoter of inflammation, effects of butyrate on its metabolism may
be one of important mechanism in its anti-inflammation function. Here, we observed satisfactory effects of butyrate on ameliorating inflammatory cells infiltration in gut epithelial cells and on reducing blood LPS and inflammatory cytokines. Interestingly, we also observed protective effect of butyrate on kidney in that butyrate significantly decreased the BUN and Cr in db/db mice.

Inflammatory cells play a central role in modulating inflammation. Here, we observed butyrate administration dramatically reduced inflammatory cytokines’ secretion and cell infiltration both in vivo and in vitro. Previous studies have observed effects of SCFAs on inflammatory cells’ migration and infiltration (Vinolo et al. 2009, López-Barrera et al. 2016). This anti-inflammatory cell infiltration effect may lie in their effect on suppressing pro-inflammatory cytokines’ secretion while promoting immune tolerance by lymphocytes (Kim et al. 2014); this is also observed in our study that butyrate reduced level of inflammatory cytokines such as IL-1β and TNF-α and reduced number of abnormal lymphocytes (ALY). Another mechanism relies on the downregulation of inflammatory adhesion molecules (such as ICAM-1 and MCP-1, which play pivotal in mediating inflammatory attracting and adhesion to inflammatory sites) on both immune cells and gut epithelial cells (Miller et al. 2005), as also observed in our present study.

Promoting gut epithelial cell proliferation and enhancing the adhesion among gut epithelial cells

In the present study, we found in db/db mice that level of serum LPS was dramatically elevated. We assume gut-leak contributes to its elevation and thereafter diabetic-endotoxemia, as LPS cannot be generated by the animal themselves. Our hypothesis is supported by a recent report (Song et al. 2015) that LPS administration to mice will result in gut-leak, and gut epithelial cell loss was accompanied with this pathological process. Therefore, the primary task for intervening gut-leak seems to preserve gut epithelial cell amount and activity.

It has been demonstrated that more than 90–95% of SCFAs can be absorbed by gut epithelial cells as energy sources (Gopal et al. 2007), and the main energy source for colon epithelial cells is butyrate; more importantly, these SCFAs may influence the normal function and generation of gut mucus (Ohira et al. 2017). Besides serving as energy source, reports indicated that butyrate is also an important regulator in the colonic cell proliferation and differentiation (Lu et al. 2015). As have been well demonstrated, hyperglycaemia and inflammation setting will result in cell death. In the present study, a severe gut epithelial cell loss accompanied with LPS elevation was observed in both in vivo and in vitro studies, and butyrate significantly reduced detected level of LPS. Another factor that contributes to gut barrier integrity is the tight junction proteins expressed on the epithelial cell membrane (Laukoetter et al. 2006). It is observed that loss of these proteins will lead to leaky barrier (Shi et al. 2014) and contribute to development of T2DM (Min et al. 2014, Wang et al. 2017). In this aspect of view, any intervention that promotes gut epithelial cell proliferation or increase the adhesion molecules’ expression between epithelial cells will help to ameliorate or reverse diabetic-endotoxemia. Obviously, butyrate is a satisfactory candidate. In our present study, although butyrate application could not increase TEER to the level as high as that in normal control group, it is comparable to that of metformin on preserving and reversing LPS-damaged cell monolayer to an ‘integrity’ state, as it is generally believed that once the value of TEER exceed 400Ω cm², the gut barrier can be regarded as in an integrity state (Akbari et al. 2015).

Reducing diabetic oxidative stress

It has been demonstrated that although proper level of intracellular ROS is helpful to strengthen the defense, hyperglycaemia-promoted over-production of ROS is involved in and will accelerate the progression of T2DM and its associated complication (Yung et al. 2016). Many factors participated in the over-production of ROS, such as hyperglycaemia and non-enzyme promoted protein glycation process (Amadori reaction) etc. As ROS can directly induce cell damage (Xie et al. 2008) and exacerbate the generation of glycated proteins and thereafter promote production of ROS (Brownlee et al. 1988), it constitutes an important joint that participates in the so-called hyperglycemic memory (Saeidnia & Abdollahi 2013).

Studies concerning butyrate and oxidative stress are abundant. Recent reports suggested that butyrate could inhibit oxidative stress in HepG2 cells via HDAC, nuclear factor erythroid 2-related factor 2 (Nrf-2) and mitochondrial modulated pathway (Xing et al. 2016, Dong et al. 2017, Mollica et al. 2017). Previously, we also observed that SCFAs including butyrate can inhibit both high glucose- and LPS-induced oxidative stress in mesangial cells (Huang et al. 2017b). From this observation, scholars believed that butyrate can be considered as novel therapeutic strategy on diabetes.
Restoring composition of gut microbiota

As have been demonstrated, T2DM is accompanied with gut dysbacteriosis (Qin et al. 2012). Studies suggest that dysbacteriosis is often accompanied with gut-leak and increased plasma LPS (Turnbaugh et al. 2006). The most important compositions for gut microbiota are Firmicutes, Bacteroidetes, Tenericutes and Cyanobacteria (Markle et al. 2013). According to a most recent report (Jandhyala et al. 2017), the abundance of Bacteroidetes was increased while corresponding Firmicutes:Bacteroidetes ratio was increased in both diabetes and diabetic pancreatitis. In this sense, decreasing Firmicutes:Bacteroidetes ratio helps to re-construct gut microbiota composition and thereafter the amelioration of diabetic endotoxemia. This beneficial effect was observed when the animals were administrated with butyrate.

In conclusion, our present study firstly investigated the effects of oral butyrate administration on diabetic-endotoxemia in db/db mice. The in vivo and in vitro study suggested that role of butyrate on restoring composition of gut microbiota, promoting gut epithelial proliferation, enhancing gut barrier integrity, reducing inflammation and ameliorating oxidative stress may be the mechanism (Fig. 10). Our present findings supply new insights to the role of gut microbiota metabolite butyrate in the development of T2DM and its associated endotoxemia.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Y-H X, C-L G, H-L G, W H, S-S T, W-Q Z and W-J G performed research. Y-H X and C-L G analyzed the data. Y-H X wrote the manuscript. Y X and H Z reviewed the manuscript. Y-H X and Q Z designed research. All the authors read and approved the final manuscript.

Butyrate supplementation ameliorates T2DM

Y-H Xu et al.

Figure 10

Butyrate ameliorates endotoxemia via enhancing gut barrier integrity in T2DM. Diabetic-endotoxemia has been recognized to be closely accompanied with T2DM, which would reduce diversity of microbiota, especially butyrate-producing bacteria, activate inflammation and oxidative stress and induce gut-leak. Butyrate has effect on T2DM by promoting gut epithelial proliferation, enhancing gut barrier integrity, reducing inflammation and ameliorating oxidative stress. A full color version of this figure is available at https://doi.org/10.1530/JOE-18-0137.
Butyrate supplementation ameliorates T2DM

Y-H Xu et al.

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