Lactobacillus casei reduces susceptibility to type 2 diabetes via microbiota-mediated body chloride ion influx

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Gut microbiota mediated low-grade inflammation is involved in the onset of type 2 diabetes (T2DM). In this study, we used a high fat sucrose (HFS) diet-induced pre-insulin resistance and a low dose-STZ HFS rat models to study the effect and mechanism of Lactobacillus casei Zhang in protecting against T2DM onset. Hyperglycemia was favorably suppressed by L. casei Zhang treatment. Moreover, the hyperglycemia was connected with type 1 immune response, high plasma bile acids and urine chloride ion loss. This chloride ion loss was significantly prevented by L. casei via upregulating of chloride ion-dependent genes (ClC1-7, GlyRα1, SLC26A3, SLC26A6, GABAAα1, Bestrophin-3 and CFTR). A shift in the caecal microflora, particularly the reduction of bile acid 7α-dehydroxylating bacteria, and fecal bile acid profiles also occurred. These change coincided with organ chloride influx. Thus, we postulate that the prevention of T2DM onset by L. casei Zhang may be via a microbiota-based bile acid-chloride exchange mechanism.

Obesity-associated T2DM has drawn much scientific attention, as evident by the rapidly increasing number of published investigations. Data showed that the world population is facing a surge in T2DM as well as individuals with prediabetes due to rapid change in lifestyle1. Thus, both strategies for both the prevention and treatment of diabetes are needed, especially in the dietary aspect.

Diet is directly associated with intestinal microbiota. There is a growing interest in understanding the changes of gut microbiota in the context of diabetes. In recent years, metagenomics has opened a new era of microbial ecology that has allowed deeper understanding of microbiome associated hyperglycemia2,3. On the other hand, it is proposed that high-fat diet induces a low-grade inflammation through modifying microflora and thus increases lipopolysaccharides (LPS) and in turn triggers the development of metabolic diseases4. More interestingly, commensal microbiota and related bile acids profile could be rapidly reshaped by dietary alteration5, but how the pathogenesis of T2DM relates with the interaction between bile acids and chloride ion is rarely studied. This aspect is of particular interest because both bile acids and chloride ions can acted as regulating signaling molecules for metabolic homeostasis6,7.

Several studies have also shown that probiotic products could regulate the blood glucose level in diabetic human8,9. Moreover, L. casei Shirota has been reported to reduce blood glucose level through reducing lipopolysaccharide-binding protein10. One research showed that B. animalis 420 could prevent mice from obesity-induced T2DM through an improvement of bacterial translocation and overall inflammatory status11. Recently, the gut microbe, Akkermansia muciniphila, exhibited an insulin resistance-reducing effect and may have potential application in T2DM12.

Our previous research showed that L. casei Zhang could improve impaired glucose tolerance in rats due to altered microbiota composition which led to an upregulation of osteocalcin level13. The aims of the present study were to investigate whether probiotic L. casei Zhang supplementation could prevent the symptoms of rat model of T2DM and identify its mechanisms.

Methods

Animals and housing. The protocol was approved by the Animal Care and Use Committee at Inner Mongolia Agricultural University in Huhhot, China. All the methods were carried out in accordance with the approved guidelines. Male sprague-dawley (SD) rats, initial weight...
Experimental design. Two separate but related rat experiments were performed to show the hypoglycemic effect of *L. casei* Zhang consumption. Firstly, the physiological change and the protective effect of *L. casei* Zhang was assessed by a short-term high fat-induced microbiota disturbance model (Fig. S1). Rats were randomly divided into high-fat-sucrose diet (HFS) group, HFS diet short-term high fat-induced microbiota disturbance model (Fig. S1). Rats were approximately 120 g (5 weeks old), were purchased from Vital River Laboratory (Contour HFS diet for 2 weeks, both M and P groups were given an intraperitoneal injection of 4% CDCA, deoxycholic acid (DCA) and lithocholic acid (LCA) was performed on fresh rat tissues by using Trizol reagents (TAKARA, Japan) on a FastPrep system (MP Biomedicals, CA, USA). Cell-free supernatants were obtained after centrifugation and filtering of the homogenates through 0.22 μm membrane.

Biochemical analysis. Blood was collected by cardiac puncture and rapidly transferred into anticoagulant tubes. Plasma was obtained by centrifuging at 3000 g for 15 min and stored at −80 °C until use. Plasma cytokines including TNF-α, INF-γ, IL-10 were respectively determined by ELISA kits (Casabio, China), and fecal total bile acids levels were determined by a commercial kit (Randox, UK) with enzymatic colorimetric method. Plasma LPS level was detected by using a kit based on Limulus amoebocyte extract (Houshiji Company, Xiamen, China).

Rat urine collection. For urine collection (1 wk after STZ injection), animals were housed individually in metabolism cages for 12 h during the daytime, with free access to drinking water. Urine volume and pH were recorded, and samples were stored at −70 °C until analysis. Urine NH4+ concentrations were determined by commercial kit (Nanjing Jiancheng Bioengineering Institute, China).

Determination of blood glucose. Fasting (12 h) and postprandial 2 h blood glucose levels were checked weekly by a portable Bayer’s Contour Blood Glucose Monitor (Contour Meter, Bayer HealthCare LLC, USA). For oral glucose tolerance test (OGTT), rats were fasted for 12 h before being administered with an oral dose of glucose (2 g/kg of body weight). Blood glucose levels were measured at 0, 15, 30, 60, and 120 min after glucose administration.

Histological evaluation. Tissues of rats were fixed in 10% neutral formalin, followed by dehydration in gradient alcohol (75%, 85%, 95% and 100%) and xylene (100%). The tissues were then embedded in paraffin and sectioned at 5 μm thickness. Sectioned tissues were stained with hematoxylin-eosin before microscopic assessment (Olympus, Japan).

Statistical analysis. All experimental data are shown as the mean ± S.E.M. Multiple groups were tested by one-way ANOVA followed by LSD test to determine if groups were significantly different from the control group. A p value < 0.05 was considered to be statistically significant.

Results

Obesity-induced pre-insulin resistance rats. No significance difference was observed in the body weight, OGTT, plasma insulin, TBA, chloride ion, TNF-α and IL-10 among the three groups (Fig. S2, p > 0.05). Two-week high fat–sucrose intake with (PB rats) or without probiotic (HF rats) treatment induced a significantly higher plasma IL-6 level compared to CT group (Fig. S2H, p = 0.0342). There was no significant difference in plasma IL-6 between PB rats and HF rats (Fig. S2H, p = 0.169). Fig. S3 showed preliminary fatty liver morphology in HF and PB rats.

The liver GlyR1, CYP7A1 and CIC3 mRNA levels in PB were greater than those in HF rats (Fig. 1A, p = 0.013, 0.010 and 0.0001), whereas TGR-5 mRNA levels in PB were much lower than HF (p = 0.020) and the CIC2, CIC4, CIC5, CD68 and F4/80 mRNA levels of PB rats were unaffected (Fig. 1A, p > 0.05). In addition, chloride ion concentrations of liver homogenates and GlyR1 and CIC3 protein levels of PB group were higher than those in HF rats (Fig. 2A and Fig. S4, p = 0.027, 0.049 and 0.0001). As shown on Fig. 2A and Fig. 2B, splenic chloride ion levels, GlyR1 and CIC3 protein expression were lower in CT and HF rats than those from the PB group (p = 0.043, 0.0019 and 0.0009).

The Fig. 1B revealed a substantial downregulation of colonic CIC2, CIC3, CIC5 and CIC7 mRNA levels in HF rats compared to CT, while PB rats displayed a 2- to 3-fold increase in the expression of those genes. In parallel, the chloride ion concentration and CIC2 protein of the small intestine in PB rats were higher than that in HF group (Fig. 2B, p = 0.035 and 0.023).

In comparison with PB rats, a 3–4 fold decrease in cardiac CFT and BEST3 mRNA was observed (Fig. 1C). Moreover, there was a significant decrease in CIC2 protein expression of HF rats (p = 0.040), but without notable changes in CT rats (Fig. 2A and Fig. S4). There was also a significant difference in chloride ion concentrations between PB and HF rats (Fig. 2B, p = 0.026).

Statistical analysis revealed that there was an opposite effect on renal GABAa1 and GABAa2 mRNA between PB and HF. Renal CIC4, CIC5, CYP7A1, SLCl26A3, SLCL26A6 and GABAa1 mRNA levels were elevated by more than 2-fold by dietary *L. casei* Zhang.
supplementation in the PB group compared to HF rats (Fig. 1C). In the muscle of HF rats, CIC1 mRNA level decreased by 3 to 5-fold compared with CT and PB rats (Fig. 1C).

The 2 week HF diet likewise induced a 5-fold reduction in the pancreatic FoxA2 mRNA level compared to CT, whereas the FoxA2 mRNA level of PB rats was significantly upregulated ($p < 0.0001$). As shown in Fig. 2A and Fig. S4, pancreatic ClC2 protein level was much lower in the PB and HF rats compared to CT ($p < 0.0001$ and 0.0055).

In hippocampus area of the brain, CT and PB rats showed more than 2 fold increased in GABAAα1 receptor and ClC2 mRNA compared to HF rats, while the GABAAα2 receptor mRNA had an opposite trend (Fig. 1B). In the prefrontal cortex area of the brain, the chloride ion concentration of PB rats was significant lower compared to HF rats (Fig. 2B, $p = 0.007$).

Fecal CA and CDCA levels were similar in the CT and HF groups ($p > 0.05$; Table 1). But these levels were significantly higher in the PB than HF group ($p = 0.0114$ and 0.0002). Compared with HF rats, the PB and CT rats exhibited lower fecal DCA and LCA levels ($p = 0.0057$ and 0.0003, HF vs PB; $p = 0.0003$ and 0.0001, HF vs CT). As a result, the PB rats showed significantly higher total bile acid level compared to HF rats ($p = 0.0335$), while the fecal bile acids level of CT rats was considerably lower.

More caecal Bifidobacterium and Lactobacillus were found in the PB rats than the HF group ($p = 0.0061$ and 0.0001, Fig. 3A and Fig. 3B). In contrast, C. coccoides–E. rectale group and C. scindens members were much higher in HF than the PB and CT rats ($p = 0.050$ and 0.0001, Fig. 3C and Fig. 3D). Similar effects of E. rectale were observed between HF and PB rats ($p = 0.0001$, Fig. 3E) but not between HF and CT rats. No significant differences in the counts were found with Clostridium IV cluster and the genus of C. sordellii ($p > 0.05$, Fig. 3F and Fig. 3G).

Short-term HFS fed rats challenged with low dose STZ. As shown in Fig. 4A, the body weight in the control rats without STZ injection (group A) continually increased during the whole experimental period, while the body weight of the rats in the treatment groups, P (probiotic plus STZ injection) and M (no probiotic but STZ injection), the body weight decreased after the STZ injection, and the body weight of P group rats started to increase after week 5.
Fig. 4B showed the OGTT results of three groups. One week after STZ injection, group P rats exhibited a significantly lower blood glucose levels at all time points compared to group M rats (\(p = 0.010\) (0 min), 0.006 (15 min), 0.012 (30 min), 0.004 (60 min), 0.002 (90 min) and 0.005 (120 min), respectively). Moreover, both fasting and postprandial 2 h blood glucose level were significantly lower in group P than group M 1 week after STZ injection (\(p = 0.010\) (4 w), 0.002 (5 w), 0.001 (6 w), 0.001 (7 w) and 0.004 (8 w), Fig. 4C.

Table 1 | Fecal composition of bile acids in rats (\(n = 8\) for each group). (1) + (2), (3) + (4) and (1) + (2) + (3) + (4) stand for primary, secondary and total bile acids, respectively

| Bile acid type                  | CT         | PB         | HF         |
|--------------------------------|------------|------------|------------|
| Cholic acid (1)                | 0.30 ± 0.018| 0.36 ± 0.012* | 0.24 ± 0.035 |
| Chenodeoxycholic acid (2)      | 0.27 ± 0.015| 1.33 ± 0.26** | 0.28 ± 0.042 |
| Deoxycholic acid (3)           | 0.27 ± 0.019| 0.36 ± 0.015* | 0.57 ± 0.080 |
| Lithocholic acid (4)           | 0.079 ± 0.0023| 0.094 ± 0.024* | 0.30 ± 0.058 |
| (1) + (2)                      | 0.56 ± 0.031| 1.69 ± 0.26** | 0.52 ± 0.083 |
| (3) + (4)                      | 0.35 ± 0.018| 0.45 ± 0.0080* | 0.87 ± 0.093 |
| (1) + (2) + (3) + (4)          | 0.91 ± 0.044| 2.14 ± 0.27** | 1.39 ± 0.15  |

*represent significant difference from CT \((p < 0.05)\) by Dunnett test.
*represent significant difference from HF \((p < 0.05)\) by Dunnett test.
As shown in Fig. 4E, round integrated pancreatic islet and tightly arranged islet cells were found in healthy rats. Group M rats showed a severe necrosis of islets (Fig. 1Ec), group P rats exhibited a mild decrease of islets (Fig. 1Eb). Importantly, more than two fold changes of liver T-BET mRNA level was observed between A and M.

L. casei significantly attenuated STZ-stimulated T-BET mRNA levels compared with M (Fig. 4F, p = 0.015). Changes of liver GATA-3 mRNA levels were not significant different among the three groups (Fig. 4G, p = 0.05).

The plasma TBA levels of L. casei Zhang-treated rats (group P) were markedly lower than groups A and M (Fig. 5A, p = 0.015 and p = 0.006, respectively). And 12 h urine chloride ion level was also significantly lower in group P than that in groups A and M (Fig. 5B, p = 0.048). The iNOS activity of STZ-injection rats significantly increased compared with normal rats (p = 0.0008), and the probiotic-treated rats (group P) had a markedly lower level of iNOS activity than the M group rats (p = 0.001) (Fig. 5C). Fig. 5D showed that the STZ-injection induced a significant increase in plasma LPS level (p = 0.047), while it is maintained at a healthy level in the group P rats (p = 0.880).

Proinflammatory cytokines (IFN-γ and TNF-α) were significantly elevated in STZ-injection groups compared with normal rats, but it was significantly lowered in the probiotic-treated rats (p = 0.030, Fig. 5E; p = 0.017, Fig. 5F). There was no significant difference in IL-10 between P and M groups (p = 0.723) (Fig. 5G). The pH of the collected urine was significantly lower in group M compared to that of groups A and P (Fig. 5H, p = 0.048). Urine NH$_4^+$ concentration in group P was significantly lower than that of group A but higher than that of group M (Fig. 5I, p = 0.027 and 0.024).
Discussion

The primary findings of the present study are that *L. casei* Zhang ingestion markedly prevents rats from the onset and development of glycemia in both fasting and postprandial 2 h blood glucose levels, as well as OGTT levels. These findings are consistent with a previous study, which showed that STZ-diabetic rats pretreated 2-week with *L. johnsonii* La1 had a significant lower blood glucose level16. It is well-established that chronic inflammation induced by gut derived endotoxin plays a key role in the onset and development of T2DM4. Our result indicated that *L. casei* Zhang reduced the endotoxin LPS production induced by STZ injection and downregulated iNOS level.

The immunomodulatory effect of probiotics is well-established and they can regulate the balance of Th1 and Th2 responses through the production of different cytokines17. In this study, *L. casei* Zhang

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**Figure 4** | The body weight (A), OGTT (B), fasting (C) and postprandial 2 h blood glucose level (D) of three groups of rats. Black triangles = M group; black circles = P group; black squares = A group. (**P < 0.01 represents significant difference between groups P and M); (E)(a), (b) and (c) are representative pancreas tissue section respectively from group A, P and M rats (×1000). (F) Liver T-bet mRNA level; (G) Liver GATA-3 mRNA level. **, p < 0.01; ns, p > 0.05.
administration significantly inhibited the Th1 associated pro-inflammatory cytokines (IFN-γ and TNF-α) as well as Th1 immune response related to T-bet gene mRNA level and thus remarkably inhibited the development of T2DM in rats. We further examined the mechanisms participate in the Th1 immunomodulatory effect of L. casei Zhang.

Several studies have reported that the blood lipid-reducing effect of probiotics (including L. casei Zhang) was mainly due to fecal bile acid elimination18,19. Our data showed that plasma bile acids level lowered by L. casei Zhang administration exhibited a notable reduction of glycemia risk in a rat model established by HFS-diet accompanied with low dose STZ-injection. Thus, it is suggested that the plasma bile acids level does not only associate with dyslipidemia but may also be related to the risk of glycemia. Our finding is supported by a previous metabolomic study showing the close correlation between the change of plasma bile acids and OGTT20. Moreover, endogenous bile acids alteration or exogenous bile acid (derivative) administration has therapeutic potential for treating metabolic diseases. Thus, the manipulation of endogenous bile acids is a potential target for diabetes prevention.

Fecal bacteria with bile acid 7α-DH activities are mostly members of the genera Eubacterium and Clostridium21,22. Moreover, C. scindens, C. hiranonis and C. hylemonae were found to have high 7α-DH activity while C. sordellii, C. leptum and C. bifermentans were of low 7α-DH activity23,24. In this study, a decrease in the fecal 7α-DH bacteria by L. casei Zhang administration appear to restrict the conversion of primary bile acids and reduce secondary bile acids production in the intestine. Consequently, liver and renal 7 alpha-dehydroxylat-
Previous research showed that a “bile acid–chloride exchanger” exists, as confirmed by the discovery of TGR5 signaling pathway which directly contributes to both bile acid uptake and chloride secretion\(^{20}\). Our data also showed the reduction of bile acid level by \textit{L. casei} Zhang could cause a significant decrease in urinary Cl\(^{-}\) excretion in the T2DM rats, as well as a tissue chloride influx and down-regulation of TGR5. Our observation supports the “bile acid–chloride exchanger” hypothesis.

Principally, the administration of \textit{L. casei} Zhang prevents the loss of pancreatic CIC-2 and FoxA2 expression in high-fat-sucrose fed rats. Low pancreatic FoxA2 expression level is proven to be positively correlated with insulin resistance and the risk of T2DM\(^{25}\). Interestingly, the liver of FoxA2-deficient mice had shown high bile acid accumulation\(^{28}\). In addition, it has been proposed that a high intracellular Cl\(^{-}\) in the β-cell of pancreas is essential to electrical activity of β-cell membrane and insulin release\(^{39}\). Considering all these, we presumed that probiotic pretreatment protects the pancreas in high-fat-sucrose fed rats by enhancing pancreatic CIC-2 expression and eliminating bile acids in feces through a bile acid–chloride exchanging mechanism.

Furthermore, we speculate that \textit{L. casei} Zhang exerts protective effect on STZ challenge and reduced the release of LPS into blood via a liver GlyRs upregulation mechanism. Incidentally, liver kupffer cells contain a glycine gated chloride channel and glycine could decrease LPS induced inflammatory TNF-alpha release from kupffer cells\(^{30,31}\). Thus, kupffer cells may play an important role in the chloride influx induced protective effect on T2DM. Additionally, it has been suggested that liver CIC-3 channel activation may also participate in the protective effect of \textit{L. casei} Zhang since CIC-3 channel is closely associated with the inflammatory nuclear factor (NF)-κB signaling\(^{32}\). In spleen, splenic macrophages also contain a glycine gated chloride channel and may participate in beneficial effect of \textit{L. casei} Zhang\(^{33}\).

In the small intestine and colon, \textit{L. casei} Zhang may participate in the maintenance of Cl\(^{-}\) secretion and chloride channel protein expression. This effect may maintain the normal function of epithelial tight junction barrier\(^{34}\). Especially, M cells containing CIC-2-CIC-7 channels might play a role in acting as epithelial barrier\(^{35}\). Likewise, probiotics such as \textit{S. boulardii} and \textit{B. breve} C50 also act as Cl\(^{-}\)secretion regulator in the intestine\(^{36,37}\).

In the skeletal muscle, CIC-1 has been reported to be responsible for muscle electric excitability which is closely related to human myotonic disorders\(^{8}\). Typically, most myotony dystrophy patients tend to comorbid with insulin resistance\(^{28}\). Therefore, it is worth testing if dietary \textit{L. casei} Zhang has any potential to improve muscle excitability and prevention of myotonia.

Clinical studies have shown that cystic fibrosis (CF) is closely related to diabetes\(^{39}\). Interestingly, our data indicate that CF and diabetes shared the same pathogenic mechanism via the downregulation of chloride dependent genes expression. \textit{L. casei} Zhang was shown to enhanced cardiac CFTR expression with the potential to prevent CF. Additionally, Bestrophin-3 (Best3) upregulation might improve microvascular perfusion and vasomotion for diabetes\(^{40}\).

Patients with T2D are characterized by low urine pH, significantly lower bicarbonate level and higher NH\(^4\)+ concentration\(^{40}\). What is more, SLC26A3 deficient mice tend to display a chloride-losing diarrhea\(^{41}\). Thus, elevated SLC26A3 expression by \textit{L. casei} Zhang was possibly involved in preventing STZ injected rats from lowering urine pH and electrolyte imbalance, which in turn alleviate diarrhea. Our result was also supported by Raheja et al who demonstrated \textit{Lactobacillus acidophilus} could upregulate SLC26A3 expression\(^{42}\).

Previous investigators have confirmed that urolithiasis and kidney stones are common complicating diseases of T2D and SLC26A6 played a major constitutive role in limiting absorption of oxalate\(^{43,44}\). Thus, stimulation of the SLC26A6 expression by \textit{L. casei} Zhang may reduce the risk of calcium oxalate formation.

Hypertension is known to be a T2D complication. GABA receptor agonists are antihypertensive through inhibiting renal sympathetic nerve activity mediated by sympathoadrenal axis\(^{45}\). Thus, the GABA receptor regulatory role of \textit{L. casei} Zhang in the kidney might protect the host from hypertension. Similar studies conducted in rats showed that \textit{L. johnsonii} La1 could reduce renal sympathetic nerve activity and enhance parasympathetic nerve activity and thereby decreased blood pressure and glucose levels\(^{14,46}\).

Several animal studies suggest that an altered GI flora affects the gut–brain axis, but the exact mechanisms are unclear\(^{47}\). In our study, GI microflora possessing 7α-DH activity varied in response to \textit{L. casei} Zhang administration and had led to an opposite chloride distribution in the prefrontal cortex and hippocampus, as compared to HF rats. This is consistent with similar study on \textit{L. rhamnosus} (JB-1) showing that the provision of JB-1 could induce opposite changes of GABA(A) and GABA(B) mRNA in the prefrontal cortex and hippocampus\(^{48}\). Accumulating evidence suggests that T2D is associated with an increased risk of Alzheimer’s disease (AD)\(^{49}\). Moreover, AD brains have low levels of GABA and a loss of functional GABA(A) receptors\(^{50}\). Thus, \textit{L. casei} Zhang may act as a potential modulator of chloride ionotropic GABA-A receptors in hippocampus of rats, and...
aid in the prevention from AD-like symptoms. The downregulation of GABA_A2s 1 and increase of GABA_A2s 2 were previously observed in hippocampus of AD[10].

In summary, the results reveal that there seemly exist some connections between the T2DM and its comorbids medical conditions/diseases characterized by mild organ chloride loss (compared to CT group, Fig. 2B), and illustrate the potential mechanisms of L. casei Zhang in preventing from T2DM onset and development. Interestingly, a mild organ chloride loss was consistent with a mild microflora change in a large-scale T2DM clinic research[1]. The preventive effect of L. casei Zhang on T2DM may be related to the reduced number of 7α-DH activity possessing bacteria, change in fecal bile acids composition, bile acids-chloride ion exchange, expression of various chloride-dependent genes and thus reduction of inflammatory response.

**Conclusion**

In conclusion, L. casei Zhang may be able to improve the onset and development of glycemia by rapidly altering gut microbiota. The short-term response to probiotic-altered microbiota may result in a chloride ion influx in multiple organs before chronic inflammation occurs. Moreover, tissue chloride ion loss and related genes suppression linked T2DM pathogenesis to its comorbid medical conditions/diseases (Fig. 6). Future studies will need to address the precise mechanisms involved in the bile acids-chloride ion exchange.

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1. Holman, R. R. Type 2 diabetes mellitus in 2012: Optimal management of T2DM remains elusive. *Nat. Rev. Endocrinol.* 9, 67–68 (2013).
2. Larsen, N. et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 5, e9005 (2010).
3. Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490, 55–60 (2012).
4. Everard, A. & Cani, P. D. Diabetes, obesity and gut microbiota. *Expert Rev. Anticancer Ther.* 12, 1459–1470 (2012).
5. Neyrinck, A. M., Possemiers, S., Verstraete, W., De Backer, F., Cani, P. D. et al. The prebiotic short-term response to probiotic-altered microbiota may result in endoplasmic reticulum stress. *Nat. Med.* 14, 828–836 (2008).
6. Ohtsubo, K., Chen, M. Z., Olefsky, J. M. & Marth, J. D. Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport. *Nat. Med.* 17, 1067–1075 (2011).
7. Bochis, I. M., Rubins, N. E., White, P., Furth, E. E., Friedman, J. R. & Kaestner, K. H. Hepatocyte-specific ablation of Foxa2 alters bile acid homeostasis and results in endoplasmic reticulum stress. *Nat. Med.* 14, 828–836 (2008).
8. Best, L. Glucose-induced electrical activity in rat pancreatic β-cells: dependence on intracellular chloride concentration. *J. Physiol.* 568, 137–144 (2005).
9. Beijima, K., Qu, W., Schlothewitz, R. F. & Thurman, R. G. Kupffer cells contain a glycine-gated chloride channel. *Am. J. Physiol. Cell Physiol.* 302, 178–187 (2011).
10. Lyn, M. et al. Glycine accelerates recovery from alcohol-induced liver injury. *J. Pharmacol. Exp. Ther.* 286, 1014–1019 (1999).
11. Lang, H. et al. Decrease of intracellular chloride concentration promotes endothelial cell inflammation by activating nuclear factor-κB pathway. *Hypertension* 60, 1287–1293 (2012).
12. Froh, M., Thurman, R. G. & Wheeler, D. Molecular evidence for a glycine-gated chloride channel in macrophages and leukocytes. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283, 856–863 (2002).
13. Nighot, P. K. & Blisklager, A. T. Chloride channel CIC-2 modulates tight junction barrier function via intracellular trafficking of occludin. *Am. J. Physiol. Cell Physiol.* 302, 178–187 (2011).
14. Kulka, M., Schwingshackl, A. & Refus, A. D. Mast cells express chloride channels of the CIC family. *Inflamm. Res.* 51, 451–456 (2002).
15. Girard, P., Pansart, Y., Coppe, M. C. & Gillardin, J. M. Saccharomyces boulardii inhibits water and electrolytes changes induced by castor oil in the rat colon. *Dig. Dis. Sci.* 50, 2183–2190 (2005).
16. Heuvelin, E., Lebreton, C., Bichara, M., Cerf-Bensussan, N. & Heyman, M. A *Bifidobacterium* probiotic strain and its soluble factors alleviate chloride secretion by human intestinal epithelial cells. *J. Nutr.* 110, 7–11 (2010).
17. Fernandez-Real, J. M. et al. Tumor necrosis factor system activity is associated with insulin resistance and dyslipidemia in myotonic dystrophy. *Diabetes* 48, 1180–1199 (1999).
18. Bridges, N. Diabetes in Cystic Fibrosis. *Pediatr. Respir. Rev.* in Press (2013).
19. Maalouf, N. M., Cameron, M. A., Moe, O. W. & Sakhaee, K. Metabolic basis for low urine pH in type 2 diabetes. *Clin. J. Am. Soc. Nephrol.* 5, 1277–1281 (2010).
20. Schweinfest, C. W. et al. slc26a3 (dra)-deficient mice display chloride-losing diarrhoea, enhanced colonic proliferation, and distinct up-regulation of ion transporters in the colon. *J. Biol. Chem.* 281, 37962–37971 (2006).
21. Rajeja, G. et al. Lactobacillus acidophilus stimulates the expression of SLC26A3 via a transcriptional mechanism. *Am. J. Physiol. Gastrointest. Liver Physiol.* 298, 395–401 (2010).
22. Taylor, E. N., Stampfer, M. J. & Curhan, G. C. Diabetes mellitus and the risk of nephrolithiasis. *Kidney Int.** 68, 1230–1235 (2005).
23. Jiang, J. et al. Calcium oxalate urolithiasis in mice lacking anion transporter Slc26a6. *Nat. Genet.* 38, 474–478 (2006).
24. Unger, T. et al. Antihypertensive effect of the GABA receptor agonist muscimol in spontaneously hypertensive rats of the sympatoadrenal axis. *Circ. Res.* 54, 30–37 (1984).
25. Tanida, M., Yamano, T., Maeda, K., Okumura, N., Fukushima, Y. & Nagai, K. Effects of intraduodenal injection of Lactobacillus johnsonii La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. *J. Nutr. Sci. Vitaminol.* 49, 109–115 (2003).
26. Foster, J. A. & McVey Neufeld, K. A. Gut-brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci.* in Press (2013).
27. Bravo, J. A. et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. U S A* 108, 16650–16655 (2011).
28. Whitmer, R. A., Karter, A. J., Yaffe, K., Quesenberry, C. P. & Selby, J. V. Hypoglycemic Episodes and Risk of Dementia in Older Patients with Type 2 Diabetes Mellitus. *JAMA* 301, 1565–1572 (2009).
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Author contributions
Y.Z., X.G., J.G., H.Q., Y.S. and L.H. contributed to experiment. X.G. analyzed the data. H.Z. designed the experiments and reviewed/edited the manuscript extensively. Y.Z. wrote the manuscript. Y.Z. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Additional information
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