Transient Receptor Potential Vanilloid 1 Activation Enhances Gut Glucagon-Like Peptide-1 Secretion and Improves Glucose Homeostasis

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Type 2 diabetes mellitus (T2DM) is rapidly becoming a serious global health problem (1,2). T2DM is characterized by a defect in insulin secretion and/or insulin sensitivity, which commonly requires multiple pharmacotherapies (3). Current strategies for T2DM treatments may cause undesirable effects, such as weight gain and hypoglycemia, but have little effect on its progression (4,5). An incretin-based therapy is currently used to manage hyperglycemia and is available in two different regimens, dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) agonists (6,7). These agents produce a glucose-dependent increase in insulin secretion and glucagon suppression, leading to lowering blood glucose (8,9). GLP-1 is a potent incretin hormone produced in L-cells of the distal ileum and colon (9). Dietary factors, including glucose, fatty acids, and fiber, are known to increase the mRNA expression of GLP-1 and stimulate the GLP-1 release (10–12). However, circulating GLP-1 is short-lived due to inactivation by the enzyme DPP-4 (13). Thus, it is a challenge to develop long-acting selective GLP-1 analogs and DPP-4 inhibitors. One option is to target selective GLP-1 secretagogues in the intestinal tract through dietary intervention.

Administration of capsaicin, a major pungent ingredient in chili peppers, regulates insulin secretion and glucose homeostasis in animal experiments and human studies (14–19). Transient receptor potential vanilloid subfamily 1 (TRPV1), a nonselective cation channel, is a specific receptor for capsaicin (20). TRPV1 is expressed in islet β-cells, neurons, rat pancreas, and rat β-cell lines RIN and INS1 (18,21–23). Both the early insulin secretory response to intravenous glucose and glucose elimination were potentiated in mice after capsaicin administration (23). Purified capsaicin caused a decrease in blood glucose concentrations in dogs during an oral glucose tolerance test and a concomitant elevation in plasma insulin levels (19). In rats, subcutaneous administration of capsaicin increased insulin secretion and plasma insulin concentrations in a dose-dependent manner (18). The oral application of capsaicin also increases glucose absorption and utilization in healthy humans (17), Ahuja et al. (24) reported that regular consumption of chili attenuated postprandial hyperinsulinemia in humans. Although several studies showed that capsaicin administration lowered blood glucose and increased insulin secretion, the capsaicin-sensitive sensory fibers in the islets of Langerhans contribute to defective insulin secretion in the Zucker diabetic rat (21). Furthermore, a mutant TRPV1 in sensory neurons initiates a chronic and progressive β-cell stress, which induces islet cell inflammation in type 1 diabetic mice (22). These studies indicated that in nonneuronal tissues, TRPV1 may regulate insulin secretion and glucose homeostasis through a distinct mechanism beyond inflammation in β-cells caused by the TRPV1α sensory neurons.

Secretin tumor cell-1 (STC-1) cells exhibit a phenotype similar to enteroglucagon L-cells and secrete several incretin hormones including GLP-1. The STC-1–mediated GLP-1 release was triggered by the initiation of calcium influx, which may involve a putative ion channel (12). Interestingly, TRPV1 has been found to be present on the rectum and distal colon (25). A human study showed that an acute lunch that contained capsaicin increased plasma GLP-1 levels (14). TRPV1 is a Ca2+-permeable cation channel that is activated by capsaicin. Physiological concentrations of insulin regulate TRPV1 protein expression and activity (26). However, it is largely unknown whether the effects of dietary capsaicin on glucose homeostasis are linked with the triggering of GLP-1 production by intestinal

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Received 26 October 2011 and accepted 12 April 2012.

DOI: 10.2337/db11-1503

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-1503/-/DC1.

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TRPV1. Therefore, we hypothesized that TRPV1 activation enhanced endogenous GLP-1 production in the intestinal tissues, which in turn promoted insulin secretion and regulated glucose homeostasis. In this study, we provide experimental evidence that TRPV1 activation by dietary capsaicin can augment GLP-1 secretion, which increases plasma insulin levels, reduces blood glucose levels in C57BL/6J mice but not in TRPV1-deficient mice, and prevents hyperglycemia in db/db diabetic mice.

**RESEARCH DESIGN AND METHODS**

**Animal treatment.** C57BL/6J mice and TRPV1–/– mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The db/db mice and age-matched lean littermate mice (C57BL/KsJ) were purchased from Model Animal Research Center (Nanjing University). Mice were housed under a 12-h/12-h day/night cycle with access to food and water. Animals were given the normal standard chow (control group) or normal chow plus 0.01% capsaicin (capsaicin group). The Institute’s Animal Care and Use Committee approved all animal protocols.

**Intrapерitoneal glucose tolerance and insulin tolerance tests.** Intrapерitoneal glucose tolerance test (IPGTT) was performed (27). After an overnight fast (14 h), a glucose solution (2 g/kg body weight) was administered via injection into the peritoneal cavity. Glucose levels were determined using the OneTouch Ultra blood glucose meter (LifeScan). Intrapерitoneal insulin tolerance test was performed in fed mice on a different cycle with free access to food and water. Animals were given the normal balanced salt solution containing varied concentrations of capsaicin. After incubation, glucose levels were determined using the OneTouch Ultra blood glucose meter (LifeScan).

**Intraperitoneal glucose tolerance test.** After an overnight fast (14 h), a glucose solution (2 g/kg body weight) was administered via injection into the peritoneal cavity, and blood was obtained from the tail vein at 0, 30, 60, and 120 min after glucose administration. Blood glucose levels were determined using the OneTouch Ultra blood glucose meter (LifeScan). Intrapерitoneal insulin tolerance test was performed in fed mice on a different day. Humulin R (0.75 units/kg body weight) (Eli Lilly) in sterile saline was administered via injection into the peritoneal cavity (27). Glucose levels were determined on the tail blood at 0, 15, 30, 45, and 60 min after insulin injection.

**Continuous monitoring of blood glucose in conscious mice.** To definitely determine daily average blood glucose level in mice, we used the Continuous Glucose Monitoring System (CGMS; Medtronic MiniMed, Northridge, CA) to continuously measure blood glucose for 24 h (detection range: 2.2–22.2 mmol/L) as described (28). CGMS is a U.S. Food and Drug Administration–approved device for 24-h recording blood glucose levels. The electrical signals were collected every 10 s, averaged, and saved to memory of the cable-tethered glucose monitor every 5 min. The data stored in the monitor were periodically downloaded into a computer for later analysis.

**Cell culture.** STC-1 cell line was purchased from the Cell Bank of Type Culture Collection of the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Cell line was maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and 1% antibiotics (11).

**GLP-1 secretion in vitro.** STC-1 cells were washed three times in Hank’s balanced salt solution (137.93 mmol/L NaCl, 5.33 mmol/L KCl, 4.17 mmol/L NaHCO3, 1.26 mmol/L CaCl2, 0.493 mmol/L MgCl2, 0.407 mmol/L MgSO4, 0.441 mmol/L KH2PO4, 0.538 mmol/L Na2HPO4, and 5.56 mmol/L D-glucose), and then incubated in growth medium for 60 min at 37°C in Hank’s balanced salt solution containing varied concentrations of capsaicin. After incubation, conditioned medium was collected, and the concentration of GLP-1 was determined using a specific GLP-1 (7–36) amide enzyme immunoassay kit (AppIygen Technologies Inc., Beijing, China) (11).

**GLP-1 and insulin secretion in vivo.** In acute studies, 8-week-old male C57BL/6J mice and age-matched male TRPV1–/– mice were gavaged (2 g/kg body weight) solution by oral gavage through a stomach tube after being deprived of food for 14 h. Sixteen mice were randomly divided into four groups: the control group given only a glucose solution; the capsaicin group given a glucose solution containing 1 μmol/L capsaicin; the resiniferatoxin (RTX) group given a glucose solution containing 1 μmol/L RTX; and the capsaicin plus 5’-ido-resiniferatoxin (iRTX) group given a glucose solution with 1 μmol/L capsaicin plus 1 μmol/L iRTX. After 30 min of oral glucose challenge, blood was taken from the central vein of mice under anesthesia with diethyl ether. Plasma was obtained by centrifugation of heparinized blood at 4°C for 20 min at 1,200 g and subjected to enzyme immunoassays (11). In chronic studies, after the treatment of dietary capsaicin, the mice were given only a glucose solution (2 g/kg body weight).

**RESULTS**

**TRPV1 and GLP-1 in STC-1 cells and mice ileum.** TRPV1 is expressed in sensory nerves, dorsal root ganglia, bladder, blood vessels, and gut (25,33). GLP-1 originates from the lower intestines, particularly from the ileum (9). STC-1 line, a mouse enteroendocrine cell line of intestinal origin, secretes GLP-1 upon nutrient intake (11,34). However, it is unknown whether TRPV1 is expressed in intestinal cells and ileum. Immunofluorescence images indicated that TRPV1 and GLP-1 colocalized in the STC-1 cell and mouse ileum (Fig. 1A and B). The expression of TRPV1 protein was clearly detected by immunoblot analysis in both cultured STC-1 cells and freshly isolated ileum from C57BL/6J mice (Fig. 1C). These results suggest that TRPV1 is present in the GLUT-1–secreting intestinal cells and tissues.

**Activation of TRPV1 stimulates GLP-1 release in STC-1 cells.** Previous studies showed that an increase in intracellular calcium levels is associated with GLP-1 secretion (11,34). We examined whether TRPV1 activation promotes GLP-1 secretion from STC-1 cells in a calcium–dependent manner. We first showed that capsaicin stimulated GLP-1 release from STC-1 cells in a dose–dependent manner (Fig. 2A), which was antagonized by the TRPV1–specific blockers capsazepine or iRTX (Fig. 2B). We next investigated whether the effects of capsaicin on GLP-1 secretion were associated with changes in the intracellular calcium levels. The removal of intracellular calcium with 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA) or extracellular calcium with EGTA inhibited the capsaicin-induced GLP-1 secretion and GLP-1 protein level in STC-1 cells (Fig. 2C and D). To examine whether voltage-dependent Ca2+ channel is involved, two L-type Ca2+ channel blockers, nifedipine and verapamil, were used. Both blockers partially attenuated capsaicin-induced GLP-1 secretion in STC-1 cells (Supplementary Fig. 1). The present results indicate that capsaicin stimulates GLP-1 secretion from STC-1 cells through TRPV1-mediated Ca2+ increase.

**Acute capsaicin administration increases GLP-1 secretion in vivo through TRPV1 activation.** Capsicum frutescens or dietary capsaicin has been shown to affect glucose homeostasis (15,16,18,19). It is unknown whether the acute effects of capsaicin on GLP-1 secretion can be detected in vivo by TRPV1 stimulation. We examined the effects of intragastric administration of capsaicin on the circulating levels of peptide YY (PYY), glucose-dependent insulinotropic polypeptide, and glucagon. Fasting mice were challenged.
with glucose (2 g/kg) and capsaicin (1 μmol/L). The plasma PYY, glucose-dependent insulinotropic polypeptide, and glucagon levels at 30 min were not different in wild-type (WT) mice with and without capsaicin administration (Supplementary Fig. 2). By contrast, administration of capsaicin or another TRPV1 agonist, RTX, increased GLP-1 secretion 30 min after glucose challenge, and this effect was inhibited by the TRPV1 antagonist iRTX in WT mice (Fig. 3A). However, the effect of increased GLP-1 secretion was absent in TRPV1−/− mice (Fig. 3B). A similar effect of capsaicin on plasma insulin levels was observed (Fig. 3C and D). In the absence of glucose challenge, capsaicin also slightly increased the GLP-1 levels, although plasma GLP-1 level is much lower than in the presence of glucose challenge (Supplementary Fig. 3). We next examined the TRPV1 action using the selective GLP-1 receptor antagonist, exendin (9–39). The plasma insulin levels were significantly decreased after intraperitoneal injection of exendin (9–39) (10 μg/mice) before glucose challenge. In addition, the effect of oral capsaicin on plasma levels of insulin was suppressed by pretreatment with exendin (9–39) in WT mice (Supplementary Fig. 4A). Oral administration of DPP-4 inhibitor sitagliptin (3 mg/kg) prevented GLP-1 degradation, which may enhance the effects of capsaicin (Supplementary Fig. 5). These results indicate that acute capsaicin administration increases GLP-1 secretion in vivo through TRPV1 activation. TRPV1 activation by chronic dietary capsaicin improves glucose tolerance. TRPV1 plays a role in the regulation of glucose homeostasis, but the results are inconsistent (35). The current study examined whether
chronic dietary capsaicin affects glucose tolerance in WT and TRPV1−/− mice. WT mice with dietary capsaicin had a lower fasting glucose levels and an improvement of IPGTT compared with WT mice without dietary capsaicin (Fig. 4A). However, there were no changes in TRPV1−/− mice with or without dietary capsaicin (Fig. 4B). The body weight was similar in WT and TRPV1−/− mice with and without dietary capsaicin (Supplementary Fig. 6). We next investigated whether a glucose challenge increased GLP-1 secretion in mice treated with dietary capsaicin. Plasma GLP-1 levels and GLP-1 protein expression in the ileum were higher in WT but not in TRPV1−/− mice with chronic dietary capsaicin (Fig. 4C). The Ca²⁺ chelators BAPTA or EGTA inhibited capsaicin-induced GLP-1 secretion from STC-1 cells. **P < 0.01 versus capsaicin; n = 6. D: The capsaicin-induced increased GLP-1 protein level was inhibited by iRTX, BAPTA, and EGTA in cultured STC-1 cells. *P < 0.05, **P < 0.01 versus capsaicin; n = 3. These data are represented as mean ± SEM and were analyzed with a Student unpaired t test.

Chronic dietary capsaicin affects glucose tolerance in WT and TRPV1−/− mice. WT mice with dietary capsaicin had a lower fasting glucose levels and an improvement of IPGTT compared with WT mice without dietary capsaicin (Fig. 4A). However, there were no changes in TRPV1−/− mice with or without dietary capsaicin (Fig. 4B). The body weight was similar in WT and TRPV1−/− mice with and without dietary capsaicin (Supplementary Fig. 6). We next investigated whether a glucose challenge increased GLP-1 secretion in mice treated with dietary capsaicin. Plasma GLP-1 levels and GLP-1 protein expression in the ileum were higher in WT but not in TRPV1−/− mice with chronic dietary capsaicin (Fig. 4C). The Ca²⁺ chelators BAPTA or EGTA inhibited capsaicin-induced GLP-1 secretion from STC-1 cells. **P < 0.01 versus capsaicin; n = 6. D: The capsaicin-induced increased GLP-1 protein level was inhibited by iRTX, BAPTA, and EGTA in cultured STC-1 cells. *P < 0.05, **P < 0.01 versus capsaicin; n = 3. These data are represented as mean ± SEM and were analyzed with a Student unpaired t test.

Chronic dietary capsaicin decreases mean blood glucose levels in a TRPV1-dependent manner. Blood glucose levels vary widely with food intake during a typical day, but routine blood glucose measurements cannot show changes in the trends of blood glucose levels. This study presented an ambulatory change in blood glucose levels in mice using a CGMS (Medtronic MiniMed) (Fig. 5A–D). The percentages of time that blood glucose levels were either >10.0 mmol/L or <3.9 mmol/L during a 24-h recording period were also calculated (Fig. 5I and J). The mean 24-h blood glucose level was lower in WT mice with chronic dietary capsaicin than those without capsaicin (Fig. 5E). In addition, a lower percentage of time >10.0 mmol/L and a higher percentage of time <3.9 mmol/L were observed in WT mice with chronic dietary capsaicin (Fig. 5G, I, and J). By contrast, no difference was observed in TRPV1−/− mice for either the mean 24-h blood glucose level or the percentage of time >10.0 mmol/L (Fig. 5F, H, and I); percentages of time <3.9 mmol/L were fewer (Fig. 5J). These results indicate that TRPV1 deletion led to an increase in the mean 24-h blood glucose levels and that dietary capsaicin decreased the mean blood glucose levels in a TRPV1-dependent manner.

Chronic dietary capsaicin improves abnormal glucose homeostasis in db/db mice. The db/db mouse is characterized by obesity, insulin resistance, and T2DM (36). We previously reported a lower expression of TRPV1 in visceral adipose tissue from these mice (37). In this study, we explored the effects of dietary capsaicin on body weight, insulin resistance, and blood glucose levels in diabetic mice.
Eight-week-old db/db mice were fed a 0.01% capsaicin diet for 14 weeks, and they responded aversively to capsaicin diet during the first 2 weeks (Supplementary Fig. 7A). Chronic dietary capsaicin reversed weight gain (Supplementary Fig. 7B), improved insulin sensitivity in db/db mice (Fig. 6A and Supplementary Fig. 7C), and lowered fasting blood glucose levels (Fig. 6B). After 30 min of an oral glucose (2 g/kg) challenge, plasma GLP-1 and insulin levels were higher in db/db mice treated with dietary capsaicin than in control db/db mice (Fig. 6C and D and Supplementary Fig. 7D). In contrast to hypertrophic and fibrosis islets with low insulin intensity in the control db/db mice, islets exhibited stronger insulin intensity and no fibrosis in capsaicin-treated db/db mice (Fig. 6E). Chronic dietary capsaicin also increased GLP-1 and TRPV1 expression in the distal ileum of db/db mice (Fig. 6E and F). These results suggest that dietary capsaicin improved the abnormal glucose homeostasis in db/db mice through a TRPV1-mediated increase in GLP-1 production.

DISCUSSION

The current study showed that TRPV1 is localized in intestinal cells and tissues that secrete GLP-1. Capsaicin administration stimulated GLP-1 secretion from STC-1 cells in a calcium-dependent manner through TRPV1 activation. We demonstrated for the first time that acute capsaicin administration by gastric gavage increased GLP-1 and insulin secretion in vivo in WT mice but not in TRPV1−/− mice. Furthermore, chronic dietary capsaicin not only increased plasma GLP-1 and insulin levels, but also improved glucose tolerance and lowered daily blood glucose profiles in WT mice, although these effects were absent in TRPV1−/− mice. In db/db mice, activation of TRPV1 by dietary capsaicin ameliorated the abnormal glucose homeostasis, elevated GLP-1 production in the distal ileum, and increased plasma GLP-1 levels.

Currently, disappointing side effects, contraindications, and minimal improvements in β-cell function highlight urgent need for searching newer therapies, although traditional antidiabetic agents play a role in the management of T2DM (4,5). A GLP-1–based therapy with good glucose control and a low risk of hypoglycemia is an attractive treatment option. GLP-1 is released by L-cells of the intestine upon food ingestion and plays an important role in glucose-dependent insulin secretion, gastric emptying, appetite control, and postprandial reduction of glucagon secretion (38). In this study, we showed oral capsaicin slightly increased the GLP-1 secretion in the absence of glucose challenge. Besides the clinical application of synthetic GLP-1 agonists, another promising option for promoting endogenous GLP-1 production is through dietary or nutrient intervention (11,39). Several studies demonstrated that consumption of dietary chili pepper may reduce blood
Capsaicin administration increases glucose absorption from the gastrointestinal tract and increases glucagon release during glucose loading in humans (17). Capsaicin is a specific activator for TRPV1, a nonselective cation channel that is highly Ca2+-permeable (20). TRPV1 is abundantly expressed in sensory neurons but is also detected in non-neuronal cells (25). This study also demonstrated that TRPV1 is expressed in STC-1 cell line and in distal ileum, both of which secrete GLP-1.

However, there are conflicting reports about the role of TRPV1 in the regulation of glucose homeostasis (35). TRPV1+ sensory neurons are important elements of the diabetes pathoetiology in type 1 diabetic mice (22). Capsaicin-sensitive sensory fibers in the islets of Langerhans contribute to defective insulin secretion in the Zucker diabetic rats (40). Capsaicin is known to stimulate the release of calcitonin gene-related peptide from perivascular sensory nerve terminals (29). The administration of a TRPV1 antagonist enhanced β-cell function and reduced

**FIG. 4.** The improvement of glucose tolerance by dietary capsaicin (Cap) is associated with TRPV1. A and B: An IPGTT (2 g/kg) and the fasting blood glucose levels in WT and TRPV1−/− mice fed chow with or without capsaicin for 24 weeks. *P < 0.05, **P < 0.01 versus capsaicin (n = 6). C and D: Plasma GLP-1 levels after 30-min oral glucose challenge in TRPV1−/− and WT mice. *P < 0.05 versus control (Cont) (n = 4). E: Immunoblot data showing GLP-1 protein levels in the mouse ileum after a 24-week administration of dietary capsaicin. *P < 0.05, **P < 0.01 versus control (n = 3). These data are represented as mean ± SEM and were analyzed with a Student unpaired t test.
plasma calcitonin gene–related peptide levels in ob/ob mice (41). By contrast, it has also been reported that capsaicin dose-dependently increased insulin secretion from RIN cells, which was blocked by TRPV1 antagonist (18). Capsaicin increased insulin secretion in incubated pancreatic minces from WT but not TRPV1²⁻/²⁻ mice (42). In addition, insulin enhanced the TRPV1 expression and function in heterologous expression systems through the phosphatidylinositol 3-kinase, mitogen-activated protein kinase, and protein kinase C pathways (26). An acute meal that contained capsaicin increased GLP-1 levels without affecting satiety, energy expenditure, or PYY levels in humans (14). Our previous studies showed that capsaicin-activated adipose TRPV1 reversed dietary obesity in mice, and stimulation of endothelial TRPV1 relaxed blood vessels and lowered blood pressure in genetically hypertensive rats (29,37,43). This evidence indicates that nonneuronal TRPV1 has functions that are distinct from neuronal TRPV1, such as glucose regulation.

Several studies showed that GLP-1 is secreted from STC-1 cells through depolarization-induced calcium influx (44). Physiological concentrations of GLP-1 stimulated the insulin secretion, which requires cytosolic calcium increase but was independent of the cAMP-dependent protein kinase (45). Our data showed that capsaicin-induced release of GLP-1 from STC-1 cells could be inhibited by several TRPV1 antagonists. Furthermore, either chelating intracellular calcium or omitting extracellular calcium abolished the capsaicin-induced GLP-1 secretion, suggesting that the GLP-1 release was causally associated with TRPV1-mediated calcium influx. This study also indicated that L-type Ca²⁺ channel is partially involved in capsaicin-mediated GLP-1 secretion, which could be related to the nonselective nature of TRPV1 for both Ca²⁺ and Na⁺ influx, then causing membrane depolarization to activate L-type Ca²⁺ channels (46). As a proof of principle, we demonstrated that both acute and chronic capsaicin administration increased plasma insulin and GLP-1 levels in WT but not in TRPV1−/− mice. The circulating GLP-1 responses following a glucose challenge were significantly reduced by 32% in TRPV1−/− mice compared with WT mice.
It is very difficult to gauge the magnitude of direct or indirect effect of TRPV1 mediated effect. Under this circumstance, we examined the plasma insulin levels in the absence of glucose challenge. Gastric gavage administration of capsaicin significantly increased the plasma levels of insulin by 30.3% compared with its control, but this effect was significantly inhibited by GLP-1 receptor antagonist-exendin (9–39). Pretreatment with exendin (9–39), capsaicin increased the plasma levels of insulin by 15.9% compared with exendin (9–39) alone (Supplementary Fig. 4B). Our study suggested that TRPV1 activation by capsaicin lowers blood glucose through both a direct action on insulin secretion in the islet and an indirect effect on the gut secretion of GLP-1.

In addition, we further determined whether capsaicin administration could lower blood glucose levels and increase plasma GLP-1 levels in db/db mice, a well-established mouse model for diabetes. A GLP-1 agonist was reported to attenuate hyperglycemia in diabetic animals (47). We showed that chronic administration of capsaicin increased GLP-1 production and insulin secretion, reduced fasting glucose levels, improved insulin sensitivity, and upregulated the expressions of TRPV1 and GLP-1 in the ileum of db/db mice. The present results indicate that a dietary capsaicin intervention can ameliorate abnormal glucose homeostasis and partially restore β-cell function in diabetic mice.

Clinical studies show that combination therapy with sitagliptin and other drugs were generally well-tolerated and safety (48). Almost no or minor drug–drug interactions has been reported between DPP-4 inhibitors and other drugs (48). It is unknown whether there is interaction between capsaicin and DPP-4 inhibitors. Several studies show that capsaicin, but not DPP-4 inhibitors (except saxagliptin), can inhibit cytochrome P450 (CYP 3A4/A5) enzymes (48–50). Thus, in general, interactions between DPP-4 inhibitors and capsaicin are absent or minor. However, more well-controlled clinical studies are needed to evaluate the safety and long-term effect of these drugs.

In summary, the current study demonstrates that chronic dietary capsaicin effectively increases GLP-1 secretion from both intestinal cells and tissues, and such benefit is related to calcium influx mediated by TRPV1 activation. Capsaicin treatment improves glucose homeostasis and insulin sensitivity in diabetic mice. Taken together, dietary capsaicin may represent a promising
FIG. 6. TRPV1 activation increases GLP-1 secretion and restores insulin secretion in \(db/db\) mice. A: An intraperitoneal insulin tolerance test in \(db/db\) mice treated with or without dietary capsaicin (cap) for 14 weeks; *\(P<0.05\) versus control (cont) (\(n=6\)). B: The fasting blood glucose levels in \(db/db\) mice treated with or without dietary capsaicin for 14 weeks. *\(P<0.05\), **\(P<0.01\) versus control (\(n=6\)). C: The GLP-1 (7–36) levels after 30-min oral glucose challenge in \(db/db\) mice; *\(P<0.05\) versus control (\(n=6\)). D: The plasma insulin levels after 30-min oral glucose challenge in \(db/db\) mice, *\(P<0.05\) versus control (\(n=6\)). E: Immunohistochemical analysis of pancreatic sections. The \(db/db\) mice and control mice were treated with or without 0.01% capsaicin at indicated dosages for 14 weeks. Pancreas consecutive sections were stained with hematoxylin and eosin. Staining of the insulin using anti-insulin antibody (green) are representative islets from each groups. F and G: Immunoblot data showing GLP-1 and TRPV1 expression levels in \(db/db\) mouse ileum tissue after 14 weeks of dietary capsaicin administration. *\(P<0.05\) versus control (\(n=3\)). These data are represented as mean ± SEM and were analyzed with a Student unpaired \(t\) test. (A high-quality digital representation of this figure is available in the online issue.)
lifestyle intervention for populations at a high risk for developing diabetes.

ACKNOWLEDGMENTS
This research was supported by grants from the National Basic Research Program of China (2012CB517805 and 2011CB503902 to Z.Zhu and D.L.) and National Natural Science Foundation of China (30870042, 81130006, 30670076, and 31071006 to D.L., Z.Y., and Z.Zhu). This work was also supported by the Program for Changjiang Scholars from the Ministry of Education in China (to Z.Zhu).

No potential conflicts of interest relevant to this article were reported.

P.W. performed most of the experiments, analyzed data, and wrote the manuscript. Z.Y. and J.C. performed some experiments and contributed to the discussion. L.L., L.M., and Z.Z. performed some experiments. D.L. edited the manuscript and contributed to the discussion. Z.Z. designed the experiments, wrote and edited the manuscript, and 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.

The authors thank Tingbing Cao, Zhidan Luo, and Lijuan Wang (Chongqing Institute of Hypertension, China) for technical assistance. The authors also thank Prof. Yu Huang and Dr. Wing Tak Wong (Chinese University of Hong Kong, China) for critical review of the manuscript.

REFERENCES
1. Danaei G, Finucane MM, Lu Y, et al. Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Blood Glucose). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370-country-years and 2·7 million participants. Lancet 2011;378:31–40
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4–14
3. Zimman B. Initial combination therapy for type 2 diabetes mellitus: is it ready for prime time? Am J Med 2011;124(Suppl.1):S19–S34
4. Earish DT, McAllister FA, Blackburn DF, et al. Benefits and harms of anti-diabetic agents in patients with diabetes and heart failure: systematic review. BMJ 2007;335:497
5. Nathan DM, Buse JB, Davidson MB, et al. American Diabetes Association; European Association for Study of Diabetes. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2009;32:193–203
6. Gupta R, Wajnberg SS, Tokala RK, Parsa KV, Singh SK, Pal M. Emerging drug candidates of dipeptidyl peptidase IV (DPP IV) inhibitor class for the treatment of Type 2 Diabetes. Curr Drug Targets 2009;10:71–87
7. Buteau J. GLP-1 receptor signaling: effects on pancreatic beta-cell proliferation and survival. Diabetes Metab 2008;34(Suppl. 2):S73–S77
8. Meece J. Pancreatic islet dysfunction in type 2 diabetes: a rational target for incretin-based therapies. Curr Med Res Opin 2007;23:933–944
9. Hira T, Mohida T, Miyashita K, Hara H. GLP-1 secretion is enhanced directly in the ileum but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate, in rats. Am J Physiol Gastrointest Liver Physiol 2009;297:G663–G671
10. Zhou J, Martin RJ, Tulley RT, et al. Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. Am J Physiol Endocrinol Metab 2008;294:E160–E166
11. Hirasawa A, Tsumaya K, Awaji T, et al. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med 2005;11:90–94
12. Toulhurst G, Reichmann F, Grable JM. Nutritional regulation of glucagon-like peptide-1 secretion. J Physiol 2009;587:27–32
13. Daoudi M, Hennuyer N, Borland MG, et al. PPARβ/δ activation induces enteroendocrine L cell GLP-1 production. Gastroenterology 2011;140:1574–1584
14. Smeets AJ, Westerterp-Plantenga MS. The acute effects of a lunch containing capsaicin on energy and substrate utilisation, hormones, and satiety. Eur J Nutr 2009;48:229–234
15. Kang JH, Goto T, Han IS, Kawada T, Kim YM, Yu R. Dietary capsaicin reduces obesity-induced insulin resistance and hepatic steatosis in obese mice fed a high-fat diet. Obesity (Silver Spring) 2010;18:785–787
16. Islam MS, Choi H. Dietary red chili (Capsicum frutescens L.) is insulinotropic rather than hypoglycemic in type 2 diabetes model of rats. Phytother Res 2008;22:1025–1029
17. Dömötör A, Szolcsányi J, Mószik G. Capsaicin and glucose absorption and utilization in healthy human subjects. Eur J Pharmacol 2006;534:280–284
18. Akiha Y, Kato S, Kabuse K, et al. Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet beta cells modulates insulin secretion in rats. Biochem Biophys Res Commun 2004;321:219–225
19. Tolan I, Ragoobirisingh D, Morrison EY. The effect of capsaicin on blood glucose, plasma insulin levels and insulin binding in dog models. Phytother Res 2001;15:291–294
20. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997;397:816–824
21. Gram DX, Ahrén B, Nagy I, et al. Capsaicin-sensitive sensory fibers in the islets of Langerhans contribute to defective insulin secretion in Zucker diabetic rat, an animal model for some aspects of human type 2 diabetes. Eur J Neurosci 2007;25:213–223
22. Razavi R, Chan Y, Afifiyan FN, et al. TRPV1+ sensory neurons control beta cell stress and islet inflammation in autoimmune diabetes. Cell 2006;127:1123–1135
23. Karlsson S, Scherrink AJ, Steffens AB, Ahren B. Involvement of capsaicin-sensitive nerves in regulation of insulin secretion and glucose tolerance in conscious mice. Am J Physiol 1994;267:E1071–E1077
24. Abuja KD, Robertson IK, Geraghty DP, Ball MJ. Effects of chili consumption on postprandial glucose, insulin, and energy metabolism. Am J Clin Nutr 2006;84:63–69
25. Matsumoto K, Kurowsawa E, Terui H, et al. Localization of TRPV1 and contractile effect of capsaicin in mouse large intestine: high abundance and sensitivity in rectum and distal colon. Am J Physiol Gastrointest Liver Physiol 2009;297:G548–G560
26. Zsombok A, Bhaskaran MD, Gao H, Derbenev AV, Smith BN. Functional plasticity of central TRPV1 receptors in brainstem dorsal vagal complex circuits of streptozotocin-treated hyperglycemic mice. J Neurosci 2011;31:14024–14031
27. Ma S, Yu H, Zhao Z, et al. Activation of the cold-sensing TRPM8 channel triggers UCPI-dependent thermogenesis and prevents obesity. J Mol Cell Biol 2012;4:88–96
28. Han BG, Hao CM, Tchekneve EE, et al. Markers of glycemic control in the mouse: comparisons of 6-h- and overnight-fasted blood sugars to Hb Alc. Am J Clinical Endocrinol Metab 2008;295:E891–E896
29. Yang D, Luo Z, Ma S, et al. Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension. Cell Metab 2010;12:130–141
30. He H, Yang D, Ma L, et al. Telmisartan prevents weight gain and obesity through activation of peroxisome proliferator-activated receptor-delta-dependent pathways. Hypertension 2010;55:869–879
31. Rodenburg W, Keijer J, Kramer E, Vink C, van der Meer R, Bovee-Oudenhoven IM. Impaired barrier function by dietary fructo-oligosaccharides (FOS) in rats is accompanied by increased colonic mitochondrial gene expression. BMC Genomics 2008;9:144
32. Mu J, Woods J, Zhou YP, et al. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. Diabetes 2006;55:1695–1704
33. Zhu Z, Luo Z, Ma S, Liu D. TRP channels and their implications in metabolic diseases. Pfluegers Arch 2011;461:211–220
34. Thomas C, Gioiello A, Noriega L, et al. TGR5-mediated bile acid sensing mediates a TLR5-mediated protective immune response in the gut. J Biol Chem 2012;287:15602–15613
35. Alevizos A, Mihas C, Mariolis A, Larios G. Insulin secretion and capsaicin. Diabetes Metab 2008;34(Suppl. 2):S87–S89
36. Tadeo F, Sánchez-Delgado M, Alguacil-G叠mez J, et al. TRPV1+ sensory neurons control beta cell stress and islet inflammation in autoimmune diabetes. Cell 2006;127:1123–1135
37. Am J Physiol Gastrointest Liver Physiol 2009;297:G548–G560
38. Rodenburg W, Keijer J, Kramer E, Vink C, van der Meer R, Bovee-Oudenhoven IM. Impaired barrier function by dietary fructo-oligosaccharides (FOS) in rats is accompanied by increased colonic mitochondrial gene expression. BMC Genomics 2008;9:144
37. Zhang LL, Yan Liu D, Ma LQ, et al. Activation of transient receptor potential vanilloid type-1 channel prevents adipogenesis and obesity. Circ Res 2007;100:1063–1070
38. Hellström PM. GLP-1: broadening the incretin concept to involve gut motility. Regul Pept 2009;156:9–12
39. Cani PD, Neyrinck AM, Maton N, Delzenne NM. Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like Peptide-1. Obes Res 2005;13:1000–1007
40. Gram DX, Hansen AJ, Deacon CF, et al. Sensory nerve desensitization by resiniferatoxin improves glucose tolerance and increases insulin secretion in Zucker Diabetic Fatty rats and is associated with reduced plasma activity of dipeptidyl peptidase IV. Eur J Pharmacol 2005;509:211–217
41. Tanaka H, Shimaya A, Kiso T, Kuramochi T, Shimokawa T, Shibasaki M. Enhanced insulin secretion and sensitization in diabetic mice on chronic treatment with a transient receptor potential vanilloid 1 antagonist. Life Sci 2011;88:559–563
42. Zhong B, Wang DH. TRPV1 Regulates Basal and Stimulated Insulin Secretion from the Pancreas [abstract]. Hypertension 2009;54:e105
43. Xu X, Wang P, Zhao Z, et al. Activation of transient receptor potential vanilloid 1 by dietary capsaicin delays the onset of stroke in stroke-prone spontaneously hypertensive rats. Stroke 2011;42:3245–3251
44. Eiki J, Saeki K, Nagano N, et al. A selective small molecule glucagon-like peptide-1 secretagogue acting via depolarization-coupled Ca(2+) influx. J Endocrinol 2009;201:361–367
45. Bode HP, Moormann B, Babew R, Goke B. Glucagon-like peptide 1 elevates cytosolic calcium in pancreatic beta-cells independently of protein kinase A. Endocrinology 1999;140:3919–3927
46. Hagenacker T, Splettstoesser F, Greffrath W, Treede RD, Busselberg D. Capsaicin differentially modulates voltage-activated calcium channel currents in dorsal root ganglion neurones of rats. Brain Res 2005;1062:74–85
47. Baggio LL, Kim JG, Drucker DJ. Chronic exposure to GLP-1R agonists promotes homologous GLP-1 receptor desensitization in vitro but does not attenuate GLP-1R-dependent glucose homeostasis in vivo. Diabetes 2004;53(Suppl. 3):S205–S214
48. Scheen AJ. Dipeptidylpeptidase-4 inhibitors (gliptins): focus on drug-drug interactions. Clin Pharmacokinet 2010;49:573–588
49. Babbar S, Chanda S, Bley K. Inhibition and induction of human cytochrome P450 enzymes in vitro by capsaicin. Xenobiotica 2010;40:807–816
50. Zhang QH, Hu JP, Wang BL, Li Y. Effects of capsaicin and dihydrocapsaicin on human and rat liver microsomal CYP450 enzyme activities in vitro and in vivo. J Asian Nat Prod Res 2012;14:382–395