Acarbose ameliorates spontaneous type-2 diabetes in db/db mice by inhibiting PDX-1 methylation

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Abstract. Pancreatic and duodenal homeobox (PDX)-1 is a gene that plays an important role in pancreatic development and function. Type-2 diabetes mellitus (T2DM) is a metabolic disease associated with insulin resistance and impaired islet β-cell function. There is evidence that methylation of PDX-1 plays a role in the development of T2DM. Acarbose is an α-glucosidase inhibitor that can effectively delay the absorption of glucose by the body. The aim of the present study was to examine the effect of acarbose on PDX-1 methylation in islet β-cells in spontaneous type-2 diabetic db/db mice. The effect of acarbose on glucose and lipid metabolism in these mice was assessed by measuring food intake, body weight, glycated hemoglobin (HbA1c), glucagon, serum total cholesterol and triglyceride levels, and fasting blood glucose (FBG). Blood glucose levels were also analyzed using intraperitoneal glucose tolerance and insulin tolerance tests. Immunohistochemistry was used to evaluate the effect of acarbose on pathological changes in the pancreas. Moreover, a BrdU assay was used to analyze cell proliferation. Lastly, the effect of acarbose on PDX-1 methylation was evaluated in mice using methylation-specific PCR and western blot analysis. In the present study, body weight significantly increased in the acarbose group, compared to the normal group. The levels of HbA1c and glucagon in the T2DM group significantly increased, compared with the normal group, but significantly decreased in acarbose-treated mice. Moreover, FBG levels significantly decreased in the acarbose groups compared with T2DM mice. Acarbose also promoted cell proliferation, compared with untreated T2DM mice. In addition, PDX-1 methylation and cytoplasmic expression levels were both downregulated in the acarbose group, compared with the T2DM group. In conclusion, these results suggested that acarbose could promote the proliferation of islet β-cells and inhibit PDX-1 methylation in islet β cells from diabetic mice. Thus, acarbose may provide a new strategy to treat T2DM.

Introduction

Diabetes mellitus is a common group of clinical syndromes that are mainly manifested in disorders of glucose metabolism and fluid imbalance (1,2). More than 400 million patients are diagnosed with the disease worldwide, and it has high mortality and disability rates (3). Notably, type-2 diabetes mellitus (T2DM) accounts for up to 90% of these patients. T2DM is characterized by hyperglycemia induced by multiple factors, including genetic inheritance, various environmental factors (such as exercise habits and work pressure) and lifestyle (4). T2DM is caused by impaired islet β-cell function and insulin resistance (5).

In recent years, researchers have carried out exploratory work on T2DM in the field of epigenetics, including DNA methylation (6) and histone acetylation (7), in an effort to explain the epigenetic mechanism of T2DM (8). Changes in dietary habits cause epigenetic changes (9). Epigenetic research related to the environment is becoming increasingly important in the prevention and treatment of diseases (10). DNA methylation is one of the most well-studied epigenetic processes in transcriptional regulation and gene silencing. PDX-1 is a gene that plays an important role in pancreatic development and function (11,12). Previous studies have suggested that the methylation of PDX-1 plays a role in the development of T2DM (13,14).

Acarbose is an α-glucosidase inhibitor that functions by competing with oligosaccharides to inhibit α-glucosidase activity of intestinal wall cells, thereby delaying the process of carbohydrate degradation and effectively delaying the absorption of glucose by pancreatic tissues (15). However, the effect of acarbose on PDX-1 methylation in islet β cells remains unclear. The purpose of the study was to evaluate the effect of acarbose on PDX-1 methylation in islet β-cells in spontaneous type 2 diabetic db/db mice.

Materials and methods

Animal studies and experimental design. Male, 8-week-old db/db mice and male -+/db mice (n=28; weight, ~18 g) were purchased from Shanghai Xipu-bikai Experimental Animal Co., Ltd. The mice were housed at 22-25˚C with 60±5%,

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12-h light/dark cycles and sufficient water and were fed a commercial diet over one week of adaptation. The mice were then randomly divided into four groups. In the normal group (n=8), male -+/db mice were fed a commercial diet. Mice with fasting blood glucose >11.1 mmol/l were considered diabetic and selected in the T2DM groups. In the T2DM group (n=8), mice were fed a high-fat diet (T2DM model group). The mice in the acarbose-treated diabetic group (T2DM+acarbose group; n=8) were given 9 g/kg/day acarbose extract (cat. no. 1906208B; Zhongmei Huadong Pharmaceutical Co., Ltd.) by gavage for four weeks. The mice in the alkylresorcinol (AR; cat. no. 3158-56-3; Sigma-Aldrich; Merck KGaA)-treated T2DM group (T2DM+AR group; n=8) received 60 mg/kg/day AR extract by gavage for 4 weeks as a positive control (16). This study was performed in strict accordance with the NIH guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23 Rev. 1985) and was approved by the Animal Care and Use Committee of Zhejiang Chinese Medical University (approval no. ZSLL-2019-102). Food intake, body weight, serum total cholesterol (TC) and triglyceride (TG) levels, glucagon, and fasting blood glucose (FBG) were measured. All mice were euthanized with 5% isoflurane. Mice were monitored until complete cessation of the heartbeat, sustained involuntary respiration for 2-3 min and absence of blinking or toe contraction reflex.

Routine measurements. During the experimental period, food intake, body weight, and FBG were recorded weekly. Before measuring FBG, the mice were fasted for 24 h (with sufficient water), blood (1 ml; total 5 ml) was collected from the tail vein, and FBG was measured with a blood glucose meter (Jiangsu Yuyue Medical Equipment & Supply Co., Ltd.) weekly until the mice were sacrificed. Four weeks after treatment, glycosylated hemoglobin (HbA1c) was measured using a DCA2000 analyzer (Bayer AG). The levels of glucagon were measured by ELISA (cat. no. MOES01072; USCN Kit, Inc.).

Lipid profiles in serum. The serum total cholesterol (TC) and triglyceride (TG) levels were measured by direct enzymatic methods using a CX-7 Biochemical Autoanalyser (Beckman Coulter, Inc.).

Glucose and insulin tolerance tests. Intraperitoneal glucose tolerance tests (IPGTTs) and insulin tolerance tests (ITTs) were performed on different days. Four weeks after treatment, all mice were fasted overnight before analysis. Glucose was administered at a dose of 2 g/kg by intraperitoneal injection, and tail vein blood glucose levels were measured using a glucose meter (Yuyue301) at 0, 30, 60, and 120 min. The mice were injected with 1 U/kg humulin R (Eli Lilly) after fasting for 4 h for the IPGTT. The glucose levels were measured at 0, 30, 60, 90 and 120 min after injection.

Methylation-specific PCR (MSP). The MSP was performed on a 7300 thermocycler (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers for Methylation PDX-1 and unmethylated PDX-1 were based on the genomic sequence (accession nos. NT_024524100 and NT_024524) of the PDX-1 gene. Primer sequences were as follows: i) Unmethylated PDX-1-forward, 5'-GCCTCGAGCGCAGACTGAGTCCAG AGTG-3' and reverse, 5'-CGAAGCTTCGGTGATTTCTTC AGGGAAA-3'; ii) Methylation PDX-1-forward, 5'-GTT TTTTTCGGAGGGAGTC-3' and reverse, 5'-TTTCCACGC GTAAACTTTAA-3'. Cellular DNA was extracted using the QIamp DNA mini kit (Qiagen GmbH) from mouse pancreatic samples according to the manufacturer's instructions. Bisulphate modification of the DNA was performed using the EZ Methylation-Gold kit (Zymo Research, Corp.) according to the manufacturer's protocol. DNA elements with methylated cytosins remained unmodified after bisulfite treatment, whereas unmethylated cytosins were completely converted into uracil nucleosides and further to thymine after PCR, thus giving different primer recognition sequences for MSP. PCR-mix (2X; 12.5 μl; Fermentas; Thermo Fisher Scientific, Inc.) was added to 2 μl bisulfonated/unmodified DNA sample followed by 0.8 μl forward and reverse primers (400 nM each) and 8.5 μl water.

The PCR reaction was run using the Taq GC Buffer amplification system (Takara Biotechnology Co., Ltd.) and the following thermocycling conditions: 94°C for 2 min; followed by 35 cycles at 94°C for 10 sec, 60-70°C for 20 sec and 72°C for 20 sec; and terminated at 72°C for 4 min followed by immediate cooling to 4°C.

After staining with 6X loading dye solution (Fermantas; Thermo Fisher Scientific, Inc.), the PCR products were analyzed on 2% agarose gels and visualized under UV illumination using a LabWorks gel imaging system (version 4.6; UVP, LLC).

Extraction of nuclear extracts and pancreatic tissue homogenate. Nuclear extracts were isolated from pancreatic tissues using NE-PER™ Extraction Reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Frozen pancreatic tissues were placed on ice and pulverized in a pre-cooled mortar with liquid nitrogen. Tissues were lysed in 500 μl RIPA buffer (Beyotime Institute of Biotechnology) supplemented with phosphatase and protease inhibitor cocktail (Thermo Fisher Scientific, Inc.) and then centrifuged at 10,000 x g for 20 min at 4°C. The soluble extracts were collected for western blotting. The determination of protein concentrations was performed using a bicinchoninic acid assay (Thermo Fisher Scientific, Inc.).

Western blot analysis. Soluble lysates (40 μg) and nuclear extracts (20 μg) were separated by SDS-PAGE on 8% gels and transferred to PVDF membranes (EMD Millipore). The membranes were blocked with 5% nonfat milk in TBS-T (10 mM Tris, pH 7.6, 150 mM NaCl, and 0.05% Tween-20) for 2 h at room temperature and probed with primary antibodies against PDX-1 (42 kDa; rabbit; 1:1,000; cat. no. 5679; Cell Signaling Technology), β-actin (45 kDa; rabbit; 1:1,000; cat. no. ab8227; Abcam) and PCNA (36 kDa; rabbit; 1:1,000; cat. no. 13110; Cell Signaling Technology) overnight at 4°C. A peroxidase-conjugated goat anti-mouse IgG secondary antibody (1:2,000; cat. no. sc-516102; Santa Cruz Biotechnology, Inc.) was then added for 1.5 h at room temperature. The bands were visualized using an enhanced chemiluminescence kit (Beyotime Institute of Biotechnology) and quantified using ImageJ software (version 6.0; National Institutes of Health).
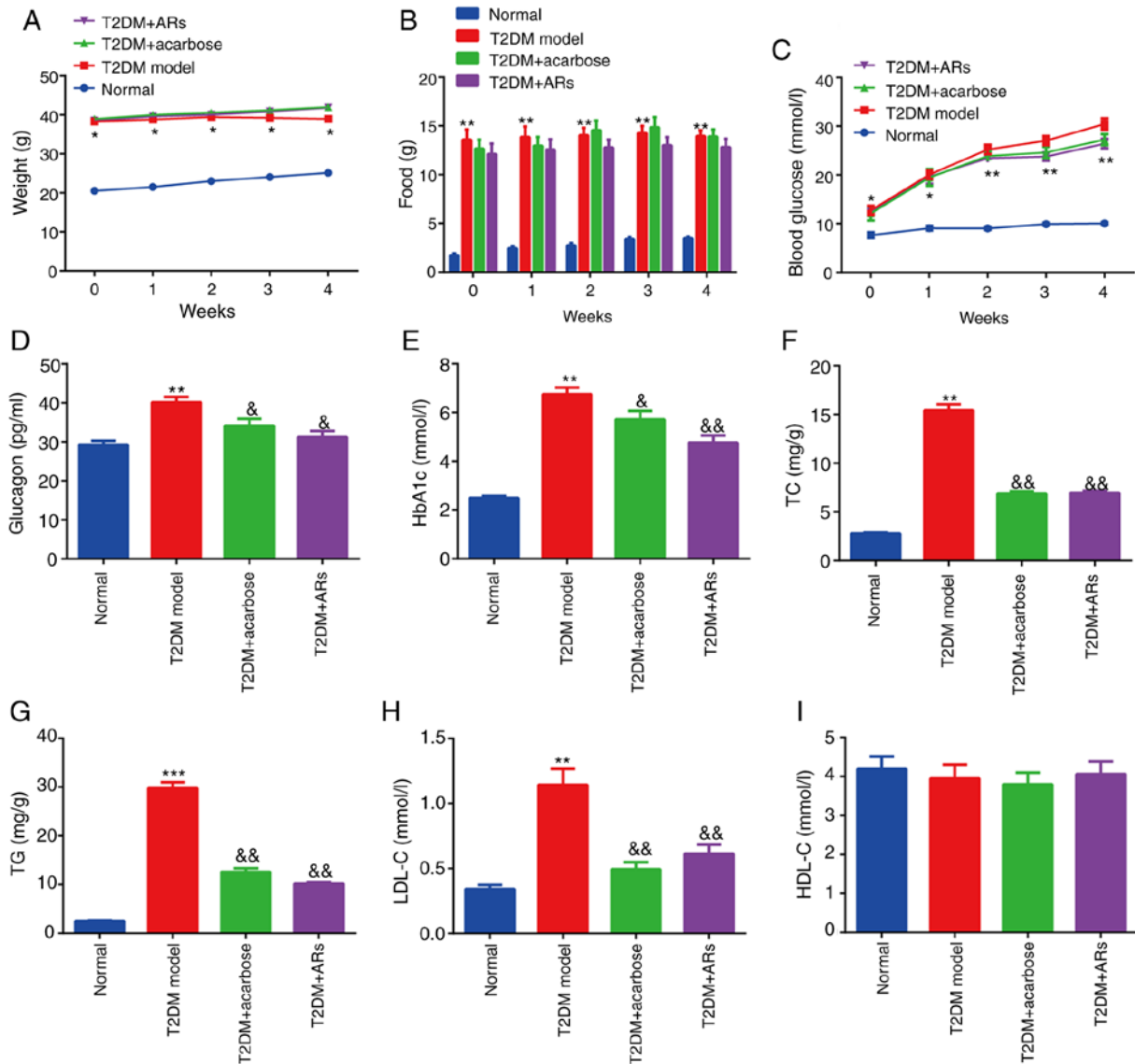
Figure 1. Effect of acarbose on glucose and lipid metabolism in diabetic mice. (A) Weight, (B) food, (C) fasting blood glucose, (D) glucagon, (E) HbA1c, (F) TC, (G) TG (H) LDL-C and (I) HDL-C levels were determined. n=8. *P<0.05, **P<0.01, ***P<0.001, vs. normal group; &P<0.05, &&P<0.01, vs. T2DM group. AR, alkylresorcinol; T2DM, type-2 diabetes mellitus; HbA1c, glycated hemoglobin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol.

β-cell proliferation assessment. After fixation by perfusion with 4% paraformaldehyde for 5 min at room temperature, the mouse pancreas was removed. After sectioning into 5-μm, dewaxing and microwave antigen recovery, the slides were briefly incubated with 0.2% Triton X-100 and 10% BSA (cat. no. A3858; Sigma-Aldrich; Merck KGaA), then kept overnight at 4˚C with guinea pig anti-insulin (cat. no. ab7842; 1:1,000; Abcam) and mouse anti-BrdU (cat. no. ab8152; 1:1,000; Abcam) antibodies followed by Alexa Fluor 568 goat anti-guinea pig (cat. no. AP108B; 1:1,000; Sigma-Aldrich; Merck KGaA) and FITC goat anti-mouse (cat. no. ab6785; 1:1,000; Abcam) secondary antibodies. Nuclei were counterstained with DAPI (cat. no. ab104139; Abcam). All BrdU+ cells and insulin+ cells (representing total β-cells) from 50 pancreatic islets per mouse were counted using a confocal microscope (Zeiss AG). The percentage of β-cell proliferation was determined by dividing the number of BrdU+ β-cells by the total number of β-cells.

Immunohistochemistry. Pancreatic tissues were fixed in 10% neutral-buffered formalin for 5 min at room temperature and embedded in paraffin. After a xylene wash to remove the paraffin and a rehydration step with serial dilutions of alcohol, the 5-μm thick sections were incubated in 0.3% H_2O_2 for 15 min to block endogenous peroxidases and heated at 98˚C for 15 min for antigen retrieval. Samples were blocked with 5% BSA for 1 h at room temperature. The sections were then incubated overnight at 4˚C with primary antibodies against inducible Neurogenin 3 (NGN3; cat. no. sc-374442; mouse; 1:60; Santa Cruz Biotechnology, Inc.), Insulin (mouse; cat. no. BM4310; 1:50; Wuhan Boster Biological Technology, Ltd.), PDX-1 (cat. no. 5679; rabbit; 1:50; Cell Signaling Technology, Inc.) and Forkhead box O1 (FoXO1; cat. no. 2880; rabbit, 1:100; Cell Signaling Technology). After a series of washes, peroxidase-conjugated goat anti-mouse IgG/goat anti-rabbit IgG secondary antibodies (cat. nos. sc-516102 and sc-2357; 1:100; Santa Cruz Biotechnology, Inc.) were applied.
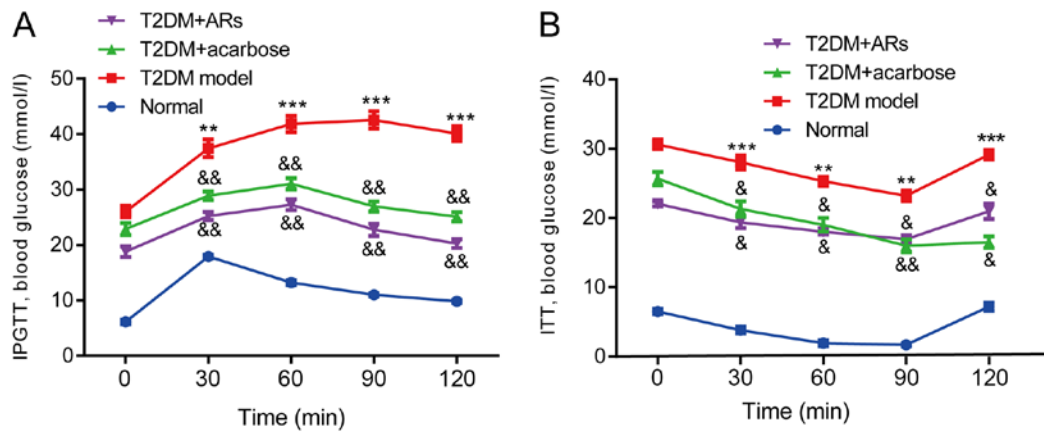
Figure 2. Effect of acarbose on fasting blood glucose. (A) Fasting blood glucose was analyzed in IPGTTs. (B) Fasting blood glucose was analyzed in ITTs. n=8. **P<0.01, ***P<0.001, vs. normal group; &P<0.05, &&P<0.01, vs. T2DM group. AR, alkylresorcinol; T2DM, type-2 diabetes mellitus; IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test.
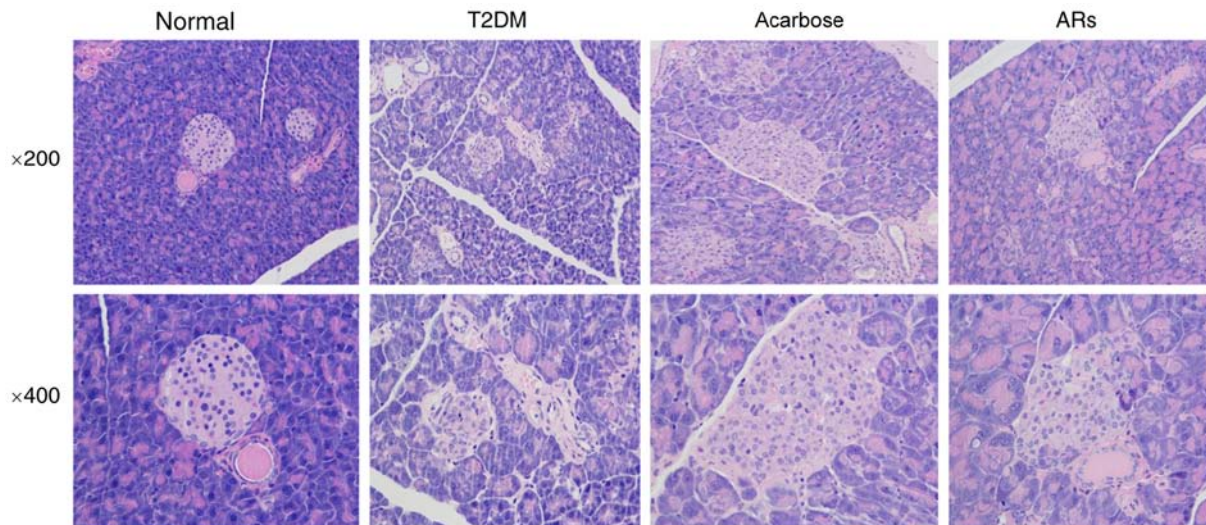

Figure 3. Effects of acarbose on pancreatic islet number and structure in diabetic mice based on hematoxylin and eosin staining. High glucose-mediated damage to pancreatic tissue were reversed by Acarbose. Magnification, x200 or x400. n=8.

The sections were stained with diaminobenzidine solution (OriGene Technologies, Inc.) washed, dehydrated, permeabilized, mounted and examined under bright-field microscopy. Images were examined using an LSM 710 laser scanning confocal microscope (Carl Zeiss AG).

Statistical analysis. Data are presented as the mean ± SEM of at least three independent experiments. Statistical analysis was performed using SPSS version 13.0 (SPSS, Inc.). One-way ANOVA followed by Bonferroni correction was used for multi-group comparisons. A t-test was performed to identify significant differences between two groups. P<0.05 were considered to indicate a statistically significant difference.

Results

Effect of acarbose on glucose and lipid metabolism in diabetic mice. T2DM mice exhibited a significant increase and body weight, compared with normal mice (Fig. 1A and B). Furthermore, T2DM mice demonstrated a significant increase in FBG compared with normal mice, and the increased FBG levels were maintained throughout the entire experiment (Fig. 1C). These results are considered common signs of diabetes. Importantly, in all diabetic mice mice, glucagon, HbA1c, TC, TG and LDL-C levels were all significantly increased, compared with the normal group (Fig. 1D-H), while HDL-C levels remained unchanged (Fig. 1I), indicating that hyperlipidemia symptoms were also successfully established in the diabetes group. Moreover, acarbose significantly decreased glucagon, HbA1c, TC, TG and LDL-C levels in serum, compared with untreated T2DM mice.

Effect of acarbose on the IPGTT and ITT. Blood glucose levels were measured at 30, 60, 90 and 120 min. In the normal group, the initial and final blood glucose levels were similar. By contrast, blood glucose levels remained high in T2DM mice at the 120-min timepoint, compared with the initial timepoint (Fig. 2A). Interestingly, blood glucose significantly decreased in the acarbose- or AR-treated groups, compared,
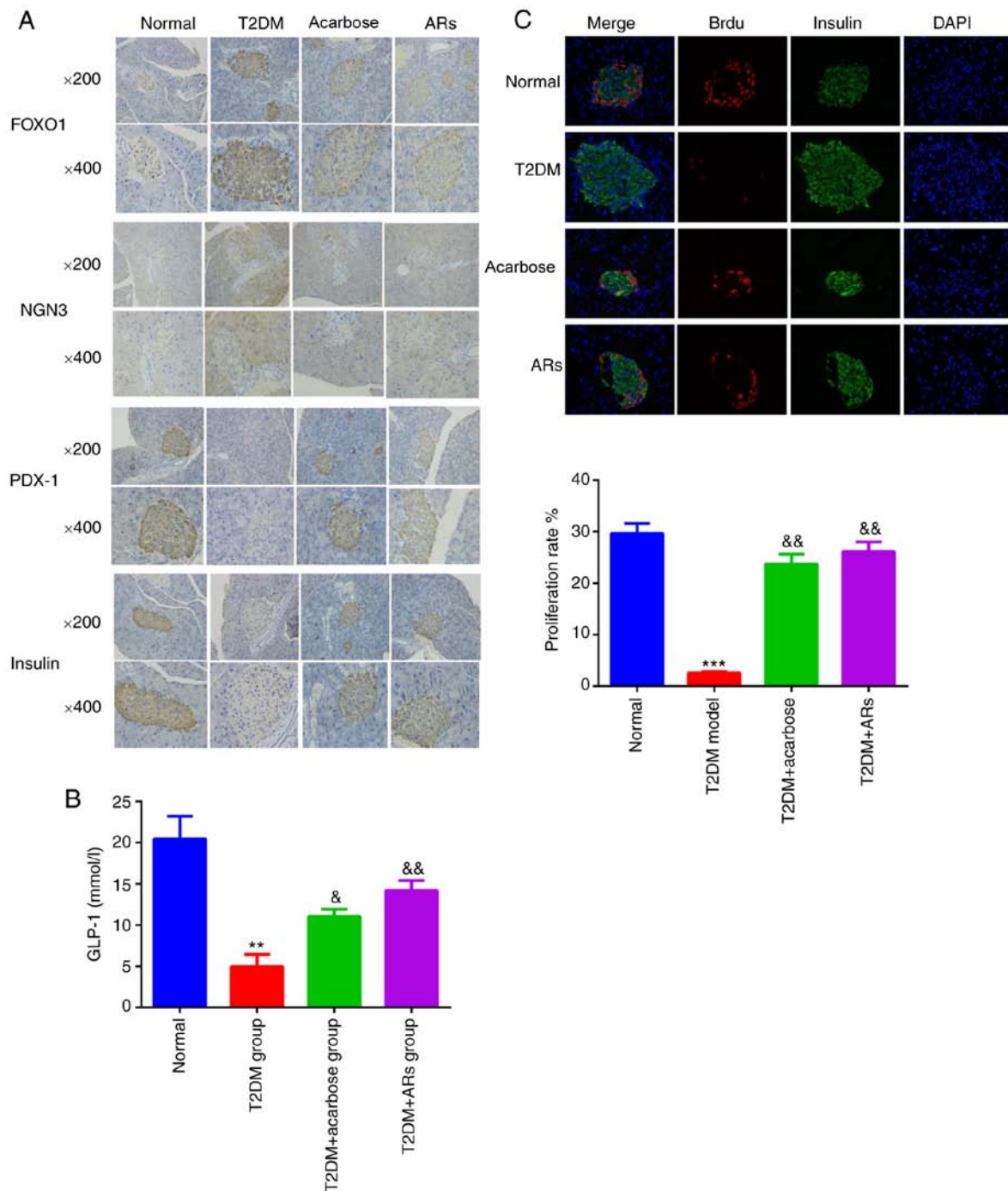
Figure 4. Effects of acarbose on insulin, PDX-1, FoxO1 and NGN 3 expression in islets. (A) Insulin, PDX-1, FoxO1 and Ngn3 expression in the islet β-cells of pancreatic tissue sections. Magnification, x200 or x400. (B) GLP-1 levels. (C) Numbers of insulin-positive and BrdU-positive β-cells. n=8. **P<0.01, ***P<0.001, vs. the normal group; &P<0.05, &&P<0.01, vs. the T2DM group. AR, alkylresorcinol; T2DM, type-2 diabetes mellitus; FOXO1, forkhead box O1; NGN3, neurogenin 3; PDX-1, pancreatic and duodenal homeobox-1; GLP-1, zinc finger GATA-like protein 1.

with T2DM mice, suggesting that acarbose could reduce glucose rapidly and stably. Similarly, in the ITT, the acarbose-treated mice displayed reduced blood glucose levels at 30, 60, 90 and 120 min compared to the T2DM model mice (Fig. 2B).

Effects of acarbose on pancreatic islet number and structure in diabetic mice. HE staining of pancreatic tissue demonstrated that high-fat diet administration elicited severe injury to the pancreas, including decreased islet cells and diminished pancreatic islet

diameter. Additionally, the structure of the pancreatic islets was disordered, vacuoles appeared, and nuclei were swollen in T2DM mice. These effects were reversed by acarbose (Fig. 3).

Effects of acarbose on insulin, PDX-1, FoxO1 and Ngn3 expression in islets. Active PDX-1 induces the efficient production of insulin. The strong nuclear expression of PDX-1 was shown in the islet β-cells of NC pancreatic tissues, while PDX-1 staining was weak in the DM group and was restored by acarbose (Fig. 4A). Conversely, strong nuclear expression of FoxO1
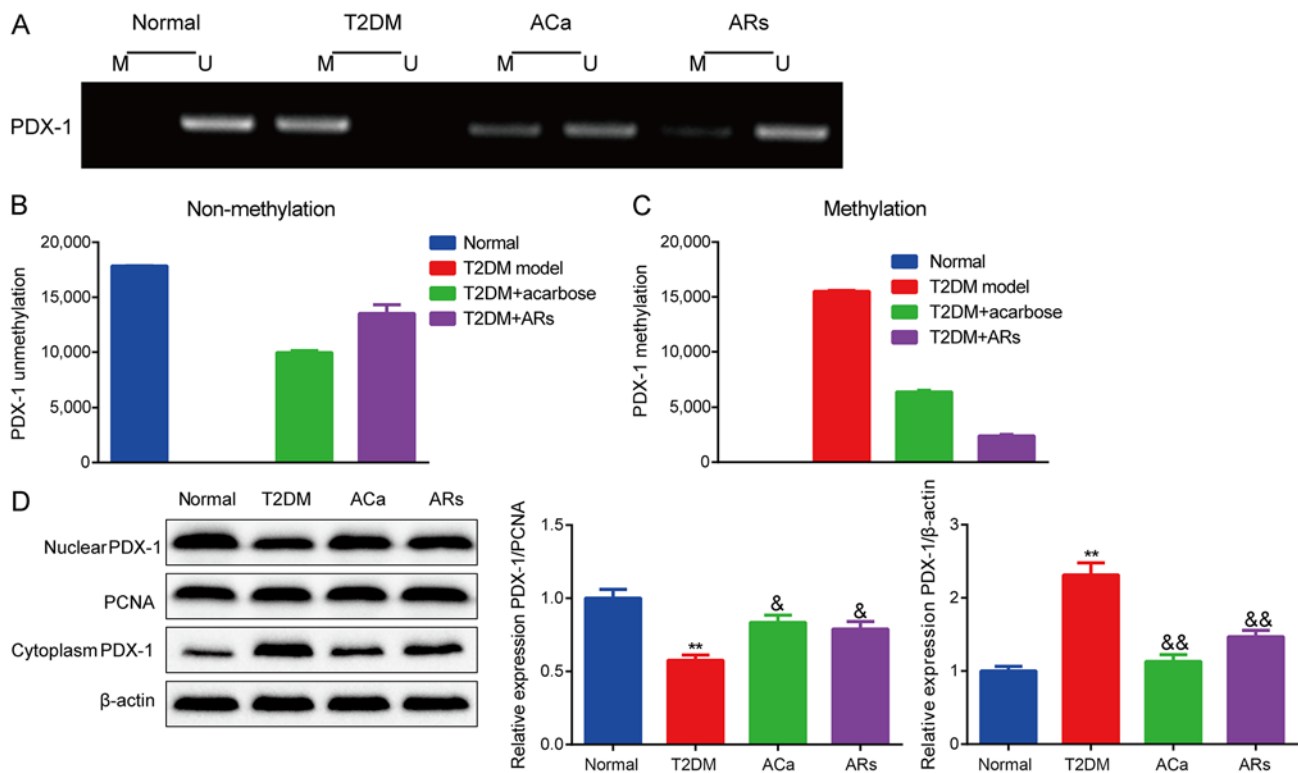
Figure 5. Effects of acarbose on PDX-1 promoter hypermethylation in diabetic mice. (A-C) PDX-1 hypermethylation was inhibited by acarbose and ARs. (D) Acarbose prevented the nuclear export of PDX-1 in T2DM model mice, PCNA and β-actin were used as loading controls for nuclear and cytoplasmic fractions, respectively. n=8. **P<0.01, vs. normal group; &P<0.05, &&P<0.01, vs. T2DM group. AR, alkylresorcinol; T2DM, type-2 diabetes mellitus; PDX-1, pancreatic and duodenal homeobox-1; PCNA, proliferating cell nuclear antigen; M methylated; U, unmethylated.

was observed in the pancreatic tissues of T2DM model mice, which was reversed by acarbose (Fig. 4A). The Ngn3 level in the pancreas was obviously increased in the T2DM model mice and was obviously decreased by acarbose (Fig. 4A). Moreover, strong insulin staining was observed in normal mice but weak staining was observed in T2DM model mice. However, acarbose increased insulin levels (Fig. 4A). the level of GLP-1 was determined, and the results showed that GLP-1 level was decreased in T2DM group compared to normal group, which partly reversed by acarbose and alkylresorcinol (Fig. 4B). Similarly, the number of insulin-positive/BrdU-positive β-cells increased dramatically in the T2DM + acarbose group compared with the T2DM group (Fig. 4C).

Effects of acarbose on PDX-1 promoter hypermethylation in diabetic mice. Hypermethylation of the PDX-1 promoter was not observed in normal mice but was significantly increased in T2DM model mice. Additionally, PDX-1 hypermethylation was inhibited by acarbose and alkylresorcinol (Fig. 5A-C). Moreover, a significant redistribution of PDX-1 from the nucleus to the cytoplasm was observed in pancreatic tissues of T2DM model mice compared with that in normal mice. In contrast, acarbose prevented the nuclear export of PDX-1 in T2DM model mice (Fig. 5D).

Discussion

The prevalence of T2DM has been increasing each year, in parallel to the increase in the population with a high risk

for diabetes development (17). Acarbose is one of the safest antidiabetic agents available and is commonly prescribed for the treatment or prevention of T2DM (18). The most important function of pancreatic β-cells is to maintain blood glucose homeostasis by secreting insulin upon glucose stimulation. Loss of pancreatic islet mass and β-cell dysfunction accompanied by a decrease in insulin synthesis are crucial factors for initiating the development of DM (19). The present study confirmed that acarbose could alleviate pancreatic β-cell damage and impaired insulin secretion in T2DM mice by reducing the methylation of PDX-1.

Insulin resistance is reported to be involved in the disorder of glucose metabolism and lipid metabolism (20). In the present study, a diabetic mouse model was established that exhibited hyperglycemia and insulin resistance. Acarbose significantly reduced blood glucose levels and improved insulin sensitivity in diabetic mice. It also reduced the elevated levels of glucagon, TC and TG seen in diabetic mice and promoted β-cell proliferation. In addition, acarbose promoted the nuclear expression of PDX-1 with a concomitant decrease in acetylated FoxO1 levels, both of which led to increased production of insulin.

PDX-1 is predominately expressed in β-cells and can induce β-cell differentiation and insulin transcription (21). Conversely, mutation or downregulation of PDX-1 results in β-cell dysfunction and glucose intolerance in humans and animals (22), indicating that the activation of PDX-1-regulated responses is an important therapeutic target for preventing the development of DM. The present study demonstrated

increased cytoplasmic PDX-1 levels and decreased nuclear PDX-1 in β-cells from diabetic mice, compared with control mice, and acarbose blocked the diabetes-mediated nucleocytoplasmic shuttling of PDX-1, suggesting that acarbose could promote insulin production by preserving nuclear PDX-1. Interestingly, in PDX-1$^+$ β-cells, FoxO1 is largely localized in the cytoplasm, but in PDX-1$^-$ β-cells, FoxO1 mainly accumulates in the nucleus (23), suggesting the mutual exclusion of FoxO1 and PDX-1. Ngn3 is a basic helix-loop-helix protein and a progenitor cell marker in islets (24). Moreover, the increase in Ngn3 resulted from the dedifferentiation of pancreatic β-cells in the islets and was probably attributed to the decrease in β-cells (25). A significant increase in Ngn3 levels was observed in the model group compared with the control group. Moreover, the levels of insulin, glucagon and PDX-1 in islets were increased in the T2DM group. These data may support the hypothesis that alterations to the genes leads to a decrease in β-cells and that acarbose could prevent β-cell proliferation.

A previous study suggested that DNA methylation may be involved in controlling cell-specific PDX-1 expression in human islets (22). In support of this, increased PDX-1 methylation was observed in T2DM mouse β-cells, leading to reduced PDX-1 levels. Moreover, acarbose blocked the increase in methylated PDX-1 in T2DM mouse β-cells.

However, our study had some limitations. First, the levels of DNA methylases Dnmt1, Dnmt3a or Dnmt3b were not measured in islets. Second, whether the Notch1/Ngn3 signaling pathway mediates dedifferentiation needs further examination *in vitro*. Third, the effect of acarbose on oxidative stress was not analyzed.

In summary, the present study demonstrated that acarbose may improve glucose metabolism in db/db mice. Furthermore, acarbose maintained β-cell identity by preventing proliferation through the regulation of Ngn3, FoxO1 and PDX-1. This process probably involved PDX-1 methylation.

Acknowledgements

Funding

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DZ and XM designed the study. LC contributed to the literature search. DZ wrote the initial draft of the manuscript. XM reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving animals were approved by The Animal Care and Use Committee of Zhejiang Chinese Medical University (approval no. ZSLL-2019-102).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sharma AK, Bharti S, Goyal S, Arora S, Nepal S, Kishore K, Joshi S, Kumari S and Arya DS: Upregulation of PPARγ by Aegle marmelos ameliorates insulin resistance and β-cell dysfunction in high fat diet fed-streptozotocin induced type 2 diabetic rats. Phytother Res 25: 1457-1465, 2011.
2. Orchard TJ, Olson JC, Erbey JR, Williams K, Forrest KY, Smithline Kinder L, Ellis D and Becker DJ: Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes: 10-year follow-up data from the Pittsburgh epidemiology of diabetes complications study. Diabetes Care 26: 1374-1379, 2003.
3. Regazzi R: MicroRNAs as therapeutic targets for the treatment of diabetes mellitus and its complications. Expert Opin Ther Targets 22: 153-160, 2018.
4. Oza MJ and Kulkarni YA: Biochanin A improves insulin sensitivity and controls hyperglycemia in type 2 diabetes. Biomed Pharmacother 107: 1119-1127, 2018.
5. Wing RR, Rosen RC, Fava JL, Bahnson J, Brancati F, Gendrano Iii IN, Kitabchi A, Schneider SH and Wadden TA: Effects of weight loss intervention on erectile function in older men with type 2 diabetes in the Look AHEAD trial. J Sex Med 7: 156-165, 2010.
6. Zhou Z, Sun B, Li X and Zhu C: DNA methylation landscapes in the pathogenesis of type 2 diabetes mellitus. Nutr Metab (Lond) 15: 47, 2018.
7. Sharma S and Taliyan R: Histone deacetylase inhibitors: Future therapeutics for insulin resistance and type 2 diabetes. Pharmacol Res 113: 320-326, 2016.
8. Malipatil N, Lunt M, Narayanan RP, Siddals K, Cortés Moreno GY, Gibson MJ, Gu HF, Heald AH and Donn RP: Assessment of global long interspersed nucleotide element-1 (LINE-1) DNA methylation in a longitudinal cohort of type 2 diabetes mellitus (T2DM) individuals. Int J Clin Pract: e13270, Oct 21, 2018 (Epub ahead of print).
9. Hudec M, Dankova P, Solc R, Bettazova N and Cerna M: Epigenetic regulation of circadian rhythm and its possible role in diabetes mellitus. Int J Mol Sci 21: 3005, 2020.
10. Paneni F, Costantino S, Volpe M, Lüscher TF and Cosentino F: Epigenetic signatures and vascular risk in type 2 diabetes: A clinical perspective. Atherosclerosis 230: 191-197, 2013.
11. Pedica F, Beccari S, Pedron S, Montagna L, Piccoli P, Doglioni C and Chilosi M: PDX-1 (pancreatic/duodenal homeobox-1 protein 1). Pathologica 106: 315-321, 2014.
12. Zhou G, Yu J, Wang A, Liu SH, Sinnett-Smith J, Wu J, Sanchez R, Nemunaitis J, Ricordi C, Rozengurt E and Brunicardi FC: Metformin restrains pancreatic duodenal homeobox-1 (PDX-1) function by inhibiting ERK signaling in pancreatic ductal adenocarcinoma. Curr Mol Med 16: 83-90, 2016.
13. Rafati R, Jalal R, Asoodeh A and Matin MM: Association of rs12255372 (TCF7L2) and D76N (PDX-1) polymorphisms with type 2 diabetes in a population living in Northeast Iran. Arch Iran Med 18: 376-378, 2015.
14. Kaneto H, Matsuoka TA, Kimura T, Obata A, Shimoda M, Kamei S, Mune T and Kaku K: Appropriate therapy for type 2 diabetes mellitus in view of pancreatic β-cell glucose toxicity: 'The earlier, the better'. J Diabetes 8: 183-189, 2016.
15. McIver LA and Tripp J: Acarbose. In: StatPearls. StatPearls Publishing Copyright © 2020, StatPearls Publishing LLC., Treasure Island (FL), 2020. https://www.ncbi.nlm.nih.gov/books/NBK493214. Accessed Aughust 10, 2020.

16. Tu J, Liu G, Cao X, Zhu S, Li Q, Ji G, Han Y and Xiao H: Hypoglycemic effects of wheat bran alkyresorcinols in high-fat/high-sucrose diet and low-dose streptozotocin-induced type 2 diabetic male mice and protection of pancreatic β cells. Food Funct 10: 3282-3290, 2019.
17. Triplitt C, Cersosimo E and DeFronzo RA: Pioglitazone and alogliptin combination therapy in type 2 diabetes: A pathophysiologically sound treatment. Vasc Health Risk Manag 6: 671-690, 2010.
18. Li FF, Xu XH, Fu LY, Su XF, Wu JD, Lu CF, Ye L and Ma JH: Influence of acarbose on plasma glucose fluctuations in insulin-treated patients with type 2 diabetes: A pilot study. Int J Endocrinol 2015: 903524, 2015.
19. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA and Butler PC: Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 52: 102-110, 2003.
20. Wang X, Zhou L, Li G, Luo T, Gu Y, Qian L, Fu X, Li F, Li J and Luo M: Palmitate activates AMP-activated protein kinase and regulates insulin secretion from beta cells. Biochem Biophys Res Commun 352: 463-468, 2007.
21. Shao S, Fang Z, Yu X and Zhang M: Transcription factors involved in glucose-stimulated insulin secretion of pancreatic beta cells. Biochem Biophys Res Commun 384: 401-404, 2009.
22. Brissova M, Shiota M, Nicholson WE, Gannon M, Knobel SM, Piston DW, Wright CV and Powers AC: Reduction in pancreatic transcription factor PDX-1 impairs glucose-stimulated insulin secretion. J Biol Chem 277: 11225-11232, 2002.
23. Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH III, Wright CV, White MF, Arden KC and Accili D: The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. J Clin Invest 110: 1839-1847, 2002.
24. Zhu Y, Liu Q, Zhou Z and Ikeda Y: PDX1, neurogenin-3, and MAFA: Critical transcription regulators for beta cell development and regeneration. Stem Cell Res Ther 8: 240, 2017.
25. Van de Casteele M, Leuckx G, Baeyens L, Cai Y, Yuchi Y, Coppens V, De Groef S, Eriksson M, Svensson C, Ahlgren U, *et al*: Neurogenin 3+ cells contribute to β-cell neogenesis and proliferation in injured adult mouse pancreas. Cell Death Dis 4: e523, 2013.