European Review for Medical and Pharmacological Sciences 2020; 24: 12808-12820

FABP4 inhibitor attenuates inflammation and endoplasmic reticulum stress of islet in leptin receptor knockout rats

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Abstract. – OBJECTIVE: Metabolic syndrome is characterized by abdominal obesity, hypertriglyceridemia and hyperglycemia. Fatty acid binding protein 4 (FABP4), as a member of intracellular lipid chaperones, is not only engaged in lipid transport but involved in inflammation and insulin resistance. The present study was to investigate the effects of BMS309403, a specific FABP4 inhibitor, on metabolic syndrome and its possible molecular mechanisms in islets.

MATERIALS AND METHODS: Leptin receptor knockout (*Lepr*$^{-/-}$) rat, a novel and representative animal model of metabolic syndrome, was adopted in this study. *Lepr*$^{-/-}$ male rats and their wild littermates were grouped and intragastrically administered with BMS309403. Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT) were performed on all rats. Serum insulin was detected by ELISA. The metabolic characters, as well as liver and kidney functions, were evaluated by serum biochemical assay. Immunohistochemistry and Western blot were adopted to detect the expression levels of FABP4, CD68, GRP78, ATF6, p-IRE1a, and Cleaved caspase-3.

RESULTS: *Lepr*$^{-/-}$ rats showed prominent characteristics of metabolic syndrome with increased FABP4, inflammatory infiltration, ER stress and apoptosis in islets. BMS309403 administration attenuated inflammation, ER stress and apoptosis in *Lepr*$^{-/-}$ rat islets while stimulating insulin secretion as well as improving manifestation of metabolic syndrome without hepatic and renal toxicity.

CONCLUSIONS: FABP4 increased in *Lepr*$^{-/-}$ rat islets and might be involved in the regulation of islet inflammation and apoptosis via ER stress. FABP4 inhibitor BMS309403 could ameliorate islet inflammation and apoptosis in metabolic syndrome through suppressing ER stress.

Key Words:
Endoplasmic reticulum (ER) stress, Fatty acid binding protein 4 (FABP4), Islet, Leptin receptor knockout (*Lepr*$^{-/-}$) rat, Metabolic syndrome.

Introduction

Metabolic syndrome is characterized by five components—abdominal obesity, hypertriglyceridemia, hypertension, hyperglycemia and reduced high-density lipoprotein cholesterol[1]. There is mounting evidence throughout recent decades implying that metabolic syndrome predicts type 2 diabetes mellitus (T2DM) and cardiovascular disease[2]. Out of many risk factors, due to World Health Organization (WHO) criteria, insulin resistance and obesity, have been identified as major risk factors of metabolic syndrome[3]; however, the pathogenesis is still from being fully understood.

Given the intertwined metabolism of fats and carbohydrates, elevated non-esterified fatty acid (NEFA) levels in plasma, along with hyperglycemia, are thought to be both a cause and a consequence of insulin resistance and T2DM with many deleterious downstream effects in-

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cluding chronic inflammation, loss of pancreatic β-cells, atherosclerosis, and heart disease, which are caused or exacerbated by elevated NEFA[4,5]. Excess NEFA or insulin-resistance environment in peripheral tissues induces a compensatory response by pancreatic β cell, which ultimately triggers enhanced insulin secretion, and then progresses to β cell dysfunction in an environment of chronic metabolic demand[6]. Although it has been proposed that decompensating endoplasmic reticulum (ER) stress responses play a crucial role in gluco/lipotoxicity[6], the specific contribution of ER stress to β cell dysfunction and apoptosis has not been fully elucidated.

Fatty acid binding proteins (FABPs), a family of intracellular lipid chaperones, are engaged in the transport of fatty acids to specific organelles in cells[7]. FABP4, one of the most active family members of FABPs, was not only found highly expressed in adipose tissue but also released by other types of tissues and cells, where FABP4 performs different functions[8]. FABP4 was reported[8] to be strongly involved in glucose and lipid metabolism, inflammation, and insulin resistance. Erbay et al[9] reported that FABP4 inhibition alleviated atherosclerosis via reducing ER stress. Our previous research also demonstrated that FABP4 expression of mesangial cells was upregulated in diabetic nephropathy and FABP4 inhibitor BMS309403 suppressed ER stress and protected human mesangial cells from glucose or fatty acids induced-apoptosis[9].

In the present study, we use leptin receptor knockout (*Lepr*$^{-/-}$) rats, an ideal metabolic syndrome model, to investigate FABP4 expression and ER stress in pancreatic islets and explore the therapeutic effects targeting FABP4 with its specific inhibitor.

Materials and Methods

Animals

Lepr$^{+/-}$ rats (Sprague-Dawley background) (SCXK 2013-0002) were kindly provided by Prof. L Zhang, Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (Beijing, China). After breeding and DNA identification, 10 male *Lepr*$^{-/-}$ rats (10 weeks) and their littermates of 10 wild type SD rats (10 weeks) were picked into the present study. All rats were housed in a condition kept at 23±2°C with a 12:12 h light/dark cycle and were provided with standard food and water *ad libitum*. All animal experiments were approved by the Animal Care and Use Committee of Hebei Medical University (IACUC-Hebmu-PD-2018023).

Animal Grouping and Administration

10 wild type SD rats were randomly divided into 2 groups: WT-Control group (WT-C, n=5), WT-Treat group (WT-T, n=5); 10 *Lepr*$^{-/-}$ rats were randomly divided into 2 groups: KO-Control group (KO-C, n=5), KO-Treat group (KO-T, n=5). All rats in WT-T and KO-T groups were intragastrically administered with FABP4 inhibitor (BMS3094030) (Bide Pharmatech Ltd, Shanghai, China.) (40 mg/kg/day) at a fixed time in the morning for 4 weeks. Rats in control groups were administered with the corresponding volume of solvent (water with 2% Tween-80 and 2% DMSO).

Insulin Tolerance Test (ITT) and Glucose Tolerance Test (GTT)

For the ITT, all rats were fasted for 4 hours. On the day of the test, the rats were weighed, and tail blood glucose was tested at 0 minutes. Then the rats were injected intraperitoneally with insulin (0.75 U/kg body weight). Blood glucose levels were tested at the tail at 15, 30, 60 and 120 min after injection. GTT was performed 3 days later after ITT. All rats were fasted overnight (14 h) but given water ad libitum before GTT. On the day of the test, the rats were weighed, and blood glucose was tested via tail vein puncture at 0 minutes. Then the rats were injected intraperitoneally with 40% glucose (2 g/kg body weight). Blood glucose levels were tested at the tail at 15, 30, 60 and 120 min after injection.

Serum and Tissue Collection

At the end of the administration course, all rats were sacrificed 2 days later after GTT. The rats were fasted overnight (14h) but given water ad libitum before euthanasia. Blood samples were collected by abdominal aorta puncture. The serum of blood samples was collected for serum insulin assay and biochemistry assay under anesthesia by 3% sodium pentobarbital (1.5 ml/kg).

Pancreas, hearts, livers, kidneys, brains and testes were collected after the rats were euthanized. Each pancreas was cut along the longitudinal axis into halves. One half of each pancreas was cut into small pieces and put in liquid nitrogen for future Western blot analysis. The other half of the pancreas was fixed

in 4% formaldehyde and embedded in paraffin blocks for histological and immunohistochemistry analysis.

Hearts, livers, kidneys, brains and testes were weighed, and the ratios of organ/brain (g/g) were calculated.

Serum Insulin Assay

Serum insulin concentration was determined by ELISA with Rat Insulin ELISA Kit (ExCell Biotech, Cat No.ER010, Shanghai, China) following the instruction of the kit.

Histopathological Observation and Immunohistochemical Staining and Analysis

For light microscopy, paraffin-embedded tissues were cut into 4 μm-thick sections and stained with H&E (Hematoxylin and Eosin) and immunohistochemistry. For immunohistochemistry of pancreas, sections were stained with primary antibodies against FABP4 (goat antibody, 1:600, R&D, AF1443, Shanghai, China), insulin (rabbit antibody, 1:1000, Proteintech, 15848-1-AP, Wuhan, Hubei, China), GRP78 (rabbit antibody, 1:1400, EARTHOX, A300087, Millbrae, CA, USA), ATF6 (rabbit antibody, 1:1000, Gene Tex, GTX30071, San Antonio, TX, USA), p-IRE1a (rabbit antibody, 1:800, Abcam, ab48187, Shanghai, China),

Cleaved caspase-3 (rabbit antibody, 1:1000, EARTHOX, A500003-01, Millbrae, CA, USA) and CD68 (rabbit antibody, 1:400, Abcam, ab125212, Shanghai, China). All pancreas sections were checked under light microscope and all islets on each section were measured. In general, 25-35 islets in each group (at least 5 islets per rat) were picked to be analyzed by Image-Pro Plus 6.0. Positive staining of FABP4, GRP78, ATF6, p-IRE1a, and Cleaved caspase-3 were measured by IOD. Cells with CD68 positive staining within islets were counted. Areas of pancreas and islets were measured by ImageJ, and the ratio of the pancreas area/islet area was calculated. Histopathological analysis was performed by an experienced pathologist blinded to the groups.

Western Blot

The rats were euthanized and total protein lysates from the rat pancreas tissues were prepared as previously described[10]. 30-50 μg of total protein lysates was loaded for Western blotting. After sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) transfer of the bands to PVDF membranes, the membranes were incubated overnight with antibodies against FABP4, GRP78, ATF6, p-IRE1α, Cleaved caspase-3 and β-actin (Proteintech, 66009-1, Wuhan, Hubei, China). After incubation with the appropriate secondary antibody for 1 h at room temperature, antibody binding was detected with Pro-light HRP chemiluminescence kit (TIANGEN, Beijing, China). For quantitative analysis, all protein expressions were normalized to β-actin. All protein bands were analyzed by ImageJ system.

Statistical Analysis

Statistical analysis was conducted using SPSS 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Armonk, NY, USA). Data with normal distribution data were expressed as means ± SDs. Statistical comparisons between experimental groups were performed by One-way ANOVA tests. Non-normal distributed data were expressed as interquartile range (M, QR). The differences between groups were analyzed using the nonparametric Wilcoxon test. A value of $p<0.05$ was considered statistically significant.

Results

BMS309403 Decreased the Weights of *Lepr*$^{-/-}$ Rats without Changing Food Intake as well as Hepatic and Renal Function

Rats of KO-C group were overweight and consumed more food when compared to rats of WT-C group. KO-T group lost weight after BMS309403 administration comparing with KO-C group ($p<0.05$, Figure 1A, 1B). But BMS309403 did not decrease the weights and food intakes of wild type rats (Figure 1A, 1B). Neither *Lepr*$^{-/-}$ rats in KO-T group nor wild SD rats in WT-T group showed hepatic or renal dysfunction (Table I).

KO-C rats showed more weights of hearts, livers and kidneys when compared to WT-C rats. Considering the increased body weights of KO rats, the ratios of organ/brain were adopted to evaluate the weights of the above-mentioned organs. Organ/brain ratios showed KO rats got enlarged hearts, livers and kidneys. Organ weights of KO-T rats showed no significant differences after BMS309403 administration comparing with KO-C group (Table II).

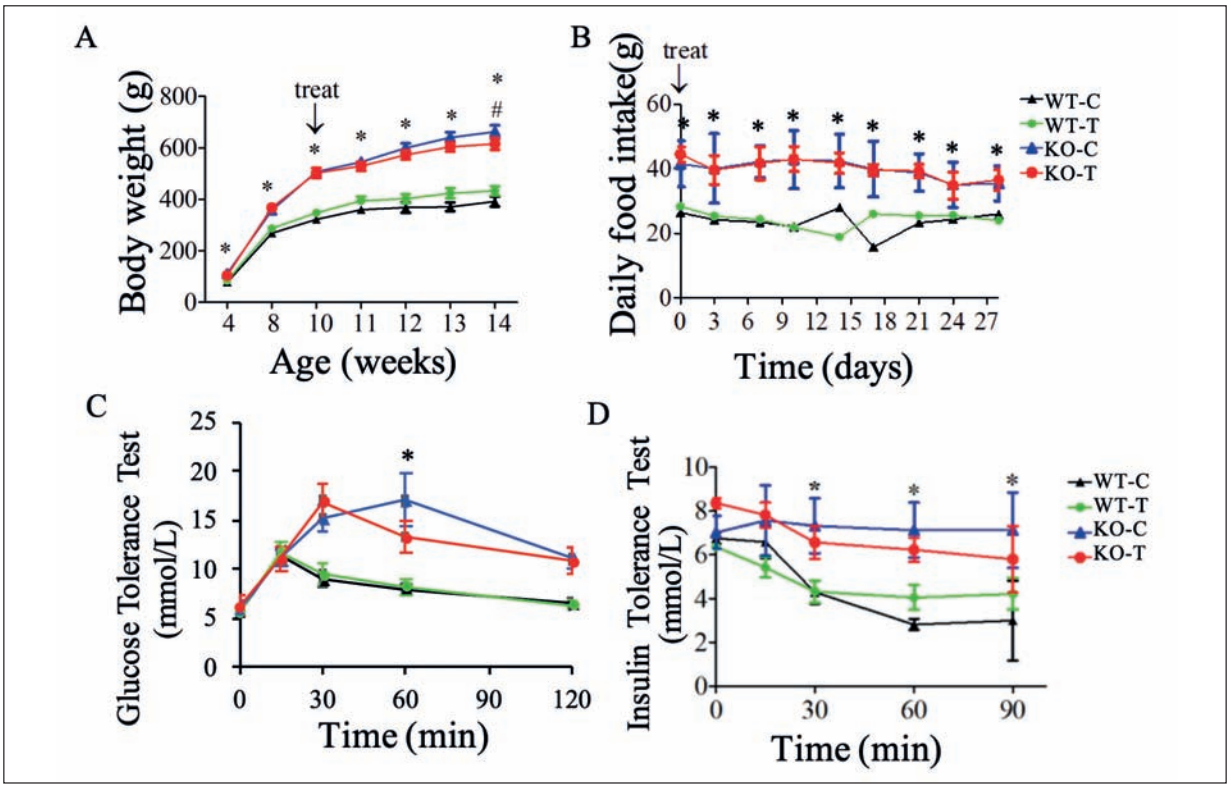

Figure 1. The comparison of the body weight, daily food intake, GTT and ITT. **(A)** body weight; **(B)** daily food intake. **(C)** Glucose tolerance test. **(D)** Insulin tolerance test. (WT-C n=5, WT-T n=5, KO-C n=5, KO-T n=5). *p<0.05 KO-C *vs.* WT-C; $^{\#}p$<0.05 KO-T *vs.* KO-C.

BMS309403 Improved Glucose Intolerance and Insulin-Resistance in Lepr$^{-/-}$ Rats

Comparing with WT-C group, rats in KO-C group showed delayed (at 60 minutes) and higher peak level of blood glucose after glucose injection. Those rats even kept high blood glucose levels at 120 minutes after injection (Figure 1C). BMS309403 administration brought an earlier peak glucose level at 30 minutes and a sharply decline in glucose level in KO-T group comparing with KO-C group (Figure 1C). Insulin injection to *Lepr$^{-/-}$* rats did not lead to an evident decline in blood glucose, implying pronounced glucose intolerance and insulin resistance while rats in KO-T group with the treatment of BMS309403

Table I. The result of serum biochemical ($\bar{x} \pm s$, mmol/L).

Groups number	WT-C n = 5	WT-T n = 5	KO-C n = 5	KO-T n = 5
TCHO	1.88 ± 0.38	1.97 ± 0.08	4.13 ± 0.85*	4.20 ± 1.32
TG	0.79 ± 0.25	0.77 ± 0.13	3.04 ± 0.75**	1.92 ± 0.44#
HDL-C	0.94 ± 0.23	0.93 ± 0.09	2.37 ± 0.46*	2.59 ± 0.76
LDL-C	0.25 ± 0.06	0.24 ± 0.03	0.32 ± 0.11	0.35 ± 0.08
NEFA	0.84 ± 0.12	0.87 ± 0.08	1.61 ± 0.47**	1.32 ± 0.20 #
ALT	52.87 ± 10.75	44.51 ± 11.02	183.06 ± 148.32	251.67 ± 171.97
AST	154.32 ± 26.18	174.09 ± 30.28	176.20 ± 44.25	245.10 ± 99.17
BUN	7.04 ± 0.78	6.25 ± 0.11	8.29 ± 1.49	7.61 ± 0.84
CREA	22.63 ± 2.21	23.12 ± 0.75	15.14 ± 1.42	14.28 ± 1.88

TCHO, Total Cholesterol; TG, Triglyceride; HDL-C, High Density Lipoprotein Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol; NEFA, Non-Esterified Fatty Acid; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BUN, Blood Urea Nitrogen; CREA, Creatinine. *p<0.05, **p<0.01, KO-C *vs*. WT-C; $^{\#}p$< 0.05, KO-T *vs*. KO-C.

Table II. Organ weight and organ coefficient ($\bar{x} \pm s$).

Groups number	WT-C n = 5	WT-T n = 5	KO-C n = 5	KO-T n = 5
Organ weight (g)				
Heart	1.35 ± 0.15	1.39 ± 0.11	1.55 ± 0.12*	1.45 ± 0.10
Liver	10.36 ± 0.90	11.89 ± 0.94	23.13 ± 1.61***	25.88 ± 7.08
Kidney	2.72 ± 0.22	2.72 ± 0.24	3.14 ± 0.24**	3.06 ± 0.29
Brain	2.08 ± 0.10	2.02 ± 0.09	1.92 ± 0.11*	1.84 ± 0.12
Testis	3.67 ± 0.35	3.43 ± 0.28	3.81 ± 0.58	3.64 ± 0.40
Organ coefficient, organ/brain ratio (g/g)				
Heart	0.65 ± 0.06	0.69 ± 0.08	0.80 ± 0.05**	0.78 ± 0.08
Liver	4.99 ± 0.49	5.91 ± 0.76	11.88 ± 0.86***	13.18 ± 3.96
Testis	1.77 ± 0.21	1.70 ± 0.18	1.86 ± 0.08	2.06 ± 0.34
Kidney	1.31 ± 0.08	1.31 ± 0.12	1.58 ± 0.07**	1.62 ± 0.11

*p<0.05, **p<0.01, ***p<0.001, KO-C *vs*. WT-C.

showed a decline tendency in glucose level after insulin injection (Figure 1C, 1D). BMS309403 had no effects on glucose tolerance and insulin sensitivity in wild type rats (Figure 1C, 1D).

BMS309403 Ameliorated Hyperlipidemia of Lepr$^{-/-}$ Rats

KO-C rats showed increased serum triglyceride, total cholesterol, HDL and NEFA comparing with WT-C group. BMS309403 decreased the blood level of triglyceride and NEFA when compared to KO-C group (p<0.05). Serum biochemistry showed no significant differences between WT-T group and WT-C group (Table I).

BMS309403 Suppressed the Inflammatory Responses in Islets from Lepr$^{-/-}$ Rats with an Increasing Insulin Secretion

All the four groups showed CD68 positive cells in pancreatic interstitium and islets. CD68 positive cells were rarely seen in islets in WT-C group (0.58±0.17 per islet) while the counts of CD68 positive cells of KO-C (1.25±0.18 per islet) were much higher (2.2 folds, p<0.001) than those of WT-C group. CD68 positive cells in KO-T rats administered with BMS309403 (0.78±0.17 per islet) were less than those in KO-C group (p<0.01, Figure 2). There were no statistical differences in CD68 positive cell counts between WT-T group and WT-C group. Islets of KO-C rats were hypertrophic and proliferated (Figure 3). The areas of islets from KO-C rats enlarged (p<0.01, Figure 3, Figure 4) when compared to WT-C group. ELISA results showed a drastic elevation of serum insulin in all *Lepr*$^{-/-}$ rats compared to their wild type control. BMS309403 administration increased serum insulin level in both wild type rats and KO rats and significantly extended the elevation of insulin in KO-T group when compared to KO-C group (Figure 4F).

FABP4 Expression of Islets from Lepr$^{-/-}$ Rats Increased and BMS309403 Had no Effects on the Expression of FABP4

FABP4 staining was seen in islet cells and capillary endothelial cells in all four groups of rats. KO-C rats showed more positive staining in islets than WT-C group (p<0.001). Both immunohistochemistry and Western blot analysis showed BMS309403 had no effects on FABP4 expression in islets or pancreatic tissues regardless of whether they come from *Lepr*$^{-/-}$ rats or wild type rats (Figure 5).

BMS309403 Decreased GRP78 Expression in Islets from Lepr$^{-/-}$ Rats

All four groups were seen with GRP78 positive staining, which was observed either in the cytoplasm of pancreatic acinar cells or in islet cells. Islets from KO-C group showed more GRP78 expression than those from WT-C group (p<0.001, Figure 6, A-E). KO-T group showed lower GRP78 expression than KO-C (p<0.01, Figure 6, A-E). BMS309403 had no effects on GRP78 expression in wild type rats. Western blot showed similar results with immunohistochemistry (Figure 6, F-G).

BMS309403 Decreased ATF6 Expression in Islets from Lepr$^{-/-}$ Rats

ATF6 was expressed in the cytoplasm of pancreatic acini and islet cells in the rats of all four groups. KO-C group expressed more ATF6 in

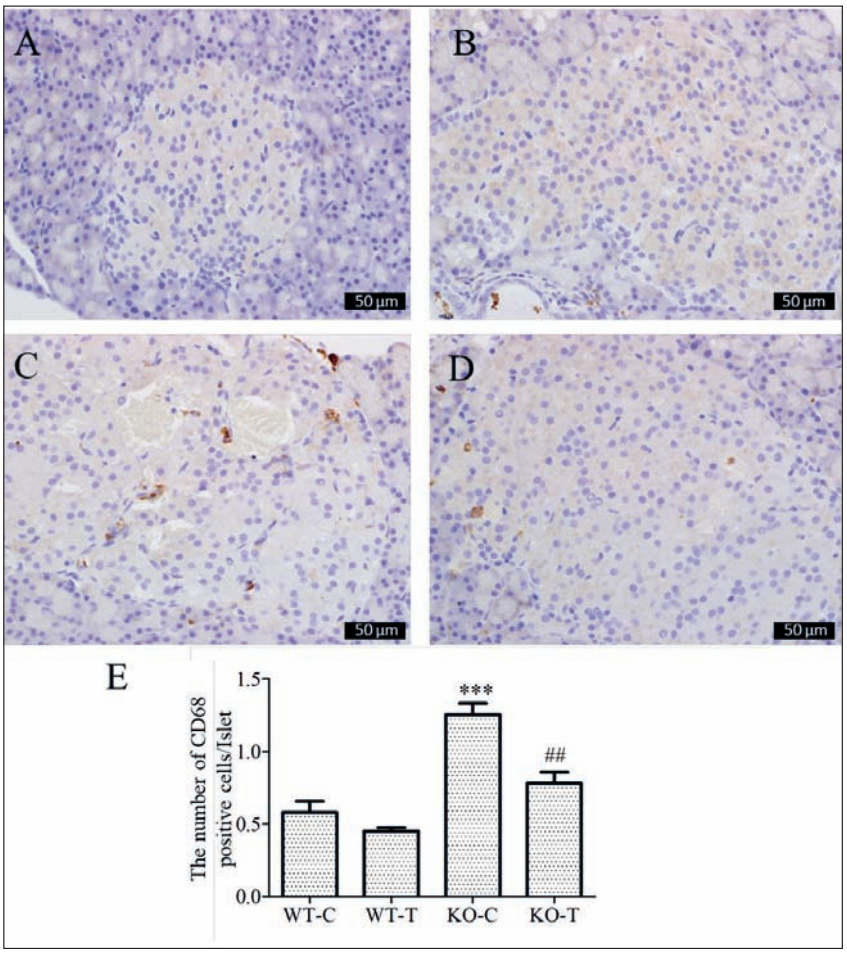

Figure 2. The expression and counting of CD68 in islet (Immunohistochemistry, 400×). **(A)** Group WT-C; **(B)** Group WT-T; **(C)** Group KO-C; **(D)** Group KO-T; **(E)** The counts of CD68-positive cells/islet of each group. (WT-C n=5, WT-T n=5, KO-C n=5, KO-T n=5)***p<0.001, KO-C *vs.* WT-C; $^{\#\#}p$<0.01, KO-T *vs.* KO-C.

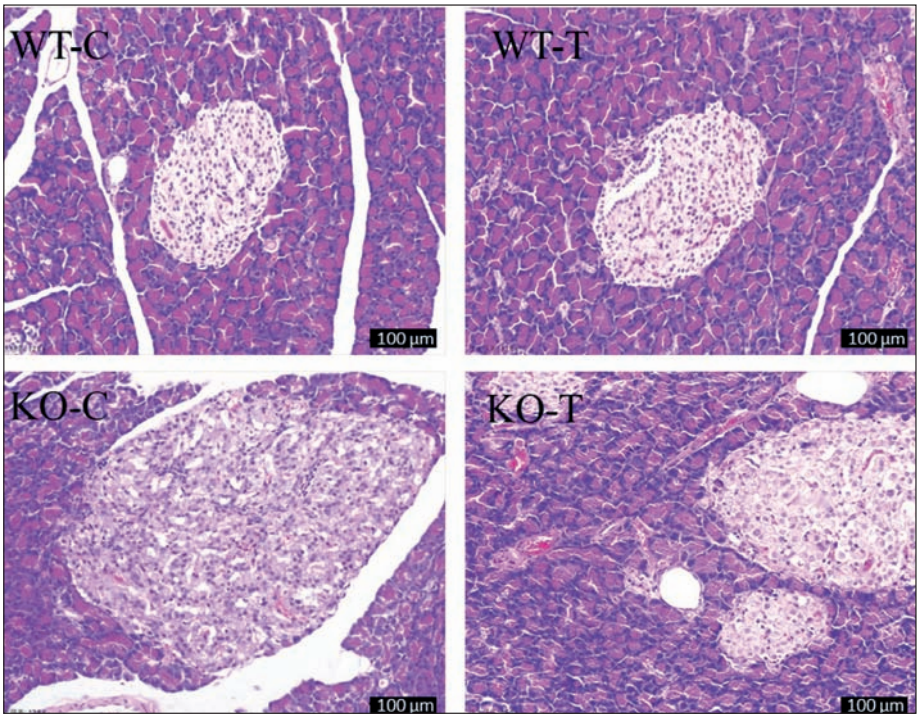

Figure 3. HE staining of pancreas (200×).

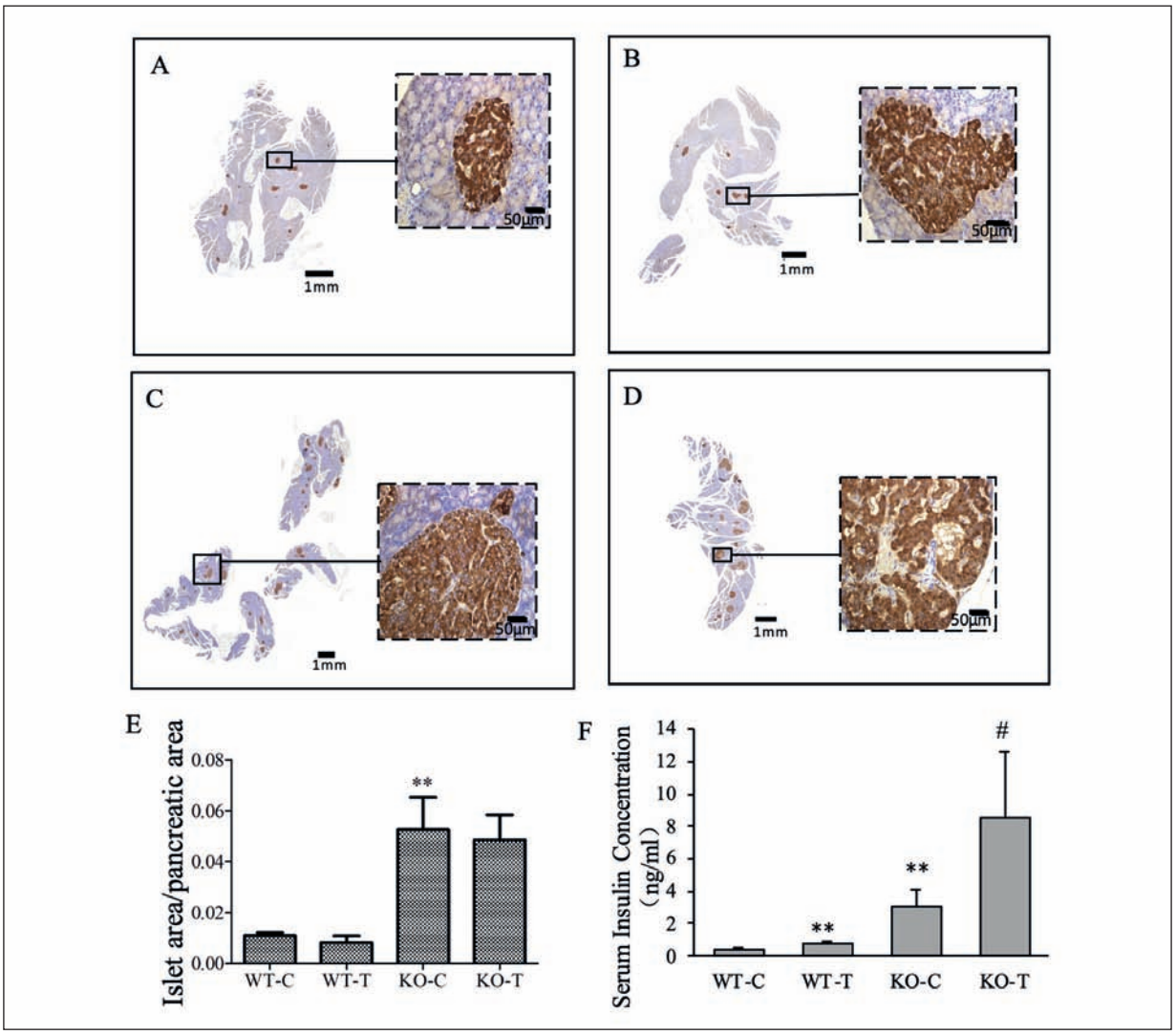

Figure 4. The measurement of islet area and insulin quantification (Immunohistochemistry, 40×/400×). **(A)** Group WT-C; **(B)** Group WT-T; **(C)** Group KO-C; **(D)** Group KO-T; **(E)** Islet area/pancreatic area of each group; **(F)** Serum Insulin level. (WT-C n=5, WT-T n=5, KO-C n=5, KO-T n=5)**p<0.01, KO-C *or* WT-T *vs.* WT-C; $^{\#}p$<0.05, KO-T *vs.* KO-C.

islets than WT-C did (p<0.001, Figure 7, A-E). *Lepr*$^{-/-}$ rats with the administration of BMS309403 (KO-T) showed weaker staining of ATF6 than KO-C (p<0.01, Figure 7, A-E). BMS309403 had no effects on ATF6 expression in wild type rats. Western blot analysis conformed to the results of immunohistochemistry (Figure 7, F-G).

BMS309403 Suppressed p-IRE1α Expression in Islets from Lepr$^{-/-}$ Rats

A few positive staining of p-IRE1α was seen in islets from WT-C group. KO-C group showed stronger staining of p-IRE1α in islets than WT-C group (p<0.001, Figure 8, A-E). p-IRE1α staining in KO-T group with the administration of BMS309403 was weaker than that in KO-C group (Figure 8, A-E). There was no significant difference in p-IRE1α expression between WT-T group and WT-C group. Western blot results were similar to those of immunohistochemistry (Figure 8).

BMS309403 Suppressed Cleaved Caspase-3 Expression in Islets from Lepr$^{-/-}$ Rats

All the rats of four groups showed cytoplasmic staining of Cleaved caspase-3 in islets. Wild type rats showed stronger peripheral staining than central region in islets. FABP4 inhibitor BMS309403 did not change the expression pattern of Cleaved Caspase-3. Islet expression of

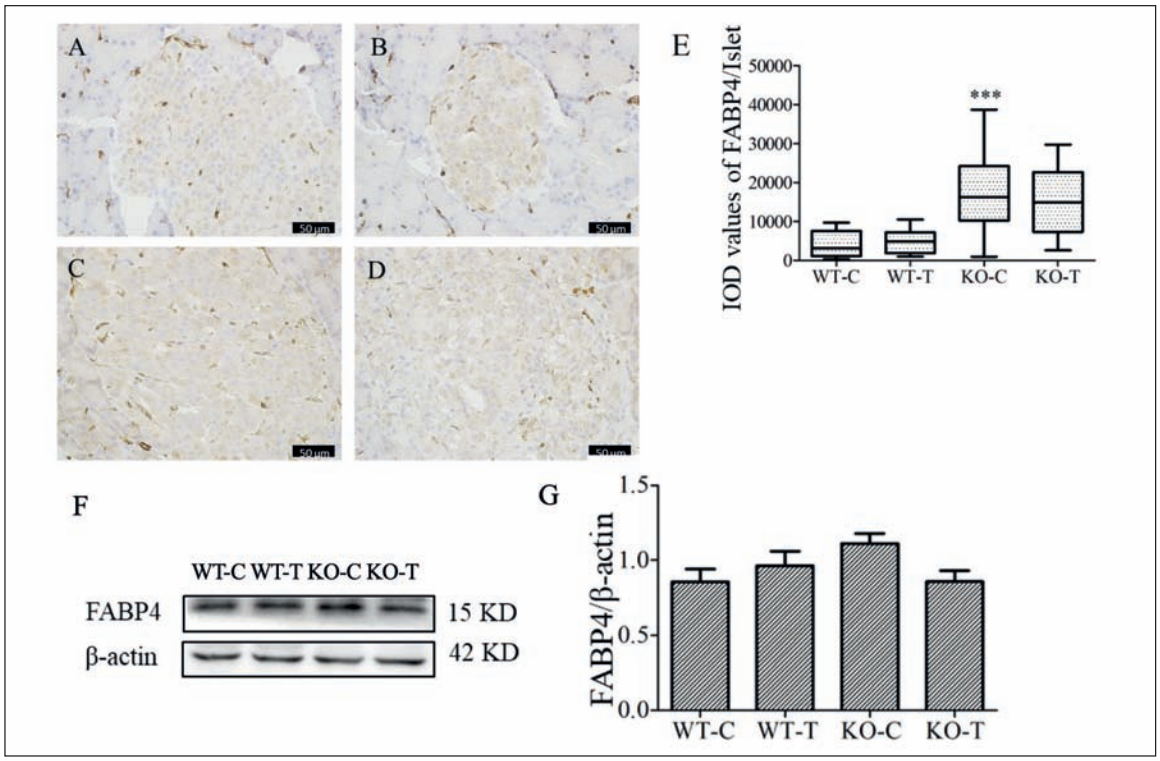

Figure 5. The expression and analysis of FABP4. **A-D**, immunohistochemical staining of islets (400×). **(A)** Group WT-C; **(B)** Group WT-T; **(C)** Group KO-C; **(D)** Group KO-T; **(E)** IOD values of FABP4/Islet. **F-G**, FABP4 protein expression in pancreas (Western blot) (WT-C n=5, WT-T n=5, KO-C n=5, KO-T n=5)***p<0.001, KO-C *vs.* WT-C.

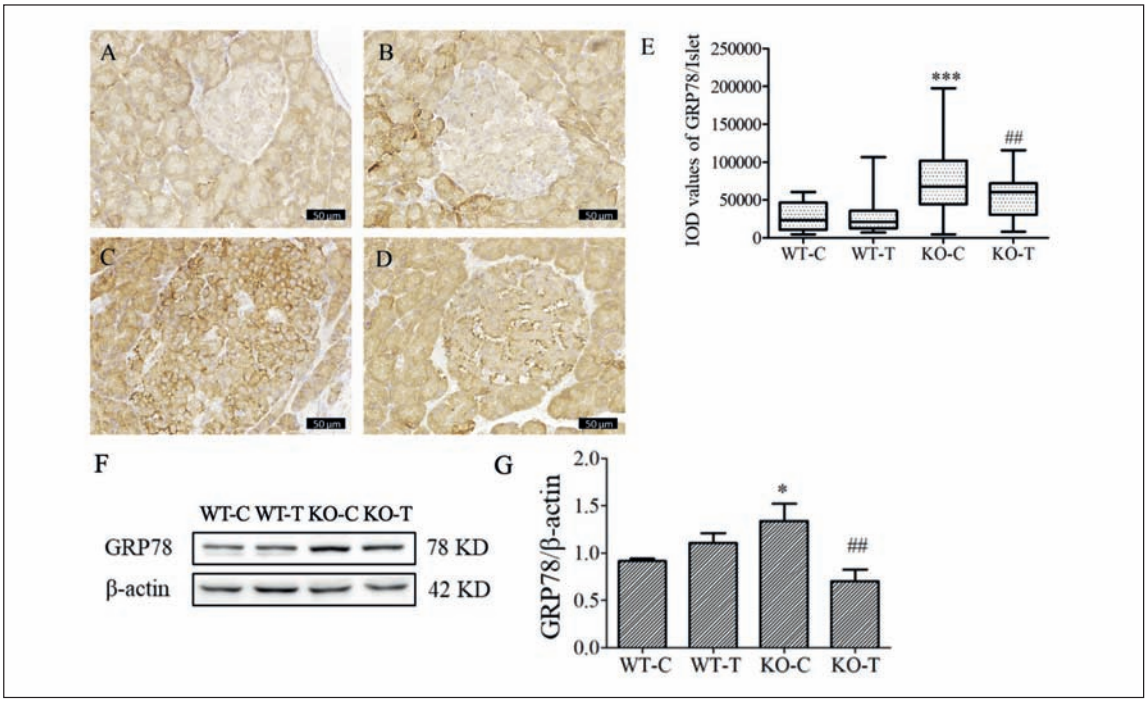

Figure 6. The expression and analysis of GRP78. **A-D**, immunohistochemical staining of islets (400×). **(A)** Group WT-C; **(B)** Group WT-T; **(C)** Group KO-C; **(D)** Group KO-T; **(E)** IOD values of GRP78/Islet. **F-G**, GRP78 protein expression in pancreas (Western blot) (WT-C n=5, WT-T n=5, KO-C n=5, KO-T n=5) *p<0.05 KO-C *vs.* WT-C; ***p<0.001, KO-C *vs.* WT-C; $^{\#\#}p$<0.01, KO-T *vs.* KO-C.

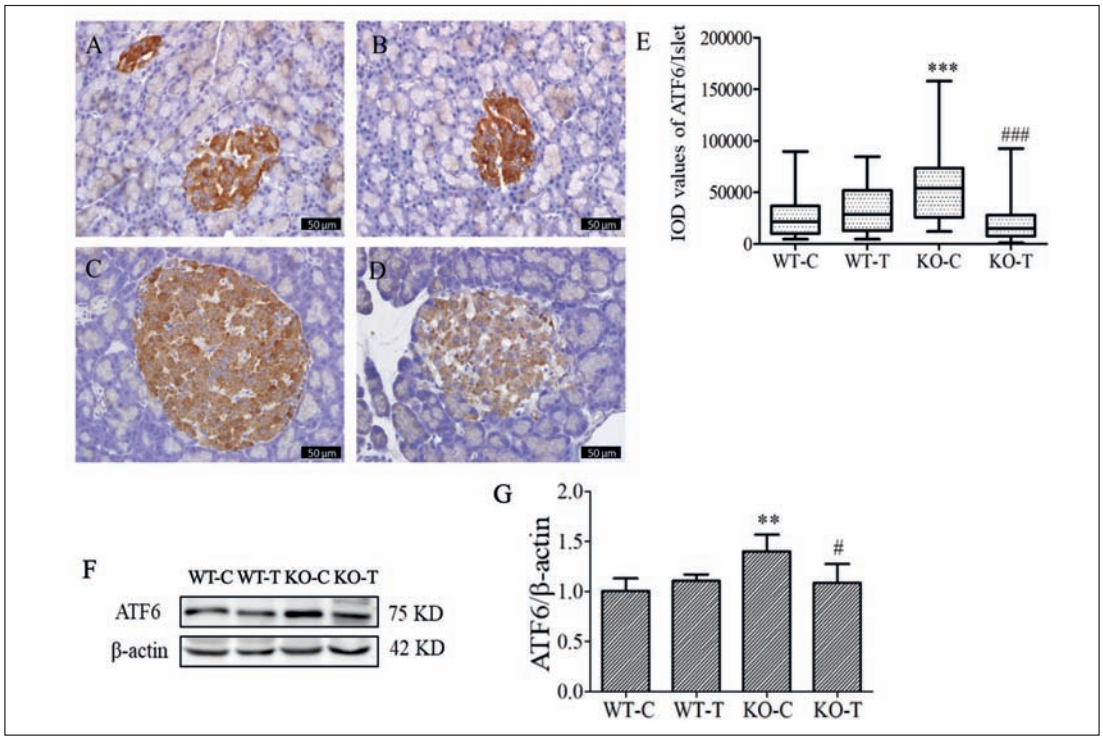

Figure 7. The expression and analysis of ATF6. **A-D**, immunohistochemical staining of islets (400×). **(A)** Group WT-C; **(B)** Group WT-T; **(C)** Group KO-C; **(D)** Group KO-T; **(E)** IOD values of ATF6/Islet. **F-G**, ATF6 protein expression in pancreas (Western blot) (WT-C n=5, WT-T n=5, KO-C n=5, KO-T n=5) **p<0.01 KO-C *vs.* WT-C; ***p<0.001, KO-C *vs.* WT-C; $^{\#}p$<0.05 KO-T *vs.* KO-C; $^{\#\#\#}p$<0.001, KO-T *vs.* KO-C.

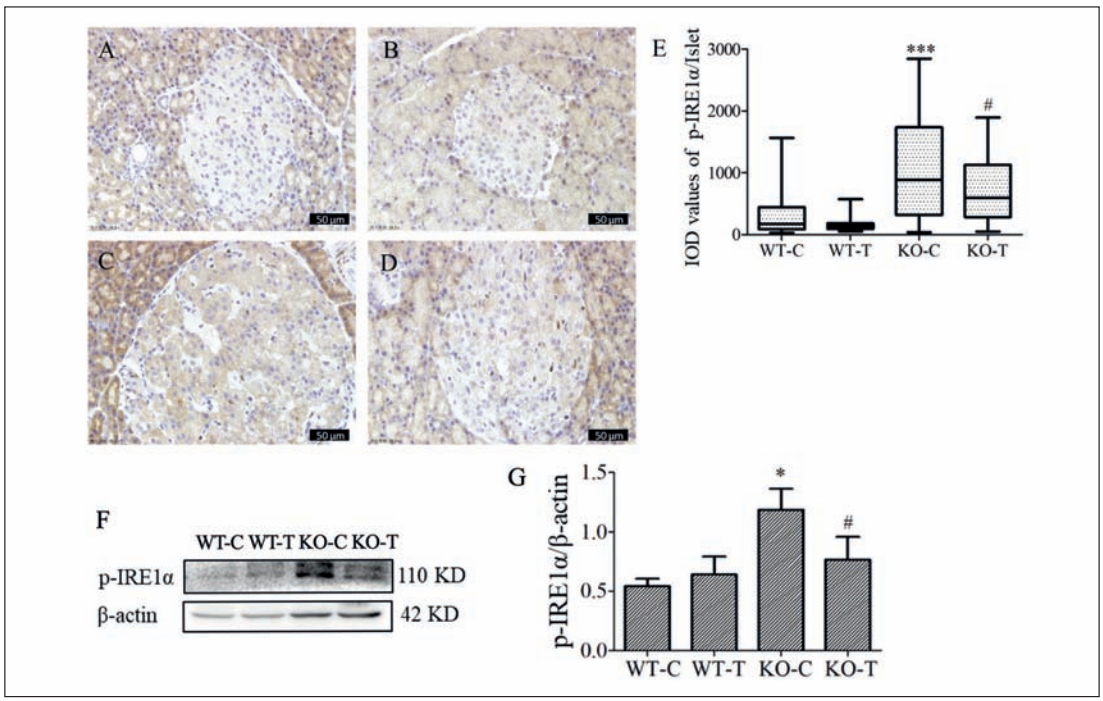

Figure 8. The expression and analysis of p-IRE1α. **A-D**, immunohistochemical staining of islets (400×). **(A)** Group WT-C; **(B)** Group WT-T; **(C)** Group KO-C; **(D)** Group KO-T; **(E)** IOD values of p-IRE1α/Islet. **F-G**, p-IRE1α protein expression in pancreas (Western blot) (WT-C n=5, WT-T n=5, KO-C n=5, KO-T n=5) *p<0.05, KO-C *vs.* WT-C; ***p<0.001, KO-C *vs.* WT-C; $^{\#}p$<0.05, KO-T *vs.* KO-C.

Cleaved caspase-3 in KO-C group was higher than that in WT-C group although the dark stained peripheral cells decreased (p<0.001, Figure 9, A-E). KO-T group showed decreased staining of Cleaved caspase-3 in islets after BMS309403 administration when compared to KO-C group (p<0.05, Figure 9, A-E). There was no statistical difference in Cleaved caspase-3 expression between WT-C group and WT-T group. Pancreatic tissue analysis by Western blot showed similar results to that of histochemistry (Figure 9, F-G).

Discussion

Autosomal recessive diabetes mutants (*db/db* mouse, and Zucker rats) are the commonly used obese and diabetic animal model for the researches of metabolic syndrome. But these models do not develop the full phenotype of metabolic syndrome. *db/db* mice usually present transient hyperglycemia or otherwise hyperglycemia with short lifetime[11]. Zucker rats do not present a typical high blood glucose level[12]. Leptin receptor knockout (*Lepr*$^{-/-}$) rat is a novel metabolic syndrome model established by CRISPR/Cas9[11]. Comparing with those autosomal recessive mutants, *Lepr*$^{-/-}$ rats present more stable phenotype of metabolic syndrome and are available for long-term observation[11].

Our previous research demonstrated that FABP4 expression in human mesangial cells was upregulated in diabetic nephropathy and FABP4 inhibitor BMS309403 suppressed ER stress and protected human mesangial cells from glucose or fatty acids induced-apoptosis[10]. The present study for the first time identified FABP4 expressed in pancreatic islet. This time we focused on whether FABP4 inhibition had effects on inflammation and ER stress in *Lepr*$^{-/-}$ rat islets. BMS309403 is an orally active small molecule which specifically interacts with the fatty acid-binding pocket within the interior of FABP4 to inhibit binding of endogenous fatty acids[13]. Treatment with BMS309403 has been shown to improve insulin resistance, diabetes mellitus, fatty liver disease and atherosclerosis

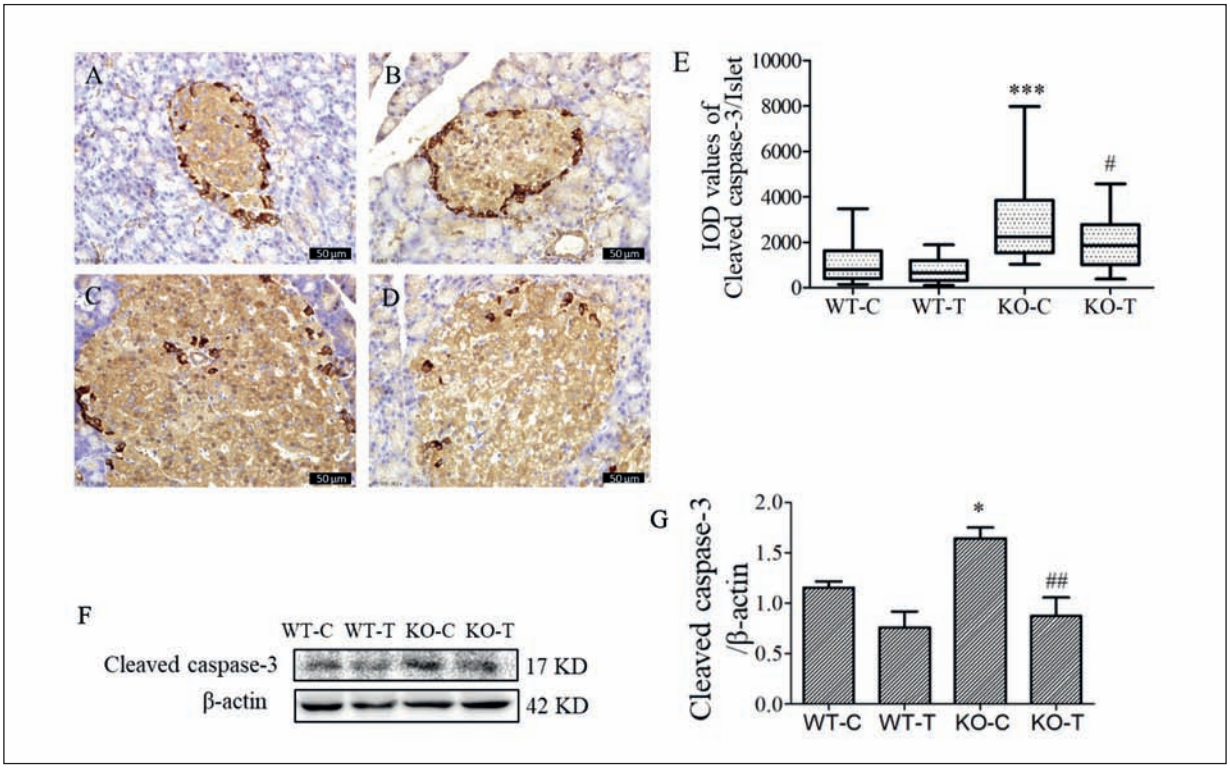

Figure 9. The expression and analysis of Cleaved caspase-3. **A-D**, immunohistochemical staining of islets (400×). **(A)** Group WT-C; **(B)** Group WT-T; **(C)** Group KO-C; **(D)** Group KO-T; **(E)** IOD values of Cleaved caspase-3/Islet. **F-G**, Cleaved caspase-3 protein expression in pancreas (Western blot) (WT-C n=5, WT-T n=5, KO-C n=5, KO-T n=5) *p<0.05 KO-C *vs.* WT-C; ***p<0.001, KO-C *vs.* WT-C; $^{\#}p$<0.05, KO-T *vs.* KO-C; $^{\#\#}p$<0.01 KO-T *vs.* KO-C.

in experimental models, indicating that chemical inhibition of FABP4 could be a therapeutic strategy against several aspects of metabolic syndrome[13]. In our study, *Lepr*$^{-/-}$ rats were adopted as an ideal metabolic syndrome model, which carried the phenotypic characteristics including conspicuous obesity, hyperlipidemia, glucose intolerance, insulin resistance, etc. The study results showed that 4 weeks of intragastric administration with BMS309403 lowered the body weights of *Lepr*$^{-/-}$ rats, as well as their blood levels of triglyceride and NEFA without hepatotoxicity and renal toxicity, indicating the dose of BMS309403 adopted in this study, was safe and effective. This FABP4 inhibitor showed a significant stimulating effect on insulin secretion by increasing serum insulin level in both wild type and *Lepr*$^{-/-}$ rats, especially causing a drastic increase of insulin in *Lepr*$^{-/-}$ rats while improving their insulin resistance as well.

The immunohistochemical assay revealed that FABP4 expression was upregulated in islets with macrophage (CD68+cells) infiltration in *Lepr*$^{-/-}$ rats compared to their wild littermates. Exogenous FABP4 was reported to activate inflammatory responses in macrophages, endothelial cells and vascular smooth muscle cells, involving chemokine signaling and TNFα-NF-κB signaling pathways[14]. Wei et al[15] suggested that obesity induced low grade inflammation in islets with the local expansion of resident macrophages, which impaired β cell function in a cell-cell contact-dependent manner within islets. Our results demonstrated that FABP4 inhibitor could suppress islet inflammation (CD68+cells decreased) in *Lepr*$^{-/-}$ rats.

Exposure to high-nutrient environment in metabolic syndrome induces adaptive unfolded protein response (UPR) in β cells, which involves three ER trans-membrane signaling proteins named IRE1α, PERK, and ATF6[6]. Accumulating evidence indicates ER stress, the subsequent event of decompensating UPR, playing a major role in β cell death, which lead to diabetes[16]. Islet inflammation might be mediated by ER stress and contributes to β cell death[17,18]. We previously found increased FABP4 expression and ER stress as well as increased apoptosis of glomerular mesangial cells in patients with diabetic nephropathy[10]. In the present study, we found, along with the increased apoptotic protein Cleaved caspase-3, UPR marker GRP78 as well as ER stress responder ATF6 and p-IRE1α were upregulated in *Lepr*$^{-/-}$ rat islets. Given β cells accounting for about 85% cells of islets, the above finding indicated β cells of *Lepr*$^{-/-}$ rat islets had undergone ER stress and apoptosis.

Inhibiting FABP4 either with specific inhibitor or neutralizing antibody were demonstrated to be effective on improving insulin resistance, atherosclerosis, diabetic/lipid-induced cardiomyopathy and T2DM[19-23]. The mechanism of these effects included attenuating the intracellular lipid content and inflammation, improving insulin signaling and insulin-stimulated glucose uptake. We previously demonstrated that FABP4 inhibition could protect human mesangial cells from NEFA or glucose-induced apoptosis by reducing ER stress *in vitro*[10]. Bosquet et al[23] also reported FABP4 inhibitor BMS309403 improved insulin resistance in high-fat-diet mice by decreasing ER stress-associated inflammation in skeletal muscle. In our study, BMS309403-treated *Lepr*$^{-/-}$ rats showed downregulated CD68, GRP78, ATF6, p-IRE1α in islets, indicating attenuated ER stress and inflammation in islets. Considering some islet preserving effects, which BMS309403 showed by improving GTT and elevating serum insulin level in rats of KO-T group, the above results implicated that the FABP4 inhibitor possibly performed to protect islets and to improve phenotypic characters of metabolic syndrome, including losing bodyweights and alleviating hyperlipidemia in *Lepr*$^{-/-}$ rats, partly due to downregulated ER stress and inflammation in islets. Given the manner of administration in our experiments, intragastric administration inevitably made the FABP4 inhibitor acted on all FABP4-expressing cells, which no doubt includes adipocytes. The detailed effects of FABP4 and the mechanism remains to be determined since it was reported that FABP4, instead of harming insulin sensitivity, benefitted and protected glucose homeostasis[25]. Wu et al[24] suggested FABP4 with linoleate potentiated glucose-stimulated insulin secretion (GSIS). And they thought this effect of FABP4 and linoleate on β cell function is likely due to synergistic action, such as the transport of linoleate to a specific cellular location or pathway, or activation of a receptor by the FABP4/linoleate complex, akin to the role of intracellular FABP4 in activating adipocyte nuclear receptors[25]. However, FABP4 does not contain a signal peptide, which would normally be required for targeting to the secretory pathway, consistent with its presence in the cytosol of adipocytes. In some researches, FABP4 was observed to activate palmitic acid-dependent inflammatory responses in macrophages, endo-

thelial cells and vascular smooth muscle cells, which just were the types of cell with ectopic expression of FABP4[14]. So endogenous FABP4, as we infer, might be crucial to the function of those types of cells. And in our study, we found FABP4 had a similar situation in pancreatic islets as it did in those types of cell. The systemic benefits of FABP4 inhibition might be partly attributed to its effects of lowering inflammation and ER stress in pancreatic islets.

Conclusions

The present study for the first time demonstrated that FABP4 was expressed in islet and the increased FABP4 in *Lepr*$^{-/-}$ rats might be involved in the regulation of islet inflammation and ER stress. FABP4 inhibitor BMS309403 could improve metabolic syndrome partly through ameliorating ER stress and inflammation in islet. Our results demonstrated the therapeutic potential of FABP4 inhibitor for metabolic syndrome.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All animal care and experimental procedures were approved by the Ethics Committee for Animals Experiments of Hebei Medical University, Shijiazhuang, China (approved document IACUC-Hebmu-PD-2018023).

Acknowledgments

This study was supported by Key Research and Development Projects of Hebei Provincial Department of Science and Technology (18274801D) and Major projection of Hebei Provincial Department of Education (ZD2016010).

Authors' Contribution

LZ and FY designed the experiments and interpreted the results. DJ, WG, LG, MG, YB and XW conducted the experiments. The final manuscript was drafted by FY, reviewed by LZ and approved by all authors.

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