Aerobic exercise regulates blood lipid and insulin resistance via the toll-like receptor 4-mediated extracellular signal-regulated kinases/AMP-activated protein kinases signaling pathway

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Abstract. Diabetes mellitus is a complicated metabolic disease with symptoms of hyperglycemia, insulin resistance, chronic damage and dysfunction of tissues, and metabolic syndrome for insufficient insulin production. Evidence has indicated that exercise treatments are essential in the progression of type-II diabetes mellitus, and affect insulin resistance and activity of islet β-cells. In the present study, the efficacy and signaling mechanism of aerobic exercise on blood lipids and insulin resistance were investigated in the progression of type-II diabetes mellitus. Body weight, glucose metabolism and insulin serum levels were investigated in mouse models of type-II diabetes mellitus following experienced aerobic exercise. Expression levels of inflammatory factors, interleukin (IL)-6, high-sensitivity C-reactive protein, tumor necrosis factor-α and leucocyte differentiation antigens, soluble CD40 ligand in the serum were analyzed in the experimental mice. In addition, expression levels of toll-like receptor 4 (TLR-4) were analyzed in the liver cells of experimental mice. Changes of oxidative stress indicators, including reactive oxygen species, superoxide dismutase, glutathione and catalase were examined in the liver cells of experimental mice treated by aerobic exercise. Expression levels and activity of extracellular signal-regulated kinases (ERK) and AMP-activated protein kinase (AMPK) signaling pathways were investigated in the liver cells of mouse models of type-II diabetes mellitus after undergoing aerobic exercise. Aerobic exercise decreased the expression levels of inflammatory factors in the serum of mouse models of type-II diabetes mellitus. The results indicated that aerobic exercise downregulated oxidative stress indicators in liver cells from mouse models of type-II diabetes mellitus. In addition, the ERK and AMPK signaling pathways were inactivated by aerobic exercise in liver cells in mouse models of type-II diabetes mellitus. The activity of ERK and AMPK, and the function of islet β-cells were observed to be improved in experimental mice treated with aerobic exercise. Furthermore, blood lipid metabolism and insulin resistance were improved by treatment with aerobic exercise. Body weight and glucose concentration of serology was markedly improved in mouse models of type-II diabetes mellitus. Furthermore, TLR-4 inhibition markedly promoted ERK and AMPK expression levels and activity. Thus, these results indicate that aerobic exercise may improve blood lipid metabolism, insulin resistance and glucose plasma concentration in mouse models of type-II diabetes mellitus. Thus indicating aerobic exercise is beneficial for improvement of blood lipid and insulin resistance via the TLR-4-mediated ERK/AMPK signaling pathway in the progression of type-II diabetes mellitus.

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Introduction

Diabetes mellitus is a severe glucose-associated metabolic disease that presents obvious genetic heterogeneity and frequently induces chronic damages of other tissues due to insulin secretion defects or/and biological function damage (1). Diabetes mellitus often results in ophthalmic diseases, cardiovascular disease and metabolic syndrome (2,3). A previous study indicated that diabetes mellitus may be regarded as a risk factor for incident chronic renal failure based upon a systematic review and meta-analysis (4). In addition, insulin resistance, glucose and lipid metabolism, and metabolic disturbance are critical indicators of the pathophysiology of diabetes mellitus (4,5). With the improvement of living standards, the incidence of diabetes mellitus is rapidly increasing and increasing complications have been observed in clinical patients with diabetes mellitus (6,7).

Recent studies have indicated that diabetes mellitus is a metabolic disease that is induced by various factors, such as inheritance, diet, immunity, inflammation, and other symptoms, including liver diseases, renal dysfunction,

stress state and various types of endocrine disease (8). Domingueti *et al* (9) demonstrated that the linkages between oxidative stress, inflammation, hypercoagulability, vascular complications and diabetes mellitus have been investigated, and inflammation and oxidative stress may be regarded as predictors for evaluating the risk of cardiovascular and renal complications in patients with diabetes mellitus. In addition, previous studies have investigated the insulin resistance and sensitivity in liver, adipose tissue, skeletal muscle and placenta samples (10-12). Furthermore, studies have indicated that diabetes mellitus may be a syndrome, which includes vascular dysfunction, insulin resistance, inflammatory responses and oxidative stress (13,14). Additionally, although studies have investigated the effects of sports on diabetes mellitus patients during treatment, the molecular mechanisms are seldom reported in previous studies (15,16). Therefore, investigating the potential mechanism(s) may contribute to understanding aerobic exercise benefits for the treatment of diabetes mellitus.

Theoretically, secretion levels of insulin (which are upregulated in the early stage and downregulated in the late stage of diabetes mellitus, and regulated by islet β-cells) are a predominant parameter for predicting insulin resistance of patients with diabetes mellitus (11). Karalliedde *et al* (17) indicated that diabetes mellitus is a complex and heterogeneous disease that presents insulin resistance as a determinant of diabetic kidney disease. Alterations of islet β-cell activity directly respond to blood glucose concentration levels in the serum, which further affects glycated serum protein and blood lipid levels (18,19). In addition, studies have found that activation of the TLR-4 signaling pathway may promote the generation of pro-inflammatory cytokines via transcription factors of activated protein 1 expression, interferon regulatory factors and the nuclear factor (NF)-κB signaling pathway in the progression of diabetes mellitus (20,21). Furthermore, accumulation and glucose output via Ca^{2+}/calcium/calmodulin dependent protein kinase kinase 2/AMP-activated protein kinase (AMPK) activation have been identified under insulin-resistant conditions in type-II diabetes mellitus (22). These investigations indicate that stimulation of islet β-cell activity regulates insulin resistance and glucose metabolism via regulation of intracellular signaling pathways.

In the present study, the influences of aerobic exercise on the activation of oxidative stress, inflammation, insulin sensitivity and glucose metabolism were investigated in a mouse model of type-II diabetes mellitus. The efficacy of aerobic exercise on ERK and AMPK activity was investigated and discussed in the liver cells of mice with type-II diabetes mellitus following a 30-day exercise treatment. Notably, the present study investigated and discussed the effects of aerobic exercise on body weight and food intake, and the TLR4-mediated ERK/AMPK signaling pathway in a mouse model of type-II diabetes mellitus.

Materials and methods

Ethical approval. The present study was performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of China. All surgical procedures were performed under anesthetic to minimize pain.

Cell culture and reagents. Islet β-cells were obtained from Fudan Institutes of Biomedical Sciences Cell Center, (Shanghai, China). The cells were cultured in minimum essential medium supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Islet β-cells were cultured for 24 h in a 5% CO_2 incubator with a humidified atmosphere at 37˚C.

ELISA. Serum was obtained from experimental mice using centrifugation at 3,000 x g for 15 min at 4˚C. In the protein detection assay, mouse interleukin (IL)-6 (cat. no. M6000B; Bio-Rad Laboratories, Inc., Hercules, CA, USA), high-sensitivity C-reactive protein (hs-CRP; cat. no. MCRP00), tumor necrosis factor (TNF)-α (cat. no. MTA00B), and soluble CD40-ligand (sCD40l; cat. no. DY617) (all from Bio-Rad Laboratories, Inc.). ELISA kits were used to determine the serum levels of inflammatory factors, respectively on day 30. The procedures were conducted according to the manufacturer's instructions. The final results were recorded at a wavelength of 450 nm on an ELISA plate reader.

Animal studies. A total of 20 six-eight-week-old db/db mice (body weight, 18-24 g) with type II diabetes mellitus were purchased from Charles River Laboratories (Sulzfeld, Germany). The mice were separately housed in a temperature-controlled room (25±1˚C). All mice with type-II diabetes mellitus were randomly divided into two groups (n=10 in each group) and received aerobic exercise treatment or no treatment according to a previous study (23). The experiments were continued 30 days. On day 30, mice were used to evaluate glucose and insulin tolerance and resistant.

Glucose and insulin tolerance tests. Mice with type II diabetes mellitus were fasted for 6 h and injected intraperitoneally with glucose at a dose of 2.0 g/kg for the glucose tolerance test. Blood glucose concentrations were analyzed by performing an ACCU-CHEK advantage glucometer (Roche Diagnostics, Indianapolis, IN, USA). The glucose tolerance tests were recorded at baseline and subsequent to glucose injections (at 0, 30, 60, 90 and 120 min). For the insulin tolerance tests, mice with type II diabetes mellitus were injected intraperitoneally with insulin (0.75 U/kg body weight). The mice were injected intraperitoneally with insulin (1 mU/kg) after a 60-min fast, and the blood glucose concentration was measured at baseline and subsequent to the insulin injection (at 0, 30, 60, 90 and 120 min).

Small interfering RNA (siRNA) transfections. Islet β-cells were cultured to 80% confluence and transfected with siRNA that targeted TLR-4 (Si-TLR-4) or Si-vector using Lipofectamine™ RNAi MAX (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. The siRNA targeting mouse TLR-4 and scrambled siRNA were obtained from Shanghai GenePharma Co., Ltd. (Shanghai, China).

Physical activity analysis by indirect calorimetry. Indirect calorimetry was performed to evaluate the physical activity of mice with type-II diabetes mellitus using the Comprehensive Laboratory Animal Monitoring System (Oxymax/CLAMS;

Columbus Instruments International Corporation, Columbus, OH, USA). On day 30, following experiments with swimming in 25-28˚C water, the physical activity of mice was monitored every 30 min for 24 h to analyze the therapeutic effects of aerobic excise. The respiratory exchange ratio, VO_2, VCO_2, heat production and physical activity were measured using a gas analyzer (ISM-3I; Danbell Equipment Co., Ltd., Beijing, China) according to the manufacturer's instructions.

Glucose-6-phosphatase, ERK and AMPK activity. The glucose-6-phosphatase activity of mice with type-II diabetes was analyzed as previously described (24). ERK and AMPK activities in mice with type-II diabetes were detected using luciferase activity according to a previous study (25). Glucose-6-phosphatase, ERK and AMPK activities were normalized by subtracting nonspecific phosphatase activity, as determined by paranitrophenylphosphate (26).

Western blot analysis. Total liver cells were isolated from the mice with type-II diabetes as described previously (27) and homogenized in 1X radioimmunoprecipitation assay buffer (Sigma-Aldrich; Merck KGaA) on day 30. Subsequently, western blotting was performed to analyze the protein expression levels. Protein concentration was measured using a bicinchoninic acid protein assay kit (Invitrogen; Thermo Fisher Scientific, Inc.). Proteins (20 μg/lane) were analyzed using 12% SDS-PAGE assays followed by transfer onto polyvinylfluoride membranes. Proteins were blocked with 5% bull serum albumin (Sigma-Aldrich; Merck KGaA) for 1 h at 37˚C. For western blotting, the following primary antibodies were used: TLR4 (1:500 dilution; cat. no. ab13556), sirtuin-1 (1:500 dilution; cat no. ab12193), microsomal triglyceride transfer protein (1:500 dilution; cat. no. ab186446), p65 (1:500 dilution; cat no. ab16502), GSH (1:500 dilution; cat no. ab26255), SOD (1:500 dilution; cat no. ab13533), CAT (1:500 dilution; cat no. ab52477), ROS (1:500 dilution; cat no. ab139476), ERK (1:500 dilution; cat no. ab17942), AMPK (1:500 dilution; cat no. ab32047), and β-actin (1:500 dilution; cat no. ab8226) (all from Abcam, Cambridge, UK) were added after blocking with 5% skimmed milk for 1 h at 37˚C. Subsequently, incubation with horse radish peroxidase-conjugated goat anti-rabbit IgG monoclonal antibody (cat. no. PV-6001; OriGene Technologies, Inc., Rockville, MD, USA) was performed for 24 h at 4˚C. The results were visualized using a chemiluminescence detection system with Quantity-One software (version 3.1; Bio-Rad Laboratories, Inc.).

Immunohistochemical staining. Liver tissue samples were isolated from experimental mice with type-II diabetes on day 30 as described previously (28). Immunohistochemical staining was used to analyze TRL-4 levels and accumulation of lipids, which was performed using an avidin-biotin-peroxidase technique, as described previously (29). Paraffin-embedded liver tissue sections (4 μm) were prepared and epitope retrieval was performed using antigen retrieval buffer (cat. no. SRE0063; Tris-EDTA buffer solution, pH 9.0; Sigma-Aldrich, Merck KGaA) for further analysis. The paraffin sections were subjected to hydrogen peroxide (3%) for 10-15 min, and were subsequently blocked with a regular blocking solution for 10-15 min at 37˚C. Finally, the sections were incubated in goat anti-mouse anti-TLR4 and anti-sirtuin-1 at 4˚C for 12 h. All sections were washed with PBS three times, incubated with secondary rabbit anti-goat antibodies (1:2,000; cat. no. PV-6001; HRP-conjugated goat anti-rabbit IgG monoclonal antibody; OriGene Technologies, Inc.) for 1 h at 37˚C, and six random views were observed by under a confocal microscope (LSM780; Carl Zeiss AG, Oberkochen, Germany).

Statistical analysis. All data were presented as means ± standard error of the mean following three independent experiments. Statistical significance was analyzed using two-tailed Student's t-test between groups. Unpaired data were analyzed by one-way ANOVA followed by Bonferroni post hoc tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Aerobic exercise decreases inflammation factors in the serum of mouse models of type-II diabetes mellitus. Type-II diabetes mellitus is a type of natural immune and chronic subclinical inflammation disease. Anti-inflammatory therapy improves sugar metabolic abnormalities and blood lipid metabolism in patients with type-II diabetes mellitus in the clinical setting. In the present study, the expression levels of inflammatory factors from the serum of mouse models of type-II diabetes mellitus were investigated. As shown in Fig. 1A, the plasma concentration of inflammatory factor, IL-6 was significantly decreased following 30 days of aerobic exercise. Plasma concentration levels of hs-CRP were downregulated in mice with type-II diabetes mellitus subsequent to treatment with aerobic exercise (Fig. 1B). In addition, the plasma concentration level of TNF-α was markedly decreased in mice after undergoing aerobic exercise treatment (Fig. 1C). Furthermore, the expression levels of leucocyte, sCD40l were markedly downregulated in the serum of experimental mice (Fig. 1D). These results indicate that aerobic exercise treatment is beneficial for downregulation of inflammatory factor serum levels in mouse models of type-II diabetes mellitus.

Aerobic exercise downregulates oxidative stress markers in the serum of mouse models of type-II diabetes mellitus. Oxidative stress contributes to diabetes mellitus-induced inflammation and apoptosis, which further leads to dysfunction of islet β-cells during type-II diabetes mellitus. Therefore, the therapeutic effects of aerobic exercise treatment on oxidative stress and antioxidant level were analyzed in mice with type-II diabetes mellitus. As presented in Fig. 2A, aerobic exercise treatment decreased the levels of reactive oxygen species in the liver cells of experimental mice. The superoxide dismutase expression levels were also upregulated in liver cells in the experimental mice following treatment with aerobic exercise (Fig. 2B). Furthermore, glutathione and catalase expression levels were markedly decreased in the liver cells of experimental mice following treatment with aerobic exercise (Fig. 2C and D). These results indicate that aerobic exercise treatment decreases oxidative stress and improves the antioxidant status in liver cells in mice with type-II diabetes mellitus.

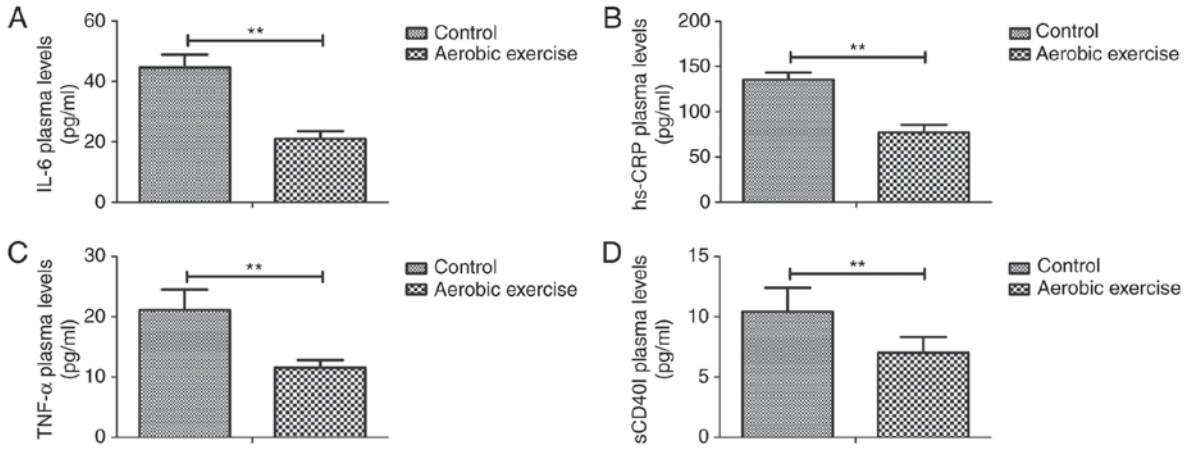

Figure 1. Effects of aerobic exercise on inflammation factors in mouse models of type-II diabetes mellitus. (A) Serum levels of IL-6 in mice with type-II diabetes mellitus following 30 days of aerobic exercise. Plasma concentration levels of (B) hs-CRP (C) TNF-α and (D) sCD40l in mice with type-II diabetes mellitus following 30 days of aerobic exercise. The results are expressed as means ± standard deviation of three independent experiments. **P<0.01 vs. control group. IL, interleukin; hs-CRP, high-sensitivity C-reactive protein; TNF, tumor necrosis factor; sCD40l, soluble CD40-ligand.

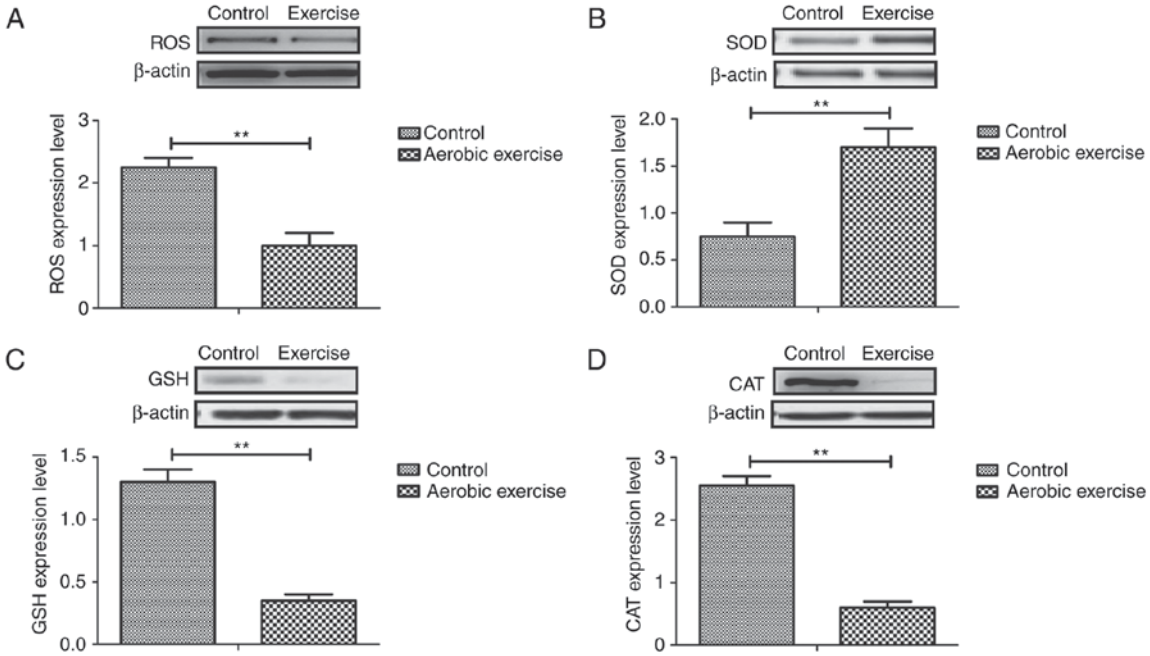

Figure 2. Effects of aerobic exercise on oxidative stress markers in mouse models of type-II diabetes mellitus. Expression levels of (A) ROS, (B) SOD, (C) GSH and (D) CAT in the liver cells of experimental mice following 30 days of aerobic exercise. The results are expressed as means ± standard deviation of three independent experiments. **P<0.01 vs. control group. ROS, reactive oxygen species; SOD, superoxide dismutase; GSH, glutathione; CAT, catalase.

Aerobic exercise improves metabolism of glucose and blood lipids, and insulin resistance in the serum of mouse models of type-II diabetes mellitus. Glucose and insulin metabolism disturbance is the most important characteristic of patients with type-II diabetes mellitus. Therefore, the efficacy of aerobic exercise on glucose and insulin metabolism was investigated in the present study. Fig. 3A demonstrated that aerobic exercise decreased the serum levels of glucose in mice with type-II diabetes mellitus after aerobic exercise (60 min). The serum insulin concentration levels were upregulated in mice with type-II diabetes mellitus after aerobic exercise (60 min) in the aerobic exercise treatment group (Fig. 3B). In addition, insulin tolerance was improved by aerobic exercise treatment in the experimental mice (Fig. 3C). Furthermore, serum levels of blood lipids in mice with type-II diabetes mellitus were downregulated by aerobic exercise treatment (Fig. 3D). Furthermore, glucose-6-phosphate and lipidoxidase activity was upregulated in liver cells in mice with type-II diabetes mellitus subsequent to treatment by aerobic exercise (Fig. 3E and F). These data indicate that aerobic exercise treatment significantly improves the metabolism of glucose, lipids and insulin in mouse models of type-II diabetes mellitus.

Aerobic exercise regulates activation of islet β-cells via the TLR-4-mediated ERK/AMPK signaling pathway. To investigate the underlying signaling mechanism of aerobic exercise on the metabolism of glucose, blood lipid and insulin, ERK

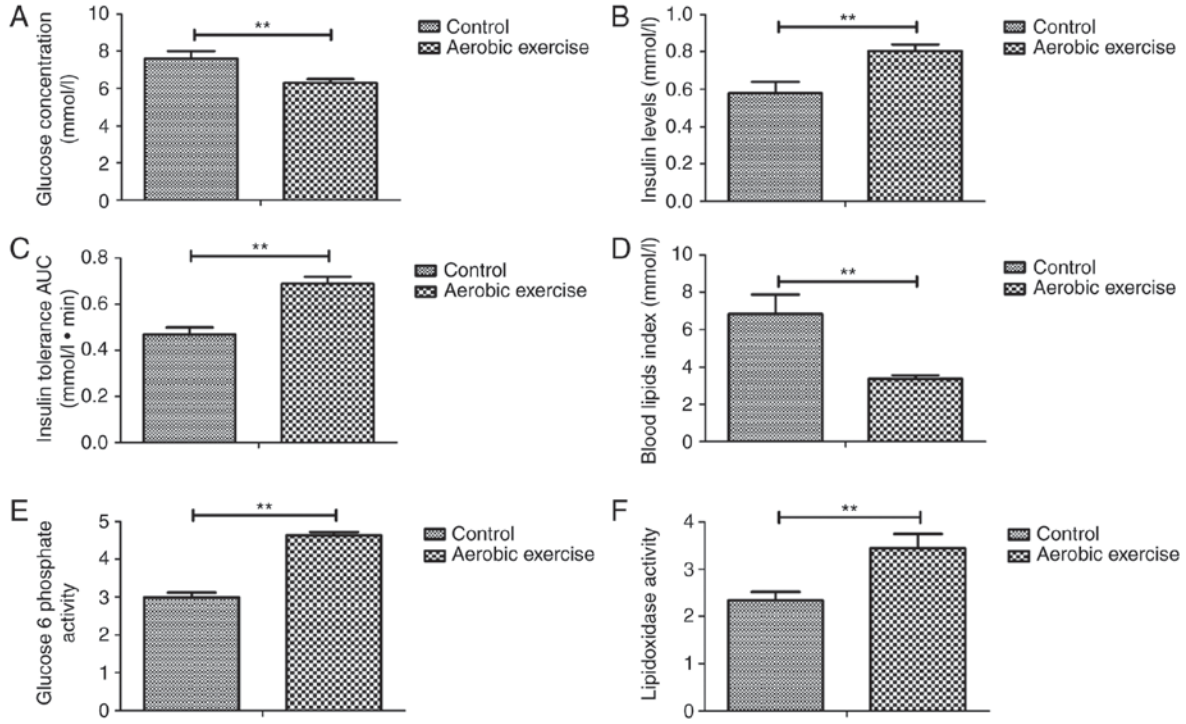

Figure 3. Effects of aerobic exercise on metabolism of glucose, blood lipid and insulin in mouse models of type-II diabetes mellitus. (A) Serum levels of glucose in mice with type-II diabetes mellitus after aerobic exercise (60 min). (B) Insulin concentration levels in serum in mice with type-II diabetes mellitus aerobic exercise. (C) Insulin tolerance in mice following aerobic exercise treatment. (D) Serum levels of blood lipids in mice with type-II diabetes mellitus following aerobic exercise treatment. Activity of (E) glucose-6-phosphate and (F) lipidoxidase was analyzed in liver cells of mice with type-II diabetes mellitus following treatment with aerobic exercise treatment. The results are expressed as means ± standard deviation of three independent experiments. **P<0.01 vs. control group.

and AMPK expression levels and activity were analyzed in the liver cells of experimental mice. Aerobic exercise treatment significantly increased the expression levels of ERK and AMPK in the liver cells in mouse models of type-II diabetes mellitus (Fig. 4A and B). The activity of ERK and AMPK was also enhanced in liver cells of mouse models of type-II diabetes mellitus (Fig. 4C and D). Results indicate that TLR-4 expression levels were upregulated in the liver cells of mice treated with aerobic exercise (Fig. 4E). An *in vitro* assay demonstrated that inhibition of TLR-4 expression by siRNA suppressed the expression of ERK and AMPK in cultured liver cells isolated from mice with type-II diabetes mellitus following treatment with aerobic exercise (Fig. 4F). Activity of ERK and AMPK was also inhibited by inhibition of TLR-4 expression by siRNA (Fig. 4G). Furthermore, activation of islet β-cells was significantly decreased following inhibition of TLR-4 expression by siRNA (Fig. 4H). These findings indicate that aerobic exercise treatment may regulate activation of islet β-cells via the TLR-4-mediated ERK/AMPK signaling pathway.

Aerobic exercise increases body weight and liver function in mouse models of type-II diabetes mellitus. Body weight and liver function were further evaluated in mouse models of type-II diabetes mellitus treatment with aerobic exercise. The results in Fig. 5A and B demonstrate that body weight was increased and food intake was decreased for mice with type-II diabetes mellitus treated by aerobic exercise. In addition, the accumulation of fat was observed to be decreased in liver tissue samples from mice with type-II diabetes mellitus treated by aerobic exercise (Fig. 5C). Furthermore, TLR-4 expression levels were upregulated in liver tissue samples from mice treated by aerobic exercise, which was determined by histological analysis (Fig. 5D). These results indicate that aerobic exercise may regulate the physiological function and body weight of mice with type-II diabetes mellitus.

Aerobic exercise relieves glucose and insulin tolerance in mouse models of type-II diabetes mellitus. Finally, the effects of aerobic exercise on glucose and insulin tolerance were analyzed in experimental mice. On day 30, experimental mice were injected with glucose and the plasma concentrations of glucose and insulin were examined. As presented in Fig. 6A, aerobic exercise treatment significantly alleviated glucose intolerance in mice with type-II diabetes mellitus subsequent to the initial glucose injection (30 and 60 min) compared with the control. In addition, insulin plasma concentration levels (0, 30 and 60 min) in the experimental mice were markedly improved by aerobic exercise treatment compared with the control (Fig. 6B). The insulin resistance test indicated that the aerobic exercise treatment significantly improved insulin resistance in type-II diabetes mellitus, as determined by the area under the curve (Fig. 6C). Furthermore, the apoptosis rate of liver cells was observed to be decreased in mice that were treated by aerobic exercise compared with the control mice (Fig. 6D). These results indicated that aerobic exercise treatment alleviated acute glucose and insulin intolerance in mouse models of type-II diabetes mellitus.

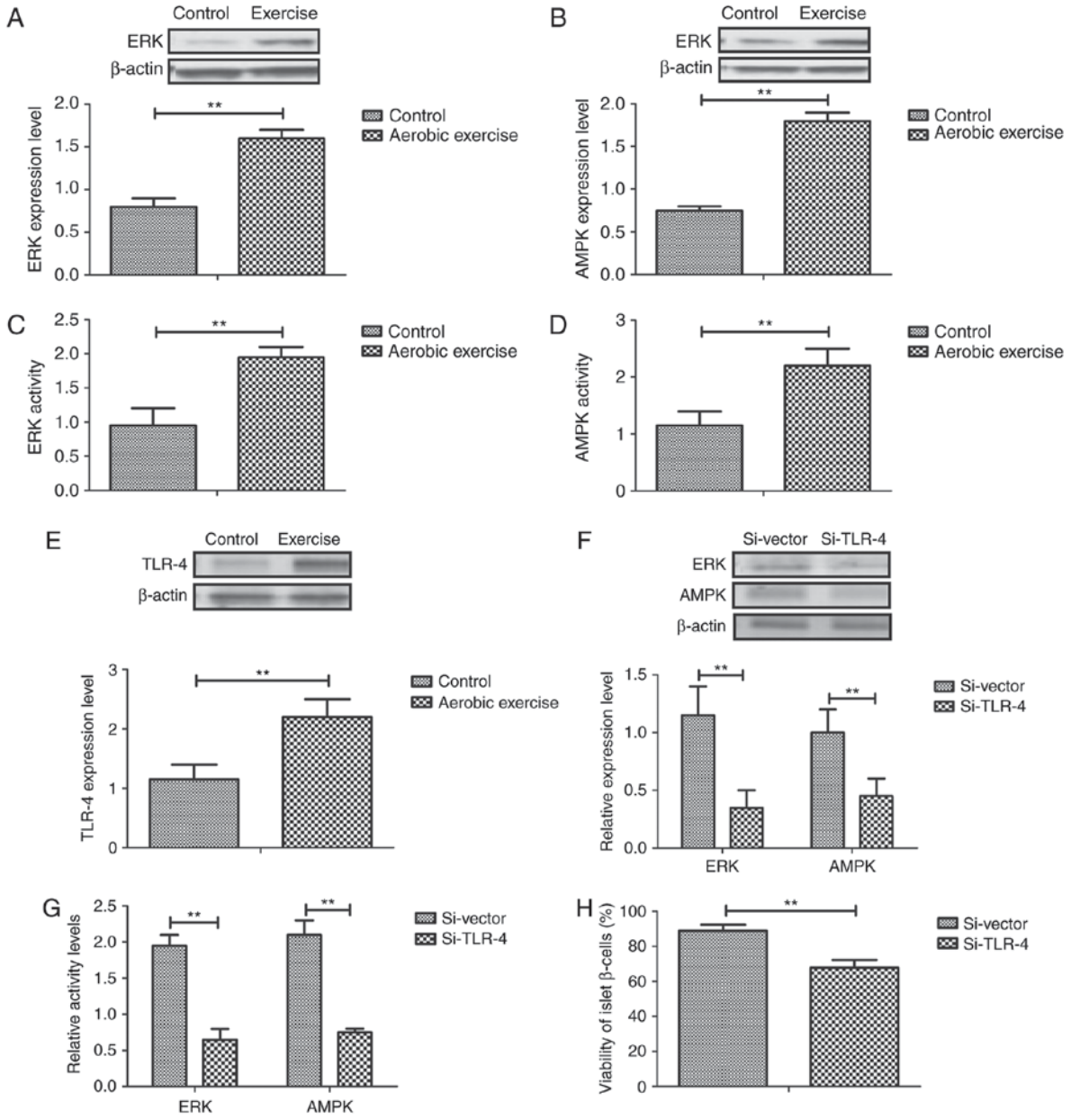

Figure 4. Aerobic exercise improves islet β-cell function via the TLR-4-mediated ERK/AMPK signaling pathway. Expression levels of (A) ERK and (B) AMPK in the liver cells of mouse models of type-II diabetes mellitus. Activity of (C) ERK and (D) AMPK in the liver cells of mouse models of type-II diabetes mellitus. (E) TLR-4 expression levels in the liver cells of mouse models of type-II diabetes mellitus. (F) Expression levels of ERK and AMPK in the liver cells treated by siRNA in mouse models of type-II diabetes mellitus. (G) Activity of ERK and AMPK in liver cells treated by siRNA in mouse models of type-II diabetes mellitus. (H) Activation of islet β-cells following inhibition of TLR-4 expression by siRNA. The results are expressed as means ± standard deviation of three independent experiments. **P<0.01 vs. control group. TLR-4, toll-like receptor 4; ERK, extracellular signal-regulated kinases; AMPK, AMP-activated protein kinase; si, small interfering.

Discussion

In the present study, the benefits of aerobic exercise treatment for type-II diabetes mellitus were analyzed in mouse models. Aerobic exercise decreased the expression levels of serum inflammatory factors and downregulated oxidative stress markers in liver cells from the mouse models of type-II diabetes mellitus. Notably, the results indicated that aerobic exercise treatment upregulates TLR-4-expression levels, and promotes ERK and AMPK expression levels and activity. Notably, the function of islet β-cells, lipid metabolism, oxidative stress insulin resistance and glucose plasma concentration have been improved in mouse models of type-II diabetes mellitus after undergoing aerobic exercise treatment. Thus, aerobic exercise may improve blood lipid metabolism, insulin resistance and glucose plasma concentration in mice model of type-II diabetes mellitus via regulation of the TLR-4-mediated ERK/AMPK signaling pathway during the progression of type-II diabetes mellitus.

Aerobic exercise treatment for patients with type-II diabetes mellitus has been identified as an efficient methodology in the clinical setting (30,31). Research has indicated that diabetes mellitus is a disease that often manifests with symptoms including hyperglycemia, insulin resistance and

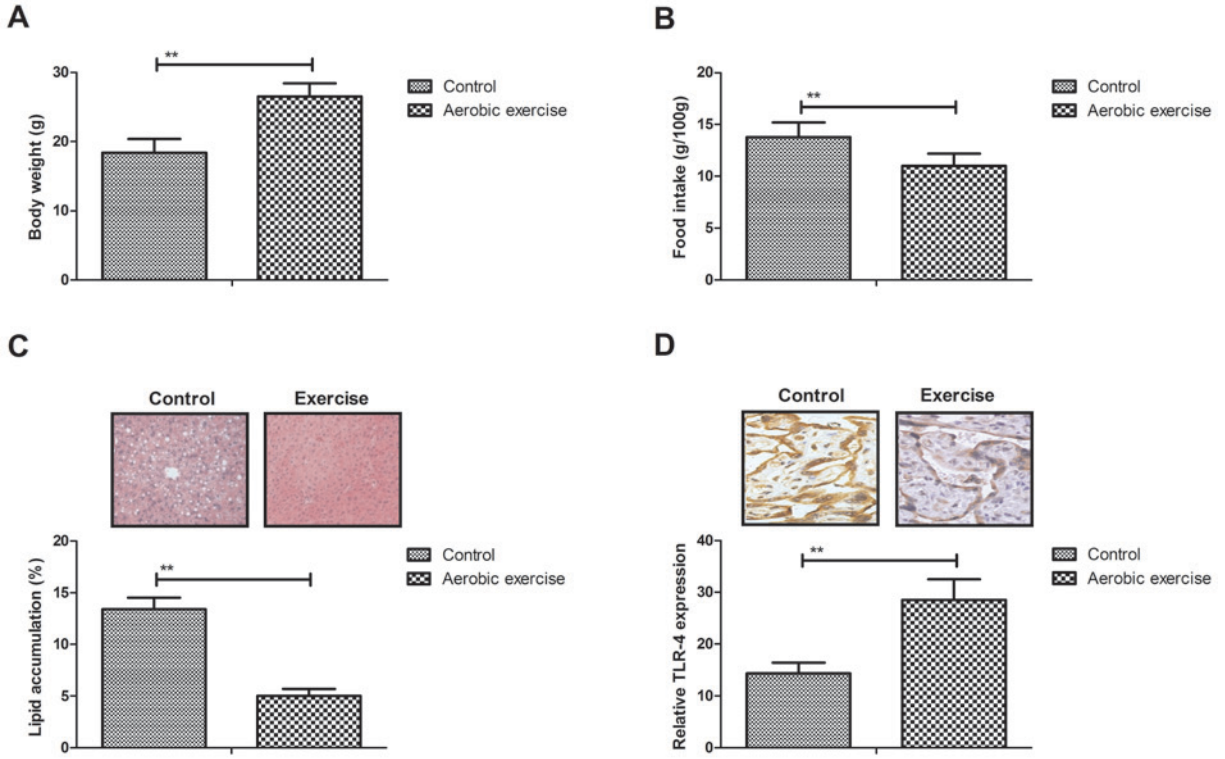

Figure 5. Effects of aerobic exercise on body weight and liver function in mice model of type-II diabetes mellitus. (A) Body weight and (B) food intake was detected in mouse models of type-II diabetes mellitus following aerobic exercise treatment. (C) Accumulation of fat in liver tissues of mice with type-II diabetes mellitus. (D) TLR-4 expression levels in liver tissues of mice with type-II diabetes mellitus. The results are expressed as means ± standard deviation of three independent experiments. **P<0.01 vs. control group. TLR-4, toll-like receptor 4.

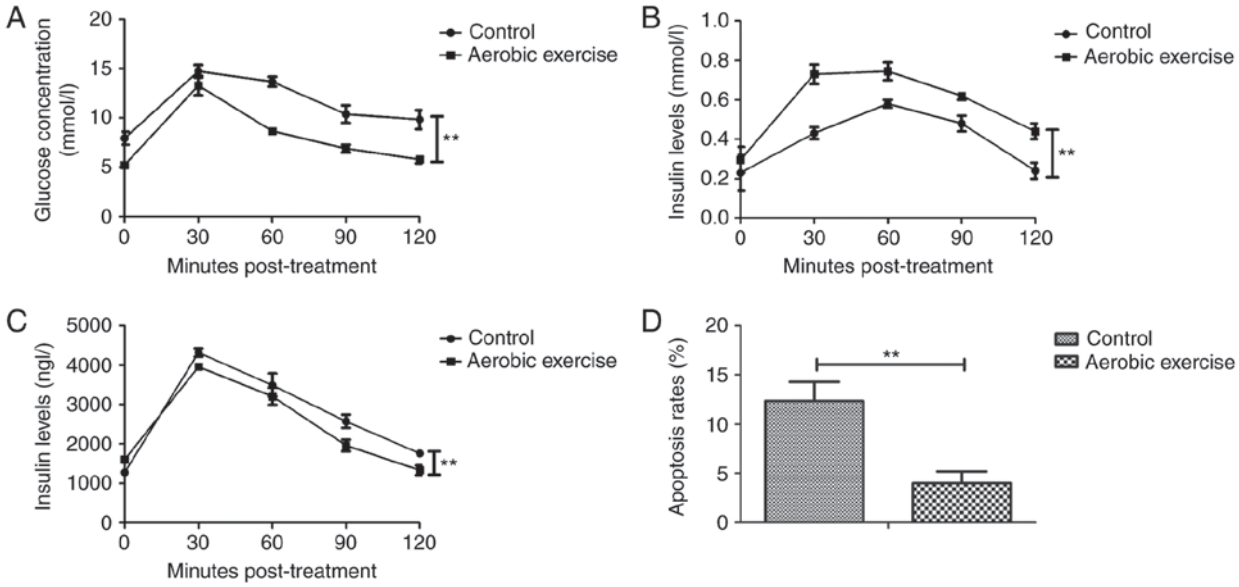

Figure 6. Effects of aerobic exercise on glucose and insulin tolerance in mouse models of type-II diabetes mellitus. (A) Effect of aerobic exercise on blood glucose concentration in mice with type-II diabetes mellitus. (B) Effect of aerobic exercise on blood insulin concentration in mice with type-II diabetes mellitus. (C) Insulin resistance and (D) apoptosis rate of liver cells in mice with type-II diabetes mellitus following aerobic exercise treatment. One-way ANOVA or Student's t-test revealed a significant effect. **P<0.01 vs. control group.

dysfunction of glucose tolerance (32). Fatone *et al* (33) verified that two-week sessions of combined aerobic and resistance exercise are beneficial for improving diabetes mellitus and metabolic syndrome. In addition, aerobic exercise and resistance exercise present ameliorative roles in glycemic control and modifiable cardiovascular risk factors in patients with type-II diabetes mellitus (34). The effects of aerobic exercise treatment on improvement of endothelial function have been investigated in women with type II diabetes mellitus during clinical investigations (35). Furthermore, a previous study

has indicated that aerobic exercise treatment affects physiological parameters and quality of life in patients with type-II diabetes mellitus (36). Furthermore, Grise *et al* reported the benefits of high intensity aerobic exercise training on cardiovascular autonomic function in a rat model of type-1 diabetes mellitus (37). In the present study, the benefits of aerobic exercise training and the molecular mechanism of aerobic exercise treatment were investigated in mouse models of type-II diabetes mellitus. Inflammatory factors and oxidative stress were observed to be markedly improved subsequent to aerobic exercise treatment.

Previous studies have identified the important association between inflammation and type-II diabetes mellitus (38,39). Inflammatory cytokines are identified as crucial regulatory signaling networks in the progression of diabetes mellitus that are mediated by different intracellular kinase signaling pathways to regulate local and system inflammatory responses following chronic diabetes mellitus (40,41). In addition, the associations between insulin resistance, low-grade inflammation and type-I diabetes mellitus have been investigated in a previous study (42). Changes in inflammatory/necrosis biomarkers following exercise treatment have been analyzed in the progression of diabetes mellitus (43,44). However, to the best of our knowledge, the molecular mechanisms involved in diabetes mellitus have not been clearly elaborated during previous studies. In the present study, the experiments analyzed the benefits of aerobic exercise training with regard to inflammatory factor expression, as well as signaling pathways in liver cells. Aerobic exercise treatment was identified to promote TLR4 expression levels to inhibit IL-6, hs-CRP, TNF-α and sCD40l in experimental mice with type-II diabetes mellitus.

Hopmans *et al* (45) indicated that diabetes mellitus may increase the risk of chronic renal failure, ophthalmic diseases and cardiovascular disease. Currently, exercise treatments combined with diets have been analyzed in patients with diabetes mellitus by kerberos authentication protocol and data mining. In addition, studies have evidenced that exercise treatments may improve diabetes mellitus-induced myocardial ischemia and failure in patients in the clinical setting (46). Currently, oxidative stress followed by excessive apoptosis frequently occurs in the liver of patients with type-II diabetes mellitus (47). Vural *et al* (48) evaluated the future atherosclerotic heart disease with oxidative stress and carotid artery intima media thickness in gestational diabetes mellitus. The severity of diabetes mellitus affecting microvascular dysfunction may be associated with increasing oxidative stress (49). The present findings have indicated that the ameliorative effects of aerobic exercise treatment on oxidative stress have been identified in the liver cells of mouse models of type-II diabetes mellitus. These findings may contribute towards improving insulin resistance and glucose-6-phosphate activity by enhancing TLR-4 expression in liver cells.

TLR-4 is important in recognizing pathogen-associated molecular patterns and endogenously derived indicators of tissue injury, and NF-κB-mediated inflammation (50,51). Previous studies have indicated that TLR-4 is expressed in various cells, including endothelium cells, syncytiotrophoblast, amnion epithelium and decidual cells that may be involved in insulin resistance and glucose metabolism (52,53). In the present study, TLR-4 expression levels were investigated in the liver cells of mice with diabetes mellitus and TLR-4 expression was identified to be downregulated in the liver cells via negative regulation of the NF-κB signaling pathway. In addition, Feng *et al* (54) propose that the expression of TLR-4/myeloid differentiation primary response gene 88/NF-κB is associated with insulin resistance in placentae in gestational diabetes mellitus. The present study is consistent with previous studies (55-57) and indicates that TLR-4 was upregulated by exercise treatment in the livers of mice with diabetes mellitus via the ERK/AMPK signaling pathway, which may contribute to improving insulin resistance and glucose metabolism for diabetes mellitus.

The AMPK signaling pathway has been identified as an important role in the progression of type II diabetes mellitus (58). Increases in AMPK expression levels are renoprotective in experimental diabetes mellitus by reducing NOX4/transforming growth factor β1 signaling, indicating that AMPK inhibition may exert a therapeutic potential in diabetic nephropathy (59). In addition, Yao *et al* (60) suggested that activation of AMPK relieves gestational diabetes mellitus in mouse models. In the present study, ERK and AMPK signaling pathways were inactivated by aerobic exercise in the liver cells of mouse models of diabetes mellitus. The activities of ERK and AMPK in islet β-cells were improved in the experimental mice subsequent to treatment by aerobic exercise. Furthermore, AMPK activation reduces hepatic triglyceride accumulation, glucose output and insulin-resistant in type-II diabetes mellitus (22). The present findings confirmed the findings of previous studies (22,59) and further emphasize the efficacy of aerobic exercise treatment for improvements of insulin tolerance and blood lipid metabolism in mouse models of type-II diabetes mellitus.

In conclusion, the present study presents the benefits of aerobic exercise for the treatment of type-II diabetes mellitus. The findings from this analysis provide additional insights into aerobic exercise treatment in improving insulin resistance and blood glucose disorders in mouse models of type-II diabetes mellitus. The detailed analyses of underlying mechanisms have found that ERK/AMPK may be novel targets in the management of type-II diabetes mellitus. Notably, aerobic exercise treatment regulates liver lipid metabolism, glucose-6-phosphate activity, the inflammatory status and oxidative stress via activation of islet β-cells, which are beneficial for improvement of insulin resistance in diabetes mellitus mice. Furthermore, inflammation and oxidative stress improvement contribute to improving the body weight, food intake and insulin secretion by promoting the TLR-4-mediated ERK/AMPK signaling pathway. Thus, the present results may provide a novel direction for the development of therapeutic strategies for the treatment of type-II diabetes mellitus in the clinical setting.

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