Quercetin modulates AMPK/SIRT1/NF-κB signaling to inhibit inflammatory/oxidative stress responses in diabetic high fat diet-induced atherosclerosis in the rat carotid artery

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Abstract. Inflammation and oxidative stress serve interrelated roles in the development of atherosclerosis and other vascular diseases. Quercetin has been previously reported to exhibit numerous beneficial properties towards several metabolic conditions and cardiovascular disease. The present study aimed to evaluate the effects of quercetin on the 5'adenosine monophosphate-activated protein kinase (AMPK)/sirtuin 1 (SIRT1)/NF-κB signaling pathway and inflammatory/oxidative stress response in diabetic-induced atherosclerosis in the carotid artery of rats. Male Wistar rats were used to create a diabetes-induced atherosclerosis model by the administration of high fat diet (HFD) with streptozotocin, which lasted for 8 weeks. Control and diabetic rats received quercetin (30 mg/kg/day; orally) for the last 2 weeks of the diabetic period. Plasma lipid profile and vascular levels of oxidative stress markers, inflammatory cytokines, NF-κB signaling proteins and SIRT1 expression were evaluated using ELISA and western blotting. Quercetin treatment in HFD diabetic rats was reported to improve the lipid profile and reduce the number of atherosclerotic lesions, atherogenic index and malondialdehyde levels, whilst increasing the activity of enzymatic antioxidants in the carotid artery. Additionally, the inflammatory response was suppressed by quercetin administration, as indicated by the reduced NF-κB and IL-1β levels, and increased IL-10 levels. Furthermore, SIRT1 expression was revealed to be significantly increased in response to quercetin treatment compared with non-treated HFD rats. However, these effects of quercetin were abolished or reversed by the administration of compound-C (0.2 mg/kg), a specific AMPK blocker, in HFD rats. Therefore, quercetin may have promising potential in ameliorating atherosclerotic pathophysiology in the rat carotid artery by inhibiting oxidative stress and inflammatory responses mechanistically by modulating the AMPK/SIRT1/NF-κB signaling pathway.

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Introduction

Diabetes mellitus is a well-known, major independent risk factor for micro- and macro-vascular diseases and subsequent complications (1,2). Micro-vascular complications are comprised of retinopathy, nephropathy and neuropathy, while macro-vascular complications involve atherosclerosis-related diseases, including atherosclerotic cardiovascular, cerebrovascular and peripheral vascular diseases (3). Atherosclerosis accelerated by diabetes is a process that has a complex pathophysiology, where dyslipidemia, hormonal abnormalities, oxidative stress, hyperglycemia and a pro-inflammatory state have all been documented to serve critical roles (4,5). These changes modulate the direct consequences of hyperglycemia on diabetic atherosclerosis and alter the pathogenesis of diabetes itself. Oxidative stress and inflammation have reciprocal interactions, such that oxidative stress directly induces the production of pro-inflammatory cytokines and mediators, which in turn promotes the production of reactive oxygen species (ROS) (5). Both inflammation and ROS pathways impair pancreatic β cell activity, insulin secretion and resistance (5). Therefore, prevention of the vascular inflammatory state and oxidative stress may prove to be a potential therapeutic strategy for improving the outcomes of diabetic atherosclerosis.

Dysregulation of 5'adenosine monophosphate-activated protein kinase (AMPK) activation also contributes to the onset and progression of diabetic atherosclerosis (6). AMPK activity has been previously demonstrated to be reduced in response to chronic inflammation in adipocytes of a type II diabetic mellitus murine model and in human adipose tissues (7,8).

AMPK suppresses the expression of NF-κB by increasing the expression of sirtuin 1 (SIRT1), thereby minimizing the inflammatory response (9). SIRT1 is a class III histone deacetylase that serves an important role in modulating the pathogenesis of chronic conditions, including diabetes and cardiovascular disease (10). Additionally, SIRT1 has been reported to increase cellular ability to remove ROS by superoxide dismutase (SOD) activation (9,11).

Quercetin is a natural flavonoid that can be found in abundance in plant-based foods, including red onions, tea, apples, capers, broccoli, parsley and red grapes (12). It has been previously revealed to mediate a multitude of physiological functions with a broad spectrum of pharmacological properties, including anti-inflammatory, anti-diabetic, lipid metabolism modulation and anti-oxidative capacities (13). Furthermore, several studies have demonstrated that quercetin reduces the plasma concentrations of total cholesterol (Chol) and triglycerides (TG), and increases the concentration of high-density lipoprotein (HDL) Chol (14,15). Additionally, previous studies have reported that quercetin exhibits protective effects against atherosclerosis in rodents (16,17) and enhances SIRT1 and AMPK activity (18,19). Therefore, due to these aforementioned protective effects of quercetin against metabolic disorders and its potential to alleviate oxidative-inflammatory responses in cardiovascular disease, the aim of the present study was to evaluate the effects of quercetin on the AMPK/SIRT1/NF-κB signaling pathway and inflammatory/oxidative stress responses in diabetes-induced atherosclerosis in rat carotid arteries.

Materials and methods

Animal maintenance and drugs. A total of 30 male Wistar rats (age, 7-8 weeks; weight, 250±20 g) were maintained and housed in stainless steel, wire-bottomed cages in a room at 12-h light/dark cycles, an ambient temperature of 23±2˚C and at 60% humidity. Water and food were provided to all animals *ad libitum*. All experiments were approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Medical University (Xian, China; approval no. 2019-1213) and performed in accordance with the Guidelines of the National Institutes of Health (publication no. 85-23; 1996) (20). Quercetin and compound-C (CC), an AMPK inhibitor, were purchased from Sigma-Aldrich, Merck KGaA.

Animal grouping. Following feeding with a normal diet and adaptation for 2 weeks, the animals were subsequently randomly allocated into five groups (n=6 rats/group): i) Control; ii) control-quercetin (control-Q); iii) high-fat diabetic (HFD); iv) HFD-quercetin (HFD-Q); and v) HFD-quercetin-CC C (HFD-Q-CC).

The control group was fed a normal diet for 8 weeks. The control-Q group was fed a normal diet for 8 weeks and received quercetin (30 mg/kg) orally each day for 2 weeks prior to tissue sampling. Quercetin was dissolved in 2% DMSO and was administered once daily at a volume of ~10 ml/kg body weight using a 16-gauge feeding tube. HFD was the atherosclerotic model group and was fed the high-fat and Chol diet for 8 weeks. By contrast, rats in the HFD-Q group were fed the high-fat and Chol diet for 8 weeks and received quercetin (30 mg/kg) orally in the final 2 weeks of the diet, similar to the control-Q group. The HFD-Q-CC group was fed the high-fat and Chol diet for 8 weeks and received quercetin (30 mg/kg) orally and CC intravenously (0.2 mg/kg) for 5 days within the period of quercetin gavage (once every 3 days), starting alongside quercetin treatment (21,22). Each rat in the HFD-Q-CC group was injected with CC 5 times in total. The food, including both high-fat and Chol diet, and normal pellets, was adjusted to 120 g. The control rats received equal amounts of saline containing 2% DMSO to minimize the effects of the procedures on the experimental results.

High-fat diet induction of diabetes development. A high-fat diet and a low-dose streptozotocin protocol was used to induce type II diabetes mellitus and the development of atherosclerosis in the rats. After 2 weeks of acclimatization, rats were fed on a high-fat Chol-saturated diet containing standard pellets supplemented with 1% Chol, 8% lard and 0.05% cholate (w/w; 62% calories from fat). Streptozotocin (35 mg/kg) dissolved in citrate buffer at pH 4.5 was then injected intraperitoneally (i.p) at the beginning of week 4. After 72 h, one drop of tail vein blood was obtained through a small scratch with lancet and fasting blood glucose (FBS) levels in the animals were measured using a glucometer (Convergent Technologies; GmbH & Co. KG), where rats with FBS >250 mg/dl were assigned into the HFD diabetic group (23). The total diabetic period was 8 weeks.

Measurement of lipid profile levels and atherogenic index (AI). Following fasting for 12 h, animals were anesthetized with an i.p administration of sodium pentobarbital (40 mg/kg) before blood samples (~3 ml from portal veins following laparotomy prior to carotid artery tissue sampling) were collected and centrifuged at 1,400 x g for 10 min at 4˚C to obtain the plasma. Subsequently, plasma levels of TG (cat. no. MBS164762; MyBioSource, Inc.), Chol (cat. no. MBS775433; MyBioSource, Inc.) and HDL (cat. no. 79970; Crystal Chem, Inc.) and low-density lipoprotein (LDL; cat. no. 79960; Crystal Chem, Inc.) were measured using specific assay kits, according to the manufacturer's protocols. The AI for each rat was calculated using the following formula: AI=Chol-HDL/HDL.

Oxidative stress marker measurements in carotid arteries. Following blood sampling, the animals underwent a surgical procedure to obtain the carotid artery samples under anesthesia. Followings tissue sampling, animals were euthanized by an overdose of sodium pentobarbital (200 mg/kg; i.p). Levels of oxidative stress in the carotid artery samples were evaluated by measuring malondialdehyde (MDA) content and the activities of glutathione peroxidase (GPX), SOD and catalase (CAT). Briefly, 100 mg carotid artery tissues were cut into small sections (~2 mm^2) and mixed with a 10X volume of pre-cooled saline before the mixture was homogenized at 4˚C. The homogenates were centrifuged at 1,400 x g for 10 min at 4˚C. The supernatants were assessed using specific assay kits or reagents (Randox Laboratories, Ltd.; GPX, cat. no. RS504; SOD, cat. no. SD125). MDA and CAT were measured according to methods designed by Aebi *et al* (24) and Ohkawa *et al* (25), respectively. The protein MDA levels were expressed as nmol/mg protein, whereas the GPX, SOD and CAT activities were expressed as U/mg protein.

Table I. Biochemical parameters of the experimental groups.

Groups	FBS (mg/dl)	Chol (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	HDL/LDL ratio	HDL/Chol ratio
Control	98±5	49.3±4.0	96.6±5.5	27.3±2.6	14.8±2.7	0.50±0.03	0.32±0.03
Control-Q	95±7	40.4±3.6	81.3±5.3	26.4±2.2	19.8±1.3	0.73±0.08[a]	0.47±0.06[a]
HFD	415±26[b]	395.3±26.5[b]	236.5±21.0[b]	261.0±16.3[b]	46.7±5.5[b]	0.18±0.04[b]	0.12±0.02[b]
HFD-Q	236±15[d]	215.7±13.4[d]	114.3±11.9[d]	202.5±14.7[c]	54.5±5.9	0.29±0.06[c]	0.26±0.04[c]
HFD-Q-CC	316±19[e]	373.8±29.1[f]	226.7±30.3[f]	256.3±20.6[e]	40.2±5.0	0.14±0.05[e]	0.10±0.04[e]

[a]P<0.05 and [b]P<0.01 vs. Control group. [c]P<0.05 and [d]P<0.01 vs. HFD group. [e]P<0.05 and [f]P<0.01 vs. HFD-Q group. FBS, fasting blood sugar; Chol, cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Q, quercetin, CC, compound-C.

Measurement of pro-inflammatory and anti-inflammatory mediators. NF-κB (cat. no. MBS265868), IL-1β (cat. no. MBS702717) and IL-10 (cat. no. MBS2700945) levels in the carotid arteries were measured using specific ELISA kits, according to the manufacturer's protocol (MyBioSource, Inc.). A coating plate was then placed in the ELISA reader to read the absorbance of each sample at 450 nm. All optical density values were converted to the final concentration based on the amount of protein (mg) of each sample and presented as pg/mg total protein.

Histological examination of atherosclerotic carotid arteries. Initially, the adipose tissues were removed from the carotid arteries of the rats prior to being fixed in 10% formalin for 24 h at room temperature. Following tissue dehydration with an ascending series of ethanol and washing with xylene, the paraffin wax-embedded carotid samples were cut (thickness, 4-μm) transversally and stained with hematoxylin for 20 min and eosin for 8 min at room temperature. Finally, an optical light microscope (magnification, x40) was used to examine atherosclerotic changes in the artery.

Western blot analysis. The artery tissue samples were homogenized in RIPA lysis buffer (cat. no. 9806S; Cell Signaling Technology, Inc.) containing protease inhibitors (cat. no. 5871S; Cell Signaling Technology, Inc.). Following centrifugation at 9,800 x g for 10 min at 4°C, equal amounts of protein (20 μg) from carotid tissue supernatants were separated on 12.5% SDS-PAGE and transferred to PVDF membranes (EMD Millipore). Following blocking with 5% skimmed milk for 1 h at room temperature, the membranes were incubated with primary antibodies against SIRT1 (cat. no. 8649S; 1:1,500; Cell Signaling Technology, Inc.) and β-actin (cat. no. 3700S; 1:5,000; Cell Signaling Technology, Inc.) overnight at 4°C. After four 5 min washes with PBS/0.1% Tween-20, membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse secondary antibodies (cat. no. 7076S; 1:2,500; Cell Signaling Technology, Inc.) for 2 h at room temperature. Following rinsing, the protein bands were visualized using the enhanced chemiluminescence reagent (Santa Cruz Biotechnology, Inc.). Protein band intensity was measured using the ImageJ software (version no. 1.6; National Institutes of Health) and normalized to β-actin.

Statistical analysis. Data are presented as mean ± SEM. Six rats were included in each group. One rat was removed from the results for western blot analysis and histological examination due to inadequate tissue sampling. Data for the remaining 5 rats were analyzed for these experiments. Comparisons between groups were performed with one-way ANOVA with Tukey's post-hoc test using GraphPad Prism software (version no. 5.0; GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Plasma lipid levels of rats in the experimental groups. The levels of Chol, TG, HDL, LDL and FBS in the control-Q group did not significantly differ compared with the control group (Table I). However, the HDL/LDL and HDL/Chol ratios were demonstrated to be significantly increased in the non-diabetic group receiving quercetin compared with the control group (P<0.05). Lipid profiles and FBS in the HFD group were revealed to be significantly increased compared with rats in the control group (P<0.01). However, treatment of diabetic rats with quercetin significantly reduced FBS, Chol, TG and LDL levels (P<0.01), while increasing the HDL/LDL and HDL/Chol ratios (P<0.05) compared with those in the HFD group. However, the lipid profile changes as a result of quercetin treatment were reversed by CC administration compared with the HFD-Q group (P<0.05 for LDL, HDL/LDL and HDL/Chol; P<0.01 for Chol and TG; Table I).

Carotid artery levels of NF-κB, IL-1β and IL-10. Rats fed on HFD exhibited significantly higher NF-κB and IL-1β levels (P<0.01) and significantly lower IL-10 levels (P<0.05) compared with the control animals (Fig. 1). However, quercetin treatment significantly suppressed the tissue levels of NF-κB and IL-1β (P<0.01) and increased IL-10 levels (P<0.05) compared with rats in the HFD group (Fig. 1). Inhibition of AMPK by CC significantly reversed the protective effects of quercetin on all three of these parameters (P<0.05; Fig. 1). Additionally, the levels of IL-1β in the HFD-Q-CC group was significantly higher than those in the HFD group (P<0.05; Fig. 1B).

Measurement of oxidative stress markers. The levels of MDA were reported to be significantly higher in the HFD group compared with the control group (P<0.01), which was significantly reversed by quercetin treatment (P<0.01; Fig. 2). Additionally, following treatment with the AMPK inhibitor

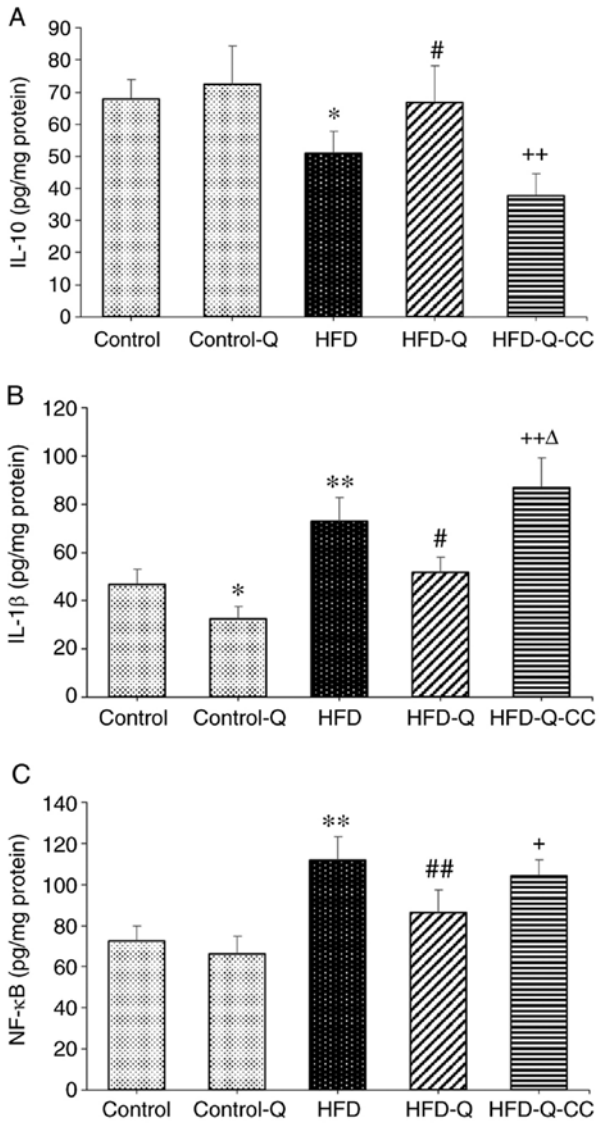

Figure 1. Comparison of the levels of inflammatory factors in the carotid arteries of five animal groups. Expression levels of (A) IL-10, (B) IL-1β and (C) NF-κB (pg/mg) in the different experimental groups. Data are presented as mean ± SEM (n=6 rats/group). *P<0.05 and **P<0.01 vs. controls. #P<0.05 and ##P<0.01 vs. HFD. +P<0.05 and ++P<0.01 vs. HFD-Q. ΔP<0.05 vs. HFD. HFD, high-fat diabetic; Q, quercetin; CC, compound C; IL, interleukin.

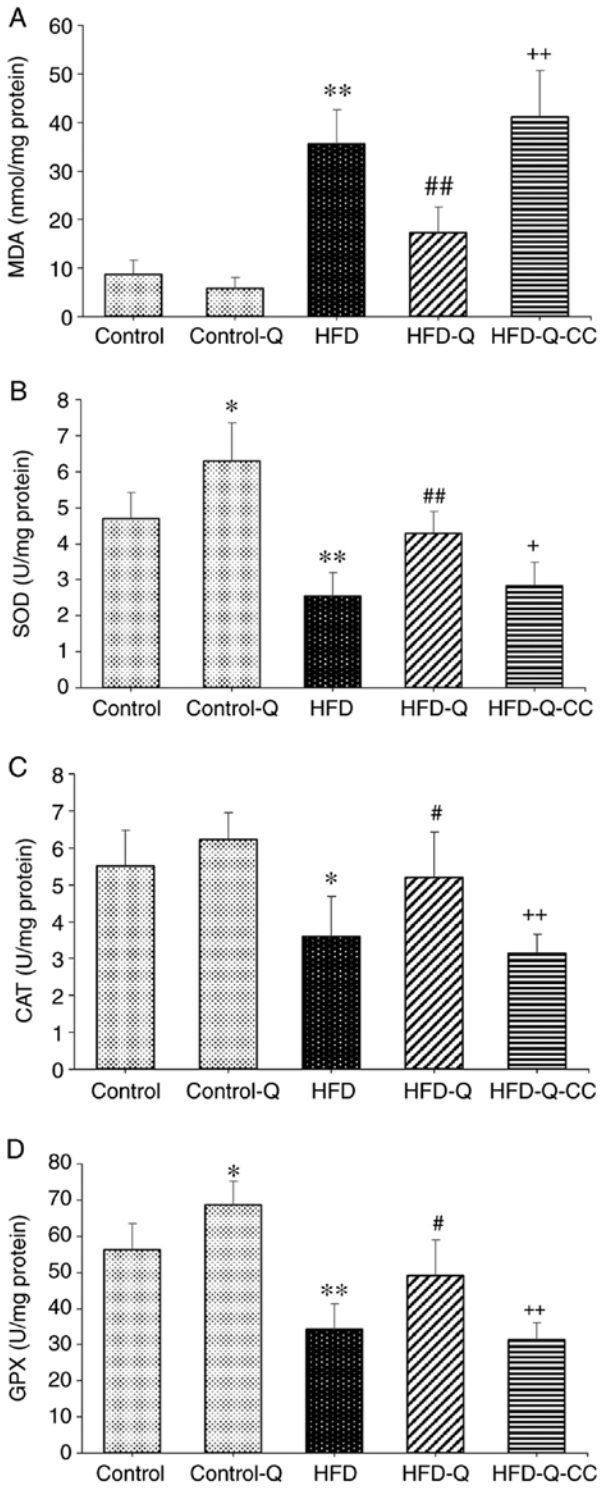

Figure 2. Comparison of oxidative stress parameters in the five animal groups. (A) MDA content and activities of (B) SOD, (C) CAT and (D) GPX in the five different experimental groups. Data are presented as mean ± SEM (n=6 rats/group). *P<0.05 and **P<0.01 vs. controls. #P<0.05 and ##P<0.01 vs. HFD. +P<0.05 and ++P<0.01 vs. HFD-Q. HFD, high fat diabetic; Q, quercetin; CC, compound C; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase.

CC in the HFD-Q group, the MDA level was demonstrated to be significantly increased (P<0.01; Fig. 2). Furthermore, HFD was revealed to significantly attenuate the activities of GPX (P<0.01), SOD (P<0.01) and CAT (P<0.05) compared with the control group (Fig. 2), all of which were significantly reversed by quercetin treatment (P<0.05). Administration of the AMPK inhibitor CC to the HFD-Q group significantly abrogated the effects of quercetin (P<0.05 for SOD; P<0.01 for GPX and CAT; Fig. 2).

Expression of SIRT1 protein. Quercetin significantly increased the protein expression of SIRT1 compared with the control group (P<0.05; Fig. 3). The expression level of SIRT1 was significantly decreased in the HFD group compared with the control group (P<0.01). Additionally, quercetin administration in rats fed on HFD resulted in a significant increase in SIRT1 protein expression (P<0.01). However, inhibition of AMPK by CC abolished the effects of quercetin on SIRT1 expression in HFD rats (P<0.01; Fig. 3).

Histological analysis and AI. Rats in the HFD group exhibited superficial erosion in the carotid artery accompanied by fat

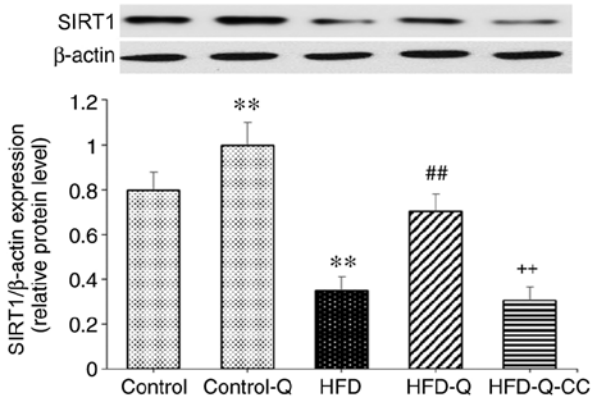

Figure 3. SIRT1 protein expression in the five different experimental groups. Data are presented as mean ± SEM (n=5 rats/group). **P<0.01 vs. controls. ##P<0.01 vs. HFD. ++P<0.01 vs. HFD-Q. SIRT1, sirtuin 1; HFD, high fat diabetic; Q, quercetin; CC, compound-C.

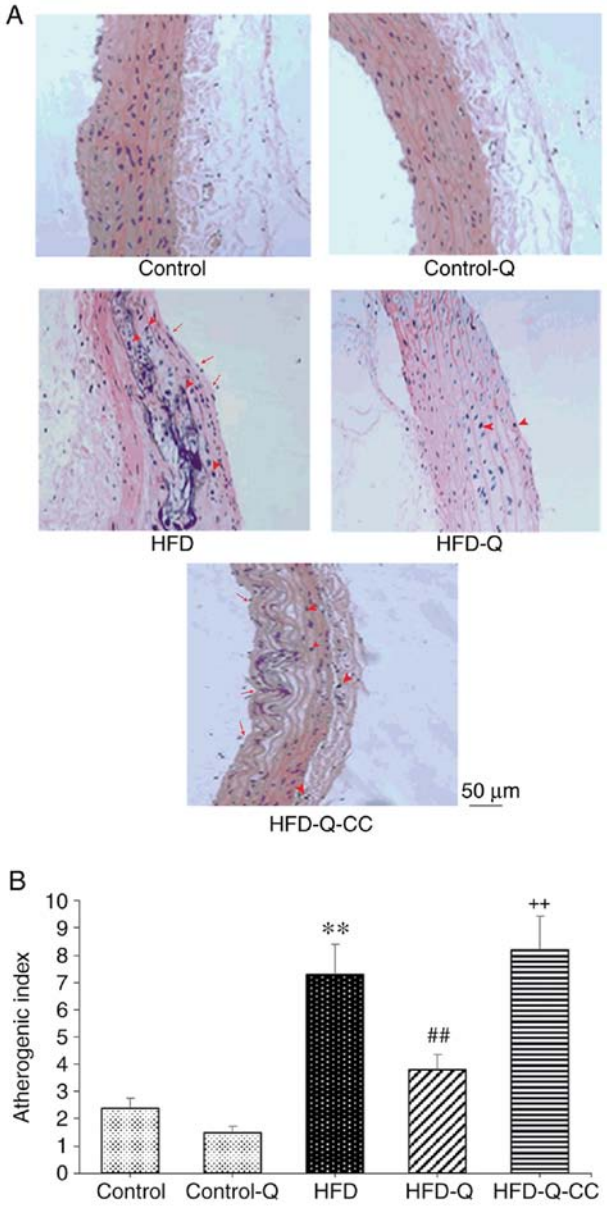

Figure 4. Analysis of atherosclerosis severity in the five different experimental groups. (A) Representative histological images of the carotid arteries displaying the atherosclerotic changes. Red arrows indicate foamy cells and fat deposition. Red arrowheads indicate macrophage accumulation. (B) Atherogenic indices in the five experimental groups. Data are presented mean ± SEM (n=5 rats/group). Scale bar length for all images is 50 μm. **P<0.01 vs. controls. ##P<0.01 vs. HFD. ++P<0.01 vs. HFD-Q. HFD, high fat diabetic; Q, quercetin; CC, compound-C.

deposition and foamy macrophage accumulation, as indicated by the arrows in Fig. 4A, and a significantly increased AI was observed compared with the control rats (P<0.01; Fig. 4). Administration of quercetin to the rats in the HFD group suppressed the formation of atheromatous plaques and foam cells, in addition to significantly reducing the AI compared with rats in the HFD group (P<0.01). However, CC treatment markedly increased the atherosclerotic changes, whilst significantly increasing the AI to suppress the protective effects of quercetin (P<0.01; Fig. 4).

Discussion

The present study demonstrated that quercetin treatment ameliorated the levels of hyperlipidemia, inflammatory cytokines and oxidative stress in the carotid arteries of diabetic rats fed on HFD. These protective effects of quercetin were mediated by the modulation of the AMPK/SIRT1/NF-κB signaling pathway.

Oxidative stress resulting from an imbalance in the redox equilibrium may induce vascular dysfunction and contribute to atherosclerotic plaque formation (5). The importance of oxidative stress in the progression of vascular complications have been previously documented in diabetes, particularly in type II diabetes (26,27). Previous reports have demonstrated that an elevation in the production of SOD, CAT and GPX antioxidants lead to a reduction in ROS levels in diabetes (5,27,28). Any reverse alterations in the levels of these enzymes trigger oxidative stress in tissues and, consequently, results in diabetic complications (28). In the present study, in addition to the elevation in MDA levels, the activity levels of SOD, CAT and GPX were demonstrated to be decreased in rats in the HFD diabetic group compared with the control group. Changes in these oxidative stress indices were revealed to be associated with increased LDL, Chol, TG, AI and increased formation of atherosclerotic plaques. Additionally, ROS has been reported to induce inflammation by increasing the levels of the pro-inflammatory cytokines IL-1β, IL-6, TNF-α and NF-κB and by upregulating the expression of adhesion molecules (29,30). Inflammatory reactions contribute to insulin resistance, hyperglycemia and, consequently, the progression of diabetic complications, followed by imbalances in redox signaling in tissues and further aggravation of diabetic atherosclerosis (31). Consistent with this notion, HFD-induced diabetes in the present study resulted in the upregulation of NF-κB and IL-1β and downregulation of IL-10.

Quercetin is a plant polyphenol with a variety of previously reported pharmacological actions (32). Administration of quercetin to HFD-receiving rats was demonstrated to markedly protect the carotid artery against diabetic atherosclerosis in the present study. The anti-atherosclerotic effects of quercetin have been associated with its suppressive effects on oxidative stress and inflammatory responses (12,17).

Quercetin reversed HFD-induced alterations in all inflammatory and oxidative stress parameters in atherosclerotic rats and significantly increased the HDL/LDL ratios, SOD and GPX levels, and reduced IL-1β levels in the carotid arteries of rats in the control group receiving the normal diet. These findings indicated that quercetin mediated significant anti-oxidative and anti-inflammatory effects under both normal and atherosclerotic conditions. Yao *et al* (33) previously deduced that quercetin intake was inversely correlated with the prevalence of diabetes in patients with type II diabetes. Additionally, quercetin was observed to control disease outcomes in a high-fructose diet model of diabetes by enhancing the PI3K/AKT signaling pathway, whilst inhibiting ROS (34). In particular, a previous *in vitro* and *in vivo* study in rats documented the antioxidant properties of quercetin, which were reported to be triggered by elevating mitochondrial activity along with the suppression of adipogenic factors (35).

AMPK serves as a major intracellular energy sensor, where dysregulations in its activity has been reported to contribute to the pathogenesis and progression of diabetic atherosclerosis (6). AMPK inhibited inflammatory responses by regulating numerous downstream signaling pathways (5,8,9). In addition, AMPK has been found to suppress TNF-α-dependent adhesion of monocytes to endothelial cells in the human aorta (36). Furthermore, it has been demonstrated that activation of AMPK reduces endoplasmic reticulum stress induced by oxidized LDL, thereby improving endothelial dysfunction and atherosclerosis in an *in vivo* model (37). This is consistent with the present study, which reported that the beneficial effects of quercetin on carotid atherosclerosis were abrogated following the inhibition of AMPK activity. This indicated that the anti-oxidative and anti-inflammatory effects of quercetin in the carotid artery of HFD rats are, at least in part, AMPK-dependent. A previous study reported that quercetin increased the phosphorylation of AMPK in cultured smooth muscle cells and aortic arteries (38), which also exhibited increased levels of acetyl CoA carboxylase, a downstream protein of AMPK, implicating the increased activity of AMPK following quercetin administration (38). By contrast, it was observed that the HFD regimen led to the downregulation of SIRT1 protein expression, which was reversed by the administration of quercetin. Subsequently, inhibition of AMPK using CC significantly reversed the effects of quercetin on SIRT1 expression in HFD rats. Therefore, this observation indicated that quercetin upregulated AMPK activity to increase SIRT1 protein expression. SIRT1 serves an important intermediary role in the anti-atherosclerotic effects of quercetin, the expression of which is high in human endothelial and vascular cells to regulate endothelial and vascular function (39). Upregulation of SIRT1 expression or increased SIRT1 activity has been previously revealed to inhibit endothelial dysfunction induced by oxidative stress (40). Additionally, AMPK has been demonstrated to upregulate the expression of SIRT1 and repress the expression of NF-κB, IL-1β and TNF-α (5,9). Furthermore, AMPK elevated the expression levels of IL-10 (40). Therefore, according to the results of the present study, modulation of the AMPK/SIRT1/NF-κB signaling pathway by quercetin was most likely responsible for its anti-hyperlipidemic, anti-inflammatory and anti-oxidative properties against atherosclerosis. However, the effects of quercetin on other candidates for the onset and progression of atherosclerosis, including nitric oxide, adhesion molecules, protein kinase C, intracellular Ca^{2+} signaling, autophagy and mitochondrial dysfunction, warrant further investigation (5). These investigations are required to further the understanding of the pathophysiology of HFD and diabetes-accelerated atherosclerosis, and the development of novel preventive and therapeutic measures.

As a limitation, quercetin was used following the confirmation of diabetes. Due to a previous report on the beneficial effects of quercetin against metabolic dysfunction and inflammatory disease (41), it was hypothesized that quercetin may have the potential to prevent or alleviate complications associated with diabetes in patients with obesity. However, further studies are required to clarify this important issue. Additionally, one of the most important end effectors of AMPK/SIRT1 activation in reducing oxidative stress, tissue inflammation and apoptosis is by promoting mitochondrial biogenesis and by altering mitochondrial physiology (42). This aspect was not addressed in the present study and requires further investigation.

In conclusion, the results of the present study demonstrated that quercetin exerted beneficial effects against atherosclerosis in the carotid artery of HFD rats. This was mediated by reducing hyperlipidemia and inflammatory and oxidative stress. These promising effects of quercetin were demonstrated to be associated with the SIRT1/NF-κB signaling pathway, where AMPK is a critical regulator.

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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

FZ, JF and DQ designed and performed the experiments. JZ and DQ aided with hypothesis conceptualization, analyzed data and wrote the first draft of the manuscript. FZ, JZ and XK assisted with experimental design and collaborated to interpret the results. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Medical University (Xian, China; approval no. 2019-1213).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zhu Y, Ye P, Chen SL and Zhang DM: Functional regulation of large conductance Ca2+-activated K+ channels in vascular diseases. Metabolism 83: 75-80,2018.
2. Chawla A, Chawla R and Jaggi S: Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum?. Indian J Endocrinol Metab 20: 546-551, 2016.
3. Kitada M, Zhang Z, Mima A and King GL: Molecular mechanisms of diabetic vascular complications. J Diabetes Investig 1: 77-89, 2010.
4. Saeid F, Aniseh J, Reza B and Manouchehr VS: Signaling mediators modulated by cardioprotective interventions in healthy and diabetic myocardium with ischaemia-reperfusion injury. Eur J Prev Cardiol 25: 1463-1481, 2018.
5. Yuan T, Yang T, Chen H, Fu D, Hu Y, Wang J, Yuan Q, Yu H, Xu W and Xie X: New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. Redox Biol 20: 247-260, 2019.
6. Almabrouk TA, Ewart MA, Salt IP and Kennedy S: Perivascular fat, AMP-activated protein kinase and vascular diseases. Br J Pharmacol 171: 595-617, 2014.
7. Mottillo EP, Desjardins EM, Crane JD, Smith BK, Green AE, Ducommun S, Henriksen TI, Rebalka IA, Razi A, Sakamoto K, *et al*: Lack of adipocyte AMPK exacerbates insulin resistance and hepatic steatosis through brown and beige adipose tissue function. Cell Metab 24: 118-129, 2016.
8. Gauthier MS, O'Brien EL, Bigornia S, Mott M, Cacicedo JM, Xu XJ, Gokce N, Apovian C and Ruderman N: Decreased AMP-activated protein kinase activity is associated with increased inflammation in visceral adipose tissue and with whole-body insulin resistance in morbidly obese humans. Biochem Biophys Res Commun 404: 382-387, 2011.
9. Chen B, Li J and Zhu H: AMP-activated protein kinase attenuates oxLDL uptake in macrophages through PP2A/NF-κB/LOX-1 pathway. Vascul Pharmacol 85: 1-10, 2016.
10. Matsuzaki T, Matsushita T, Takayama K, Matsumoto T, Nishida K, Kuroda R and Kurosaka M: Disruption of Sirt1 in chondrocytes causes accelerated progression of osteoarthritis under mechanical stress and during ageing in mice. Ann Rheum Dis 73: 1397-1404, 2014.
11. Kume S, Uzu T, Kashiwagi A and Koya D: SIRT1, a calorie restriction mimetic, in a new therapeutic approach for type 2 diabetes mellitus and diabetic vascular complications. Endocr Metab Immune Disord Drug Targets 10: 16-24, 2010.
12. Xiao L, Liu L, Guo X, Zhang S, Wang J, Zhou F, Liu L, Tang Y and Yao P: Quercetin attenuates high fat diet-induced atherosclerosis in apolipoprotein E knockout mice: A critical role of NADPH oxidase. Food Chem Toxicol 105: 22-33, 2017.
13. Kobori M, Takahashi Y, Sakurai M, Akimoto Y, Tsushida T, Oike H and Ippoushi K: Quercetin suppresses immune cell accumulation and improves mitochondrial gene expression in adipose tissue of diet-induced obese mice. Mol Nutr Food Res 60: 300-312, 2016.
14. Egert S, Bosy-Westphal A, Seiberl J, Kürbitz C, Settler U, Plachta-Danielzik S, Wagner AE, Frank J, Schrezenmeir J, Rimbach G, *et al*: Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: A double-blinded, placebo-controlled cross-over study. Br J Nutr 102: 1065-1074, 2009.
15. Pfeuffer M, Auinger A, Bley U, Kraus-Stojanowic I, Laue C, Winkler P, Rüfer C, Frank J, Bösch-Saadatmandi C, Rimbach G and Schrezenmeir J: Effect of quercetin on traits of the metabolic syndrome, endothelial function and inflammation in men with different APOE isoforms. Nutr Metab Cardiovasc Dis 23: 403-409, 2013.
16. Cao H, Jia Q, Shen D, Yan L, Chen C and Xing S: Quercetin has a protective effect on atherosclerosis via enhancement of autophagy in ApoE-/-mice. Exp Ther Med 18: 2451-2458, 2019.
17. Omar HM, Almaeen AH, Elghaffar SKA, Ragab SM, El-Metwally TH and Ahmed EA: Atherosclerotic rat model after a high-fat, high-sucrose diet: Protective role of quercetin, O-coumaric, and berberine. Anal Quant Cytol Histol 40: 76-84, 2018.
18. Feng K, Chen Z, Pengcheng L, Zhang S and Wang X: Quercetin attenuates oxidative stress-induced apoptosis via SIRT1/AMPK-mediated inhibition of ER stress in rat chondrocytes and prevents the progression of osteoarthritis in a rat model. J Cell Physiol 234: 18192-18205, 2019.
19. Peng J, Li Q, Li K, Zhu L, Lin X, Lin X, Shen Q, Li G and Xie X: Quercetin improves glucose and lipid metabolism of diabetic rats: Involvement of Akt signaling and SIRT1. J Diabetes Res 2017: 3417306, 2017.
20. National Research Council (US) Institute for Laboratory Animal Research: Guide for the Care and Use of Laboratory Animals. National Academies Press (US), Washington, DC, 1996.
21. Kim YM, Kim MY, Kim HJ, Roh GS, Ko GH, Seo HG, Lee JH and Chang KC: Compound C independent of AMPK inhibits ICAM-1 and VCAM-1 expression in inflammatory stimulants-activated endothelial cells in vitro and in vivo. Atherosclerosis 219: 57-64, 2011.
22. Hasanvand A, Amini-Khoei H, Hadian MR, Abdollahi A, Tavangar SM, Dehpour AR, Semiei E and Mehr SE: Anti-inflammatory effect of AMPK signaling pathway in rat model of diabetic neuropathy. Inflammopharmacology 24: 207-219, 2016.
23. Bayrami G, Karimi P, Agha-Hosseini F, Feyzizadeh S and Badalzadeh R: Effect of ischemic postconditioning on myocardial function and infarct size following reperfusion injury in diabetic rats pretreated with vildagliptin. J Cardiovasc Pharmacol Ther 23: 174-183, 2018.
24. Aebi H: Catalase in vitro. Methods Enzymol 105: 121-126,1984.
25. Ohkawa H, Ohishi N and Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351-358,1979.
26. Pham-Huy LA, He H and Pham-Huy C: Free radicals, antioxidants in disease and health. Int J Biomed Sci 4: 89-96, 2008.
27. Bigagli E and Lodovici M: Circulating oxidative stress biomarkers in clinical studies on type 2 diabetes and its complications. Oxid Med Cell Longev 2019: 5953685, 2019.
28. Asmat U, Abad K and Ismail K: Diabetes mellitus and oxidative stress-A concise review. Saudi Pharm J 24: 547-553, 2016.
29. Oguntibeju O: Type 2 diabetes mellitus, oxidative stress and inflammation: Examining the links. Int J Physiol Pathophysiol Pharmacol 11: 45-63, 2019.
30. Najafi M, Noroozi E, Javadi A and Badalzadeh R: Anti-arrhythmogenic and anti-inflammatory effects of troxerutin in ischemia/reperfusion injury of diabetic myocardium. Biomed Pharmacother 102: 385-391, 2018.
31. Becatti M, Mannucci A, Taddei N and Fiorillo C: Oxidative stress and inflammation: New molecular targets for cardiovascular diseases. Intern Emerg Med 13: 647-649, 2018.
32. Bule M, Abdurahman A, Nikfar S, Abdollahi M and Amini M: Antidiabetic effect of quercetin: A systematic review and meta-analysis of animal studies. Food Chem Toxicol 125: 494-502, 2019.
33. Yao Z, Gu Y, Zhang Q, Liu L, Meng G, Wu H, Xia Y, Bao X, Shi H, Sun S, *et al*: Estimated daily quercetin intake and association with the prevalence of type 2 diabetes mellitus in Chinese adults. Eur J Nutr 58: 819-830, 2019.
34. Xu D, Hu MJ, Wang YQ and Cui YL: Antioxidant activities of quercetin and its complexes for medicinal application. Molecules 24: 1123, 2019.
35. Funakoshi T, Kanzaki N, Otsuka Y, Izumo T, Shibata H and Machida S: Quercetin inhibits adipogenesis of muscle progenitor cells in vitro. Biochem Biophys Rep 13: 39-44, 2017.
36. Ewart MA, Kohlhaas CF and Salt IP: Inhibition of tumor necrosis factor alpha-stimulated monocyte adhesion to human aortic endothelial cells by AMP-activated protein kinase. Arterioscler Thromb Vasc Biol 28: 2255-2257, 2008.
37. Dong Y, Zhang M, Wang S, Liang B, Zhao Z, Liu C, Wu M, Choi HC, Lyons TJ and Zou MH: Activation of AMP-activated protein kinase inhibits oxidized LDL-triggered endoplasmic reticulum stress in vivo. Diabetes 59: 1386-1396, 2010.
38. Kim SG, Kim JR and Choi HC: Quercetin-induced AMP-activated protein kinase activation attenuates vasoconstriction through LKB1-AMPK signaling pathway. J Med Food 21: 146-153, 2018.

39. Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB, DeRicco J, Kasuno K and Irani K: SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci USA 104: 14855-14860, 2007.
40. Guo H, Chen Y, Liao L and Wu W: Resveratrol protects HUVECs from oxidized-LDL induced oxidative damage by autophagy upregulation via the AMPK/SIRT1 pathway. Cardiovasc Drugs Ther 27: 189-198, 2013.
41. Chen S, Jiang H, Wu X and Fang J: Therapeutic effects of quercetin on inflammation, obesity, and type 2 diabetes. Mediators Inflamm 2016: 9340637, 2016.
42. Herzig S and Shaw RJ: AMPK: Guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol 19: 121-135, 2018.