European Review for Medical and Pharmacological Sciences 2021; 25: 6660-6669

# Decreased serum SIRT6 as a novel predictor of coronary artery disease

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**Abstract. – OBJECTIVE: SIRT6 is an NAD-dependent histone deacetylase known to regulate aging, inflammation and energy metabolism, and might play an important role in atherosclerosis. However, whether it also plays a role in coronary artery disease (CAD) remains unclear.**

**PATIENTS AND METHODS: In this study, we detected the expression of SIRT6 in serum by Western blotting. The concentrations of SIRT6 in serum specimens from 69 patients with CAD [30 with stable angina (SA) and 39 with acute coronary syndrome (ACS)] and 16 controls were analysed using the enzyme-linked immunosorbent assay (ELISA) method.**

**RESULTS: Western blotting analysis of the serum samples found that SIRT6 expression was decreased in the SA group ($p$=0.000) and ACS group ($p$=0.000) compared with the control group. Significantly lower levels of serum SIRT6 were observed in SA patients (18.80±9.14 ng/mL) and ACS patients (16.85±9.66 ng/mL) than in healthy controls (25.79±14.23 ng/mL). SIRT6 concentrations were positively correlated with other markers of CAD, such as high-density lipoprotein cholesterol (r=0.362, $p$<0.01) and age (r=0.265, $p$<0.05), and negatively correlated with blood glucose (r=-0.284, $p$<0.05). Multivariate logistic regression analysis demonstrated that lower SIRT6 levels were independently associated with the presence of CAD in men (OR=0.817, 95% CI 0.694-0.962, $p$=0.015). Receiver operating characteristic (ROC) curve analysis showed that lower serum SIRT6 could distinguish CAD patients (AUC, 0.726; 95% CI, 0.508-0.943; $p$=0.041) from controls. SIRT6 is found downregulated in blood vessels of atherosclerotic APOE-/- mice and human aorta arteries.**

**CONCLUSIONS: We demonstrated that SA and ACS patients had lower serum concentrations of SIRT6. The decreased serum SIRT6 level was independently associated with the diagnosis of CAD. SIRT6 may play a cardioprotective role in CAD patients, and future research is required to address this issue.**

*Key Words:*
SIRT6, High-density lipoprotein cholesterol, Acute coronary syndrome, Stable angina, Coronary artery disease.

## Introduction

Coronary artery disease (CAD), in which atherosclerotic lesions form in arteries and obstruct blood flow, is the most common type of cardiovascular disease. Left untreated, impaired blood flow can lead to cardiac ischaemia and the development of acute coronary syndrome (ACS), which includes life-threatening conditions such as unstable angina and acute myocardial infarction. Myocardial infarction remains the leading cause of death and disability worldwide.

SIRT6, a NAD-dependent histone deacetylase and ADP-ribosyltransferase from the sirtuin protein family. It plays many important roles in ageing, cancer, obesity, insulin resistance, inflammation, and energy metabolism[1]. Several lines of evidence indicate that SIRT6 plays an important regulatory role in cardiovascular diseases. In myocardium, SIRT6 deficiency can promote insulin-like growth factor signal transduction, leading to cardiac hypertrophy and heart failure, and SIRT6 overexpression can prevent myocardial hypertrophy, heart failure, myocardial and hypoxia damage[2-4]. Hepatocyte specific SIRT6 knockout significantly increased PCSK9 gene expression and plasma LDL cholesterol, a high risk factor for atherosclerosis, while SIRT6 overexpression could improve lipid metabolism by reducing plasma LDL cholesterol and PCSK9 levels[5]. SIRT6 also acts as a co inhibitor of c-myc transcription activity[6]. It is noteworthy that c-MYC is related to scavenger receptor expres-



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sion and foam cell formation in human coronary smooth muscle cells[7]. SIRT6 deletion in bone marrow-derived cells promotes atherosclerosis, and the absence of SIRT6 protein expression enhances atherosclerosis through increased inflammation[8,9]. Genetic variants of SIRT6 are known to be associated with the severity of CAD in humans, and increasing SIRT6 expression in mouse atherosclerosis models inhibited heart failure and reduced atherosclerotic lesion formation[2,9-11]. SIRT6 expression was reduced in atherosclerotic ApoE knockout mice and patients with diabetes[8,12,13]. SIRT6 is also downregulated in endothelial cells exposed to atherogenic factors such as chronic hydrogen peroxide stimulation, high glucose levels, and lipopolysaccharide[14-16]. In general, the protective effect of SIRT6 in cardiovascular diseases such as atherosclerosis and myocardial hypertrophy is relatively clear. These studies[14-16] have described the function of SIRT6 in cells or tissues, but the clinical value of SIRT6 in serum is not clear, and whether SIRT6 can be used as a biomarker for CAD is not clear.

SIRT6 was originally described as a nuclear protein, but it has since been shown to localize to cytoplasmic stress granules in response to stress, where it may associate with different target proteins depending on extracellular stimuli[17-20]. In this study, we report that SIRT6 is also expressed in human blood serum and that SIRT6 serum levels are significantly lower in CAD patients than in controls with normal coronary artery function. We further demonstrated a positive correlation between SIRT6 serum levels and other markers of cardiovascular risk, such as high-density lipoprotein cholesterol (HDL-C). These findings provide further evidence that SIRT6 plays an important role in cardiovascular disease in patients and identify the decreased serum SIRT6 as a useful biomarker for the diagnostic value of CAD.

## Patients and Methods

### Ethics and Informed Consent

This retrospective study was approved by the Ethics Committee of the Second Hospital of Shandong University (approval number KYLL-2018(LW)020), and all participants provided written informed consent before enrolment.

### Patients

69 CAD patients (age range: 36-85 years) diagnosed with SA (stable angina) or acute coronary syndrome (ACS) were recruited from December 2017 to June 2018 in the Cardiovascular Department of the Second Hospital of Shandong University. SA was defined as no change in the frequency, duration, or intensity of angina symptoms in the previous four weeks, combined with normal levels of cardiac enzymes[21]. ACS was diagnosed based on typical clinical symptoms, electrocardiograph changes and positive (acute myocardial infarction) or negative (unstable angina) troponin I[22,23]. CAD was diagnosed by the presence of coronary lesions (≥50% obstruction of the vessel) in at least one major artery segment, as assessed by three independent cardiologists. Patients were excluded if they had tumours, autoimmune disease, serious liver or renal dysfunction, myocardiopathy or valvular heart disease. Patients were assessed at admission for CAD type and severity using the SYNTAX score, an angiographic tool grading the complexity of coronary artery disease (http://www.syntaxscore.com/)[24]. The control group consisted of 16 individuals with normal coronary artery function confirmed by angiography. Controls were age-matched to patients.

### Serum Sampling and Analysis

Serial blood samples were taken after admission and analysed for routine laboratory parameters, including liver function, kidney function, lipids, lipoproteins and myocardial enzymes. Levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), HDL-C, low-density lipoprotein cholesterol (LDL-C), blood glucose (BG), creatinine (CREA) and hypersensitive troponin I (hs-TnI) were measured using a biochemical analyser (Cobas c702, Roche, Basel, Switzerland). Human atherosclerotic aortic tissues were obtained from patients with coronary artery disease during coronary artery bypass grafting and normal aortic tissues were obtained from patients during heart valve replacement.

### Animals

All animal protocols were reviewed and approved by the Animal Care and Use Committee of Shandong University. C57BL/6 and ApoE-/- mice 5 weeks old were purchased from the Viewsolid biotech and maintained on a 12-hr day/night cycle. After adaptive feeding for 1 week, the ApoE-/- mice were fed with high fat diet, and the C57BL/6 mice were fed with normal diet for 3 mouth.

### Reagents and Antibodies

Antibodies against SIRT6 (Catalogue No. 12486) and β- tubulin were purchased from Cell Signaling Technology (Danvers, MA, USA).

Antibody against Transferrin were purchased from Proteintech Biotechnology (Catalogue No. 66171-1-Ig, Wuhan, China). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit and anti-mouse IgG antibodies were purchased from Beyotime Biotechnology (Shanghai, China), and a diaminobenzidine (DAB) chromogenic reagent kit was purchased from Millipore Technology (Danvers, MA, USA).

### Western Blotting

Highly abundant interfering proteins were first removed from serum samples from the control, SA and ACS groups using Multiple Affinity Removal Spin Cartridge Human 7 (Agilent Technologies, Santa Clara, CA, USA). Mice aorta tissues were lysed in RIPA buffer [150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% deoxycholic sodium salt, 50 mM Tris HCl (pH 7.4), 2 mM EDTA, 2 mM Na3VO4, 10 mM NaF, and Roche Complete inhibitor cocktail]. The protein concentration was then determined using the bicinchoninic acid assay (BCA; Sigma-Aldrich, St Louis, MO, USA). Equal amounts of protein (50 μg) were loaded onto a 10% SDS-PAGE gel (Invitrogen, Carlsbad, CA, USA) for electrophoretic separation, transferred to a nylon membrane and immunoblotted with rabbit primary antibody (1:1000) against SIRT6 and mouse primary antibody (1:1000) against tubulin and transferrin (Cell Signaling Technology, Danvers, MA, USA). Membranes were washed with Tris-Buffered Saline containing Tween-20 (TBS-T), incubated with horseradish peroxidase-conjugated anti-rabbit second antibody or anti-mouse second antibody (1:5000) (Cell Signaling Technology) for 2 h at room temperature, and washed three times with TBS-T. Bands were visualized using the Enhanced Chemiluminescence Plus kit (EMD Millipore, Billerica, MA, USA). The densitometry analysis system Quantity One (Bio-Rad, Hercules, CA, USA) was used to quantify band intensities. The internal control was transferrin for Western blot of serum samples and β-tubulin for mice aorta arteries.

### Immunohistochemistry (IHC)

Mice and human paraffin embedded aorta tissue sections were deparaffinized and then incubated with antibodies against SIRT6, overnight at 4°C. For IHC, slides were washed 3 times with phosphate-buffered saline (PBS), incubated with secondary antibody, then treated with a streptavidin derivative coupled to alkaline phosphatase, stained with diaminobenzidine (DAB) Chromogen A, and counterstained with hematoxylin. Slides were examined at 20 × magnification under an Olympus BX-UCB microscope (Tokyo, Japan).

### Enzyme-Linked Immunosorbent Assay (ELISA) of Serum SIRT6

Assays were performed by a researcher blinded to the treatment group. Blood samples from subjects who had fasted for 8 h were centrifuged at 1,500 g for 15 min at 4°C and then stored at -80°C before use. The levels of SIRT6 were measured from 10-mL peripheral venous blood samples in duplicate using commercially available human enzyme-linked immunosorbent assay (ELISA) kits according to the instructions of the manufacturer (Catalogue No. CSB-E17018h, CUSABIO BIOTECH CO., LTD, Wuhan, China).

### Real-time PCR

RNA was isolated from mice aorta tissue by using Trizol Reagent. The expression was determined in cDNA synthesized from total mRNA by quantitative RT-PCR (qRT-PCR) on a 7500 real-time PCR machine with a Duplex Real-time PCR instrument (Bio-Rad, USA) by using SYBR Green Supermix (Bio-Rad, USA). Results were calculated with Ct value and normalized to 18 s mRNA level. mSIRT6 primer sequences are 5'-TGACACCACCTTCGAGAATGCT-3', 5'-AGACAAATCGCTCCACCAAC-3'. m18S primer sequences are 5'-TTGACTCAACACGGGAAACC-3', 5'-AGACAAATCGCTCCACCAAC-3'.

### Statistical Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 20.0 (IBM, Chicago, IL, USA). Data were expressed as the mean ± SD (standard deviation) or n (%). The chi-squared test was used for categorical data comparisons between 2 groups using Student's *t*-test when the variables were normally distributed, and the Mann-Whitney U test was used for not normally distributed data. Variables that could be a predictor of CAD with a significant *p*-value were entered into multivariate analysis. The results of univariate and multivariate regression analyses were reported in terms of odds ratios (ORs) and associated 95% confidence intervals (CIs). Potential associations between serum SIRT6 levels and clinical characteristics were tested by Pearson linear analysis. Receiver operating characteristic (ROC) curves were used to evaluate the value of using SIRT6 as a predictive marker for the diag-

nosis of CAD. Two-tailed *p*-values <0.05 were considered statistically significant.

## Results

### Clinical and Biological Characteristics

A total of 69 CAD patients (54 men) were recruited with an average age of 58.2 ± 10.6 years, of whom 30 had SA and 39 had ACS. In the control group (n=16), 8 were men, and the mean age was 57.9±7.7 years. No significant differences in age, current smoking, or CREA, were observed among the three groups. TG, TC, HDL-C, ALT, AST, BG, and hs-TnI were significantly higher in the ACS group than in the control group. The proportions of hypertension, increased LDL-C levels and high SYNTAX scores were higher in the SA and ACS groups than in the control group. The proportions of diabetes, increased ALT, AST, BG, and hs-TnI levels and high SYNTAX scores were higher in the ACS group than in the SA group (Table I).

### Circulating SIRT6 Levels in Patients with CAD

To understand whether serum SIRT6 alteration in CAD patients compared with control. Western blotting and ELISA methods were used to analysis the SIRT6 level in serum. The Western blotting results indicated that serum SIRT6 expression was significantly decreased in SA, ACS compared to control groups (Figure 1). The ELISA result indicated that SIRT6 levels were significantly lower in patients with SA (18.80±9.14 ng/mL, $p< 0.05$) or ACS (16.85±9.66 ng/mL, $p< 0.01$) than in controls (25.79±14.23 ng/mL) (Figure 2). Serum SIRT6 levels tended to be lower in patients with ACS than in those with SA, but this difference was not significant.

### Predictive Accuracy of the SIRT6 Assay

ROC analysis was performed to further evaluate the value of using SIRT6 as a predictive marker for the diagnosis of CAD (Figure 3). SIRT6 levels < 27.25 ng/mL had 63% sensitivity and 85% specificity in predicting CAD.

### Univariate and Multivariate Logistic Regression

Univariate and multivariate logistic regression analyses were used to evaluate the effects of different variables on CAD (shown in Table II). In univariate analysis, male sex (OR = 3.6, 95% CI: 1.157-11.197, $p$=0.027), older age (OR = 1.066, 95% CI: 1.003-1.133, $p$=0.039), increased CREA level (OR = 1.068, 95% CI: 1.013-1.126, $p$=0.015), increased LDL-C level (OR = 4.634, 95% CI: 1.785-12.029, $p$=0.002), increased TC level (OR = 2.76, 95% CI: 1.346-5.659, $p$=0.006), and decreased SIRT6 level (OR = 0.933, 95% CI:

**Table I.** Clinical and biological characteristics of the three groups.

| | Control (16) | SA (30) | ACS (39) |
|---|---|---|---|
| Age (years) | 57.9 ± 7.7 | 64.7 ± 8.7 | 62.7 ± 10.7 |
| Male sex (%) | 8 (50) | 21(70) | 33 (84) * |
| Current smoking (%) | 4 (25) | 14 (46.7) | 16 (41) |
| Hypertension (%) | 0 | 19 (63.3)* | 18 (46.2)* |
| Diabetes (%) | 0 | 0 | 10 (25.6)*# |
| ALT (U/L) | 16.13 ± 5.84 | 22.12 ± 14.24 | 41.97 ± 26.88*# |
| AST (U/L) | 17.06 ± 4.43 | 18.54 ± 6.06 | 152.69 ± 142.10*# |
| CREA (mmol/L) | 63.31 ± 9.65 | 73.15 ± 12.15 | 72.03 ± 13.73 |
| BG (mmol/L) | 5.42 ± 0.54 | 5.53 ± 0.68 | 7.95 ± 3.53*# |
| TC (mmol/L) | 3.59±0.95 | 4.03±1.07 | 4.62±0.87*# |
| HDL-C (mmol/L) | 1.22 ± 0.31 | 1.03 ± 0.25* | 1.03 ± 0.26* |
| LDL-C (mmol/L) | 2.14 ± 0.67 | 2.80 ± 0.90* | 3.21 ± 0.81* |
| TG (mmol/L) | 0.756 ± 0.30 | 1.58 ± 0.70*# | 1.36 ± 0.75*# |
| hs-TnI (ng/ml) | 0 | 0 | 20.90 ± 18.31*# |
| SYNTAX score | 0 | 16.17±2.59* | 31.46±10.34*# |

Data are expressed as the mean ± SD or n (%). ACS, acute coronary syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BG, blood glucose; CREA, creatine; TC: total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SA, stable angina; TG, triglyceride; hs-TnI, hypersensitive troponin I. *p < 0.05 vs. control, #p<0.05 ACS vs. SA.

**Table II.** CUnivariate and multivariate logistic analysis for CAD.

| | Univariate | | | Multivariate in men | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *p*-value | OR | 95% CI | *p*-value |
| Male | 3.6 | 1.157-11.197 | 0.027 | | | |
| Age | 1.066 | 1.003-1.133 | 0.039 | 1.291 | 0.983-1.696 | 0.066 |
| Current smoking | 0.433 | 0.127-1.479 | 0.182 | | | |
| CREA | 1.068 | 1.013-1.126 | 0.015 | 1.025 | 0.888-1.182 | 0.737 |
| LDL-C | 4.634 | 1.785-12.029 | 0.002 | 5.171 | 0.061-438.00 | 0.468 |
| BG | 1.929 | 0.957-3.888 | 0.066 | | | |
| TG | 2.758 | 0.929-8.191 | 0.068 | | | |
| TC | 2.76 | 1.346-5.659 | 0.006 | 2.236 | 0.056-88.64 | 0.668 |
| HDL-C | 0.205 | 0.33-1.284 | 0.09 | | | |
| SIRT6 | 0.933 | 0.885-0.934 | 0.01 | 0.817 | 0.694-0.962 | 0.015 |

Variables that could be a predictor of CAD with a significant p-value were entered into multivariate analysis. ACS, acute coronary syndrome; BG, blood glucose; CAD: coronary artery disease; CREA, creatine; TC: total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SA, stable angina; TG, triglyceride..

0.885–0.934, $p$=0.001) were predictors for CAD. A multivariable logistic regression model, including age, CREA, LDL-C, TC and SIRT6, was established by using CAD as the dependent variable in the male group. In multivariate logistic regression analysis, only decreased SIRT6 levels (OR = 0.817, 95% CI: 0.694–0.962, $p$=0.015) were predictors of CAD.

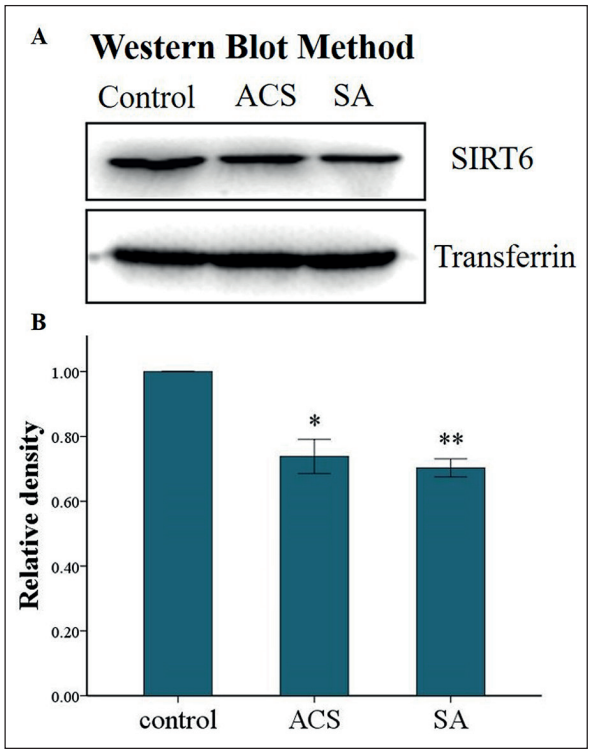


**Figure 1.** Western blotting in different groups (SA, ACS and control) (n=6) (**A**). SIRT6 expression (42kd) was significantly decreased in the ACS group (*$p$<0.01 *vs.* control) and SA group (**$p$<0.01 *vs.* control) (**B**). No significant difference was found between the SA and ACS groups.

### *Correlations of SIRT6 Levels with Clinical and Laboratory Parameters*

The pathogenesis of coronary heart disease is atherosclerosis. Atherosclerosis is a disease process caused by abnormal lipid metabolism, blood coagulation disorder, aging and chronic vascular inflammation related to genetic and environmental factors. Hypercholesterolemia is one of the main risk factors for the development of atherosclerosis. Among the various lipoprotein classes, however, high density lipoproteins (HDL) are inversely associated with the incidence of atherosclerosis, since they are able to exert a series of atheroprotective functions. In the control and both CAD patient groups, serum SIRT6 levels were positively associated with HDL-C levels (r=0.362, $p$<0.01). But no significant association was found between SIRT6 and triglycerides (r=-0.189, $p$=0.103), LDL-C (r=-0.177, $p$=0.126).

Aging is an important internal cause of disease, which can play a pathogenic role through the following mechanisms: aging causes dysfunction of vascular endothelial cells and affects the regeneration of damaged endothelial cells; aging causes lipid metabolism disorder and induces the production of oxidized low-density lipoprotein; aging can also stimulate the migration of vascular smooth muscle cells, change the cell phenotype and reduce the cell proliferation ability. It leads to the thinning of plaque fibrous cap, which in-

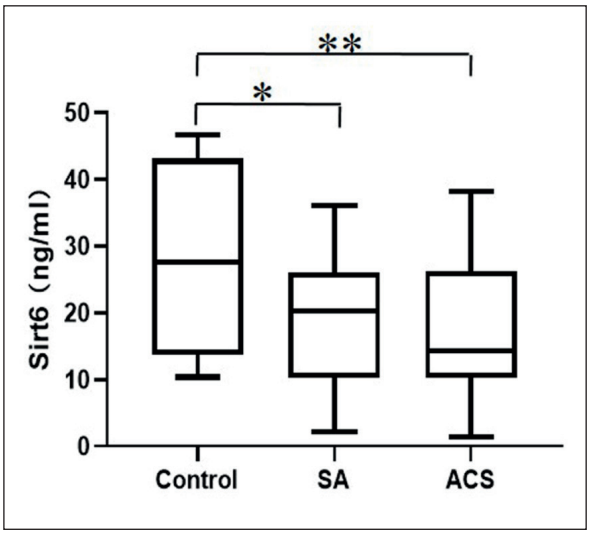


**Figure 2.** Serum SIRT6 levels were lower in patients with SA (*$p$<0.05 *vs.* control) and patients with ACS (**$p$<0.01 *vs.* control). No significant difference was found between the SA and ACS groups.

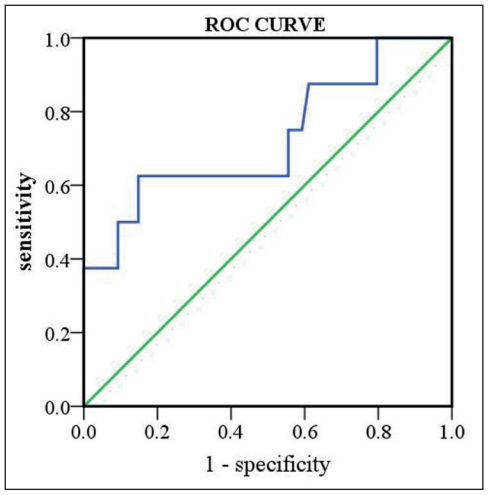


**Figure 3.** Receiver operator characteristic (ROC) curve analysis of SIRT6 in predicting the diagnosis of CAD (AUC, 0.726; 95% CI, 0.508-0.943; $p$=0.041).

creases the vulnerability and aggravates the occurrence and development of atherosclerosis. In the control and both CAD patient groups, serum SIRT6 levels were positively associated with age (r=0.265, $p$<0.05). In our study, we also found that serum SIRT6 levels were negatively associated with blood glucose (r=-0.284, $p$<0.05), which is a high risk factor for atherosclerosis or CAD. However, no relationship was found between serum SIRT6 and SYNTAX score (r=-0.155, p=0.156). (Figure 4)

Previous studies[14-16] have shown that SIRT6 regulates endothelial cell function and lipid me-

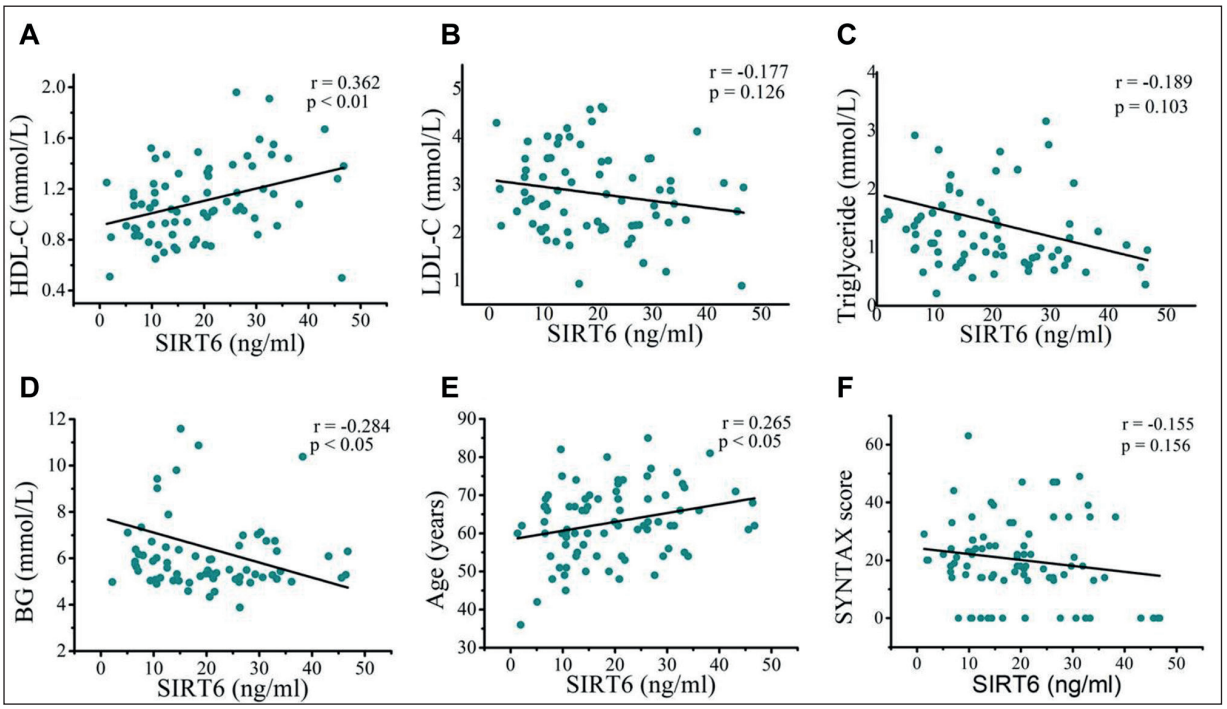


**Figure 4.** Correlation of serum SIRT6 levels with high-density lipoprotein cholesterol (HDL-C) (**A**), low-density lipoprotein cholesterol (LDL-C) (**B**), triglyceride (**C**), blood glucose (BG) (**D**), age (**E**) and SYNTAX score (**F**).

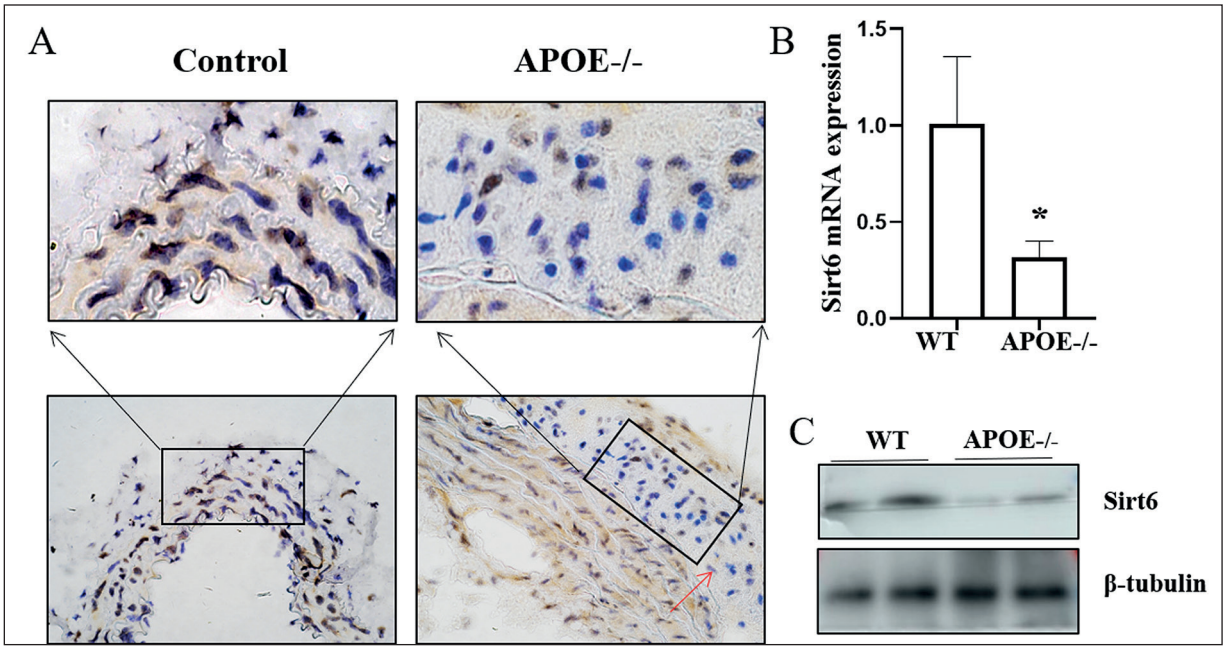


**Figure 5.** The expression of SIRT6 in atherosclerotic vessels decreased significantly. **A** IHC staining showed that the expression of SIRT6 in atherosclerotic ApoE-/- mice arteries were significantly decreased (magnification: 200×). **B, C,** real time PCR and WB results showed that the mRNA and protein levels of SIRT6 in atherosclerotic ApoE-/- mice were significantly decreased. (*$p<0.05$ *vs.* control). (the arrow indicated the atherosclerotic plaque).

tabolism, which are key factors affecting atherosclerosis. This further proves the important value of SIRT6 in coronary heart disease.

### *SIRT6 is Downregulated in Atherosclerotic Vascular Tissue*

The SIRT6 immunohistochemical staining in mice (Figure 5A) and human aorta arteries (Figure 6) showed that SIRT6 expression is markedly downregulated in atherosclerotic vascular tissue compared to non-atherosclerotic vascular tissue. Western blot and Real-time PCR analysis of mice arteries showed that SIRT6 expression is markedly downregulated compared to non-atherosclerotic vascular tissue (Figure 5B, 5C).

## Discussion

In this study, we investigated serum SIRT6 levels in SA and ACS patients and patients with normal coronary arteries. We found that reduced serum SIRT6 may be a risk factor for cardiovascular disease. Serum SIRT6 level was decreased significantly in SA and ACS patient compared with control. Serum SIRT6 levels were positively correlated with HDL-C levels in patients with SA or ACS, as well as in controls with normal coronary artery function. The novelty of this study was that this is the first study to evaluate the relationship between circulating SIRT6 and serum HDL-C in patients with CAD. Our results suggested that serum SIRT6 levels < 27.25 ng/mL may be a threshold value for CAD diagnosis.

SIRT6 expression has been reported to reduce atherosclerosis in ApoE-/- mice and diabetes patients[9,12,13]. However, it is not yet known what the role of serum SIRT6 level plays in CAD patients. In the present study, we report that circulating SIRT6 is decreased in patients suffering from coronary atherosclerosis. ROC analysis also indicated that SIRT6 has a good value as a predictor of coronary artery disease. Whether the reduced serum SIRT6 levels in CAD patients are a cause or result of the atherosclerotic process remains to be seen. To elucidate this phenomenon, we detected the SIRT6 expression in the atherosclerotic ApoE-/- mice vessel. And found that SIRT6 expression was decreased significantly in atherosclerotic vascular tissue compared to non-atherosclerotic vascular tissue. This result indicated the reduced serum SIRT6 level may be caused by the decreased expression in atherosclerotic vascular tissue. However, further studies may explore the

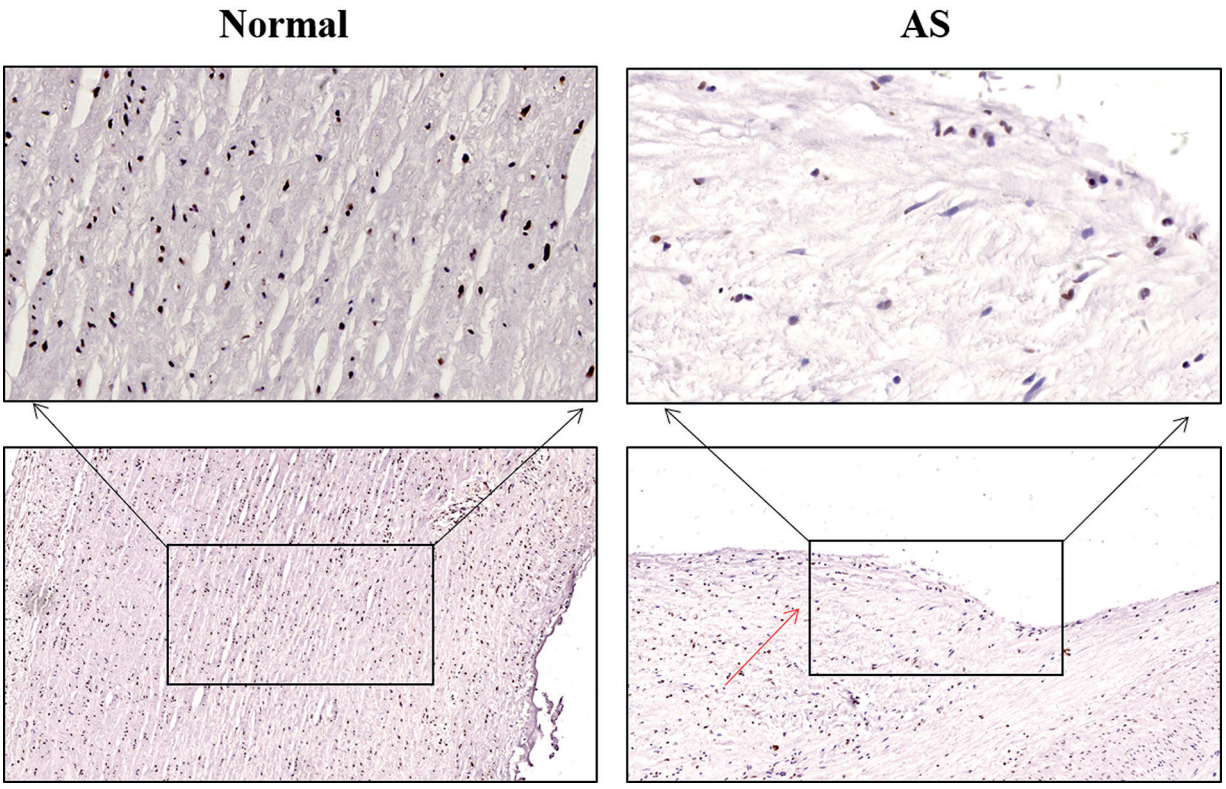


**Figure 6.** The expression of SIRT6 in atherosclerotic vessels decreased significantly. IHC staining showed that the expression of SIRT6 in atherosclerotic human aorta arteries were significantly decreased (magnification: 200×). (AS, atherosclerosis, the arrow indicated the atherosclerotic plaque).

hypothesis that increasing SIRT6 expression exerts anti-atherogenic properties in CAD patients.

In order to explore whether SIRT6 is an independent influence factor of CAD, we analyzed the correlation between SIRT6 level and CAD risk or protection factors. The results indicated that SIRT6 appears to regulate lipid metabolism by controlling cholesterol homeostasis, synthesis of TG and cholesterol, and β-oxidation[1,25,26]. SIRT6 knockout caused fatty liver formation in mice[25]. A link between SIRT6 and lipid metabolism was supported by our observation that serum SIRT6 positively correlated with HDL-C in all study groups. However, SIRT6 levels did not correlate with the levels of TG or LDL-C. Future work should explore in detail whether SIRT6 may affect cardiovascular function *via* lipid metabolism. Another pathway by which SIRT6 may affect cardiovascular function is through glucose metabolism. In animal models, SIRT6 plays an important role in glucose production and uptake, insulin signalling, and metabolism[27-29]. In our study, circulating SIRT6 negatively correlated with blood glucose. Disruption of glucose homeostasis is an established CAD risk factor, and future studies should investigate whether such disruption underlies the observed correlation between SIRT6 and CAD[30]. It is also possible that SIRT6 exerts anti-atherogenic effects through one or more of the pathways influenced by HDL-C, which has been shown to protect against atherosclerosis by reducing the efflux of cholesterol from cells and promoting antioxidant and anti-inflammatory responses[31-33].

## Conclusions

Our results should be interpreted with caution in light of the limited sample size and the fact that some clinical characteristics, such as sex, diabetes and hypertension, varied significantly among the three groups, which may result in bias. Second, we cannot exclude possible confounding factors due to differences in treatment or diet.

In conclusion, we demonstrate that SIRT6 may exert cardioprotective effects against atheroscle-

rosis and may be a useful biomarker for CAD. Further research is required to dissect the processes underlying the relationship between SIRT6 and CAD.

### Conflict of Interest

The authors declare that they have no competing interests.

### Acknowledgements

This work was supported by the Key Research and Development Project in Shandong, China (2017G006027), Shandong Medical and Health Science and Technology Development Program Project, China (2013WS0243) and National Natural Science Foundation of China (NO. 81600344).

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