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Association between expression of AMPK pathway and adiponectin, leptin, and vascular endothelial function in rats with coronary heart disease

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Abstract. – OBJECTIVE: The aim of this study was to explore the association between the expression of adenosine monophosphate-activated protein kinase (AMPK) pathway and adiponectin (APN), leptin, and vascular endothelial function in rats with coronary heart disease (CHD).

MATERIALS AND METHODS: Experimental rats were divided into three groups, including: control (Col) group, CHD model (CHD) group, and CHD+AMPK activator (CHD+AICAR) group. Except those in Col group, all rats were fed with high-fat diet and intraperitoneally injected with pituitrin to establish the CHD model. The levels of serum APN, leptin, and endothelin-1 (ET-1) were determined via enzyme-linked immunosorbent assay (ELISA). The content of serum nitric oxide (NO) was detected using the nitrate reductase method. Meanwhile, the expression of AMPK pathway-related protein AMPKα in vascular endothelial tissues was detected via Western blotting (WB). Aortic vascular endothelial cells (VECs) were cultured with AICAR or ET-1 in vitro. Subsequently, the expressions of AMPK pathway and protein kinase B (AKT) pathway-related proteins were determined through co-immunoprecipitation and WB. Moreover, the expression level of NO in VECs was determined using the DAF-FM DA fluorescence probe.

RESULTS: Compared with Col group, CHD group showed significantly decreased levels of serum APN and NO ($p<0.05$), significantly increased the levels of leptin and ET-1 ($p<0.05$), as well as remarkably decreased protein expression of p-AMPKα in vascular endothelial tissues ($p<0.05$). After injection of AMPK activator AICAR (200 mg/kg), the protein expression of p-AMPKα in CHD rats was significantly activated ($p<0.05$). The levels of serum APN and NO were remarkably upregulated ($p<0.05$), while the levels of leptin and ET-1 were significantly reduced ($p<0.05$). Besides, AICAR could evidently activate the activity of AMPK pathway in VECs in vitro, upregulate the protein levels of p-eNOS (Ser1177) and p-AMPKα, and promote the secretion of NO ($p<0.05$). In addition, AICAR remarkably inhibited ET-1-induced expression of AKT pathway ($p<0.05$).

CONCLUSIONS: Activating the AMPK pathway may play a positive role in the normal function of VECs and exert a certain curative effect on CHD in rats.

Key Words:
AMPK pathway, Coronary heart disease (CHD), Adiponectin, Leptin, Vascular endothelial function.

Introduction

According to the statistics of the British Heart Foundation and the American Heart Association, cardiovascular disease (CVD) is still the leading cause of individual death[1]. Estimated by the Global Burden of Disease Study in 2010, CVD has caused 15.6 million deaths around the world, accounting for 29.6% in all deaths. This is twice the number of cancer-related deaths. Meanwhile, it exceeds the total number of deaths due to infectious disease and nutritional disorders in pregnant and lying-in women and neonates[2,3]. Among all CVD deaths, coronary heart disease (CHD) is the biggest killer, accounting for more than one-fifth. The mortality rate of CHD in males and females is 19% and 20%, respectively far higher than that of breast cancer in females (2%) and lung cancer in males (6%)[2].

Adenosine monophosphate-activated protein kinase (AMPK) is a member of the serine/threonine kinase and SNF1/AMPK protein kinase family. It is a functional enzyme composed of three protein subunits α, β, and γ[4]. AMPK is expressed in many tissues, including liver,

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brain, and skeletal muscles. Currently, Shirwany and Zou[5] have found that it is allosterically activated by increased AMP/ATP ratio[5], such as hypoxia, glucose deprivation or inhibition on ATP anabolism[6]. Moreover, AMPK plays an important physiological role in the cardiovascular system. Increasingly, more reports have shown that AMPK may also act as a sensor by responding to oxidative stress[5].

Coronary atherosclerosis is a major pathological basis of CHD, and the body's lipid metabolism is closely related to CHD. Adiponectin (APN) and leptin, the proteins secreted by adipocytes, exert a regulatory effect on fat anabolism. They have been found closely related to the development of CHD[7,8]. Endothelial cells can sense the changes in hemodynamics, oxygen content, and local blood-borne signals. Meanwhile, they can maintain homeostasis through appropriate control and regulatory processes as well. Such reactions include the release of paracrine mediators, such as nitric oxide (NO), endothelin-1 (ET-1), and prostacyclin[9]. The role of AMPK pathway in related diseases under stress conditions has been comprehensively explored. However, there are few studies on the AMPK pathway expression during the progression of chronic CHD, as well as its effects on related proteins and cell functions. Therefore, the aim of the present work was to explore the association between the expression of AMPK pathway and APN, leptin, and vascular endothelial function in CHD rats. Our findings might help to provide a theoretical basis for the research on CHD and a potential method for the clinical treatment of CHD.

Materials and Methods

Main Materials

Sprague-Dawley (SD) rats (Changzhou Cavens Laboratory Animal Co., Ltd., Changzhou, China), pituitrin injection (Nanjing Xinbai Pharmaceutical Co., Ltd., Nanjing, China), APN, leptin, and ET-1 detection kits (BioLegend; San Diego, CA, USA), ET-1 (Sigma, St. Louis, MO, USA), NO detection kit, DAF-FM DA (NO fluorescence probe), and protein A agarose (Beyotime, Beijing, China), p-AMPKα, AMPK, endothelial nitric oxide synthase (eNOS), p-eNOS (Ser1177), protein kinase B (AKT), p-AKT, and β-actin antibodies and horseradish peroxidase (HRP)-labeled secondary antibodies (Abcam, Cambridge, MA, USA), AICAR (Selleck, Shanghai, China), and 0.22 μm pinhole filter (Millipore, Billerica, MA, USA).

Animal Grouping and Establishment of CHD Model

Healthy SD rats weighing 160-180 g were divided into three groups, including: control (Col) group (n=20), CHD model (CHD) group (n=20), and CHD+AMPK activator (CHD+AICAR) group (n=20). The rat model of CHD was established in CHD group and CHD+AICAR group, as follows: the rats were fed with high-fat diet (87.3% of basal feed, 10% of lard oil, 2% of cholesterol, 0.5% of sodium cholate, and 0.2% of propylthiouracil) for 6 weeks. Subsequently, AICAR was intraperitoneally injected (200 mg/kg) daily in rats of CHD+AICAR group. Meanwhile, an equal volume of phosphate-buffered saline (PBS) was intraperitoneally injected in rats of CHD group, and pituitrin was also intraperitoneally injected (30 U/kg) once daily in the last 3 d. The rats in Col group were fed with normal basal feed and injected intraperitoneally with the same amount of PBS at the appropriate time. This investigation was approved by the Animal Ethics Committee of the Nanjing Medical University Animal Center.

Detection Indexes

After the last intraperitoneal injection of pituitrin, the rats were deprived of food for 24 h. Subsequently, the blood samples were collected from the femoral artery under anesthesia, and the serum was separated. The levels of serum APN, leptin, and ET-1 were determined *via* enzyme-linked immunosorbent assay (ELISA). Meanwhile, the content of serum NO was detected according to the instructions of the nitrate reductase method.

Culture of Vascular Endothelial Cells (VECs)

VECs were thawed and inoculated into a gelatin-coated petri dish at a density of 2×10^5 cells/mL. All VECs were cultured in Dulbecco's Modified Eagle's Medium/nutrient mixture F-12 (DMEM/F-12; Hyclone, South Logan, UT, USA) medium containing 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA) in an incubator with 5% CO_2 at 37°C. When the cells covered about 90% of petri dish, cell passage was performed. After washing with PBS twice, the cells were digested with an appropriate amount of 0.25% trypsin containing ethylenediaminetetraacetic acid (EDTA) at 37°C for 3 min. The digestion was then terminated using DMEM/

F12 medium containing 10% FBS, followed by passage (1:1). The fifth-generation cells were used for subsequent experiments.

Related Protein Expressions in VECs Cultured In Vitro Under Different Treatments

The fifth-generation VECs in good growth condition were cultured with 0 mM of AICAR, 0.5 mM of AICAR, 1 mM of AICAR, and 2 mM of AICAR for 40 min. After discarding the medium, the cells were lysed using cell lysis buffer, and total protein was extracted. Protein expression of p-AMPKα was finally detected *via* Western blotting (WB).

The fifth-generation VECs in good growth condition were cultured with 0 mM of AICAR, 1 mM of AICAR and an equal amount of dimethyl sulfoxide (DMSO) as control for 40 min. After discarding the medium, the cells were lysed using cell lysis buffer, and total protein was extracted. Protein expressions of p-eNOS (Ser1177) and eNOS were finally detected *via* WB.

The fifth-generation VECs in good growth condition were cultured with 0 nM of ET-1 + 1 mM of AICAR, 10 nM of ET-1, and 10 nM of ET-1 + 1 mM of AICAR. After discarding the medium, the cells were lysed using cell lysis buffer, and the total protein was extracted. Protein expressions of phosphate adenosine 5'-monophosphate (AMP)-activated protein kinase α (p-AMPKα), adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK), protein kinase B (AKT), and phosphate protein kinase B (p-AKT) were finally detected *via* WB.

Detection of NO in VECs

The fifth-generation VECs in good growth condition were cultured with 0 mM of AICAR, 1 mM of AICAR and an equal amount of DMSO as control for 1 h. The expression of NO was detected according to the instructions of the DAF-FM DA (NO fluorescence probe) kit. DAF-FM DA was diluted (1:1000) to a final concentration of 5 μM using the diluent. After removal of cell culture medium, an appropriate volume of diluted DAF-FM DA was added for incubation at 37°C for 20 min. After washing with PBS for 3 times, the cells were photographed under a fluorescence microscope.

Co-Immunoprecipitation

The fifth-generation VECs in good growth condition were cultured with 0 mM of AICAR, 0.5 mM of AICAR and an equal amount of DMSO as control for 0 min, 20 min, 40 min, 60 min, and 120 min, respectively. The cells were lysed using cell lysis buffer, and total protein was extracted. 1 μg of corresponding antibodies were added into cell lysate and slowly shaken, followed by incubation at 4°C overnight. Subsequently, 10 μL of protein A agarose beads were washed with an appropriate amount of lysis buffer for 3 times, followed by centrifugation at 3000 rpm for 3 min. After pretreatment, 10 μL of protein A agarose beads were added into cell lysis buffer incubated with p-AMPKα antibody overnight. After slowly shaken and incubation at 4°C for 4 h, p-AMPKα was coupled with protein A agarose beads. After immunoprecipitation, the mixture was centrifuged at 3000 rpm and 4°C for 3 min to precipitate agarose beads to the bottom of tube. Next, the supernatant was carefully removed, and agarose beads were washed with 1 mL of lysis buffer for 3 times. Finally, 15 μL of 2×dodecyl sulfate, sodium salt (SDS) loading buffer was added and boiled for 5 min, followed by mass spectrometry.

Western Blotting

After the blood was drawn from rats in each group, vascular endothelial tissues were quickly collected and ground in liquid nitrogen. The cells were lysed using cell lysis buffer, and the total protein was extracted. The concentration of extracted protein was determined using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). After separation *via* 8% dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE), the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After sealing with 5% skimmed milk powder and 0.1% Tween-20 in Tris-Buffered Saline, the membranes were incubated with primary antibodies of p-AMPKα, AMPK, eNOS, p-eNOS (Ser1177), AKT, and p-AKT at 4°C overnight. On the next day, the membranes were incubated again with HRP-labeled secondary antibodies. Immuno-reactive bands were finally exposed by the enhanced chemiluminescence (ECL) reagent. β-actin was used as an internal reference.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) software was used for all statistical analysis. Experimental data were expressed as mean ± standard deviation ($\chi \pm s$). Independent-samples *t*-test was performed for inter-group comparison. $p<0.05$ was considered statistically significant.

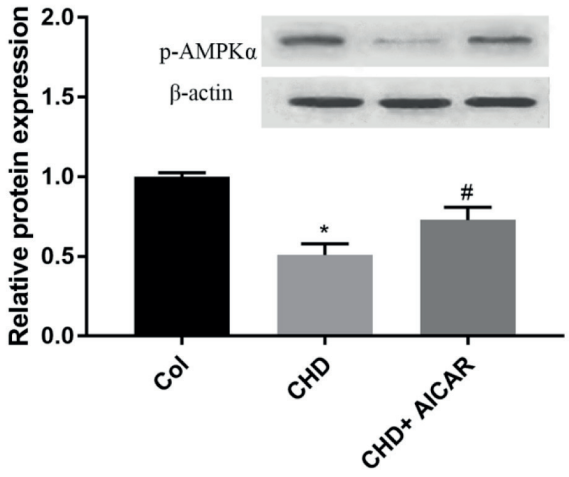

Figure 1. Expression of AMPK signaling pathway protein p-AMPKα in vascular endothelial tissues in each group detected using WB. Compared with Col group and CHD+AICAR group, the expression level of p-AMPKα protein in CDH group is evidently down-regulated (p<0.05). The expression level of p-AMPKα protein in CHD+AICAR group is evidently higher than that in CDH group (p<0.05), whereas lower than Col group (p<0.05). *p<0.05: Significant differences in CHD group *vs*. Col group and CHD+AICAR group, #p<0.05: Significant differences in CHD+AICAR group *vs*. Col group and CHD group.

Results

Expressions of APN and Leptin in Rats

Compared with Col group, CHD group showed significantly decreased level of serum APN (p<0.05). After injection of the AMPK pathway activator AICAR, it was effectively improved (p<0.05). However, it was still significantly lower than that in the Col group (p<0.05) (Table I). Besides, compared with Col group, CHD group exhibited significantly increased level of serum leptin (p<0.05), which was effectively reduced after the injection of the AMPK pathway activator AICAR (p<0.05). However, it was still higher than that in the Col group (p<0.05) (Table I).

Vascular Endothelial Function in Rats

Compared with Col group, CHD group showed significantly decreased level of NO secreted by vascular endothelium (p<0.05). This could be effectively stimulated after injection of the AMPK pathway activator AICAR (p<0.05). However, it was still lower than that in Col group (p<0.05) (Table II). Besides, compared with Col group, CHD group exhibited remarkably upregulated level of ET-1 secreted by vascular endothelium (p<0.05). Similarly, this was effectively reduced after the AMPK pathway activator AICAR was injected (p<0.05). Meanwhile, it was still higher than that in Col group (p<0.05) (Table II).

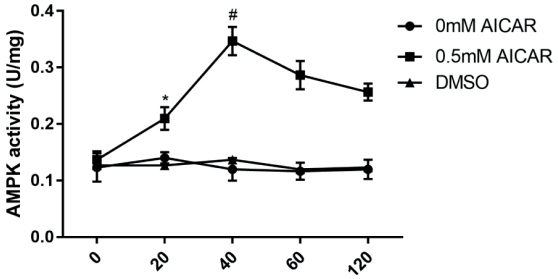

Figure 2. Effect of AICAR on AMPK activity. With the prolongation of incubation time, AMPK activity rises evidently and reaches peak at 30 min. *p<0.05: Significant differences under incubation with 0.5 mM of AICAR for 20 min *vs*. other incubation time, #p<0.05: Significant differences under incubation with 0.5 mM of AICAR for 40 min *vs*. other incubation time.

Table I. APN and leptin expressions in Col group, CHD group and CHD+AICAR group (χ±s, n=20).

Group	Serum APN concentration (ng/mL)	Serum leptin concentration (ng/mL)
Col	7.23±0.7	0.43±0.05
CHD	5.2±0.67*	1.24±0.08*
CHD+AICAR	6.2±0.36#	0.62±0.07#

The serum APN content in CHD group is significantly lower than that in Col group and CHD+AICAR group (p<0.05). The serum APN content in CHD+AICAR group is markedly higher than that in CHD group (p<0.05), but lower than that in Col group (p<0.05). The serum leptin content in CHD group is significantly higher than that in Col group and CHD+AICAR group (p<0.05). The serum leptin content in CHD+AICAR group is remarkably lower than that in CHD group (p<0.05), but higher than that in Col group (p<0.05). *p<0.05: significant differences in CHD group vs. Col group and CHD+AICAR group, #p<0.05: significant differences in CHD+AICAR group vs. Col group and CHD group.

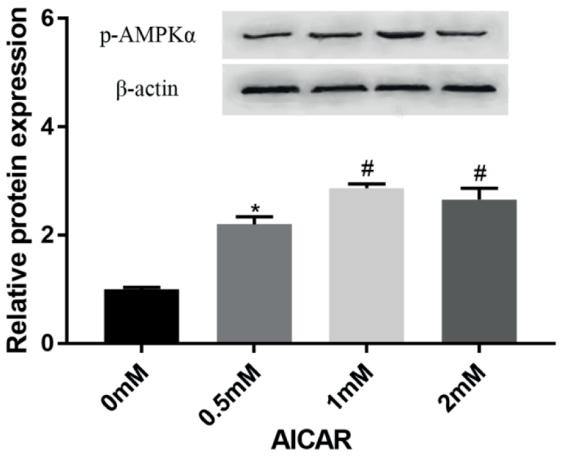

Figure 3. Effect of AICAR at different concentrations on p-AMPKα protein expression in VECs detected using WB. With the increase of AICAR concentration, p-AMPKα protein expression evidently rises ($p<0.05$). Meanwhile, it is no longer increased when AICAR concentration reaches 1 mM. *$p<0.05$: Significant differences under 0.5 mM of AICAR *vs.* other concentrations, #$p<0.05$: Significant differences under 1 mM and 2 mM of AICAR *vs.* other concentrations.

Effect of AICAR on AMPK Pathway Protein in VECs

The expression of AMPK signaling pathway protein p-AMPKα was detected *via* WB. Compared with Col group, the protein expression of p-AMPKα evidently declined in the CDH group ($p<0.05$). Moreover, p-AMPKα protein expression in CDH rats significantly increased by AICAR treatment ($p<0.05$) (Figure 1).

Effect of AICAR on AMPK Pathway Activity in VECs Cultured In Vitro

Transient AMPK pathway activity in VECs cultured with AICAR for different time points was determined using p-AMPKα co-immunoprecipitation. The results found that AMPK pathway activity showed an increasing trend with time. It reached peak at 40 min, and then, gradually declined (Figure 2). Besides, the effect of AICAR at different concentrations on the protein expression of p-AMPKα was de-

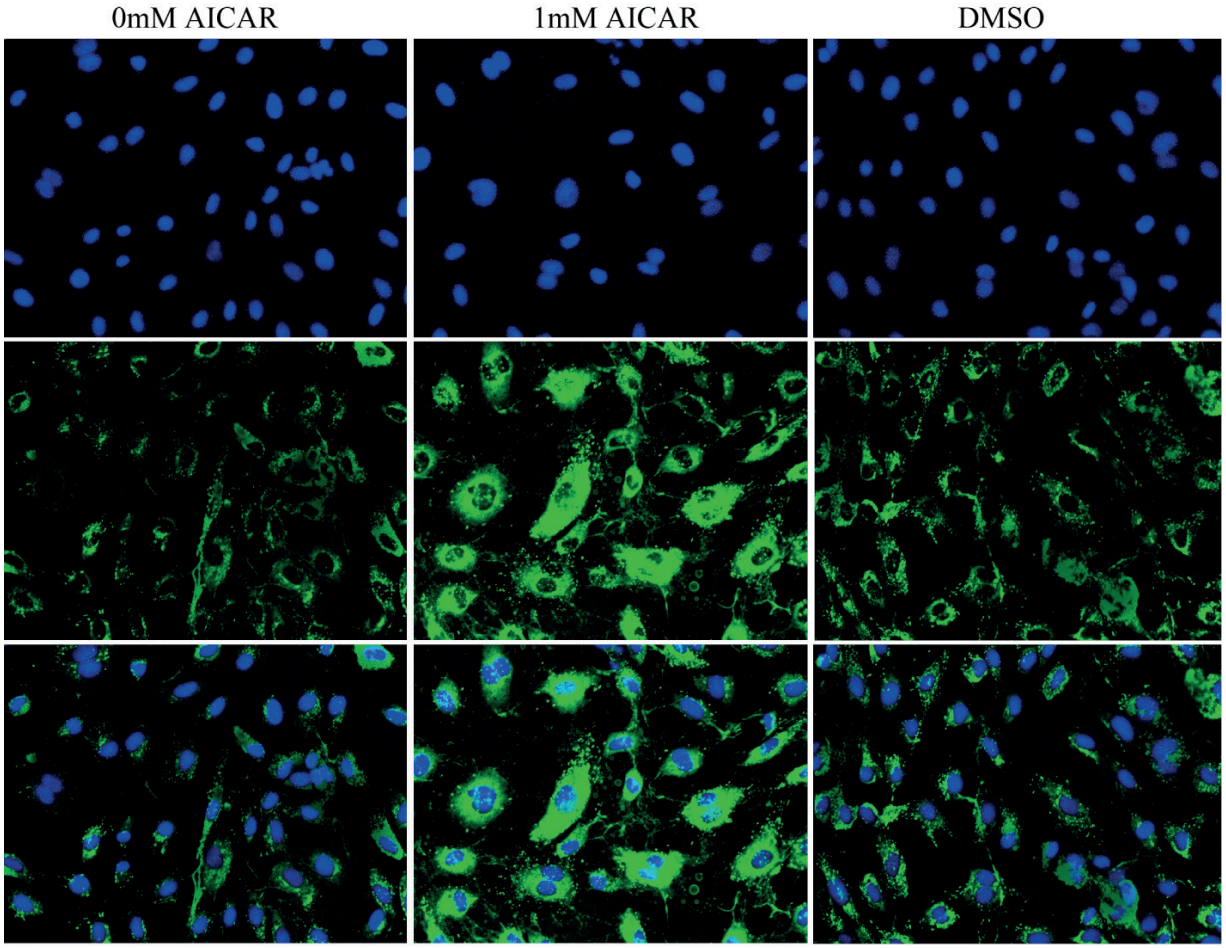

Figure 4. Effect of AICAR on the production of NO detected using DAF-FM DA fluorescence probe (200X). The fluorescence intensity of VECs cultured with 1 mM of AICAR is markedly higher than that of VECs cultured with DMSO or 0 mM of AICAR.

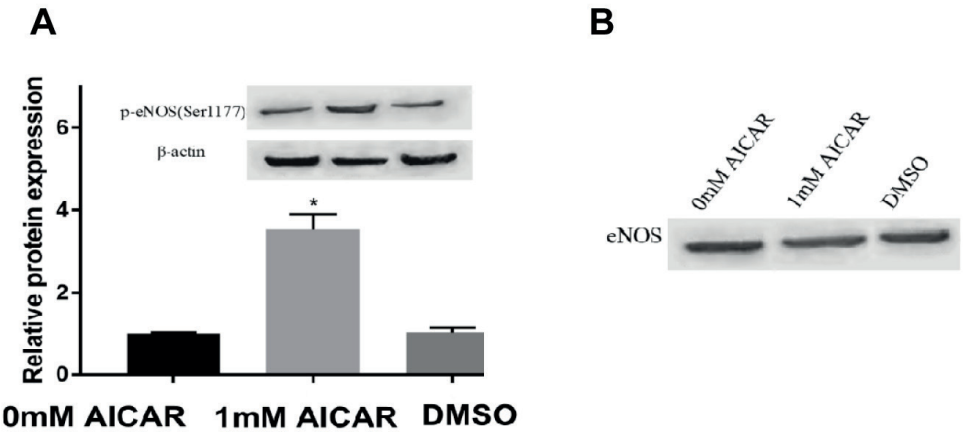

Figure 5. Effect of AICAR on the production of eNOS and p-eNOS (Ser1177) in VECs determined using WB. **A,** 1 mM of AICAR can remarkably promote p-eNOS (Ser1177) protein expression compared with 0 mM of AICAR or DMSO ($p<0.05$). **B,** There is no significant difference in the protein expression of eNOS among groups ($p>0.05$). *$p<0.05$: Significant differences under 1 mM of AICAR *vs*. 0 mM of AICAR and DMSO.

tected using WB. The results revealed that the expression of AMPK in VECs rose with the increase of AICAR concentration. However, it no longer increased when AICAR concentration reached 1 mM (Figure 3).

AICAR Stimulated VECs to Produce NO

To confirm the effect of AICAR on the production of NO, VECs were incubated with 1 mM of AICAR for 1 h. The intensity of DAF-FM DA fluorescence probe was detected *via* fluorescence imaging. The results manifested that the fluorescence intensity of VECs cultured with DMSO or 0 mM of AICAR was weak. However, it was markedly enhanced after culture with 1 mM of AICAR. These findings indicated that AICAR could stimulate VECs to produce more NO (Figure 4).

Effect of AICAR on the Production of eNOS in VECs

According to WB results, the protein expression of p-eNOS (Ser1177) was remarkably upregulated after culture with 1 mM of AICAR for 40 min in VECs ($p<0.05$) (Figure 5A). However, it had no significant effect on the expression level of eNOS ($p>0.05$) (Figure 5B).

Effects of ET-1 and AICAR on AKT and AMPK Pathway Expressions in VECs

WB results showed that ET-1 incubation could significantly increase p-AKT protein expression in VECs ($p<0.05$) (Figure 6A) and reduce p-AMPKα protein expression ($p<0.05$) (Figure 6B). However, it had no significant effects on the protein expressions of AKT and AMPK ($p>0.05$) (Figure 6). After culture with 1 mM of AICAR, the protein

Table II. NO and ET-1 content in Col group, CHD group and CHD+AICAR group ($\chi\pm s$, n=20).

Group	NO content (μmol/L)	ET-1 content (pg/mL)
Col	12.60±1.67	34.43±5.05
CHD	6.70±1.48*	47.24±3.08*
CHD+AICAR	10.62±2.07#	40.62±4.07#

The serum NO content in CHD group is markedly lower than that in Col group and CHD+AICAR group ($p<0.05$). The serum NO content in CHD+AICAR group is markedly higher than that in CHD group ($p<0.05$), but lower than that in Col group ($p<0.05$). The serum ET-1 content in CHD group is markedly higher than that in Col group and CHD+AICAR group ($p<0.05$). The serum ET-1 content in CHD+AICAR group is remarkably lower than that in CHD group ($p<0.05$), but higher than that in Col group ($p<0.05$). *$p<0.05$: Significant differences in CHD group *vs*. Col group and CHD+AICAR group, #$p<0.05$: Significant differences in CHD+AICAR group *vs*. Col group and CHD group

expression of p-AKT was significantly inhibited (p<0.05) (Figure 6A), while p-AMPKα was significantly upregulated (p<0.05) (Figure 6B).

Discussion

In 1995, AMPK was first studied in the heart in the Lopaschuck laboratory. It was proved that stress conditions, such as heart ischemia, could effectively induce the activation of AMPK, inhibit acetyl-CoA carboxylase, and reduce malonyl-CoA, accompanied by the increase in the fatty acid oxidation rate[10,11]. Under non-stress conditions, such as chronic CVD, AMPK pathway is inhibited during the progression of CHD. Besides hypoxia, glucose deprivation or inhibition on ATP anabolism, AMPK can also be activated by some activity regulators, including APN, leptin, IL-6[12], ciliary neurotrophic factor[13], metformin[14], thiazolidinedione[15], berberine[16], and resveratrol[17]. Related studies have shown that obesity is closely related to metabolism and CVD. APN is a kind of adipose-derived plasma protein that is downregulated in subjects with obesity-related diseases. Low-level APN has a correlation with increased morbidity rate of obesity related CVD[18]. Moreover, APN protects cardiovascular tissues under stress conditions through many mechanisms, such as inhibition on the pro-inflammatory and hypertrophic responses. It can stimulate endothelial cell response as well. The effects of APN are mainly attributed to the regulation of signaling molecules, including AMPK[8]. In this study, the level of plasma APN in CHD rats was detected. The results showed that the level of plasma APN

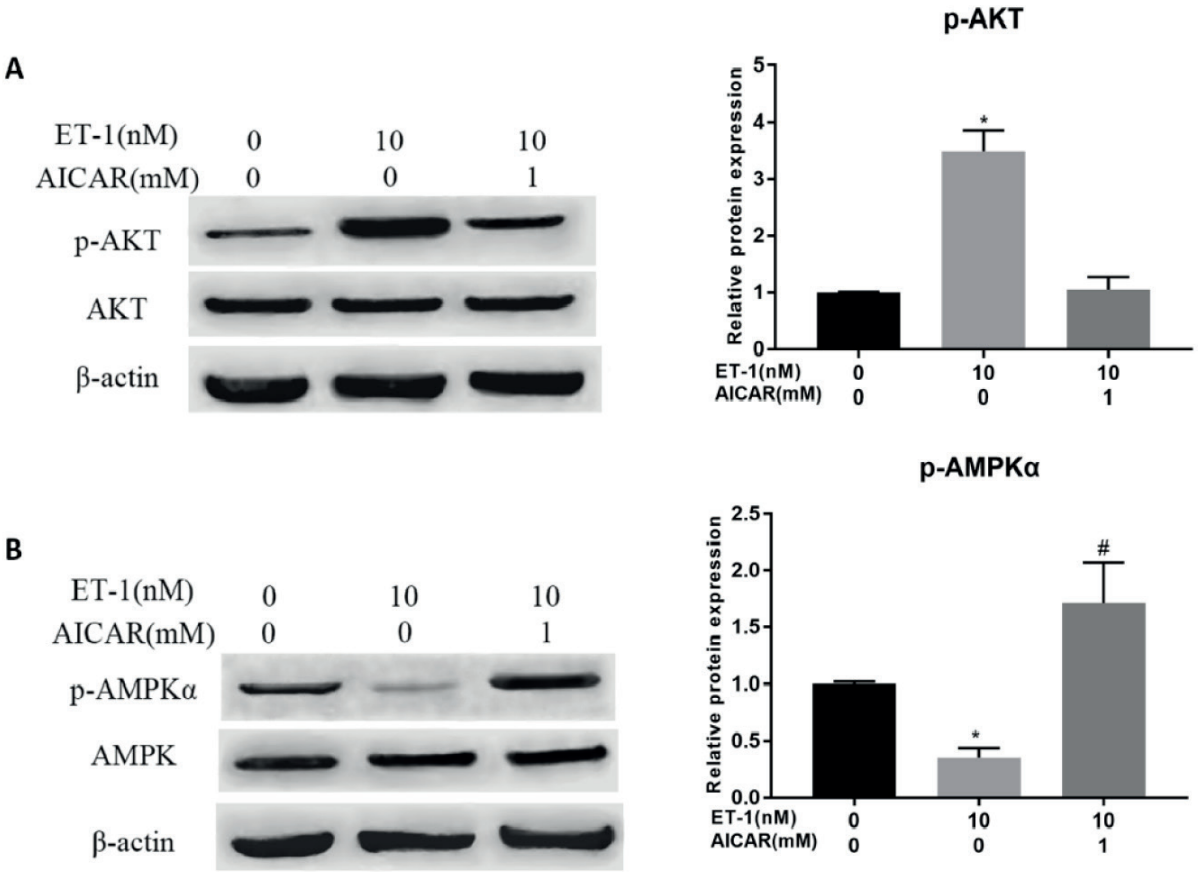

Figure 6. Effects of ET-1 and AICAR on AKT and AMPK pathway expressions in VECs. **A,** Effects of ET-1 and AICAR on AKT pathway-related proteins AKT and p-AKT in VECs detected *via* WB: ET-1 can markedly promote p-AKT protein expression (p<0.05), but it has no significant effect on the protein expression of AKT (p>0.05). AICAR can significantly inhibit p-AKT protein expression (p<0.05). **B,** Effects of ET-1 and AICAR on AMPK pathway-related proteins AMPK and p-AMPKα in VECs detected *via* WB: ET-1 can remarkably suppress p-AMPKα protein expression (p<0.05). However, it has no significant effect on the protein expression of AMPK (p>0.05). AICAR can significantly up-regulate p-AMPKα protein expression (p<0.05). * & #p<0.05: A significant difference in this group *vs.* the other two groups.

in rats with high-fat diet-induced CHD declined p-AKT, and the AMPK pathway was inhibited. After the AMPK pathway was activated by AICAR, the level of APN was significantly upregulated. APN can enhance the body's sensitivity to insulin and declined APN level indicates decreased sensitivity to insulin[19]. In this study, CHD rats were fed with high-fat diet, causing obesity. Obesity is the basis of pathogenesis of CHD. Therefore, it may lead to low insulin sensitivity to a certain extent. Activating AMPK can effectively enhance the body's insulin sensitivity[20] mainly by upregulating APN level.

Leptin is a kind of adipocyte-secreted protein composed of 167 amino acids. It possesses physiological effects of reducing individual energy intake, promoting the increase of exercise, inhibiting fat synthesis, and consuming the fat[21]. Scholars[22,23] have demonstrated that leptin facilitates the proliferation of vascular smooth muscle cells, induces VEC oxidation and promotes platelet aggregation, thereby enhancing atherosclerosis development. Minokoshi et al[24] have observed that the AMPK pathway is able to stimulate fatty acid oxidation in skeletal muscles mainly through the hypothalamic sympathetic nervous system, reducing fat deposition. In this study, elevated level of leptin in CHD rats was probably due to leptin resistance caused by high-fat diet, which is a distinguishing feature of dietary obesity[22]. Ropelle et al[25] have proved that the reactivity of leptin in the hypothalamus can be increased by regulating the AMPK pathway. In this study, the level of leptin in CHD rats was effectively reduced by activation of the AMPK pathway. The possible reason is that activating AMPK pathway reduces the body's resistance to leptin, thus reducing leptin level through the negative feedback regulation.

NO is the central signaling molecule involved in vascular endothelial function, which is synthesized by NOS and L-arginine in VECs. AMPK can phosphorylate and activate eNOS at multiple sites[26]. AMPK-dependent activation of eNOS occurs after drug stimulation against VECs, such as AICAR. In this study[27], the AMPK pathway was remarkably activated in CHD rats after injection of AICAR. *In vitro* experiments confirmed that the activation of AMPK pathway was mainly realized by stimulating phosphorylation of eNOS, further promoting the expression of NO in VECs. This is consistent with the conclusion that NO release increases by AMPK-dependent phosphorylation of eNOS[5]. In addition, the elevated level of ET-1 due to CHD revealed that vascular endothelium-dependent vasomotor dysfunction was caused by the disease. Shi-Wen et al[28] have indicated that ET-1 can upregulate the expression level of p-AKT, leading to vascular endothelial contraction *in vivo*. Besides, there is a negative feedback regulation in AKT expression for the AMPK pathway expression[29]. *In vitro* experiments have demonstrated that activating the AMPK pathway can effectively reduce ET-1-induced up-regulation of AKT and inhibit the abnormality of vascular endothelial contraction caused by AKT expression. This may eventually alleviate the symptoms of CHD and restore the normal function of VECs.

Conclusions

In summary, activating the AMPK pathway may play a positive role in the normal function of VECs, and exert a certain curative effect on CHD in rats.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

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