Inhibiting mTOR enhanced Cardiac STAT3 Phosphorylation at Site Ser 727 and Attenuated Myocardial Ischemia Reperfusion Injury in Diabetic Rats

Xiang Xie
Wenzhou Medical University
Zhongbao Zhao
Perking university Shenzhen Hospital
Danyong Liu
Affiliated Hospital of Guangdong medical University
Dengwen Zhang
Guangdong Provincial People's Hospital
Yi He
Guangdong Provincial People's Hospital
Liangqing Zhang
Affiliated Hospital of Guangdong Medical University
Han Lin
Wenzhou Medical University
Sheng Wang
Guangdong Academy of Medical Sciences: Guangdong Provincial People's Hospital
Zhengyuan Xia (zyxia@hku.hk)
Hong Kong University: University of Hong Kong

Research Article

Keywords: Diabetes, myocardial ischemia reperfusion injury, mTOR, STAT3

Posted Date: May 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-266485/v1

Abstract

Background Reduced levels of myocardial STAT3 activity in diabetic hearts may contribute to the increased susceptibility to ischemia-reperfusion injury (I/RI). The protein mammalian target of rapamycin (mTOR) can regulate metabolism and cell processes and plays major roles in the dynamics of I/RI. However, the role of mTOR in regulation of myocardial STAT3 and thereby affect myocardial I/RI in diabetes at relatively late stages of the disease is unknown.

Methods Diabetes was induced by Streptozotocin in Sprague-Dawley rats. Myocardial I/RI was achieved with coronary occlusion for 30 minutes and reperfusion for 2 hours in absence or presence of the mTOR inhibitor rapamycin. In vitro cardiomyocyte hypoxia/re-oxygenation (H/R) was established within H9C2 cells.

Results In diabetic rats, the levels of troponin-I (Tn-I), lipid peroxidation products 15-F2t-Isoprostane (15-F2t-Iso) and MDA, and the expression of protein mTOR were all significantly increased,and SOD releasing, the expression of protein phosphorylation of STAT3(p-STAT3-Ser727) were both significantly decreased compared to non-diabetic rats. Myocardial I/RI significantly increased the infract size (IS) and further increased the mTOR activation and decreased p-STAT3-Ser727 compared to diabetic rats. The selective mTOR inhibitor rapamycin reversed these changes and conferred cardioprotective effect. In H9C2 cells, high glucose (HG) significantly increased lactic dehydrogenase (LDH) release, apoptosis cells, ROS release, activation of mTOR, and decreased p-STAT3-Ser727. H/R further increased cellular injury, mTOR knock-down significantly reduced H/R injury.

Conclusion Myocardial mTOR was enhanced in diabetes and contributed to I/RI. mTOR inhibition attenuated myocardial I/RI through increasing p-STAT3-Ser727.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

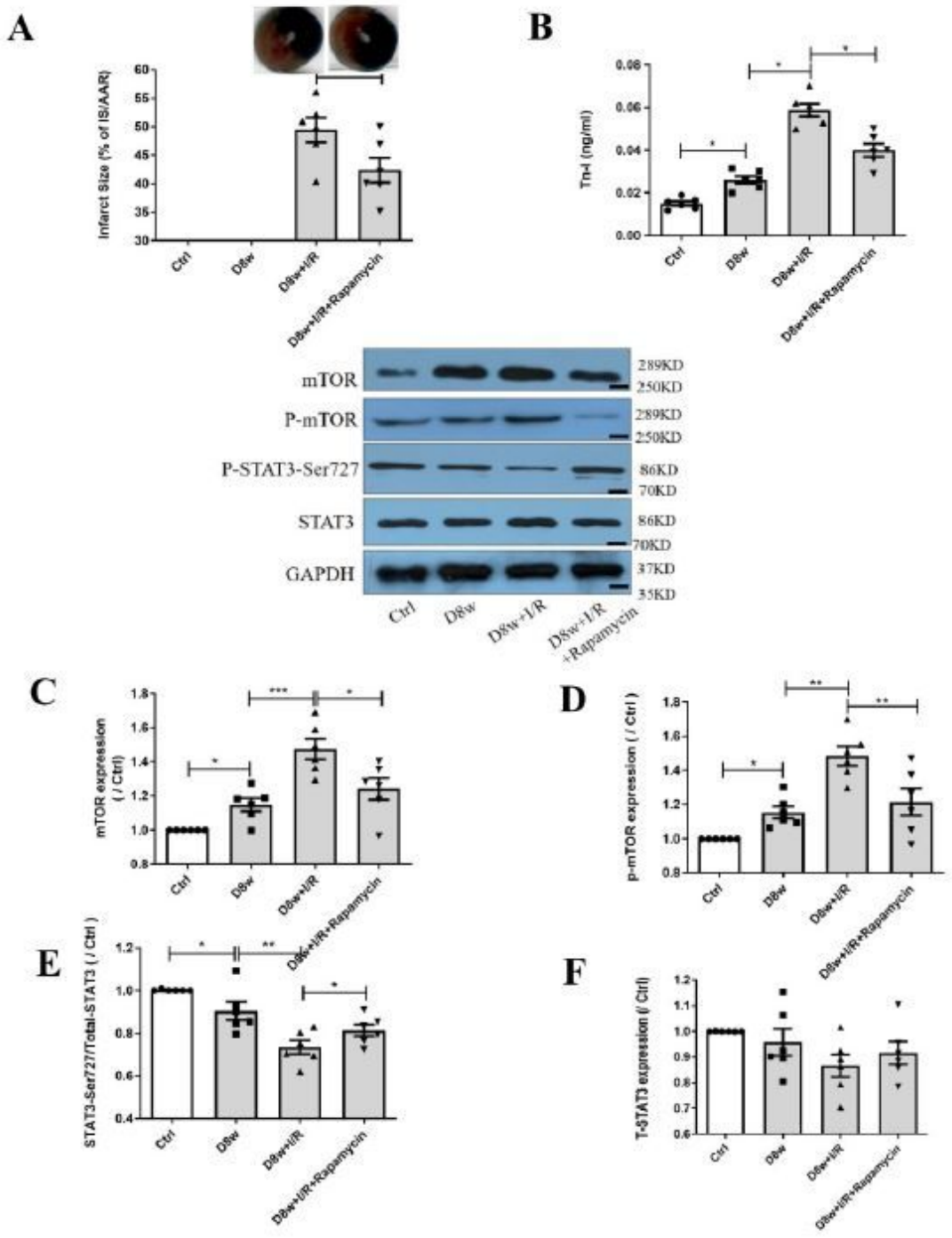

Figure 1

The effects of mTOR inhibitor Rapamycin on post-ischemic myocardial injury. A: infarct size (IS); B: serum Tn-I release; C: mTOR protein expression; D: phosphorylated mTOR (p-mTOR) protein expression; E: protein expression of p-STAT3(Ser727); F: STAT3 protein expression. Ischemia reperfusion (I/R) was achieved by 30 minutes ischemia followed by 2 hours reperfusion in diabetic rats. Date are expressed as mean ± SEM (n = 6 per group), *p<0.05.

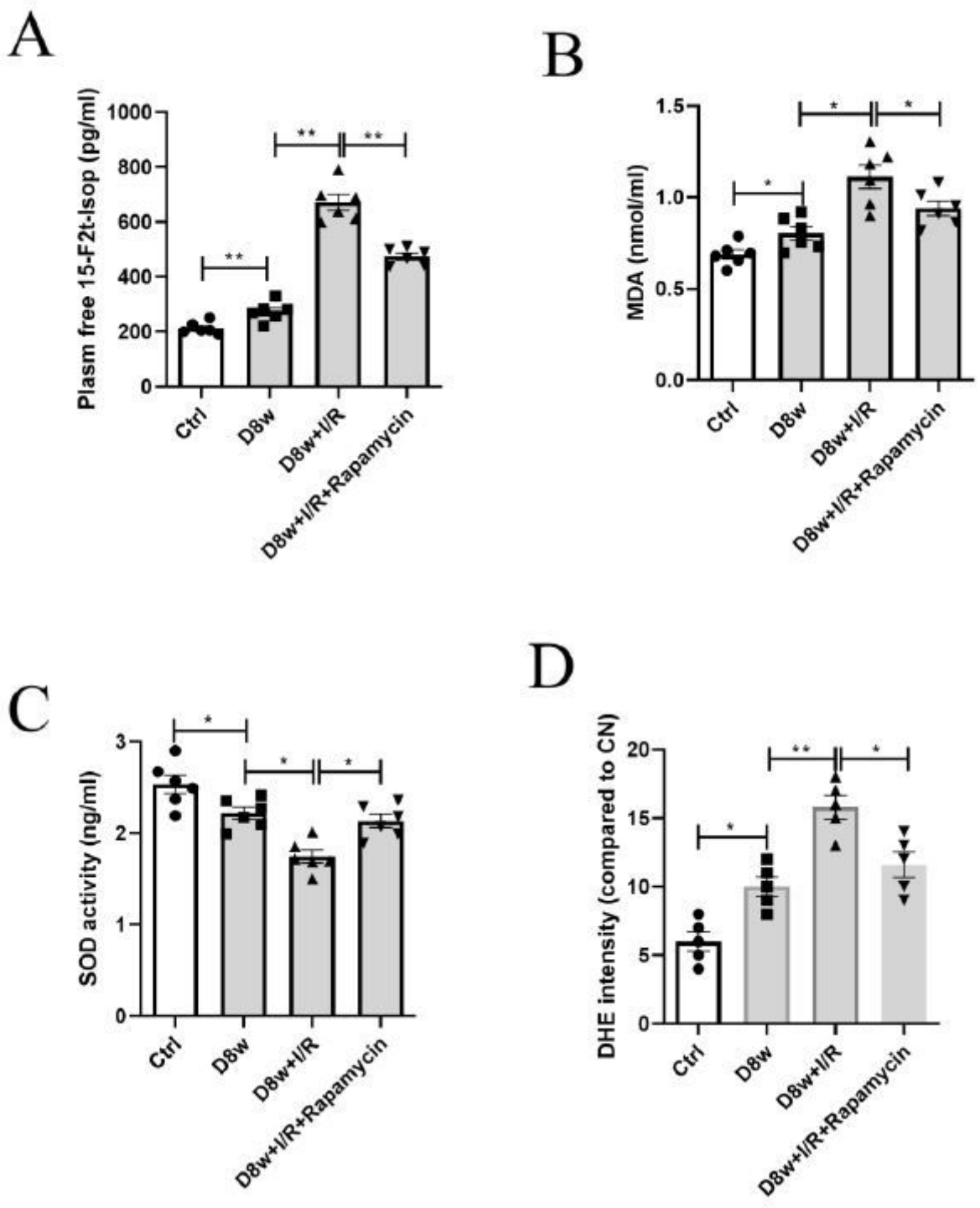

Figure 2

mTOR inhibition with Rapamycin decreased post-ischemic myocardial ROS production. A: 15-F2t-Isoprostane(15-F2t-Iso) release; B: MDA release; C: SOD activity; D: DHE staining. Ischemia reperfusion (I/R) was achieved by 30 minutes ischemia followed by 2 hours reperfusion in diabetic rats. Date are expressed as mean ± SEM (n =6 per group), *p<0.05.

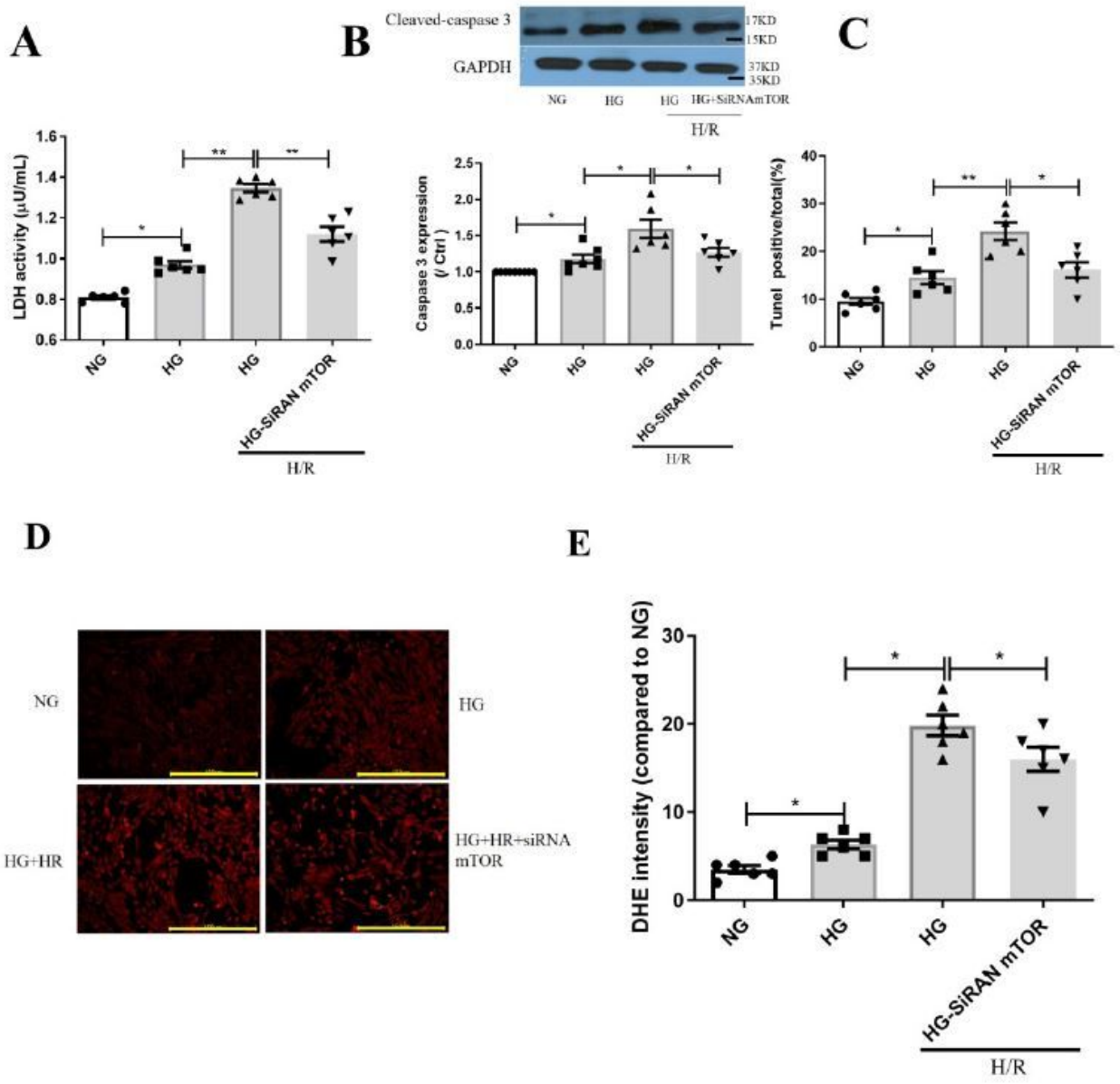

Figure 3

Effects of mTOR gene knockdown on hypoxia re-oxygenation(H/R) injury in H9C2 cells. A: LDH releasing; B: protein cleaved-caspase3 expression; C: TUNEL staining; D: DHE staining. In HG group, H9C2 cells were subjected to 30 mM high glucose for 48 hours, and then all cells subjected to 6 hours hypoxia and 12 hours reperfusion. Data are expressed as mean ± SEM of two independent experiments each performed in triplicate. n=6 per group. *p < 0.05.

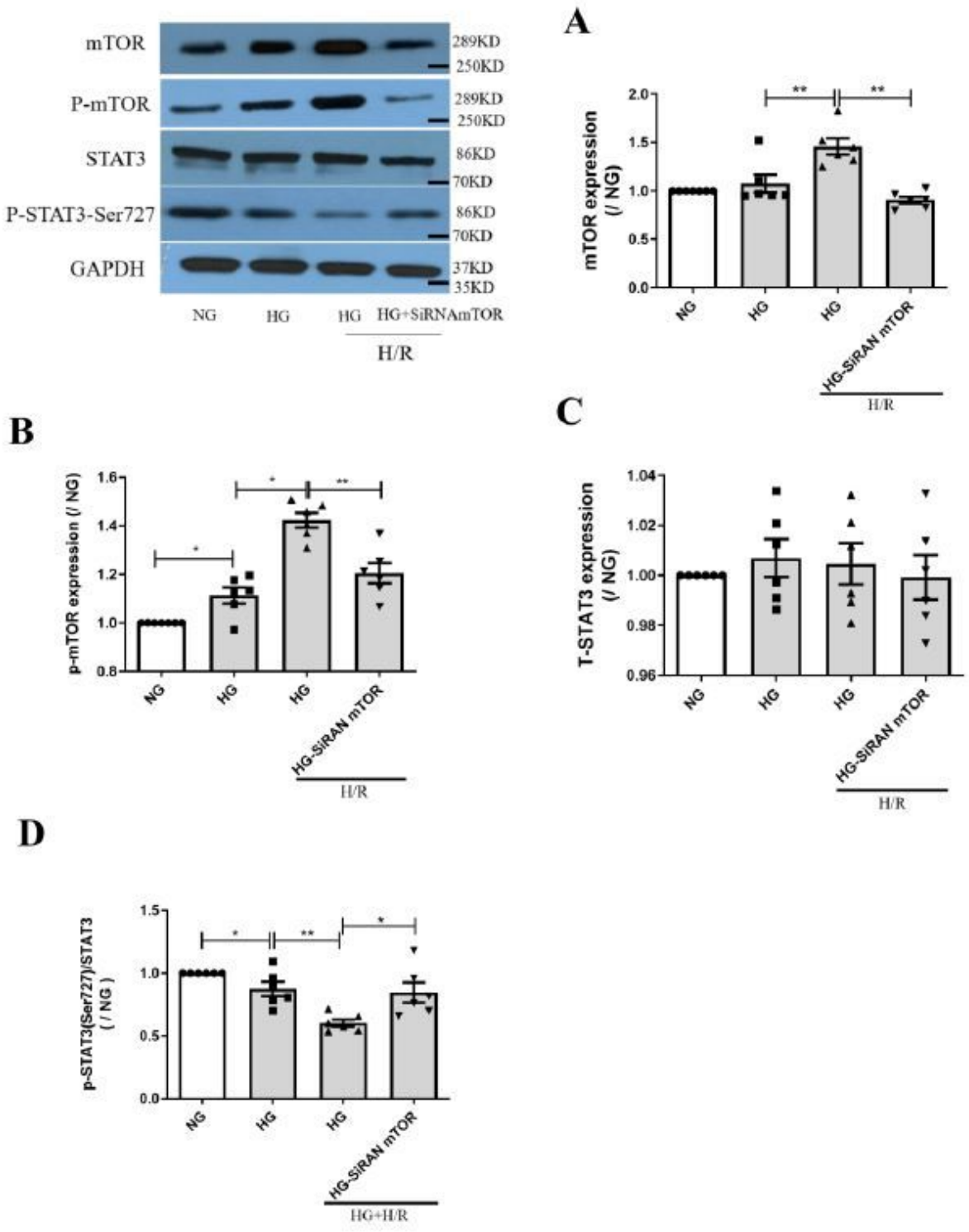

Figure 4

Effects of mTOR gene knockdown on mTOR and STAT3 protein expressions. A: protein mTOR expression; B: protein p-mTOR expression; C: protein STAT3 expression; D: protein p-STAT3(Ser727) expression. In HG group, H9C2 cells were subjected to 30 mM high glucose for 48 hours, and then subjected to 6 hours hypoxia and 12 hours reperfusion. Data are expressed as mean ± SEM of two independent experiments each performed in triplicate. n=6 per group. *P <0.05.